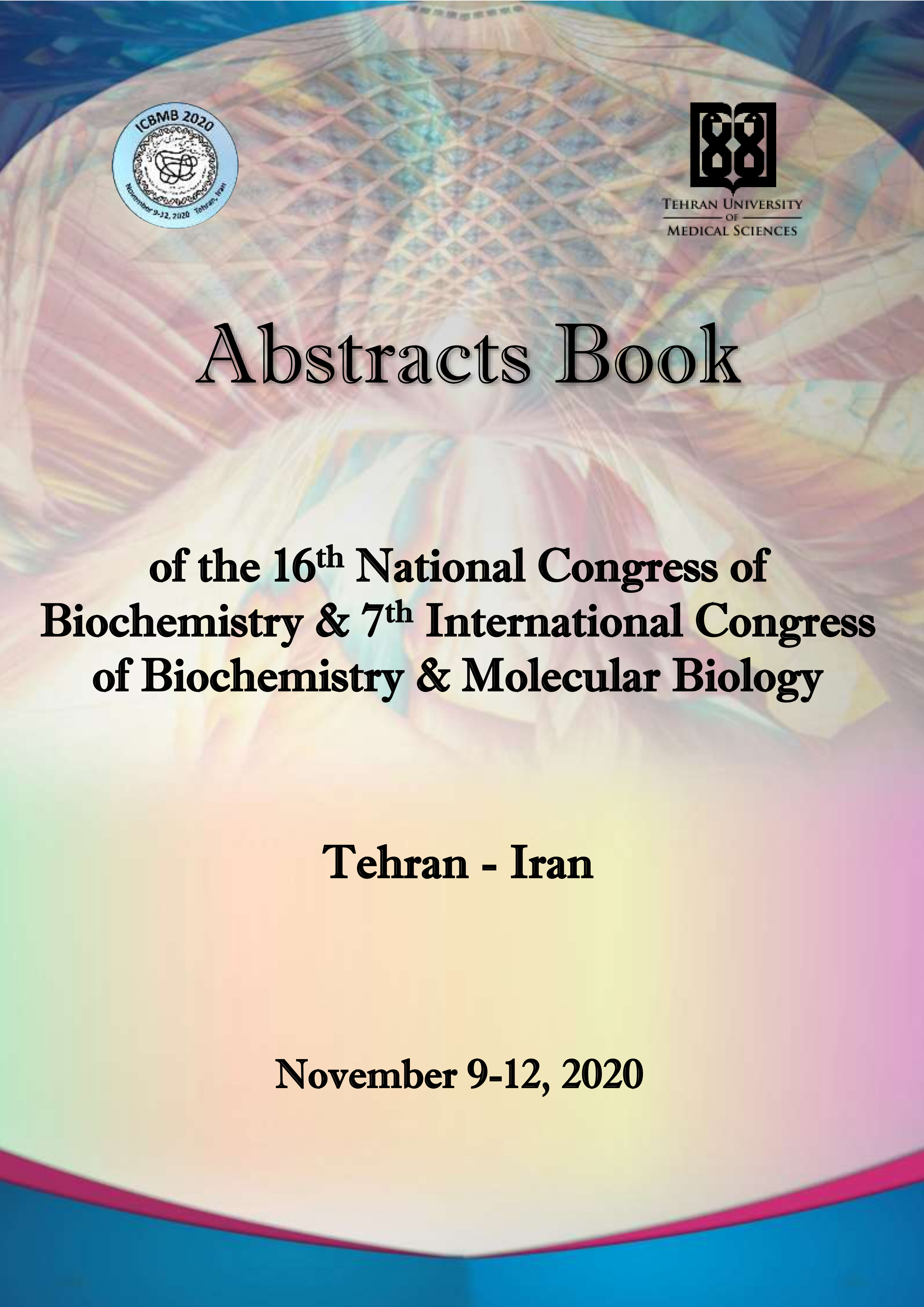

ICBMB 2020

TEHRAN UNIVERSITY
OF
MEDICAL SCIENCES

Abstracts Book

of the 16th National Congress of Biochemistry & 7th International Congress of Biochemistry & Molecular Biology

Tehran - Iran

November 9-12, 2020

‘In The Name of God’

بسم الله الرحمن الرحيم

Dear Colleagues

On behalf of the organizing committee, I am honored and delighted to welcome you to the Virtual 16th National and 7th International Congress of Biochemistry and Molecular Biology (ICBMB2020) which is going to be held on November 9-12, 2020. Following the cancellation of the ICBMB2020 on September in Tehran University of Medical Sciences, due to the COVID-19 pandemic, the organizing committee of the congress has decided to shift to a virtual meeting space with the goal of providing an engaging and meaningful experience for those who had expected to attend. The ICBMB2020 covers a wide range of critically important scientific sessions from basic and clinical research to innovations in the field of biochemistry and molecular biology. More than 20 sessions will be included in the program, which will start with IFCC Guidelines on Covid-19 Testing symposium. We have also tried to provide an opportunity for all researchers to display/talk about their research achievements and contradictions in front of the experts and budding scientists. Our online program offers state-of-the-art lectures, oral presentations of abstracts, panel discussions, and poster sessions. We are proud to offer the opportunity in continuing medical education for the attendances.

I hope that this virtual congress would provide valuable, useful and informative ideas to the participant students, researchers and other experts. I convey my best wishes for the success of the event.

With Kind Regards

Dr. Mohammad Ali Sahraian

Congress President

Dear Colleagues and Friends,

On behalf of the Biochemical Society of Iran and the organizing committee, it is our great pleasure to welcome you to the fully virtual 16th National and 7th International Congress of Biochemistry and Molecular Biology which will be held from November 9-12, 2020 (ICBMB2020). After canceling of the physical congress due to the COVID-19 pandemic, the organizing committee of the congress, has decided to put the best energy in organising the virtual congress. Virtual ICBMB2020 is the most important Iranian event in Biochemistry and Molecular Biology and it is an opportunity to show the active participation of the Biochemical Society of Iran founded about 60 years ago. The congress is held in collaboration with the International Federation of Clinical Chemistry (IFCC) and the International Union of Biochemistry and Molecular Biology (IUBMB). The scientific program will include Biochemistry and Molecular Biology. In particular, this virtual event in this very hard situation, provides an apportunity for us to strengthen our knowledge on the key role of Biochemical testing in monitoring of patients with COVID-19.

We have been greatly encouraged by an outstanding number of abstract submissions; under these difficult circumstances, it is extremely appreciated. We hope that the scientific program of the virtual congress provide a wonderful opportunity to learn and hear from experts and to share your knowledge in the field of biochemistry and molecular biology.

We are looking forward to see all of you online on the 19-22th of November 2020.

With Warmest Regards

Dr. Reza Meshkani **Dr. Mohammad Taghi Goodarzi**

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Keynote Speakers

Molecular, serological, and biochemical diagnosis and monitoring of COVID-19: IFCC taskforce evaluation of the latest evidence

Khosrow Adeli [1], Giuseppe Lippi[2], Andrea Horvath[3]

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2. Department of Neuroscience, Biomedicine and Movement, University of Verona, Verona, Italy
3. Department of Clinical Chemistry and Endocrinology, New South Wales Health Pathology, Prince of Wales Hospital, Sydney, Australia

The global coronavirus disease 2019 (COVID-19) has presented major challenges for clinical laboratories, from initial diagnosis to patient monitoring and treatment. Initial response to this pandemic involved the development, production, and distribution of diagnostic molecular assays at an unprecedented rate, leading to minimal validation requirements and concerns regarding their diagnostic accuracy in clinical settings. In addition to molecular testing, serological assays to detect antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are now becoming available from numerous diagnostic manufacturers. In both cases, the lack of peer-reviewed data and regulatory oversight, combined with general misconceptions regarding their appropriate use, have highlighted the importance of laboratory professionals in robustly validating and evaluating these assays for appropriate clinical use. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Task Force on COVID-19 has been established to synthesize up-to-date information on the epidemiology, pathogenesis, and laboratory diagnosis and monitoring of COVID-19, as well as to develop practical recommendations on the use of molecular, serological, and biochemical tests in disease diagnosis and management. This review summarizes the latest evidence and status of molecular, serological, and biochemical testing in COVID-19 and highlights some key considerations for clinical laboratories operating to support the global fight against this ongoing pandemic. Confidently this consolidated information provides a useful resource to laboratories and a reminder of the laboratory’s critical role as the world battles this unprecedented crisis.

Developing a novel radiation cancer therapy by employing monochromatic X-ray and nanoparticles

Fuyuhiko Tamanoi

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We are trying to develop a new type of radiation therapy that combines synchrotron-generated monochromatic X-rays with mesoporous silica nanoparticles (MSNs) loaded with high Z elements such as gadolinium, iodine, gold or silver. Our approach is to deliver high Z elements to the tumor by the use of nanoparticles and irradiate with monochromatic X-ray generated at Spring-8. This is based on the Auger effect proposed by Pierre Auger in 1923. It is postulated that irradiation of an element such as gadolinium with a monochromatic (monoenergetic) X-ray at an energy level that corresponds to the K-edge energy results in photoelectric effects that includes the release of Auger electrons that causes double strand DNA breaks which then leads to apoptosis of cancer cells. Our approach will provide a solution to the problem with current radiation therapy regarding adverse effect on normal tissues, as our approach is expected to have less effect on non-cancerous tissues. As a proof-of-principle study, we used tumor spheroids incubated with gadolinium-loaded MSNs to distribute gadolinium throughout the tumor spheroids and accumulation of gadolinium in perinuclear regions inside cancer cells. Irradiation of the tumor spheroids with 50.25 keV monochromatic X-ray resulted in complete destruction of tumor spheroids, while irradiation with 50.0 keV X-ray had no effect. The tumor destruction was dependent on the presence of gadolinium, as no spheroid destruction was observed only in the presence of gadolinium. Significance of our results will be discussed.

Artemisinin and derivatives against viruses: *in vitro*, *in vivo* and clinical evidences

Thomas Efferth

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Traditional Chinese medicine commands a unique position among all traditional medicines because of its 5000 years of history. Our own interest in natural products from traditional Chinese medicine was triggered in the 1990s, by artemisinin-type sesquiterpene lactones from *Artemisia annua* L. As demonstrated in recent years, this class of compounds has activity against malaria, cancer cells, and schistosomiasis. Interestingly, the bioactivity of artemisinin and its semisynthetic derivative artesunate is even broader and includes the inhibition of certain viruses, such as human cytomegalovirus and other members of the Herpesviridae family (e.g., herpes simplex virus type 1 and Epstein-Barr virus), hepatitis B virus, hepatitis C virus, and bovine viral diarrhea virus. A role against SARS-CoV-2 mediated COVID-19 is discussed. Analysis of the complete profile of the pharmacological activities and molecular modes of action of artemisinin and artesunate and their performance in clinical trials have further elucidated the full antimicrobial potential of these versatile pharmacological tools from nature.

November 9-12, 2020 Tehran, Iran

Natural C20 Carotenoids: Biochemical, Pharmacological and Therapeutic Potential in Different Diseases

Seyede Zahra Bathaie

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There are at least two types of carotenoids in the nature. The C40 lipid soluble carotenoids such as β-Carotene and C20 carotenoids that can be lipid or water soluble. Crocin is a C20 water soluble and crocetin is a C20 lipid soluble carotenoids. The main source of C20 carotenoids in the nature is saffron. Saffron is the most expensive spice in the world, which is mainly cultivated and produced (> 85% of the world production) in Iran. It has been used as a home remedy from the ancient time in many nations, including Iran, Greece, Egypt, India, China and etc. Nowadays, saffron is cultivated in many countries. But, its quality is different and the percentages of the constituents are not the same. We isolated different saffron constituents in my Lab., from 20 years ago and investigated the biological and pharmacological properties of these constituents.

All of these constituents, especially saffron carotenoids showed the anticancer, antidiabetic, antioxidant/ oxidant activities. They can be used to kill the cancer cells, while they can protect the nerve cells in some disorders such as Alzheimer diseases. These components are also very useful to improve the atherosclerotic lesions in both animal models and human.

The main question is how can these components exert such contradictory effects? What are the mechanism of their action? And how can they distinguish these different situations? To answer these questions, we investigated the signaling pathways before and after treatment of cells, animal models or patients with the mentioned diseases; and any alterations in the expression of target proteins were considered. We hope to find the main target of these components and to solve this mystery of the nature. In this presentation, I will discuss some of our findings in this regard.

Epigenetic modifications in cancer: Implications for epi-drug and epigenetic editing

Zohreh Rahimi

Department of Clinical Biochemistry, Kermanshah University of Medical Sciences, Kermanshah, Iran

Epigenetic modifications including alteration in DNA methylation, histone modification and changes in non-coding RNA gene expression are known to be involved in the pathogenesis, progression and metastasis of cancer. Due to the reversibility and the plasticity of epigenetic processes they are targeted in cancer therapy. DNA hypermethylation is associated with silencing of tumor suppressive and differentiation genes. So, DNA methyltransferase inhibitors have emerged as first epi-drugs for cancer treatment. Also, histone deacetylase inhibitors, histone methyltransferase inhibitors, histone demethylase inhibitors and inhibitors of epigenetic readers are among epigenetic modulators. In this paper, three epigenetic mechanisms will be discussed and drugs targeted these mechanisms and also epigenetic editing technologies, zinc finger proteins and transcription activator-like effector proteins, and the clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease 9 (CRISPR/Cas9) system as a revolutionary genome-editing technology, as strategies to reverse epigenetic changes in cancer will be explained.

Convalescent plasma and hyperimmune globulins in fight against SARS-CoV-2

Aliasghar Rahimian[1], and Mahdi Aminian [1]

1. Department of Clinical Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

The outbreak of COVID-19 has become a major health issue all over the world. No specific medicine against SARS-CoV-2 infection has been proven so far and the supportive cares and using existing anti-viral therapies remain as the main effort for treatment of mild to severely ill patients. To date, the clinical research efforts are mainly focused on repurposing the existing anti-viral therapies, immunomodulatory agents and anti-inflammatory drugs. As a historical approach, convalescent plasma therapy has been used for treatment of 1918 flu outbreaks and other viral respiratory syndromes. In 2014, World Health Organization (WHO) has published a comprehensive guideline for the use of convalescent blood or plasma collected from recovered patients as an empirical treatment during Ebola outbreaks. This approach has been reported to improve the survival rate and to shorten the hospitalization time of infected patients in previous outbreaks of respiratory infections similar to COVID-19 such as SARS coronavirus during 2004 outbreak. Health authorities and medical leaders consider this approach as a promising research area for COVID-19 treatment and, Food and Drug Administration (FDA) has approved the use of convalescent plasma for investigational treatment of critically ill patients with COVID-19. Here, we explain a roadmap for rapid establishment of convalescent-plasma-based COVID-19 therapies. This study explains two major approaches. First, being the main requirements for urgent initiation of plasma transfusion from recovered patients to critically ill patients. The second one provides an overview of purification and manufacture of hyperimmune globulins against SARS-CoV-2 from convalescent plasma. It should be noted that plasma transfusion and hyperimmune globulins in treatment of infectious diseases have their own advantages or disadvantages. Therefore, local health authorities should decide the more applicable approach regarding the local status of disease outbreak and available facilities.

Keywords: COVID-19, Transfusion therapy, Plasma derivatives.

Exocytosis Proteins and Diabetes: link to pathophysiology, prevention, complication and treatment

Mohammad Taghi Goodarzi

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Insulin resistance and defective insulin secretion are the two leading pathological causes in type 2 diabetes. Exocytosis proteins mediate insulin secretion and glucose uptake. Among the wide range of proteins that are involved in exocytosis, the soluble N-ethylmaleimide-sensitive factor activating protein receptors or SNAREs play important roles in membrane trafficking, docking and fusion. Deficiencies in these proteins are involved in diabetes incidence. The isoforms of VAMP-2, syntaxin-4 and SNAP-23 play important roles in regulating GLUT-4 trafficking and vesicle fusion in adipocytes. GLUT4 is important factor for glucose uptake into muscle cells and adipocytes. Therefore, dysfunction of the SNARE complex inhibits translocation of GLUT4 to the cell surface and subsequently leads to increased blood glucose and insulin resistance.

Here the mechanisms of exocytosis proteins in health and disease, particularly in diabetes, will be discussed. Since exocytosis proteins might be ideal candidate targets for prevention and treatment of type 2 diabetes, the capabilities of these proteins in improvement of metabolic dysregulation will be described. Furthermore, the findings of some reports about using natural factors such as resveratrol in SNARE protein gene expression will be presented.

The impact of N-and C- terminal peptide fragments on IMPDH1 interaction with specific DNA and on the formation of super-macromolecular structures of cytoophidia

Razieh Yazdanparast

Institute of Biochem. Biophys. University of Tehran, Tehran, Iran

It is by now well accepted that mutations in the retinal inosine monophosphate dehydrogenase1 (IMPDH) gene is one cause of inherited redtina-specific disorder named RP10. The main structural difference between the retinal IMPDH1 isforms and the canonical form resides in the C- and N- terminal extensions with as yet undefined functional role(s). Besides, it has recently been documented that IMPDH1, the committing enzyme in the *de novo* purine biosynthetic pathway, is a moonlighting enzyme involving in many other biological functions namely acting as a transcription factor, implying its interaction with specific DNA sequence(s). In this report, I aimed to report on the impact(s) of these terminal extensions of the mouse retinal IMPDH1, mainly 603 and 546 isofroms, on the specific /non specific ssDNA-binding activities compared to that of the canonical isoform. In addition, regarding the recent reports on the effect of GDP/GTP cellular content on the mode of enzyme activity regulation via the novel approach of cytoophidia (membrane less cellular organelles) formation, we also decided to evaluate the impact of the terminal extensions on this event using *in vitro* approaches. Our results clearly indicated that the binding of the recombinant mouse retinal isoform, IMPDH1 (603), to specific target gene was significantly augmented, while its binding to non-specific ssDNAs was lower than that of the canonical form. Additionally, it became evident that the end-terminal extensions attenuated the extent of GDP/GTP inhibitory response on the macromolecular complex (cytoophidia) formation.

These results would certainly provide the ground for future physiological evaluation of these results and hopefully to a link to RP10 disease. Details will be discussed in the presentation.

Biomaterial Conjugations for Theranostic Applications

Nosratollah Zarghami [1], Nasrin, Mohajeri [1]

[1] Department of Clinical Biochemical Biochemistry, Tabriz University of Medical Sciences, Tabriz Iran

Background: Biomaterial conjugation with other moieties is an appealing target-tracking strategy. Tracking and imaging the function of biomolecules, especially biomarkers, plays a pivotal role in disease identifications. With increasing advancement in cancer cell biomarker detection and new capabilities of nucleic acid and saccharide biomaterials in form of nanomaterials, Epidermal Growth Factor Receptor (EGFR) and miR-21 tumor markers are main targets for theranostic implementations.

Methods: Plant extract and nucleic acid sequence used as a fluorescence probe and synthesized through the hydrothermal method and characterized by HR-TEM, EDAX analysis, FTIR, size, and zeta potential. Chitosan with saccharide source used as a polymeric carrier (nanogel) of miR-21 and probe agents produced by the ionic method. To EGFR diagnosis, the covalent conjugation of DNA-dot to EGFR antibodies was done EDC/ imidazole chemistry by covalence linkage. Also, fluorescent theranostic imaging of biomarkers was done under fluorescent microscopy.

Results: The average size of fluorescence probes was 4.5-5 nm which was conjugated to EGFR antibody and chitosan polymer. DNA-dot@EGFR conjugates identified lung cancer cells with 84–92% specificity and 100% sensitivity. Moreover, the chitosan nanoparticles contain miR-21 and fluorescence probe showed uniform size distribution of (104 nm). Nanoparticles without toxicity transferred antimiR sequence to cancer cells with 80-90% entrapment efficiency and good serum stability. The nanogel targeted with folate can detect the ovarian cancer cell line.

Conclusion: DNA-dot@ antibody is potentially a toxic-free and efficient method of antibody labeling that opens up new horizons in fluorescent nanoimaging in the field of cancer cell detection. Also, chitosan nanogels provide cancer-specific imaging with the potential of theranostics agents

The pathogenic role of the SP/NK1R system in glioblastoma multiforme through the modulation of redox status

Seyed Isaac Hashemy

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Altered redox balance is among the main contributing factors developing glioblastoma multiforme (GBM), a highly aggressive grade IV brain tumor. Neuropeptide substance P (SP) plays a key role in modifying the cellular redox environment through the activation of neurokinin-1 receptor (NK1R). In this study, we aimed to investigate the redox-modulating properties of both SP and a commercially available NK-1R antagonist, aprepitant in GBM cells. To detect the effect of aprepitant on the viability of U87 glioblastoma cells, resazurin assay was applied. The level of intracellular malondialdehyde (MDA), thiol content, total anti-oxidant capacity (TAC), and reactive oxygen species (ROS) were also assessed. The expressions of thioredoxin (Trx), thioredoxin reductase (TrxR), and glutaredoxin were measured by quantitative real-time polymerase chain reaction (qRT-PCR). Concurrently, the activity of these redox-active proteins was also analyzed. Our results showed that SP-mediated activation of NK1R not only increased the intracellular level of MDA and ROS, but also reduced the concentration of thiol in U87 cells. We also found that upon SP addition, there was a significant reduction in TAC of the cells, which shed more lights on the probable participation of the SP/NK1R axis in the regulation of oxidative stress in glioblastoma cells. Aprepitant remarkably decreased these effects. Besides, SP reduced both expression and enzymatic activity of Trx, TrxR, and glutaredoxin, and these effects were significantly decreased by aprepitant. In conclusion, our results suggest a possible involvement of SP/NK1R signaling in GBM pathogenesis through oxidative stress and offering new insight for the application of aprepitant as a redox modulating strategy in GBM patients.

Nicotinamide adenine dinucleotide (NAD$^+$) in cancer and its treatment potentials

Mitra Nourbakhsh, PhD

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Cancer cells have an exceptional energy metabolism for supporting rapid proliferation needed for cancer development. There is a preference for anaerobic metabolism characterized by enhanced glycolysis which apart from energy provision, supports the production of nucleotides, lipids, and amino acids required for cancer cell proliferation. Nicotinamide adenine dinucleotide (NAD$^+$) is a coenzyme that facilitates redox reactions in several metabolic pathways, including glycolysis. NAD$^+$ is also used as a substrate for poly (ADP-ribose) polymerase (PARP), many sirtuin enzymes, and NAD$^+$ glycohydrolase (CD38 and CD157). Unlike metabolic reactions in which NAD$^+$ is recycled, the activity of these enzymes lead to the degradation of NAD$^+$ and its depletion inside the cell. Therefor a constant production of NAD$^+$ is vital for survival of cancer cells and these cells critically rely on the continuous intracellular provision of NAD$^+$. NAD$^+$ is synthesized from either *de novo* pathways using aspartic acid or tryptophan as the substrate, or salvage pathway using nicotinamide or nicotinic acid as the starting material. The salvage pathway predominates in humans, with nicotinamide phosphoribosyltransterase (NAMPT) serving as the rate limiting enzyme.

NAD$^+$-consuming enzymes carry on a wide range of reactions including epigenetic modifications and transcriptional regulation, DNA repair, calcium mobilization, and life span regulation. Thus, NAD$^+$ is involved in cancer pathogenesis beyond energy metabolism and affects cancer progression and its response to treatment.

NAMPT overexpression is a common phenomenon in cancer cells, fueling NAD$^+$ to guarantee cell survival and boost tumorigenic properties. The underlying mechanisms that are responsible for the increased expression of NAMPT are not clearly understood; however, down-regulation of suppressive microRNAs, has been suggested as a plausible mechanism. Therefore, microRNA-based strategies for the attenuation of NAMPT are currently under investigation. Inhibition of NAMPT by chemical inhibitors have also shown promising results for induction of apoptosis in cancer cells and increasing treatment efficiency.

Phytoestrogen Effects on Female Reproductive System: Disorders and Cancers

Fatemeh Zal[1,2], Zohreh Mostafavi-Pour[1], Mohammad Samare-Najaf[1], Ayeh Bolouki [1], Sina Vakili[1], Asma Neisy[1], Navid Jamali[1], Majid Jafari Khorchani[1]

[1]Biochemistry Department, Medical School, Shiraz University of Medical Sciences, [2]Infertility research center, Shiraz University of Medical Sciences, Shiraz, Iran.

Introduction: Phytoestrogens are found in various types of plants and are able to bind to estrogen receptor. Quercetin is one of the phytoestrogens that is found in fruits and vegetables. The useful effects of quercetin on diseases associated with estrogen deficiency have been found in the past years. For example, the prevalence of breast cancer that is significantly lower in regions where people use flavonoid containing foods. As phytoestrogens mimic the estrogen's activity, in recent studies we focused on preventive effects of quercetin on female reproductive system disorders like doxorubicin-induced toxicity in the ovary and uterus, ovariectomy-induced osteoporosis, DHEA-induced PCOS and insulin resistance, endometriosis and decrease in the implantation rate and uterine receptivity in diabetes. **Methods:** The techniques and experiments that were used include: histopathological-stereological methods, the expression of ERα, GLUT4, nesfatin1, aromatase and adipoR1 genes that were quantified with real-time PCR and also measuring serum adiponectin and sex-steroid levels. In ovariectomized rats, osteoporosis markers and mRNA expression of autophagy related genes were analyzed in serum and tibia of rats. In endomeriosis model, the size and histoarchitecture of the endometrial implants, serum levels of 17-β estradiol, progesterone and Tumor necrosis factor (TNF)-α, markers of oxidative stress and autophagy were assessed. In diabetes model blastocysts were recovered at fourth day of pregnancy for protein and mRNA expression changes. Uterus was harvested at day four of pregnancy for implantation rate assays. **Results:** Our studies showed the protective role of this phytoestrogen against doxorubicin-induced toxicity in uterine and ovarian tissues and improve the diabetic, obesity and infertility symptoms of PCOS (reduction of PCOS-IR and uterus GLUT4 and ERα expression). Quercetin prevented osteoporosis by regulating the total number of bone cells, maybe through regulating autophagy and apoptosis. Administration of quercetin prior to pregnancy alleviated reproductive problems in diabetic mice likely via its estrogenic and antihyperglycemic effects. In addition, we could show the quercetin, metformin and their combination had potential therapeutic effect in the rat model of endometriosis.

Conclusion: Therefore, this review article provides evidence that quercetin could be considered as a potential agent to attenuate female reproductive disorders and fertility complications.

Keywords: Phytoestrogen, Reproductive System, Quercetin, Estrogen deficiency

Oral Presentations

O-1

Comparison between binding affinity of spike-glycoprotein and ACE2 receptor in SARS-CoV and SARS-CoV2

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Background: Improved binding affinity within SARS-CoV2 and human angiotensin-converting enzyme 2 (hACE2) was suggested for correlating with the incremented disease severity and virus transmissibility in humans. In this study, we focused on protein-protein interaction of spike protein and ACE2 receptor by in silico approach. Therefore, the analyze them are widely applied to discover the inhibitor and new drugs for control of corona virus debases.

Methods: In this study, various computational methods were utilized including molecular dynamics simulation and docking. Every molecular detail could be explained by these methods from conformational alterations over the interaction of the virus with its receptor to molecular binding phenomena within the virus-receptor system at the atomic level.

Results: Based on our results, the highest binding energies were related to SARS-CoV2 with -31.5759 ±2.4425 kcal.mol^{-1} when compared to Sars-Cov and also showed that, electrostatic interactions have an important role in binding affinities between SARS-CoV2 and ACE2. We also used PyContact for analysis of non-covalent interaction between spike and ACE2 receptors in MD simulation to discover more detail about spike-ACE2 contact during the MD. The results of this comparison show that the sars-COV2 attaches to much more specific positions of the receptor than the SARS-COV.

Conclusion: In our work, an intensive structural assessment was performed to understand the dynamics and conformational motions occurring in the interaction of SARS-CoV and SARS-CoV2 with its receptor. The results of this study helped to introduce a new therapeutic target for drug and especially peptide design, for the treatment of patients with covid19.

Keywords: Key world: spike-glycoprotein, ACE2 receptor, SARS-CoV, SARS-CoV2, binding affinity

O-2

Evaluation of total antioxidant capacity and oxidative stress markers in COVID-19 infected individuals

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Background: Coronavirus disease 2019 (COVID-19) has reached a universal pandemic posing a great risk to public health. Viral infections caused by respiratory viruses are often associated with increased ROS production. Although ROS such as H2O2 and superoxide anion (O2 -.) are naturally produced during cellular metabolic processes, an excessive amount of them due to viral infections may result in uncontrolled oxidative stress and depletion of specialized antioxidant systems. The present study aims to investigate the total antioxidant capacity (TAC) and oxidative stress markers in patients with COVID-19 infection by determining serum levels or activities of oxidative stress markers.

Methods: Totally 120 patients with COVID-19 infection and 60 healthy controls were enrolled in the study. The patient group consisted of 60 COVID-infected individuals with advanced disease and 60 cases without any related symptoms. Determination of serum concentration of nitric oxide (NO), serum activities of catalase (CAT) and superoxide dismutase (SOD) as well as total antioxidant capacity were done by ELISA kits (Zellbio) under the manufacturer's instructions.

Results: Our data revealed that serum levels of NO were significantly higher in COVID-19 infected individuals particularly in patients with severe acute respiratory syndrome (SARS) ($P < 0.05$). A reductive trend was also seen in TAC due to COVID-19 infection. By contrast, serum activities of SOD and CAT were higher in healthy subjects compared with infected individuals ($P < 0.05$).

Conclusion: In conclusion, we demonstrated that COVID-19 infection may be associated with reduced antioxidant defense and increased oxidative stress.

Keywords: COVID-19, Anti-oxidant, Oxidative stress, SOD, CAT

O-3

3D-QSAR Pharmacophore Modeling, Virtual Screening, Molecular Docking, Molecular dynamics Simulation and QM-MM studies of Angiotensin-converting Enzyme 2 and Main Protease of Sars-Cov-2: An Effort for Finding Treatment of COVID-19

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Background: The angiotensin-converting enzyme 2 (ACE2) and main protease (MPro), are the putative drug candidates for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Methods: In this study, we performed 3D-QSAR pharmacophore modeling and screened 1264479 ligands gathered from Pubchem and Zinc databases. Following the calculation of the ADMET properties, molecular docking was carried out. Moreover, de novo ligand design was performed. MD simulation was then applied to survey the behavior of the ligand-protein complexes. Furthermore, MMPBSA was utilized to re-estimate the binding affinities. Then, a free energy landscape was applied to find the most stable conformation of the complexes. Finally, the hybrid QM-MM method was carried out for the precise calculation of the energies.

Results: The Hypo1 pharmacophore model was selected as the best model. Our docking results indicate that the compounds ZINC12562757 and 112260215 were the best potential inhibitors of the ACE2 and MPro, respectively. Furthermore, the Evo_1 compound enjoys the highest docking energy among the designed de novo ligands. Results of RMSD, RMSF, H Bond and DSSP analyses have demonstrated that the lead compounds preserve the stability of the complexes' conformation during the MD simulation. MMPBSA results are in accordance with the molecular docking results. The results of QM-MM showed a stronger Evo_1 potential in MPro-specific inhibition, as compared to the 112260215 compound. **Conclusion**: In conclusion, our results showed that the de novo designed Evo_1 compound has the potential to be used as a drug for the treatment of COVID-19; however, further in vitro and in vivo validations are required.

Keywords: COVID-19, SARS-CoV-2, main protease, ACE2, molecular dynamics simulation

O-4

Umbelliprenin suppresses angiogenesis signaling in SKBR-3 cell line by downregulation of EGF / $CoCl_2$ -mediated PI3K / AKT / MAPK

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Background: Umbelliprenin (UMB), a prenylated coumarin of different species of Ferula, has demonstrated anti-cancer effects in various types of cancer cells, but the potential molecular mechanisms to the anti-angiogenic activity of UMB in breast cancer cells have not yet been studied. In this study, we investigated the possible molecular pathways involved in the anti-angiogenic effect of UMB in EGF and $CoCl_2$ stimulated SKBR-3 breast cancer cells.

Methods: Effects of UMB on the changes in EGFR signaling genes (EGFR, PI3K, AKT, mTOR, S6K, 4EBP1, ERK1/2, HIF-1α, HIF-1β, VEGF, VEGFR) and proteins (VEGF/HIF-1α) expression were assayed in SKBR-3 via Quantitative PCR and Western blotting assays.

Results: UMB decreased dramatically the living cells in a concentration manner (103.9 ± 27.43 µM) and non- toxic doses of UMB IC_5 and IC_{10} (10 and 20 µM, respectively) were used for *in vitro* anti-angiogenic effects. UMB significantly reduced pro-angiogenic AKT, ERK1, ERK2, mTOR, S6K, HIF-1α, HIF-1β, VEGF and VEGFR mRNAs in EGF-treated, and AKT, ERK2, S6K, HIF-1α, HIF-1β, VEGF and VEGFR mRNAs in $CoCl_2$-treated cells. UMB significantly increased anti-angiogenic 4EBP1 mRNA in EGF / $CoCl_2$-treated cells. UMB significantly decreased the levels of HIF-1α and VEGF proteins, in $CoCl_2$-treated cells.

Conclusion: Our findings shown that UMB exhibits anti-angiogenic effects by decreasing the expression of AKT/mTOR/MAPK angiogenesis pathways in EGF or $CoCl_2$ treated SKBR-3 breast cancer cells.

Keywords: Umbelliprenin, angiogenesis, EGF, $CoCl_2$, breast cancer

O-5

Zerumbone Promotes Cytotoxicity and Suppress Migration and Invasion in Human Malignant Glioblastoma Cells

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Background: Glioblastoma multiforme (GBM) is the deadliest and most prevalent tumor in the central nervous system. Unfortunately, the prognosis of GBM patients is poor following surgical interventions, chemotherapy, and radiotherapy. Consequently, more efficient and effective treatment options for the treatment of GBM need to be explored. We investigate for the first time the substantial apoptotic, anti-migration, and invasion activities of Zerumbone, as a sesquiterpene derived from Zingiber zerumbet Smith, on U-87 GBM cells.

Methods: The cellular toxicity was measured by the MTT. Apoptosis was evaluated by cell cycle analysis and Annexin V/PI staining kit. Moreover, to determine the Bax. Bcl2, P53, MMP-2, MMP-9, and MCP-1 gene expression, we used real-time polymerase chain reaction. The protein expression of NF-kB, MMP-2, MMP-9 was analyzed using western blotting. Visualizing and quantitating the generation of ROS was performed by the DCFDA/H2DCFDA kit. To determine the cellular metastasis of U87 cells, we used gelatin zymography (matrix metalloproteinase [MMP]-2/-9 enzymatic activity), wound healing, and a QCM ECMatrix cell invasion assay, respectively.

Results: Our results showed that Zerumbone caused significant growth inhibition in a concentration-dependent manner. Zerumbone also induced apoptosis and caused cell cycle arrest in the G2/M phase. In detail, the apoptotic process triggered by Zerumbone involved the upregulation of pro-apoptotic Bax and the suppression of anti-apoptotic Bcl-2 gene expression. Moreover, Zerumbone activated NF-κB p65 and enhanced the generation of reactive oxygen species (ROS), and N-acetyl cysteine (NAC), as an antioxidant, reversed the ROS-induced cytotoxicity of U-87 MG cells. Our data also revealed that Zerumbone also could significantly reduce the migration and invasion of U-87, possibly via suppression of MMP-2 and MMP-9 protein expression and activity.

Conclusion: Collectively, the study raises the possibility of Zerumbone as a potential natural agent for treating GBM due to its ability to induce cytotoxicity and suppresses invasion and migration.

Keywords: Glioblastoma multiforme, Zerumbone, Apoptosis, Migration and invasion

O-6

Signaling Crosstalk of FHIT, p53, and p38 in etoposide-induced apoptosis in MCF-7 cells

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Background: FHIT (Fragile Histidine Trail) is a tumor suppressor in response to DNA damage that has been deleted in various tumors. However, the signaling mechanisms and interactions of FHIT concerning apoptotic proteins including p53 and p38 in the DNA damage-induced apoptosis are not well described. In the present study, we used etoposide-induced DNA damage in MCF-7 as a model to address these crosstalks.

Methods: MCF-7 cells or p53-Knockdown (KD)-MCF-7 treated with etoposide (10 and 100µM) for 1, 3, and 6h. The cell is transfected with FHIT Overexpression or FHIT siRNA. After treatment, the cells were lysed and protein extracts were probed for FHIT, phospho-p38 (p-p38), p53, and MDM2 by western blotting. P21 and BAX expression levels are determined by Real-time PCR. Finally, apoptosis and cell cycle measured by Flow cytometry, and caspase-9 activity was measured by ELISA.

Results: The time course study showed that the expression of FHIT, p53, and p38MAPK started after 1h following etoposide treatment. FHIT overexpression led to an increase in p53 expression, p38 activation, and augmented apoptosis following etoposide-induced DNA damage compared to wild-type cells. However, FHIT knockdown blocked p53 expression, delayed p38 activation, and completely inhibited etoposide-induced apoptosis. Inhibition of p38 activity prevented the induction of p53, FHIT, and apoptosis in this model. Thus, activation of p38 upon etoposide treatment leads to an increase in FHIT and p53 expression. In p53 knockdown MCF-7, the FHIT induction was hampered but p38 activation was induced in lower doses of etoposide. In p53 knockdown cells, inhibition of p38 induced FHIT expression and apoptosis.

Conclusion: Our data demonstrated that the exposure of MCF-7 cells to etoposide increases apoptosis through a mechanism involving the activation of the p38-FHIT-p53 pathway. Moreover, our findings suggest signaling interaction for these pathways may represent a promising therapy for breast cancer.

Keywords: FHIT, p53, p38, etoposide, DNA damage

O-7

Suppression of SIRT1/p53/BAX signaling pathway by miR-590 reduces cell survival and induces cell death in breast cancer cells

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Background: Sirtuin1 (SIRT1), a NAD-dependent protein deacetylase, is one of the regulators of cell survival and is suggested as a promoting factor in tumor progression by deacetylation and suppression of several tumor suppressors including p53. In this study, we explored the role of miR-590 in SIRT1/p53/BAX pathway and its influence on survival and apoptosis of breast cancer cells.

Methods: Breast cancer cells were transfected with miR-590 mimic, inhibitor, and their negative controls. Gene expression of miR-590, SIRT1, p21, and BAX was evaluated with real-time PCR and SIRT1 protein expression and its activity were respectively assessed by Western blot analysis and a fluorometric method in breast cancer cells. The effects of miR-590 upregulation on the acetylation of p53, apoptosis, and cell viability were assessed by Western blot analysis, flow cytometry, and WST-1 assay, respectively. Luciferase reporter assay was used to evaluate targeting of 3´-UTR of SIRT1 mRNA by miR-590.

Results: In response to miR-590, there was an increase in p53 and its acetylated form, and BAX and p21 expression, suppression of cell survival, and induction of apoptosis in breast cancer cells. SIRT1 3´-UTR was recognized to be directly targeted by miR-590. Protein expression and activity of SIRT1 were considerably inhibited by the miR-590.

Conclusion: These findings suggest that miR-590 through increasing in p53 and its acetylated form, targeting SIRT1 and the subsequent decline in cell survival may serve as new candidate targets for the more efficient therapeutic strategy for breast cancer.

Keywords: miR-590, breast cancer, SIRT1, p53, BAX, apoptosis

O-8

Synergistic toxic effects of Metformin and AICAR against Breast cancer cells mediated by the down regulation of antiapoptotic proteins

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Background: Despite the development of chemotherapy options for Breast cancer, treatments have not been completely successful because of numerous side effects and recurrence. Therefore, to overcome these obstacles, especially minimizing the side effects, the identification of safer drugs is of tremendous importance. It has been discovered that Metformin and AICAR as respectively indirect and direct AMPK activators have anti-cancer properties, albeit at very high concentrations. These anti-diabetes drugs act through downregulation of mTOR pathway through different mechanisms. Consequently, we decided to investigate the potential of combining Metformin and AICAR in the treatment of Breast cancer.

Methods: MDA-MB-231 breast cancer cell line was employed as a breast cancer model to investigate the toxicity of metformin and AICAR either alone or in combination against this cell line. MTT assays, microscopic analyses of the morphological changes of the cells as well as Western Blotting were applied. Phospho-S6, Phospho-AMPK, BCL-2, cIAP1, Phospho-4EBP1, procaspase3, procaspase9 and p62 expression were used to investigate the mechanism of action of the drugs following 48h treatment. Combination index (CI) method was used to calculate the synergistic effects.

Results: The combination of Metformin and AICAR exhibited high toxic with synergistic effects against MDA-MB-231 cancer cells. The CI was found to be 0.54 for MDA-MB-231 cells. Microscopic studies revealed morphological alterations including rounding, loss of adhesion, and sporadic distribution suggesting the mode of cell death is apoptosis (the characteristic feature of apoptosis). The combination effect was the result of reduced expression of BCL-2, cIAP1, p62, Phospho-S6, and Phospho-4EBP1.

Conclusion: Metformin in combination with AICAR showed better anti-cancer effects in breast cancer cells due to the suppression of anti-apoptotic proteins mediated by the upregulation of AMPK/mTOR pathways.

Keywords: Breast cancer, combination therapy, Metformin, AICAR

O-9

Investigation of silver nanoparticles effects on tumor growth, angiogenesis and apoptosis in mouse breast tumor model induced by 4T1 cell line

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Background: Considering the increasing use of nanoparticles in various products and the ease of entering these particles into living organisms, the present study was aimed to study the toxicity of silver nanoparticles (AgNPs) in BALB/c mice. On the other hand, some studies have shown that toxic effects of nanoparticles could control cancer.

Methods: Study designed in two separate phase includes: toxicity phase (4 groups of 5 mice) and tumorigenesis phase (7 groups of 4 mice). In toxicity phase, the best possible dose, which had the lowest toxic effects, was selected for the second phase and its effects on tumorigenesis were investigated. Finally, at second phase, tumor was induced by 4T1 cell line then biochemical markers, angiogenesis markers, and apoptosis markers were measured.

Results: AgNPs at concentration >0.25 mg/kg resulted in pathological changes different tissues; as well as it led to significant change in sperm quality and quantity, and blood brain barrier permeability. AgNPs at 0.25 mg/kg significantly changed the oxidative stress levels in serum and liver tissues but did not change the level of liver enzymes and renal markers in serum. When AgNPs have been injected into mice before induction of cancer, leading to tumorigenic effects, while exhibiting anti-tumor effects when injected after induction of cancer.

Conclusions: The current research results showed clearly the toxic effects of AgNPs at very low concentration and suggest that further in vivo investigation are required to be able to confirm the safety of nanoparticle derived application to use in life. Based on the results of the tumorigenesis, it can also be concluded that AgNPs have dual effects on tumorigenesis, and more studies are needed to confirm or reject this claim.

Keywords: Silver nanoparticles, toxicity, tumorigenesis, matrix metalloparoteinase, Apoptosis

O-10

Silibinin: An apoptotic inducer and autophagy modulator in colon cancer cells

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Background: One effective strategy to overcome the unlimited proliferation of cancer cells is promoting cell death. Factors capable of inducing both apoptosis and autophagy, as two main pathways of cell death, as well as inhibiting cell proliferation could be promising candidates for increasing drug sensitivity. Silibinin, an effective chemopreventive agent, has been shown anti-cancer properties against different types of cancers. In the present study, we investigated the anti-cancer activities of silibinin on CT26 mouse colon cell line.

Methods: CT26 cells were used to investigate anti-cancer properties of Silibinin on cancer cell proliferation and programmed cell death by using MTT and colony-forming assay, Annexin V/PI flow cytometry, RT-qPCR and Western blot. One-way variance analysis (ANOVA), followed by Tukey–Kramer pairwise comparison, was performed to determine the significance of the difference between groups, using SPSS 25.0 software.

Results: Silibinin showed no toxicity in Vero cells while 50 µM of this agent significantly reduced CT26 cells survival ($p<0.001$). Moreover, Silibinin remarkably induced apoptosis and autophagy through the up-regulation of the mRNA and protein expression of Bax ($p<0.001$) and Caspase-3 ($p<0.001$), the mRNA expression of Atg5 ($p<0.01$), Atg7 ($p<0.01$) and BECN1 ($p<0.001$) and down-regulation of the mRNA and protein expression of Bcl-2 ($p<0.05$). Furthermore, the conversion of LC3-I to LC3-II ($p<0.001$) was observed in the cells treated with Silibinin.

Conclusion: The findings of the present study revealed that Silibinin target proliferation, cell survival of CT26 cells. These results suggested that Silibinin induced colon cell apoptosis along with autophagy which might be due to the down-regulation of Bcl-2, as a mutual factor in these two processes.

Keywords: Silibinin, Cell proliferation, Apoptosis, Autophagy, Colon cancer

O-11

Inhibitory effects of bilirubin on colonization and migration of A431 and SK-MEL-3 skin cancer cells compared with human dermal fibroblasts (HDF)

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Background: Bilirubin, an endogenous antioxidant compound, induces apoptosis in cells especially in tumors. This study evaluated the effects of bilirubin on colony formation and cell migration of none melanoma (A431 skin cancer cells), and melanoma (SK-MEL-3 skin cancer cells), compared with normal human dermal fibroblasts (HDF).

Methods: HDF cells were isolated from normal newborn foreskin tissue. After viability was determined by MTT assay, the colony formation of cells was tested by clonogenic assay in bilirubin (100 µM). Acellular scaffold tissue was prepared and confirmed by Hematoxylin and Eosin (H&E) and Hoechst staining. The cells adjacent to acellular scaffold tissue were investigated by scanning electron microscopy (SEM). The q-RT-PCR was used to investigate the gene expression of matrix metalloproteinase-2 (MMP-2), vascular endothelial growth factor-A (VEGF-A), very late antigen-4 (VLA-4), and vascular cell adhesion protein 1 (VCAM-1). The molecular docking studies were done by Autodock V.4:2 software.

Results: The Ic50 obtained from MTT assay was 125, 100, and 75 µM of bilirubin for HDF, A431, and SK-MEL-3 cells, respectively. The colony formation of cancer cells, treated with 100 µM bilirubin, was reduced significant ($p<0.05$). Bilirubin inhibited the cell migration via high tendency interaction with MMP-2, and VEGF. In the presence of bilirubin, VLA-4 disrupted the VCAM-1 function despite gene overexpression thus the inhibition of cell migration occurred.

Conclusion: Bilirubin decreased the cell adhesion, and inhibited the cell colonization via inducing apoptosis and cell death. With high tendency of interaction with migration main factors, bilirubin caused inhibition the cell migration.

Keywords: bilirubin, skin cancer, acellular tissue, cell migration

O-12

Cytosolic and mitochondrial ROS production resulted in apoptosis induction in breast cancer cells treated with Crocin: The role of FOXO3a, PTEN and AKT signaling

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Background: Different groups have reported the Crocin anticancer activity. We previously showed Crocin-induced apoptosis in rat model of breast and gastric cancers, through the increased Bax/Bcl-2 ratio and caspases activity, as well as the cell cycle arrest in a p53-dependent manner. Since Crocin antioxidant activity has been shown under different conditions, it is interesting to elucidate its apoptotic mechanism. **Methods**: Here, we treated two breast cancer cell lines, MCF-7 and MDA-MB-231, with Crocin. MTT and ROS assays, cell cycle arrest, Bax/Bcl-2 ratio and caspase3 activity, AKT/FOXO3a/BIM and PTEN were determined. PARP cleavage and expression of some proteins were studied using Western blotting and immunofluorescence.

Results: The results indicated stepwise ROS generation in cytosol and mitochondria after Crocin treatment. Attenuating the early ROS level, using diphenyleneiodonium, diminished sequent mitochondrial damage (decreasing Dy) and downstream apoptotic signaling. Crocin induced ROS production, FOXO3a expression and nuclear translocation, and then, elevation of the expression of FOXO3a target genes (Bim and PTEN) and caspase-3 activation. Application of N-acetylcysteine blocked AKT/FOXO3a/Bim signaling. FOXO3a knockdown resulted in a decrease of Bim, PTEN and caspase 3, after Crocin treatment. PTEN knockdown caused a decrease in FOXO3a, Bim and caspase 3, in addition to an increase in p-AKT and p-FOXO3a, after Crocin treatment. In conclusion, Crocin induced apoptosis in MCF-7 and MDA-MB-231 human breast cancer cells. The ROS-activated FOXO3a cascade plays a central role in this process. FOXO3a-mediated upregulation of PTEN exerted a further inhibition of the AKT survival pathway.

Conclusion: These data provide a new insight into applications of Crocin for cancer therapy.

Keywords: Cytosolic ROS, Mitochondrial Damage, FOXO3a, PTEN, AKT Survival Pathway.

O-13

Effects of sFLT01 overexpression on angiogenesis and migration of DU145 prostate cancer cell line

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Background: About 90% of cancer-related deaths are due to metastasis of cancer cells and angiogenesis is a critical step in this process. sFLT01 is a novel fusion protein and a dual targeting agent that neutralizes both VEGF and PlGF proangiogenic activities. GRP78 dual effect in tumor growth and angiogenesis could be activated under VEGF stimulation. The current study was designed to investigate the inhibitory impact of sFLT01 protein on VEGF/GRP78 axis.

Methods: To this point, sFLT01 construct was synthesized, recombinant plasmid was expressed in eukaryotic host cells, sFLT01-HisTag protein was extracted and analyzed. The functional activity of sFLT01 on VEGF-enhanced tube formation and angiogenesis of HUVEC cells was examined. Eventually, the inhibitory impact of sFLT01 on growth and invasiveness and migration of human prostate cancer cell line, DU145, was assessed. Real-time PCR evaluated the level of GRP78 and its effect on the downstream factors; matrix metallopeptidase proteins 2&9 (MMP2&9) along with tissue inhibitor of metalloproteinase proteins1&2 (TIMP1&2).

Results: sFLT01 protein showed modulatory impact on proliferation, invasion, and migration of DU145 cells along with the potential of HUVECs angiogenesis. Real-time PCR analysis depicted a significant downregulation in GRP78, MMP2 and MMP9 transcripts' levels, and a subsequent elevation of TIMP1 and TIMP2 expression under sFLT01 stimulation was detected. Overall, these data indicated that the inhibitory impact of sFLT01 on cancer cells growth and invasiveness could be mediated through the modulation of VEGF/GRP78/MMP2&9 axis and activation of TIMPs.

Conclusion: This study highlighted some anticancer aspects of sFLT01 as a next-generation antiangiogenic agent and showed that the inhibitory impact of sFLT01 on angiogenesis, growth and invasiveness and migration of cancer cells.

Keywords: Prostate cancer, Angiogenesis, sFLT01, VEGF, GRP78

O-14

A potent herbal combination for treatment of triple negative breast cancer

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Background: Due to the highly aggressive nature and inferior prognosis of triple negative breast cancer, chemotherapy has become its predominant treatment strategy. Co-administration of chemotherapeutic agents and herbal medicines provide beneficial impacts, however, they may have adverse interactions. In this study, the anti-metastatic potential of combinations of two bioactive carotenoids of saffron, crocin and crocetin, on breast cancer cells and on murine model of TNBC was evaluated. The effect of the most potent combination on the Wnt/β-catenin pathway was also assessed.

Methods: The effects of the carotenoid combinations on the viability of 4T1 cells were determined by MTT assay. The effects of the nontoxic doses on migration, mobility, invasion and adhesion to ECM were examined by scratch assay, Transwell/Matrigel-coated Transwell chamber and adhesion assay respectively. Tumors were inoculated by injecting 6-8 week-old female BALB/c mice with 4T1 cells. The weights and survival rates of the mice and tumor sizes were monitored. Histological analysis of the tissues was conducted. The expression levels of Wnt/β–catenin pathway genes were measured by Real-time PCR and western blotting.

Results: Treatment of 4T1 cells with combination doses inhibited viability in a dose-dependent manner. The nontoxic combinations significantly inhibited migration, cell mobility and invasion, also attenuating adhesion to ECM. The combination therapy mice possessed more weight, higher survival rates and smaller tumors. Histological examination detected remarkably fewer metastatic foci in their livers and lungs. It was also demonstrated that the combinations exerted anti-metastatic effects by disturbing the Wnt/β-catenin target genes in the liver and tumors.

Conclusion: Our findings propose a carotenoid combination as an alternative potent herbal treatment for TNBC, lacking the adverse effects associated with either chemotherapeutic agents or herb-chemotherapeutic drugs.

Keywords: Combination, Triple negative breast cancer (TNBC), Anti-metastatic, 4T1, Mice model, Wnt/β-catenin

O-15

Aprepitant Promotes Caspase-dependent Apoptotic cell death and G2/M arrest through PI3K/Akt/NF-κB axis in Cancer Stem-like Esophageal Squamous Cell Carcinoma spheres

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Background: The antagonists of the neurokinin-1 receptor (NK1R) are known for their anti-inflammatory, anxiolytic, antiemetic, and anticancer activities. Aprepitant, a nonpeptide NK1R antagonist, is being used in nausea and vomiting, the most frequent side effects of cancer chemotherapy in patients. It has been established that NK1R activation by Substance P (SP), which links cancer promotion and progression to a neurokinin-mediated environment, became one mechanism that corresponds to the mitogenesis of tumor cells.

Methods: This study aims to explain and evaluate the anticancer impacts of aprepitant on esophageal squamous cancer cell (ESCC) spheres by using in-vitro experiments, such as Resazurin, ROS, Annexin-V binding, RT-PCR, and Western blot analysis.

Results: As a result, we have shown that aprepitant had strong antiproliferative and cytotoxic effects on ESCC cell-spheres. In addition, aprepitant caused significant G2-M cell cycle arrest depending on concentration increase. Further, exposure of cells to this agent has resulted in caspase -8/-9-dependent apoptotic pathway activation by modifying the expression of genes involved in apoptosis. Besides, treatment of the cells by aprepitant abrogates of PI3K/Akt pathway, as shown by reducing the level of Akt, which induces apoptotic cell death.

Conclusion: In summary, pharmacological inhibition of NK1R with aprepitant seems to have a significant chance of treating ESCC as a single agent or in conjunction with other chemotherapeutic drugs.

Keywords: NK1 receptor, Aprepitant, Substance P, ESCC, Apoptosis

O-16

Anti-diabetic effect of an isolated oligosaccharide via modulation of angiogenesis in diabetic rats: modulation of Notch signaling pathway

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Background: Diabetes mellitus (DM) is a well-known clinical entity with various late complications. Islet vascularization appears to be a key element in the control of β-cell mass expansion to increased insulin demands. Furthermore, diabetic complications are suggested to be related to the disturbances in angiogenesis. One of the main signaling pathways in the modulation of angiogenesis is the Notch signaling pathway. The objective of this study is to isolate the oligosaccharide fraction from Rosa canina and evaluate its effect on the angiogenesis in the pancreas from diabetic rats.

Methods: Oligosaccharide fraction was isolated from Rosa canina and structurally characterized using HPLC, FTIR, NMR, and MS/MS techniques. Rats were sorted into control, diabetic, Metformin, and oligosaccharide-treated groups. The level of angiogenic and notch signaling factors was evaluated by using quantitative real-time PCR and immunohistochemistry.

Results: Structural analyses of the isolated oligosaccharide indicated an oligosaccharide fraction with antidiabetic effects. The appearance of the pancreas showed an increased level of vasculature in the oligosaccharide-treated rats compared to diabetic ones. The levels of Hes1, CD34, CD31, and VEGF in immunohistochemistry analysis of rats exposed upon oligosaccharide were enhanced relative to STZ-induced diabetic rats. The mRNA levels Notch1, Hes1, VEGF were in line with immunohistochemical analysis.

Conclusion: Data clearly indicated that the notch signaling pathway plays a causal role in the regulation of angiogenesis in the pathogenesis and remedy of diabetes. In addition, the isolated oligosaccharide seems to relieve diabetes and its complications through modulation of notch-downregulated angiogenesis and vasculature in diabetes.

Keywords: Diabetes, Angiogenesis, Notch signaling, Oligosaccharide, Rosa canina

O-17

Trehalose and N-Acetyl Cysteine alleviate inflammatory cytokines and oxidative stress in LPS-stimulated human peripheral blood mononuclear cells

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Background: Inflammation and oxidative stress have been found to be implicated in the development of a large number of human diseases. Trehalose, a non-reducing disaccharide, exerts a variety of biological effects including antioxidant and anti-inflammatory activities. However, there is little data on the effects of trehalose on human cells including PBMCs. Here, we aimed to examine whether trehalose could attenuate oxidative stress and inflammation induced by LPS in PBMCs.

Methods: ELISA and Real-time PCR were used to assess the levels of inflammatory cytokines. For investigating the phosphorylation of JNK and P65-NF-κB, western blot was utilized. Oxidant-antioxidant markers were assayed using ELISA and colorimetric procedures.

Results: The results showed that trehalose significantly mitigated the effect of LPS on the phosphorylation of JNK MAPK and NF-κB-P65, which was associated with significant reduced inflammatory cytokines IL-6, TNF-α and IL-1β and increased anti-inflammatory cytokine IL-10. The antioxidant N-Acetyl Cysteine also showed similar effects on JNK/MAPK and NF-κB-P65 phosphorylation and inflammatory cytokines. Further, trehalose alleviated oxidative stress in LPS-stimulated PBMCs, as it reversed the altered levels of malondialdehyde, total thiols and restored a decline in the activity of antioxidant enzymes GPX and MnSOD.

Conclusion: The results of this study indicated that trehalose prevented inflammation and oxidative stress in the LPS-stimulated PBMCs, providing an evidence for trehalose as a therapeutic potential in inflammatory conditions.

Keywords: Trehalose, N-Acetyl Cysteine, Inflammation, Oxidative stress, Peripheral Blood Mononuclear Cells

O-18

Synergistic effects of metformin/ astaxanthin on oxidative stress markers in type 2 diabetic patients: a randomized, double-blind, placebo-controlled clinical trial

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Background: Oxidative stress plays a key role in pathogenesis and complications of diabetes. The effective role of antioxidants in controlling T2DM complications has been reported in experimental and clinical studies. The aim of this study was to determine whether Astaxanthin supplementation may enhance the effects of metformin due to its similarity to metformin in activating AMPK and affects oxidative stress markers in a randomized, placebo-controlled, double-blind clinical trial.

Methods: Forty-seven patients with T2DM were treated just with metformin, aged 18-60 years old and on stable medication were recruited were randomly assigned into two groups. Patients in the Astaxanthin treatment group received 10 mg/day, and those in the placebo group received identical placebos for 3 months. Data pertaining to weight, height, blood pressure and BMI, as well as food consumption, were collected at baseline and at the conclusion of the study. Plasma total antioxidant capacity, malondialdehyde concentration, catalase activity, superoxide dismutase activity and anthropometric parameters were measured at the baseline and at the trial end.

Results: Compared with the placebo/metformin group, Astaxanthin/metformin significantly increased plasma total antioxidant capacity ($p < 0.05$), catalase activity ($p < 0.05$) and superoxide dismutase activity ($p < 0.05$). There was not a significant reduction in malondialdehyde levels ($p > 0.05$). Astaxanthin had no significant effects on the anthropometric parameters except for a significant reduction in blood pressure levels. Astaxanthin was well tolerated, and no serious adverse event occurred.

Conclusions: Our study demonstrated that 12 weeks of supplementation with 10 mg/day Astaxanthin with metformin may act synergistically on AMP-activated protein kinase-dependent pathways, leading to increased antioxidant markers, which may reduce the complications of diabetes and reduce the therapeutic doses of metformin necessary in the treatment of diabetes.

Keywords: Oxidative stress, Type 2 diabetes, Metformin, Astaxanthin

O-19

Combination of metformin and chlorogenic acid attenuates hepatic steatosis and inflammation in high fat diet fed mice

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Background: Non Alcoholic Fatty liver disease (NAFLD) has become an important health problem in the world. Natural products, with anti-inflammatory property, are potential candidates for alleviating NAFLD. Metformin (MET) and chlorogenic acid (CGA) has been reported to be effective in the improvement of NAFLD, however, their combination effects on NAFLD have not been investigated.

Methods: 50 C57BL/6 male mice were divided into two groups, one fed a standard chow diet (n=10) and the other was fed a high fat diet(HFD) (n=10) for 10 weeks. Animals in the HFD group were randomly divided into a 4 groups (HFD, HFD + MET (0.25%), HFD + CGA (0.02%) and HFD + MET + CGA (0.25%+0.02%)) for 12 weeks.

Results: MET and CGA combination decreases fasting blood glucose (FBG) and improves glucose intolerance. Decreased hepatic triglyceride level was associated with lower expression levels of sterol regulatory element-binding protein-1c (SREBP-1c) and fatty-acid synthase (FAS) in MET+CGA treated mice. MET and CGA combination treatment resulted in the polarization of macrophages to the M2 phenotype, reduction of the expression of pro-inflammatory cytokines (IL-1β, TNF-α and IL-6) and decreasing protein level of NF-kB p65. It was found that the lowering effect of combined MET and CGA on the expression of gluconeogenic genes was accompanied by promoting phosphorylation of Glycogen synthase kinase 3β (GSK-3β). Treatment of HFD mice with combination of MET and CGA was found to be more effective at alleviating inflammation and hyperglycemia by increasing phosphorylation of AMPK.

Conclusion: These results demonstrate that the MET + CGA combination might exert therapeutic effects against NAFLD.

Keywords: Metformin, Chlorogenic acid, Non-alcoholic fatty liver disease, Liver inflammation, macrophage polarization, AMPK, High Fat Diet .

O-20

Saffron Carotenoids Change the Structure-Activity of Superoxide Dismutase in Breast Cancer: in vitro, in vivo, and in silico Studies

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Background: Superoxide dismutase (SOD) is an important balancer of oxidative stress conditions in cells, which has been proposed as a target for developing new therapies against ROS-mediated diseases. Crocin and (Cro) and crocetin (Crt) are antioxidants, reported to be effective against cancer.

Methods: In the present study, we used multi-spectroscopic techniques along with in silico analysis to investigate the molecular details of Cro/ Crt interaction with SOD in breast cancer. For this purpose, docking analysis is used to predict the binding sites of the ligands within SOD. Circular dichroism, fluorescence quenching, and spectrophotometry are used to obtain the binding parameters as well as conformational changes. Enzyme activity in the presence of different concentrations of Cro/Crt was assessed in the cell-free system and also cell line and in vivo cancer models.

Results: UV- Vis and fluorometry proposed that both Cro/Crt strongly attached to the protein; the ΔG° of binding at 310 °K was -8.6 and -4.4 kcal/mol, respectively. Both phytochemicals acted as ROS scavengers and inhibited enzyme activity in a dose-dependent manner both in cell-free and MCF-7 models. Computational analysis predicted the sites near the active site channel as the Cro/Crt binding sites. Our data suggest that Cro inhibits SOD activity by scavenging superoxide (O2•), while Crt inhibits SOD by affecting the copper-binding site. Surprisingly, one month of treatment with saffron carotenoids increased SOD activity in breast tumors of balb/C mice.

Conclusion: Collectively, Cro/Crt induce structural and functional changes in SOD and thereby affect the cancer hemostasis. Our findings suggest different mechanisms of Cro/Crt actions on SOD activity in breast cancer at long or short term courses of treatment.

Keywords: SOD, Breast Cancer, Crocin, Crocetin, Spectroscopy

O-21

Renoprotective effects of Origanum majorana methanolic L and carvacrol on ischemia/reperfusion-induced kidney injury in male rats

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Background :The most important cause of acute renal failure in normal kidneys is ischemia-reperfusion (I/R) injury. It seems that reactive oxygen species play a vital role in the pathogenesis of I/R damage in the kidney. The aim of the current study was to investigate the protective effects of Origanum majorana methanolic extract, carvacrol and vitamin E on I/R-induced kidney injury in male rats .

Methods :Thirty Wistar male rats were randomly allocated into 5 groups; sham, I/R, I/R + Origanum majorana methanolic extract (300 mg/kg), I/R + carvacrol (75 mg/kg), and I/R + vitamin E (100 mg/kg). Renal function markers, oxidant- antioxidant parameters, and histopathological examination were evaluated in the plasma and renal tissues at the end of our experimental study.

Results: It was exhibited that the urea, creatinine, and oxidant markers such as protein carbonyl significantly augmented in the I/R untreated group, while glomerular filtration rate and antioxidant indices such as total thiol, and ferric reducing antioxidant power markedly decreased. However, these findings markedly reversed in the treatment groups with Origanum majorana extract or carvacrol in comparison to I/R merely group. Besides, with regard to histopathological results, methanolic extract of Origanum majorana or carvacrol could prevent the destructive effects of IR on kidney tissues.

Conclusion: We concluded that Origanum majorana extract or its ingredient, carvacrol, exerts renoprotective impacts in I/R-induced kidney injury possibly by scavenging of free radicals and increasing of antioxidant power.

Keywords: Origanum majorana, Ischemia-reperfusion, renal injury, Carvacrol

O-22

Protective features of Curcumin on obesity-induced skeletal muscular inflammation via modification of immune cells population

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Background: Curcumin, as a natural polyphenol has been introduced to suppress the inflammatory process. There is increasing evidence that obesity is associated with increased inflammation. However, the role of curcumin on High fat diet-induced muscular inflammation remains unknown.

Methods: 30 sixth-week-old male C57BL/6 mice were fed normal chow diet, high fat diet (HFD 60%) or HFD+curcumin (150mg/ kg diet) for 26 weeks to induce obesity. We evaluated the percentage of macrophages, macrophage phenotypes and T-cell subsets in skeletal muscle (gastrocnemius) by flow cytometry assay. In addition, the effect of curcumin on inflammatory cytokines and chemokines were analyzed by qRT-PCR. The protective effects of curcumin were investigated by assessment of AMPK-JNK signaling pathway using Western blot analysis of total and phosphorylated proteins.

Results: Our results showed that curcumin intervention attenuated body weight gain and insulin resistance in response to high fat diet. Curcumin significantly diminished macrophage recruitment into skeletal muscle of HFD-treated mice. Curcumin treatment also led to the switching of macrophages to the M2 phenotype and enhanced percentage of regulatory T cells (Treg cells) population in skeletal muscle of HFD-treated mice. We confirmed these data by flow cytometry, immunohistochemistry and real-time PCR analysis. Treatment with curcumin was accompanied by reduction of skeletal muscle lipid content and decreased expression of pro-inflammatory-related genes such asTLR2, TLR4, F4/80, MCP1, TNF-α, IL-1β, IL-6, iNOS, and enhanced mRNA level of adiponectin, adiponectin receptor and IL-10. Interestingly, feeding curcumin to the HFD fed mice, controlled inflammation by inhibiting the upsurge of JNK phosphorylation and at the same time, reversing blunted phosphorylation of AMPK.

Conclusions: These findings indicate that curcumin may be beneficial in the management of diet-induced inflammation and could be explored as a therapeutic adjuvant against complications accompanied by obesity.

Keywords: Curcumin, high fat diet, obesity, inflammation, skeletal muscle tissue

O-23

The effect of gallic acid on the expression of thioredoxin gene in liver tissue and serum oxidative stress markers in rat models of hypothyroidism

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Background: Hypothyroidism is a metabolic disease that is associated with mental disorders, heart diseases and infertility in men and women. Disturbance of oxidative balance and induction of oxidative stress can be one of the important causes of this disease. In this study, for the first time, the effect of gallic acid, as an antioxidant agent, has been investigated on oxidative status in hypothyroid rats.

Methods: A total of 28 four-week-old male Wistar rats were randomly assigned into 4 groups including: control group, hypothyroid, hypothyroid treated with dose 50 mg/kg/day and hypothyroid treated with dose 100 mg/kg/day of gallic acid. After one week of co-administration with the propylthiouracil tablets to induce hypothyroidism, gallic acid was also administered in two different doses at 1.5 cc daily for four weeks. T3 and T4 hormones, serum uric acid (UA), total antioxidant capacity (TAC), lipid peroxidation index (MDA) and thiol group were measured in serum via calorimetry. To evaluate expression of thioredoxin (TXN1) gene in liver, real-time PCR were used.

Results: Our results demonstrated significant reduction of TAC, MDA, and UA index in different hypothyroid groups than the control group (except the hypo+ GA100 that was increased). However, the mean of THIOL index was significantly increased in the different hypothyroid groups compared with the control group. The expression of the TXN1 gene meaningfully increased in the hypothyroid groups and decreased in two gallic acid-treated groups relative to the control group.

Conclusion: As a potent antioxidant agent, gallic acid may be effective in improving oxidative stress caused by hypothyroidism. It can be used as a regulator of oxidative conditions, although further investigation is required.

Keywords: Gallic acid, Hypothyroidism, TXN1, Oxidative stress

O-24

Simultaneity metformin and sitagliptin effect on expression levels of proteins involved in insulin resistance Glucose Transporter 4, Protein Kinase B(Akt) Insulin-like Receptor Substrate-1, phosphatidylinositol 3 kinase and Mammalian Target Of Rapamycin in adipose tissue of diabetic patients: A Clinical Trial

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Background: A cause of persistent insulin resistance and type II diabetes is obesity, which finally leads to a wide range of metabolic alterations, the most important of which is insulin resistance. In insulin resistance, the mechanism of insulin signal to intra-cells, chiefly in adipose tissue cells happens, which by identifying the proteins this pathway, can be suitable targets for therapeutic approaches Patients and

Methods: In this clinical trial study, we investigated 6 persons of type 2 diabetic patients that were 3 months' treatment with simultaneity metformin and sitagliptin, that 4 persons from them returned after treatment and 8 persons in a control group. To determine the content of proteins involved in insulin resistance GLUT4, Akt, PI3K, IRS-1, and mTOR in adipose tissue of diabetic patients with the use of SDS-PAGE and western blot analyses.

Results: Following simultaneity metformin and sitagliptin treatment, we noticed increases in glucose disposal and decreases in serum glucose levels ($p<0.05$) and insulin resistance ($p<0.05$), and change in serum insulin levels. On the other, increase the content of proteins involved in insulin resistance increase of GLUT4, Akt, IRS-1, PI3K, and mTOR in adipose tissue of diabetic patients were observed ($p<0.05$).

Conclusion: Simultaneity metformin and sitagliptin treatment significantly improved proteins involved in insulin resistance GLUT4, Akt, IRS-1, PI3K, and mTOR in type2 diabetic adipose tissue.

Keywords: Insulin Resistance, Metformin, Sitagliptin, Glucose Transporter, Target of Mammalian Rapamycin

O-25

Phytochemical and biochemical properties Oliveria decumbence essential oil

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Background: Oliveria decumbence is traditionally used to relieve thirst in children, ingestion, abdominal pain, stomach pain, stomach inflammation, fever, and cold therapy. The primary objective of this study was to explore the phytochemical and biochemical properties of O. decumbence essential oil (ODEO).

Methods: ODEO was extracted by hydro-distillation and analyzed by GC-MS method. In vitro, the antioxidant capacity of ODEO was analyzed by superoxide ion, and nitric oxide (NO) scavenging. Ex-vivo studies of ODEO were examined by analyzing the levels of NO and reactive oxygen species (ROS) in lipopolysaccharide (LPS) -stimulated macrophages. The expression of NOX (NADPH oxidase) and iNOS (inducible nitric oxide synthase) genes were determined using real-time polymerase chain reaction. In-silico studies were performed by molecular docking to investigate the ODEO effect on NOX and iNOS. MTT assay, fluorescence staining and flow cytometry were performed to confirm the anticancer effect and determine the death mode in treated A549 lung cancer cell line.

Results: GC-MS analysis revealed thymol, carvacrol, p-cymene, and γ-terpinene as basic ingredients. ODEO exhibits strong in-vitro antioxidant capacity against biological oxidants. In LPS-stimulated macrophages treated with ODEO, the reduction of oxidants such as ROS, and NO was considerable. Down-regulation of NOX and iNOS mRNA expression by ODEO in LPS-stimulated macrophages was significant. Molecular docking results indicated that thymol/carvacrol can inhibit iNOS activity by blocking the arginine binding site. However, they did not show any evidences for NOX inhibition. MTT assay showed that despite having a limited effect on L929 normal cells, ODEO induces cytotoxicity in A549 cells. Apoptotic death in ODEO-treated cells was confirmed.

Conclusion: Our results indicated that ODEO is effective as an antioxidant, and anti-cancer agent, suggesting the ODEO as a potent candid in cancer therapy and the development of novel antioxidant agents in the preservation and the treatment of related diseases.

Keywords: Oliveria decumbens, phytochemical, inflammatory, antioxidant, cancer

O-26

Effect of Theophylline on Tumor Necrosis Factor Alpha and Reactive Oxygen Species Levels in Infertile men

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Background: Theophylline acts as a cyclic-AMP phosphodiesterase inhibitor which elevates cAMP dependent sperm motility. It is used in treatment of male infertility, including asthenoteratozoospermia. The aim of this study was to evaluate any possible effect of theophylline on sperm characteristics,TNF-α (Tumor Necrosis Factor) and ROS (Reactive Oxygen Species) levels in seminal plasma of asthenoteratozoospermic men.

Methods: 120 infertile men with asthenoteratozoospermia were allocated to this study. Patients were randomly divided into two groups: Placebo, and theophylline (200 mg/day) groups were administered twice a day for 3 months. Two semen samples (one before and one after the theophylline therapy) were evaluated under double-blind condition. Semen parameters, TNF-α and ROS levels (by human TNF-α ELISA kit and Chemiluminescence assay- Agarwal, respectively) of seminal plasma were analyzed (pre and post intervention) for each sample. The study data was analyzed using T-Test setting and means difference was considered significant at $p<0.05$.

Results: Theophylline increased significantly the mean sperm count and normal morphology in the men with asthenoteratozoospermia compared to preadministration. Theophylline was significantly effective on the progressive motility of sperm ($P<0.01$). Based on the results, mean concentration of TNF-α in the seminal plasma was significantly increased in patients treated with theophylline compared to preadministration. Moreover, ROS levels in seminal plasma of patients with asthenoteratozoospermia decreased significantly compared to before administration of theophylline.

Conclusion: Although our results demonstrate that oral therapy of theophylline significantly increase the quality of sperm, especially motility from infertile men with asthenoteratozoospermia, but more molecular studies are needed to elucidate the safety of theophylline administration.

Key words: Theophylline, Tumor Necrosis Factor alpha, Reactive Oxygen Species, Male Infertility

O-27

How false positives can be misleading in functional studies using CRISPR-Cas9

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Background :CRISPR-Cas system has proved to be a powerful gene manipulation system with significant benefits in biomedical studies including gene activation, silencing, and knockout. In recent years, the use of the CRISPR-Cas9 system in functional genome-wide studies has increased where researchers look for the genes involved in a special pathway. However, the results of the studies done by CRISPR-Cas system cannot be taken as guaranteed and special cares need to be taken in follow-up experiments in order to avoid false-positive reports.

Methods :A genome-wide CRISPR-Cas9 library was used in Panc1 pancreatic cancer cell line to identify the genes involved in gemcitabine modulation. A list of the candidate sensitizer genes was acquired. Panc1 cells carrying sgRNAs targeting top candidate genes were prepared. A dose-response assay of gemcitabine was performed. Western blotting was performed to measure the level of the targeted protein. SURVEYOR assay was performed to confirm the on-target act of the sgRNA. Monoclonal cell lines were prepared and the targeted genomic region was sequenced.

Results: DNAJB12 appeared as the second top gemcitabine sensitizer in our study. Polyclonal cells carrying a sgRNA targeting DNAJB12 showed more sensitivity to gemcitabine compared to ones carrying control sgRNAs. SURVEYOR assay confirmed the on-target activity of the sgRNA and some of the monoclonal cell lines showed more sensitivity to gemcitabine compared to control cells. However, checking the targeted region of the DNAJB12, none of the sensitive monoclonal cell lines showed on-target gene manipulation.

Conclusion: Our results show that the sensitizing activity of the DNAJB12 was a false-positive result due to the off-target activity of the DNAJB12 targeting sgRNA. This could not be discovered unless extensive follow-up experiments had been performed. We suggest that in any studies using CRISPR-Cas9, exhaustive care should be taken not to report false-positive results.

Keywords: CRISPR-Cas9, Genome-wide screening, DNAJB12, False Positive

O-28

The relationship between NFL with ATG5 and Parkin markers in patients with multiple sclerosis

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Background: Multiple sclerosis (MS) is the most common inflammatory neurodegenerative disease. Neurofilament light chain (NFL) is a novel biomarker of axonal damage that has been suggested as a useful assistant in the monitoring of MS patients. It has been shown that the auto/mitophagy associated with MS pathogenesis.

Method: In this study, we aimed to study correlation between ATG5 and Parkin, as markers of autophagy and mitophagy respectively, with NFL in serum and cerebrospinal fluid (CSF) in MS subjects. ATG5, Parkin, and NFL levels were measured in a cross-sectional study of 40 MS patients compared with gender, age and BMI matching healthy volunteers.

Results: Based on our results, levels of ATG5, Parkin, and NFL were significantly elevated in both serum and CSF of MS patients compared to control individuals ($p < 0.0001$). The correlation indices between NFL, ATG5, and Parkin in both case and control groups showed a direct and moderate correlation between NFL and ATG5 in the CSF level of the control group ($r = 0.554$, $P = 0.011$).

Conclusion: Our data support the feasibility of quantifying NFL as a sensitive and clinically meaningful serum/CSF biomarker to follow up the nerve tissue injury in MS condition.

Keywords: Multiple sclerosis, Autophagy, Mitophagy, Neurofilament light chain, Novel biomarkers

O-29

PVA-Gelatin-CQD/Ag Scaffolds Loaded with Vitamin C Promote Skin Wound Repair in Rats

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Background: Electrospinning of nanofibers is a new strategy in wound healing. This three-dimensional and temporary scaffold structure provides a convenient space for cells and repairs agents in the process of wound healing.

Methods: In this study, CQD/Ag Nanoparticle was prepared. Then, PVA-gelatin solution in combination with nanoparticles was used in electrospinning process. Characterization of scaffold was analyzed with some tests including FTIR, zeta potential, dynamic light scattering, water contact angle, mechanical properties and biodegradability. Additionally, In vitro analysis such as antibacterial activity, drug release, cytotoxicity and cell immigration were done. Finally, In vivo study was investigated.

Results: Incorporation of CQD/Ag nanoparticles significantly enhanced the tensile strength and water contact angle. Degradation rate of the nanofibers was evaluated 54% at 10 days. The results of antibacterial tests (MIC and disc diffusion) imply that the nanofiber has high antibacterial activity. The release of vitamin C by PVA- gelatin-CQD/Ag nanofibers corresponds to the Higuchi model. The cytotoxicity and scratch surface by MTT method revealed that samples did show no cytotoxicity on fibroblast cells. Scratch surface was healed entirely after 24 h. During the animal test, on the 14th day, the nanofiber exhibited 94.36% wound-healing effect,

Conclusion: Based on in vitro and in vivo studies, the composite scaffold is able to accelerate the wound healing process and can be potential materials for chronic wound treatment (approximately 30% higher than PVA-gelatin alone).

Keywords: Carbon Quantum dot/Ag nanoparticles, Wound Healing, Polyvinyl Alcohol, Electrospinning, Vitamin

O-30

The efficiency of miRNAs and encapsulation-based insulin-producing cell therapy to control hyperglycemic condition

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Background: Type 1 diabetes involves the destruction of over 75% of pancreatic islet beta-cells mediated by autoimmune reactions, absolute insulin deficiency, and hyperglycemia. Such issues can be overcome by the identification of stem cells with and using this renewable source of cells to differentiate into insulin-producing cells (IPCs).

Methods: Functional beta-like cells were differentiated from adipose-derived stem cells (ADSCs) in presence of anti-miR-7 and over-expression of miR-375. The microfluidic system was developed to support the survival of IPCs.

Results: The contribution of miR-375 and, or anti-miR-7 in the differentiation of ADSCs to mature beta-like cells revealed that the cell response to glucose challenge was not elicited by the up-regulation of miR-375 alone, but suppression of miR-7 caused significant changes in glucose response. The beta-like cells encapsulation by Collagen-Alginate made by the microfluidic system were supported in terms of morphology and biological function and proved to be efficient for transplantation to control the hyperglycemic condition.

Conclusion: For the first time, this study shows that the use of the efficiency of the two-component system, miRNAs-based cell therapy, and Collagen-Alginate fiber-entrapped IPCs can control hyperglycemic condition with enhancing the sensitivity of IPCs derived from stem cells to glucose.

Keywords: Anti-miR-7, IPCs, Diabetes, Encapsulation, Microfluidics, miR-375

O-31

Transforming growth factor–β1 mediated CHST11 and CHSY1 mRNA expression is ROS dependent in vascular smooth muscle cells

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Background: Transforming growth factor (TGF)-β mediates glycosaminoglycan (GAG) chain hyperelongation on secreted proteoglycans and these modifications are associated with increased lipid binding in the vessel wall and the development of atherosclerosis. In vascular smooth muscle cells, TGF-β regulated GAG elongation via extracellular signal-regulated kinase (ERK) and p38 as well as Smad2 linker region phosphorylation. In this study, our aim was to identify the TGF-β mediated signaling pathway involving reactive oxygen species (ROS) and Smad2 linker region phosphorylation that regulate the mRNA expression of GAG synthesizing enzymes, chondroitin 4-O-sulfotransferase 1 (C4ST-1) and chondroitin sulfate synthase 1 (ChSy-1) which are the rate limiting enzymes involved in GAG chain elongation.

Methods: Signaling molecules were assessed by western blotting; quantitative real-time PCR was used for analysis of gene expression and intracellular ROS level was measured by a fluorescence based assay.

Results: TGF-β induced ROS production in VSMCs. nicotinamide adenine dinucleotide phosphate oxidase (Nox) inhibitors, DPI (diphenyleneiodonium) and apocynin blocked TGF-β mediated Smad2 linker region phosphorylation. TGF-β treatment increased the mRNA levels of C4ST-1 and ChSy-1. Pharmacological inhibition of Nox blocked TGF-β mediated mitogen activated protein kinases (MAPKs) phosphorylation and TGF-β stimulated C4ST-1 and ChSy-1 mRNA expression.

Conclusions: These findings demonstrated that TGF-β mediated expression of C4ST-1 and ChSy-1 can occur via Nox-dependent pathways and Smad2 linker region phosphorylation.

Keywords: TGF-β1, CHSY1, Atherosclerosis,

O-32

Evaluation of a novel cell penetrating protein for in vitro DNA delivery

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Background: The inability of vaccines to transfer cargoes via cell membrane is one of the limitations in vaccine development against human immunodeficiency virus-1 (HIV-1). The supercharged proteins were shown to be effective for delivery of various types of cargoes including DNA and protein. Herein, we used B1 protein, a positively supercharged protein, which it is a product of a frameshift in the gene that encodes enhanced green fluorescent protein (eGFP).

Methods: B1 protein was generated in bacterial expression system under native conditions. In order to evaluate the DNA delivery efficiency of B1 protein, firstly, we formed nanoparticle complexes between B1 and two different multi-epitope DNA constructs such as nef-vif-gp160-p24, and nef-vpu-gp160-p24 at N: P ratio of 1:1. Then, the zeta potential and size of nanoparticles were determined by scanning electron microscopy (SEM) and ZetaSizer. To check the cytotoxicity of complexes in vitro, we performed MTT assay in HEK-293T cell line. Finally, to investigate the efficiency of DNA delivery by B1 protein, the nanoparticles were transfected into HEK-293T cells and the gene expression was evaluated by flow cytometry and fluorescent microscopy.

Results: Our findings showed that B1 protein could form non-covalent stable nanoparticles as confirmed by SEM and ZetaSizer (~80-110 nm) at N/P ratio of 1:1 with both multi-epitope DNA constructs. Furthermore, the MTT results confirmed that the nanoparticles did not have cytotoxicity effects. In addition, the flow cytometry results indicated that in vitro transfection efficiency of B1 protein for DNA delivery into HEK-293T cells was about 32-35% in comparison with TurboFect commercial reagent.

Conclusion: Altogether, these findings indicated the ability of B1 protein as a potent DNA delivery system into living cells. Although, further evaluation is essential to investigate the potency of B1 in induction of effective immune responses.

Keywords: Human immunodeficiency virus-1, DNA delivery, Supercharged protein, B1 protein

O-33

Urinary proteomic approach for identification of potential diagnostic biomarkers in renal antibody mediated rejection

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Background: Allograft rejection is one of the devastating complications after renal transplantation. Development of noninvasive biomarkers for precise and timely detection of rejection is a critical unmet need. This study investigated the urinary proteome of renal transplant recipients to identify novel diagnostic biomarkers of antibody-mediated rejection (AMR) of allografts.

Methods: Twenty-two kidney transplant patients with biopsy proven AMR, and 14 subjects with stable graft function were enrolled in this study. Urine samples were collected on the day of biopsy and label free quantitation (LFQ) proteomics was applied to each sample (n=36). LFQ data was subjected to Random Forest (RF) algorithm with the setting of sensitivity and specificity greater than 70% to predict main candidate proteins contributing to the diagnosis of AMR. Bioinformatics analysis was performed to determine biological functions and possible relevant metabolic pathways of candidate proteins.

Results: Proteomics analysis revealed 377 differentially expressed proteins, where 177 and 200 proteins were found up- and down-regulated, respectively, in AMR samples compared to those with stable graft function. Up-regulated proteins were mostly engaged in complement pathway and the majority of down-regulated proteins were involved in metabolic pathways. Twenty proteins with the highest sensitivity and specificity were identified as potential biomarkers, among which over-expressed immune response elements and acute phase proteins together with down-regulated extracellular matrix (ECM) related proteins were found as main candidate biomarkers.

Conclusion: Proteomics analysis revealed differential expression of proteins between groups. Bioinformatics analysis showed significant changes in the expression of ECM-related proteins such as Nidogen-1, Collagen alpha-1(XV) and epidermal growth factor in AMR which may be representative of ECM remodeling during AMR process and might be considered as candidate biomarkers for early diagnosis of AMR. However, further studies are needed to validate their clinical applicability in diagnosis of AMR.

Keywords: antibody mediated rejection, urinary proteomics, biomarker, renal allograft

O-34

Neural Differentiation of Retinal Pigment Epithelial Cells and Mesenchymal Stem Cells by Optogenetic Stimulation

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Background: In retinal degenerative disorders, such as age-related macular degeneration (AMD) and retinitis pigmentosa neural retinal cells are damaged. Neural retinal cell transplantation is one of the most promising therapeutic approaches in treatment of vision loss. Retinal pigment epithelium (RPE) cells and bone marrow mesenchymal stem cells (BMSCs) were recruited for optogenetic study due to their differentiation ability into retinal specific neurons. This research explored the potential role of blue light stimulation on retinal neural differentiation of Opto-mGluR6 engineered human RPE and BMSCs. **Methods**: hRPE and BMSCs transduced by AAV-MCS-IRES-EGFP-OptomGluR6 viral vector at multiplicities of infection (MOIs) of 1 and 10, respectively. 48h after transduction, sample cultures were immunostained by melanopsin marker to pursue for melanopsin expression. Transduced cultures were also treated with 2 µM all trans retinal and stimulated 5 days with blue light (470 nm) in a humedified CO2 incubator. Total RNA was isolated and RT-qPCR for retinal specific neuron genes were performed. Imunnocytochemistry (ICC) assay for Rhodopsin, PKCa, Thy1, CRX, CD73, OPN1, recoverin and CRABP as retinal specific neuron markers were performed.

Results: hRPE and BMS cells were successfully transduced. Transducted cells expressed Opto-mGluR6 protein in cells membrane. RT-qPCR represented that Pou4f1, Dlx2, Eomes, Barlh2, Neurod2, Neurod6, Rorb, Rora, Rxrg, Nr2f2, Ascl1, Hes5 and Sox8 overexpressed 4.7, 8.5, 10.9, 23.8, 1.6, 3.1, 5.2, 0.5, 6, 2.2, 3.4, 5.2 and 5.2 fold in BMS cells culture and 0.1, 0.6, 0.4, 2.2, 0.4, 0.5, 2.3, 1.5, 0.7, 0.6, 1.5, 1.2 and 45.3 fold in hRPE cells culture. ICC assay confirmed expression of retinal specific neuron markers in BMSCs and hRPE cells culture.

Conclusion: Optogenetic stimulation induce dominant differentiation of ganglion, amacrine, photoreceptor, bipolar precursors and muller precursor’s cells in BMS treated culture when compared to the mRPE. While mRPE cells represented dominant terminal müller glial differentiation compared to BMS.

Keywords: Optogenetic stimulation, Retinal differentiation, Retinal pigment epithelial cell line

O-35

The protective effect of α7nACh receptor and its interaction with 5-HT1B/1D receptors in acute intestinal ischemia-reperfusion injury in rats

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Background: Over the past decades, there is great attention to the nervous system modulating effects on the immune response in inflammation-associated injuries such as acute intestinal ischemia-reperfusion (IR). Recently we proved anti-inflammatory and antioxidant effects of 5-hydroxytryptamine (5-HT)1B/1D receptors in intestinal IR induced by 30min occlusion of the superior mesenteric artery followed by 2h reperfusion in rats. Also, alpha7 nicotinic acetylcholine (α7-nACh) receptor has anti-inflammatory effects in different inflammation-associated injuries. Starting from these premises, we aimed to examine the function of the α7-nACh receptors and the functional interactions between the anti-inflammatory and antioxidant effects of α7-nACh and 5-HT1B/1D receptors in acute intestinal IR injury.

Methods: to confirm the expression and localization of α7-nACh receptors on the ileum nerves, an immunofluorescence-based method was applied. Then, Acute systemic administration of α7-nACh receptor agonist PNU-282987 and antagonist methyllycaconitine, and 5-HT1B/1D receptors agonist (sumatriptan) and antagonist (GR127, 935) were used in the model of intestinal IR injury. Finally, glutamate level as a molecular mediator between 5-HT1B,1D and α7-nACh receptors, superoxide dismutase and glutathione peroxidase as critical antioxidant enzymes, serum pro-inflammatory cytokines level such as tumor necrosis factor-alpha and interleukin-beta as systemic inflammation indicators, malondialdehyde level as a lipid peroxidation parameter, myeloperoxidase activity as a neutrophil cumulation index, and histology of the ileum were assessed.

Results: The α7-nACh receptors were expressed by 9% on the ileum nerves. Likewise, activation of the α7-nACh receptor showed anti-inflammatory and antioxidant effects in intestinal IR injury but not as well as 5-HT1B/1D receptors. Interestingly, 5-HT1B/1D receptors via attenuation of glutamate release indirectly activated the α7-nACh receptor and its protective effects against inflammation and oxidative stress.

Conclusions: The protective effect of the α7-nACh receptor on intestinal IR injury was activated indirectly through the 5-HT1B/1D receptors modulatory impact on Glu release.

Keywords: α7-nACh receptor. 5-HT1B/1D receptor. Intestinal ischemia-reperfusion. Inflammation. Glutamate

O-36

SFN0011: A new developed next-generation anti-angiogenic molecule.

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Background: Age-related macular degeneration (AMD) is the most common cause of irreversible blindness among the elderly population. The current treatment options for AMD include intravitreal injection of anti-VEGF. However, clinical experiences demonstrate that the efficacy of such therapies is limited due to overlapping and compensatory alternative angiogenic pathways which culminate to escape mechanisms. sFLT01 is a novel fusion protein that consists of the VEGF/PlGF (placental growth factor) binding domains of human VEGFR1/Flt-1 (hVEGFR1) which are fused to the Fc portion of human IgG (1) through a polyglycine linker.

Methods: We investigated sFLT01 molecule structural components via bioinformatics tools and achieved to its amino acid and nucleotide sequences. We augmented the nucleotide sequence of sFLT01′s by another genetic syntax, against to a nominated antigenic factor. So, we analyzed the secondary and tertiary structures of the cognate tri-specific molecule with swiss-model and i-tasser. The best models were applied in docking analysis with cluspro. The cloning process of the construct was performed in the AAV2 vector and the result was confirmed by conventional PCR and restriction enzyme digestion. RNA extraction and culture condition media collection were performed following the transfection of HEK293T cell line by AAV2-SFN0011.

Results: We designed, constructed, and produced SFN0011 that targets VEGFA, PLGF, and a third party angiogenic factor that could efficiently inhibit tube formation, Tie2 phosphorylation, and Ang2-Tie2 interaction in vitro.

Conclusion: We propose that targeting various angiogenic pathways by SFN0011 may be a fundamental approach in development of next-generation antiangiogenic therapeutic drugs for AMD and other related diseases.

Keywords: Age-related macular degeneration, Angiogenesis, sFLT01, SFN0011, VEGF, Resistance

O-37

Immediate stearoyl-CoA desaturase-1 activity is essential for endodermal differentiation of human induced pluripotent stem cells

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Background: Stearoyl-CoA desaturase 1 (SCD1) is required for de novo synthesis of fatty acid and is associated with protein posttranslational modification through fatty acid acylation process. In this study, we evaluated whether the dynamic of SCD1 activity is important in differentiation of human induced pluripotent stem cells (hiPSCs) to endoderm lineage using a chemical inhibitor, biochemical methods and immunostaining.

Methods: The hiPSCs were cultured in an endoderm inducing medium containing activin A and low defined fetal bovine serum in the presence of an SCD1 inhibitor at different time points. The yield of three germs layers' endoderm, mesoderm and ectoderm was assessed by measuring the surface markers CXCR4, KDR, SSEA-3 and NCAM using flow cytometry. The expression of endoderm specific markers Sox17 and CXCR4 and pluripotency markers Sox2 and Oct4 was assessed by means of Western blotting. Total protein acylation was evaluated using click chemistry reaction and the cell cycle analysis was performed using flow cytometry. The study was approved by the local ethics committee (No. IR.TBZMED.REC.1395.680).

Results: The population of cells with endodermic features decreased at the end of differentiation when SCD1 was inhibited at the first day and effectively rescued by oleate supplementation. Moreover, SCD1 inhibition at day 0 preserved hiPSCs stem cell properties without a shift toward mesoderm or ectoderm. Only, treatment of cells with SCD1 inhibitor at first day decreased the intensity of fluorescent in the click chemistry. Treatment at two subsequent days of endoderm induction induced no significant effect on endoderm specific markers and fluorescent intensity. Reproducible results were also obtained with a human embryonic stem cell line.

Conclusion: SCD1 activity is required for the initiation and commitment of endodermal differentiation from hiPSCs. The requirement for SCD1 activity in endodermal differentiation of ESCs may be eminent in disorders of endoderm-derived organs and dysregulated metabolism.

Keywords: Human iPS cells, endoderm, stearoyl-CoA desaturase, stem cells

O-38

Reduced hypertrophy of chondrogenic differentiated dental pulp stem cells using cobalt chloride

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Background: Degenerative articular cartilage resulting from trauma, inheritance, or aging remains a major problem worldwide. The application of MSCs may potentially make cartilage repair more broadly available. DPSCs are easily accessible multipotent stem cells with high expansion potential. Under appropriate conditions, MSCs are able to differentiate and form cartilage-like tissue while it accompanied with unfavorable hypertrophic properties. Cobalt chloride ($CoCl_2$) a broadly used chemical for mimicking hypoxia, has been proved that triggers the HIF-1α signaling pathway and affect chondrogenic differentiation. The aim of our study was to investigate the hypertrophy of DPSC chondrogenic pellets after $CoCl_2$ pretreatment.

Methods: MTT test was used to determine the optimum concentration and exposure time of $CoCl_2$ for DPSCs. $CoCl_2$ uptake by cells was quantified by inductively coupled plasma mass spectrophotometry (ICP). Chondrogenic differentiation was performed using the standard pellet culture. The GAG content of the pellets was detected by Alcian blue staining to confirm chondrogenic differentiation. Hypertrophic marker, collagen X, was evaluated by Immunohistochemistry.

Results: Concentration of 100 µM for 24 hours was selected as optimum condition for $CoCl_2$ pretreatment ICP results revealed uptake of $CoCl_2$ by DPSCs which in turn leads to successfully differentiated into cartilage like tissues that was confirmed by existing of cartilage specific marker, GAGs. The designed two-step directed chondrogenic differentiation in this study with transient $CoCl_2$ pretreatment led to decreased expression of collagen X in DPSC-derived pellets.

Conclusion: Reduced hypertrophy in DPSC chondrogenic pellets, using a simple chemical agent for mimicking hypoxia, could be favorable for future researches and probable clinical applications.

Keywords: Dental pulp stem cell, Chondrogeninc differentiation, Hypoxia, Cobalt chloride

Poster Pretentions

P-1

Design and Application of a PCR Assay to Detect Francisella tularensis

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Background: Francisella tularensis is the causative agent in tularemia for which the high prevalence of treatment failure and relapse is a major concern. This highly infectious (<10 bacteria) and high-mortality rate pathogen is naturally resistant to penicillin and has been classified as a class A bioterrorism agent by the Centers for Disease Control and Prevention (CDC). The purpose of the present work was to design of a Polymerase Chain Reaction (PCR) assay to detect Francisella outer membrane protein A (FopA) gene as a protective gene for tularemia.

Materials and Methods: The specific primers were designed based on the sequence of the FopA gene of *F. tularensis*. Analytical specificity and sensitivity of the FopA-PCR were evaluated.

Results: The results demonstrated that the primers against FopA gene amplified a 196 bp fragment. The PCR assay was highly specific and no amplification were observed from the non- Francisella organisms.

Conclusion: Designed FopA-PCR has a high sensitivity and specificity compared to other conventional methods, which could be useful for detection of tularemia.

Keywords: Francisella tularensis, Tularemia, PCR, FopA.

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P-2

Efficacy of Biogenic Novel Curcumin Nanoparticles on Leishmania major Promastigotes

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Background: In many parts of Iran and the world, Leishmaniasis is a major problem and also there are several side effects of pentavalent antimony for the treatment of the disease. Curcumin, the therapeutic vehicles in the Nano-scale such as liposome are applied as innovative drug delivery systems instead of the traditional systems. Accordingly, this survey designed a new approach to the investigation of therapeutic efficiency of novel Nanoparticles Curcumin against Leishmania major Promastigotes, in vitro.

Methods: Leishmania (L) major [MRHO/IR/75/ER] cultured in NNN and were transferred to RPMI-1640 medium, supplemented with Antibiotics and 10% fetal calf serum (FCS) then grown at 25±2°C. In plateau phase of parasite growth, effect of different concentrations of Curcumin Nanoparticles (CNPs) in comparison to Glucantime and control, using the Badu (chemiluminescence) technique, on L Major Promastigotes were assessed by means of ELISA reader.

Results: After preparation of CNPs, it confirmed with various tests. The obtained data showed a significant difference between groups treated with various concentrations of CNPs and negative control group at 24 h ($P=0.0001$) in the logarithmic and stationary phases of the parasite. Yet, it showed no significant difference at 48 h ($P>0.05$). Application of Glucantime was also showed a significant difference with negative control group at 24 h ($P= 0.0002$), ($P=0.0001$) in the logarithmic and stationary phases of the parasite, respectively. But Glucantime application after 48 h and 72 h ($P>0.05$) were not effective for two phases of parasite.

Conclusion: The results showed that the CNPs induced a better and more tangible effect on survival of Leishmania major Promastigotes.

Keywords: Leishmania major, Curcumin, Nano-liposome, in vitro

P-3

The radio-sensitizing effect of pharmacological concentration of Ascorbic acid on human pancreatic cancer cells

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Background: Previous studies have reported the inevitable destructive effects of radiotherapy on normal adjacent cells. Ascorbic Acid (AA) has been proposed as an effective anti-cancer agent with no obvious effects on normal cells. Objective: The effects of AA in combination with radiotherapy on human pancreatic carcinoma cell line were studied.

Methods: The human pancreatic cancer cells were cultured and then, divided into four groups. The control group (A) without any treatment; group B that received 2Gy radiotherapy alone; group C that was treated with 4 mM AA alone; and group D that was co-treated with AA and radiotherapy. Cell viability, DNA fragmentation, expression of apoptotic genes, and reactive oxygen species (ROS) production were determined in treated cells.

Results: There was a noticeable decrease in cell viability after treatment with AA (and/or) radiotherapy. All treated groups showed elevated ROS production, Bax/Bcl2 expression, DNA fragmentation, and cytotoxycity compared with the control group. Cells under combination therapy showed the most cytotoxicity.

Conclusion: The results suggest that AA at a dose of 4 mM may be used as an effective radio-sensitizing agent in pancreatic cancer cell line.

Keywords: Ascorbic acid, Radiotherapy, pancreatic cancer

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P-4

Exemestane-Delivery by some Nano-Cages Effective in the Breast Cancer: Molecular Modeling Computations

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Background: Aromatase inhibitor used to treat breast cancer in postmenopausal women. Aromatase plays an important role in the development of estrogen-positive breast cancer. This study had two important aimes. First, to discover an efficient drug with improved potency in the inhibition of aromatase enzyme, which is effective in the breast cancer treatment. In this regard, Exemestane (EMS) was chosen among the studied drugs. Second, the evaluation of the adsorption behavior of EMS onto some fullerene-like cages such as: B12N12, B12P12, C20 and C24 to minimize the toxic side effects of this drug. A high dose of EMS are using for its anticancer activity. Thus, the mentioned cages can be used for delivery and release of the drug into target.

Methods: Molecular docking was used to estimate the binding of all three generations of aromatase inhibitors to the molecular structure of this protein by using Lamarkian algorithm in AutoDockTools software. All quantum computations were performed using the MPW1PW91 and PBE generalized gradient approximation (GGA) for the exchange correlation of density functional theory by Gaussian 09 package.

Results: In this study, EMS exhibits the highest binding energy (-8.77 kJ mol-1) and the lowest inhibition constant (373 nM) than other drugs. The adsorption energies for B12N12 (-1.24eV), B12P12 (-0.67 eV), C20 (-0.18 eV) and C24 (-0.12 eV) fullerene-like nano-cages were calculated. The data showed better value for B12N12 in compare with other nano-cages.

Conclusion: The calculated adsorption energies revealed that B12N12 nano-cage was better than the other mentioned nano-cages. Thus, it can possibly function as a drug delivery carrier and may be used in the therapy of postmenopausal patients with advanced breast cancer.

Keywords: Nano-cages, Breast Cancer, Exemestane (EMS), Density functional theory (DFT).

P-5

Expression and purification of engineered IL17RA protein in E. coli

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Background: Interleukin 17A (IL17A) is a pro-inflammatory cytokine that increases cell division and cancer progression. Overexpression of IL17A plays a pathogenic role in increasing the rate of growth, angiogenesis, and metastasis of cancer cells. It also causes inflammatory responses and autoimmune diseases by binding to its receptor (IL17RA). The purpose of this study is designing and producing a recombinant protein with increased binding affinity to IL17A and also very similar to IL17RA. This protein will occupy the IL17A binding sites and block their binding to the natural receptor so it can prevent or control the development of autoimmune, inflammatory, and cancerous diseases.

Methods: In this study, IL17RA protein was mutated in three positions, and the best variant was selected from 1000 mutated sequences. For this purpose, three-dimensional structures of IL17A and IL17RA and their complexes were obtained from PDB database. The mutations were performed at the positions of 31, 91 and 265 IL17RA by using R software. The binding energy between IL17RA mutant samples and IL17A in each case was calculated by FoldX software. The best variant was expressed in SHuffle (T7) strain of E. coli and purified by Ni+2–NTA chromatography. Protein expression and purification were confirmed by SDS-PAGE and Western blotting techniques.

Results: The variant containing the W31K, N91T, and R265N point mutations had the lowest binding energy between 1000 protein models, and bound to the IL17A protein with high affinity. This protein was expressed and then successfully purified.

Conclusion: This engineered protein had lower binding energy than wild-type protein. So that, it could bind more strongly to IL17A protein.

Keywords: Mutation, E. coli, protein expression, IL17A Protein

P-6

Association of ins/del Genetic Polymorphism of CCR5 in Men with Bladder Cancer in Comparison with Healthy Iranian Men

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Background: Chemokines are involved in the development of effective immune responses, and chemokine receptors can be involved in tumor pathogenesis. Thus, CC Chemokine receptor 5 (CCR5), a member of the CC chemokine receptor class may be related to tumor initiation and progression. Numerous tumor cell types can express chemokines and chemokine receptors. Bladder cancer is also associated with infection-induced inflammation. Thus, our study aimed to realize the possible association between insertion/deletion (ins/del) polymorphism of CCR5 and bladder cancer risks in Iranian population.

Methods: We evaluated the association between ins/del polymorphism in CCR5 gene and bladder cancer in 148 cases and 151 controls of Iranian men. Having used a salting-out method for DNA extraction from blood samples, we designed a quick and simple Polymerase Chain Reaction (PCR) for the detection of ins/del polymorphism in CCR5 gene.

Results: There was no significant association between CCR5 ins/del polymorphism and susceptibility to bladder cancer because the frequency distribution of insertion/insertion, insertion/deletion, and deletion/deletion genotypes were 140, 8 and 0 in cases, respectively; and were 145, 6 and 0 in controls, respectively.

Conclusion: Our proofs, for the first time, propose that the ins/del polymorphism in CCR5 gene was not associated with bladder carcinoma. Further studies on wider populations with various ethnicities are required to confirm this finding.

Keywords: CCR5, insertion/deletion polymorphism, bladder cancer.

P-7

Comparison of Recombinant IL17RA Expression in two Strains of E. coli; SHuffle T7 and Rosetta-gami

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Background: IL17RA is a receptor for inflammatory cytokine IL17A. The binding of IL17A to its receptor activates transcription factors involved in cell division, inflammatory and autoimmune responses. In this study, IL17RA was mutated in three amino acids to increase its binding affinity for IL17A and then produced as a recombinant drug to block natural receptor-ligand interaction. IL17RA has five disulfide bonds, so its expression should be done in the strains with the ability to form disulfide bonds. For this purpose, the expression of recombinant engineered IL17RA in SHuffle and Rosetta-gami strains was simultaneously and comparatively performed. Thioredoxin reductase (trxB) and glutathione reductase (gor) in the cytoplasm keep cysteines in their reduction state and prevent the formation of disulfide bonds. Rosetta-gami strain can form the necessary disulfide bonds in the cytoplasm because of the mutations in the trxB and gor genes. In SHuffle strain, with the deletion of the trxB and gor genes and expression of an isomerase (DsbC), the formation of disulfide bonds in the cytoplasm can be done.

Methods: After induction of the promoter with 1 mM of IPTG, protein expression was done and confirmed with dot blotting, SDS-PAGE, and Western blotting techniques. Image J software was used to quantify the data.

Results: Recombinant protein was expressed in both strains successfully. According to image J results, the expression of recombinant IL17RA in SHuffle T7 strain was higher than in the Rosetta-gami strain.

Conclusion: In conclusion, SHuffle T7 is a suitable strain for the expression of recombinant IL17RA.

Keywords: Rosetta-gami, SHuffle T7, Protein expression, IL17RA

P-8

Production of a fusion apta-toxin against VEGFR and investigating its inhibitory effect on angiogenesis.

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Background: When the size of a tumor reaches about 1 mm, it encounters food and oxygen deficiency (hypoxia). In this situation, tumor cells secrete angiogenic factors to create a new vessel from preexisting one. Increased angiogenesis, increases the risk of metastasis due to the entrance of tumor cells to bloodstream and spreading to other organs. Targeting and induction of endothelial cell death for angiogenesis inhibition was investigated by the fusion of QK peptide aptamer, as a VEGF receptor identifier with Pseudomonas exotoxin A, as an apoptotic agent.

Methods: The fusion protein containing toxin (PE), G4S linker, and QK peptide were designed; and the bioinformatics of the gene and coding usage were performed to optimize the expression in E. coli system. Sub cloning of genes in the expression plasmid pcoldIII and its transformation into E. coli, BL21 (DE3), and Rosetta2 (DE3) was performed. Then, the best expression conditions with proper strain and optimal temperature was determined. The conjugated protein was examined by SDS-PAGE and Western blot methods and then, was purified using the His-Tag column under gradient conditions. The HUVEC cell was isolated from human umbilical cord, cultured and the fusion protein was added to the cell and its toxicity was measured by MTT assay.

Results: The results demonstrated that the fusion QK-PE was successfully cloned in pcoldIII vector and expressed, solubilized and refolded. Then, it was purified by gradient affinity chromatography and the LPS contamination of the purified fusion protein was depleted by triton X-114 treatment. The viability of HUVEC cells under QK-PE treatment was dose dependent up to 0.5 µg/ml, as determined by MTT assay.

Conclusion: The protein offered the potentials to be used as an anti-angiogenesis agent. The novelty of present study is the application of a small VEGF biomimetic peptide (QK) for cell targeting that has the advantage of easier cell penetrating potency.

Keywords: VEGFR, QK Peptide, pseudomonas exotoxin, Angiogenesis, Metastasis

P-9

Effect of Co-Administration of Theophylline and Zinc Sulfate on Total Antioxidant Capacity and Malondialdehyde Levels in Seminal Plasma of Asthenoteratozoospermic Men

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Background: Theophylline is a dimethylxanthine that reduces oxidative stress. Zinc sulfate as a strong antioxidant commonly is prescribed in treatment of male subfertility. Therefore, this study aimed to investigate the effect of co-administration of theophylline and zinc sulfate on Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA) Levels in Seminal Plasma of Asthenoteratozoospermic men.

Methods: 120 asthenoteratozoospermic patients participated in a double-blind, randomized clinical trial study and were randomly divided into four groups: Placebo, theophylline, theophylline + zinc sulfate and zinc sulfate. After 3 months of oral administration, the TAC level was analyzed using the ferric-reducing ability of plasma assay (FRAP). The MDA level was also analyzed using Tiobarbiotic acid (TBA) method at the beginning and at the end of the of the trial. Data was analyzed statistically using the repeated measurements ANOVA and the means were considered significantly different at $P<0.05$.

Results: The TAC level of seminal plasma increased significantly in all three groups compared to the placebo; but, this increase was significantly higher in the theophylline + zinc sulfate group than the theophylline and zinc sulfate groups. The MDA level of seminal plasma reduced significantly in all three groups compared to the placebo; but, this reduction was significantly higher in the theophylline + zinc sulfate group than the theophylline and zinc sulfate groups. The increase of TAC level and decrease of MDA level of seminal plasma was significantly higher in the zinc sulfate group than the theophylline group. There was no significant difference in the mean TAC and MDA of seminal plasma in the placebo group, before and after administration.

Conclusion: Co-administration of theophylline and zinc sulfate, as two antioxidants, could reduce oxidative stress in seminal plasma. Because of the synergism of these drugs, this combination may be prescribed in the case of infertile men with asthenoteratozoospermia.

Keywords: Theophylline, Zinc sulfate, Asthenoteratozoospermia, TAC, MDA

P-10

Association between of vitamin D deficiency and prevalence of metabolic syndrome in female population: A systematic review

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Background: The increasing prevalence of metabolic syndrome (MetS) especially in the female population, has become a major problem in health care systems. In this regard, it is necessary to identify risk factors. Vitamin D deficiency is now proposed as one of the possible risk factors for MetS, we investigated the relationship between vitamin D status and MetS in the female.

Methods: We searched observational studies with keywords vitamin D, metabolic syndrome, metabolic syndrome X, insulin-resistance syndrome, metabolic cardiovascular syndrome, reaven syndrome X, and female in PubMed, Scopus, Science Direct, Cochrane, Web of Science, Google scholar, and SID databases, regardless of publication time. 295 studies were found, and finally, only 12 articles were selected according to exclusion and inclusion criteria.

Results: In 9 studies that reported the prevalence of MetS, the prevalence of MetS among women with vitamin D deficiency was higher than the female with normal vitamin D (34.5% vs. 30.2%). The prevalence of abdominal obesity, high blood pressure, high triglyceride (TG), and HDL deficiency were higher in women with vitamin D deficiency. Also, the mean waist circumference, blood pressure, fast blood sugar (FBS), TG, and body mass index (BMI) were higher in this group. The most incident factor was high blood pressure (61.4% vs. 56.5%) and the lowest prevalence was associated with high FBS (32.2% vs. 33.5% in the other group).

Conclusion: The prevalence of MetS is significantly associated with vitamin D deficiency, and among related factors, HDL, TG, and blood pressure were statistically associated with vitamin D status.

Keywords: Metabolic syndrome, Female, Vitamin D

P-11

The Effects of Malvidin on Oxidative Stress Parameters and Inflammatory Cytokines in LPS-induced Human THP-1 cells

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Background: Malvidin is an anthocyanin, which is involved in inhibiting inflammatory-related mediators in inflammatory diseases. However, its mechanism of action in THP-1 cells is not yet known. THP-1 is a human monocytic cell line that is derived from patients with acute monocytic leukemia.

Methods: The present study aimed to investigate the effect of malvidin on inflammatory responses and oxidative stress in lipopolysaccharide (LPS)-induced inflammation in THP-1 cells. THP-1 cells were stimulated with LPS (50 ng/ml) to induce inflammation in the presence or absence of malvidin. The anti/proinflammatory cytokines were evaluated by real-time polymerase chain reaction and enzyme-linked immunosorbent assay. Total protein levels/phosphorylation of c-Jun N-terminal kinase (JNK), P65-NF-κB, and IKKα/IKKβ were evaluated by Western blot analysis. Malondialdehyde (MDA) and nitric oxide (NO) metabolite levels, ferric reducing antioxidant power (FRAP), total thiol (T-SH) content, and superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity were measured to evaluate the antioxidant activity of malvidin in THP-1 cells.

Results: Treatment of LPS-stimulated THP-1 cells with malvidin (100 and 200 µM) led to the significant inhibition of interleukin-6 (IL-6), tumor necrosis factor-α, and IL-1β messenger RNA (mRNA) expression and protein levels as well as a significant increase in the IL-10 mRNA expression and protein secretion. Moreover, 200 µM malvidin treatment reduced the phosphorylation of JNK, IKKα/IKKβ, and P65-NF-κB. These findings showed that malvidin not only decreased the MDA and NO metabolite levels but also increased the FRAP and T-SH content, as well as SOD and GPx activities.

Conclusion: The findings of the present study demonstrated the potential role of malvidin in blocking inflammation and oxidative stress induced by LPS in THP-1 cell line, suggesting that malvidin is likely to be a therapeutic agent for inflammatory diseases.

Keywords: cytokines, inflammation, malvidin, oxidative stress, THP-1 cells

P-12

Evaluating the co-effect of theophylline and zinc sulfate on the expression of Bcl-2, Bax and Caspas-3 proteins and preventing DNA fragmentation in infertile men with asthenoteratozoospermia: A double-blind, clinical trial study

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Background: Theophylline acts as a cyclic-AMP phosphodiesterase inhibitor which elevates cAMP dependent sperm capacitation, however it can also damage sperm DNA integrity and change the proteins expression. Moreover, Zinc sulfate as an antioxidant can prevent sperm DNA damages. Objective: We investigated the effect of co-administration of theophylline and zinc sulfate on Bcl-2, Bax and Caspase-3 proteins expression and DNA integrity in infertile men with asthenoteratozoospermia.

Methods: 120 asthenoteratozoospermic patients participated in a double-blind, randomized clinical trial study and were randomly divided into four groups: Placebo, theophylline (200 mg/day), theophylline (200 mg/day) +zinc sulfate (220 mg/day) and zinc sulfate (220 mg/day). After 3 months of Oral treatment, the expression of proteins was analyzed using western blotting and the DNA fragmentation was also analyzed using sperm chromatin dispersion test at the beginning of the trial and at the end of the treatment. Data was analyzed statistically using the repeated measurements ANOVA and the means were considered significantly different at $P<0.05$.

Results: The expression of Bax and caspase-3 proteins increased significantly in the theophylline received group, while it showed a significant reduction in the zinc sulfate received group compared to the placebo. The expression of Bcl-2 protein increased significantly in the zinc sulfate received group, while it showed a significant reduction in the theophylline received group compared to the placebo. Sperm DNA fragmentation increased significantly in the theophylline received group, while it showed a significant reduction in the zinc sulfate received group compared to the placebo. There was no significant difference in the expression levels of Bcl-2, Bax and caspas-3 proteins and the mean sperm DNA fragmentation in the theophylline+zinc sulfate group compared to the placebo.

Conclusion: Co-administration of theophylline and zinc sulfate, as an antioxidant, could protect the sperm DNA from the undesirable effects of theophylline. Therefore, this drug combination may be prescribed in cases of infertile men with asthenoteratozoospermia.

Keywords: Theophylline, Zinc sulfate, Asthenoteratozoospermia, protein expression, DNA integrity

P-13

Reduced secretory form of Klotho fails to alter calcium and phosphate concentrations in the cerebrospinal fluid of patients with relapsing-remitting multiple sclerosis

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Background: It has been reported that calcium and phosphate metabolism is disturbed in the serum of multiple sclerosis (MS) patients. Moreover, previous studies have shown that a multi-functional protein, namely Klotho, participates in the regulation of calcium-phosphate and vitamin D metabolism. We hypothesized that Klotho changes in the central nervous system (CNS) of MS patients may lead to alteration of calcium and phosphate concentrations. Thus, we aimed to evaluate the alteration of calcium and phosphate levels along with Klotho concentration in the cerebrospinal fluid (CSF) of patients with newly diagnosed MS.

Methods: In this study, 15 patients with newly diagnosed relapsing-remitting MS (RRMS) along with 15 control individuals with non-inflammatory neurological disorders were enrolled. The CSF was obtained by lumbar puncture (LP) method. The Klotho, calcium, and phosphate concentrations were measured in the CSF using commercial kits.

Results: The results showed that the mean concentration of secretory Klotho in the CSF of MS patients was lower than controls ($P < 0.05$). Furthermore, we found no significant changes in the CSF concentration of calcium and phosphate between MS patients and control individuals ($P > 0.05$). Reduced Klotho in CSF failed to alter the metabolism of calcium and phosphate.

Conclusion: Klotho is involved in calcium-phosphate metabolism in the kidneys. However, it seems that secretory Klotho does not contribute to the metabolism of calcium and phosphate in the CNS at the early stages of MS. Further studies are required to understand the exact mechanism(s) behind the role of Klotho in the CNS of healthy individuals and MS patients.

Keywords: Multiple Sclerosis, Relapsing-Remitting , klotho, Phosphates, calcium

P-14

Modeling of Immunotoxin Constituent Effect on Breast cancer

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Background: cancer biochemistry focuses on the growth of tumors and chemotherapy in cancer research, so breast cancer, which is the leading cause of death in the world, requires serious attention to diagnostic and therapeutic

Methods: in this regard, the development of new immunotoxin drugs aimed at intelligent treatment. The type of disease can be debilitating. Therefore, after obtaining the desired antigen, by examining the structural quality of Cituximab antibody in terms of location of amino acids, 99% of amino acids were in the allowed and appropriate position, and also by examining ERRAT and examining the crystallographic structure of protein. Amino acids were examined at the site, and the chart was obtained with 100% accuracy and a quality factor of 79,503

Results: the results of this study led to the acquisition of a new immunotoxin structure with optimal structural quality after being placed in real quasi-real conditions, as well as the study of the antibody structure after dominoes.

Conclusion: Cancer is a group of diseases that include abnormal increases in the number of cells with the potential to invade and spread to other parts of the body. In this regard, the development of new immunotoxin drugs aimed at intelligent treatment of these diseases can be a way forward. The results of this study led to the achievement of a new structure of immunotoxin with the desired structural quality after exposure to quasi-real conditions and also the study of antibody structure after domain domination

Keywords: Cancer Biochemistry, Targeted Treatment, Antibody, Toxin, Demin

P-15

Evaluation of promoter methylation of PPAR-γ and PGC-1α genes in adipose tissue of obese women

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Background: Epigenetic mechanisms regulating lipogenesis and adipogenesis pathways in the fight against obesity recently come into focus. This study aimed to investigate the gene expression and promoter methylation of PPARγ and PGC1α genes in adipose tissue of obese women compared to normal-weight women.

Methods: This case-control study was composed of 23 obese women ($BMI \geq 35$ kg/m^{2}) and 23 normal-weight ones ($18.8 \leq BMI \leq 24.9$ kg/m^{۲}) aged between 20 to 55 years. The transcript levels of PPARγ and PGC-1α were evaluated in visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) of all participants. After DNA extraction from SAT and treatment with bisulfite, the methylation promoter of PPARγ and PGC-1α genes was also examined.

Results: Decreased expression of PGC1α genes and increased expression of PPARγ genes were observed in subcutaneous adipose tissue of obese people compared to controls. However, the expression of these genes in the visceral adipose tissue of obese individuals was not different from that of healthy individuals. Increased methylation of PGC1α promoter in the SAT significantly correlated with obesity indices; BMI, hip circumference, waist circumference, and insulin resistance. However, PPARγ methylation promoter rates had an inverse correlation with anthropometric indices and insulin resistance value.

Conclusion: Epigenetic regulation in the regulatory regions of PPARγ and PGC1α gene seems to be important in the etiology of obesity and related disorders. However, further studies are needed to prove this concept.

Keywords: Obesity, PPARγ, PGC1α, Lipogenesis, Adipogenesis, Adipose tissue, Methylation.

P-16

The effect of different concentrations of high glucose to induce Liver fibrosis in the human LX-2 Cells

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Background: In the process of liver fibrosis (chronic liver injury), the transforming growth factor-beta (TGF-β) is increased, consequently hepatic stellate cells (HSCs) will be activated. These changes increase the expression of proteins such as alpha-smooth muscle actin (αSMA) and collagen-1 in activated HSCs. The deleterious effect of hyperglycemia on the biology of the liver is supported by clinical evidence. High glucose can lead to the development of liver injury but its role alone on human HSCs and liver fibrosis has not been studied. In this study, we investigated the effect of high glucose as a factor in the progression of Liver fibrosis on human HSCs.

Methods: LX-2 cells (an immortalized human HSC cell lineage) were cultured in Dulbecco's modified Eagle's Medium (DMEM) + 10% fetal bovine serum (FBS) at 37 °C in 5% CO2 and treated with different concentrations of glucose including 22.2, 32.2-44.4 µM for 48 hours. Total RNAs were extracted and Quantitative Real-time PCR (qRT-PCR) was performed for determination of TGF-β, α-SMA and collagen-1 expression.

Results: Our results indicated no significant changes in the level of TGF-β, αSMA, and collagen-1 in the cells treated by different concentrations of high glucose compared to the control group which was treated by 5.5 µM glucose ($P > 0.05$).

Conclusion: Overexpression of TGF-β, αSMA, and collagen-1 is used as standard markers for HSC activation that leads to liver fibrosis. The results demonstrate that the levels of TGF-β, αSMA, and collagen-1 were not increased by treating different concentrations of high glucose. So, high glucose alone cannot cause the progression of liver fibrosis in LX-2 cells.

Keywords: Liver fibrosis, glucose, TGF-β, LX-2

P-17

Quercetin Reduces Liver Fibrosis in the TGFβ –Treated human LX-2 Cell line

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Background: In the process of liver fibrosis (chronic liver injury), the transforming growth factor-beta (TGF-β) is increased, consequently hepatic stellate cells (HSCs) will be activated. These changes increase the expression of proteins such as alpha-smooth muscle actin (αSMA) and collagen-1 in activated HSCs. Quercetin belongs to an extensive class of polyphenol flavonoid compounds almost ubiquitous in plants and plant food sources. Quercetin is found in various food products and plants, including fruits, seeds, vegetables, tea, coffee, bracken fern, and natural dyes. Quercetin is one of the most extensively studied flavonols that possesses strong anti-cancer, anti-inflammatory, antioxidant, antifibrogenic, and as well as cardio- and neuroprotective effects. We investigated the effect of Quercetin on LX-2 human liver cells, as a potential therapeutic for liver fibrosis.

Methods: LX-2 cells (an immortalized human HSC cell lineage) were cultured and treated with 2 ng/ml of TGFβ to induce liver fibrosis for 48 hours. Next, Quercetin (25 mM) was added into the medium of the cells treated by TGFβ for 24 hours. Then, total RNAs were extracted and Quantitative Real-time PCR (qRT-PCR) was performed to investigate the expression of some markers.

Results: Our results indicate the expression of αSMA and collagen-1 are increased in the cells which were treated by TGF-β. Quercetin can reduce the expressions of αSMA and collagen-1 in the cells treated by TGF-β compared to the control group ($P < 0.05$).

Conclusion: Overexpression of αSMA and collagen-1 is used as a standard marker for HSC activation that leads to liver fibrosis. Quercetin attenuates TGFβ -induced liver fibrosis via reducing the levels of αSMA and collagen-1 in LX-2 cells. These data show Quercetin may be a useful agent for the treatment of liver fibrosis.

Keywords: Liver fibrosis, Quercetin, TGF-β, α-SMA, collagen-1, LX-2

P-18

The possible Association of Adipose Tissue Gene Expression of MALAT1, TUG1 with Abdominal Volume Index, Conicity Index, and Renal Injury Markers in the Context of Obesity

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Background: An impaired adipose tissue function caused by a dysregulated gene expression contributes substantially to obesity**.** There is fresh evidence about the regulatory roles of two lncRNAs; MALAT1 and TUG1 as emerging epigenetic players in regulating energy metabolism and insulin signaling. However, the clinical pertinence of these lncRNAs in the field of obesity research in humans is not yet obvious.

Methods: Here, we investigated mRNA expression of MALAT1 and TUG1 in visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) of obese female participants (n=20) and normal-weight women (n=19). We also evaluated the association of their gene expressions with central obesity indices, insulin resistance, and kidney damage indices.

Results: The results showed lower mRNA levels of TUG1 in both the VAT and SAT of obese women, compared to the controls. Furthermore, TUG1 expression in SAT and VAT positively correlated with central obesity indices including BMI, waist to height ratio, abdominal volume index (AVI), and conicity index (CI) and with HOMA-IR in all participants. Moreover, TUG1 expression in SAT positively correlated with creatinine levels and total protein in the obese group even after adjusting with HOMA-IR. The association of TUG1 transcript levels with obesity indices remains significant after adjusting HOMA-IR. The expression of MALAT1 did not differ between the two groups for any tissue. However, it was positively correlated with HOMA-IR in the whole population.

Conclusions: It seems likely that transcript levels of TUG1 in VAT and SAT are involved in kidney abnormalities and central obesity parameters in the context of obesity.

Keywords: lncRNAs, MALAT1,TUG,Adipose Tissue,Obesity

P-19

COVID-19 treatments: A narrative review of the reported clinical trials which may close to the operational phase.

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Background: The recent pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread so rapidly and severely affected the people of almost every country in the world. The highly contagious nature of this virus makes it difficult to take control of the present pandemic situation.

Methods: It is reviewed the published clinical features, symptoms, complications, and treatments of patients with COVID-19. It was searched PubMed for all published articles regarding COVID-19 up to May 2020 (more and less). Keywords used were “COVID-19,” “2019 novel coronavirus,” “SARS-CoV-2,” “2019-nCoV,” “Wuhan coronavirus,” and “Wuhan seafood market pneumonia virus.”

Result: In this study, the clinical trial results gathered based on phase four of laboratory procedures, mostly. Also, it is presented set out the latest achievements of sciences to help health workers around the world to combat the future waves of coronavirus infections. Most of the introduced products approved by the FDA, however, conditional approving can be found such as hydroxychloroquine and chloroquine. This authorization allows for the unapproved use of these medications in light of a public health emergency. It is believed that the second wave of coronavirus infections is inevitable, considering no specific treatment available, the coronavirus disease 2019 (COVID-19) presents a threat to people of all ages including the elderly people and people with other medical complications as a vulnerable group to this disease.

Conclusion: The development of drugs against SARS-CoV-2 has become an urgent necessity to combat the COVID-19 pandemic. With about 15% of COVID-19 patients suffering from severe disease and hospitals being overwhelmed, treatments are desperately needed in this situation. Although there is no specific drug or vaccine available which can be used to treat the COVID-19till now, this study tries to list the most potential ones in a short story that targeting physician societies.

Keywords: Corona virus, COVID-19, Wuhan coronavirus, Treatments, Vaccines

P-20

Bromelain-cisplatin chemo-herbal combination effect on PC3 human prostatic cancer cells: in-vitro study

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Background: Bromelain enhances anticancer impacts to chemotherapeutic agents. The question as to whether bromelain does promote in-vitro cytotoxic and proapoptotic effects of cisplatin on human prostatic PC3 line was investigated. PC3 human prostatic cancer cells were treated with cisplatin alone or in combination with bromelain.

Methods: MTT, clonogenic assay, flow cytometry and real time quantitative polymerase chain reaction were used to investigate cell viability, colony formation, proapoptotic potential and P53 gene expression.

Results: Cisplatin (IC10) combined with bromelain (IC40) significantly affected PC3 cell viability, inhibited colony formation, and increased P53 proapoptotic gene expression compared to cisplatin alone.

Conclusion: Nevertheless, bromelain-cisplatin chemo-herbal combination did not display any additive proapoptotic effect compared to single treatments. Bromelain-cisplatin chemo-herbal combination demonstrated synergistic in-vitro anti-cancer effect on human prostatic cancer PC3 cell line that drastically reduced required cisplatin dose.

Keywords: PC3 Cells, Bromelain, Cisplatin, Synergistic Effect

P-21

Investigating the role of Spirulina platensis on NMRI mouse ovary following silver nanoparticles-induced toxicity: a biochemical, hormonal, and stereological study

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Background: The female fertility can be effected by silver nanoparticles (Ag NPs); because of their ability to cause toxicity in the ovaries. The purpose of this research is investigating the toxicity of Ag NPs, in addition to the antioxidant and protective role of Spirulina platensis (SP) in mouse ovaries.

Methods: In this study, 24 female NMRI Mice were divided into four groups as follows: control; Ag NPs (500 mg/kg daily); SP (300 mg/kg daily) and Ag NPs+SP (n = 6 per group). After 30 days' treatment orally via gavage, plasma biochemical and hormonal parameters were measured and the left ovaries were estimated stereologically. The results were analyzed by one-way ANOVA and Tukey's test, using SPSS, and the means were considered significantly different at $p \leq 0.05$.

Results: The Ferric Reducing Antioxidant Power (FRAP) values, estradiol, progesterone, LH, and FSH concentrations, the mean volume of corpus luteum, and the number of primary, secondary, and graffian follicles were significantly lower ($p \leq 0.05$), whereas malondialdehyde (MDA) concentration was significantly higher ($p \leq 0.05$) in Ag NPs treated group compared with control group. No statistically significant difference was found between the mean total volume of ovary, cortex, medulla, oocyte and its nucleus in different types of follicles, the number of primordial follicles, and zona pellucida, granulosa and theca layer thickness in any group. SP in the Ag NPs+SP treated group considerably improved adverse effects of Ag NPs on above mentioned parameters. On the other hand, FRAP values, reproductive hormone concentrations, the mean volume of corpus luteum, and the number of primary, secondary, and graffian follicles were significantly higher ($p \leq 0.05$), whereas MDA concentration was significantly lower ($p \leq 0.05$) in the SP treated group compared with control group.

Conclusion: The results of this research showed that SP can greatly improve ovary damages induced by Ag NPs, through reducing oxidative stress.

Keywords: silver nanoparticles, Spirulina, ovary, mouse, stereology.

P-22

Bioinformatic assessment of the role of Trp102 residue on structure and function of mnemiopsin 2 by site-directed mutagenesis

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Background: Bioluminescence in photoproteins is an intriguing phenomenon of light emission. Mnemiopsin 2 is a member of Ca+2-regulated photoproteins which has ability of blue light emission through cooperation of calcium ions, coelenterazine (substrate). Unique advantages, like high sensitivity and low-level background signal, make photoproteins to have impressive application in in vivo and in vitro studies. Photoproteins contain EF-hand motifs and a hydrophobic cavity for binding substrate. The Trp102 is one of the residues of cavity. In the current study, we have tried to investigate the role of aromatic residues in the structure and function of mnemiopsin 2 by designing Trp102Tyr mutation.

Methods: For this purpose, the three-dimensional structure of mutant was made with MODELLER program V. 9.23 and the best structure was evaluated using ModEval, SAVES and SPdbViewer software. PIC and ProtParam servers were used to calculate the interactions and physico-chemical properties of protein. ProtScale server showed Kyte & Doolittle hydropathy plot. Finally, the graphical form of the desirable model was drawn using the UCSF Chimera software.

Results: The assessment of ProtParam results showed that Trp102Tyr mutation reduced the molecular weight and protein stability. According to the interactions obtained from PIC server, hydrophobic interactions were reduced in mutant as compared to wild type photoprotein. It seems that the increation of ionic and hydrogen interactions were unfavorable, which has led to a reduction in protein stability. Based on the Kyte & Doolittle hydropathy, this mutation decreases the affinity to coelenterazine mainly because of the change in the geometry and hydrophobicity of coelenterazine-binding cavity.

Conclution: In conclusion, all analyses imply that the whole structure of Trp102Tyr mutant was preserved and it has probable activity as similar as wild type protein. Also, the Trp102 was shown as a key residue that plays important roles in binding and stabilization of substrate to the photoprotein cavity.

Keywords: Mnemiopsin 2, Modelling, Photoprotein, Site-directed mutagenesis, W102Y

P-23

Expression of MT1 receptor in patients with gastric adenocarcinoma and its relationship with clinicopathological features

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Background: Gastric cancer accounts 8% of the total cancer cases leading to 10% of total cancer deaths worldwide. The indoleamine N-acetyl-5-methoxytryptamine, better known as melatonin, is the principal hormone produced by the pineal gland. Recently, it has been well documented some anti-cancer roles of melatonin in some malignancies as breast and colon cancer; as well as some its protective roles in the GI tract that have been known as free radical scavenger, antimitogenic and apoptotic properties. According to the anti-cancer effects of melatonin, wide distribution of this neurohormone in GI tract and some proposed physiologic and pharmacologic roles for this neurohormone and following our previous study which has shown expression of MT2 receptor in gastric adenocarcinoma, this study initially scheduled to determine the expression of melatonin receptor MT1 in tissue samples of adenocarcinoma cancer patients.

Method: A total of 10 gastric adenocarcinoma patients and 10 normal individuals were examined for MT1 gene expression by real-time PCR. Additionally, for screening of different alleles of MT1 in our samples, the SSCP-PCR procedure was developed. Our results have shown interestingly high expression for MT1 receptor in cancer and marginal cancer groups comparing with normal group.

Results: Our findings also have shown that a remarkable association between MT1 receptor mRNA levels and grade in individuals over age 50. PCR-SSCP analysis results showed a variation between individuals which may be effective on their gene expression patterns.

Conclusion: According to our knowledge, for the first time this study evaluated the expression of MT1 receptor gene in gastric adenocarcinoma tissues which consistent with our previous study but with some difference in comparisons between kind of tissue expression and difference in polymorphisms. Moreover, these results show the defending role of melatonin in the GI system.

Keywords: Melatonin, Gastric adenocarcinoma, MT1 receptor, Gene expression, polymorphism.

P-24

Reducing dose of docetaxel using a novel small molecule to achieve efficient inhibition of cancer cell growth

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Background: N-cadherin plays a key role in prostate and breast cancer metastasis and progression. This study aimed to investigate the effects of a novel small molecule, as a N-cadherin inhibitor, in combination with Docetaxel (DTX) on cell proliferation in the prostate and breast cancer cell lines.

Methods: In pervious in silico study we found the appropriate small molecule can inhibit N-cadherin function in cell-cell adhesion and metastasis. Human prostate cancer (PC-3, DU-145) and breast cancer cell lines (MDA-MB468) were used. The cells were treated with various concentrations of DTX (0.001-1000 nM) and small molecule (0.001-100 µM) separately or their combination at time periods of 48 h compared to the control groups. After that the cell proliferation was determined by MTT assay. Also the IC50 values of DTX and small molecule for different cell lines were determined.

Results: Treatment of the above mentioned cancer cell lines with DTX and small molecule, significantly reduced the survival of the cells. Cell viability assay showed a dose-dependent inhibition of PC-3, DU-145 and MDA-MB 468 cell viability by DTX and small molecule treatment ($p < 0.001$). Also, combination therapy by DTX (0.01 nM) and small molecule (0.01 µM) decreased prostate cancer cell proliferation to 53%. While using the drugs separately could decrease cell proliferation to 70 % after 48 h incubation ($p < 0.001$). Besides, combination therapy by DTX (0.5 nM) and small molecule (0.5 µM) inhibited proliferation of MDA-MB468 cells by 70% but using drugs alone reduced the cell proliferation to 64%.

Conclusion: Our data suggest that combination treatment of prostate cancer and breast cancer cells lines with small molecule and DTX could be beneficial in cancer treatment by using reduced doses of DTX.

Keywords: Cancer, Small molecule, Docetaxel, N-cadherin

P-25

Experimental Studies to Understand Basement Membrane Function on Differentiation of the Mesenchymal Stem Cells

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Background: The basement membrane is a dense extracellular matrix that is located between the epithelial cells and connective tissue. The epithelial cells attach to one surface of the basement membrane and the other surface to the connective tissue. The basement membrane is broadly similar between different types of tissues but they may differ in function and effect on different cells in different tissues. The basement membrane enables a variety of cells and multicellular orangs to reconstruct the tissue structure and orangs of their cells that have been destroyed. In the case of damage to the basement membrane, tissue dysfunction occurs. The basement membrane helps to maintain tissue order and make cells in the tissues. Common disorders and those that cause pathogenesis including: emphysema (Enlargement and enlargement), Scars, adhesions, cirrhosis of liver, and excessive accumulation of basement membrane material occurs in patients with diabetes, are examples of cases reported so far. The purpose of this study was to conduct experimental studies to evaluate the interactions between mesenchymal stem cells and the basement membrane in several different orangs and in vitro.

Materials and methods: Preparation of scaffolds from the esophagus-trachea-skin-blood vessels in a chick model based on the following procedure: slow and snap freezing at zero degrees Celsius and liquid nitrogen respectively, treatment with Triton x-100 and SDS and final rinse in ethanol 70%, sterile distilled water and PBS. Then the mesenchymal stem cell-like cells dived from mouse and were cultured on the prepared scaffolds.

Results and conclusion: Evaluate the interactions between the cells and the scaffold indicated morphological or cytochemical changes such as cell division, migration and differentiation. So these changes in the behavior of the implanted cells shown that the inducible effect of the basement membrane.

Keywords: Extracellular Matrix ‹Mesenchymal Stem Cells ‹Decellularization.

P-26

Effect of bioactive nanofillers on the biological properties of bio-based composite scaffolds

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Background: It is well-known that mechanical strength of the natural bone results from the complex integration of hydroxyapatite (HAp) into a fine protein template which is in the form of collagen strands. Artificial nanofibrous composites have therefore been fabricated and served as a temporary tissue engineered scaffold. The aim of this study was to evaluate the biological properties of nanofibrous scaffolds made of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) combined with HAp nanoparticles having different geometries, surface chemical groups, and concentrations.

Methods: The tested scaffolds were PHBV nanofibers, PHBV nanofibers containing different concentrations of surface-modified as well as surface-unmodified spherical nanoparticles, and PHBV nanofibers containing different concentrations of both surface-modified and surface-unmodified rod-like nanoparticles. The effect of the different parameters corresponding to HAp nanoparticles on the MG63 osteoblast-like cell line, in terms of biocompatibility, viability, and proliferation was evaluated by MTT assay.

Results: Cell-viability evaluation through the MTT assay revealed no cytotoxic effects. To put it another way, MTT assays indicated that the evaluated nanofibrous scaffolds were completely biocompatible and all scaffolds show a good proliferation behavior. According to the biological assays, PHBV nanofibers containing modified spherical HAp and PHBV nanofibers containing untreated rod-like particles, with concentration of 25% with respect to the polymer, had better cell viability and proliferation compared to other experimental groups.

Conclusion: The results reflected that nanofibrous scaffolds containing HAp nanoparticles provided the ECM-like environment necessary for the cells to proliferate. In vitro cell assays also demonstrated that while particle geometry cannot significantly affect the cellular behavior, the surface modification and particle concentration have the highest importance for the cellular functions among the studied factors.

Keywords: Cell culture, MG63 cell line, MTT assay, cell viability, biocompatibility

P-27

Investigation of Interleukin-38 circulating level as a biochemical marker for disease activity in multiple sclerosis

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Background: Both pro- and anti-inflammatory cytokines have in recent years been studied as biochemical markers of disease activity. Multiple sclerosis (MS) is a chronic autoimmune disease of central nervous system. The aim of this study was to investigate the possible role of Interleukin (IL)-38, a newly-discovered cytokine, as an anti-inflammatory biochemical marker in multiple sclerosis.

Methods: Our study groups were composed of 44 newly-diagnosed and 43 previously-treated MS patients, and a sex and age-matched healthy group. IL-38 serum levels were assessed by enzyme-linked immunosorbant assay (ELISA). This work was supported by Elite Researcher Grant Committee under award number [971223] from the National Institute for Medical Research Development (NIMAD).

Results: The results of ELISA showed a significant higher level of IL-38 in newly-diagnosed MS patients (206±38pg/ml) compared to those previously treated (158±39pg/ml, P<0.0001). Among the patients, 76.4% presented with relapsing remitting (RRMS), 12.4% had primary progressive (PPMS) and 11.2% were in secondary progressive (SPMS) course of disease at the time of sampling. IL-38 levels did not differ between these groups of patients (169±47pg/ml, 168±24pg/ml, 149±19pg/ml, respectively, P=0.4). No significant association with expanded disability status scale was detected. The progression index showed no correlation with IL-38 levels in previously-treated patients.

Conclusion: Although, the higher serum levels of IL-38 in new cases of MS compared to treated patients and healthy controls suggest that this cytokine might play a role in disease development, it seems that it cannot be considered as a biochemical marker for disease activity and type of MS.

Keywords: IL-38, biochemical marker, multiple sclerosis

P-28

Reduced levels of IL-38 in vitiligo and its possible role as a biochemical marker for the quantity of melanin pigments

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Background: Vitiligo is a progressive disorder, caused by melanocytes dysfunction and chronic depigmentation. Cytokines, including interleukins have a variety of pro- and anti-inflammatory effects in the body through a number of biochemical pathways. These small molecules are candidates to act as biochemical markers for various diseases. In this study, we investigated the potential role of IL-38, a newly-introduced cytokine with a proposed anti-inflammatory profile, in vitiligo,

Methods: A group of patients with generalized vitiligo (69 patients) and 72 healthy individuals were enrolled in this study. Serum IL-38 level was evaluated in all subjects using capture ELISA. The medical information of the patients was collected. This work was supported by Elite Researcher Grant Committee (award no.971223) from the National Institute for Medical Research Development (NIMAD).

Results: IL-38 serum quantity in vitiligo patients (159±39pg/ml) was lower than healthy controls (166±34pg/ml, P=0.039). We found a significant difference in IL-38 level in both male and female patients according to skin type. The quantity of this cytokine in female patients with skin type III (168±49pg/ml) was higher than those with skin type IV (135±9.6pg/ml) (P=0.018). In addition, IL-38 level in male patients with skin type III (149±21pg/ml) was significantly lower than the female patients with this type of skin (168±49 pg/ml) (P=0.046).

Conclusion: The lower level of IL-38 in the patients implies the role of this cytokine in vitiligo, and its relationship with different skin types, candidate this cytokine for more studies as a biochemical marker for the quantity of melanin pigments in the skin.

Keywords: Biochemical markers, IL-38, vitiligo

P-29

Glucose concentration significantly affects the non-glycosylated heavy chain levels of monoclonal antibody produced in a CHO cell line

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Background: Glycosylation of therapeutic proteins has a significant impact on their safety and efficacy. Many factors shape the glycosylation of bio therapeutics, ranging from expression systems and cell culture processes to downstream purification strategies. In a Chinese hamster ovary (CHO) cell culture process, it was seen that the amount of non-glycosylated antibody was correlated to the extent of time the cells deprived of glucose. It has been shown that due to the lack of glucose in the feed, nearly half of the product was non-glycosylated. Therefore, in this study different glucose concentration set points were checked to control glycosylation of a glycoprotein-based therapeutic drug.

Methods: In this research four experiments were designed in a 5L bioreactor to examine the effect of different glucose set points (0, 0.5-1, 1-2 and 2-3 g/L) during fed-batch culture on the level of non-glycosylated IgG1. The effect of glucose set points were determined to be significant using ANOVA analysis (p values <0.05). Each experiment was harvested on the day 15 and the non-glycosylated heavy chain (NGHC) levels were evaluated by the method of capillary electrophoresis.

Results: According to the results, increasing glucose concentration set points from 0 to 0.5-1, 1-2 and 2-3 g/L, reduced NGHC levels of the monoclonal antibody (70±5% p value = 0.069); however, increasing glucose concentrations led to the increase in lactate accumulation (from 9±1 to 15±4 mM, at the end of the culture) and subsequently decreased protein expression (from 3500±200 to 3000±300 mg/L p Valus = 0.08).

Conclusion: Process parameters such as glucose availability can affect non-glycosylated level of monoclonal antibodies. Furthermore, nutrients consumptions and by-products accumulations are affected by glucose concentrations. Therefore, optimization of glucose concentration is integral part of CHO cell culture.

Keywords: CHO cell cultures, Glucose concentration, Monoclonal antibody, Non-glycosylated heavy chain (NGHC)

P-30

Spirulina Platensis counteracts the silver nanoparticles-induced reproductive system toxicity by regulating oxidative stress in testis of male NMRI mice.

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Background: Silver nanoparticles (AgNPs) are used in industrial and medical applications widely. humans may be exposed risk of toxicity through different routes. This study was performed by examining the probable protective role of Spirulina Platensis (SP) against the effect of AgNPs on the male reproductive system.

Methods: Adult male NMRI mice were divided into four groups (n=6): control group, SP group (300 mg/kg.bwt/day orally for 35 days), AgNPs (20 nm) group (500 mg/kg.bwt/day orally for 35 days), co-treated group SP+AgNPs (administered SP300 mg/kg.bwt/day with AgNPs 500 mg/kg bwt/day orally for 35 days). At the end of the trial period, sperm and serum samples were collected. Subsequently, Body and testes weights, epididymal sperm count, motility, vitality, morphology, tail length, sperm DNA damage, daily sperm production (DSP), Sexual hormone serum levels, malondialdehyde (MDA), total antioxidant capacity (TAC) were evaluated. In addition, histopathology evaluation of testis tissue was performed using tissue processing, hematoxylin-eosin (H&E) staining and Stereology techniques respectively.

Results: Sperm parameters reduction were observed in the AgNPs treated group. serum levels of testosterone and TAC were significantly decreased following AgNPs treatment. MDA, LH were significantly incremented in serum of AgNPs treated mice. histopathology analysis revealed AgNPs exposure induced histopathological changes and alterations of the seminiferous tubules, degeneration and dissociation of spermatogenic cells. SP co-administration counteracted significantly AgNPs reproductive toxicity impacts. SP co-exposure caused an increase in TAC serum level, a decrease in lipid peroxidation product (MDA serum level) and improvements in histopathological changes of the testes tissue and spermatozoa abnormalities. In parallel, SP modulated the serum levels of testosterone, FSH, LH hormones.

Conclusion: Spirulina Platensis as a natural antioxidant by regulating oxidative stress, prevented the AgNPs-induced testicular and male reproductive system injuries. the obtained data demonstrated that SP co-administration elicits both protective and therapeutic potential against the AgNPs-induced reproductive toxicity.

Keywords: Silver nanoparticles, Spirulina Platensis, Oxidative stress, male Reproductive toxicity, Testis tissue, Spermatogenesis

P-31

Synthesis and characterization of 3D dandelion-like particles for application in bone tissue engineering

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Background: Bioactive hydroxyapatite (HAp) particles as reinforcing filler have widely been used to produce polymer composite scaffolds suitable for application in bone tissue engineering. HAp phase has high surface activity and bioactive structure, similar to the minerals found in human bone tissue. It is well known that biological and mechanical properties of HAp depend greatly on various factors such as crystalline phase and morphology. Therefore, a particular importance lies in selecting a suitable method for synthesizing HAp to precisely engineer the morphology and microstructure of particles.

Methods: Herein, HAp microstructures with dandelion-like morphology were synthesized by hydrothermal method under relatively new conditions in the presence of ethylene diamine tetra-acetic acid (EDTA) and urea reagents. The morphology and structure of the as-synthesized microparticles were characterized by Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

Results: The morphology of HAp particles was significantly affected by both the pH and EDTA/Ca ratio. While the low pH values could promote the formation of dandelion-like morphology, the EDTA/Ca ratio affected the degree of self-assembly. SEM observations showed that the as-precipitated powder consisted of dandelion-like particles with dimension of around 5 µm. Moreover, characteristic IR absorption bands of HAp corresponding to phosphate and hydroxyl groups were detected in FTIR spectrum. In brief, while the typical peaks of phosphate are seen at low wavenumbers (below 1400 cm–1), the absorptions of hydroxyl were at about 3575 and 632 cm–1.

Conclusion: In conclusion, this study represents a successful attempt to synthesize the HAp phase with dandelion-like morphology for application in bone tissue engineering. The fabricated particles as an assembled construct can reinforce the polymer-based composites more efficiently than the conventional structures.

Keywords: Keywords: Hydroxyapatite, dandelion-like particles, bone, crystalline phase.

P-32

Synthesis, detect and investigates the catalytic activity of the V_2O_5 metal complex as synthetic enzymes

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Background: Natural enzymes (typically proteins) catalyze different kinds of reactions in the body and speed up the rate of chemical reactions. However, these biological molecules have some drawbacks such as low stability and high cost of extraction and purification. So, developing alternatives to natural enzymes (artificial enzymes) has gained much more attention among the researchers. Vanadium oxide (V_2O_5) nanoparticle is an inorganic semiconductor material. These nanoparticles, with very low toxicity, show good physical and thermal stability. Also, these particles have shown great application due to its redox-activity and layered structures.

Methods: In this work, V_2O_5 nanoparticles were synthesized and characterized by X-ray diffraction (XRD), UV-Vis absorption spectroscopy, Fourier transformed infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The enzyme-like activity of prepared nanoparticles was investigated spectrophotometrically using colorimetric methods.

Results: The obtained results indicated that V_2O_5 has a peroxidase-mimic activity. Kinetic parameters (Km and Vm) were calculated to be 0.035×10^{-3} M and 74.6×10^{-4} Ms^{-1}, respectively. Also, the ability of the prepared nanoparticles to decolorize malachite green was investigated and the results indicated that V_2O_5 nanoparticles completed the decolorization process of MG within 2 h.

Conclusion: This study indicated that V_2O_5 nanoparticles, with intrinsic peroxidase-mimic activity, can be used for the decolorization and detoxification of malachite green in wastewater.

Keywords: Vanadium oxide (V_2O_5) nanoparticle, Peroxidas-mimic activity, Colorimetric method

P-33

The effect of diabetic serum free fatty acids on the stemness markers of adipose tissue-derived mesenchymal stem cells

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Background Diabetes mellitus (DM) is caused by deficient insulin secretion or insulin resistance leading to dyslipidemia. Human adipose tissue-derived mesenchymal stem cells (AdMSCs) are crucial for the regeneration process of adult tissues. Since regenerative capacity is impaired in DM, this study aimed to evaluate the effect of diabetic serum free fatty acids (FFAs) on stemness of AdMSCs in vitro.

Methods AdMSCs were retrieved from abdominal adipose tissue samples. Pooled diabetic and non-diabetic serums were then prepared from 7 and 8 women in menopausal ages (up to 65 year), respectively. AdMSCs were treated with diabetic serum (DS), non-diabetic serum (NDS), diabetic FFA-enriched serum (DFS) and non-diabetic FFA-enriched serum (NDFS) for 48 hours. FFA composition was analyzed using gas-liquid chromatography. The expression of stemness markers CD49e and CD90 were also analyzed by quantitative PCR. Cell proliferation was evaluated by BrdU assay. The study was approved by the local ethics committee (No. IR.TBZMED.REC.1398.659).

Results A significantly higher oleate level was observed in DS (1.4-fold, $p<0.05$) compared to NDS. Compared to NDS, DS significantly decreased CD49e expression (0.4±0.24 versus 1.05±0.43-fold change, $p<0.05$, respectively). However, no significant differences were observed in CD90 expression between the groups. Compared to NDFS, DFS significantly decreased the CD49e and CD90 expression (4.14±0.08 versus 6.2±0.46-fold change, $p<0.05$, and 1.34±0.17 versus 2.74±0.5, $p<0.05$, respectively). Additionally, DFS significantly decreased AdMSCs proliferation rate in contrast to NDFS (68.0%±1.0 versus 105.36%±0.65, $p<0.05$).

Conclusion The negative effect of DS on AdMSCs stemness maintenance may be due to the higher oleate level. Therefore, controlling this fatty acid level in DM can be considered as a strategy for improving regeneration capacity in diabetic complications such as ulcers and delayed wound healing.

Keywords: free fatty acids, mesenchymal stem cells, diabetes, gas-liquid chromatography

P-34

Evaluation of Curcumin Effects on Post-Operative Peritoneal Adhesion in Rats

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Background: The purpose of this study holds, for the first time, an evaluation of the intraperitoneal curcumin lavage on the development of post-operative intra-abdominal adhesions.

Methods: Thirty male Wistar rats were randomized into five groups. The rats were administered anesthesia and underwent surgery in order to create intra-abdominal adhesions. Before the abdomen was closed, five lavage solutions of normal saline (control group), curcumin 1, 3, and 5% and hydrocortisone 1% were used for 1 min. After five days, the rats underwent laparatomy. Based on a histopathology evaluation and serum levels of hs-CRP, TNFα and Isoprostane, peritoneal adhesion severity was compared in different groups.

Results: The groups that received curcumin 3% and 5% showed a significant decrease in TNFα, hs-CRP and Isoprostane serum concentrations compared to the normal saline group, however, these differences were not significant, between the other groups. The intensity of adhesions in the different groups of curcumin 1, 3 and 5% concentrations and hydrocortisone 1% were compared to the normal saline control group and no significant statistical difference was recorded.

Conclusion: Curcumin was not effective in post-operative peritoneal adhesion; however, further studies on curcumin lavage in higher concentrations are recommended.

Keywords: Curcumin, Post-operative Peritoneal Adhesion, Rat

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P-35

Essential micronutrients during pregnancy

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Vitamins and minerals, collectively known as micronutrients. Many women are deficient in these essential nutrients for good body health. Besides that, during pregnancy, the need to provide nutrients for the fetus is added to it and exacerbates the deficiency of these nutrients; and this can affect the health of mothers and their babies. Iron deficiency causes disorders such as iron deficiency anemia, low birth weight, premature delivery, impaired physical and cognitive development in infants and young children, and weakness and fatigue in adults. This deficiency may also increase the risk of dying from bleeding during childbirth and as well as prenatal death. Folic acid deficiency and as well as zinc deficiency can be associated with increased risks of premature delivery, low birth weight, fetal growth retardation, and complications of pregnancy and childbirth such as placental abruption and preeclampsia. Iodine deficiency during pregnancy leads to cretinism, hearing loss, premature delivery, possible lesions, and even fetal death. Deficiency of other minerals such as selenium, copper, potassium, magnesium and calcium, and as well as deficiencies of vitamins other than folate, are associated with complications of pregnancy and childbirth, and as well as impaired fetal growth. Further research is needed to find an appropriate and cost-effective solution to prevent, diagnose, treat, and assess the consequences of micronutrients deficiencies during pregnancy.

Keywords: Pregnancy ،Micronutrients ،Vitamins ،Minerals ،Fetal growth ،Low birth weight ، Complications of pregnancy and childbirth

P-36

Evaluation of apoptosis in the Regorafenib-resistant SW48 colon cancer cell line treated with β1 integrin siRNA using DDAB-mPEG-PCL hybrid nanoparticles

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Background: Colorectal cancer is the third most common cancer globally and Regorafenib as an RTKI agent disrupts tumor angiogenesis. Unfortunately, drug resistance occurs through different mechanisms such as β1 integrin overexpression. Using siRNA targeting β1 integrin as a combination therapy is an effective and specific method to combat such resistancy. Lipid-polymer hybrid nanoparticles are a new series of nanocarriers for efficient siRNA encapsulation and delivery. Herein the apoptosis rate was assessed in the Regorafenib resistant SW48 cell lines using a complex of siRNA and HNPs (DDAB-mPEG-PCL) in combination with Regorafenib/HNPs.

Methods: Regorafenib resistant SW48 cell line and non-resistant cells were transfected with different doses of siRNA/HNP complexes in combination with Regorafenib/HNPs. Apoptosis assessment was conducted after 56 hours of transfection according to the protocol of FITC Annexin V Apoptosis Detection Kit using the BD FACSCalibur instrument. Both early apoptotic (annexin V-positive and propidium iodide-negative) and late apoptotic (annexin V-positive and propidium iodide-positive) cells were considered as the determinant of cell death. The results were analyzed by FLOW JO software.

Results: The apoptosis rate in the non-resistant and the resistant cells were 26.9±0.56% and 13.12±0.62% respectively ($p<0.01$). Downregulation of β1 integrin gene expression induced by siRNA/HNP complex in combination with Regorafenib/HNPs resulted in a significant enhancement of apoptosis in the resistant cells ($p<0.001$).

Conclusion: Our results indicated that combination therapy using siRNA/HNP and Regorafenib/HNPs complex increases the apoptosis rate which may induce drug sensitivity.

Keywords: Colorectal cancer, Regorafenib, β1 integrin, SiRNA, lipid-polymer hybrid nanoparticle, apoptosis.

P-37

Antioxidant and anticancer properties of Zataria multiflora essential oil

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Background: Zataria multiflora (Avishan Shirazi) is a thyme-like aromatic plant from the Lamiaceae family. Z. multiflora essential oil (ZMEO) is one of the essential oils possessing broad biological activities. The primary objective of this study was to explore the phytochemical and biochemical properties of ZMEO.

Methods: The chemical composition of ZMEO was and analyzed by gas chromatography. In vitro, the antioxidant capacity of ZMEO was analyzed by hydrogen peroxide and nitric oxide (NO) scavenging. The ability of ZMEO on the inhibition of NADH oxidase (NOX) and nitric oxide synthase (NOS) expression and activity and modulation of NF-kB and NRF2 transcription factor expression in LPS-stimulated macrophages were investigated. MTT assay, fluorescence staining, flow cytometry were performed to confirm the anticancer effect and determine the death mode in treated epithelial breast cancer cell line (MCF-7).

Results: The main components of ZMEO were carvacrol, thymol, p-cymene, γ-terpinene, and linalool. ZMEO exhibited good antioxidant activity against hydrogen peroxide and NO at low concentrations. ZMEO strongly reduced intracellular hydrogen peroxide and NO production and NOX and NOS expression in LPS-stimulated macrophages. NF-kB expression was declined while NRF2 expression was increased by the ZMEO in LPS-stimulated murine macrophages. ZMEO increasingly suppressed viability in MDA-MB-231, MCF-7, and T47D Breast cancer cells while nontoxic to L929 normal cells in monolayer cell cultures, whereas MDA-MB-231 multicellular spheroids were more resistant to inhibition. ZEO significantly induced cell apoptosis confirmed by fluorescent staining, flow cytometry analysis, and DNA fragmentation in MDA-MB-231 cell cultures.

Conclusion: ZMEO is effective as an antioxidant and anti-cancer agent, suggesting the ZMEO as a potent candid in cancer therapy and the development of novel antioxidant drugs in the preservation and the treatment of oxidative related diseases. The decrease in NF-kB and increase in NRF2 conducted that ZMEO by modulation of transcription factor changes the oxidative stress.

Keywords: Zataria multiflora, essential oil, antioxidant, cancer, NRF2, NF-kB

P-38

The effect of diabetic serum free fatty acids on stemness markers of adipose tissue-derived mesenchymal stem cells

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Background Diabetes mellitus (DM) is caused by deficient insulin secretion or insulin resistance leading to dyslipidemia. Human adipose tissue-derived mesenchymal stem cells (AdMSCs) are crucial for the regeneration process of adult tissues. Since regenerative capacity is impaired in DM, this study aimed to evaluate the effect of diabetic serum free fatty acids (FFAs) on stemness of AdMSCs in vitro.

Methods AdMSCs were retrieved from abdominal adipose tissue samples. Pooled diabetic and non-diabetic serums were then prepared from 7 and 8 women in menopausal ages (up to 65 year), respectively. AdMSCs were treated with diabetic serum (DS), non-diabetic serum (NDS), diabetic FFA-enriched serum (DFS) and non-diabetic FFA-enriched serum (NDFS) for 48 hours. FFA composition was analyzed using gas-liquid chromatography. The expression of stemness markers CD49e and CD90 were also analyzed by quantitative PCR. Cell proliferation was evaluated by BrdU assay. The study was approved by the local ethics committee (No. IR.TBZMED.REC.1398.659).

Results A significantly higher oleate level was observed in DS (2.08-fold, $p<0.05$) compared to NDS. Compared to NDS, DS significantly decreased CD49e expression (0.4±0.24 versus 1.05±0.43-fold change, $p<0.05$, respectively). However, no significant differences were observed in CD90 expression between the groups. Compared to NDFS, DFS significantly decreased the CD49e and CD90 expression (4.14±0.08 versus 6.2±0.46-fold change, $p<0.05$, and 1.34±0.17 versus 2.74±0.5, $p<0.05$, respectively). Additionally, DFS significantly decreased AdMSCs proliferation rate in contrast to NDFS (68.0%±1.0 versus 105.36%±0.65, $p<0.05$).

Conclusion The negative effect of DS on AdMSCs stemness maintenance may be due to the higher oleate level. Therefore, controlling this fatty acid level in DM can be considered as a strategy for improving regeneration capacity in diabetic complications such as ulcers and delayed wound healing.

Keywords: free fatty acids, mesenchymal stem cells, diabetes, gas-liquid chromatography

P-39

Effects of opium on the expression of CD36 gene in Coronary Artery Disease

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Background: In some Asian countries, the traditional belief among people is that opium may has a positive effect on coronary artery disease (CAD) health, but researches have shown that this substance may play a potential role in the development and progression of CAD. The molecular mechanism of this substance in relation to CAD has not yet been determined exactly. The aim of the study was to explore the role of opium on the expression of scavenger receptor, CD36, in the coronary artery disease (CAD) patients.

Methods: This case-control study was conducted on three groups: CAD opium-addicted (n=30); CAD non-opium-addicted (n=30); and non-opium-addicted with no CAD individuals as a control group (n=17). The protein and mRNA levels was evaluated by flow cytometry and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) methods, respectively.

Results: A significant decrease was found in the mRNA level in both CAD and CAD-addicted patients compared to the control group ($P = 0.002$ and $P > 0.001$, respectively). No significant difference was found in protein level among the three study groups.

Conclusions: The results of the present study demonstrated that opium has not shown a significant effect on the expression of CD36 at the gene and protein levels.

Keywords: Keywords: Opium, Coronary artery disease, Cytokines, CD36

P-40

A new approach to metal drugs with the help of artificial cell synthesis science

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Background: Cisplatin is the primary drug of chemotherapy. It has many side effects on humans and animals. In this study, we used modern methods to synthesize artificial cells of the liposome type so that we could reduce the side effects of the drug.

Methods: The preparation of the optimal formulation of liposome was improved and developed as a new treatment to achieve greater drug efficacy.

Results: In order to optimize cis-platinum with a nanoliposome layer, we examined the effects of different molecular ratios of SPC80: cholesterol in size, dispersion index (PDI), and livestock efficiency. The mean smaller average and maximum bed efficiency were obtained in the formula without DSPE-mPEG (F1) of 4.4 nm 2.3 and 243 and 8.38 80 3.80%, respectively. Trapping efficiency has decreased with increasing cholesterol content (F1 → F4). In order to evaluate the effect of adding DSPE-mPEG2000, 5% DSPE-mPEG2000 has been added to the selected F1 formula. The optimal size of nanoliposome, PDI, zeta potential and frost efficiency was 2.1nm 119.7 n 119, 0.01 0.2 0.203, 1.34mV 1.26--26.03 and 3.65 3 3, based on which the F5 formula was selected as the best formulation. Determination of the release rate of cisplatin from PEGylated nanoliposomes had acceptable results. The FTIR spectroscopy investigated the functional groups of the nanoliposomal surface and chemical interaction between drug and nanoliposome, in which the spectrum before cisplatin loading is shown. There were distinctive peaks of a phospholipid, cholesterol, and DSPE-mPEG at 3449 cm−1 (O-H stretching), and 1636 cm-1substantiated the existence of C=O stretching. These peaks were repeated, which it illustrates the FTIR spectrum after cisplatin loading. These findings indicated no chemical interaction between cisplatin and carriers (core and wall materials).

Conclusions: This study suggests that nanoliposome-loaded cisplatin plays a vital role in improving drug efficacy and reducing the dosage.

Keywords: Solid cancer, Cisplatin, Chemotherapy, Nanoliposome

P-41

Gallic Acid Mitigates Diclofenac-Induced Liver Toxicity by Suppressing Inflammatory Response and Modulating Oxidative Stress in Male Rats

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Background: Diclofenac (DIC) is an NSAID and consumption of this drug creates side effects such as liver injury. Gallic acid (GA), a natural component of many plants, is used as an antioxidant agent. Objective: This study assesses the hepatoprotective effects of GA in the rat model of DIC-induced liver toxicity.

Materials and methods: In this research, the male Wistar rats were separated into five groups (n¼6). Group 1, control, received normal saline (1mL/kg bw, i.p); Group 2 received DIC-only (50mg/kg bw, i.p.); Groups 3, received DIC (50mg/kg bw, i.p.) plus silymarin (100mg/kg bw, po), groups 4 and 5 received DIC (50mg/kg bw, i.p.) plus GA (50 and 100mg/kg, po, respectively)

Results: The data demonstrated that the liver levels of the GSH, GPx, SOD, and CAT significantly reduced and the levels of the serum protein carbonyl, AST, ALP, ALT, total bilirubin, MDA, serum IL-1b, and the liver IL-1b gene expression were remarkably increased in the second group compared to the control group. On the other hand, treatment with GA led to a significant elevation in GSH, GPx, SOD, CAT, and a significant decrease in protein carbonyl, AST, ALP, ALT, total bilirubin, MDA, serum IL-1b, and gene expression of IL-1b in comparison with the second group. Histological changes were also ameliorated by GA oral administration. Discussion and

Conclusions: The data found that the oral administration of GA could alleviate the noxious effects of DIC on the antioxidant defense system and liver tissue.

Keywords: Gallic acid, Diclofenac, Liver toxicity, Oxidative stress,

P-42

Methods for evaluating cell-cell interactions in vitro

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Background: Cellular interactions are significant biological phenomena, which play an essential role in cell differentiation and even the daily activities of cells at the beginning of the embryo's period and after. Introducing and developing some of the indicators of cell-cell interaction evaluation is one of the important things that has attracted the attention of cellular and molecular biologists in recent years. In this article, we first introduce some related methods. Appropriate indexes for evaluating cells that interact with each other have also been investigated. In the final section, examples of empirical studies in this research are presented, and a suitable and available technique is investigated.

Methods: Techniques used in studies of cell-cell interactions: Cell isolation differentiation Proliferation Assays Analysis of radioactive proteins by electrophoresis on two-dimensional polyacrylamide gels Labeling with [35S] methionine Monolayer cultures of EECs Histotechnique Cell Culture in this research, techniques 7 and 8 were used to study the cell-cell interactions between blastema tissue of a New Zealand male rabbit's pinns and rabbit liver hepatocytes of the same race. Therefore, rings with 2mm diameter in rabbit auricle were created by punch, and again after two days, punch with 4mm in diameter was performed. Then, the obtained blastema rings were assembled with liver hepatocytes, and the assembled samples were transferred to the culture medium. At 7, 10, 14, and 21 days post-culture, the samples were transferred to 10% formalin fixative for histological examination (complete culture medium includes FBS, Penstep, and DMEM).

Evaluation indexes, results, and conclusion: Evaluation indexes in this study are based on morphological and cytochemical studies, including changes, differentiation, or behavior of blastema cells such as cell division, apoptosis, migration. It seems that the methods used in this study could also be a suitable method for evaluating cell-cell interactions.

Keywords: Cell-cell interactions, Blastema cells, Hepatocytes, In vitro conditions, Cell culture, Cytotechnique.

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Blastema Tissue Ideal Model for Commercialization

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Background: Cell studies and researches on blastema tissue have long been the subject of an investigation by cellular and molecular science laboratories. Matin and Mahdavi in 2011 reported oct4 genes and their activity. Hashemzadeh investigated wound healing under in vitro conditions in 2015. The purpose of this study is to prepare, extract, and technology blastema tissue to freezing and commercialize it as transferable tissue and isolated for research laboratories and maintain in a tissue bank.

Method: In this study, the cryopreservation protocol of tissues using liquid nitrogen is selected among cryopreservation protocols. After that, blastema rings obtained from New Zealand male rabbit's ear punch were frozen in two different groups using the liquid nitrogen. In the first group, the blastema rings were transferred to cryotubes containing 0.05 ml DMSO, 0.5 ml FBS, and 0.45 ml of culture medium before being placed in liquid nitrogen. In the second group, the blastema rings were first placed in cryotubes containing 0.5 ml DMSO and 0.5 ml FBS and transferred to liquid nitrogen. In each group, some rings were placed as control samples in the culture medium. Before, the thawing technique was performed, and the vitality of the blastema tissue was determined on certain days.

Results: At 7, 10, 15, and 21 days post-culture, blastema cell vitality was assessed by trypan blue. Microscopic observations showed that between the two groups of cultured blastemas as control and frozen blastemas in two different groups, cells with lower DMSO concentration were more viable.

Discusstion: Based on the results of this study, it seems that blastema tissue can be maintained for a longer time using the cryopreservation method with liquid nitrogen as a tissue model. Therefore, this research will continue to increase shelf life by using different materials for freezing and investigate new methods of tissue freezing.

Keywords: Blastema Tissue - stem cell-like Cells - In vitro Freezing - Commercialization - Animal Tissue Bank

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Determination of kinetic and thermodynamic parameters of amyloid-β 1–42 interaction with astaxanthin

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Background: The abnormal folding and aggregation of the amyloid-β is a hallmark of neurodegenerative disorders such as Alzheimer's disease (AD). The use of natural compounds with anti-aggregation properties is an attractive strategy to treat the neurodegenerative diseases. Astaxanthin (ATX), a xanthophyll carotenoid with powerful antioxidant activity, has been found to have the protective effect on amyloid β (Aβ) aggregation. The aim of this study was to obtain the kinetic and thermodynamic parameters of ATX interaction with Aβ1-42.

Methods: For this purpose, Aβ1-42 was immobilized on the activated surface plasmon resonance (SPR) gold chip surface and then ATX at various concentrations (1-25 µM) was injected with flow rate 30 µl/min for 2 min. The values of arbitrary response unit (RU) were determined at four different temperatures. The dissociation (kd) and association (ka) rate constants were calculated using the SPR Navi™ Data viewer and Trace Drawer™ Software. The Gibbs energy change (ΔG°), enthalpy change (ΔH°), and entropy change (ΔS°) were estimated from van't Hoff analysis.

Results: The negative values of ΔH° (-165.72 kJ mol−1) and ΔS° (-658.63 J mol−1 K-1) suggested that the hydrogen bonds and van der waals interactions were the main forces governing Aβ1-42/ATX interaction. The SPR results also showed that the binding of ATX to Aβ1-42 is a non-spontaneous, exothermic and enthalpy-driven process with KD= 0.31 × 10-6 M at 310 K. Furthermore, the docking of Aβ1-42 structures (PDB IDs: 1IYT, 1Z0Q, and 5OQV) with ATX (extracted from PDB ID: 1GKA) were performed using AutoDock Vina and LigPlot+ molecular docking software. The docking results revealed that hydrophobic and hydrogen bond forces have an important role in the interaction of Aβ1–42 with ATX.

Conclusion: Overall, Aβ1–42 seems to have high affinity with ATX and this binding is temperature-independent over the temperature range (298-310 K).

Keywords: Amyloid β-peptide1-42 (Aβ1-42), Astaxanthin, Molecular docking, Surface plasmon resonance, Neurodegenerative diseases.

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Evaluation of fermented soybean meal by bacillus pumilus on Glutathione Reductase (GR), Nitiric Oxide (NO), Total Antioxidant Capacity (TAC) levels and cyclooxygenase-2 (COX-2) gene expression in liver tissue of CLP-induced septic rats

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Background: Sepsis is a complex illness resulted from a systemic inflammatory response to infection and is the leading cause of death in critically ill patients. Cyclooxygenase-2 (Cox-2) is an inducible enzyme responsible for the formation of inflammatory prostanoids. Its role in the pathophysiology of inflammatory states like sepsis is increasingly recognized. Bioactive peptides can be released by the microbial activity of fermented food or through enzymes derived from microorganism. Soy-based foods are known to have good nutritional and functional qualities. Bacillus spp. has been used to produce fermented soy based foods. In this study, the effect of bioactive peptides produced by bacillus pumilus on oxidative factors and Cox-2 gene expression in septic rats were evaluated.

Methods: A total number of 60 male Wistar rats were randomly divided into six experimental groups. Rats were treated with fermented soybean meal by i.p. injection with dose of 5T 10 and 20%for 48hafter CLP (Cecal ligation and puncture) injury. Then, liver samples were collected to determine levels of Glutathione Reductase (GR), Total Antioxidant Capacity (TAC) and Nitric Oxide (NO).

Result: The result revealed that treatments significantly improved antioxidant and liver enzymes by in increasing remarkably($P<0.05$) TAC and GR level. Moreover, COX-2 gene expression in the liver tissue was decreased remarkably($P<0.05$) in the treatment group compared to the CLP group.

Conclusion: Our result suggests CLP induced oxidative hepatic damage and fermented soybean meals have the potential for the treatment of liver damage consecutive to chemical intoxication.

Keywords: Sepsis, Anti-oxidant, Fermented soybean meal, bacillus pumilus, Real-time PCR

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Evaluation of fermented soybean meal by Bacillus pumilus on Glutathione peroxidase, Catalase, Myeloperoxidase (MPO) and MPO gene expression in lung tissue of septic rats

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Background: Sepsis is a complex illness resulted from a systemic inflammatory response to infections caused by the invasion of microorganisms and toxins. The observed symptoms in sepsis are resulting from an imbalance between the immune system and the production of oxygen free radicals. Myeloperoxidase (MPO) is a heme-containing peroxidase that catalyzes the formation of hypochlorous acid to carry out their antimicrobial activity. It is abundantly stored in azurophilic granules of neutrophils. The extracellular release of MPO from activated neutrophils triggers the inflammation and tissue damage. Soy bioactive peptides that are specific fragments of major soy proteins can be released by enzymatic hydrolysis, food processing, and/or fermentation. Here, the impact of bioactive peptides produced by bacillus pumilus on the activity of antioxidant enzymes, Glutathione peroxidase (GPX), Catalase (CAT) and MPO, and expression of MPO gene in lung samples of septic rats were evaluated.

Methods: A total number of 60 male Wistar rats were randomly divided into six experimental groups. Rats were treated with fermented soybean meal by intraperitoneal injection with a dose of 5T 10 and 20%for 48hafter CLP (Cecal ligation and puncture) injury. Then, lung samples were collected to determine levels of GPX, CAT, and MPO.

Result: The obtained result indicates that treatments significantly induce the activity of GPX, the activity of MPO was decreased and CAT activity was not remarkably altered in lung tissues. Moreover, the expression of MPO was decreased in the treatment group compared to the CLP group.

Conclusion: Our result suggests that CLP induced the activity and expression of GPX-related oxidative lung damage, whereas had a negative effect on MPO expression and function. Furthermore, we showed that fermented soybean meals have the potential for the treatment of lung damage following the chemical intoxication.

Keywords: Sepsis, Fermented soybean, Myeloperoxidase, Glutathione peroxidase, Catalase

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Helicobacter pylori affect the gastric expression and serum levels of ghrelin in H. pylori-positive subjects

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Background: Helicobacter pylori (H. pylori), infects more than half of the population worldwide. This bacterium can affect the production of some peptides produced by the stomach, such as gastrin, somatostatin, and probably, ghrelin. Ghrelin has a range of functions including, regulation of appetite and energy homeostasis, regulation of gastric secretion and emptying, cardiovascular protection, anti-inflammatory action and etc. The Relationship between H. Pylori and ghrelin levels is still unclear.

Methods: In this study, we enrolled 68 adult people, referred for upper gastrointestinal endoscopy at Kosar hospital, Semnan, Iran, between February 2018 and January 2019. Individuals were classified into three groups based on two factors: H. pylori infection and Gastritis. Blood sampling and endoscopy operation were performed after overnight fasting at 8-10 AM for all participants. Diagnosis of H. pylori infection was determined by the RUT test and microscopic observation, also gastritis status was classified according to the updated Sydney System by pathologists. Total ghrelin levels were measured by the ELISA Sandwich method using a commercially human ghrelin ELISA kit. The expression level of ghrelin mRNA was evaluated by using a real-time quantitative RT-PCR method.

Results: Out of the 68 subjects, 31, 28 and 9 subjects were H. pylori-positive with gastritis, H. pylori-negative with gastritis, and H. pylori-negative without gastritis respectively. Serum ghrelin levels and mRNA expression were significantly lower in H. pylori-positive with gastritis patients compared with both H. pylori-negative with and without gastritis, (P-value = 0.008, 0.01). Based on gastritis, no significant difference was seen in H. pylori-negative patients, neither in serum nor in mRNA expression of ghrelin. Based on our findings, Serum ghrelin levels correlated with BMI in all three groups (P-value <0.05).

Conclusions: Our results revealed H. pylori infection may affect the level of the ghrelin, thereby affecting human health through its participation in ghrelin level regulation.

Keywords: Helicobacter pylori, Ghrelin, Gastritis, Body Mass Index

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Quercetin Reduces Liver Fibrosis in the fructose –Treated human LX-2 Cell line

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Background: In the process of liver fibrosis (chronic liver injury), the transforming growth factor-beta (TGF-β) is increased, consequently hepatic stellate cells (HSCs) will be activated. These changes increase the expression of proteins such as alpha-smooth muscle actin (αSMA) and collagen-1 in activated HSCs. Quercetin belongs to an extensive class of polyphenol flavonoid compounds almost ubiquitous in plants and plant food sources. Quercetin is found in various food products and plants, including fruits, seeds, vegetables, tea, coffee, bracken fern, and natural dyes. Quercetin is one of the most extensively studied flavonols that possesses strong anti-cancer, anti-inflammatory, antioxidant, antifibrogenic, and as well as cardio- and neuroprotective effects**.** We investigated the effect of Quercetin as a potential therapy on liver fibrosis in the fructose Treated human lx-2 Cell line

Methods: LX-2 cells (an immortalized human HSC cell lineage) were cultured in Dulbecco's modified Eagle's Medium (DMEM) + 10% fetal bovine serum (FBS) at 37 °C in 5% CO2. Cells were treated with 16 micromolar of fructose (amount of fructose in the serum of diabetic persons to induces liver fibrosis for 48 hours. Next, Quercetin (25 mM) was added into the medium of the cells treated by TGFβ for 24 hours Then total RNAs were extracted, reversely transcribed into cDNA and Quantitative Real-time PCR (qRT-PCR) was performed

Results: Our results indicate the expression of αSMA and collagen-1 are increased in the cells which were treated by fructose. Quercetin can reduce the expressions of αSMA and collagen-1 in the cells treated by fructose compared to the control group. ($P< 0.05$)

Conclusion: Overexpression of αSMA and collagen-1 are used as a standard marker for HSC activation that leads to liver fibrosis. Quercetin attenuates fructose. -induced liver fibrosis via reducing the levels of αSMA and collagen-1 in LX-2 cells. These data show Quercetin may be an agent for the treatment of liver fibrosis.

Keywords: Liver fibrosis, Quercetin, fructose., α-SMA, collagen-1, LX-2

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The effect of noise pollution on anxiety in adult male Wistar rats and the effect of lemon essential oil on reducing it

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Background: As cities expand and increase in population, humans are exposed to a lot of noise pollution on a daily basis. The purpose of this experiment is to measure the effect of noise pollution on human anxiety.

Methods: The study was performed on Wistaran rats. Mice were treated with 100 dB of noise pollution for 8 hours daily for a period of one month. Then the level of anxiety was measured using the EPM Elevated plus maze by counting the number of times he entered and stayed in the closed arm of the maze. In cases where the test result showed increased anxiety, the effect of lemon juice spray was tested for 20 minutes and then the anxiety level was measured again.

Results: With the effect of noise pollution, the amount of time to stay and the number of times to enter the open arm decreased and vice versa in the closed arm, which indicates the effect of noise pollution on the increase of anxiety. In this experimental group, the effect of lemon juice on the rate of recurrent anxiety and the results showed that the average amount of OAT and CAT after the effect of inhaling the aroma of sour lemon increased the amount of time spent, which indicates a decrease in anxiety and the average time spent. In the closed arm, the smell of lemon has decreased after inhaling.

Conclusion: Exposure to daily noise pollution causes anxiety in humans and inhaling sour lemon essential oil reduces this anxiety.

Key word: Noise pollution, anxiety, Wistar rat, lemon, maze

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Targeted treatment of immunotoxins and breast cancer

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Background: Cancer is one of the main reasons of death in the most countries and in Iran. Immunotherapy quickly became one of the best methods of cancer treatment, along with chemotherapy and radiation. "Immunotoxin Therapy" is a promising way of cancer therapy that is mentioned in this field. Immunotoxins are made from a toxin attaching to an antibody target proteins present on cancer cells

Methods: in this research, the use of The three dimensional structure of the required protein sequences, including antigens as well as immunotoxin components, was determined using the modeling model based on the Swiss-Model program and using the RCSB protein database. In addition, the modeling and assembling of immunotoxic structures was performed with version 9.15 of the Modeller program. For this purpose, the components of the immunotoxin structure were first linked together by selective linker. Then, the structural models of the components that make up the immunotoxin structure were transferred to the aligment.seg file, and the operation was performed by rereading the sequence of each structure in the Paymoul software. Finally, according to the program command in the structural modeling path, 6 structures related to each immunotoxin structure were prepared and evaluated. The structures were displayed using version 2.2 of the Pimwell program

Results: The purpose is to connect the components of the immunotoxin structure through the selected linker and then measure the structure modeled by the application software.

Conclusion: Cancer is one of the leading causes of death in most countries and in Iran. Immunotoxins are made from toxins bound to antibody target proteins in cancer cells, thus modeling and assembling immunotoxin structures to bind structural components.

Keywords: Cancer, Immunotherapy, Toxin, Linker, Antibody

P-51

A new model to study the impact of cholesterol in vitro: Cholesterol can induce Liver fibrosis

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Background: Liver fibrosis is a reversible wound-healing response occurring in most forms of chronic liver injury characterized by the activation of hepatic stellate cells (HSCs). During Liver injury, the transforming growth factor-beta (TGF-β) is increased consequently hepatic stellate cells (HSCs) will be activated and change to myofibroblasts. These changes increase the expression of proteins such as alpha-smooth muscle actin (αSMA) and collagen-1 in activated HSCs that lead to hepatic fibrogenesis. Studies showed that higher dietary consumption of cholesterol was associated with a higher risk of cirrhosis or liver cancer and improve liver fibrosis in patients with hypercholesterolemia. In this study, we investigated the effect of high cholesterol as a factor in the progression of Liver fibrosis on human HSCs.

Materials and Methods: LX-2 cells (an immortalized human HSC cell lineage) were cultured in (DMEM) + (FBS) at 37 °C in 5% CO2. Cells were treated with different concentrations of cholesterol including 25, 50-100 micromolar for 48 hours. Then total RNAs were extracted, reversely transcribed into cDNA and Quantitative Real-time PCR (qRT-PCR) was performed.

Results: Our results indicated that the levels of TGF-β, αSMA, and collagen-1 in LX-2 cells were significantly increased by treating 50 and 100 micromolar cholesterol compared to control group ($P< 0.05$), but when the cells simultaneously treated with 25 micromolar cholesterol, no significant change were observed in the level of TGF-β, αSMA and collagen-1

Conclusion: Overexpression of TGF-β, αSMA, and collagen-1 are used as a standard marker for HSC activation that leads to liver fibrosis. The results demonstrate that high cholesterol may result in the progression of liver fibrosis in LX-2 cells via increasing the levels of TGF-β, αSMA, and collagen-1(as a new fibrotic cell model) so controlling cholesterol consumption and knowing the mechanism of cholesterol is important to treat and reduce liver injury.

Keywords: Liver fibrosis, cholesterol, TGF-β, α-SMA, collagen-1, LX-2

P-52

Bioinformatics comparison and discussion of COVID-19 protein sequence with other human coronaviruses

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Background: COVID-19 is an acute respiratory disease of coronavirus origin that has spread around the world. Genomic analysis of the virus shows that part of its genome, which is involved in the expression of S protein, is important for bioinformatics and structural studies. This virus, which is a derivative of SARS-related coronaviruses, has a very high prevalence and transmission power compared to other coronaviruses. One of the causes of rapid COVID-19 transmission could be a genetic mutation in the viruschr('39')s S protein.

Methods: In this study, we tried to use BLAST and Clustal Omega servers to study the homology of this virus according to the protein sequence and then using bioinformatics servers such as Chimera, ESPript and Uniprot to examine the protein sequence of the virus and compare points of functional significance with similar coronaviruses.

Results and Conclusion: After examining the COVID-19 sequence and comparing its RBD and RBM regions with SARS-COV, it was concluded that these two viruses, despite their general similarities in sequence, have significant differences in the regions involved in binding to the ACE2 receptor. The differences and similarities can be a way to come up with an idea for modeling COVID-19 or applying multiple mutations to test for functional changes in the virus.

Keywords: Bioinformatic ‹Coronaviruse ‹Homology.

P-53

The assessment of neuronal protective effect of carvacrol in the experimental modal of hippocampal injury induced by TMT in adult male rat

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Background: Hippocampal neurodegeneration causes abnormalities at the molecular and behavioral levels. Carvacrol is a monoterpene and of strong antioxidant that protect the nervous system from neuronal degeneration. In this study, we evaluated the neuronal protective effect of carvacrol in the experimental model of hippocampal injury induced by trimethyltin chloride (TMT), at the level of hippocampus expression of Caspase-3, Bcl2, Bax and BDNF genes.

Methods: Twenty four adult male rats were randomly divided into four groups of six animals each as given below: (1) TMT group: (n=6) the animal were injected intraperitoneally with a single dose of (8 per/kg) of TMT, (2) DMSO group: (n=6) the animal were injected intraperitoneally with a single dose of (DMSO 30%) (3) CAR group (CAR40): (n=6) rats were treated vehicle intraperitoneally CAR at doses of (40 mg/kg) for 21 day (4) normal group:(n=6) rats received saline intraperitoneally with a single dose (physiological saline 0.1 ml/100 g). In the third week, the brain was removed from the skull to perform real-time PCR.

Results: The present study showed that TMT toxin enhances the relative expression of Caspase-3 and Bax genes also reduces relative expression of Bcl-2, BDNF genes in the hippocampus of the experimental groups ($p<0.05$). In addition, comparison of treatment groups carvacrol and TMT showed Carvacrol increased and improved the relative expression of Bcl-2, BDNF ($p <0.05$). But decreased relative expression of Bax, Caspase-3 genes and neuronal damage in the hippocampus of animals

Conclusion: Carvacrol may improve the neurodegenerative disorders and damage caused by TMT at the histopathological, cellular and molecular levels, and memory and learning.

Keywords: TMT trimethyltin chloride, carvacrol, apoptotic genes, Caspase-3, BDNF

P-54

A new approach to breast cancer therapy with nanodrugs

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Background: Breast cancer is one of the leading causes of death in women worldwide. Researchers have studied new and targeted methods of cancer by studying nanoparticles such as liposomes. Their clinical use of chemotherapy drugs is limited due to their dangerous side effects. The primary purpose of this study was to investigate the effectiveness of the use of nanoparticles for the treatment of breast cancer and to reduce the side effects of anti-tumour drugs.

Methods: The nanoliposomes, which contained cis-platinum, were prepared by the thin film layer hybridization method, and then the anti-tumour effect of cis-platinum enclosed on the MCF7 cell line was investigated.

Results: The cellular uptake experiments were performed the in vitro localization of free cisplatin and liposome-loaded cisplatin on MCF-7 cell lines monitored by a fluorescence microscope. The findings represent that the rate of the entry of cisplatin-containing liposome formulations into cancer cells is much higher than the free form of cisplatin. These results are aligned with the cytotoxicity experiments. The cell survival using MTT method showed that the free cisplatin and nano-liposome-loaded cisplatin reduced the growth of breast cancer cells in a dose-dependent manner. As indicated in, cisplatin demonstrated higher levels of toxicity in nanoliposomal form in comparison to the free form of the drug. The IC50 values of free cisplatin solution and cisplatin-containing liposomes were 56.47 and 34.70 μg/ml, respectively. This revealed that the formulated cisplatin is at least ~1.62-fold more potent than the free form of cisplatin ($P < 0.05$).

Conclusions: the results of this study showed that drugs such as synthesized nanocomposites play an essential role in drug effectiveness and dose reduction. Nanoparticles containing cisplatin can also be used as a suitable carrier with appropriate and valuable efficacy for future studies.

Keywords: Breast Cancer, MCF7, Nano-liposome, Cisplatin.

P-55

The Relationship between Circulating Cystatin C level and Tumor Size in Breast Cancer Patients

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Background The Cystatin C (Cys-C) is an endogenous inhibitor of lysosomal cysteine proteinase, which has been shown to play a role in several normal and pathological processes. This relatively small protein is abundant in various body tissues and fluids and it is suggested to be a better renal functional marker than Creatinine. The aim of this study is investigating the relationship between serum Cys-C level and tumor size in female patients with breast cancer.

Methods This study was a case-control study and was carried out on women with breast cancer and healthy women as controls who referred to the Reza Radiotherapy and Oncology Center in Mashhad. The blood collected from 40 healthy volunteers and 40 patients diagnosed with breast cancer who had undergone only a biopsy, without metastasis or any other cancer nor any underlying diseases. Serum Cys-C level were measured using ELISA and the tissue levels were measured using immunohistochemistry, in Innovative Medical Research Center. A statistical analysis was performed using SPSS version 20 software and the significance level of the tests was considered to be less than 0.05.

Results In this study, the mean age of subjects in breast cancer and control groups were 52 and 48 years, respectively. The serum Cys-C level in breast cancer patients was higher compared to the control group and its measurement in different tumor size: greater than 5 cm, between 2 to 5 cm and less than 2 cm, was therefore obtained 0.699, 0.645 and 0.278 (ng/ml), respectively. There was a significant relationship between serum Cys-C level and tumor size of the patients ($p=0.002$).

Conclusion According to the results, Cys-C may be a suitable non-invasive biomarker to detect the progression of breast cancer in relation to tumor size.

Keywords: Breast Cancer, Cystatin C, Tumor size, Biomarker, Blood

P-56

Evaluation of the effect of soil pollution with cadmium on root and shoot growth in sunflower and watercress

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Background: Heavy metals enter the body of living organisms and endanger their health by consuming crops grown in contaminated soils. One of the ways to purify the soil is phytoremediation. Sunflower and watercress are among the plants known for their phytoremediation properties. In this study, the effect of cadmium metal on the growth rate of these plants has been investigated.

Methods: In order to investigate the effect of cadmium on the growth of sunflower and watercress plants, a pot study was performed in cadmium-contaminated soil with two plants, with a control group over a two-month period. Soil was contaminated with 0.63 mg of cadmium nitrate Cd (NO3) 2 per kg of soil. After the end of the period, the plants were completely harvested and the size of root and shoot length and growth rate in pots were examined. Data were analyzed by SPSS software.

Results: The results showed that the amount of root growth in the royal treatment group compared to the control group (without cadmium) shows about 4 times. This difference in stem and sunflower stem length was 0.84 and 0.81 times in the control group, respectively, and the rate of root growth in the sunflower group did not show a significant difference. In the study of the appearance of the leaves and stems of the sunflower plant, less vegetables and freshness were observed in the treatment group than in the control group. In the herb, cadmium also weakened the stem

Conclusion: Cadmium affects the rate of root growth in the plant, causing the plant to wear out and die. For this reason, it seems that the imperial plant, despite the removal of heavy metals from the soil, is not suitable for phytoremediation due to its intolerance to pollution.

Key Word: Phytoremediation, sunflower, watercress, cadmium

P-57

Alteration in gene expression of IGF1 in endometrial tissues of women with endometriosis

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Background: Endometriosis is a common, estrogen-dependent chronic inflammatory disease which is defined as the presence of endometrial tissue outside the uterine cavity. As a multifactor disease, genetic and epigenetic factors, are involved in pathogenesis of endometriosis. Endometrial cells proliferation, is a key element in endometriosis. So growth factors such as insulin-like growth factor-1(IGF1) may have important roles in its pathogenesis. IGF-1 plays main roles in growth and differentiation of endometrial cells.

Methods: In this case-control study, 12 endometrial samples (eutopic) and 12 endometriotic lesions (ectopic) of women with endometriosis and 12 endometrial control samples were analyzed. Control samples were obtained from women who had no evidence of endometriosis during diagnostic laparoscopy in Royan Institute. Control and eutopic endometrial samples were obtained by pipelle. Ectopic samples were obtained during laparoscopy. All women signed the informed consent form and did not receive any hormonal treatments during the last three months. After endometrial tissues collection, RNA extraction and cDNA synthesis were done. Real-time PCR technique was used for quantitative gene expression. P value less than 0.05 was considered statistically significant.

Results: Gene expression profile of IGF1 was decreased in eutopic and ectopic endometrial lesions compared with control samples. In addition, gene expression level of IGF1 was lower in ectopic lesions in compare to eutopic samples in endometriosis women. , although these differences were not statistically significant.

Conclusion: It seems altered gene expression of IGF1 could play role in the etiology of endometriosis. because of its role in regulatory pathways of cellular proliferation. This preliminary data suggests that endometriotic tissue undergoes an impairment of cellular growth regulation during the disease.

Keywords: Endometriosis, IGF1, Ectopic endometrium, eutopic endometrium

P-58

Gene expression of AIRE in endometrial tissues of women with endometriosis

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Background: Endometriosis is an inflammatory disease of women in reproductive age. In this disease uterine endometrial tissue grows outside of uterine cavity and could cause infertility and/or pelvic pain although some affected women are asymptomatic. One of etiologic factors of endometriosis is immunologic alterations. Autoimmune regulator (AIRE) as a transcription factor, plays a role in central immunological tolerance via regulation the expression of tissue-specific antigens. This regulatory protein is involved in the negative selection of autoreactive T cells and also the production of regulatory T lymphocytes. This study was carried out to compare gene expression of AIRE in endometrial tissue of women with endometriosis in compare to controls.

Methods: Four women with endometriosis (endometriosis group) and six women without endometriosis (control group) were enrolled after diagnostic laparoscopy in this study till now. Ectopic endometrial samples were collected from women with endometriosis during laparoscopy. Eutopic endometrial tissue of endometriosis and control groups were taken by pipelle. RNA extraction and cDNA synthesis were done and real-time PCR was used for gene expression analysis. GAPDH gene was used as housekeeping gene.

Results: Primary results showed that AIRE gene expression was higher in both eutopic and ectopic tissues of women with endometriosis in compare to controls. In addition, its gene expression in ectopic tissues of endometriosis group was higher than eutopic samples.

Conclusion: It seems overexpression of AIRE in endometriotic tissue of women with endometriosis may play role in pathogenesis of endometriosis through induction of regulatory T cells and decrease the clearance of endometriotic lesions.

Keywords: Endometriosis, AIRE, Endometrium, Gene expression, Ectopic, Eutopic

P-59

Overexpression of B-cell-specific Moloney murine leukemia virus integration site 1 and Fas ligand in colorectal cancer patients and their correlation with cancer progression and prognosis

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Background: B-cell-specific Moloney murine leukemia virus integration site 1 (BMI1) and Fas ligand (FasL), two important stemness genes, are presumed to involve in the pathogenesis of colorectal cancer (CRC). In the current study, we investigated the expression levels of the two genes in primary tumors of CRC patients to determine their association with cancer progression and prognosis.

Methods: Relative expression level of BMI1 and FasL, was first assayed by real-time polymerase chain reaction in 100 primary cancerous and paired adjacent non-cancerous tissues. Then, the association of the expression value of the genes in primary cancerous tissue with clinico-pathological features and overall survival of patients was assessed.

Results: Cancerous tissues had higher expression levels of BMI1 and FasL compared to their adjacent non-cancerous counterparts. The relative expression value of BMI1 and FasL was revealed to be correlated with tumor size, grade, TNM stage, metastasis ($P=0.0001$ for all), and shortened overall survival time ($P=0.00001$). Furthermore, BMI1 and FasL were appeared as an independent prognostic factors in the multivariate Cox regression analysis.

Conclusion: The overexpressed levels of BMI1 and FasL in cancerous tissue of patients with CRC are associated with cancer

Keywords: CRC, BMI1, FasL, Cancerous tissue

P-60

A Review of Relationship between Oxidative Stress and Cardiovascular Disease

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Background: Oxidative stress is caused by an imbalance among the production of free radicals of oxygen and the defense mechanism of anti-oxidants in the body. An increase of free radicals can result in a structural and functional alteration among the bodily molecules of lipids, proteins and nucleic acids and eventually causes damage to the tissues. Bodies have evolved with an elaborate defense network against oxidative stress, in which multiple antioxidant compounds and enzymes with different functions exert their respective roles. Anti-oxidants such as catalase, glutathione peroxidase, and superoxide dismutase can cause balance between the production and elimination of free radicals through the elimination of active oxygen forms.

Methods: in the current study, key words including oxidative stress, free radicals, anti-oxidants and cardiovascular disease were reviewed from the list of Mesh and other credible websites including PubMed, Science Direct and Google Scholar over the past two decades and the data was organized.

Result: The link between oxidative stress and atherosclerosis has been confirmed in a number of studies that have measured elevated markers of oxidative stress such as reactive oxygen species, super oxide radical, hydroxyl radical and hydrogene proxide in patients and shown that they are predictive for coronary artery disease. Lipid hydroperoxides are also recognized as a marker of increased oxidant stress and are an independent predictor for major adverse cardiovascular events. Among the circulating proteins with antioxidant function, such as uric acid, albumin, haptoglobin, transferrin, ceruloplasmin, and reduced glutathione, levels of reduced glutathione have been inversely related to atherosclerosis. Oxidative stress promotes atherosclerosis through a number of complementary mechanisms.

Conclusion: The existing evidence supports the view that oxidative stress may play a crucial role in cardiac and vascular abnormalities in different types of cardiovascular diseases and that antioxidant therapy may prove beneficial in combating these problems.

Keywords: including oxidative stress, free radicals, anti-oxidants, cardiovascular disease

P-61

Protective Effect of Nigella Sativa Against Mercury, Arsenic and Lead Toxicity

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Background: Nigella sativa (black seeds) is a small shrub under the botanical family, Ranunculaceae. The use of Nigella Sativa seeds and oil in traditional remedies goes back more than 2,000 years. The black seeds contain protein, fat, carbohydrates, crude fiber, total ash, volatile oil, fatty oil, cellulose and moisture. Many active components of Nigella Sativa have been identified, including thymoquinone, dithymoquinone and thymohydroquinone. Thymoquinone utilization could prevent many disorders, such as neurobehavioral, kidney and liver disorders. toxic metals can be exposed to humans and environment through numerous ways, such as the air, food, water, waste and industries and the accumulation of their ions lead to serious environmental and health hazards.

Methods: in the current literature review key words including Nigella sativa, thymoquinone, black seed, toxicity arsenic, Mercury and Lead from the list of MeSH and other credible scientific websites such as Science Direct, PubMed and Google Scholar were used to compile the Protective Effect of Nigella Sativa Against Mercury, Arsenic and Lead Toxicity.

Result: Articles show that in brain preparations of male Wistar rats, significant reduction in the arsenic-induced neurotoxicity has been observed upon pre-treatment with thymoquinone. in mercury toxicity study confirmed that thymoquinone improves the renal proliferative reaction and decreased histological injury like renal cell apoptosis and proliferative responses owing to Hg exposure in rats. In lead toxicity thymoquinone significantly reduced the harmful effect of Pb acetate in male rats through reducing DNA damage and alterations in the gene expression, levels of malondialdehyde and protein carbonyl and also increased glutathione levels.

Conclusion: meanwhile, medicinal plant like Nigella sativa is a widely used as an antidotal and protective agent due to its effective constituent, thymoquinone. There are various reports on its biological activities and protective effects in different organs and tissues including brain, genome, liver, kidneys, lungs, etc.

Keywords: Nigella sativa, thymoquinone, black seed, toxicity arsenic, Mercury, Lead

P-62

Controlling Culture pH to drive Lactate Switch in Chinese Hamster Ovary cells

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Background: CHO cells consume glucose at a high rate and produce lactate to replenish the NAD+ which is essential for glycolysis to continue. However, according to the previous studies high levels of lactate concentration during the culture can lead to a reduced protein production. One of the key changes that can occur during a production run is the transition from a lactate producing to a lactate consuming stage, demonstrating a switch in metabolism. H+ or lactate gradients can induce lactate consumption. This work aims at studying the effect of pH control on lactate metabolism in CHO cells.

Methods: An IgG2 producing CHO-DG44 cell line was cultured in a chemically defined media as a fed-batch process. Briefly, two experiments in 10L bioreactor were run with an initial cell density of (0.6-0.7) × 106 cells. mL-1 and incubated at 37 °C for 15 days. In the first condition pH was not controlled and in the second condition pH was closely limited between 6.9-7.0 using NaOH (0.5 M) throughout the culture.

Results: In both experiments a significant pH drop was observed. pH decreased to 6.75 (on day 7) in pH un-controlled condition; while it was limited to the range of 6.9-7.0 in pH controlled condition. The maximum lactate concentration of 16 and 23 mM were observed in pH-uncontrolled and pH controlled conditions, respectively. Moreover, lactate consumption profile was different between two experiments. Accordingly, lactate accumulated from day 6 to the end of the culture in pH controlled condition, whereas lactate started to consume around day 4 in pH-uncontrolled condition. Consequently, the protein expression was enhanced around 30% ($p<0.05$) as the result of lactate consumption during the un-controlled culture.

Conclusion: The current results indicated that pH gradient applying can induce lactate consumption resulting in the shifting metabolism and improving cellular productivity.

Keywords: Culture pH, IgG2, Lactate metabolism, Protein expression

P-63

Oxidative stress status in antibody mediated renal allograft rejection

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Background: Antibody mediated rejection (AMR) is one of the major causes of allograft dysfunction that may lead to chronic damage and cell apoptosis. One of the initiators of apoptosis is oxidative stress. Markers of oxidative stress were evaluated in this study.

Methods: Thirty-six transplant patients (22 with biopsy proven AMR and 14 with stable graft function) with the mean age of 40 years were enrolled. All patients were receiving the same immunosuppressive medicines during the study. Demographic characteristic of patients and relevant clinical data were recorded. Serum samples were collected and levels of oxidative stress markers including total oxidant status (TOS), total antioxidant capacity (TAC), 8-isoprostane (8-IP), total thiol groups, nitric oxide level (NO), and the activity of antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured and oxidative stress index (OSI) was calculated.

Results: Data analysis showed that hemoglobin, hematocrit, calcium and estimated glomerular filtration rate (eGFR) were significantly lower in patients with AMR compared to those with stable graft function. Significant increase in TOS, 8-IP, and TAC together with marked reduction in NO and total thiol groups were detected in AMR patients. CAT, GPx, and OSI were not significantly different among studied groups, however lower SOD activity was detected in AMR group.

Conclusion: The findings pointed out the increased oxidative stress in patients with AMR yet, further studies are required for a more in-depth validation of the results.

Keywords: antibody mediated rejection, Oxidative stress, renal allograft

P-64

The impact of different $NaHCO_3$ concentrations on culture pH and monoclonal antibody charge variants level

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Background: Charge heterogeneity is considered as one of the critical quality attributes of monoclonal antibodies. The origin of this characteristic could be various enzymatic reaction or process parameters such as pH. This study attempted to control culture pH and subsequently acidic charge variant of a mAb by using different concentrations of bicarbonate buffer in basal media.

Methods: In this work, four experiments were designed to examine the effect of NaHCO3 concentration on charge variants of a mAb during the fed-batch culture operating in a 5L bioreactor. In each experiment, NaHCO3 concentrations of 0.8, 1.2, 1.6 and 2.2 g/L were added to a chemically defined culture media. Eventually, pH pattern and charge heterogeneity of target mAb were determined at the end of the fed-batch experiments (day 15). The impact of different buffer concentrations on pH and charge variant levels were evaluated using the statistical paired T-test (p values <0.05).

Results: According to our observations, the pH increased by about 0.1 unit during stationary (production) phase at elevated buffer concentrations (dose–dependent manner). pH of culture with the lower buffer concentration (0.8 g/l) was 6.8 while by using more concentrated buffer (2.2 g/L) in media, pH of culture was 7.1. Moreover, the results of cation exchange chromatography of target mAb indicated that the culture with lower pH of 6.8 resulted in lower acidic charge variants, about 35%, in comparison with the culture with higher pH of 7.1 and acidic charge variant of 48%.

Conclusion: Process parameters such as pH can affect acidic charge variants. Decreasing pH of culture by means of changing buffering capacity of basal media can be a practical method in reducing acidic charge variants.

Keywords: $NaHCO_3$, pH, Monoclonal antibody, Charge Variants

P-65

Evaluating the effect of EDTA on supernatant clarification and protein activation

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Background: Turbidity and color of cell culture supernatant makes purification procedure difficult by subjecting filtration process. In addition, the color complex can co-purify with the target protein and have a negative impact on resin lifetime. It has been shown that the presence of metal ions in harvested cell culture supernatant has detrimental effect on supernatant color and target protein activation process. EDTA (ethylene diamine tetra acetic acid) is used as a chelating agent that binds to ions and decreases their availability in cell culture media. In this study, the efficacy of EDTA was evaluated in reduction of supernatant turbidity and then the activation of protein.

Methods: To check the efficacy of EDTA on clarification process and activation of target protein, several experiments were carried out by adding different concentrations of EDTA (1mM, 5mM and 10mM) in cell culture supernatant. Following addition of EDTA, supernatant was kept overnight at 4°C and filtered. Afterwards, color and turbidity of the supernatants were evaluated. Subsequently, affinity and anion exchange chromatography were carried out to purify the target protein and finally protein activation was investigated using SDS-PAGE.

Results: The results showed that EDTA addition is effective to decrease the turbidity of cell culture supernatant compared to untreated samples (without EDTA). Accordingly, the protein activation process was enhanced by addition of EDTA. However, increasing concentrations of EDTA from 1 mM to 10 mM did not have significant effect on target protein activation time.

Conclusion: EDTA addition in cell supernatant is beneficial to improve clarification process and consequently target protein activation.

Keywords: Clarification, EDTA, Protein activation, Turbidity

P-66

Association between Infants' Serum Ghrelin and Growth in Breast Feeding Infants, A Meta-Analysis

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Background: Ghrelin increases appetite in infants. Increased appetite leads to using more food and breast milk. As a result, weight gain and proliferation of fat cells in the baby`s body will follow. It also increases the production of other adipokines in the baby. The purpose of this study was to evaluate association of serum ghrelin with growth in breast milk fed infants by using a Meta-analysis.

Methods: The PRISMA checklist was used for this study. From 1994 to April 2019, 130 articles were collected from database searches (Scopus, Cochrane Library, Science, PubMed and EMBASE) and other sources for the association of infants` serum ghrelin and their weight gain and growth. The results of six articles were reported by meta-analysis. In this study, the articles were used on infants under two years of age that receiving breastfed exclusively. The results were evaluated by meta-analyzing using Stata software.

Results: Meta-analysis showed a positive and significant correlation between infant serum ghrelin and infant growth (r = 0.163). Newcastle-Ottawa Scale (NOS) was equal and higher than seven scores for six meta-analysis articles.

Conclusion: The results of random-effect model demonstrated a positive and significant correlation between infant serum ghrelin and the growth of infants.

Keywords: ghrelin, growth, weight gain, meta- analysis

P-67

Selecting an appropriate CIP solution for regeneration and cleaning an in-house coupled affinity chromatography media

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Background: Impurities can remain on the affinity media and block pores. Therefore, finding an appropriate cleaning solution is always considered as a critical parameter. In this experiment, a 96-well throughput screening method was used to investigate the cleaning and regeneration efficiencies of five different CIP solutions.

Methods: In the present study, the media was equilibrated with 7-10 CV of equilibration buffer and then the target protein was loaded. After re-equilibrating, target protein was eluted by 5-7 CV of elution buffer. Then, affinity chromatography media (resin) was equilibrated again and cleaned with 4 CV of CIP solution. This experiment was continued up to 100 runs in a way that in run 1, 10, 20, 40, 60, 80 and 100 recovery and HCP content were determined using Bradford and ELISA methods respectively. Silver nitrate staining was performed to evaluate the cleaning efficiency of each solution.

Results: Based on the obtained results, 0.01 M NaOH did not efficiently remove impurity from surface of resin. On the other hand, 0.1 M NaOH or 6 M Gn-HCl had a strong ability in cleaning of media but the recovery capacity of media was dramatically decreased. Moreover, drop in recovery capacity after using 2 M Gn-HCl and 0.025 M NaOH + 1 M NaCl were less than the rest of solutions even after 100 cycles. Additionally, HCP content of eluates were slightly less than control sample.

Conclusion: Results indicated that cleaning using harsh solutions such as 0.1 M NaOH decreases the recovery capacity of the media. Furthermore, although most of the CIP solutions can reduce the HCP amounts but the efficiency of these solutions should be investigated in column phase. Collectively, 2 M Gn-HCl and 0.025 M NaOH + 1 M NaCl were considered as the most effective CIP solutions for regeneration and cleaning of affinity chromatography media.

Keywords: Cleaning, HCP, Recovery, Media

P-68

The effect of gradient elution on IgG1 charge variants reduction compared to step mode of elution in cation exchange chromatography

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Background: Monoclonal antibodies are large proteins susceptible to multiple post translational modifications (PTMs) that may induce charge heterogeneity. The monitoring and characterization of antibody charge heterogeneity are important because their potential influence on quality attributes. In this study, to obtain the acceptable method for separation of charge variants, the elution step of cation exchange chromatography (CEX) was conducted in step and gradient modes and the results were compared together.

Method: To follow this purpose, IgG1 mAb eluate with 30 mg/ml of resin binding capacity was loaded onto XK column containing SP Sepharose resin with bed height 28 cm. Two different elution step methods with 25-75 % gradient in 5 CV and 10 CV were conducted and the results were compared with step mode of elution as control. Fractionation were performed with 20-30 % deletion from first and 10-15 % removal from end part of the CEX eluate peak. Selected fractions were pooled to investigate of charge variant contents via IEX-HPLC.

Result: The results showed acceptable acidic and basic charge variants reduction were achieved in gradient mode. Maximum reduction of acidic charge variants were occurred when 25-75 % gradient in 5 CV was applied with 30 % and 10 % deletion from first and end of eluate peak respectively. Also, basic charge variants were noticeably reduced when 25-75 % gradient was used in 10 CV and 20 % and 15 % from first and end parts of the eluate were removed respectively.

Conclusion: Based on the results, charge variants reduction in cation exchange chromatography is depended on the elution condition and better results were achieved in gradient mode. However, eluting the target protein with gradient method is time consuming, but the best charge variants reduction is obtained compare to step mode.

Keywords: Cation exchange chromatography, Charge heterogeneity, IgG1, SP Sepharose

P-69

Relationship between Methylenetetrahydrofolate Reductase C677T and A1298C Gene Polymorphisms with Hypertension in Patients with Thrombosis

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Background: High blood pressure damages blood vessels and may promote the formation of thrombosis. One of the most important risk factors for thrombosis is increase of blood homocysteine (Hcy). The Hcy is converted to methionine by the activity of the enzyme methylene tetrahydrofolate reductase (MTHFR). The most common mutations in MTHFR are C677T and A1298C with different polymorphisms. The aim of this study was to investigate the relationship between the C677T and A1298C MTHFR gene polymorphisms with hypertension in patients with thrombosis in an Iranian population.

Methods: In this case-control study a total of 60 patients (24 males, 36 females), containing 30 patients with thrombosis with mean age 41±8 years and 30 healthy controls without any vascular pathology with mean age 40±7 years, were included. The MTHFR C677T and A1298C gene polymorphisms of the samples were identified using the real-time polymerase chain reaction and measurement of Hcy levels was done by enzyme immunoassay method.

Results: Subjects with thrombosis had significantly higher Hcy levels than control subjects, despite of adjusting effect of age and gender ($p<0.01$). The results of the repartition of the blood pressure showed that among the patients with thrombosis, 43.3% have a high blood pressure, while this result was only 16.6% for the healthy subjects ($p=0.02$). Interestingly, there was more heterozygous mutation in MTHFR A1298C in thrombosis group (76.7% AC) and there is a significant difference in allele frequencies between the hypertensive group vs control groups (77.8% AC, 22.2% AA; $p=0.034$), conversely, there were not significant for MTHFR C677A allele (44% CC, 38.9% CT, 16.7% TT).

Conclusion: These results showed that genetic polymorphisms related to the MTHFR gene are in relationship with the risk of hypertension accompanied with thrombosis and could be used in future studies to assess the effect of the antihypertensive supplement in this population.

Keywords: Methylene tetrahydrofolate reductase, Gene polymorphism, Thrombosis, Hypertension, Homocysteine

P-70

The effect of L-Arginine on aggregation content of cation exchange chromatography eluates

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Background: Removal of aggregates of monoclonal antibodies therapeutics is a key objective of bioprocessing because of their perceived impact on immunogenicity. Cation exchange chromatography (CEX) is one of the polishing step used for achieving effective reduction of aggregates. On the other hand, L-Arginine as the most commonly used additives for preventing protein aggregation is recommended during this process to reduce aggregation. In this study, the efficacy of L-Arginine elution buffer on reducing the aggregation of eluate was investigated during chromatography and the results were compared to the elution buffer without this component.

Methods: In this study, two CEX elution buffers containing 9 mM phosphate with same ionic strength (8 mS/cm) were designed in a way that 50 mM of L-Arginine was added to one of them. The desired amount of a IgG1 monoclonal antibody was loaded on to the Capto SP ImpRes media packed in Tricorn 10/300 column. Next, the protein was eluted with each elution buffer separately in 0-100% gradient applied in 5 CV with 16 min residence time. The aggregation content of both Capto SP ImpRes eluates were assessed using SE-HPLC and the results were compared together.

Result: Comparison the aggregation content of both Capto SP ImpRes eluates in recovery about 60% demonstrated that aggregation content of eluate conducted with 50 mM L-Arginine elution buffer decreased about 94% and reached to less than 0.5% which was 2 folds less than the aggregation content of Capto SP eluate eluted with elution buffer without L-Arginine supplement (about 1%).

Conclusion: As the previous studies reports, adding L-Arginine as a stabilizer is able to decrease the aggregation content of CEX eluates more than 60-70%. In the present study, the aggregation content was decreased more than 90% using L-Arginine elution buffer while it was reduced about 75% using elution buffer without L-Arginine in recovery about 60%.

Keywords: Aggregation, Capto SP ImpRes, IgG1 monoclonal antibody, L-Arginine

P-71

Evaluating antibody monomer separation from associated aggregates using Capto adhere media

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Background: The aggregation might affect the biological activity of biopharmaceutical product, so aggregate in drug substance should be kept in minimum level. Capto adhere as multimodal strong anion exchanger for polishing of monoclonal antibodies (mAb) has high capability in removal of aggregates. In this study, the efficacy of Capto adhere media was evaluated in reduction of aggregate form antibody.

Methods: To check the efficacy of this media, some experiments were designed in flow through mode in a way that different pH (5.6, 7.1, 6.0 and 6.5) and ionic strength were investigated separately. In all experiments, Tricorn 5/200 column containing 4 ml media was equilibrated with 2 CV of buffer. Eluate of mAb was loaded onto the column after adjusting pH and ionic strength of eluate similar to the equilibration buffer. Next, the column was equilibrated and bounded protein such as aggregate forms etc., was eluted using elution buffer containing 100 mM Na_3Citrate, pH 3.

Results: The results cleared that acceptable aggregation reduction and recovery were not obtained when the buffer was used with pH 5.6 and 20 mS/cm ionic strength. With increasing the pH to 7.1 in 20 mS/cm ionic strength, the results were same as pH 5.6. Therefore, in the next step, ionic strength of buffer was decreased to 12.5 mS/cm, which caused to increase in recovery up to 78% and 80% reduction of aggregation. To obtain better recovery, in next runs pH 6.5 and 6.0 were investigated with 6.5 mS/cm ionic strength. The recovery of process was increased to 96 % and 98% respectively besides acceptable reduction of aggregation forms were achieved.

Conclusion: Capto adhere media has an acceptable capacity to reduce aggregate forms of eluate with equilibration buffer in pH 6.0. The recovery of process in flow through mode increases with decreasing the pH and ionic strength.

Keywords: Aggregation, Capto adhere media, Monoclonal antibody, Recovery

P-72

Effect of Dichloroacetate on an IgG2 producing CHO cell culture performance

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Background: Lactate is one the metabolites produced as a byproduct in mammalian cell cultures. In the most cases over production of lactate is attributable to a high aerobic glycolysis rate or Warburg effect. Such elevated level of lactate can adversely affect the culture performance through negative impact on growth rate and productivity. Considering studies indicated that DCA diminishes aerobic glycolysis through inhibiting the activity of pyruvate dehydrogenase kinase (inhibitor of pyruvate dehydrogenase complex). The present study has conducted to investigate the ability of DCA to modulate lactate production and improving CHO cell culture performance.

Methods: An IgG2 expressing CHO cell line was cultured in a chemically defined media at 37℃ with 5% CO_2. Fed batch experiments were run, in the absence or presence of different concentrations of DCA (5 and 10 mM). The final expression level of monoclonal antibody along with the relevant acidic/basic charge variants were determined at the end of batch experiments.

Results: The results indicated that, DCA neither affects lactate production and nor improves cell culture performance (expression level and acidic/basic charge variants). Additionally, the cultures supplemented with DCA exhibited a sharp drop in viable cell density and expression level.

Conclusion: According to the obtained results, DCA cannot be considered as an affective supplement to control lactate accumulation in CHO cell culture.

Keywords: CHO cells, DCA, lactate reducing

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P-73

The effect of galactose supplementation on IgG1 glycosylation

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Background: Glycosylation can be changed directly under the influence of galactose concentrations. It has an important role in determining protein structure, function and stability. Galactose in culture usually causes an increases G1, G1F and the rest of the G1 compositions. This study demonstrated, for the first time, the effect of galactose concentrations on the levels of G0, G0F and mannose-5 (Man5) glycoform levels during mAb production.

Methods: Galactose concentrations of 25 mM and 50 mM were evaluated during shake flask experiment. Shake flask cultures were conducted using 500 ml baffled shake flasks. The flasks were incubated at 37° C and 5% CO_2 on an orbital shaker with the same agitation speed for three experiments. The final expression levels of antibody were determined by mAb selected affinity chromatography. The glycan profile was determined by hydrophilic interaction chromatography.

Results: According to the results, the proportion of G0, G0F and Man5 varied between control condition (with no galactose addition) and galactose supplemented cultures. The predominant glycoform was Man5 (1.92 %) and G0F (65.46%) in control culture; however, the addition of galactose with a final concentration of 50 mM and 25 mM decreased the Man5 (0.85% and 1.1%, respectively) and G0F content (30% and 46%, respectively). Meanwhile, galactose supplementation caused a large increase of up to 30% and 17.2% (50 mM and 25 mM galactose, respectively) in G0 glycoform (compared to the 1.17 % in control culture).

Conclusion: The results of the present study demonstrate that galactose supplementation can have a significant impact on Man5, G0 and G0F content in monoclonal antibodies.

Keywords: CHO cell line, Galactose, Glycosylation, Supplementation

P-74

Caffeic acid treatment could effect on Nrf2 regulated enzyme activities in human endometriotic and endometrial stromal cells

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Background: Endometriosis is one of the most prevalent gynecological disorders and the major cause of pain and infertility that is characterized by extra-uterine endometrial cell proliferation. To date, the pathogenesis of endometriosis has not been completely understood; however, several studies have shown that oxidative stress plays a pivotal role in the progression and development of endometriosis. The nuclear factor erythroid-derived 2-like 2 (Nrf2) fights against oxidative stress via the upregulation of antioxidant enzyme genes. Caffeic acid is an antioxidant and anti-inflammatory compound found in many foods and vegetables. The present study aimed to evaluate the effect of caffeic acid treatment on Nrf2 regulated enzyme activities in human endometriotic and endometrial cells.

Methods: Endometriotic and endometrial stromal cells were isolated from women with endometriosis (N=10) and normal women (N=10), respectively, and cultured in a proper medium. Then, cells were treated with the proper dose of caffeic acid (determined by MTT assay) followed by antioxidant enzyme activities of NAD (P) H: quinone oxidoreductase 1 (NQO1) and heme oxygenase (HO-1) were measured in endometriotic and endometrial cells.

Results: Our data revealed that NQO1 and HO-1 enzyme activities significantly decreased in endometriotic cells when compared with eutopic ones. Furthermore, caffeic acid treatment of endometriotic cells notably enhanced antioxidant enzyme activities of NQO1 and HO-1. However, in endometrial cells, no significant difference was observed in the above-mentioned parameters among caffeic acid-treated and non-treated cells.

Conclusion: In conclusion, caffeic acid could induce cytoprotective response, and enhancement the NQO1 and HO-1 enzyme activities might be through induction of Nrf2 gene expression in endometriotic cells. Further in vitro and in vivo studies must be done to determine the safety and efficacy of caffeic acid treatment.

Keywords: Caffeic acid, Endometriosis, HO-1, NQO1, Nrf-2.

P-75

Modulation of IgG high mannose glycoform using CHO cell culture supplementation with $MnCl_2$

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Background: Understanding the influence of cell culture process parameters on produced antibody and quality attribute is important particularly considering that antibodies containing high mannose glycoforms have become a concern due to its impact on clearance and biological activity. In the present study a series of fed batch experiment were conducted to decrease the high mannose level of monoclonal antibody produced by a CHO cell line.

Methods: A CHO cell line producing monoclonal antibody was cultured in a chemically defined medium. The effect of basal media supplementation with different concentration of $MnCl_2$ (0, 10, 20 and 40 µM) on high mannose glycoform was investigated by experiments run fed batch mode. The final expression levels of monoclonal antibody along with the glycan profile were assessed at the end of experiments.

Results: The results illustrated that the glycosylated forms of IgG were significantly improved ($p<0.05$) as media supplemented with $MnCl_2$. However, final titer and maximum viable cell count were diminished as concentration of $MnCl_2$ increased. Moreover, the maximum percentage of high mannose glycoform was observed when cells were cultured without $MnCl_2$.

Conclusion: Accordingly, the glycosylation rate of IgG was not necessarily increased as concentration of $MnCl_2$ increased in cell culture.

Keywords: CHO cells, high mannose glycoform, Manganese dichloride

P-76

Paper based analytical devises for ABO blood grouping: A systematic review

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Background: The clinical importance of blood group antigens is linked to their ability to stimulate immune antibodies that can cause hemolysis. Serological typing is the most commonly used typing method, based on hemagglutination reactions like tube typing, gel and microplate methods. In several clinical situations, rapid and accurate blood grouping plays an important role. In recent decades, paper-based microfluidics has gained much interest in wide application areas like point-of-care (POC) diagnostics. Microfluidic paper-based analytical device (μPADs) is one of the POC technologies. In this study we evaluate μPADs that are performed for blood grouping and its recent progresses. Finally, we critically discuss the current challenges, limitations and future perspectives of blood typing μPADs.

Methods: An exhaustive literature search was carried out using databases including PUBMED, SCOPUS, Web of Science and GOOGLE. Keywords were blood grouping, paper microfluidic, rapid test and etc. Only English articles were included. Finally studies references were reviewed for more relevant articles.

Results: After screening, 16 documents included. They were from 2010 to 2020. Most of them worked on primary antigens but 3 articles investigate on secondary antigens. Forward typing method was the main approach but 4 studies worked on reverse typing as well. The mean accuracy of tests was 99%. Other information's were classified in Table 1.

Conclusion: After 2010 studies on the μPADs for blood typing begins and progressed. μPADs are cheap, fast and simple to use but some limitation such as stability, clarity and sensitivity of test require more investigations.

Key words: μPADs, Blood group, POCT

P-77

Interaction of Memantine with Homocysteine on the Apoptosis in the Rat Hippocampus cells

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Background: It has been hypothesized that elevated plasma Homocysteine (Hcy) plays a role in the pathogenesis of Alzheimer’s disease (AD) and age-related cognitive decline. The mechanism of Hcy neurotoxicity in the brain is controversial; as well Hcy is a ligand of N-Methyl-D-aspartate (NMDA) receptor. Memantine, a competitive antagonist of NMDA receptors approved for the treatment of moderate to severe Alzheimer's disease.

Methods: Hcy was injected 0.5 µmol/µl in the hippocampus of the rat brain and meantime hydrochloride was injected 10mg/kg intraperitoneally 1 hour prior to Hcy injection. After five days, rats were killed and whole brain were taken out, fixed, and embedded in paraffin. The slices of the rat brain were prepared and immunohistochemical analysis was done to reveal the protein expression of Bax, Bcl-2, and the activation of Caspase 3 in the rat hippocampus layers.

Results: The data showed significant increase of Bax and Caspase-3 immunoreactivity in hippocampus of rat brain in Hcy group. Also an increase in Bax/Bcl-2 ratio in rat hippocampus cells. Memantine pretreatment could not change the levels of Bax, Bcl-2, Caspase-3 significantly in rat’s hippocampus cells.

Conclusion: These findings suggest that Memantine could not antagonize Hcy – induced apoptosis. Hcy may induce apoptosis via the other oxidative stress mechanism in the rat brain.

Keywords: Memantine, Homocysteine, Apoptosis, Hippocampus

P-78

A Report of Three Cases with Specific Neuromuscular Disorders in Female Rats Induced by Vitamin E-Deficient Diet

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Background: Rats fed with poor diets of vitamin E for a prolonged period manifest many ranges of disorders from neuromuscular to skin ulcers.

Methods: We report three cases of female Sprague-Dawley rats with severe neuromuscular disorders that had experienced more than three gestations. The dams were accidentally found during working on their male offspring for embryology purposes in the Anatomy Laboratory of Shiraz University. The clinical signs include ataxia, nystagmus, incoordination, cervical deviation (scoliosis) concaved to the left or right and circling toward the side of the lesion, while the other dams with the same age but fewer deliveries or male parents were not affected. The cases were unresponsiveness to injection with two doses of Neurobion, vit D and steroidal anti-inflammatory (Betamethasone) agents. To test whether the neuromuscular disorders observed in dams are due to vitamin E deficiency, the blood sample were collected and then dams killed and necropsied for histopathologic purposes. Also for comparison, the blood samples were taken from the three normal dams as the control.

Results: The serum vitamin E levels in the affected dams were determined to be several folds lower (1.78 µg/mL vs 4.64 µg/mL) compared to that of the control. On histological examination, H&E-stained slides of the cerebellar cortex showed encephalomalacia characterized by variable-sized spaces with indefinite forms filled with cellular debris, whereas no marked changes were observed in other sites of the central nervous system.

Conclusion These specific signs in female rats induced by a prolonged vitamin E-deficiency have not been described previously. It is important to note that vitamin E is relatively unstable and may not be retained well when added to the laboratory chow. Hence, the chow produced by companies cannot provide the nutritional needs of rats for a prolonged period at least during pregnancy and lactation.

Keywords: Vitamin E deficiency, Neuromuscular disorders, Rats

P-79

Effective Role of Bilirubin in the Pathogenesis of Non-alcoholic Fatty Liver Disease through Regulation of the Autophagosome Formation

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Background: Non-alcoholic fatty liver disease (NAFLD) is a chronic hepatic disorder which becomes a global concern. One of the most important processes involved in NAFLD pathogenesis is Autophagy; Atg5, an autophagy-related gene, is a crucial gene for the formation of autophagosomes through autophagy. Bilirubin, a non-polar tetra-pyrrole compound, is the final product of heme catabolism which can ameliorate NAFLD via autophagy. Thus, in this study, the effective role of bilirubin, as an endogenous antioxidant compound, was investigated in the pathogenesis and treatment of NAFLD.

Methods: HepG2 cell line was cultured in the proper medium; by using the MTT assay, 50 and 100μM concentrations of bilirubin were determined as nontoxic concentrations for these cells. NAFLD model was performed by treatment of HepG2 cells with 50mM of D-glucose solution. The treatment was done in 24- and 48-hour periods. Finally, RNA content of the treated and untreated cells was extracted and quantitative RT-PCR was employed to investigate the mRNA expression levels of Atg5 gene. Statistical significance was analyzed using Mann–Whitney and Kruskal–Wallis tests and p-values below 0.05 considered statistically significant.

Results: mRNA expression level of Atg5 gene was increased in fatty liver cells after 24h treatment with 100μM of bilirubin compared to both controls ($p<0.05$). After 48 hours, a significant increase was observed in the expression level of Atg5 gene in NAFL cells treated with both concentrations of bilirubin ($p<0.05$). This increase in expression was also observed in all groups treated with bilirubin compared to the normal control group ($p<0.05$).

Conclusion: Conceivably, autophagy is one of the most important mechanisms in protection and improvement of NAFLD in the patients with Gilbert syndrome. These in vitro findings suggest the bilirubin potential role as an endogenous antioxidant and its activity in the protection against and/or treatment of NAFLD by the regulation of autophagic flux.

Keywords: Non-alcoholic fatty liver disease, Bilirubin, Autophagy

P-80

Therapeutic Role of SIRT1 in Non-alcoholic Fatty Liver Disease through Regulation of Lipophagy

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Background: NAD-dependent histone/protein deacetylase sirtuin-1 (SIRT1) is the best characterized member of sirtuins in mammalian cells. Most of its functions are enzymatic activities that mainly regulate metabolic pathways in different organs, especially in liver. Non-alcoholic fatty liver disease (NAFLD) is the most prevalent hepatic disorder all over the world and characterized by accumulation of triglyceride droplets in the cytoplasm of liver cells. One of the most important processes that can confront with lipid accumulation is lipophagy, which can be regulated by SIRT1 activity.

Methods: The following information has been obtained by using of the protein database of National Center for Biotechnology Information (NCBI), UniProt bioinformatics database, The Human Protein Atlas, and also the related papers from Google Scholar, PubMed and Scopus databases.

Results: Lipophagy, is a dynamic process, which connects the autophagy and lipid metabolism in liver cells. Through lipophagy, hepatocellular lipid droplets are surrounded by bilayer membranes and transferred to autolysosomes, which finally become degraded. Therefore, lipophagy, like lipolysis, would have a beneficial role in the reduction of lipid accumulation. SIRT1, a well-recognized protein in the regulation of auto- and lipophagy, can modulate lipophagy by deacetylation of ATG5, ATG7 and ATG8/LC3, as the major mediator proteins of lipophagy, and also via down-regulation of mTOR, which is involved in the control of lipophagy. One of the most significant pathways is SIRT1-LKB1-AMPK, which Liver kinase B1 (LKB1) becomes deacetylated by SIRT1 and leads to AMP kinase activation and so, the regulation of lipophagy as its result.

Conclusion: Nowadays, finding an excellent way to treat NAFLD becomes a challenge! Lipophagy has a crucial role in NAFLD pathogenesis and helps to protection against the disease. This catabolic process can be regulated by SIRT1 protein through different pathways; Thus, SIRT1 may be a therapeutic target in patients with NAFLD, via activation of lipophagy.

Keywords: SIRT1, Non-alcoholic Fatty Liver Disease, Lipophagy

P-81

NAMPT has drawn ever-increasing attention in biomedical fields

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Background: Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the reaction of nicotinamide with 5-phosphoribosyl-1-pyrophosphate (PRPP) to provide nicotinamide mononucleotide (NMN). NAMPT is the rate-limiting enzyme in the biosynthetic pathway of NAD^+. NAD is an essential cofactor for the activity HDACs (histone deacetylases) known as SIRTs. Human NAMPT consists of 491 amino acids with a molecular weight of 52 kDa and dimerization is essential for the catalytic activity of the enzyme. NAMPT expressed in large amounts in bone marrow, liver tissue, and muscle. Also present in the heart, placenta, lung, and kidney tissues. NAMPT has two forms of intracellular (iNAMPT) and extracellular (eNAMPT) in mammals. iNAMPT is as an essential and rate-limiting NAD biosynthetic enzyme. eNAMPT has been reported to act as a cytokine, also known as pre-B-cell colony-enhancing factor 1 (PBEF1), promotes B cell maturation and inhibits neutrophil apoptosis, or visfatin. NAMPT has a post-translation and modification, which includes N-acetylmethionine, Phosphotyrosine, and Phosphoserine.

Methods: Three online databases PubMed, ExPASy (http://www.expasy.org/tools/findmod), UniProt (https://www.uniprot.org/), and related articles were looked through comprehensively.

Results: Dysregulation of NAMPT activity has been implicated in the human diseases such as acute lung injury, aging, atherosclerosis, breast cancer, diabetes, obesity-related disease, rheumatoid arthritis and sepsis. In cancer, first NAMPT inhibits apoptosis of tumor cells, second, reported that NAMPT could activate an IL-6/STAT3 survival-signaling pathway via a nonenzymatic mechanism, third increased NAMPT activity has been associated with angiogenesis and neovascularization. Chemical inhibitors of NAMPT are FK866, CHS 828, other chemical inhibitors (MPC-9528, CB30865), siRNA/miRNA (miR-494, miR-206), antisense oligonucleotide to NAMPT, and antibody to NAMPT.

Conclusion: Inhibition of NAMPT/PBEF/visfatin activity is considered as a novel antitumor therapeutic Strategy.

Keywords: NAMPT, visfatin, Therapeutic Strategy

P-82

MicroRNA-494 Reduces NAD Levels and Cell Survival and Induces Apoptosis by Targeting Nicotinamide Phosphoribosyltransferase

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Background: Breast cancer is frequently associated with elevated levels of Nicotinamide phosphoribosyltransferase (NAMPT) in their tumor and blood tissues. NAMPT is a rate-limiting enzyme in NAD synthesis pathway. MicroRNA-494 has been described to play key anti-tumor roles in human cancers. The objective of this research was to explore the inhibitory effect of miR-494 on NAMPT mRNA and activity as well as cell viability, NAD+ levels, and cell apoptosis in breast cancer cells.

Methods: The sense and antisense oligonucleotides of miR-494, as its mimic and inhibitor, respectively, were transfected into MCF-7 and MDA-MB-231 breast cancer cell lines. RT -PCR and western blot were used to evaluate the NAMPT mRNA and protein levels respectively. Subsequently, intracellular NAD+ levels were determined by a colorimetric method. Finally, cell apoptosis was examined by flow cytometry. Bioinformatics evaluations predicted NAMPT as a miR-494 target gene that was confirmed by luciferase reporter assay.

Results: Our results showed an inverse relationship between the expression of miR-494 and NAMPT in breast cancer cell lines. The NAMPT mRNA and protein levels were significantly reduced by miR-494 and it was also able to reduce the cellular NAD content. Cell viability was decreased following miR-494 up-regulation. Furthermore, miR-494 mimic induced apoptosis in MCF-7 and MDA-MB-231 cells.

Conclusion: Our findings indicate that miR-494 acts as a tumor suppressor and has an important effect in suppressing the growth of BC cells through NAMPT. In this manner, miR-494 may be a promising new approach in the management of breast cancer.

Keywords: Breast cancer, NAMPT, NAD, miR-494, microRNA

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Evaluation of Serum Substance P Level and Tissue Distribution of NK-1 Receptor in Papillary Thyroid Cancer

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Background: Papillary thyroid carcinoma (PTC) is the most frequent malignancy of the endocrine system. This study was aimed to evaluate the serum substance P (SP) levels, the tissue distribution of NK-1 receptors (NK1-R), and their possible diagnostic value in PTC.

Methods: This was a case-control study on 31 cases (age range: 25-64 years, 40.26 ±12.77) who were primarily diagnosed with PTC and were candidates for total thyroidectomy. Pre-operative serum level of SP was measured using a commercial ELISA kit. The tissue distribution of NK1-R was evaluated immunohistochemically.

Results: The serum level of SP in the patient group was higher than the control (p-value = 0.005). Besides, tumoral tissues had a higher expression of NK1-R than their normal surroundings (p-value = 0.005). However, no significant correlation was found between neither SP level nor NK1-R expression and the disease stage or lymph node involvement.

Conclusion: Substance P level and NK1-R expression are upregulated in PTC patients, showing the involvement of SP/NK1R complex in PTC pathophysiology. However, proposing SP/NK1R as a diagnostic factor demands further studies since no correlation was found between SP/NK1R and clinical stage or the lymph node involvement.

Keywords: Thyroid Carcinoma, Substance P (SP), NK1R, Tachykinin, Cancer.

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Protective effects of N-acetyl cysteine against diclofenac-induced nephrotoxicity in rats via attenuation of oxidative stress and inflammatory response

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Background: Diclofenac (DIC), a phenylacetic acid derivative, can lead to nephrotoxicity. In the present study, the protective effect of N-acetyl cysteine (NAC) on DIC-induced nephrotoxicity was investigated.

Methods: Thirty-two Wistar rats were divided into four groups. Group 1, the control group; group 2, rats that received DIC only; group 3, rats that received DIC plus NAC, and group 4 was treated with DIC and silymarin. Then, the serum and tissue parameters of the oxidative stress were examined.

Results: In group 2, DIC caused a significant increase ($p<0.05$) in the levels of serum biochemical parameters (GOT, GPT, urea, Cr, uric acid), TNF-α, protein carbonyl content, MDA, and liver TNF-α gene expression as opposed to control group. In treated groups with NAC and silymarin, a significant reduction ($p<0.05$) was seen in levels of serum biochemical parameters, TNF-α, protein carbonyl content, MDA, and liver TNF-α gene expression as well as ameliorated renal histopathological changes compared with group 2.

Conclusion: Alterations in the levels of biochemical, antioxidant assays, and histopathological changes in the kidney proved the toxic effects of diclofenac. NAC showed that it could reduce these changes and was able to restore normal antioxidant status in the rats.

Keywords: N-acetyl cysteine, Diclofenac, Nephrotoxicity, Oxidative stress, TNF-α

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Quercetin through mitigation of inflammatory response and oxidative stress exerts protective effect in rat model of diclofenac-induced liver toxicity

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Background: Diclofenac (DIC) is known for its anti-inflammatory and analgesic attributes but liver toxicity is one of the main concerns for this agent. Previous studies have demonstrated that quercetin has antioxidant and anti-inflammatory properties. We aimed to assess the protective effect of quercetin against DIC-induced liver toxicity in rats.

Methods: The rats after exposure to DIC were treated with different doses of quercetin (20, 40, and 80 mg/kg). The levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), intracellular glutathione (GSH) and catalase (CAT) in the liver tissue were assessed.

Results: The results indicated significantly declined in the abovementioned factors in DIC-alone treated group compared to the control group. Furthermore, the levels of the triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), total bilirubin, alkaline phosphatase (ALP), nitrite content, alanine aminotransferase (ALT), malondialdehyde (MDA), serum tumor necrosis factor-α (TNF-α), serum interleukin -1β (IL-1β), aspartate aminotransferase (AST), and inflammatory cytokines were evaluated. The results indicated remarkable elevation in all aforesaid factors in DIC-alone treated group compared to the control group. We showed that treatment with quercetin caused a noticeable elevation in GPx, SOD, GSH, CAT and a remarkable reduction in levels of TG, TC, LDL-C, VLDL-C, total bilirubin, ALP, nitrite content, ALT, MDA, serum TNF-α, serum IL-1β, AST and inflammatory cytokines compared to the DIC-alone treated group. Furthermore, histopathological alterations were also improved following quercetin administration.

Conclusion: Our findings indicated that quercetin through mitigation of oxidative stress and inflammatory response exerted a protective effect against diclofenac-induced liver toxicity in rats.

Keywords: Quercetin, Diclofenac, Liver toxicity, Oxidative stress, TNF-α, IL-1β

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The Effect of Coenzyme Q10 Treatment on Gene Expression of Nrf2 and HO-1 in Streptozotocin-Induced Diabetic Rats

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Background: Oxidative stress, by the formation of reactive oxygen species (ROS), contributes to the complications of diabetes mellitus. Coenzyme Q10 is a natural antioxidant with a strong free radical scavenging activity and may promote the expression of antioxidant factors. Nuclear factor-E2-related factor-2 (Nrf2)/ heme oxygenase 1 (HO-1) antioxidant pathway is an important endogenous antioxidant system, which may be dysregulated in diabetes. Therefore, we hypothesized that the up-regulation of Nrf2 and HO-1 genes in the diabetic liver may reduce the complications in diabetes. In this study, we investigated the effects of Co Q10 on the expression of Nrf2 and HO-1 in the liver tissues of normal, diabetic, and diabetic rats treated with Co Q10.

Methods: Thirty male rats were randomly divided into five groups: Groups 1 and 2 received normal saline (control) and Sesame Oil (sham), respectively. Group 3 was treated with CoQ10 (10 mg/kg), Groups 4 and 5 were diabetic rats (induced with STZ:55 mg/kg) that subsequently received normal saline and CoQ10 (40 mg/kg), respectively. After five weeks of treatment period, liver tissues were isolated from all rats. The mRNA expression of Nrf2 and HO1 was determined by qRT-PCR.

Results: The results showed that the expression levels of Nrf2 and HO-1 gene were significantly suppressed in the liver of diabetic rats compared to normal control groups. Treatment with Co Q10 significantly increased the levels of Nrf2 and HO1 mRNAs in comparison to diabetic control rats ($P < 0.05$).

Conclusion: This study showed that Co Q10 has beneficial effects on diabetes-induced oxidative stress by the regulation of Nrf2 and HO1 mRNA expression in the liver. Consequently, Co Q10 can reduce the complications of diabetes mellitus through activating anti-oxidative defense signaling pathway in diabetic rat liver.

Keywords: Coenzyme Q10, Diabetes mellitus, Gene expression, Nrf2, HO1

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Development and Optimization of Denosumab RANKL Binding Assay by ELISA Method

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Background: Denosumab, a human monoclonal antibody decreases bone resorption and increases bone mass by inhibiting RANK/RANK-L. Binding activity of biopharmaceuticals should be determined if the mechanism of action requires binding to a receptor. Binding activity of monoclonal antibodies could be determined using an immune assay such as ELISA. An ELISA binding test has been developed in Aryogen Pharmed for determination of Denosumab potency in preventing RANK-L from activating its receptor, RANK in comparison to standard. The designed test has problems such as signal reduction and improper response curve. In this study, these problems were solved and the test was optimized.

Method: The changes were made in test method for getting better signals were as follows: increasing in the amount of bovine serum albumin (BSA) in blocking solution from 3% to 5% (g/100ml); as well as the volume that used to fill the plate wells was 300µl instead of 100 µl. Also, the plate incubation time with the blocking buffer increased from 60 to 75 minutes. The optimizations performed during washing steps are also included in content of wash buffer and the number of washing steps at different stages of the test. BSA amount was increased in wash buffer to 1.5 g/1 L. Five wash steps were added after plate coating and 10 steps were fixed for TMB addition step. In order to solve the upper plateau in response curve 200 ng concentration was added to serial dilution.

Results: The obtained signals from Densumab binding to RANK-L increased significantly and showed conformity with Denosumab concentration dilution. Test result analysis with PLA software showed all features of four parametric logistic curves.

Conclusion: Modification of blocking and washing steps of this ELISA test can correct response signals related problems and are considered significantly important steps.

Keywords: Denosumab, RANK, RANK-L, ELISA, Binding activity

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Determination of Residual EDTA in Protein Formulations by Mixed Mode Chromatography (MMC) and UV detector

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Background: Ethylenediaminetetraacetic acid (EDTA) is a chelating agent that forms stable complexes with most metal ions. It is used in recombinant protein manufacturing to inhibit product degradation by host cell-derived metalloproteases. This chelating agent is cytotoxic, weakly genotoxic, but not carcinogenic. Although residual EDTA in monoclonal antibodies' drug products is below toxic levels, considering the importance of determining residual EDTA, an analytical method was developed to quantify residual EDTA in drug product of mAbs. The method iswas based on Mixed Mode Chromatography using UV Detector.

Methods: Residual EDTA was determined on a mixed mode HPLC column (SiELC, PrimesepB column, 150×4.6mm×5um) with two mobile phases including MeCN30%/Water70%/H_2SO_4 0.002%/$CuSO_4$ 0.02%(A) and (MeCN50%/Water50%/H_2SO_4 0.02%/$CuSO_4$ 0.02%(B) with a gradient from A to B in 36min. The flow rate was 0.7ml/min with column oven temperature of 30ºC. The UV wavelength was set to 254nm. The sample preparation involved spiking 0.1 mM EDTA in final volume of 200µl drug product. The protein of drug products was first removed using 10KD amicon. 20µl volume of the final solution was injected into the HPLC.

Results: The results showed that EDTA could produce a suitable UV signal, which can be used to quantify this impurity in LOQ of 0.05mM. The specificity test revealed that there was no interference from buffer formulation ingredients. This method can be used to determine residual EDTA in protein formulations with good precision (RSDs<5%) and accuracy (Recoveries: 90-110%). The proposed HPLC method was successfully applied to quantify residual EDTA in different batches of monoclonal antibodies' drug products. Residual EDTA was not detected in tested batches.

Conclusion: in this work, we introduced a simple and fast analytical method for the quantitative determination of residual EDTA in protein formulations. The proposed method is sufficiently accurate and precise for determination of this compound in protein drug products such as monoclonal antibodies.

Keywords: Mixed Mode Chromatography, EDTA, Protein Formulation, Biopharmaceuticals, Monoclonal antibodies

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Increase DNA Recovery in Host Cell DNA (HCD) Analysis of Biopharmaceutical Products

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Background: The expression of biological products using recombinant DNA technology has enabled the use of monoclonal antibodies and proteins for therapeutic usage. Host cell DNA contamination occurs during the production of biopharmaceuticals and must be controlled and monitored for the purity and safety of the drug products. A sodium iodide-based DNA extraction and a subsequent real time PCR assay were developed and validated for the quantitative measurement of residual host cell DNA impurity in monoclonal antibody therapeutic products.

Methods: In this experiment, initially, we aimed to use NaI and sarcosinat to dilute the monoclonal antibodies to reach concentration of 10 mg/ml. Two kinds of samples were used (spiked 10 pg/µl sample and non-spiked sample). Then, 25 µl of proteinase K and lysis buffer were used to denature all proteins. In the next step, DNA precipitated with isopropanol and ethanol 70%. After extracting DNA, absolute Real-Time PCR was done to detect and quantify HCD.

Results: We could extract and quantify of 0.001 pg/µl HCD in mAb. Also the recovery percent of our procedure was 91.35 % according to the DNA concentration in the spiked sample. The efficiency of our PCR was 94% and R2 of standard curve was 0.999.

Conclusion: HCD is one of the biggest concern in the biopharmaceutical industry. Therefore, most of the health organization make a specification for the level of HCD impurity in biopharmaceutical drugs. The WHO go on to dictate that the levels of residual host cell DNA that should not exceed 10 ng/dose for administered drugs. However, biopharmaceutical drugs would have passed several stages of purification, so we expected the low concentration of HCD in final product. Finally, existence of the accurate and precise method to measure the HCD is essential in any biopharmaceutical company.

Keywords: Biopharmaceutical drugs, Monoclonal antibodies, HCD, Real-Time PCR

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Optimization of SDS-PAGE Gel Electrophoresis Method for Denosumab as an IgG2 Monoclonal Antibody

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Background: One of the widely used, fast and inexpensive method for protein characterization is Polyacrylamide Gel Electrophoresis (PAGE). The procedure starts by denaturation of the proteins with SDS, an anionic detergent that causes a constant charge to weight proportion for all proteins such as monoclonal antibodies. Denosumab is a novel, fully human IgG2 monoclonal antibody that consists of 2 heavy and 2 light chains. Because of the structural differences, the SDS PAGE test for Denosumab should be modified to have sharper bands with higher resolution and less smearing compared to other mAbs.

Method: 6- 20% gradient resolving gel with 4% stacking gel was prepared. Nine µl of 0.8 mg/ml test sample and reference standard was mixed with 3µl sample buffer. Ten µl of samples, reference standard and 5 µl stained protein ladder were loaded in wells. Gel was fixed and stained with 0.1% coomassie blue; then de-stained and scanned and analyzed using Image Lab TM software. To optimize the SDS PAGE test for Denosumab, the LDS 4X sample buffer (6.82 g Tris (hydroxymethyl) aminomethane, 8.00 g LDS, 6.66 g Tris (hydroxymethyl) aminomethane hydrochloride, 40 g glycerol, 0.06 g EDTA, up to 100 ml UPW) was substituted with 5X sample buffer (1.5 mL of 1M Tris-HCl pH= 6.8, 6.25 ml of 100% (v/v) Glycerol, 5 ml of 10% (w/v) SDS solution, 2.5 ml of 2% (w/v) bromophenol blue). Samples were prepared with 5X sample buffer in the proportion of 1 to 3 and then heated for 2 minutes before run.

Results: Sample buffer alteration resulted in a noticeable improvement in band resolution and position of Denosumab in SDS PAGE.

Conclusion: As the result of present study showed, using the 5X sample buffer would result in a better band positioning and resolution in SDS PAGE test for Denosumab.

Keywords: SDS-PAGE, Denosumab, Monoclonal Antibody, Electrophoresis

P-91

Zinc supplementation ameliorates type 2 diabetes mellitus through the enhancement of superoxide dismutase-mediated antioxidant status: A randomized, double blind, controlled trial

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Background: Diabetes Mellitus type 2 is one of the major health concerns around the globe due to its widespread prevalence. Chronic hyperglycemia, followed by glucose autoxidation and lipids peroxidation can cause oxidative damages. Despite the current guidelines for the management of type 2 diabetes mellitus, patients still struggle with the hyperglycemia consequences. Imbalance in zinc homeostasis, in particular, renders diabetic patients more susceptible to the damages of oxidative stress. Aims: This study aimed to evaluate the effects of zinc supplementation on the superoxide dismutase gene expression and enzyme activity and oxidative stress status in overweight individuals with T2DM.

Methods: In this randomized, double blind, placebo-controlled trial, 70 overweight (BMI>25) T2DM patients were selected based on the inclusion criteria. They were divided into two groups for supplementation of daily 50 mg zinc gluconate or placebo for 8 weeks. Blood samples were collected from all the individuals and superoxid dismutase (SOD) genes expression and activity and oxidative stress indices and, biochemical parameters such as fasting blood glucose, insulin, glycated hemoglobin, homeostasis model of assessment-insulin resistance, serum levels of zinc and lipid profile, were assessed in the two groups.

Results: The results showed that, in comparison with the control group, zinc supplementation increased both gene expression and enzyme activity of SOD as well as the levels of insulin among the patients in the zinc group. Moreover, there was a meaningful reduction in the levels of malondialdehyde, nitrotyrosine, FBG, HbA1c and HOMA-IR value, triglycerides and total cholesterol after the zinc treatment.

Conclusions: The results of evaluated assumptions showed that the zinc supplementation positively affect both gene expression and SOD activity. Also zinc treatment caused significant improvement in oxidative stress indices, lipid and glycemic markers. These data suggest that daily supplementation with 50 mg of zinc gluconate could be beneficial for the management of overweight T2DM.

Keywords: Type 2 diabetes mellitus, Zinc supplementation, Antioxidant therapy, Superoxide dismutase, Nitrotyrosine, Malondialdehyde.

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Effects of Matricariachamomilla on acetylcholinesterase activity and anxiety behaviors in a rat model of scopolamine-induced cognitive impairment

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Background: Matricariachamomilla is a well- known medicinal plant for the treatment of neurodegenerative diseases such as Alzheimer's disease (AD). Acetylcholinesterase (AChE) has proven to be the most viable therapeutic target for symptomatic improvement in AD. The present study aimed to investigate the effects of hydroalcoholic extract of M. chamomilla on AChE activity of healthy rats and scopolamine models.

Methods: We attempted to investigate in vivo studies of M. chamomilla extract on rat anxiety behaviors, and acetylcholinesterase activity. The elevated plus maze (EPM) test was done for anxiety behaviors. Sixty-three adult male Wistar rats (250 -300 g) were used in this study. Animals were randomly divided into 6 groups (10 in each) as follows: (i) group 1 (control group) in which the normal rats were administrated 10 mg/kg saline throughout the experiment; (ii) group 2 (negative control) in which the rats were given intraperitoneally 1 mg/kg scopolamine; (iii) group 3: rats given intraperitoneally 200 mg/kg extract and 1 mg/kg scopolamine; (iv) group 4: rats received intraperitoneally 500 mg/kg extract and 1 mg/kg scopolamine; (v) group 5: rats given intraperitoneally 200 mg/kg extract; (vi) group 6: rats received intraperitoneally 500 mg/kg extract.

Results: The significant increase in percentage entries in open arms and time spent in the open arms was observed in treated groups in the presence of the extract in a dose-dependent manner. Furthermore, the AChE activity and the values of the Vmax, Kcat, and Kcat/Km were significantly higher in treated groups in the presence of extract in a dose-dependent manner. However, the Km value for group 4 was significantly less than those in the other treated groups.

Conclusion: These data highlight the beneficial effects of M. chamomilla extract on AChE activity, which affects memory dysfunction and provide an improved therapeutics for AD.

Keywords: AChE activity, memory function, Matricariachamomilla, Alzheimer's disease.

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In vivo study on the alteration of brain tubulin structure and tubulin assembly affected by hydroalcoholic extract of Matricariachamomilla in a rat model of scopolamine-induced cognitive impairment

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Background: The assembly of tubulin and microtubules are important in the memory mechanism. Microtubule depolymerization during axon and dendrites plays an essential role in the nervous system degeneration in Alzheimer's disease (AD). Matricariachamomilla extract as a neuroprotective agent is widely applied for the treatment of AD. Since the inhibitory effect of AD by M. chamomilla extract is attributed to its antioxidant properties, other factors can also be involved in this study. We decided to evaluate the effects of M. chamomilla extract on microtubule activity and its relationship with inhibition of AD.

Methods: Sixty-three adult male Wistar rats (250 -300 g) were randomly divided into six groups (n=10 per group) comprising of group 1: the normal rats were administrated 10 mg/kg saline as the control group; group 2: the rats were given 1 mg/kg scopolamine as negative control; group 3: rats were given intraperitoneally 200 mg/kg extract and 1 mg/kg scopolamine; group 4: rats received 500 mg/kg extract and 1 mg/kg scopolamine; group 5: rats were given 200 mg/kg extract;and group 6: rats received 500 mg/kg extract. Treated groups were given intraperitoneally the extract and scopolamine. The microtubule and tubulins were extracted from rats, and microtubule polymerization, secondary and tertiary structures of α/β-tubulins, were evaluated in the presence and absence of M. chamomilla extract in the experimental groups. **Results**: The significantly highest optical density, and the initial rate of microtubule polymerization ($p<0.05$) was observed in group 6 as compared to other groups. The content of secondary and tertiary structures of tubulins was significantly ($p<0.05$) affected in treated groups.

Conclusion: These data concluded that M. chamomilla extract is beneficial effects on microtubule assembly and tubulin structures as a molecular target for memory dysfunction and provides improved therapeutics for neurodegenerative disease.

Keywords: Microtubule assembly, Memory function, Matricariachamomilla, Alzheimer's disease.

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The effect of α- Synuclein in dysfunction of mitochondria in Parkinson’s disease

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Background: Synuclein proteins, which include three members: alpha, beta, and gamma synuclein, are small and soluble structures that are involved in neurological diseases and certain cancers. Parkinson’s disease (PD) one of the most common neurodegenerative diseases in the senescent that affected millions of people worldwide. α-syn. has a critical role in pathogenesis of PD and it is detectable in plasma, serum and cerebrospinal fluid (CSF). The interplay of mitochondrial and bioenergetics dynamics is particularly important in the function of the nervous system. It appears that accumulation of α-syn and dysfunction of mitochondria are related, and particularly involved in PD pathogenesis.

Methods: A search was performed in Universal Protein Resource (UniProt) and Nextprot database and published data collected in searching database (PubMed, Web of Science and Scopus) by following keywords: “α Synuclein (α-syn), Parkinson’s disease, mitochondrial dysfunction, cellular mechanism.”

Results: α-syn which is coded by the SNCA gene, is a presynaptic neuronal protein that can interfere with mitochondrial proteins with binding to the outer membrane or enter that under certain conditions. Experimental and human studies of PD have shown abundant evidence of mitochondrial damage, owing to the fact that most of the genes associated with Parkinson's play a key role in mitochondrial homeostasis. α-syn may exert indirect effect on complex I activity through the interaction with cardiolipin that is a protein necessary for its function in respiratory chain. The interaction between α-syn and cardiolipin can disrupt electron transfer and consequence serious damage in neurons.

Conclusion: Mitochondrial dynamics impairment is associated with functional damage to neurons, nerve cells, and PD. α -syn protein has been identified as one of the factors affecting the mitochondrial dynamics of neurons. Identifying the detailed molecular mechanisms between α -syn and mitochondrial dysfunction can provide new insights into the pathophysiology of PD.

Keywords: α Synuclein, Parkinson’s disease, Mitochondrial dysfunction, Cellular mechanism

P-95

Optimization of protein expression of wild type-survivin in E. coli C41 strain

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Background: Apoptosis is the mechanism responsible for the physiological deletion of cells and appears to be intrinsically programmed. Apoptosis is an active and regulated cell death process that occurs in the body. The inhibitor of apoptosis protein (IAP) family functions as inhibitor of apoptotic pathways. Several proteins negatively regulate the function of the IAP family looks like Smac/DIABLO. All of the IAP molecules are well-known with the ability to suppress apoptosis and presence of a Zinc-binding fold termed the baculoviral IAP repeat (BIR domain). Survivin is the smallest member of the IAPs family which inhibits apoptosis and regulates mitosis. Survivin is expressed in most cancers, fetal development and embryonal tissues, but not in most normal adult tissue. In order to study the recombinant protein of survivin, its protein expression level was investigated in a bacterial host.

Methods: In this research, the pET28a vector included the wild-type of survivin gene was transformed in to E. coli C41 expression strain. The expression of protein solution with a final concentration of 0.5 mM IPTG and/or 4 mM of lactose was induced and then placed in incubator-shaker at different times and temperatures, and the expression level was investigated by SDS-PAGE.

Results: In all conditions at E. coli C41, the expression of survivin protein was seen as a solution, but part of the protein was insoluble in sediment remains. The best condition was observed when C41 host incubated at 30 °C for 14 h by adding 0.5 mM IPTG.

Conclusions: The expression of survivin was observed at all conditions in E. coli C41, but some of the protein accumulated in the bacteria as inclusion body.

Keywords: Survivin, Expression, E. coli.

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Magnetic nanocomposites for isolation of breast cancer cells in blood

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Background: Engineering magnetic nanomaterials to possess a high affinity toward cancer cells opens new horizons in capturing circulating tumor cell (CTCs) in blood without need to aptamers and antibodies. To functionalize magnetic nanocomposites, small molecules such as folic acid has received attentions owing to high affinity (k_d= 0.1-1 nM) toward folate overexpressed cancer cells (i.e., MCF-7) and also low cost. Herein, we fabricated a novel magnetic composite using CoFe2O4 and folic acid to capture breast cancer cells.

Methods: We used hydrothermal method to fabricate magnetic nanocomposite. Co $(NO_3)_2.6H_2O$ and Fe $(NO_3)_3$. 9H2O were dissolved in deionized water. Next, they added to NaOH solution and dark brown color indicated fabrication of magnetic $CoFe_2O_4$ nanoparticles followed by addition of folic acid and hydrothermal treatment. To capture cancer cells with a magnet, DIL tracker mixed with FCA for 12h. After that, MCF-7 cells were added to the 1mL of media or blood containing FCA-DIL and cell capture efficiency was evaluated by counting cells.

Results: Chemical structure of CFA studied by FTIR and UV-Vis spectroscopy. FTIR analysis of CFA showed the peak related to Fe-O at around 590 cm^{-1}. Furthermore, –CH2 and –CH= vibration that are associated to folic acid was implied in the range of 800 to 900 cm^{-1}. UV-Vis spectroscopy was carried out that indicated two signature peaks of folic acid. FESM images showed the morphology of CFA. Vibrating sample magnometer analysis depicted the saturation magnetization (Ms) at 37.339 emu g^{-1}. After 48 treatments of hFF cells with CFA, MTT indicated that CFA has no toxicity in the range of 0 to 300 µg/mL.

Conclusion: Fabrication of CFA with innovative approach was confirmed according to characterization approaches. CFA was able to capture MCF-7 cells in media and blood, with cell capture efficiency 88% and 80%, respectively.

Keywords: Magnetic nanocomposite, Breast cancer cells, $CoFe_2O_4$, Cell capturing

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Analysis of XIAP and Caspase-9 Interaction using Split-Luciferase Complementation Assay

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Background: Human inhibitor of apoptosis proteins, which includes eight members, act as key regulators of cell fate. XIAP as an inherent regulator of caspases, inhibit Caspase-3/-7 and -9 through binding to their IAP binding motif via its BIR domains directly. Over-expression of XIAP leads to elevated inhibition of caspases and then a serious imbalance between cell death and cell life, which contributes to oncogenesis. XIAP as a potent cytosolic drug target and its interaction with Caspase-9 has been studied extensively. Here, we developed a biosensor to monitor interaction between XIAP and Caspase-9 for the first time, which may also suite for high-throughput screening of small molecules.

Methods: Our method was based on protein complementation assay (PCA) approach using split-luciferase. For this purpose, XIAP and Caspase-9 fused to the N-terminal and C-terminal moieties of firefly luciferase with a flexible linker. A mutant form of Caspase-9 was produced by Quick-Change PCR. The plasmids introduced into HEK293T cells by transfection to monitoring interaction of XIAP with both native and mutant forms of Caspase-9 using luciferase activity. Protein expression level was also confirmed with immunoblotting. Caspase-3 like activity was measured using Ac-DEVD-AMC to assess inhibition effect of XIAP on endogenous Caspase-3.

Results: Split luciferase-activity showed that XIAP interacts with Caspase-9; however, it was not affected much by introducing inhibitory mutation(s) on processing site and/or active site of Caspase-9, unexpectedly. These results probably show that only one inhibitory mutation does not work for proteins with multi-lateral interactions and unavoidable proximity in multi-protein complexes.

Conclusion: It seems that multiple mutations are necessary to omit proximity effects of XIAP with Caspase-9.

Keywords: XIAP, Caspase-9, Luciferase assay

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The effect of N-acetylcysteine on glutathione and lipid peroxidation levels in testicular tissue of adult male mice

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Background: N-acetylcysteine (NAC) is a small molecule containing a thiol group and is a precursor to the amino acid L-cysteine. NAC was discovered to have an antioxidant effect and acting as a free radical scavenging agent. In this study, the effect of N-acetylcysteine on glutathione and lipid peroxidation levels of testicular tissue in adult male mice was investigated.

Methods: Twenty healthy adult male mice weighing 25-30 g were randomly divided into 2 groups of 10 each. The mice were maintained in a standard plastic cages with easy access to food and water and also subjected to photoperiod of 12-h light/12-h dark. The experiment was performed according to the guidelines and approval of institutional animal ethics committee. The first group (control) received only basal diet and the second group received NAC 150mg/kg for 7 days. Twenty-four hours after the last treatment, the testis was carefully excised. Testis extracts were prepared by freezing the samples in liquid nitrogen, homogenizing with a hand homogenizer, and suspending the homogenates in 100 mM sodium phosphate buffer, pH 7.4. The suspensions were centrifuged for 10 min at 4,000 rpm.

Results: The results showed that administration of 150 mg/kg NAC for 7 days significantly can reduce lipid peroxidation levels and increase glutathione levels in the testicular tissue of adult male mice ($p<0.05$).

Conclusion: NAC may reduce lipid peroxidation by increasing glutathione levels in the testicular tissue of adult male mice.

Keywords: N-acetyl cysteine, glutathione, oxidative stress

P-99

Vitamin E Improves Ovarian Injury Caused by Doxorubicin in Rats

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Background: Recently, the exposure to Doxorubicin (DOX), a widely used chemotherapeutic agent, is increased among reproductive-age women due to elevated rates of cancer incidence, which led to insulting ovarian reserve. The present study seeks to assess the potential protective effect of Vitamin E (Vit.E), an antioxidant, in rat ovarian toxicity caused by doxorubicin.

Methods: Female animals were divided into 4 groups as follows: Control, Vitamin E (200mg/Kg), Doxorubicin (accumulative 15mg/Kg), and DOX+Vit.E. Each group contained 8 rats treated for 3 weeks. After treatment, alterations in ovarian follicles, apoptosis, and nitric oxide levels were evaluated by histopathological-stereological, RT-qPCR, and colorimetric analysis.

Results: The results revealed ameliorative effects of Vit.E on DOX-induced dramatic decline in the number of primordial follicles, an increase in the number of atretic follicles, elevated tissue NO levels (p-value<0.001), and elevation and decrease in Cas-3 and Bcl-2 gene expression, respectively (p-value<0.05).

Conclusion: Our findings suggest a promising preventive role of this dietary supplement on DOX-induced ovarian toxicity.

Keywords: Cancer, Chemotherapy, Antioxidant, Vitamin, Ovary.

P-100

Quercetin Alleviates Doxorubicin-Induced Uterine Toxicity, a Rat Model Study

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Background: In recent years, exposure to chemotherapeutic agents such as Doxorubicin (DOX) has become a main cause of uterine toxicity due to elevated rates of incidence of cancer in women with reproductive ages. Therefore, the present study sought to assess the ameliorative effect of Quercetin (QCT), a phytoestrogenic flavonoid, on uterine toxicity caused by doxorubicin in a rat model.

Methods: Thirty-two adult female rats were divided into four groups including control, QCT (at 20 mg/Kg), DOX (at accumulative doses of 15 mg/Kg), and DOX+QCT. After 21 days of treatment, alterations in uterine histoarchitecture, apoptosis, and nitric oxide (NO) levels were evaluated by stereological-histopathological, RT-qPCR, and colorimetric analysis.

Results: The findings demonstrated ameliorative effects of QCT on the doxorubicin-induced decline in the thickness of myometrium and uterine vessels volume (p-value<0.05), increase in uterine levels of NO (p-value<0.001), and increase and decrease in Cas-3 and Bcl-2 gene expression, respectively (p-value<0.05).

Conclusion: Taking together, our findings indicate a promising protective role of this dietary phytoestrogen on DOX-induced uterine toxicity.

Keywords: Cancer, Chemotherapy, Phytoestrogen, Antioxidant, Uterus.

P-101

Anti-proliferative effect of docetaxel in combination with quercetin in breast cancer cell line MDA-MB-231

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Background: Breast cancer is the most common cancer among women and one of the most important causes of death among them. Docetaxel is a chemotherapy drug that is widely used for the treatment of breast cancer. However, some side effects and development of resistance by the cancer cells may complicate treatment and lead to chemotherapy failure. Therefore, there is an increasing interest in the use of combination therapy consisting of natural compounds and traditional chemotherapeutic agents. Quercetin is a natural flavonoid with strong antioxidant and anticancer activities. This study aims to evaluate the cytotoxic effects of docetaxel and quercetin as single agents or in combination together on human breast cancer cell line, MDA-MB-231.

Methods: MDA-MB-231 cell line was treated with different concentrations of docetaxel (0-50 nM) and quercetin (0-200 μM) for 48 h. The effects of docetaxel and quercetin were measured on cell proliferation by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Then, the combined effect of docetaxel and quercetin was examined on cell proliferation using IC10 and IC40 of them, respectively.

Results: Docetaxel and quercetin decreased the growth of MDA-MB-231 cells as a single treatment at 48 h. Nevertheless, a combination of these two drugs was more effective in the reduction of cell viability with a combination index (CI) value of 0.76.

Conclusion: Our results suggest that docetaxel combined with quercetin exhibits a synergistic effect against MDA-MB-231 cells proliferation with dose reduction thus minimizing toxicity to normal cells.

Keywords: Breast Cancer, Cell viability, Docetaxel, Quercetin

P-102

An Investigation of the Effect of Calcium Nanofluoride on the Breast Cancer Cell lines

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Background: Breast cancer is known to be the second leading cause of death in women. Nano-drugs are known as a new treatment way for cancer by circulating the drug in the bloodstream for a longer period of time. In this study, the effect of calcium nanofluoride, as a new treatment strategy, is examined on the breast cancer cell lines, as a pattern of cancer cells, for the treatment and reduction of cancer symptoms.

Methods: Calcium nanofluoride was synthesized by combining calcium carbonate with hydrogen fluoride and converted to nanoparticles by sonication. The nanoparticle was confirmed by measuring zeta potential (DLS) and by electron microscope (TEM) devices. The MDA-MB-231, MCF-7 and BT cell lines were cultured in DMEM culture medium and tested using bromophenol blue and MTT assay. The vital power of cells and IC50 for calcium nanofluoride was obtained after 24, 48 and 72 hours and then, apoptosis and the cell cycle tests were performed on the breast cancer cell lines. Untreated cancer cells were considered as controls.

Results: Calcium nanofluoride caused apoptosis by 2.8% MDA-MB-231, 71% of MCF-7 and 4% of BT breast cancer cells lines. In the MDA-MB-231 cell line, the G1 and G2 stages of the cell cycle showed an increase of 6.7 and 1.01 percent, respectively, and in the S stage, a decrease of 5.79 percent. Also, in MCF-7 cell line, in stages G1 and G2 showed a decrease of 11.46 and 6.43 percent, respectively, and in stage S, an increase of 22.52 percent. In BT cell line, G1 and G2 increased by 14.64 and 5.95 percent, respectively, and decreased by 16.1 percent in the S stage.

Conclusion: The percentage of apoptosis was increased by calcium nanofluoride in MDA- MB-231, MCF-7 and BT cell lines. The levels were confirmed by examining the cell cycle.

Keywords: Breast cancer, Calcium Nanofluoride, Apoptosis, Cell Cycle, IC 50

P-103

A potent cancer-preventive effect of citrus Auraptene against colorectal cancer

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Background: Colorectal cancer (CRC) has an increasing rate in the last decades in Iran. Resistance to chemotherapy led to the search for alternative treatments such as medical herbs. Auraptene (7-geranyloxycoumarin) is the most abundant prenyloxycoumarin that exists mainly in Rutaceae and Umbeliferae (Apiaceae) families and also in a variety of citrus fruits. Quite recently, considerable attention has been paid to the chemopreventive nature of auraptene (AUR) against CRC. In this review, the effects of AUR on the prevention of colon cancer were discussed.

Methods: A systematic literature search was conducted using the PubMed and google scholar database published between 2001 and May 2020.

Results: Six studies were included in the review. In vivo studies showed that ulcerative colitis, obesity, and inflammation are some risk factors for CRC. The inhibition of the formation of aberrant crypt foci was seen in the colitis mouse model induced with dextran sodium sulfate (DSS), which received AUR in their diet. Recent research focused on the dietary use of auraptene on the mouse model of colon cancer initiated with azoxymethane (AOM). The results revealed the inhibition effects of AUR on chronic inflammation, especially in the large boweldue to reduce the level of pro-inflammatory cytokines, such as Stat3, nuclear factor-kB, NF-E2-related factor 2, tumor necrosis factor-α, interleukin-1b, and interleukin-6. In HT-29 colorectal cancer cells, AUR prohibited the production of pro-MMP7 protein through an interruption in phosphorylation of 4EBP1 and eIF4B via ERK1/2 pathway. Moreover, AUR can inhibit the formation and growth of colonospheres in the FOLFOX-resistant HT-29 cell line. Importantly, it can decline the phospho-epidermal growth factor receptor (pEGFR) in these cells, so it affects the re-emergence of colon cancer stem cells.

Conclusion: Auraptene has shown a remarkable role in the suppression mechanism of colon carcinogenesis, but further research will be required.

Keywords: Auraptene, colorectal cancer, chemopreventive

P-104

Assessment of the relationship between serum vitamin D and potassium levels in diabetic nephropathy: A case control study

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Background: Diabetic patients are at risk for microvascular disease, including retinopathy, neuropathy and nephropathy. Vitamin D seems to play a role in diabetes and since its depletion increases the activity of the renin-angiotensin system, it would appear that it is responsible for the regulation of certain electrolytes and blood pressure. The aim of this study was to assess the relationship of serum vitamin D and potassium levels in diabetic nephropathy (DN).

Methods: In this case-control study, 83 patients were included who had type 2 diabetes. The samples were assessed based on proteinuria and glomerular filtration rate (GFR) calculation. Among them, 41 patients were without nephropathy and 42 patients with nephropathy. The serum 25-hydroxyvitamin D level was measured by ELISA and the serum potassium levels was measured by the Flame Emission Spectroscopy, Ion-selective Electrode. Data were analyzed by SPSS v.26 software. The significance level of the tests is less than 5%.

Results: The gender composition was 54.2% male and 45.8% female. The mean age of the DN and control groups were (63.45±8.33), (59.98±11.08) year, respectively. However, 83.3% of ND group and 41.5% of the control group had hypertension ($p < 0.0001$). The average proteinuria of DN group was 936 ± 452.25 mg/24h. In addition, the means of GFR was (37.45±14.43), (86.51±25.33) ml/min for DN and control groups, respectively ($p<0.0001$). The mean of serum vitamin D and serum potassium of DN and control groups were (22.06±8.85), (28.92±11.92) ng/ml and (4.80±0.73), (4.25±0.36) mEq/l respectively, ($p=0.004$; $p<0.0001$).

Conclusion: The results of this study showed that there was a significant relationship between the decrease in serum vitamin D levels and the increase in serum potassium levels accompanied with hypertension in DN. Thus, it is therefore possible to suggest different therapeutic approaches, such as hypoglycemic agents, antihypertensive drugs, renin-angiotensin system inhibitors and vitamin D supplement, to slow the progression of DN.

Keywords: Diabetic Nephropathy, Vitamin D, Potassium, Hypertension, Glomerular filtration rate, Proteinuria

P-105

LC3II/LC3I ratio in relation to proliferation in breast cancer cells

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Background: Breast cancer ranks first in term of incidence and the second leading cause of cancer death after lung cancer among women. Autophagy is a homeostatic degradation process whereby material is delivered to lysosomes, digested and recycled to sustain cellular metabolism. Autophagy has dual roles in cancer as interventions to both stimulate and inhibit autophagy have been recommended as cancer therapies. During Autophagy process, a cytosolic micro-tubule-associated protein 1A/1B-light chain 3 (LC3-I) is altered to LC3-II that can be used as an autophagy marker. The aim of this study was evaluation of correlation of LC3II/LC3I ratio expression as an autophagy marker with cell proliferation in breast cancer cell line MDA-MB-231.

Methods: MDA-MB-231 cells were cultured in presence of different dose of tunicamycin or N-acetyl cysteine as activator and inhibitor of autophagy respectively in different times. Then, MTT assay was carried out for evaluation of tumor cells proliferation. As well as that western blot was performed for evaluation of LC3II/LC3I ratio expression in MDA-MB-231.

Results: Data showed that tunicamycin resulted in inhibited cell proliferation in a time and dose dependent manner; but N-acetyl cysteine led to increased cell proliferation in concentration of 2mM. The cells treated with tunicamycin showed increased ratio of LC3II/LC3I, whereas the cells treated with N-acetyl cysteine led to decreased LC3II/LC3I ratio.

Conclusion: Activation of autophagy pathway is correlated with inhibited cell proliferation. Therefore, autophagy is a mechanism that leads to decrease cell proliferation in breast cancer cell line and could be used as a target in cancer therapy.

Keywords: Autophagy, Breast Cancer, Proliferation

P-106

Umbelliprenin suppresses COCL2/ EGF-induced HIF1α/VEGF production in MDA-MB-231 cell line

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Background: Breast cancer is one of the most common malignancy in women in the world. Umbelliprenin is a naturally occurring component derived from plant species that shows anti-cancer properties.

Methods: In this study, we evaluated the effect of umbelliprenin on HIF1α/VEGF production in MDA-MB-231 cell line. The cytotoxic effect of umbelliprenin on MDA-MB-231 cells was evaluated using MTT assay. Later, treatment of the cells with EGF and COCL2 and the effect of umbelliprenin on the genes and proteins involved in angiogenesis pathway in the EGF/COCL2-treated cells was evaluated using real time PCR and western blot analysis, respectively.

Results: Our results showed that umbelliprenin exerts its cytotoxic effect on the MDA-MB-231 cells in a concentration-dependent manner. Moreover, umbelliprenin in a concentration -dependent manner decreased the expression of VEGF and HIF-1α in the cells treated with EGF and COCL2 at both gene and protein levels.

Conclusion: Our results indicated this compound could be considered as a potential anti-cancer drug.

Keywords: Umbelliprenin, MDA-MB-231, COCL2, EGF, HIF1α, VEGF, Angiogenesis, Cancer

P-107

Design, Construction and Cloning of HIV-1 Nef-Vpr Fusion DNA into Mammalian Expression Vector

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Background: HIV-1 Nef and Vpr antigens have been described as suitable candidates for therapeutic HIV vaccine development. The aim of this study was to generate Nef-Vpr fusion gene construct and to clone the construct into pcDNA3.1, a eukaryotic expression vector.

Methods: HIV-1 Nef and Vpr genes were PCR-amplified from the pNL4-3 plasmid using specific primers and Pfu DNA polymerase. Results of PCR amplification were visualized by electrophoresis on 0.8% agarose gel. At first, the amplified Nef fragment was cloned into NheI and BamHI restriction sites of pcDNA3.1 expression vector. Next, cloning of Vpr gene was performed into BamHI and HindIII restriction sites of the pcDNA-Nef vector. Finally, the recombinant fusion pcDNA-Nef-Vpr construct was generated in large scale and as endotoxin-free DNA. The purity of DNA construct was determined by NanoDrop spectrophotometry.

Results: PCR amplification of Nef and Vpr genes was confirmed by detection of 620 bp and 291 bp bands, respectively. Cloning of the Nef-Vpr construct into the vector was confirmed by detection of a 911 bp fragment following enzymatic digestion with NheI and HindIII, PCR amplification with Nef forward primer and Vpr reverse primer, and sequencing.

Conclusion: The successful construction of recombinant fusion plasmid encoding a chimeric Nef-Vpr gene was performed in a eukaryotic expression vector for HIV-1 DNA vaccine development in near future.

Keywords: HIV-1, Nef, Vpr, pcDNA, Cloning, Recombinant plasmid

P-108

Designing a fusion peptide construct harboring L1, L2 and E7 epitopes from human papillomaviruses 16 and 18 using immunoinformatics tools

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Background: Human papillomavirus (HPV) is the major cause of cervical cancer in women worldwide. Among high-risk HPV types, HPVs 16 and 18 had the most prevalent types associated with cervical cancer. HPV L1 and L2 capsid proteins as well as E7 oncoprotein play a crucial role in HPV-related diseases, and can be used as target antigens for preventive-therapeutic vaccines.

Methods: In this study, different bioinformatics and computational tools were used to design novel immunodominant epitopes of the L1, L2 and E7 proteins from HPV16 and 18 for vaccine development against HPV infection. For this purpose, prediction of MHC-I and MHC-II peptide presentation pathways for the L1, L2 and E7 conserved regions were analyzed using NetMHCpan 4.0 and NetMHCIIpan, respectively. In each protein, peptides with the highest binding affinity scores were selected. Then, immunogenicity and allergenicity was determined by the IEDB tools. Next, population coverage was estimated separately for each putative epitope in world. After that, GalexyPepDock server was used for peptide-protein flexible Docking to predict docking scores between MHC alleles and peptides.

Results: A fusion peptide construct harboring L1 (416-430 and 12-21), L2 (11-20 and 281-297), E7 (43-57) from HPV-16, and L1 (8-22, 461-471), L2 (11-20, 274-290), E7 (78-91) from HPV- 18 were designed based on immunoinformatics tools. These peptides were non-allergen, conserved, and immunogenic.

Conclusion: We found the immunodominant and conserved epitopes of HPV16 and 18 L1, L2 and E7 proteins for design of a multiepitope peptide vaccine candidate against HPV infection.

Keywords: HPV, Capsid protein, Oncoprotein, Immunoinformatics tools

P-109

Alpha-lipoic acid supplementation effects on serum values of catalase, thiol groups, oxidative stress index and homeostasis model assessment of beta-cell function (HOMA-B) in women with gestational diabetes

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Background: Alpha-lipoic acid (ALA) is a short-chain fatty acid derived from octanoic acid. ALA is a natural antioxidant in the body that has a sulfuric part and can destroy different kinds of free radicals. This trial aims at studying the effect of ALA supplementation in some oxidative stress indexes and factors in women with gestational diabetes mellitus (GDM).

Methods: The current study was a randomized double-blind placebo-controlled clinical trial. Sixty women with GDM at 24-28 weeks of gestation were chosen and randomly categorized into two groups including drug (n=30) and placebo (n=30). The drug group received an ALA capsule (300 mg) daily. The biochemical factors before and after the intervention were measured.

Results and Conclusion: The results showed that FBS and OSI decreased whereas TAC, HOMA-B and catalase increased after 8 weeks of ALA supplementation in women with GDM.

Keywords: Key Words: Gestational diabetes mellitus, Alpha-lipoic acid, oxidative stress.

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Association between anthropometric and glycemic status with proxidant-antioxidant balance among patients with diabetic foot ulcer

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Background: Diabetic foot ulcer (DFU) is a major complication of diabetes mellitus which affect more than 25 percent of diabetic patients and the first cause of non-traumatic lower extremity amputation. Oxidative stress happens as a result of imbalance between pro-oxidant and anti-oxidant within the body and play a significant role in diabetes associated complications including DFU. PAB is a simple measure for pro-oxidant and anti-oxidant balance in the serum that can be helpful in monitoring patient's oxidative status. Here we hypothesized that there may be a link between prooxidant-antioxidant balance and anthropometric and glycemic status in patients with DFU.

Methods: Twenty-five patients with DFU enrolled in this study. Fasting blood sample were taken from patients for biochemical assessment. Serum level of PAB, fasting blood sugar (FBS), insulin, HbA1c, quantitative insulin sensitivity check index (QUIKI) and Homeostatic model assessment for insulin resistance (HOMA-IR) was measured. Furthermore, anthropometric measure for the fasting weight, height and BMI of the patients were calculated. Bivariate correlations between parameters and serum PAB value were performed using Spearman's rank correlation.

Results: Our data showed a significant correlation between serum PAB value (169.5± 47.4) and HbA1C (9.6±1.6%), insulin (15.8±13.6%) and also BMI (24.9±3.9) ($p<0.05$). However, there was no significant association between serum PAB value and FBS (135.1±40.7mg/dl), QUIKI (0.31±0.03) HOMA-IR (5.1±4.2) and weight (76.8±12.5g).

Conclusion: We found that there was a significant correlation between serum value of PAB and HbA1C, insulin and BMI ($p<0.05$). Higher level of PAB is associated with high level of glycosylated hemoglobin, insulin and BMI, which are among the main risk factor of DFU.

Keywords: Diabetic foot ulcer, Proxidant-antioxidant balance, Glycemic status, Oxidative stress

P-111

Upregulation of SREBP-1c expression in patients with gastric adenocarcinoma

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Background: SREBP-1c is a transcription factor that indeed leads to lipid homeostasis in mammals. It has a regulatory role during mitosis, showing that the expression of lipogenic genes is a prerequisite for cell cycle progression. The objective of this study was to investigate the expression of SREBP-1c in gastric adenocarcinoma (GA) patients compared to control subjects.

Methods: A total of 112 patients with GA and 156 non-tumoral control cases were enrolled in this study. Immunohistochemical method was employed to analyze SREBP-1c expression in Paraffin-embedded tissues. Serum insulin concentration was measured by ELISA, and tissue mRNA expression of SREBP-1c determined by qRT-PCR.

Results: SREBP-1c gene expression was higher in GA vs. controls, and SREBP-1c protein level enhanced in GA tissues compared to controls. However, there was no statistically significant difference in fasting blood glucose and insulin serum levels between the two groups.

Conclusion: Normal mammalian cells typically acquire fatty acids from the circulation owing to insulin, which is the most lipogenic hormone in the subset of adult tissues. In contrast, some cancer types like GA depend on de novo fatty acid biosynthesis that could be regulated by the transcription factor SREBP-1c. As a result, unrestricted fatty acid anabolism could be observed in cancers, unaffected by the extracellular lipid availability and regulatory hormonal motivation.

Keywords: Gastric adenocarcinoma, SREBP-1c, Fatty acid biosynthesis

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P-112

Optimizing the recombinant production of the antibody fragment against EpCAM in a chemically defined minimal medium using Response Surface Methodology

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Background: The epithelial cell adhesion molecule (EpCAM) is an important cancer-associated marker that overexpressed in epithelial tumors, cancer stem cells, and circulating tumor cells. This molecule is interesting for targeted cancer therapy. In recent years single-chain variable fragments (scFvs), have been developed for EpCAM targeting. In this study, scFv against EpCAM extracellular domain (EpEX) was expressed in E. coli k12 strain and Response Surface Methodology (RSM) was employed to obtain optimum culture conditions in a chemically defined minimal medium.

Methods: RSM based on Central Composite Design (CCD) was used to evaluate the effect of four variables (cell density before induction, IPTG concentration, post-induction temperature, and post-induction time) on antiEpEX-scFv production. The experimental data were analyzed by Design-Expert software.

Results: Based on the results of 30 experiments, a quadratic model was developed which was used to illustrate the correlation between the production of antiEpEx-scFv and four independent variables. According to the developed model, the optimum culture conditions of recombinant antiEpEX-scFv expression were induction at cell density 0.8 with 0.8 mM IPTG for 24 h at 37°C. These conditions were applied for the production of antiEpEx-scFv in chemically defined minimal medium leading to a protein yield of 197.33 µg/ml that significantly consistent with the prediction of the model.

Conclusion: The optimized culture conditions obtained here for efficient production of antiEpEX-ScFv in shake flask cultivation on a chemically defined minimal medium could lead to more cost-effective industrial production of recombinant antiEpEx-scFv protein.

Keywords: Response Surface Methodology, single-chain variable fragment, epithelial cell adhesion molecule, Escherichia coli

P-113

Benzo[a]pyrene effects on the telomerase activity of peripheral blood mononuclear cells: an in vitro study.

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Background: Aging at the cellular level is a process of telomere shortening and increment of apoptosis in peripheral blood mononuclear cells (PBMCs). Telomere length is maintained by the activity of telomerase, a specialized RNA Reverse transcriptase enzyme. Benzo[a]pyrene B[a]P, a polycyclic aromatic hydrocarbon, is an environmental pollutant which induces apoptosis. The aim of this study was to investigate the effect of B[a]P exposure on the cell viability and telomerase activity in human PBMCs.

Methods: Whole blood was taken from healthy volunteers and PBMCs isolated by Ficoll-Paque density gradient. The cells were cultured at density of 1×10^6 cells in a 3 Cm^3 plate. After treatment with various concentration of B[a]P (0-10µM) for 72h, cell viability was determined by MTT Assay. Telomerase activities was also determined in PBMCs treated with 1, 2.5 and 5 µM of B[a]P, by Telomeric Repeat Amplification Protocol (TRAP assay). PCR-amplification of RNA element in telomerase enzyme was performed by specific primers (TS, ACX, TSNT and NT). PCR product separated by 12.5% polyacrylamide gel electrophoresis (PAGE), then telomerase activity was analyzed by densitometry of PCR amplified ladder of amplified bands using ImageJ 1.52a software.

Results: Our result showed that B[a]P caused a significant reduction of PBMCs viability in a dose-dependent manner (IC50 = 1.593 ± 0.14µM), but telomerase activity did not change at any concentration used in this study, as compared to the vehicle (DMSO)-treated control cells.

Conclusions: The findings of present study suggest that B[a]P is a cytotoxic agent for PBMCs at low concentration, but it does not alter the telomerase activity even at three-fold higher concentration.

Keywords: Benzo[a]pyrene, Telomerase activity, PBMCs

P-114

Aberrant DNA methylation alteration in the PBMCs exposed to benzo[a]pyrene

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Background: DNA methylation is one of the epigenetic modifications that can alter gene expression. Previous studies suggested that benzo[a] pyrene (B[a]P), an important carcinogenic compound, and developmental toxin might alter DNA methylation (1). In the present study, we investigated the changes in the promoter DNA methylation of several tumor suppressor genes in peripheral blood mononuclear cells (PBMCs) exposed to different concentrations of B[a]P.

Methods: PBMCs from healthy volunteer was isolated by Ficoll density gradient; then cultured in RPMI1640 containing 10% deactivated FBS. PBMCs stimulated by Phytohaemagglutinin (PHA) subsequently treated with various concentrations (1, 2.5, and, 5μM) of B[a]P. Methylation-specific PCR (MSP) was performed to detect DNA methylation status in the promoter region of 8 genes (p16INK4a, p14ARF, COX-2, p21Cip1, P53, MLH1, APC, and hTERT). In this regard, extracted DNA from PBMCs was treated with sodium bisulfite for 16 h, which converts unmethylated cytosine to uracil but leaving 5-methylcytosine (5mC) unaffected. Polymerase chain reaction (PCR) was carried out with a specific primer for methylated and unmethylated DNA to determine methylation status in each concentration.

Results: We found no change in the promoter methylation of any of the above-mentioned genes except for Cyclooxygenase-2 (COX-2). COX-2 promoter was methylated in the untreated control cells, but B[a]P treatment decreased COX-2 methylation at a concentration of 5 μM as compared to the control cells.

Conclusion: Our study demonstrated that COX-2 promoter demethylation may occur due to B[a]P exposure in PBMCs. COX-2 is an enzyme that catalyzes the synthesis of prostaglandins and plays an important role in the inflammation process. Induction of COX-2 promoter demethylation can be considered as a possible toxic effect induced by B[a]P exposure.

Keywords: Benzo[a]pyrene, PBMCs, DNA methylation, Epigenetic

P-115

Evaluation of anti-heat shock protein antibody in HIV-infected patients

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Background: The diagnosis of HIV infection is usually performed by serologic detection of antibodies against HIV proteins. Moreover, HIV infection may facilitate the presence of human complement, so different types of specific antibodies can be detected. Some studies found differences in the detection of IgG antibodies to heat shock proteins (Hsps) between HIV-infected patients and healthy individuals. In this study, we generated the recombinant small Hsps in E.coli to determine whether small Hsps might be used as potential markers in HIV-infected patients.

Methods: At first, the recombinant small Hsps 20 and 27 proteins were expressed in E.coli expression system and purified by affinity chromatography using nickel-nitrilotriacetic acid (Ni-NTA) agarose column under native conditions. Then, serum samples were prepared from HIV-infected patients and healthy individuals. Finally, detection of antibody responses was performed against the recombinant Hsp20 and Hsp27 proteins as potential markers using indirect ELISA.

Results: Our data showed that both recombinant proteins could be successfully purified under native conditions. Furthermore, circulating levels of anti-Hsp27 antibodies were significantly elevated in HIV-infected patients as compared to those in HIV-negative individuals/ healthy individuals. In contrast, no significant differences were observed in the levels of anti-Hsp20 antibodies between these groups.

Conclusion Generally, Hsp27 may have potential value as a diagnostic marker for HIV-1 infections along with p24 antigen as a common method.

Keywords: HIV, Heat shock proteins, Diagnosis, Seroreactivity

P-116

Evaluating Glargine soluble expression using different tags

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Background: Insulin Glargine is a long-acting insulin, used in the management of diabetes. It contains 53 amino acids that is different from human insulin as it contains glycine instead of asparagine in position 21 of the A-chain and by carboxy-terminal extension of B-chain by 2 arginine residues. Insulin Glargine is highly important due to its application. However, producing Glargine is known as a complicated task. For this reason, to achieve soluble Glargine with high yield of production, we used several constructs containing different tags including GB tag (which increases the expression level) and SUMO tag (which increases the solubility). Achieving soluble protein is indeed important as it decreases number of stages involved in the production of a protein.

Methods: To evaluate and determine the best expression condition for GB-Glargine, SUMO Glargine, and Glargine with no tag, these proteins were expressed in LB and M9 medium in different conditions, and different concentrations of Isopropyl β-D-1-thiogalactopyranoside. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the expression difference between Glargine with no tag, GB-Glargine, and SUMO-Glargin proteins.

Results: Results showed that after inducing the expression under different conditions, SDS-PAGE illustrated that in similar conditions, the expression of GB-Glargine is significantly higher in comparison to the Glargine with no tag. Following inducing the expression of SUMO-Glargine, results have displayed that while GB-Glargine and the Glargine with no tag are expressed in the pellet, the soluble protein can be achieved after inducing the SUMO-Glargine protein.

Conclusion: The finding of our study shows that GB-tag enhances the expression of Glargine comparing to the Glagine with no tag. Additionally, we have shown that the SUMO tag increases the expression of the protein in the soluble form. In general, we conclude that the SUMO tag has greatly increased protein expression.

Keywords: insulin glargin, SUMO tag, GB tag, expression

P-117

Identification of stable reference genes in exosomes derived from breast and hepatic cancer cell lines

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Background: Exosomes have been identified as mediators of cell-to-cell communication by transferring bioactive molecules such as nucleic acids, proteins and lipids into recipient cells. Exosomal RNAs have recently been studied as the potential diagnostic marker for various common cancers such as colorectal, hepatocellular carcinoma (HCC), gastric, breast and lung. Real time quantitative polymerase chain reaction (RT-qPCR) is the most widely used approach for gene expression profiling. The accurate data interpretation in RT-qPCR is desperately related to selection appropriate reference genes. GeNorm, NormFinder, BestKeeper and Delta CT algorithms are several commonly used algorithms that help in selecting the best reference genes. Here, the selection of superior reference genes for RT-qPCR in gene expression analysis for exosomal RNAs extracted from several cell lines was firstly investigated.

Methods: Determining the most stable reference genes, GAPDH, 18SrRNA, TBP, YWHAZ and B2M genes were examined as candidate reference genes in exosomes extracted from SK-BR-3, HepG2, PLC and Huh cell lines using GeNorm, NormFinder, BestKeeper and Delta CT algorithms.

Results: Analysis by geNorm program ranked GAPDH as the most stable reference gene, while BestKeeper demonstrated UBC as the most stable one. Moreover, YWHAZ was assessed as the most stable one by Delta CT and NormFinder, implying the importance of the employed algorithm in comparative interpretation of the data.

Conclusion: Cumulative data suggested that GAPDH, YWHAZ, UBC, B2M and 18SrRNA have the highest stability to the lowest one respectively.

Keywords: Reference genes, Exosome, RT-qPCR, GeNorm, NormFinder, BestKeeper

P-118

Investigating the correlation between the rs1800624 and rs1800625 polymorphisms of RAGE gene and nephropathy in patients with type 2 diabetes mellitus

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Background: Interactions of advanced glycation end products with RAGE, as their cellular receptor, is a key point toward the initiation of inflammatory and microvascular outcomes in this group of patients. The present study aimed at evaluating the association between the rs1800625 (-429T/C) and rs1800624 (-374T/A) polymorphisms of RAGE gene promoter and diabetic nephropathy as well as examining the possible application of these polymorphisms as candidate markers of diabetic nephropathy among the population of Qazvin, Iran.

Methods: This was a case/control study to perform SNP genotyping technique in which the diabetic patients were divided into two groups of with or without nephropathy. The frequency of genotype and -429T/C and -374T/A polymorphisms allele were determined using TETRA-Primer ARMS-PCR technique. Hardy-Weinberg equilibrium test and correlation of polymorphisms, odds ratio (OR), and haplotype analysis were performed by FAMHAP software.

Results: Based on our data, the CC genotype of -429T/C polymorphism may play a protective role against the development of nephropathy (OR=0.586, 95%; CI: 0.158-2.167) while the AA genotype may be associated with increased risk of the disease (OR=1.889, 95%; CI: 0.454-7.854). Alleles analysis revealed that the C allele of -429T/C polymorphism maybe protective against the appearance of nephropathy (OR=0.794, 95%; CI: 0.48-1.314) whereas the A allele may be related to increased risk for nephropathy (OR=1.452, 95%; CI: 0.783-2.695). Haplotype analysis demonstrated that there was no significant correlation between the two -429T/C and -374T/A SNPs ($\chi2=5.125$, p value=0.135).

Conclusion: According to our findings, no correlation between the -374T/A and -429T/C polymorphisms and the haplotypes in RAGE gene and the occurrence of diabetic nephropathy among the study population in Qazvin, was established.

Keywords: Diabetes Mellitus, Diabetic Nephropathy, Receptor for Advanced Glycation End Products, polymorphism, Qazvin

P-119

Evaluating Cytotoxicity of Chloroform and Petroleum Ether Fractional Extracts of Galium verum L. in HepG2 and HT29 Cell Lines

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Background: Studies on fractional extracts of Galium verum L. have confirmed their cytotoxicity and anti-cancer effects on different cancer cell lines. The aim of the present study was to investigate the cytotoxic effects of Galium verum extracts on liver and colon cancer cell lines.

Methods: In the present study, colon cancer (HT29) and liver (HepG2) cell lines were randomly divided into control and test groups that were exposed to 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL of the extract. MTT assay was used to evaluate the viability of the cells. The groups were finally compared using GraphPad Prism software (Tukey's Post Hoc Test).

Results: The chloroform fractional extract of Galium verum had cytotoxic effect on HT29 at concentration of 100 and 50 µg/ml, it increased cell viability of HepG2 cancer cells; whereas, the petroleum ether's fraction showed cytotoxic effects on HT29 at all determined concentrations and also on HepG2 at concentration of 3.125 µg/mL. Treatment of HT29 with different concentrations of petroleum ether fractional extract and also HepG2 treatment with the same extract at concentration of 3.125 µg/mL showed a significant decrease in cell viability, compared with the control. IC50 concentration was determined at >100 µg/mL.

Conclusion: The results indicated that the fractional petroleum ether extract had cytotoxic effect on HT29 cancer cells at all concentrations, but it affected HepG2 cancer cells only at concentration of 3.125 µg/mL.

Keywords: Galium verum, MTT assay, HT29, HepG2, Viability

P-120

Biochemical and Histological Effects of Sustanon 250 on Rat Testis

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Background: AAS (anabolic-androgenic steroid) are a large group of artificial derivatives of the testosterone sex hormone, that used in young men to increase muscle mass. Sustanon as an example of AAS drug is an oil-based injectable anabolic– androgenic steroid that consists of four different testosterone esters. It constantly releases testosterone into the bloodstream and produces stability testosterone levels for 3-4 weeks. In the present study, we examined the Histological and biochemical effect of Sustanon on the Testis of Rats.

Methods: Male Wistar rats were divided into 4 groups. The first group (control group) injected intramuscularly with sesame oil, group2, group3 and group4 were injected intramuscularly with different doses of Sustanon 250 of 10, 20, and 30 mg/kg body weight (BW) weekly for 8 weeks respectively. After the treatment period, rats were sacrificed. The blood samples were obtained from the animals by heart puncture. The serum level of FSH was determined. The right testis was removed for histological study.

Results: Testis weight had significantly decreased in Sustanon treated groups, compared to the control group. Plasma FSH concentrations were reduced significantly in treated rats. Histological examination of the testes revealed degeneration in spermatogonia and reduced the number of spermatocytes, spermatids, and sperm in treated groups. Also, the diameter of seminiferous lumen and thickness of the germinal epithelium was significantly reduced.

Conclusion: The use of Sustanon by Athletes for better muscular development is increasing. It is suggested that Sustanon can induce an adverse effect on the male reproductive system and decreased fertility.

Keywords: Sustanon, Infertility, Hormones, Histopathology

P-121

Investigating the effects of Galium verum methanolic extract on the anticancer effect of HT29 colon cancer cells and AGO1522 fibroblast cells

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Background: several studies have reported that gallium verum extract due to rich component like polyphenolic, flavonoieds, terpens interfere in different cellular activity. In this study cytotoxicity and anticancer effect of gallium verum extract investigated on HT29 colon cancer cell line and AGO normal fibroblast cell line.

Methods: MTT assay, flow cytometry and RT-PCR were used for measurement of toxicity, apoptosis and necrosis induction, cell cycle modification and the expression of Bax and Bcl2 genes.

Results: the concentration of IC50 was reported at 400µg/ml in treatment of HT29 and AGO cell lines with different concentration of methanolic extract of Galium verum by MTT assay. Apoptosis induction was observed by increasing ROS production, and increasing cell arresting in the G0 / G1 phase; also decreasing the S phase was observed in colon cancer cells compared to fibroblasts. A further increase in the ratio of BAX/Bcl2 expression was reported in cancer cells compared to normal cells.

Conclusion: Methanolic extract of gallinm verum at concentration 400µg/ml (IC50) caused anticancer effects on colon cancer cells compared to normal cells. However, fibroblasts cells also have shown an increase effect on cell growth at concentration less than 400 µg/ml, so in the future it is recommended that subsequent studies investigate its effects on wound healing.

Keywords: Galium verum , MTT assay, HT29, AGO1522 , Viability

P-122

Bioinformatics data mining of microRNAs regulating the SLC4A4 gene and their association with colon cancer

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Background: MicroRNAs are epigenetic regulators and are important in reducing gene expression values; our aim in this research study was to identify microRNA regulatory SLC4A gene and its relationship to the development of colorectal cancer.

Methods: We first examined the SLC4A4 gene at TargetScan, miRTarbase, miRWalk, DIANA, miRbase, and other databases, and selected miRNAs capable of connecting nucleotides with the SLC4A4 target gene. Then, we shared them through the Venny webtool and prioritized the miRNAs based on the most frequently reported databases. Furthermore, we examined the tissue expression of selected miRNAs at the AtlasTissue database separately. We also identified other target genes at the miRTargetLink database and took a brief look at the signaling pathways associated with the SLC4A4 gene and related miRNAs. Finally, we searched for articles related to these miRNAs to determine the relationship with CRC disease, with the keywords miR-, cancer, in reputable scientific databases such as PubMed, Scopus, etc. and studied them.

Results: Out of 2505 miRNAs extracted from the database, there were 2,262 miRNAs in the miRWalk, 1,055 in the TargetScan, 13 in the DIANA, and 19 in the miRTarBase databases. Of these, 4 miRNAs were shared between miRWalk, TargetScan, miRTarBase, 13 between miRWalk and miRTarBase, and 1 between TargetScan and miRTarBase. Our findings demonstrated that selected microRNAs have a good expression in the colon, and according to our information, these microRNAs can bind to the target gene and can affect its expression. A review of articles in scientific journals related to selected miRs indicated that they were associated with various signaling pathways involved in diseases and some cancers of the gastrointestinal tract.

Conclusion: According to our findings, the association of selected miRs with the SLC4A4 gene in CRC may also be significant, and confirmatory molecular tests should be performed to confirm these findings.

Keywords: miRNAs, SLC4A4, CRC, Bioinformatics

P-123

The Anticancer Efficacy of Platiunm Azidothymidine on Hepatocellular Carcinoma Via Affecting the Telomerase and the Bcl-2 Genes Expression

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Background: The study of correlation between cancer biomarkers after treatment with anticancer drugs would represent a promising insight into the effectiveness of the drug.

Methods: In this study, after induction of hepatocellular carcinoma, rats were divided into four groups; groups A and B as healthy or control group, and negative untreated cancer group respectively, groups C and D were treated with platinum azido-thymidine (0.9 mg/kg/day), a novel anti-cancer drug, and azidothymidine (AZT)(0.3mg/kg/day) respectively. After induction of cancer, the telomerase and Bcl-2 expression were evaluated by real time PCR(RTqPCR), and also Bcl-2 concentration and telomerase activity were measured by enzyme-linked immunosorbent assay (ELISA) and telomerase repeat amplification protocol (TRAP) respectively.

Results: A significant correlation was observed between telomerase and Bcl-2 in untreated HCC induced rats as compared to the control group in untreated cancer group. A direct significant correlation between telomerase activity and expression ($r=0.453$, $p=0.022$) and a negative significant correlation between telomerase activity and Bcl-2 concentration ($r=-0.43$,$p=0.034$) and also between telomerase and Bcl-2 expression ($r=-0.088$,$p=0.006$) was observed. In drug treated groups, there was a significant negative correlation between telomerase expression and Bcl-2 concentration ($r=-0.45$, $p=0.025$) only in the AZT-treated group.

Conclusion: Our results indicated a correlation between cancer factors in untreated cancerous group B and in treated groups only limited to the azidothymidine-treated group (group D). Hence, it may be possible to use this strategy to develop remarkable anticancer drugs in future studies, though this hypothesis requires more in depth research.

Keywords: Bcl-2, Telomerase, Azidothymidine, Platinum azidothymidine, Hepatocellular carcinoma

P-124

Bisphenol A as a carcinogen agent and protective role of Adiponectin

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Background: The human body is constantly exposed to chemical compounds, including Bisphenol A. Bisphenol A (BPA) [4, 4'_dihidroxy_2,2 di phenyl propane] as an estrogenic monomer disrupt endocrine system. Because of similarity to estrogen, it could increase the risk of breast, endometrial, ovarian and prostate cancers in human body. BPA can exert its physiological effects through multiple physiological receptors, including estrogen and androgen receptors. In white adipose tissue, BPA can accumulate and deregulate metabolic homeostasis via inhibition of adiponectin (APN) secretion and induction of proinflammatory IL-6 and TNFα. According to the literature, low concentrations of BPA inhibit APN secretion in human adipocyte through the PPARγ-dependent pathway. APN is involved in energy homeostasis and could improve insulin resistance. APN levels play an important role in protection against carcinogen agents.

Methods: Literature searches using the PubMed, Scopus, Scholar data bases were conducted from 1995 to 2020 using keywords BPA, adiponectin and estrogen receptor. Several studies met the criteria.

Results: BPA can lead to various cancers through several signaling pathways. By reducing the expression of aromatase and estrogen receptors in tumor cells, APN can prevent the development of cancers such as breast, lung, thyroid and colon cancers. Furthermore, APN could exert anti-tumor potential by regulating cell signaling, apoptosis and cell cycle pathways.

Conclusion: It seems induction of APN synthesis could have protection against harmful effects and carcinogenicity of BPA.

Keywords: BPA, Adiponectin, Cancer, Estrogen receptor

P-125

Comparative analysis of host effect on solubility of antiEpCAM single-chain antibody fragment

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Background: EpCAM (epithelial cell adhesion molecule) is one of the first cancer-associated antigens considered as a suitable target for cancer immunotherapy. Due to EpCAM exclusive overexpression in epithelial-derived neoplasms, it can be considered as a suitable target for many solid tumors and cancer stem cells. Advances in genetic engineering techniques could facilitate producing recombinant antibody fragments of various sizes and shapes such as scFvs (single-chain variable fragments). ScFv is a non-immunogenic, small arrangement of the functional recombinant antibody fragment which is made up of a VH and a VL domain of an antibody with high specificity and affinity. Due to the small size, the scFvs are suitable for recombinant expression in E. coli. However, the proteins expressed in large amounts in E. coli tend to form inclusion bodies that need to be refolded which may result in the poor recovery of bioactive proteins. Here, we studied the impact of four E. coli strains on the soluble level of antiEpCAM-scFv protein.

Methods: E. coli SHuffleTM T7, E. coli OrigamiTM (DE3), E. coli RosettaTM (DE3), and E. coli BL21TM (DE3) strains were used as hosts for pET22b (+)-antiEpCAM-scFv expression. The expression of antiEpCAM-scFv was evaluated via the standard SDS-PAGE. The antiEpCAM-scFv expression was also confirmed as a hexahistidine-tagged protein using western blotting.

Results: The data showed that the amount of soluble antiEpCAM-scFv obtained in BL21TM (DE3) (93.96 ± 3.65 mg /L) was significantly higher to those produced in the same condition in E. coli RosettaTM (DE3) (36.21 ± 10.56 mg /L), and SHuffleTM T7 (36.17 ± 3.83 mg /L) strains. Furthermore, the highest volumetric productivity of protein reached 318.29 ± 26.38 mg /L in BL21TM (DE3).

Conclusion: Despite the reducing cytoplasm, BL21TM (DE3) can be a very efficient strain for the soluble production of antiEpCAM-scFv protein.

Keywords: EpCAM, scFv, Solubility, E. coli

P-126

Study of the protective effect of the Elaeagnus angustifolia (Oleaster) aqueous extract on Carbon tetrachloride-induced liver injury in rats

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Background: The purpose of this research was to explore the beneficial effects of an aqueous extract of Elaeagnus Angustifolia (Oleaster) on liver damage caused by carbon tetrachloride (CCl_4). Oleaster has active polyphenolic compounds, flavonoids, and polar glycosides which may induce antioxidant and anti-inflammatory properties.

Methods: In this study, 28 rats were divided into four groups: control, CCl_4 for liver injury, and groups obtaining Oleaster aqueous extract with doses of 100 mg/kg and 400 mg/kg. after the 28-day intervention, hepatic damage was induced with CCl_4 and 72 hours later blood was taken from rats, and analysis of biochemical markers including AST, ALT, Urea, and Creatinine as well as antioxidant and antioxidant stress indicators including FRAP and MDA was performed on serum samples and FRAP and MDA were performed on urine samples.

Results: We found that in the groups receiving Oleaster extract, the ALT and AST liver markers were significantly decreased, and this decrease was more significant in the group receiving 100 doses than in 400. The Urea and Creatinine markers also had significant reductions as compared to the injury group in the groups treated with Oleaster. The results of serum antioxidant indicators of the groups receiving Oleaster extract in the FRAP test were significantly increased compared to the Injury group, and in the MDA test, a significant decrease compared to the Injury group was observed only at a dose of 400. In the urine sample, the MDA test was significantly reduced in the treated groups compared to the injury group.

Conclusion: Based on these findings, Oleaster aqueous extract may have a beneficial effect against liver damage and it suggests that these benefits are introduced by improving the antioxidant mechanism and reducing oxidative stress, according to the findings of oxidative stress measures.

Keywords: Oleaster aqueous extract, CCl_4, Liver injury

P-127

Stabilization of G quadruplex DNA of Bcl2 promoter via isoquinoline alkaloids

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Background: Today, DNA is becoming an interesting target for cancer drug designing. Among all the structures that DNA can have, G-quadruplex is the most interested one. It presents at chromosomal extremities, in promoter of oncogenes and untranslated regions (UTR) of several important genes. Once it formed cellular processes like transcription, translation and replication can be hindered. There is a huge interest in drug design to develop small molecules which can bind and stabilize such G-quadruplex structures. The human Bcl2 gene includes a 39bp GC-rich region upstream of the P1 promoter that is involved in the regulation of Bcl2 gene expression.

Methods: Based on these findings, Bcl2 oligonucleotides and its complementary oligonucleotide were synthesized. In order to understanding the interactions between ligands and the G-quadruplex structure, FRET and t-FRET methods were carried out in presence of some isoquinoline alkaloids.

Results: A qualitative analysis of FRET method data reveals a concentration dependent increasing in the melting temperature (Tm) of G-rich sequences of Bcl2 promoter in presence of palmatine. Also t-FRET method data showed its preferential binding to the folded form of G-rich strand when compared with the related double strand DNA.

Conclusion: Isoquinoline alkaloids are attractive compounds for G quadruplex structure stabilization. Recognition of efficient compounds for selective stabilizing quadruplex structures may open novel aspects in drug development.

Keywords: G quadruplex, Alkaloid, FRET and t-FRET methods, Palmatine

P-128

Urinary KIM-1 as a potential biomarker in high salt diet induced CKD

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Background: Chronic kidney disease (CKD), describes the gradual loss of kidney function. 10% of the population worldwide is affected by CKD. In its early stages, symptoms of CKD are usually not apparent. Significant reduction of the kidney function is the first obvious sign of disease. If diagnosed early (stages 1 to 3), the progression of CKD can be altered and complications reduced. In stages 4 and 5 extensive kidney damage is observed, which usually results in end-stage renal failure.

Methods: High NaCl diet is known as one of the most important risk factors in development of CKD. NaCl is the major determinant of osmolarity of extracellular fluid. High NaCl is known to induce osmotic stress in renal medullary epithelial cells. Osmotic stress may also act on the genome via a direct biophysical pathway and induce endoplasmic reticulum (ER) stress. ER stress initiates apoptotic signaling pathways. During ER stress, three death-inducing signals are generated. The first triggers the transcriptional induction of CHOP/GADD153, the second is mediated through phosphorylation of the translation initiation factor eIF-2, and the third involves activation of caspases.

Results: During apoptosis, the cell membrane is destroyed. Kidney injury molecule-1 (KIM-1) is mainly expressed in the apical membrane of tubular epithelial kidney cells after kidney injury. The extracellular domain ok KIM-1 is shed from the cell surface into extracellular space by a metalloproteinase-dependent process from injured kidney, which releases the soluble KIM-1 and it is excreted in the urine.

Conclusion: Based on recent studies, urinary KIM-1 can be a useful biomarker in diagnosis of CKD in early stages.

Keywords: CKD, High salt diet, ER stress, Apoptosis, KIM-1

P-129

Circulating mRNA and serum levels of osteoprotegerin and receptor activator of NF-κB ligand in non-alcoholic fatty liver disease

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Background: Pathogenesis of the beginning and progression of non-alcoholic fatty liver disease (NAFLD) has not been clarified exactly. The osteoprotegerin (OPG)/ receptor activator of NF-κB ligand (RANKL) axis seems to play an imperative function in the onset and progression of this disease. The goal of the present study was to investigate the PBMC expression and plasma levels of RANKL and OPG cytokines in NAFLD subjects and compare them with healthy group.

Methods: Plasma levels of OPG and RANKL were determined with ELISA kits in 57 men with NAFLD, and 25 healthy men as controls. Biochemical and anthropometric parameters tests were evaluated in the study groups. RANKL and OPG mRNA content was evaluated by quantitative RT-PCR.

Results: OPG contents were markedly decreased in NAFLD patients as compared with healthy subjects [1.43 (1.05-5.45)] vs [2.94 (1.76-4.73)] ng/ml; P=0.007). The level of RANKL were significantly reduced in NAFLD patients [74.00 (56.26-203.52) ng/ml] than in healthy subjects [119.37 (83.71-150.13) ng/ml]; (P=0.03). In addition, OPG and RANKL gene expression in significantly decreased in NAFLD patients in comparison to control group (P <0.05). Moreover, ROC curve indicated that OPG might has a good capability to discriminate between NAFLD patients and normal subjects. A positive correlation was observed between OPG and RANKL in serum sample (r = 0.495) (P = 0.000).

Conclusion: Decreased plasma levels and gene expression of RANKL and OPG cytokines in NAFLD patients indicate that there is a relationship between these cytokines and the pathology of NAFLD disease. Confirmation of this association as well as the mechanism and role of these cytokines in NAFLD require further studies.

Keywords: Receptor activator of nuclear factor-kB ligand, Osteoprotegerin, Non-alcoholic fatty liver disease, Gene expression

P-130

MiR-122 as a hepatic differentiator: How it can compete with admissible growth factors based protocols

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Background: MicroRNAs (miRs) as endogenous gene regulators can act as powerful differentiation tools. As each tissue has its specific miR signature, so they can be used for inducing differentiation in stem cells. While using growth factors as differentiator agent is costly, time consuming, and not permanent, miRs potentially could be good substitute for them. MiR-122 is the most abundant and specific miR in the liver that play an important role in liver development. Here we have shown that how miR-122 can compete with growth factors for inducing hepatic differentiation in induced pluripotent stem cells (iPSCs).

Methods: iPSCs were transduced with lentiviruses containing miR-122 and scrambled as negative control; another group was treated with growth factors according to the protocols. After miRs expression evaluation for making sure of efficient transduction, hepatic differentiation was evaluated by analyzing some biochemical markers. Albumin and urea secretion were measured by photometric methods, and glycogen deposition was detected by Periodic acid–Schiff staining (PAS).

Results: It was shown that miR-122 upregulation can make hepatic differentiation more effectively than standard utilized growth factors. Albumin production was significantly higher in miR-122 group compare with growth factors group ($P<0.001$), and urea secretion had the same pattern ($P<0.01$). Positive staining was detected for glycogen depositions in both group. All three markers were undetectable in scrambled group.

Conclusion: Altogether these contents indicate that miR-122 can induce hepatic differentiation in iPSCs like standard growth factors, even more effective, so it could be considered as a good candidate for embedment costly and time consuming growth factor differentiation based protocols.

Keywords: Growth factor, Hepatic differentiation, iPSCs, miR-122

P-131

Regulatory Potential of Jujube Gold Nanoparticle on Keap1 gene in Nrf2 / Keap1 pathway in Diabetic Rats

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Background: Oxidative stress due to hyperglycemia plays an important role in the development of diabetes complications. The key role in controlling diabetes complications has been identified. In recent years, activation of this pathway by AUNPs has been considered as a therapeutic strategy. The aim of this study was to investigate the role of gold nanoparticles synthesized by jujube extract on the expression of Nrf2 and Keap1 genes in the liver of Streptozotocin (STZ)-induced diabetic rats.

Methods: Twenty male Sprague-Dawley rats were randomly divided into 4 groups of 5 each; normal, diabetic (induced with STZ: 55 mg/kg), diabetic rats + AuNPs doses (0.5, mg/kg b.w), diabetic rats + AuNPs doses (1, mg/kg b.w). At the end of treatment (three weeks), liver tissues were collected from all rats. The Nrf2 and Keap1 genes expression were determined by QRT-PCR.

Results: Results of QRT-PCR showed that the expression of hepatic Nrf2 was significantly decreased while, hepatic Keap1 gene showed increase in expression ($p < 0.05$). Treatment of diabetic rats with jujube gold nanoparticles doses (1 mg/kg b.w) for 3 weeks significantly reduced the relative expression of keap1 gene in comparison with diabetic group ($p < 0.05$). In the event that it had no significant effect on the relative expression of the Nrf2 gene ($p > 0.05$).

Conclusions: The results of this study suggest that activation of the Nrf2 / Keap1 antioxidant pathway by gold-jujube nanoparticles may be involved in reducing the complications of diabetes. In molecular level, it seems jujube gold nanoparticle has notable effect on Keap1 gene as a regulator of Nrf2 gene.

Keywords: Diabetes mellitus ،Gold-jujube nanoparticles ،Nrf2 ،Keap1

P-132

Effect of proline and glycine on some biochemical parameter of recombinant urate oxidase from Aspergillus flavus

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Background: Urate oxidase with a high specificity toward uric acid is usually needed in large quantities for medical uses including the analysis of urine or human serum for uric acid and as a protein drug to reduce toxic urate accumulation. Due to the instability of the urate oxidase to temperature, various methods, including the use of osmolytes, have been used to increase the stability of this enzyme. In this study, the effect of two osmolyte such as proline and glycine was investigated on optimum temperature, optimum pH, Km and Vmax of recombinant urate oxidase from Aspergillus flavus.

Methods: The coding sequence of recombinant uricase, was cloned, expressed in E. coli BL21, and purified by Ni–NTA agarose affinity chromatography. Uricase activity in the presence and absence of proline and glycine was determined by measuring the decrease in absorbance at 293 nm resulting from the oxidation of uric acid to allantoin.

Results: Only in the presence of proline, temperature and optima pH of recombinant urate oxidase increased and decreased, respectively. The kinetic parameters of Km and Vmax of the enzyme were reduced in the presence of both additives.

Conclusion: The results of this study suggest that among used additives, proline had the most stabilization effect on recombinant urate oxidase.

Keywords: Aspergillus flavus, Urate oxidase, kinetic parameters, Proline, Glycine

P-133

Evaluation of the combined effect of Quercetin Nano drug and Doxorubicinin on MDA-MB231 cell lines in breast cancer treatment

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Background: Breast cancer is the most common death in women worldwide. Doxorubicin is commonly used to treat cancer and as an anti-cancer drug in chemotherapy. Quercetin with flavonoid compounds found in fruits and vegetables that here was used as the anti-cancer agent in combination with doxorubicin on breast cancer cell line.

Methods: Nano quercetin was first prepared from a combination of corticosteroids in a microfluidic instrument, and DLS was used to characterize nanostructure. The AO/ PI test was then performed to confirm the LC50 we obtained from other articles. Flow cytometry apoptosis analysis was used to find cell death.

Results: The survival rate of MDA-MB-231 cancer cells treated with a combination of quercetin and nano quercetin with doxorubicin was significantly decreased in comparison with doxorubicin lonely. We detected a similar result for apoptosis assay. The apoptosis rate of MAD-MB-231 cells under treatment with quercetin, nano quercetin, and doxorubicin increased when compared to the control cell that was 67.4, 63.5, and 66 percent, respectively.

Conclusion: A combination of quercetin, nano quercetin, and doxorubicin on MDA-MB231 breast cancer cells showed significantly reduced cell viability and increase apoptosis. This study illustrated the sensitized role of quercetin, nano quercetin in cancer cell toxicity when used chemotherapy like doxorubicin.

Keywords: Breast Cancer, Quercetin Nano drug, Doxorubicin, Drug Resistance

P-134

Evaluation of gene expression levels of Aquaporine 3 and Caspase 3 accompany with chromatin damage in the infertile men with asthenozoospermia

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Background Asthenozoospermia is characterized when total sperm motility is less than 40% or the sperm progressive motility is below 32% in semen. Some studies showed that differences in gene expression levels of the aquaporin (AQP) channels in human sperm may appear to be associated with infertility. Aquaporin 3 (AQP3) channel is a mediator in volume homeostasis, water, and energy transferring in the sperm tails. This study aimed to evaluate the expression levels of AQP3 and caspase 3 (CASP3) genes and chromatin damage in the infertile men with asthenozoospermia in comparison with normal individuals.

Methods: Thirty-five Asthenozoospermia and 35 fertile individuals, participated in this study. Semen samples were collected in sterile containers after 2-4 days of sexual abstinence. The expression of AQP3 and CASP3 genes in the sperm of individuals assessed with qPCR technique. The sperm chromatin structure assay (SCSA) test was applied for assessment of chromatin damage using flow-cytometry technique.

Results: The mean of AQP3 gene expression levels in asthenozoospermia significantly lesser than normal individuals. In contrast, the CASP3 gene expression levels and SCSA test in the infertile men with Asthenozospermic were significantly higher than the normal group.

Conclusion: These results may reflect the hypothesis that increasing the CASP3 gene may degrade the AQP3 structure and its activity following by reducing the available glycerol for energy production, which can eventually decrease sperm motility. Meanwhile, the SCSA test may forecast suitable information about the status of AQP3 and CASP3 genes expressions in Asthenozoospermia, indirectly.

Keywords: Aquaporin, Caspase, Asthenozoospermia, Sperm, Motility, SCSA

P-135

Synergistic antioxidative effects of ceftriaxone and resveratrol against pyelonephritis-induced oxidative damage

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Background: Pyelonephritis is the E. coli infection of the kidney that affects more than 250,000 cases and causes 100,000 hospitalizations each year. The present study was conducted to assess the protective effects of resveratrol or in combination with ceftriaxone on pyelonephritis induced- oxidative stress in the kidney and serum samples of male rats.

Methods: 30 male rats were divided into five groups of six. The rats in group 1 were undergone surgery with no infection induction. Group 2, 3, 4, and 5 were undergone surgery and exposed to bacterial infection. Groups 1, as healthy control, and 2, as pyelonephritic animals, were treated with normal saline for a week. Groups 3 and 4 received ceftriaxone at the dose of 50 mg/kg and resveratrol at the dose of 10 mg/kg, respectively, for a week. Group 5 was treated with the combination of ceftriaxone (50 mg/kg) and resveratrol (10 mg/kg) for a week. Pyelonephritis was induced by injecting 0.3 ml of bacterial cell suspension into three sites in the kidneys. After 24 h, the disease was confirmed and the treatments were started in the next day. At the end of treatments, the serum samples and kidney tissues were collected to measure the serum activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) as well as the levels of malondialdehyde (MDA) in the kidneys.

Results: Ceftriaxone or resveratrol-treated rats had lower renal MDA levels and higher GPx and SOD serum activities compared to the pyelonephritis group ($P < 0.001$). Interestingly, the combination of resveratrol and ceftriaxone had more significant effects on the reduction of the MDA level and the induction of GPx and SOD activities.

Conclusion: Our data suggested the antioxidative activities of ceftriaxone or resveratrol and their synergism in alleviating pyelonephritis- induced oxidative stress.

Keywords: Pyelonephritis, Oxidative stress, Malondialdehyde, Glutathione peroxidase, Superoxide dismutase

P-136

Niosomal Virosome derived by vesicular stomatitis virus glycoprotein as a new gene carrier: The first report

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Background: Virosomes as membranous vesicles with viral fusion protein in their membrane are versatile vehicles for cargo delivery. The vesicular stomatitis virus glycoprotein (VSV-G) is a common fusogenic protein used in virosome preparation. This glycoprotein has been used in liposomal systems so far, but in this study, we have tried to use the niosomal form instead of liposome for the first time. Niosomes are vesicular systems composed of non-ionic surfactants.

Methods: Niosomes were constructed by the thin-film hydration method. VSV-G gene in pMD2.G plasmid was expressed in the HEK293T cell line and then was reconstituted in the niosome bilayer. The formation of niosomal virosomes was confirmed with different methods such as SDS-PAGE gel, western blotting, and transmission electron microscopy (TEM). The efficiency of niosomal virosome was investigated with the pmCherry reporter gene.

Results: SDS-PAGE and western blotting proved the expression and successful insertion of protein into the bilayer. The TEM images showed the spike projection of VSV-G on the surface of niosomes. The transfection results showed high efficiency of niosomal virosomes as a novel carrier.

Conclusion: This is the first report that has verified that niosome could be used as an efficient bilayer instead of liposome to construct virosomes.

Keywords: vector, VSV-G, gene delivery, niosome, virosome, protein reconstitution

P-137

Monitoring of XIAP-BIR2 and Caspase3 Interaction by Split-Luciferase complementary assay

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Background: Apoptosis is an essential mechanism for the removal of damaged and unnecessary cells under the control of many activating and inhibiting proteins. An imbalance between the activation or inhibition of death pathways and cell survival leads to the loss of homeostasis and the consequence of various illnesses. For this reason, it is important to study this process. XIAP and Caspase3 are two important proteins that play a regulatory role in the apoptosis pathway. The purpose of this study was to investigate the interaction of the BIR2 domain from XIAP with Caspase3 using a split-luciferase assay.

Methods: In this study, two constructs were designed and then amplified by PCR and ligated in pET-28a (+). The expression of both constructs was performed in E. coli Bl21(DE3) and purification process carried out with the affinity chromatography. Protein purity and concentration were determined by SDS-PAGE and Bradford. Luciferase activity was measured by a luminometer.

Results: The result of sequencing showed that the BIR2 domain from XIAP connected to N-Luc and Caspase3 connected to C-Luc split fragments of luciferase. The expression of constructs was optimized in the condition of 2XYT medium at 20 °C for 16 h with IPTG 0.5 mM. Luciferase activity of purified constructs showed that they interacted with each other.

Conclusion: Cloning of both constructs was confirmed by double digestion and sequencing. The activity of luciferase showed they impinged on each other, but inhibitor compounds and/or mutations are necessary to confirm it in the futures.

Keywords: XIAP-BIR2, Caspase3, Split-Luciferase, Cloning.

P-138

Impact of obesity in mitochondrial and endoplasmic reticulum dysfunction: relationship with the aging process.

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Background: Under the obesity condition, the production of reactive oxygen species (ROS) increases which is more than capable of an antioxidant system that ultimately disrupts the hemostasis of mitochondrial energy and function. ROS plays as a local messenger between mitochondria and the endoplasmic reticulum (ER). Oxidative stress disrupts the synthesis of proteins and homeostasis of the ER, which mutually harms mitochondrial function. The inefficiency of these two organelles reduces beta-cells capacity and Insulin resistance creates. The mitochondrion is an important organ to cell aging. With cell aging, more ROS is produced, ROS exacerbates ER stress, and the UPR creates a set of signals that induces the aging process. Interestingly with aging, conversion of preadipocytes to mature adipocytes, and the ability of adipose tissue to store free fatty acids, is reduced which causes the prevalence of metabolic syndrome at old age.

Method: We reviewed a number of articles from PubMed and Scopus databases to investigate the relationship between obesity and signaling pathways created in both mitochondrial and ER organelle.

Result: In obesity, mitochondria of hypertrophied adipocytes are an important source of ROS, and impair WAT function by altering the production of adipokine. Studies show, which Signals that activate sequentially under ER stress and mitochondrial dysfunction, reduce adiponectin (adipokine that is mainly produced in adipose). Adiponectin regulates glucose levels and the breakdown of fatty acids. Moreover, improves ROS production as a second messenger, as well as neutralizes excessive accumulation of oxygen products, thus inhibiting the production of intracellular oxidative stress which induces the aging process

Conclusion: Obesity and the ROS production in mitochondria and mutually its effect on the endoplasmic reticulum is associated with a decrease in adiponectin, which induces aging.

Keywords: Reactive oxygen species, Endoplasmic Reticulum, Mitochondria, aging, obesity

P-139

The Study of Association between Fibroblastic Growth Factor 21 48758426G>T Polymorphism, its Plasma Concentration, and Chitotriosidase Activity with Coronary Artery Disease

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Background: Cardiovascular disease (CVD) is the first leading cause of disease-related mortality in developed and developing countries. Coronary artery disease (CAD) is the most common cardiovascular disease. Various factors including lifestyle and genetics are involved in the etiology of this disease. It is also associated with activation of the immune system and inflammatory processes. Fibroblast growth factors (FGFs) are important factors in regulating the metabolism of carbohydrates and lipids, and their various polymorphisms have been studied in a variety of diseases. The chitotriosidase enzyme, a chitinase, is secreted by macrophages and is an indicator of the activity of these cells.

Methods: Angiography was performed in Kermanshah's Imam Ali hospital and people were divided into two groups of patients and control based on their angiography results. Polymorphism was determined by the PCR-RFLP method, FGF21 concentration was measured by ELISA, Chitotriosidase activity assay was measured by the fluorimetric method. Statistical analysis was performed using SPSS 16.0 software.

Results: No association was found between polymorphism rs11665896 and coronary artery disease or the severity of the disease (p=0.386). The activity of the chitotriosidase in the patient group was statistically higher than the control group, respectively 17.98±17.25 and 13.73±14.55 (nmol/h/ml of plasma, Mean±SD, p=0.022). The concentration of FGF21 protein in the plasma of both control and patient groups was measured 331.43±320.67 and 263.50±198.98 (pg/ml), respectively, which has no difference (p=0.352).

Conclusion: Polymorphism rs11665896 has nothing to do with the occurrence or severity of coronary artery disease, although larger and more genetically diverse populations should be considered. The activity of the chitotriosidase as an indicator of macrophage activity was higher in the patient group because of the activation of the immune system. The FGF21 protein, which is involved in the metabolism of lipids and carbohydrates, was not associated with the incidence or severity of coronary artery disease in the subjects.

Keywords: Coronary Artery Disease, Fibroblast Growth Factor 21, Chitotriosidase, rs11665896 Polymorphism.

P-140

Effect of Vitamin D3 on serum lipid profile and HbA1C levels in type 2 diabetes mellitus: a randomized controlled trial

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Background: Epidemiological studies show that low levels of vitamin D3 have related to decreased insulin sensitivity and increased risk of type 2 diabetes (T2DM). Aims: This study looks at whether taking vitamin D3 (Vit D3) can be effective in controlling serum glucose levels and lipid profile in T2DM.

Methods: The study was designed as a randomized controlled trial. Participants included 64 people with T2DM [hemoglobin A1C (HbA1c) > 6.5(, and 25- hydroxyvitamin D levels less than 30 ng / ml]. They were randomly divided into two groups: placebo (n= 32), and intervention (n= 30). The intervention group received a Vit D3 supplementation (50,000 IU per week) for 8 weeks. Serum glucose levels, HbA1c, and lipid profiles were evaluated using an auto analyzer.

Results: Between-group analysis showed that levels of 25 hydroxyvitamin D significantly increased in the intervention group compared to the placebo group (40.05 ± 9.69 ng/mL vs. 20.07 ± 6.79ng/mL, P <0.001). Vit D3 decreased the serum levels of HbA1C, and FBS in the intervention group compared with the placebo group, although the differences were not significant [HbA1C; (7.94 ± 1.48 % vs. 7.79 ± 1.52 %) (P=0.697), and FBS, (154.116 ± 53.75vs. 135.84 ± 46.97 P=0.159)]. In addition, total cholesterol (TC), and LDL-c levels slightly decreased in the intervention group compared to the placebo group [TC; (155.90 ± 29.53 vs.157.96 ± 32.37) (P=0.844), and LDL-c; (82.90 ± 22.69 vs.88.34±30.60) (P=0.428)].

Conclusions: The study showed that vitamin D3 was able to reduce serum levels of TC, LDL-C, FBS, and HbA1C in patients with T2D, but changes between groups were not significant. Likely, the effect of vitamin D3 on improving hyperglycemia and reducing the lipid levels would increase with increasing intervention time and increasing sample size.

Keywords: Vit D3 supplementation, HbA1c, lipid profile

P-141

microRNA-141 is associated with hepatic steatosis by downregulating the sirtuin1/AMP-activated protein kinase pathway in hepatocytes.

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Background: MicroRNAs (miRNAs) are a class of small non-coding RNAs that function as critical gene regulators by targeting mRNAs. Sirtuin 1 (SIRT1) and its activators have been shown to play an important role in the pathophysiology of various metabolic diseases including non-alcoholic fatty liver disease (NAFLD). This study aimed to investigate the effect of miR-141 on SIRT1 and AMPK and lipid accumulation in HepG2 cells.

Methods: Liver hepatocellular cells (HepG2) were treated with a high concentration of glucose to be subsequently used for the assessment of miR-141 and SIRT1 levels in a model of hepatic steatosis. On the other hand, cells were transfected with miR-141 to investigate its effect on hepatocyte steatosis and viability as well as SIRT1 expression and activity along with AMPK phosphorylation. The targeting of SIRT1 by miR-141 was evaluated by bioinformatics tools and confirmed by luciferase reporter assay.

Results: The expression of miR-141 was upregulated while SIRT1 was downregulated as a result of the transfection of HepG2 cells with miR-141. SIRT1 protein level, as well as AMPK phosphorylation, was decreased due to the overexpression of miR-141 The activity of SIRT1 and cell viability was also declined in cells transfected with miR-141mimic. Intracellular lipid was increased as a result of the transfection of HepG2 cells with miR-141. The results of luciferase reporter assay verified SIRT1 to be directly targeted by miR-141. miR-141 could effectively suppress SIRT1 and lead to decreased AMPK phosphorylation in HepG2 cells.

Conclusion: miR-141/SIRT1/AMPK signaling pathway may be considered a potential target for the therapeutic management of NAFLD.

Keywords: miR-141, SIRT1, AMPK, liver, Non-alcoholic fatty liver disease

P-142

Pharmacophore and QSAR Studies to Design Novel Tumor necrosis factor Inhibitors

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Background: TNF (Tumor necrosis factor) is a key cytokine in regulator of the inflammatory such as Crohn’s disease, rheumatoid arthritis, ankylosing spondylitis, and autoimmune response. TNF demonstrates to modify multiply signaling pathways with wild-ranging downstream effects.

Methods: In this investigation, pharmacophore-based, QSAR modeling, and virtual screening strategy were developed to explore new direct inhibitors of TNF by Schrodinger module and molecular docking. One pharmacophore model and three quantitative structure-activity relationship models were developed on a known series of TNF inhibitors.

Results and Conclusion: The goodness of the hit score value of the best pharmacophore model was -8.8, which indicated that it is reliable to be used for virtual screening. The built pharmacophore model was used to search the zinc 15 database. Based on the QSAR results and detailed binding mode analyses with docking simulation two compounds (ZINC000005839581) and (ZINC000004214419) had the best score and passed the Lipinski's rule and Lead-likeness. We hope that this in silico study could serve as lead compounds for the development of novel treatments for inflammatory and autoimmune diseases.

Keywords: Tumor necrosis factor, QSAR Studies, molecular docking, Drug Discovery

P-143

Investigating the effect of natural deep eutectic solvents on lactate dehydrogenase activity and stability

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Background: Lactate dehydrogenase (LDH; NAD oxidoreductase, E.C 1.1.1.28), an important enzyme that plays a critical role in the final step of glycolysis, has been expressed as a recombinant enzyme because of its importance in the industry. Nonetheless, the low stability of LDH limits the industrial application. Several methods have been suggested to overcome this issue such as applying additives, protein engineering, and chemical modifications. Studies have shown that natural deep eutectic solvents (NADES) such as Glycerol-Betaine (GlyB) and Glycerol-Choline Chloride (GlyC) increase the stability of several enzymes and can decrease thermal denaturation. Here, we investigated the effect of GlyB and GlyC on LDH conformation in addition to its thermal stability and activity.

Methods: GlyB and GlyC were obtained by thermal mixing procedure. To investigate the effect of GlyB and GlyC on thermal stability and activity of LDH, UV visible spectroscopy was applied. Fluorescence spectroscopy was used to further examine the conformational alteration of LDH in the absence and presence of NADES.

Results: Succeeding NADES preparation using the thermal mixing procedure, a significant difference in thermal stability and kinetics parameters in the absence and presence of each NADES was observed. Moreover, GlyB and GlyC were seen to affect the conformation of LDH.

Conclusion: GlyB and GlyC affect the conformation, thermal stability, and activity of LDH, which can be considered for storage stability.

Keywords: Lactate dehydrogenase, Deep eutectic solvents, Stability, Activity

P-144

Soluble expression of enterokinase light chain using DSB fusion tag

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Background: Enterokinase light chain is an important enzyme that recognizes (Asp)4-Lys sequence. Fusion systems have been designed to increase soluble protein. As the enterokinase light chain has disulfide bonds, the native form is aggregated in cytoplasmic space and cannot be folded correctly. At the presence of the DSBA (disulfide bond) sequence in the design of the enterokinase gene, the protein is transferred to the periplasmic space, in which the disulfide bond is formed and the protein can be folded correctly. Here, we investigated the effect of the DSB tag on enterokinase solubilization.

Methods to obtain the enterokinase gene, the PCR technique was performed using proper primers to remove the DSB sequence from recombinant fusion enterokinase. The amplicon and DSB-enterokinase construct then were subjected to double digestion using NdeI and XhoI. Digested products were ligated using T4 DNA ligase and then were transformed into E. coli BL21 using heat-shock method. To evaluate and determine the best expression condition for both constructs, each protein was expressed in different concentrations of Isopropyl-β-D-1-thiogalactopyranoside and lactose and various temperatures and time to find the optimal condition for expression. Cold osmotic shock method was used to isolate DSB-enterokinase from periplasmic space. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the soluble expression of enterokinase and DSB-enterokinase.

Results Enterokinase containing DSB tag was previously available in pET-21. To delete the DSB tag, PCR was successfully performed, which resulted in the generation of enterokinase. Double digestion was conducted successfully on enterokinase construct and amplicon using NdeI and XhoI. SDS-PAGE illustrated a difference between the soluble expression of enterokinase and DSB-enterokinase, which will be discussed.

Conclusion A significant difference was observed between the soluble expression of enterokinase and DSB-enterokinase.

Keywords: Enterokinase, Escherichia coli, Solubility

P-145

Efficacy of Curcumin on Serum Levels of Adipokines in Patients with Non-Alcoholic Fatty Liver Disease: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Background: One of the chronic liver disorders is a non-alcoholic fatty liver disease (NAFLD), which is associated with changes in adipokines levels, and insulin resistance. Aims: The purpose of the current research was to evaluate the effect of phytosomal curcumin on the serum adiponectin and leptin levels in patients with NAFLD.

Methods: For this randomized double-blind, placebo-controlled experiment, 65 eligible patients were distributed randomly to groups of curcumin and placebo recipients using a blocked randomized technique. Weight, height, body mass index (BMI), fasting blood sugar (FBS), lipid profile, aspartate aminotransferase (AST), alanine aminotransferase (ALT), adiponectin, leptin, and the leptin: adiponectin ratio were assessed at the baseline, and eight weeks after intervention.

Results and Conclusion: NAFLD was associated with reduced adiponectin levels, and increased levels of leptin. Phytochemical curcumin was able to significantly increase adiponectin levels and decrease leptin levels, with a decline in the leptin: adiponectin ratio. Curcumin efficacy can be increased with the use of higher doses of this substance over the long term.

Keywords: NAFLD ,leptin,adiponectin, curcumin

P-146

Evaluation of FBS Substitution with Platelet Rich Plasma and Adding Vitamin C to the Culture Medium on Human Limbal Stem Cell Proliferation

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Background: Limbal stem cell deficiency (LSCD) is one of the most common diseases of the cornea which is characterized by the loss or deficiency of the stem cells in the limbus area that are vital for the re-population of the corneal epithelium and could lead to blindness. Corneal epithelial cells fall out naturally, but in LSCD there is no substitute for the lost cells. LSC therapy is one of the newest and most effective ways to treat LSCD. The current study aims to optimize the process of LSC culture by Substitution of FBS with Platelet-Rich Plasma (PRP) and adding vitamin C to the culture medium.

Methods: Eye samples were collected from the Khatamolanbya Hospital affiliated with the Mashhad University of Medical Sciences, Iran. After Limbal dissection, Limbal tissue was cultured by explants culture method on the amniotic membrane in the presence of (PRP) or fetal bovine serum (FBS). The proliferation of limbal stem cells was evaluated by immunofluorescent (P63 marker) and resazurin assay. Also, the effect of vitamin C on LSC proliferation was investigated. Graphpad Prism software was used to analyze the results.

Results: LSCs proliferations mean in PRP and FBS culture medium were 1.6×106 and 7×105, respectively. The addition of vitamin C to the culture medium results in an increased proliferation rate by 50% in 200µM culture media.

Conclusion: Our results showed that FBS can be replaced by PRP. Also, we showed that vitamin C has a beneficial effect on cell proliferation. PRP and vitamin C can be used in a cell culture medium for LSC therapy purposes in LSCD patients

Keywords: Limbal stem cells, LSCD, Vitamin C, Platelet-Rich Plasma, FBS

P-147

Adjuvant therapy with Quercetin and Vitamin C: A mutual interplay between antioxidant enzymes suppressing and ROS production in prostate cancer cells

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Background: Induction of oxidative stress in cancer cells activates apoptotic pathways in the cells and leads to cell death. One of the proper approaches to induce oxidative stress in cancer cells is inhibiting the expression and reducing the levels of antioxidant enzymes. Various studies have shown that natural compounds have great potential for suppressing antioxidant enzymes in cancer cells. In this study, the effects of treatment with vitamin C (VC) and Quercetin (Q) on antioxidant enzymes activity in prostate cancer cell lines (PC3 and DU145) were evaluated.

Methods: Cell viability in the presence and absence of treatment with Q and VC was assessed by MTT assay. 2'-7'-dichlorodihydrofluorescein diacetate fluorescent was used as a probe to assess the generation of intracellular reactive oxygen species (ROS). The activities of antioxidant enzymes including glutathione peroxidase (GPx) and glutathione reductase (GR) were determined by spectrophotometric methods. All data were analyzed by GraphPad Prism software version 8.0.2.

Results: The IC50 values for VC and Q were 260-370 µM and 140-190 µM respectively for DU145 and PC3 cells and dose 100 µM VC + 75 µM Q was selected. After the treatment of PC3 cells with VC and Q, the activity of GPx and GR enzymes decreased significantly to compare with the control. The intracellular ROS level also increased significantly. While the same treatment of DU145 cells reduced the activity of GR enzyme and increased the level of intracellular ROS although these increments were not significant.

Conclusion: These findings indicate that the treatment of these two prostate cancer cells with VC and Q might have beneficial effects as an anticancer approach however needs further studies in the future.

Keywords: Vitamin C, Quercetin, prostate cancer, GPx, GR

P-148

Dialogue between Klotho and microRNAs and cell signaling pathway in cancers

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Background: Developing data manifested that Klotho as a circulating factor can control the function of manifold signaling pathways such as Wnt. The anti-tumor ability of this secreted protein has been described in miscellaneous malignancies including lung cancer, cervical cancer, and melanoma. Besides, some lines of evidence implied that the dialogue between microRNAs (miRNAs) and Klotho partakes in different cancers pathogenesis like gastric cancer and colorectal cancer. Therefore, this review article was aimed to investigate the crosstalk of Klotho with the Wnt signaling and miRNAs in various cancers.

Methods: Fifty articles were reviewed from 2008 to 2018 in PubMed and Google scholar.

Results: Abnormal activation of the Wnt cascade has been reported in many cancers. Interestingly, increased expression of Klotho performs an inhibitory role in the activation of Wnt cascade in the different malignancies. Klotho is the target gene of a number of miRNAs. The interplay of miRNAs and Klotho plays a serious impact on the pathogenesis of cancer. In addition, the relationship between miRNAs and Klotho-associated pathways has a noticeable role in cancer-related processes.

Conclusion: Klotho as an anti-tumor factor takes part in the cancers pathophysiology via the regulation of key pathways like Wnt. Also, miRNAs play as the oncogenic miRNAs or tumor suppressor miRNAs in cancers through targeting Klotho. The possible link between Klotho, miRNAs, and signaling pathways proposes that this protein as a tumor suppressor may display an encouraging therapeutic approach in cancer.

Keywords: Klotho, cancer, microRNAs, signaling pathways

P-149

Insilico derived synthetic MMP-9 and the reactive antibody towards the native protein

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Background: Matrix metalloproteinases (MMPs) like MMP-9 is an important factor in the pathogenesis of dry eye disease and causes extracellular matrix degradation and pain in DED. Pro-MMP-9 consists of propeptide as a truncated domain, fibronectin domain, catalytical domain, uniquely a linker, and hemopexin domain. We confirmed that continuous peptide "……. FPFTFLGKEYST" predicted by using epitope mapping in silico study can stimulate the rabbit, s immune system reactive towards the whole protein.

Methods: The protein sequence (FASTA) of MMP-9 was obtained using UniProt Knowledgebase at http://www.uniprot.org. For determining the best region of MMP-9 containing B cell epitopes IEDB server was routinely used at http://tools.iedb.org/bcell/. The epitopic synthesized peptide was injected into a female New Zealand white rabbit of 8 weeks old for preparing polyclonal antibodies. The titers of antibody were measured using the indirect ELISA method and then were purified by affinity chromatography column Protein-A.

Results: The polyclonal antibody against synthetic epitopic peptide can react towards the whole native protein of MMP-9.

Conclusion: The epitopic sequence in fibronectin domain confirmed by IEDB database) can immunize the rabbit serum, so that the prepared rabbit polyclonal antibody can react towards the whole native protein of MMP-9 in high affinity.

Keywords: MMP-9, polyclonal antibody

P-150

Cloning, expression, and purification of Brain-derived neurotrophic factor (BDNF) using N-His SUMO vector in Escherichia coli

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Background: Growth factors are polypeptides or proteins that regulate many cellular functions, including survival, proliferation, migration, differentiation regulating neuronal survival, determining cell fate, and establishing proper connectivity. In non-neuronal cells, growth factors stimulate proliferation. On the other hand, mature neurons are postmitotic and cannot re-enter the cell cycle. Brain-derived neurotrophic factor (BDNF), the second member of the “neurotrophic” family. The most important functions of BDNF include developmental processes, regulation of neuron, synaptogenesis, neuroprotection, and control of short and long-lasting synaptic interactions that influence mechanisms of memory and cognition. According to the aforementioned importance, we decided to clone, express, and purify this protein.

Methods: After codon optimization and gene synthesis using the SUMO tag, the optimized BDNF gene was subcloned into the expression vector, pET21, and transformed into E.coli BL21.The SUMO- BDNF expression was optimized in Luria-Bertani (LB) at a different temperature, IPTG concentration, growth medium, and time. The protein purity was assayed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Results: BDNF-SUMO gene was digested and cloned into BamH1 and Xho1 restriction site of an expression vector (pET21). The highest protein expression level was determined with 1 mM of IPTG, at 25°C for 18h of incubation. The expressed protein was purified by Ni-agarose affinity chromatography and was illustrated by SDS-PAGE.

Conclusion: In this study we have subclone and then expressed SUMO- BDNF protein in E. Coli strain BL21 and the purified protein then was achieved by Ni-agarose affinity chromatography.

Keywords: Brain-Derived Neurotrophic Factor, Escherichia coli, SUMO-1 Protein

P-151

Natural deep eutectic solvents affect the stability and activity of malate dehydrogenase

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Background: Malate dehydrogenases (MDH, L-malate: NAD oxidoreductase, E.C.1.1.1.37), catalyzes the NAD+ /NADH-dependent interconversion of the substrate's malate and oxaloacetate. MDH plays a crucial role in the tricarboxylic acid cycle. This enzyme is used in the aspartate transaminase diagnostic kit that is used in the diagnosis of liver disease. Due to its importance, it was decided to produce and stabilize this enzyme. Protein instability is one of the main limitations in this regard. The use of stabilizing additives, protein engineering, and chemical modification of enzymes are common strategies to overcome this problem. It has been proven that NADES (natural deep eutectic solvents) such as Glycerol-Betaine (GB) and Glycerol-Choline Chloride (GC) increase thermal stability and they also have an effect on the structure. Consequently, it was decided to investigate the effect of these two additives on thermal stability and activity, as well as the structure of malate dehydrogenase.

Methods: GB and GC were achieved by thermal mixing procedure. To evaluate the effect of GB and GC on the activity as well as thermal stability and conformational changes, UV visible spectroscopy and fluorescence spectroscopy were used, respectively.

Results: Following the NADES preparation using the thermal mixing procedure, in the presence and absence of additives, significant changes were seen in the structure, kinetic parameters, and thermal stability of malate dehydrogenase.

Conclusion: As a result of this study, it was found that GB and GC affect the activity, thermal stability, and structure. Hence, they can be used in storage stability.

Keywords: Malate dehydrogenase, Deep eutectic solvents, Stability, Activity

P-152

Reducing the effect of insulin resistance on alpha-synuclein gene expression in skeletal muscle

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Background: Alpha-synuclein (SNCA) as the presynaptic protein is expressed in different tissues and prevents insulin-resistance (IR) through increasing glucose-uptake by adipocytes and muscles. However, the effect of insulin metabolism on SNCA expression has scarcely elucidated. In the present study, we assessed the probable effect of insulin resistance on SNCA expression in muscle C2C12 cells and also skeletal muscle tissues of type 2 diabetic mice.

Methods: Sixteen male C57BL/6 mice were divided into two experimental groups, including control and type 2 diabetic mice with IR (induced by high-fat diet + low-dose streptozotocin). The animals of the study involved the measurements of fasting blood glucose, oral-glucose-tolerance-test, as well as fasting plasma insulin. Moreover, insulin-resistant and insulin-sensitive muscle C2C12 cells were prepared. The insulin-resistance was confirmed by the glucose-uptake assay. Comparative quantitative real-time PCR was used to assess the SNCA expression.

Results: The obtained results have shown a significant ~ 27% decrease in SNCA expression level in muscle tissue of diabetic mice ($P = 0.022$). Moreover, there was a significant change of SNCA expression in insulin-resistant C2C12 cells ($P < 0.001$).

Conclusion: Type 2 diabetes due to insulin-resistance can decrease SNCA gene expression in muscles. In addition to the role of SNCA in cell susceptibility to insulin and glucose uptake, the SNCA expression can also be affected by insulin metabolism.

Keywords: Insulin resistance, Alpha-synuclein, Muscle tissue, C2C12 muscle cells, Type 2 diabetes

P-153

Study of changes in blood factors in pumpkin seeds exposed rats

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Background: This study aims to investigate blood factors, including glucose, cholesterol, triglycerides, HDL, LDL, albumin, and total protein in rats, which were directly exposed to pumpkin seeds.

Methods: The study was performed on 72 rats with four groups in three repetitions and each repetition contains 6 male rats in 60 days period. During the first week, all groups used a basic diet to adapt to the environment and the experimental diets were then given to the groups daily for 9 weeks. The first group received orally the basic ration; the second group of them includes 0.1 g/kg weight of pumpkin seed in the diet. The third group includes 0.3 g/kg weight of pumpkin seed in the diet, and the fourth group includes 0/6 g/kg weight of pumpkin seed in the diet. At the end of the eighth week of the experiment, after 12 hours of food deprivation, 6 rats were randomly selected from each group and blood samples were taken from them. Glucose, cholesterol, triglycerides, total protein, albumin, HDL, and LDL levels were determined in the laboratory.

Results: The results showed that the effect of pumpkin seeds on glucose, cholesterol, triglycerides, and LDL concentration was significantly reduced ($p<0.05$) and HDL concentration has increased significantly ($P<0.05$), But it had no significant effect on total protein and albumin concentrations. The use of 0.1 g/kg experimental ration had the highest reduction in glucose concentration, but the 0.6 g/kg ration had the highest reduction in cholesterol, triglycerides, and LDL concentrations, in HDL the 0.6 g/kg has also the highest increase.

Conclusion: Based on the results, it can be said that the use of pumpkin seeds due to its reducing power in glucose, cholesterol, triglycerides, and LDL can be achieved for people who have high blood sugar and have diabetes or high blood fats.

Keywords: Pumpkin seed, Blood factors, Rats.

P-154

Evaluation of the effect of curcumin extract on doxorubicin-induced cardiotoxicity

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Background: chemotherapy one of the most widely used treatment strategies for cancer. Of course, this treatment strategy faces major challenges. Doxorubicin is an effective chemotherapeutic agent used to treat various cancers. However, several studies have shown that the use of doxorubicin in therapeutic concentrations is associated with dangerous side effects, such as cardiac toxicity. Currently, the use of medicinal plants in combination with chemotherapeutic agents to reduce side effects is a new approach that diverse studies have shown promising results.

Methods: We examined here the effect of hydroalcoholic curcumin extract on doxorubicin-induced cardiotoxicity in rats and H9c2 cell line. The in vitro and the in vivo models were established by doxorubicin. MTT assay, total antioxidant capacity (TAC), total oxidant capacity (TOC), the activity of catalase (CAT), Malondialdehyde (MDA), LDH, CK, reactive oxygen (ROS), DNA damage, and apoptosis condition were measured in H9C2 cell line in the presence and absence of curcumin. And TAC, TOC, CAT activity, MDA, LDH, CK, and ROS were assessed in an animal model of cardiotoxicity in the presence and absence of curcumin.

Results: Our results showed a significant increase in cell viability, TAC, and CAT activity in presence of curcumin in comparison with the absence of curcumin (p= 0.04; p= 0.001; p= 0.01, respectively) in the H9C2 cell line. And TOC, MDA, LDH, CK, ROS, cleaved caspase 3, and DNA damage was statistically decreased in the presence of curcumin rather than absent of curcumin in the cell line. Further, results revealed that TOC, MDA, ROS, LDH, and CK levels significantly decreased in rats that were treated with curcumin in comparison with the control group (p=0.001, p=0.04, p=0.01, p=0.002, and p=0.01, respectively).

Conclusion: Our data indicate that the hydroalcoholic extract of curcumin extract attenuated doxorubicin-induced cardiotoxicity in rat and H9c2 cell line by decreasing oxidant parameters as well as increasing antioxidant parameters.

Keywords: Doxorubicin, Cardiotoxicity, Comet assay, oxidative stress

P-155

Selenium and Selenoproteins implications in breast cancer patients

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Background: Epidemiologic evidence supports an inverse relationship between selenium levels and cancer mortalities. Selenium is incorporated into Selenoproteins, such as S and P. Selenoprotein P (Sepp) serves in homeostasis and distribution of selenium through the body. Selenoprotein S (SelS) involves the reduction of endoplasmic reticulum stress and protects cells from oxidative damage. The aim of our study was analyzing of Se, selenoprotein S, and P roles in breast cancer.

Methods: our study considered 60 tissue specimens (30 tumor and 30 tumor margin tissues), and 30 blood serum from primary breast cancer patients. Tissue samples were homogenized, and the supernatant was collected. All selenium measurements were carried out using an atomic absorption spectrometer, and selenoproteins were measured by the ELISA method.

Results: SEPP and SelS levels were lower in tumoral tissue, and there was no association between serum and tumoral tissue level, but there was a noticeable correlation between serum and tumor margin levels of selenoproteins. Selenoprotein S and P were associated entirely with plasma, and there was a meaningful correlation among selenium and selenoproteins range in both serum and tumor margin samples. Also, Se accumulation in tumor tissues was significantly higher than tumor margins.

Conclusion: Selenoprotein P is associated with selenoprotein S level in serum but not tissues. Selenoproteins ranges reduce in tumoral tissue. Selenium accumulation in 1 region plays an essential role in the development of tumoral disorders, and the difference in serum and tissue samples indicates tissue accumulation happens regardless of serum concentration.

Keywords: Breast cancer, selenium, selenoprotein S, selenoprotein P

P-156

Exposure to 4-methylimidazole as a food pollutant induces neurobehavioral toxicity in mother and developmental impairments in the offspring

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Background: 4-methylimidazole (4MI) is a toxin that is first found in ammoniated forage. Spectrum widely exposures and applications of this compound in industry and food products caused concern developmental health. The goal of this study was the assessment of histological and behavioral changes made by 4MI in maternal and fetus using behavioral tests and H&E staining.

Methods: In the present study doses of 4MI (100,200,300 mg/kg) given orally to pregnant mice from gestational day (GD) 1 to GD14; then on the day 15, Behavioral experiments including (open field, passive avoidance) were used to assess changes, memory, anxiety in pregnant mice. After behavioral testing fetuses were dissected on day 15 of gestation and morphological and histological studies on the fetus and brain pregnant mice were carried out using hematoxylin-eosin staining.

Results and Conclusion: Our findings showed that 4MI can induce neurotoxicity in pregnancy mice by abnormalities in the dentate gyrus of the hippocampus and disrupt neurobehavioral functions. Also in the fetus, 4MI produced significant changes in tissues of the brain, liver, uterus, and thymus of treatment groups compared with the control group. Conclusion: 4MI can cause developmental and behavioral toxicity in both maternal and fetus.

Keywords: 4-Methylimidazole, Developmental toxicity, Neurobehavioral toxicity, Abortion, Embryo-fetal toxicity

P-157

Evaluation of the effect of hydroalcoholic extract of Achillea wilhelmsii on the rate of cellular death and expression of four important genes in the HIIPO signaling pathway in A549 non-small cell lung cancer cell line

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Background: Nowadays the use of plant compounds for prevention, control, and treatment of different cancers has received increasing attention. Achillea wilhelmsii is a medicinal plant that has been widely used for treatment and alleviation of different digestive, vascular, and neurological illnesses. The present study aimed to evaluate the effect of the hydroalcoholic extract of this plant on the survival and mRNA expression of LATS1 and LATS2 tumor suppressor genes and TAZ and YAP1 oncogenes associated with the Hippo signaling pathway in the A549 lung cancer cell line.

Methods: After preparation of the hydroalcoholic extract of A.W, its effects on the A549 lung cancer cell line survival and mRNA expression of LATS1, LATS2, TAZ, and YAP1 genes were assessed and was compared to the control group using MTT and RT-PCR, respectively.

Results: The survival rate was decreased significantly after 24 hours treatment with hydroalcoholic extract of A.W at concentrations of 200 and 1000 µg/ml and after 48 hours treatment at a concentration of 100-1000 µg/ml. The mRNA expression of LATS1 and LATS2 did not change while the expression of TAZ and YAP1 decreased significantly as compared to the control group.

Conclusion: The results of this study showed that the hydroalcoholic extract of A.W decreased the expression of TAZ and YAP1 oncogenes in the A549 lung cancer cell line.

Keywords: Lung cancer, Hydroalcoholic extract of A.W, MMT, HIPPO pathway, A549 cell line

P-158

Protective effects of silymarin on paraquat-induced nephrotoxicity in rats

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Background: Paraquat is a quaternary nitrogen herbicide which induces kidney toxicity due to producing oxidative stress. We have investigated the potential protective effects of silymarin on paraquat-induced renal toxicity.

Methods: Twenty-four male rats were divided into six groups, group 1, control group; group 2, rats that received paraquat only (25 mg/kg b.w./day, PO); groups 3, were treated with paraquat (25 mg/kg b.w./day, PO) and silymarin (50 mg/kg b.w./day, PO). Then, the serum and tissue parameters of the oxidative stress and renal histopathological changes were examined.

Results: In group 2 which received paraquat only, a remarkable increase ($P<0.05$) was observed in the serum creatinine, urea, malondialdehyde, protein carbonyl, and TNF-α. Also, there was a significant decrease in renal superoxide dismutase, catalase, the ferric reducing ability of plasma and vitamin C in the second group. Oral administration of silymarin significantly decreased serum urea, creatinine, protein carbonyl, malondialdehyde, and TNF-α as well as renal histopathological changes.

Conclusion: The present study suggests silymarin has nephroprotective effects against nephrotoxicity caused by paraquat.

Keywords: Silymarin, paraquat, kidney injury, oxidative stress, TNF-α

P-159

Association of FOXE1 gene expression with the development of thyroid neoplasm in Iranian population

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Background: Thyroid neoplasm is rare cancer with a growing rate of incidence worldwide. As one of the most important genetic factors of thyroid gland growth and development, FOXE1 gene is a key regulator of follicular cells' proliferation and differentiation. The main goal of this study was the assessment of FOXE1 mRNA expression in neoplastic thyroid tissues in the Iranian population.

Methods: Bioinformatics analysis of FOXE1 gene via GeneCards, STRING, and KEGG databases were done. Forty thyroid neoplastic tissues and their adjacent normal tissues were collected from Sina hospital, Isfahan, Iran. Normal and neoplastic tissues were confirmed by an expert pathologist. RNA extraction was followed by cDNA synthesis and genomic DNA contamination was overcome using DNaseI treatment. Specific primers were designed for RT-qPCR gene amplification. REST software would be used for statistical analysis. Bioinformatics analysis of FOXE1 gene declared its potential role in the development of thyroid neoplasm. RT-qPCR specific primers were specific and unable to make primer dimers and hairpins. The purified RNAs were detected with the least proteins and DNA contamination. RT-qPCR triplicates were with the least standard deviation.

Results: Analysis of the first 10 samples showed an up-regulation of FOXE1, but it was not significant ($p=0.1$). The role of FOXE1 as a sensitive gene in thyroid cancer was established before and extensive studies confirmed the association of the two SNPs (rs955113 and rs1867277) with papillary thyroid cancer and follicular thyroid cancer. FOXE1 is inversely correlated with the differentiation stage of tumors, therefore anaplastic tumors showed less expression of FOXE1 mRNA.

Conclusion: In this study, we would determine the expression levels of FOXE1 gene in malignant and neighboring healthy tissues to investigate the role of the gene in thyroid neoplasm development. It would be suggested that the study be performed on larger populations to express the result more accurately.

Keywords: Bioinformatics, RT-qPCR, papillary, follicular

P-160

The early diagnosis of gastrointestinal cancers by fingerprint patterns

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Background: Gastrointestinal cancers are malignant with a high mortality rate. Early diagnosis of patients could improve the results of treatment.

Methods: This study assessed the association between gastrointestinal cancers and particular fingerprint patterns, using the ink method, for early diagnosis of these malignancies. The study was conducted on 153 gastrointestinal cancer patients and 299 healthy persons.

Results: Dermatoglyphics analysis showed that whorl and loop patterns significantly changed in the cases group as compared to control. However, the odds ratio suggested that whorl pattern in 6 or more fingers might be a risk factor for developing gastrointestinal cancers, and so may aid in the early diagnosis of these cancers.

Conclusion: To conclude, we showed that the whorl and loop patterns significantly changed in gastrointestinal cancer patients as compared to control healthy people. However, our results showed that only a whorl pattern might be a risk factor for developing gastrointestinal cancer. These findings could prove the association between dermatoglyphic patterns and gastrointestinal cancers. Shortly, the dermatoglyphic analysis can be used as a biological marker in the early diagnosis of malignancies such as gastrointestinal cancers.

Keywords: Dermatoglyphic pattern, gastrointestinal cancer, Uterine cancer

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Functional profiling of circulating extracellular vesicle-free microRNAs reveals the prostate cancer diagnostically important miRs

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Background: MicroRNAs are interesting as cancer diagnostic and prognostic biomarkers because of their unique expression profile in each tissue, higher stability in blood than mRNAs, and reliable quantification. In the case of prostate cancer (PCa), it is currently emphasized to explore new biomarkers, particularly the microRNAs available in the bloodstream in free form.

Methods: In this study, a repository of microarray data for PCa circulating extracellular vesicle-free microRNA profiling in Gene Expression Omnibus was searched for differentially expressed miRNAs (DE-miRs) based on expression fold changes. The microRNA-gene networks were constructed for the sets of positively or negatively regulated miRNAs. Gene ontology annotations for target genes were also extracted and analyzed.

Results: Human miR-1587, miR-223-3p, miR-3125 and miR-642b-3p were highly significant DE-miRs in PCa. Human miR-4459, miR-1273g, miR 642a-3p and miR-642b-3p were hubs in relevant miRNA-gene networks. FOXK1, PML, CD24, ATN1, BAZ2A, CDKN1A, NUFIP2, and HARNPU were miR target genes with significant dysregulation.

Conclusion: By emphasizing the diagnostic potential, miR-4459, miR-1273g-3p, miR-3135b, miR-5001-5p, and miR-1587 were proposed as novel microRNAs with the capability to be used as significant biomarkers for detection of PCa using circulating vesicle-free miRNAs.

Keywords: Prostate Cancer, Diagnosis, Biomarker, Vesicle-free microRNA, Gene ontology

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Phyto-chemistry; Ever-growing insights on interdisciplinary collaborations with pharmacology and Medicine in the Era of Viral Pandemics

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Background: Phytochemistry combines the basics of chemistry and botany with the discovery of natural plant products with commercial value in various industries such as traditional and modern complementary biological and medicinal sciences, pharmaceuticals, nutrients, and dietary supplements which may result in major effective activities against viral and bacterial pandemics. Aims: Our investigation developed to evaluate the multipotent opportunities and value of combinatory researches between different scientific disciplines like chemistry, medicine, and pharmacology and to overcome the world pandemics and their health and social consequences, and to propose new insights in realizing a practical network.

Methods: The paper makes a statistical brief at functions of phytochemical knowledge in the form of scientometrics findings reviews in the era of the huge new viral pandemics which showed potential solutions for semi-solved issues in the case of worldwide problems.

Results and Conclusion: There are ultimate potentials to develop vast frame on basic and applied interdisciplinary researches between medicine, pharmacology, and phytochemistry which ends in practical suggestions for improving social health status through scientific collaboration in issues such as developing new herbal antiseptics against surface lipoproteins of microorganism capsid, enhancing human immune system resistance through prescribing herbal immune-boosting materials and the new generation of antibiotic drugs against the micro-organism surface epitopes and or genetic DNA or RNA materials inside its nucleus utilizing viro-static herbal components.

Keywords: Phyto-chemistry, Viral Pandemics, Scientometrics

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Curcumin-Crocetin Loaded Nanoparticles in Combination with 17-AAG Exerts Cytotoxic Effects and Upregulates caspase 9 Gene Expression Level in Colorectal Cancer cells

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Background; we aimed to evaluate the possible anti-cancerous impacts of curcumin-crocetin-loaded nanoparticles combined with 17-AAG in colorectal cancer cells. HCT-116 colorectal cancer cells exposed with, or without, various concentrations of synthesized nanoparticle and 17AAG and cell inhibitory effects were assessed.

Methods; The cellular viability of low concentrations of 17-AAG with two concentrations of curcumin-crocetin loaded nanoparticles lower than IC50 were examined. The cytotoxicity induction was evaluated by MTT assay. Likewise, the relative gene expression level of caspase 9 was evaluated by Real-Time PCR.

Results; Low concentrations of 17-AAG in combined with curcumin-crocetin loaded nanoparticles at lower doses than IC50 of both agents induces cell toxicity and inhibited the HCT-116 cell growth. Also, a significant decrease in cell viability was seen in all double combinations versus monotherapies. Certainly, curcumin-crocetin loaded nanoparticles increases the effects of 17-AAG and act as chemosensitizing agent. In addition, combination treatments displayed increase the caspase 9 gene expression ratio is compared with single agents. In double treatments, caspase 9 mRNA was upregulated in comparison to single treatments of 17-AAG and curcumin-crocetin loaded nanoparticles.

Conclusion; Overall it could be concluded that very low concentrations of 17-AAG when combined with curcumin-crocetin loaded nanoparticles tend to exert anti-cancerous impacts.

Keywords: nanoparticle, caspase 9, colorectal cancer

P-164

Study on Repositioning of Spironolactone Drug on Human Glioblastoma U87MG Cells

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Background: Glioblastoma cancer stem cells (GCSCs) are one of the major reasons for resistance to treatment and tumorigenesis in glioblastoma cancer by inducing a double-stranded DNA break (DSB) repair mechanism. Recently researchers showed Spironolactone, an old FDA-approved drug that's clinically applied in heart failure and hypertension therapy, caused cancer stem cell death by inducing DSB. in this study we aimed to assess the cytotoxicity effect of spironolactone on human U87MG glioblastoma cells.

Methods: Human glioblastoma cell line (U87MG) was cultured. The spironolactone gradient concentrations have been applied to assess the cytotoxicity effect in dose-answer dependent manner. Absorbance was measured by using ELISA Plate reader stat FAX 303 in at 570 nm. Obtained results were analyzed by Spss, v21 software, LSD test.

Results: Results revealed that the Spironolactone could be inhibited U87MG glioblastoma cells growth in 30 μM and 50 μM concentrations at 72h. also, in 30 μM concentration at 48h the spironolactone have been growth inhibitory effect on U87MG cells. results were significantly confirmed in a p-value less than 0.05.

Conclusion: Hence, probably spironolactone could be a newcandidate for repurposed as an efficient drug in glioblastoma treatment by effecting on GCSCs growth inhibition. The spironolactone inhibitory function may via DSB repair mechanism in GCSCs inhibition. Nevertheless, required more investigation to approval.

Keywords: DNA Double-Strand Break, Cancer Stem Cell, Drug repositioning, drug resistance

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Evaluation of the Effect of 6-shogaol on the Expression of Insig1, in Acute Lymphoblastic Leukemia cell line

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Background: Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. Our group has recently shown that ginger has strong anti-cancer activity against ALL cells. Moreover, it is reported that 6-shogaol (6Sh), a derivative of ginger, may bind to PPARγ in cancerous cells and triggers its transcriptional activity. This project aims to evaluate the effect of 6-shogaol on the mRNA expression level of Insig1, the gene targeted by PPARγ, in B-ALL cell line, Nalm-6, in order to investigate the mechanism by which it may inhibit leukemic cells.

Methods: 2 × 104 Nalm-6 cell line was treated with 6-shogaol (200µM) or solvent (0.4% DMSO) for 96h, and cell viability was determined using MTT assay. Furthermore, 12h after treatment with 200µM 6Sh or 0.4% DMSO, total RNA was extracted using TRIzol reagent and cDNA was synthesized. The effect of 6-shogaol on the expression of Insig1 gene was assessed by the Real-time PCR technique. Briefly, 1 µl (4ng/ µl) of the synthesized cDNA was added to 9 µl of the master mix solution (including 0.5 µl of each primer, 4.7µl SYBR green Master mix, and 3.3 µl Rnase free water). Each sample was analyzed and normalized to the level of the housekeeping gene (GAPDH). The threshold cycle values of the samples were calculated, and the gene transcription levels were analyzed using the 2−ΔΔCt method.

Results: It was demonstrated, for the first time, that 6-shogaol had a significantly higher growth inhibitory effect on the 6Sh treated cells than their relative controls [47.82 ± 0.99% vs 100%, respectively). Moreover, the expression level of Insig1 was increased in cells treated with 6-shogaol compared with the vehicle-treated cells by 1.55±0.43 fold.

Conclusion: It is postulated that 6-shogaol may play its role against leukemic cells by increasing the expression levels of Insig1 gene.

Keywords: Acute lymphoblastic leukemia, 6-Shogaol, PPARγ, Insig1

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Investigation of 3T3-L1 cell differentiation to adipocyte, affected by aqueous seed extract of Phoenix dactylifera L.

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Background: Obesity can result in dyslipidemia and hyperglycemia, which are associated with insulin resistance and type 2 diabetes. Reports about hypoglycemic and hypolipidemic properties of Phoenix dactylifera L. seed extract are available in the STZ-induced diabetic rat model, but there have been no studies to assess the anti-diabetic effect in other diabetic models. This study aimed to find out the possible effects of Phoenix dactylifera L. seed on adipogenesis and glucose homeostasis.

Methods: 3T3-L1 cells were cultured in adipocyte differentiation media with or without the treatment of different Phoenix dactylifera L. Seed extract (0.312-1 mg/ml). The assays were performed at 5, 8, and 12 days after induction of differentiation.

Results: Analysis of results showed that TG contents of treated groups were significantly low compared to control. The results indicated that the treatment of 3T3-L1 cells with Phoenix dactylifera L. seed extract can reduce adipogenesis via down-regulation of PPAR-γ and CEBP-α, and some of the adipocyte-specific genes involved in fatty acid metabolisms such as ap2, ACACA and FAS.

Conclusion: Phoenix dactylifera L. seed has the potential to inhibit adipogenesis and it might be able to inhibit obesity. This is the first study that exhibits the inhibitory effect of phoenix doctylifera L. seed extract on adipogenesis of 3T3-L1 cells

Keywords: adipocyte differentiation, adipogenesis, glucose homeostasis, obesity, Phoenix dactylifera seed, 3T3-L1 cell

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Phenotypic and Genotypic Detection of AmpC Enzymes (MOX and CIT) in Clinical Isolates of Escherichia coli

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Background: AmpC β-lactamase enzymes cause multi-drug resistance. The diagnosis of these enzymes in clinical isolates of bacteria is important in epidemiological, research Studies and hospital infections control because plasmid genes can be transmitted to other organisms in the hospitals The purpose of this study is the detection of AmpC enzymes (MOX and CIT) in the isolated strains of E. coli in three educational hospitals in Hamadan (Iran).

Methods: 102 strains of E. coli and were isolated from the three hospitals in Hamadan from March to September 2017. The isolated gram-negative bacteria are identified by using common biochemical tests. Antibiotic sensitivity of isolated strains was studied by the standard disk diffusion method and based on CLSI protocol. To detection of phenotypic AmpC activity, the AmpC detection disks were used. The clinical isolates with MIC ³ 8 µg/ml for cefoxitin were included for evaluation by AmpC diagnosis disks. To detect genes encoding for AmpC PCR method was used.

Results: Sixty-eight isolates (66.6%) were resistant to third-generation cephalosporin and of these 61 (59.8%) isolates had MIC ³ 8 µg/mL to cefoxitin. All 68 isolates were analyzed by AmpC detection disks, of which 10(14.7%) isolates were AmpC- β-lactamase producers. By PCR method, 24 (35.2%) isolates had cit, 46(67.6%) for mox genes.

Conclusion: High resistance to cephalosporins has been observed among the clinical isolates. Due to the possibility of plasmid transferring of ampC genes between bacteria, changing consumption patterns of antibiotics and the treatment protocol is necessary. The results of this study suggested that physicians should pay attention while prescribing antibiotics and send the sample to the laboratory for the antibiogram tests so that the best medical choice is given to the patients. The emergence of plasmid-mediated AmpC and ESBL β-lactamase producing E. coli and attitude possible risk to the spread of antibiotic resistance in clinical situations.

Keywords: Escherichia coli, AmpC, Multi-Drug Resistance, β- Lactamase

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The molecular dynamic role of metformin in the induction of apoptosis and cancer treatment

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Background: Metformin, a biguanide drug, is widely used as the first-line pharmacologic treatment of type II diabetes. To date, there is increasing evidence suggesting that metformin can inhibit cancer cell growth and induce apoptosis. Bad, Bak, and Bim are three major factors that mediate apoptosis induction in cells. This study investigated the effect of metformin on Bad, Bak, and Bim pro-apoptotic factors using docking and molecular dynamics simulation.

Methods: Information about the three-dimensional structure and Protein Data Bank (PDB) files of three pro-apoptotic factors, Bad Bak and Bim, were obtained from the www.rcsb.org database, and then optimization, simulation, molecular binding, and molecular dynamic calculations were performed using AutoDock v.4.2, VMD v.1.9.2, and Gromacs v.4.5.4 software.

Results: The present study showed that metformin binds to three pro-apoptotic factors with a high tendency. This binding occurred through hydrogen and hydrophobic bonds between the 5, 4, and 8 residues with the binding energy of -5.56, -5.70, and -4.93 kJ/mol at the Bad, Bak, and Bim binding sites, respectively. Docking of metformin to Bad, Bak, and Bim decreased Total Energy, increased Radius of gyration, and induced the conformational changes in the secondary structure of these pro-apoptotic factors.

Conclusion: Due to the high tendency of metformin to interact with Bad, Bak, and Bim pro-apoptotic factors, it can affect their molecular dynamics and increase their conformational changes which subsequently can lead to dimerization and activation of apoptosis.

Keywords: Apoptosis, metformin, pro-apoptotic factors, Simulation

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Macrophage migration inhibitory factor as a potential biomarker of COVID-19 progression

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Background: Coronavirus disease 2019 (COVID-19) has reached a universal pandemic and poses a great risk to public health. Acute lung injury caused by covid-19 virus triggers an immune response against infection and subsequent activation of multiple signaling pathways leads to enormous production of inflammatory proteins such as macrophage inhibitory factor (MIF). This protein activates pro-inflammatory cytokine secretion (TNF alpha and IL-8) and overrides the anti-inflammatory effects of glucocorticoids in a concentration-related fashion. The present study aims to investigate the diagnostic and prognostic value of MIF in Patients with covid-19 infection by determining serum levels of MIF.

Methods: Totally 60 patients with Covid-19 infection and 30 healthy control were enrolled in the study. The patient group consisted of 30 COVID-infected individuals with advanced disease and 30 cases without any related symptoms. The determination of serum concentration of MIF protein was done by the ELISA kit (Zellbio) in accordance with the manufacturer's instructions.

Results: Our data revealed that serum levels of MIF were significantly higher in COVID-19 infected individuals particularly in patients with the severe acute respiratory syndrome (SARS) ($P < 0.05$). By contrast, healthy subjects showed no changes in normal blood concentrations of MIF protein.

Conclusion: In conclusion, we demonstrated that serum levels of MIF increase during COVID-19 infection which may provide valuable diagnostic and prognostic information validating a potential biomarker. Additionally, developing anti-MIF strategies may represent a new therapeutic approach in inflammatory diseases like SARS.

Keywords: COVID-19, MIF, Immune response

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A novel blood-based biomarker for evaluation of the severity of respiratory failure caused by COVID-19

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Background: Coronavirus disease 2019 (COVID-19) has reached a universal pandemic and poses a great risk to public health. Acute lung injury caused by the COVID-19 virus triggers an immune response against infection and subsequent activation of multiple signaling pathways leads to the enormous production of surfactant protein D (SP-D). This multimeric protein is involved in innate immune responses in the lungs and various non-pulmonary organs as well as modulation of host cell responses against pathogens via multiple cellular receptors. The present study aims to investigate the diagnostic and prognostic value of SP-D in Patients with COVID-19 infection by determining serum levels of SP-D.

Methods: Totally 60 patients with COVID-19 infection and 30 healthy control were enrolled in the study. The patient group consisted of 30 COVID-infected individuals with advanced disease and 30 cases without any related symptoms. The determination of serum concentration of SP-D protein was done by ELISA kit (Zellbio) in accordance with the manufacturer's instructions.

Results: Our data revealed that serum levels of SP-D were significantly higher in COVID-19 infected individuals particularly in patients with the severe acute respiratory syndrome (SARS) ($P < 0.05$). By contrast, healthy subjects showed no changes in normal blood concentrations of SP-D protein.

Conclusion: In conclusion, we demonstrated that serum levels of SP-D increases during COVID-19 infection which may provide valuable diagnostic and prognostic information validating a potential biomarker.

Keywords: COVID-19, Biomarker, Immune response

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Anti-diabetic effect of an oligosaccharide fraction from Momordica charantia via modulation of notch signaling in diabetic rats

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Background: Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia. Therapeutic strategies to remedy diabetes and its complications have been designed based on targeting dysregulated signals involved in the initiation and progression of diabetes. The objective of this study is to isolate the active biomolecule from M. charantia and evaluate the molecular mechanism of the fraction on the differentiation of pancreatic cells by targeting the Notch signaling pathway in diabetic rats.

Methods: Samples of M. charantia was supplied from Kermanshah and extracted using different methods. The extracted fraction was isolated, evaluated, and characterized by HPLC and mass spectroscopy methods. The rats will be segregated into 5 groups including control, diabetic and groups 3, 4, and 5 where diabetic rats were taken three different doses of oligosaccharide fraction of M. charantia, respectively. The gene expression was determined by real-time PCR technique using temperature cycles according to the protocol provided by the Qi gene company. In addition, IHC was performed to visualize the level of Hes1 and cyclin D1.

Results: Extracted fraction from M. charantia is an oligosaccharide by lowering the blood-glucose effect in STZ-diabetic rats. The weight of diabetic rats exposed to MCF soared compared to untreated diabetic rats. Treatment by MCF in diabetic rats brought about a decrease in the expression of hes1, and an increase in the level of cyclin d1 in the pancreas tissues compared to untreated diabetic rats. Furthermore, MCF- treated diabetic rats showed a decline in the mRNA expression of signaling factors in Notch 1 pathway, an escalate in the expression of pdx1 and Ins1 as well as a plunge in the ratio of Bax/Bcl2.

Conclusion: Evaluating the underlying ant-diabetic molecular mechanism of MCF compound indicated that involvement of Notch signaling pathway, suggesting the causal role of MCF in differentiation and proliferation of pancreatic islet cells.

Keywords: Diabetes, Momordica Charantia, Oligosaccharide fraction, Notch signaling

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The effect of Fe3O4@NFC- ONSM nano-particle on the viability of the cell line MAD-MB-231

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Background: Fe3o4 nanoparticles have as of late been considered in the clinical and clinical fields because of their numerous alluring properties, including biocompatibility, degradability, and simplicity of synthetase. Iron oxide nanoparticles have both semiconductor and magnetic conduct features that naturally prompt biomedical applications for multi-reason. The effective utilization of these nanoparticles in clinical fields, for example, antibacterial, antifungal, and against disease can be referenced. This investigation inspects the survival rate of the MDA-MB-231 cell line against Fe3O4 @ NFC-ONSM nanoparticle.

Methods: The MDA-MB-231 cell were treated with various concentrations of Fe3O4@ NFC-ONSM (6.25, 12.5, 25, 50 µg/ml) for 48, 24, and 72 hours. The MTT method was used to determine the viability of the cell. Of staining, Hoechst33285 was used to test for the induction of apoptosis.

Results: The survival rate of MDA-MB-231 cell against Fe3O4@NFC-ONSM decreases, based on the results of the MTT study. Hoechst's staining results showed that DNA heterogeneity in the treated cell was growing. As the results show, Fe3O4 @ NFC-ONSM progressively reduces cancer cell growth and apoptosis according to time and dose.

Conclusion: The results reveal a new path for the biosynthesis of Fe3o4 MNPs which can contain various applications in the field of biomedicine, particularly in cancer treatment.

Keywords: Survival value, Fe3O4 @ NFC-ONSM nano-particle, MAD-MB-231 cell line, Apoptosis.

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Oxidative stress and inflammation variables as markers of coronary artery disease

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Background: Coronary artery disease (CAD) are the major cause of death worldwide. The association of CAD with inflammation is well established. Recently, it has been confirmed that the C1q/TNF-related protein 12 (CTRP12) has a great anti-inflammatory effect. The present study was conducted for the first time to assess the association between CTRP12 serum levels and the severity of CAD.

Methods: This case-control study was performed on 200 suspected coronary artery disease (CAD) patients and 50 healthy matched controls. According to angiography results, the patients were divided into CAD+ (n=150) with any major coronary arteries stenosis ≥50% and CAD− (n=50) with <50% stenosis of the arteries. And also, the CAD+ patients were classified into one-vessel disease (1VD), two-vessel disease (2VD), and three-vessel disease (3VD) in regarding the number of affected vessels. CTRP12, Interleukin-6 (IL6), tumor necrosis factor α (TNF-α), total antioxidant capacity (TAC), total oxidant capacity (TOC), malondialdehyde (MDA) levels, and Catalase activity were evaluated. Also, lipid profiles, hsCRP, and demographic factors were analyzed and measure mRNA expression levels of Nrf2 in the groups.

Results: The IL6, TNF-α, MDA, LDL, and BMI, hsCRP, and TOC levels in both CAD groups were significantly higher than for the controls ($P < 0.05$). CTRP12, TAC levels and the activity of Catalase were significantly lower in both CAD groups when compared with control subjects ($P < 0.05$). And also, expression levels of Nrf2 gene were significantly higher in cases when compared with healthy control subjects. Moreover, both patients with 3VD and 2VD had higher MDA and hsCRP levels compared with 1VD subgroups. And also, patients with 3VD had lower CTRP12 in comparison to 2VD and 1VD patients.

Conclusion: Our data revealed that CTRP12 serum level has inversely associated with the severity of coronary artery disease. And it may be used as markers of CAD evaluation.

Keywords: Oxidative stress, inflammation, coronary artery disease(CAD), C1q/TNF-related protein 12 (CTRP12), Nrf2.

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Molecular docking and screening based on myricetin-like inhibitors for sortase A in Streptococcus mutans isolates

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Background: Oral and dental infections are mainly caused by bacterial biofilms, and Streptococcus mutans via activity of streptococcal sortase A enzyme is the most common bacterium that cause this infection. The aim of this study was to perform virtual docking screening based on myricetin -like inhibitors for sortase A in S. mutans isolates from pupils.

Methods: This study was done on specimens isolated from teeth of pupils (n =150) in Golestan province during 2019. Biofilm formation was assessed using the microtiter plate assay. After identifying of SrtA gene using polymerase chain reaction (PCR) with specific primers, broth microdilution test was used according to CLSI M100-S25(2015) criteria to determine the minimum inhibitory concentration(MIC) of penicillin, erythromycin and myricetin. For bioinformatics analysis, molecular docking method between S. mutans sortase A enzyme and 4 ligands that had a high affinity for protein (sortase A) was used by AutoDock software.

Results: The frequency of S. mutans isolates containing srtA was 87 % of which, 63% were capable of biofilm production. Determining MIC showed that 54.3 isolates of S. mutans tested were susceptible to penicillin (MIC, ≤0.12μg/mL), while 67% (MIC, ≤0.25μg/mL) and 79% (MIC, ≤16μg/mL) of isolates were categorized as susceptible to erythromycin and myricetin, respectively. Bioinformatics study also showed that the studied ligands were able to effectively inhibit the enzyme sortase A, and in the meantime, the inhibitory ability of ligand 2 was even higher than that of myricetin.

Conclusion: The results showed that in vitro myricetin has a relative effect in comparison with conventional antibiotics in the control of dental biofilm. Also, due to the fact that ligand 2 has higher inhibitory power than myricetin it can prevent the formation of S. mutans biofilm by inhibiting the enzyme sortase A.

Keywords: S. mutans, Sortase A, Biofilm, myricetin, Molecular Docking

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Frequency of macroprolactinomas and its comparison with true hyperprolactinemia in infertile women

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Background: Prolactin measurement is one of the most important tests requested in the diagnosis of primary infertility. The formation of macroprolactin and IgG complex is one of the causes of hyperprolactinemia in several patients. There are different methods for diagnosing hyperprolactinemia and macroprolactinomas. Among them, the polyethylene glycol (PEG) precipitation method is a rapid, accurate, reliable, simple, inexpensive, and cost-effective method. This study aimed to determine the frequency of macroprolactinomas in infertile patients who have been reported as hyperprolactinemia and do not require drug treatment of hyperprolactinemia.

Methods: Among 80 patients who requested prolactin measurement 24 patients with hyperprolactinemia (prolactin greater than 35 mg/L) were evaluated using the PEG precipitation method. If more than 60% of prolactin is precipitated, the patient is considered as macroprolactinomas and does not require hyperprolactinemia-related drug treatments.

Results: In this study among 24 patients, (prolactin above 35 mg/L), 10 patients (41.6%) were considered by the PEG test as macroprolactinomas and 14 patients (58.3%) with true hyperprolactinemia.

Conclusion: To avoid wasting time and money and impose psychological stress on patients before receiving certain medications from infertility specialists for further diagnostic examination, the PEG precipitation method is emphatically recommended.

Keywords: prolactin, hyperprolactinemia, macroprolactinomas, PEG precipitation method

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A novel OAT mutation causes vitamin B6-responsive GACR in Iran

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Background: Gyrate atrophy of the choroid and retina (GACR) is a rare congenital metabolic disorder characterized by progressive visual loss, myopathy, cataract, night blindness. It has been found mostly in Finnish and the OAT (ornithine aminotransferase) gene mutations are specified as underlying causes. Patients have a high level of ornithine in body fluids and the disease could be controlled by vitamin B6 as supplementation and low protein diets but not in most cases.

Case presentation: Here we report the first characterized case of vitamin B6-responsive GACR in Iran with novel splicing mutation in the OAT gene. The clinical features, imaging, biochemical findings, and whole-exome sequencing (WES) analysis have confirmed the diagnosis. WES data revealed the splicing mutation in exon 5 of the OAT gene (NM_001322967(OAT): c.425-1G>A).

Conclusion: Identifying novel mutations causing GACR and follow up with them to evaluate the treatment response improves knowledge about the diagnosis and treatment of GACR. Authors: Samira Molaei Ramshe, Safoura Zardadi, Hossein Darvish (Corresponding author)

Keywords: Gyrate atrophy, OAT, vitamin B6, Iran

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Investigating the effects of Salvia chorassanica Bunge and shoot extracts on gastric cancer cells: evidence of different behavior on various tumor grades

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Background: Different Salvia species demonstrated anti-proliferative effects on various cancer cells. Owing to the still high mortality in gastric cancer patients and poor literature on the anti-proliferative effects of Salvia species on gastric cancer cells, it was aimed to determine the anticancer effects of a local Iranian Salvia, Salvia chorassanica, on two gastric cell lines with different grades.

Methods: Root, stem and leaves of Salvia chorassanica extracts were prepared and treated in different concentrations on two AGS and MKN-45 cell lines which have been harvested in an appropriate culture medium. MTT assay was employed to determine the toxicity of three types of the extract on two studied cell lines and the expression of Bax, BCL-2, Caspase3, MMP2, and MMP9 genes were identified through reverse transcription Real-time PCR (RT-Real time PCR).

Results: Bunge and shoot extracts demonstrated toxicity to both cell lines which were more pronounced in AGS cells and root extract treatment. On contrary to AGS cells, the Caspase3 gene was up-regulated while the MMP2 and MMP9 genes were down-regulated in all types of treatment. Except of MKN-45 cells treated with leaves extract, Bax/BCL-2 expression ratio was decreased in treatment with three types of extract.

Conclusion: Remarkable low IC50 concentration of root extract in MKN-45 cell line compared to other Salvia species is indicating the considerable cytotoxicity of Salvia chorassanica against gastric cancer cells. In addition, gene expression analysis in MKN-45 needs further confirmation on potential anti-metastatic roles of leaves and root extracts in higher grades of gastric cancer.

Keywords: MKN-45, AGS, cytotoxicity, Salvia chorassanica, gene expression

P-178

Apoptosis Effect of Chamomile on Breast Cancer Cell Line MCF-7

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Background: Breast cancer is the most common cancer among women in the world. and also chamomile plant contains several effective compounds with different biological effects. According to investigated studies, in the current thesis cleared that flavonoid compounds in chamomile have a killing effect on MCF-7 cancer cells.

Methods: To determine chamomile alcohol- aquast extract concentration and IC50 measurement; the enzymatic method was applied as reaction substrate from tetrazolium solution salts and MTT is the main case and the killing dose was obtained 45/5 µg/ml and it treated with cell dose, RNA extraction was implemented by special kit. After RNA concentration, nanodrop was used to RNA extraction quality and it was measured by using electrophoresis. Therefore, c DNA synthesis was done by related kit and synthesized c DNA quality and quantity investigations were implemented in nanodrop/ designed primers were applied to c DNA connection optimum temperature for synthesized cases and we have been used PCR and electrophoresis methods. By using PCR (Real-Time PCR) results, genes expression quantity amount was determined. The effect of the extract on the apoptosis pathway in three genes BAX, BCL-2, p53

Results and Conclusion: The results demonstrate that the extract decreases the growth rate of the cancer cell line through inducing the apoptosis mechanism. As long as the expression of the anti-apoptosis Bcl-2 gene reduced dramatically, an over-expression in Bax and p53 genes were monitored indicating activation of the apoptosis pathway. Conclusions: This research was done to evaluate the induction of apoptosis in MCF-7 breast cancer cell line by Chamomile extract. The expressions of genes through which apoptosis pathway are involved Bax, Bcl-2, p53 were examined by Real-time PCR. demonstrated an effective chamomile extract in preventing or treating breast cancer cell line MCF-7.

Keywords: Chamomile, Breast Cancer, MCF-7, Bax, Bcl-2, p53, MTT.

P-179

The effects of hydroalcoholic extract of Teucrium polium on serum level of ANGPTL8 (Betatrophin) in hypertriglyceridemic rats

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Background: Hypertriglyceridemia is a risk factor for many diseases, including cardiovascular disease, diabetes, and nonalcoholic fatty liver disease. The family of ANGPTLs, especially ANGPTL3, ANGPTL4, and ANGPTL8, which regulate lipoprotein lipase, play a pivotal role in triglyceride metabolism and its associated diseases and complications. Teucrium polium belongs to the Labiatae family with the local name of Teucrium. Various cases of therapeutic effects of this plant, including lowering blood sugar, lowering triglycerides, etc. have been reported so far. The aim of this study was to evaluate the effects of hydroalcoholic extract of Teucrium on serum levels of ANGPTL8 in hypertriglyceridemic mice.

Methods: This study is a case-control study that was performed on 48 male Wistar rats in the weight range of 180-250 g. Mice were divided into 6 groups of 8 under the groups of treatment (1, 2, 3, and 4), fat control and virgin control. The virgin control group received standard nutrition, but groups 1 to 5 were injected with Triton X-100 to induce hypertriglyceridemia. After confirmation of hypertriglyceridemia, the treatment groups (1, 2, and 3) were placed on gavage with 25, 50 and 100mg/kg of an extract of Teucrium Polium over 22 days. The treatment group 4 was gavaged with 10 mg/kg of gemfibrozil for 22 days, too. serum samples obtained from all groups and were used to measure various parameters including triglyceride, cholesterol, HDL, LDL, ALP, CPK, SGOT, SGPT and ANGPTL8.

Results and Conclusion: Consumption of Teucrium extract and gemfibrozil could not show a significant correlation between betatrophin and TG levels after the intervention. However, there is an inverse relationship between ANGPTL8 and ALP levels with the use of gemfibrozil. Teucrium polium extract can reduce TG, cholesterol, LDL, and ALP and increase HDL levels.

Keywords: Teucrium Polium, Betatrophin, ANG-PTLs, Hypertriglyceridemia, Cardiovascular disease

P-180

The role of substance P/NK1R in the pathogenesis of colorectal cancer through constitutively active PI3K/Akt/NF-κB signal transduction pathways

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Background: Colorectal cancer (CRC) is the third most common malignancy with a high mortality rate, supporting the necessity for an effective antitumor drug. Substance P (SP) is the most important member of the tachykinins family, binding to the specific receptors on the surface of tumor cells, named neurokinin-1 receptor (NK1R), to exert its different effects such as tumor cell proliferation and angiogenesis. Aprepitant, as a specific NK1 receptor antagonist, is suggested as a novel antitumor agent. However, the exact molecular mechanism of the NK1R/SP system and aprepitant in CRC is not completely known and requires to be deciphered.

Methods: To show the cytotoxic effects of aprepitant on the viability of the SW480 cells, the resazurin assay was conducted. The level of ROS was evaluated after 24-hour treatment with SP and aprepitant. PI / Annexin V-FITC staining was performed to evaluate the apoptosis. Also, the expression of Bax, p53, Bcl-2, and Survivin genes was measured by Real-Time PCR assay. Western blotting was used to measure the expression of PI3k/AKT/NF-κb proteins.

Results: The half-maximal inhibitory concentration (IC50) value for SW480 cells treated with aprepitant was found to be approximately 18 after 24 hours. The level of oxygen free radicals (ROS) was significantly increased and decreased after 24 hours of treatment with SP and aprepitant, respectively. Induction of apoptosis by tumor cells was also observed by the aprepitant. the expression of NF-κB anti-apoptotic target genes, including Bcl-2 and Survivin, decreased under the influence of aprepitant. According to Western blotting results, the expression of PI3K / Akt / NF-κB pathway proteins was also significantly reduced by the aprepitant.

Conclusion: Towards this end, this study suggests that SP/NK1R system plays an important role in the development of CRC, and pharmaceutical targeting of NK1R using aprepitant might be a promising treatment against CRC.

Keywords: Colorectal cancer, Substance P, Aprepitant, PI3k/AKT, NF-κb, Apoptosis

P-181

The Therapeutic Potential of MicroRNAs in regulating the mTOR signaling pathway in Cancer: A Review

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Background: The mammalian target of rapamycin (mTOR) is a large Ser/Thr protein kinase that belongs to the phosphoinositide 3-kinase (PI3K) family and mediates various physiological and pathological processes, especially cell proliferation, protein synthesis, autophagy, and cancer development.

Methods: This study reviewed The Therapeutic Potential of MicroRNAs in Regulating the mTOR signaling pathway in Cancer. In this regard, we searched databases such as PubMed, Science Direct, Google Scholar, and Scopus databases by using these keywords “MicroRNAs ”, “Signaling Pathways”, “Tumor cells”, “mTOR”, and “pharmacological inhibitor” without any time limit. The relevance of studies was identified by reviewing the titles and the abstracts. A total of 100 English language articles including experimental, observational, molecular, and cellular studies were reviewed.

Results: The mTOR expression is transient and tightly regulated in normal cells, but it is overactivated in cancer cells. Recently, several studies have indicated that microRNAs play a critical role in the regulation of mTOR and mTOR-associated processes, some acting as inhibitors and the others as activators.

Conclusion: Although it is still in infancy, the strategy of combining both miRNAs and mTOR inhibitors provides an approach to selectively sensitizing tumor cells to chemotherapy-induced DNA damage and then attenuating the tumor cell growth and apoptosis.

Keywords: mammalian target of rapamycin, phosphoinositide 3-kinase, microRNAs, autophagy

P-182

The Emerging Role of Substance P/Neurokinin-1 Receptor Signaling Pathways in Growth and Development of Tumor Cells

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Background: Tachykinins (TKs) include an evolutionarily conserved group of small bioactive peptides that possess a common carboxyl-terminal sequence, Phe-X-Gly-Leu-Met-NH2. TKs also have been shown to have implications in different steps of carcinogenesis, such as angiogenesis, mitogenesis, metastasis, and other growth-related events.

Methods: This study reviewed The Emerging Role of Substance P/Neurokinin-1 Receptor Signaling Pathways in Growth and Development of Tumor Cells. In this regard, we searched databases such as PubMed, Science Direct, Google Scholar, and Scopus databases by using these keywords “Tachykinins”, “Signaling Pathways”, “Tumor cells”, “Substance P”, and “pharmacological inhibitor” without any time limit. The relevance of studies was identified by reviewing the titles and the abstracts. A total of 100 English language articles including experimental, observational, molecular, and cellular studies were reviewed.

Results: The biological actions of substance P (SP), as the most important member of the TK family, are mainly mediated through a G-protein coupled receptor named neurokinin-1 receptor (NK1R). More recently, it has become clear that SP/NK1R system is involved in the initiation and activation of signaling pathways involved in cancer development and progression. Therefore, SP may contribute to triggering a variety of effector mechanisms including protein synthesis and a number of transcription factors that modulate the expression of genes involved in these processes.

Conclusion: The overwhelming insights into the blockage of NK1R using specific antagonists could suggest a therapeutic approach in cancer therapy. In this review, we focus on evidence supporting an association between the signaling pathways of the SP/NK1R system and cancer cell proliferation and development.

Keywords: Tachykinins, Substance P, Neurokinin-1 Receptor, Signaling Pathways, Cancer.

P-183

The Role of Tachykinins in the Initiation and Progression of Gastrointestinal Cancers

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Background: Tachykinins (TKs) are an evolutionarily conserved family of peptide hormones, which are widely distributed within the peripheral and central nervous systems. TKs exert their biological actions in many processes via three subtypes of transmembrane G-protein coupled receptors, which are NK1R, NK2R, and NK3R.

Methods: This study reviewed the role of tachykinins in the initiation and progression of gastrointestinal cancers. In this regard we searched databases such as PubMed, Science Direct, Google Scholar, and Scopus databases by using these keywords “Tachykinins”, “Gastrointestinal cancer”, “Metastasis”, “G-protein coupled receptors”, and “pharmacological inhibitor” without any time limit. The relevance of studies was identified by reviewing the titles and the abstracts. A total of 100 English language articles including experimental, observational, molecular, and cellular studies were reviewed.
Results: The administration of the gastrointestinal cancer cells with Tk receptor antagonists induces apoptotic cell death through the tachykinin-mediated pathway. The findings showed that the pharmacological inhibition of TKRs with its selective antagonists has a promising prospect for the GI cancer treatment approach, either as a single agent or in combination with other chemotherapeutic agents.

Conclusion: In this review, we have presented different effects of TKs in the initiation and progression of GI cancer cells. Blocking the TK receptors with their specific antagonists is also reviewed as a potential therapeutic approach in GI cancer therapy.

Keywords: Tachykinins, Gastrointestinal cancer, Metastasis

P-184

The critical roles of Circular RNAs in Diabetes mellitus: a systematic review

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Background: Circular RNAs (circRNAs), as an emerging member of non-coding RNAs (ncRNAs), have received scholarly attention since recent evidence indicating that these novel ncRNAs are implicated in various biological processes. Due to their lack of 5 ′ and 3' ends, circRNAs' two ends are covalently bonded together, and circRNAs are synthesized from pre-mRNAs in a process called back-splicing, which makes them more stable than linear RNAs. Moreover, the involvement of circRNAs in the regulation of transcription, splicing, translation, and post-translational levels has been reported.

Methods: We systematically searched the PubMed, google scholar, and Scopus database to explore the role of circRNAs in DM between March 2010 and February 2020. After performing the literature search and review, 150 eligible studies were identified.

Results: Not only is there accumulating evidence showing that circRNAs play a critical role in the pathogenesis of different diseases such as cancer, neurological disorders, and diabetes mellitus (DM), but it has also been indicated that dysregulation of circRNAs has made them promising diagnostic or prognostic biomarkers for early detection. Recently, DM has emerged as the main concern to human health worldwide, and increasing attention has been paid to investigate the mechanisms underlying the DM process. In the meantime, it has been demonstrated that there is a strong correlation between expression levels of circRNAs with DM and their complications.

Conclusion: CircRNAs play a critical role in the pathogenesis of diabetes. It is denoted that dysregulated circRNAs affect pancreatic β-cell structure and function, glucose-stimulated insulin secretion, insulin signaling, as well as peripheral tissues such as skeletal muscles, adipocyte, and other pathways that are implicated in cellular proliferation, autophagy, and inflammation. Besides, circRNAs as emerging ncRNAs, have offered the new opportunity as tools for diagnosis and prognosis when there is a correlation between their expression and progression of diabetes.

Keywords: Circular RNAs, Diabetes mellitus, Biomarkers

P-185

Investigation of antibiotic resistance profile and biofilm formation in Escherichia coli isolates with carbapenem resistance

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Backgrounds: Escherichia coli, a gram-negative bacillus, is a member of the Enterobacteriaceae family that is capable of causing various infections. Infections caused by E. coli with carbapenem resistance as an important treatment problem are increasing worldwide. The present study aimed to investigate the antibiotic profile and the ability of biofilm production of E. coli isolates to have carbapenem resistance in 1398.

Methods: 73 isolated E. coli were collected from patients in hospitals and laboratories in Tehran province, and after confirmation of the samples with standard biochemical experiments, the samples with the enzyme method of carbapenemase enzyme were screened with the help of the phenotypic method. Then, the antibiotic profile of the obtained samples, along with the ability to form biofilm by microplate assay in laboratory conditions were examined.

Results: Of the 73 samples, 23 (31%) had a carbapenemase enzyme. The highest antibiotic resistance was observed with ampicillin, chloramphenicol, and ciprofloxacin antibiotics, and the lowest resistance was related to gentamicin, amikacin, and cotrimoxazole. Most samples were able to form a biofilm.

Conclusion: The present study suggests that, unfortunately, the insulin resistance of E. coli is increased compared to carbapenem antibiotics, and that the samples studied have a higher resistance to antibiotics such as phosphomycin compared to previous studies. It is recommended that these isolates be evaluated for the presence of resistance genes to colistin and phosphomycin.

Keywords: Escherichia coli, carbapenemase, biofilm, antibiogram

P-186

Evaluation of antibiotic resistance profile and biofilm formation in Escherichia coli isolates with broad-spectrum beta-lactamase (ESBL) resistance

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Backgrounds: Escherichia coli is a gram-negative bacterium that can cause a variety of infections, including nosocomial infections. Various infections caused by Escherichia coli with annual carbapenem resistance cause high mortality and treatment costs in various countries around the world. The aim of the present study, which was conducted as part of the dissertation project, is to investigate the antibiotic profile and the antibiotic resistance profile and biofilm formation of Escherichia coli isolates with ESBL resistance in 1398.

Methods: 73 isolated Escherichia coli were collected from patients in hospitals and laboratories in Tehran province and after confirmation of the samples with standard biochemical experiments, ESBL isolates were screened by a combined phenotypic combination method. Then, the antibiotic profile of the obtained samples, along with the ability to form biofilm by microplate assay in laboratory conditions were examined.

Results: Of the 73 samples, 52 (71%) had broad-spectrum beta-lactamase enzymes. The highest antibiotic resistance was observed with ampicillin, chloramphenicol, and ciprofloxacin antibiotics, and the lowest resistance was related to gentamicin, amikacin, and cotrimoxazole. Most samples were able to form a biofilm.

Conclusion: Comparing the present study with previous studies, we saw an increase in antibiotic resistance in the studied isolates, and unfortunately, the prevalence of isolates with high beta-lactamase resistance was high. It is recommended that the isolates be tested for ESBL resistance genes.

Keywords: Escherichia coli, ESBL, biofilm, antibiogram

P-187

The Effect of Intermittent Fasting and water deprivation on Brain Growth Factors

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Background: Intermittent fasting has beneficial effects on health by changing the level of growth factors (Gfs). Regarding the controversy in the effect of fasting on GFs and the possible role of age and gender in this association, we aimed to investigate the association between fasting (with or without water deprivation) and Gfs such as BDNF, proBDNF, and GDNF.

Methods: In this experimental study, 84 Wistar rats (21 male immature, 21 male mature, 21 female immature, and 21 female mature) were divided into 12 groups (7 rats each) based on the method of treatment (control/fasting with access to water/fasting without access to water). Control groups had free access to food and water; fasting groups were deprived of food or food and water for 12h a day. After 3 weeks of treatment, serum levels of BDNF, proBDNF, and GDNF were determined using the ELISA method and analyzed by SPSS software.

Results: fasting was associated with significantly higher levels of BDNF, proBDNF, and GDNF but this effect was gender-dependent and was mainly seen in male rats. Also, there was not any noticeable difference when water deprivation was added to fasting.

Conclusion: The effect of fasting on the level of BDNF, proBDNF, and GDNF is gender dependent and could be explained by structural differences of tissues especially the brain between male and female rats.

Keywords: intermittent fasting, water deprivation, growth factors, BDNF, proBDNF, GDNF, rat

P-188

Codon Optimization and Expression of Recombinant Human Tissue Factor in Pichia pastoris, and application in a prothrombin time reagent

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Background: Tissue factor (TF) is a membrane-bound glycoprotein, acting as a cofactor for coagulation factor VII (FVII), and widely used as the core reagent in the prothrombin time (PT) test. Currently, the major source of thromboplastin is animal tissue such as the rabbit brain; which has some ethical issues including the possibility of infectious disease transmission and also frequent changes in the International Sensitivity Index (ISI). To overcome these limitations, we expressed the recombinant human TF (rHTF) protein in Pichia pastoris to be used for the preparation of PT reagent.

Methods: The amino acid sequence of TF, obtained from the UniProt database and was back-translated and optimized for expression in Pichia pastoris. The optimized coding sequence for TF was synthesized and inserted into the expression vector (pPICZαA) and electroporated into KM71H. The cell lysate of the yeast expressing rHTF was analyzed using Dot-blotting and Western-blotting. Additionally, the biological function of the product was verified by measuring the time needed for clotting citrated plasma.

Results: rHTF was expressed in the KM71H and the protein expression was confirmed using Dot-blotting and Western-blotting techniques (three protein band 30, 97, and 245 KDa). After rHTF protein extraction from the yeast Pichia pastoris through the glass bead method, the results demonstrated no biological activity of negative control sample in plasma coagulation (more than 300 seconds). However, the cells precipitate containing rHTF protein and PMSF, in 46±6 seconds and the supernatant in 66.67±3.786 seconds was able to coagulate the plasma sample.

Conclusion: The yeast Pichia pastoris is a suitable source for the production and extraction of high amounts of TF to use in the PT kit.

Keywords: Tissue factor, Pichia pastoris, PT kit

P-189

Investigation of miRNA-153 and miRNA-223 involvement in Mn2+-imposed Parkinson like disorder

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Background: Overexposure to manganese ion (Mn2+) induces some neurological and behavioral abnormalities known as manganism. Patients and animal model of manganism manifests oxidative stress, catecholamine dysregulation, and cognitive deficits similar to Parkinson's disease. Mn2+ toxicity also could be considered an important risk factor in neurological disorders. This study was designed to investigate Parkinson's disease involved miRNAs in the rat model of manganism to advance our understanding of miRNAs role in the common pathophysiological process.

Methods: In this study rats were administrated intraperitoneally by a high dose of MnCl2 (15 mg/kg) and behavioral assessment such as forced swimming test and sucrose preference test was done. The rats that manifested Parkinson like behavioral signs were sacrificed and hippocampus tissue was isolated from the scalp. Total RNA was extracted from the hippocampus tissue and microRNA-153 (miR-153) and miR-223 content were evaluated by using quantitative RT-PCR.

Results: In comparison with the control that was treated by saline as a vehicle, the rats that received MnCl2 manifested significantly increased immobility time and decreased sucrose preference which could be interpreted as Parkinson's disease symptoms. Interestingly, the behavioral abnormalities were accompanied by a remarkable reduction in miR-153 and miR-223 in hippocampus tissue ($P<0.05$).

Conclusion: By considering the miR-153 role in SNAP and α-synuclein expression, its possible molecular mechanism of Mn2+ toxicity is similar to Parkinson's disease so Mn2+ exposure could intensify pathophysiological signs of disease and also could be mentioned as an effective risk factor especially in miners. Reduced miR-223 also revealed extensive neurodegeneration in Mn2+ neurotoxicity.

Keywords: Manganism, Parkinson's disease, miR-153, miR-223, Behavioral assessment

P-190

Evaluation of the antiproliferative effect of hydrolyzed olive protein on the human breast cancer cell line (MCF-7)

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Background: Plant components, including proteins, play a crucial role in reducing the risk of diabetes, cardiovascular disease, high blood pressure, obesity, and prevent various types of cancer. Enzymatic hydrolysis is one of the common methods to produce bioactive peptides with antiproliferative properties. This study aimed to investigate the antiproliferative effect of hydrolyzed olive protein on the human breast cancer cell line (MCF-7) in vitro.

Methods: After protein extraction from olives, enzymatic hydrolysis was performed using trypsin under conditions, time 5h, PH 8.5, and the temperature 39°C. Then, to evaluate the antiproliferative effect of hydrolyzed proteins, it was freeze-dried and prepared at various concentrations of (5, 10, 20, 40, and 80 mg/ml) and using MTT assay for 24 and 48 hours. The amino acid analysis was performed by RP-HPLC to determine the amino acids in the hydrolyzed protein. One-way ANOVA using SPSS Version (23.0) and Tukey HSD with a level of statistically significant $P < 0.05$ were used to analyze the data.

Results: These results indicated by increasing protein hydrolysis concentrations from (5-80 mg/ml) % Cell Viability was reduced significantly ($P < 0.05$). At a concentration of 80 mg/ml, the lowest %cell viability was observed 9.71±1.32 at 48h. IC50 (half maximal inhibitory concentration) was achieved 28.77mg/ml. The results of the amino acid analysis indicate a high content of hydrophobic amino acids. Also, the presence of positively charged amino acids has an effective role in creating the antiproliferative properties of hydrolyzed proteins.

Conclusion: According to the results of the present study, protein hydrolysis of olive with trypsin plays an important role in reducing the viability of human breast cancer cells (MCF-7).

Keywords: olive, Enzymatic hydrolysis, antiproliferative effect, MCF-7

P-191

Effects of chamomile extract on the induction of apoptosis and cell proliferation in A-375 cell line

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Background: Today, cancer is one of the most common diseases and the number of patients is increasing day by day. So far, many studies have been done on different types of flavonoids and their effects. These compounds can be used as complementary therapies in Along with other treatments, it may be a treatment in itself. Melanoma accounts for only 1% of all skin cancers, but it is the cause of death in 60% of skin cancer patients. In this study, the effect of total hydroalcoholic extract of chamomile extract on the survival of A-375 cells related to skin melanoma cancer cells was investigated.

Methods: After treatment of melanoma cell line with concentrations of 45.5, 34.1, 25.6, 0.23, 19.2, 14.4 µg / ml of aqueous/alcoholic extract of chamomile by MTT method and relevant calculations, IC50 was measured.

Results: According to the MTT test data for 24 hours and its graph, a concentration of 27.3 µg / ml (luteolin concentration) was measured as the IC50 level for chamomile extract. The results showed that A-375 cell treatment reduced cell viability by decreasing the concentration of chamomile extract.

Conclusion: The results of this study indicate that the treatment of cell line A-375 with a combination of total aqueous/alcoholic extract of chamomile can have effective results on cell proliferation and cell death and at IC50 dose, it is effective on melanoma cell survival.

Keywords: Apoptosis, Melanoma, Flavonoids, Chamomile, MTT

P-192

Study of the effect of chamomile extract on ovarian cancer.

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Background: Ovarian cancer accounts for about 4% of all women's cancers, and is the fifth leading cause of cancer deaths in women. Control of the disease involves the removal of a tumor-associated with chemotherapy using drugs such as Taxol and Platinum. Although 70 to 80 percent of patients respond to the first course of chemotherapy, the disease eventually regenerates with resistance to chemotherapy, and the patient dies within two years due to disease metastasis. Typically, chemotherapy drugs are cytotoxic and cause severe toxicity in normal cells, so developing therapies to overcome this resistance and finding natural drugs with low toxicity for healthy tissues is a necessary requirement for the successful treatment of ovarian cancer and overall improvement and Survival is sick. Chamomile has many properties, including relaxing, analgesic, anti-inflammatory, antimicrobial and antispasmodic properties. Apart from information on the effects of chamomile on the improvement of various diseases in recent years, many studies have been conducted on the antioxidant properties of both hypochlostromy and anti-aging. Chamomile extract has anti-proliferative activity and apoptosis in various human cells that its effect on normal body cells is low.

Methods: 10 thousand cells of the ovarian cortex were treated in concentrations of 71,162, 56.875, 45.5, 34.125, 25.5892 µg / ml of chamomile extract for 24 hours, and the lethality of the drug was measured by MTT test and IC50 was calculated.

Results: According to the results of the MTT test and the calculations performed, the highest lethality of the drug was in class A-2780 at a concentration of 11.466 µg / ml, which is the determinant of IC50 drug.

Conclusion: The results of this study show that total chamomile extract can have positive results in cell death of 2780 ovarian cancer cells.

Keywords: Apoptosis, Chamomile, MTT, Ovarian cancer

P-193

Anti-cancer effect of Chamomile extract on lung cancer

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Background: Lung cancer is one of the most dangerous types of cancer for men and women, which has an increasing number of deaths each year and its survival rate is lower than other types of malignancies, ie with overall survival of 5 years. -20% In most countries, according to GLOBOCAN 2018, it is estimated that lung cancer is the most common cancer diagnosed (11.6% of all cases) and the leading cause of cancer death (18.4% of all deaths). Caused by cancers). Despite advances in treatment options including surgery, radiography, chemotherapy, and specific targeted therapies, metastatic disease is unsatisfactory due to the late diagnosis of this cancer.

Methods: To perform MTT, the cells were counted and seeded on a plate and then treated with concentrations of 9.1, 10.92, 13.65, 18.2, 22.75 µg / ml of chamomile extract. After 24 hours, the light absorption of the plate was measured. Using relevant calculations and plotting the IC50 scale was determined.

Results: MTT test data showed a dose of 13.19 µg / ml (luteolin concentration) as IC50 for total chamomile extract on A549 lung cancer class. Accordingly, by reducing the concentration of total chamomile extract, we see a decrease in the process of cell proliferation of lung cancer.

Conclusion: Based on the data of this study, it can be concluded that cell line treatment A-549 with a combination of total aqueous/alcoholic extract of chamomile has effective results in reducing cell survival and reducing the proliferation of lung cancer cells.

Keywords: Lung cancer, cell line A-549, the whole extract of chamomile, apoptosis

P-194

Molecular docking studies of Lysine-Specific Demethylase 1 (LSD1) Inhibitors as anticancer agents

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Background: Histone lysine demetylases1 (LSD1) is a promising medication to treat cancer, which plays a crucial role in epigenetic modulation of gene expression. Inhibition of LSD1 with small molecules has emerged as a vital mechanism to treat cancer, also, the design of LSD1 inhibitors is an ongoing topic in drug design.

Methods: The molecular docking process was performed using Molecular Operation Environment (MOE) software to predict the mode of interaction between the best possible biological conformations of compounds in the active site of LSD1 enzyme. The 2D structures of compounds were prepared by Chem Draw ultra 8.0 software and converted into 3D format by Hyper Chem7 using AM1 semi-empirical method. The compounds were docked into the active site of LSD1 (PDB ID: 2Z5U) by MOE software. The best pose of compounds with the higher score was selected for ligand-target interaction analysis by the LigX module in MOE software.

Results: The docking results showed a high docking score (-12.57 kcal/mol) for the most active compound of tranylcypromine (TCP) in comparison to that of the least active compound (-10.36 kcal/mol) and also, TCP derivatives forming N5, C4a or cyclic adduct with cofactor FAD in LSD1 enzyme and could form π-π stacking and π-cation interactions with Tyr761, Val 811 and Arg316 and hydrogen bond with backbone acceptor hydrogen bond of His 812 and electrostatic interactions with Gly 314, Thr 335, Glu 559.

Conclusion: The dockings showed the possible binding mode in selected compounds in the LSD1 active site that Arg 316, Tyr 761, and Val 811 are possibly crucial residues, whereas some hydrogen bonding and hydrophobic interactions are vital in the active site of the protein and might be helpful to the stability of compounds in the active site of LSD1. Such findings indicated appropriate dockings for prediction for the reasonable formation of new LSD1 inhibitors to treat cancer.

Keywords: Lysine-Specific Demethylase 1, Tranylcypromine, Molecular Docking, LSD1 inhibitors

P-195

Evaluation of culture media on production of β-carotene and growth of the microalga Dunaliella salina

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Background: Carotenoids, e.g. β-carotene and astaxanthin, are the important photosynthetic metabolites of the algae. They have several properties such as antioxidant, antidiabetic, anti-obesity activity, etc. The microalga *Dunaliella salina* (*D. Salina*) is known as the only biological producer of the valuable pigment β-carotene with the advantage of providing 9-Cis-β-carotene that enhances antioxidant activity. In this work, the effect of f/2 and modified Johnson medium was examined on the production of β-carotene.

Methods: *D. Salina* CCAP 19/18 was cultivated in f/2 and modified Johnson (with tris-base and $NaHCO_3$) media, separately. The autoclaved media in 250 mL Arlene mayers were inoculated with 6×10^5 cells mL^{-1}. The prepared media were kept on a shaker incubator (150 rpm, 25 ± 1 °C) with 60 μmol $m^{-2}s^{-1}$ light intensity in 16:8 light: dark cycle. Growth was measured directly by cell counting, β-Carotene, and chlorophyll contents were determined using a spectrophotometer. All the experiments were carried out in triplicate.

Results: *D. Salina* cultivation in f/2, Johnson with $NaHCO_3$, and Johnson with tris-base media reached stationary phase after 8, 9, and 12 days, respectively. Johnson medium with $NaHCO_3$ had the best growth condition and contained higher cell density (4.88×10^6 cells mL^{-1}) rather than other media. β-Carotene augmentation from highest to lowest in Johnson with $NaHCO_3$, tris-base, and f/2 media were 9.5, 9, and 6.61 mg L^{-1}, respectively. The pH difference in all media was not significant. The chlorophyll to the β-carotene ratio in three investigated media was not noticeable, but it was higher in Johnson medium with $NaHCO_3$ (1.85) rather than the others.

Conclusion: Since the production of β-carotene from the natural resources is increasing, therefore enhancing the production of this useful pigment through biological methods is necessary. *D. Salina* was better adopted in Johnson medium (with $NaHCO_3$) and showed the highest β-carotene production of 9.5 mg L^{-1}.

Keywords: β-carotene, Dunaliella salina, modified Johnson medium, f/2 medium

P-196

Helminthes c-type lectin proteins and host-parasite interactions: Focus on Toxocara canis

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Background: C-type lectins are a family of calcium dependent glycoproteins whose carbohydrate recognition domain (CRD) binds to mono and oligosaccharides. Recently, C-type lectins have been identified from parasitic worms. Lectins are extremely diverse in amino acid sequences. The aim of the present study was to explain differences or similarities of CRD domain of Toxocara canis CTL and other nematodes, mouse and human.

Methods: The female Toxocara canis was retrieved from the 2-6 month puppies and the collected eggs were cultured until they were embryonated, then the larvae were cultured for a week. A cDNA library was made from the total mRNA of Toxocara canis second stage larvae. After PCR amplification of the C-type lectin gene and analysis of the amino acids using the alignment method the phylogenetic tree was constructed.

Results: Toxocara canis CTL gene was 657 bp in length and encoded a protein with 219 amino acids. The CTL of species of Strongylida order were closely placed in the tree, whereas the members of Ascaridida orders were located in a separate branch. High levels of similarity (36%-44%) and conservation of C-type lectin from T. canis with mouse and human C-type lectins were observed. Its C-type lectin showed a higher similarity with asialoglycoprotein receptor (ASGPR), macrophage lectin, dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), and MINCLE receptor of mouse and human.

Conclusion: The analysis of CRD domain of C-type lectin protein of Toxocara canis could expand our understanding of host-parasite interaction and immune responses in toxocariasis. Toxocara canis CTL has been considered an important protein in immunogenicity of Toxocara canis. CTL of Toxocara canis shares similar sequence with proteins of immune system of mouse and human.

Keywords: C type Lectin proteins, Toxocara canis, Strongylida, Ascaridida, Human, Mouse

P-197

Quercetin may inhibit prostate cancer metastasis through beta1 containing integrins

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Background: Prostate cancer becomes resistant to conventional therapies at later stages and metastasizes to bone and brain commonly. Nevertheless, the factors involved in their metastatic pathways are not well identified. Beta1 containing integrins such as alpha4beta1 and alpha5beta1 are the major regulators of cancer cell migration and metastasis in prostate cancer. The aim of our study was to investigate the effect of quercetin as dietary supplements on some microenvironment factors including beta1 containing integrins in metastasis of prostate cancer cells.

Methods: The effect of quercetin (75µM) on PC3 and DU145 cell lines, established from bone and brain, was investigated. Expression level of alpha4, alpha5 and beta1 integrin subunits was evaluated in the presence and absence of quercetin using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Statistical analysis was done using SPSS software version 16.

Results: In DU145 cells treated with quercetin, relative mRNA expression of beta1 and alpha4 integrin subunits was reduced by 78% and 88%, respectively, and integrin alpha5 subunit decreased by 61% compared to untreated control group. While relative mRNA expression of beta1 and alpha4 subunits of integrin in PC3 cells treated with quercetin, decreased by 33% and 56%, respectively, and alpha5 subunit of integrin by 84% compared to untreated control group.

Conclusion: Our results indicated that quercetin could reduce the expression of beta1 containing integrins that are involved in migration and metastasis of prostate cancer cells. Quercetin may be useful as a dietary supplement, in prevention of prostate cancer, however, further investigation is needed.

Keywords: Integrins, Quercetin, Prostate cancer cells, DU145, PC3

P-198

Biochemical study of the interaction of nano-berberine with transferrin and calf thymus DNA by spectroscopic methods

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Background: In this work the interactions of nano-berberine with transferrin and calf thymus DNA were investigated.

Methods: Fluorescence quenching, synchronous fluorescence, resonance light scattering were used to investigate the interactions between nano-berberine and transferrin. The conformational changes were investigated by fluorescence spectroscopy. The thermodynamic parameters ΔH, ΔS, and ΔG were investigated at temperatures in the range of 298- 308 K. Fluorescence quenching, resonance light scattering, transition temperature, viscometer, and UV-visible spectroscopy were used to investigate the interaction of nano-berberine with calf thymus DNA.

Results: Data disclosed that the drug-protein complex formation occurred through a remarkable static quenching procedure and the maximum emission wavelength shifted toward a shorter wavelength indicating changes of the tertiary structure of the protein. The synchronous fluorescence study indicated that nano-berberine induced structural changes on transferrin. The thermodynamic study showed that van der Waals forces and hydrogen bonding are involved in the interaction between Tf and nano-berberine and that hydrophobic forces are involved in the interaction between nano-berberine and ct-DNA. Fluorescent assay at different temperatures revealed that the quenching mechanism in both interactions was a Dynamic kind. Due to negative ΔG^0, the system of interaction of nano-berberine with ct- DNA was thought to be spontaneous.

Conclusion: The competitive fluorescence experiment indicated that nano-berberine does not compete with AO and EB for binding to DNA. Viscometer studies showed that increasing the concentration of nano-berberine makes no clear changes in solution viscosity. Also, the absence of an effect of NaCl and KI in interaction of DNA with nano-berberine verified the groove mode.

Keywords: Nano-berberine, Calf thymus DNA, Transferrin, Fluorescence spectroscopy

P-199

The role of salt for autoimmune disease: Multiple sclerosis

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Background: An autoimmune disease is a condition arising from an abnormal immune response to a normal body part. In recent decades, scientists have observed a steady rise in the incidence of autoimmune diseases in the world.

Methods: This essay was a systematic review of English articles published in PubMed, Nature, Semantic scholar and Research gate since 2010. Being up to date, matching with keywords and accessing the full text were incoming metrics.

Results: Interleukin (IL)-17 producing T helper cells (Th17 cells) play a pivotal role in autoimmune diseases exactly in multiple sclerosis (MS). Researchers show that increased salt concentrations found locally under physiological conditions in vivo dramatically boost the induction of murine and human Th17 cells. Most researches demonstrated that excessive salt enhances the differentiation of Th17 cells, inducing a highly pathogenic phenotype that aggravates experimental neuroinflammation. First human studies revealed an association between increased MS disease activity with elevated salt consumption, while more recent epidemiology studies in larger cohorts suggest no correlation between salt intake and MS. Recent data from long-term studies with volunteers subjected to constant sodium intake have shown that urine Na^+ concentrations oscillate between day to day measurements. However, it is known that ordinary urinary sodium analyses and nutritional questionnaires do not necessarily correspond to the actual sodium load and more sophisticated analyses are needed.

Conclusion: Therefore, only further studies under less extreme conditions can show the extent to which increased salt intake actually contributes to the development of autoimmune diseases. Although more research is needed on the mechanisms underlying the impact of lifestyle on MS, studying the combined lifestyle factors simultaneously would help to enhance our understanding of the multifactorial impact on MS etiology.

Keywords: Autoimmunity, Sodium chloride, T helper (TH) 17 cells, Multiple sclerosis (MS)

P-200

Autophagy modulation can change differentiation capacity of immature pericytes toward different cardiac cells

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Background: To date, many attempts are made to increase the regenerative potential of stem cells. In this study, we evaluated the hypothesis of whether an autophagy modulation could alter differentiation potency of CD146+ cells into pericyte, endothelial, and cardiomyocyte lineage.

Methods: CD146+cells were enriched from the human bone marrow aspirates and trans-differentiated into endothelial cells, pericytes, and cardiomyocytes after exposure to autophagy stimulator/inhibitor. The protein levels of autophagy proteins were monitored by Western blotting. We also monitored the lineage protein and gene levels. Pro-inflammatory cytokine and angiocrine factors were measured by ELISA. The exosome secretion capacity was measured by AChE activity and real-time PCR assay.

Result: Data revealed the modulation of autophagy factors in differentiating CD146+ cells after exposure to Met and HCQ ($p < 0.05$). Real-time PCR analysis showed that the treatment of CD146+ cells with autophagy modulators altered the expression of VE-cadherin, cTnI, and α-SMA ($p < 0.05$). Met increased the expression of VE-cadherin, α-SMA, and cTnI compared to the HCQ-treated cells ($p < 0.05$) while Western blotting revealed the protein synthesis of all lineage-specific proteins under the stimulation and inhibition of autophagy. The treatment of cells with HCQ increased the levels of TNF-α and IL-6 compared to the Met-treated cells. Data revealed the increase of exosome biogenesis and secretion to the supernatant in cells treated with HCQ compared to the Met groups ($p < 0.05$).

Conclusions: In summary, autophagy modulation could alter differentiation potency of CD146+ cells which is important in cardiac regeneration.

Keywords: Human bone marrow CD146+ cells, Autophagy modulation, Nitrosative assay, Lineage-specific differentiation, Exosome activity

P-201

The production of astaxanthin and growth of the microalga Haematococcus pluvialis: Effect of sodium acetate in culture medium

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Background: Astaxanthin is a reddish-orange carotenoid, which has several properties, e.g. antioxidant, anticancer, antidiabetic, anti-inflammatory, and etc. One of the most important sources of astaxanthin is the green unicellular microalga *Haematococcus pluvialis* (*H. pluvialis*). The production of astaxanthin by this microalga contains two steps of green and red phase. The first stage is increasing the cell density under favorable conditions and in the second phase, accumulating the astaxanthin by performing stress conditions, e.g. nutrient limitation, salinity, light intensity, etc. Bold's Basal Medium (BBM) have been used as an optimal and repetitive medium to growing *H. pluvialis* in both stages. In this paper, the effect of sodium acetate as carbon source in BBM (BBM+SA) on the production of astaxanthin and growth of *H. pluvialis* was examined.

Method: The autoclaved BBM and BBM+SA media in 500 mL Erlenmeyer flasks were inoculated with 7.5×10^5 cells/mL. These media were kept on shaker incubator (350 rpm, 25±1°C) exposed to 70 μmol.m^{-2}s^{-1} light intensity. *H. pluvialis* was grown until late exponential phase and then harvested by centrifuging at 5500 rpm for 5 min at 5°C. Next, it was suspended in 500 mL Erlenmeyer flask of nitrogen free BBM (BBM-N) and BBM+SA (BBM+SA-N), exposed to high illumination intensity of 200 μmolm^{-2}s^{-1}. All samples were carried out in triplicate.

Results: Cell density in BBM and BBM+SA media reached 1.2×10^6 cell/mL (after 6 days) and 1.3×10^6 cells/mL (after 4 days), respectively. In the second phase, the amounts of astaxanthin produced in BBM-N and BBM+SA-N media were 36±1.01 mgL^{-1} (after 10 days) and 40±0.9 mgL^{-1}, respectively. BBM+SA showed the best growth with higher cell density and astaxanthin augmentation.

Conclusion: The culture medium of BBM+SA showed the maximum cell density 1.3×10^6 cells/mL and astaxanthin production of 40±0.9 mgL^{-1}. BBM+SA also reduced the growth period of *H. pluvialis* from 16 to 11 days.

Keywords: Astaxathin, Haematococcus pluvialis, BBM, BBM+SA

P-202

Evaluation of SELENBP1 gene expression in colorectal cancer cell line upon treatment with beta-lactoglobulin nano-particles carrying chemotherapeutic drugs.

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Background: Cancer is known as the second cause of death around the world. One of the most common cancers is colorectal cancer, which is considered as the fourth leading cause of cancer-related death. Tumor progression occurs as a consequence of changes in the expression of tumor suppressor genes or oncogenes. Irinotecan and 5-fluorouracil are two major drugs in the systemic chemotherapy of colorectal cancer. However, numerous side effects of these drugs undermine the effectiveness of the treatment. Encapsulation of these drugs into a beta-lactoglobulin nano-carrier, which is conjugated with folate receptor, not only makes the oral chemotherapy possible but also allows their specific targeting to the cancerous cells through receptor-mediated endocytosis.

Methods: In this study, the effects of these co-loaded protein nanocarriers on the viability of the HCT116 cell line were assessed using MTT assay and the IC50 values were determined. Moreover, the expression of one of the leading genes, SELENBP1, that changes considerably in colorectal cancer was also analyzed by Real-Time PCR before which the RNA was extracted and then used for cDNA synthesis.

Results: MTT assay analysis indicated that the encapsulation process reduced the IC50 value of these drugs. Besides improving cytotoxicity, the designed nano-drugs effectively decrease cell proliferation in a time- and concentration-dependent manner. The Real-Time melting curve showed specific annealing of the primers, and the data revealed that the new nano-particles can effectively increase the expression of SELENBP1.

Conclusion: All in all, upon using beta-lactoglobulin as a carrier, the anti-proliferative characteristic of the combination of Irinotecan and 5-fluorouracil was improved. Furthermore, the expression of the gene changed toward its normal level which probably makes the protein nano-particles loaded with chemotherapeutic agents a good candidate for colorectal cancer treatment.

Keywords: Colorectal cancer, Cancer, Beta-lactoglobulin, Irinotecan, Fluorouracil.

P-203

Antioxidant effects of c-phycocyanin against cisplatin-induced hepatotoxicity in Balb/c mice

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Background: Chemotherapy is associated with complications as a therapeutic approach. Cisplatin as a highly impressive medicine agent has side effects and hepatotoxicity at high doses. The phycobiliprotein c-phycocyanin(C-PC), extracted from Spirulina platensis, has been demonstrated to have a series of physiological and pharmacological attributes without leading to harm and toxicity. The aim of this study was to investigate the protective effects of C-PC anin on cisplatin-induced hepatotoxicity in Balb/c mice.

Methods: Fifty male Balb/c mice were tested in five groups. Group 0 was considered as control. Group 1 and 2 were fed by C-PC (1mg/kg) daily for 4 weeks via gavages. For induction of hepatic injury in groups 2-4, cisplatin (3mg/kg) was injected once every five days intraperitoeally. Group 3 received silymarin (100mg/kg) daily for 4 weeks via intraperitoneal route. Finally, serum levels of malondialdehyde, total antioxidant capacity, total billirubin, total protein and albumin and the activities of glutathione peroxidase, catalase, superoxide dismutase, alanine transaminase, aspartate aminotransferase, and lactate dehydrogenase were assessed.

Results: In treated group with cisplatin the levels of malondialdehyde (lipid peroxidation index) and billirubin and biomarkers of hepatic hurt increased and antioxidant enzymes (such as catalase), albumin and proteins decreased. In group 2, C-PC significantly ($P<0.001$) reduced the elevated amounts of alanin and aspartate transaminase, lactate dehydrogenase, billirubin and malondialdehyde and enhanced the diminished amounts of total proteins, albumin and hepatic antioxidant enzymes($P<0.001$). All to all, these data revealed that C-PC acted an effective scavenger and antioxidant agent in the C-PC-treated mice.

Conclusion: According to the results, due to its antioxidant properties, C-PC has a potent protective property against hepatotoxicity in Balb/c mice. These data suggest that the human nutrition of C-PC can be beneficial for prevention of liver diseases correlated to oxidative stress.

Keywords: C-Phycocyanin, Cisplatin, Toxicity, Antioxidant, Balb/c mice.

P-204

Determination of microbiota from the reared *Aedes aegypti* in insectary to introduce a suitable paratransgenic candidate for controlling the Aedes-borne diseases

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Background: The vector borne diseases are one important health challenges in all over the world and causes numerous morbidity and mortality annually. Vectors have a crucial role in development and distribution of some dangerous diseases such as: Malaria, Leishmaniasis, Dengue fever, etc. Vector control is one of the main aspects of fighting against Vector borne diseases and has long been considered by researchers and authorities as crucial elements to control these diseases from the past. Different approaches have been used for combatting against vectors. Latest WHO recommendations are designing the new insecticides, creation of transgenic insects and applying the paratransgenic method. Paratransgenic is a technique that insect-symbiont microorganisms are used to impact on the insect life duration or pathogen life cycle in the insect's body. One of the important vectors is *Aedes aegypti*, which is involved in transmission and distribution of a wide range of diseases such as zika, dengue fever and chikungunya. Therefore, we decided to identify the symbiont bacteria in the midgut of *Aedes aegypti* in this study and introduce the best ones for using them in the subsequent paratransgenetic studies to limit the transmitted diseases by this insect.

Methods: In this study, in controlled and sterile condition, the midgut of *Aedes aegypti* was dissected and a homogenous suspension was prepared in the sterile normal-saline solution. In the next step, Gram-positive and Gram-negative bacteria were isolated by using the culture dependent methods and their genus and species were determined by biochemical tests. Moreover, in cases that it was not possible to accurately determine the genus and species, sequencing the 16S rRNA gene and comparing the acquired sequences with the submitted sequences in the GenBank was performed.

Results: In this study, *Escherichia coli*، *Serratia marcescens* ، *Klebsiella michiganensis*، *Klebsiella oxytoca*, *Stenotrophemonas maltophilia*, *Asaia krungthepensis* ,*Asaia bogorensis* ، *Klebsiella pasteu*rii,*Enterobacter homaechei Hafnia (bacterium)*, *Salmonella enterica*, bacteria were identified in the midgut of the *Aedes aegypti* mosquitoes by using the culture dependent method and sequencing the 16s rRNA gene.

Conclusion: According to the previous performed studies and isolated bacteria, *Asaia* and *Seratia* have good properties for using in the paratransgenetic technique.

Keywords: paratransgenic, Microbiota, midgut, *Aedes*

P-205

Effects of PD-1/PD-L1 Blockers and Selenium on Augmentation of Anti-Tumor Immune Responses in the Colorectal Cancer

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Background: In colorectal cancer (CRC), interactions of immune/tumor cells through immunosuppressive proteins such as programmed-death1 (PD-1) and programmed-death ligand1 (PD-L1) reduce anti-tumor immune responses and increase tumor survival. In addition, inflammation and inflammatory factors such as cyclooxygenase-2 (COX-2) are the main factors in the tumorigenesis of CRC. Blockade of the PD-1/PD-L1 pathway by anti-PD-1/PD-L1 antibodies could inhibit the interaction of these immunosuppressive proteins in cancer. Additionally, anti-oxidant/anti-cancer elements such as selenium could decrease inflammation and inflammatory products. This review discusses the combination effects of PD-1/PD-L1 blockade along with selenium supplementation on the anti-tumor immune responses in CRC.

Methods: Relevant studies in electronic databases between 2000–2020 were selected by searching MEDLINE Library. Only relevant English published articles were included.

Results: Available evidence proposed that selenium and PD-1/PD-L1 blockade augment anti-tumor immune responses through multiple mechanisms. Selenium in the selenoproteins reduces inflammation via the reduction of reactive-oxygen-species (ROS) and DNA damages. In the other pathway, selenium decreases expression of the inflammatory protein, COX-2, by activation of AMP-activated protein kinase as a cellular-energy sensor. In addition, inhibited HIF-1α by selenium reduces expression of the PD-1/PD-L1 in tumor and immune cells. In the immune system, selenium upregulates phagocytosis of macrophages and dendritic cells, the proliferation of lymphocytes and, cytotoxicity of CD8+T lymphocytes and natural killer cells. Blockade of the PD-1/PD-L1 pathway suppresses PD-1 signaling and reactivates stimulatory signaling of CD28, which increases antitumor activities such as IFNγ and perforin/granzyme release by cytotoxic CD8+T lymphocytes and natural killer cells. Therefore, stimulatory signaling of CD28 is reactivated and cytotoxic activities such as IFNγ, perforin/granzyme releasing are enhanced.

Conclusion: The combination of PD-1/PD-L1 blockade with selenium supplementation might be a powerful anti-cancer therapy through increasing the IFNγ and perforin/granzyme release and decreasing the ROS, DNA damages, COX-2 and PD-1 and PD-L1 levels in CRC.

Keywords: Colorectal cancer, Selenium, PD-L1, PD-1, Inflammation, Immune Response.

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Effect of Tumor Lysate and Selenium on Anti-Tumor Immune Responses in Colorectal Cancer

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Background: In Colorectal cancer (CRC), high levels of inflammatory molecules such as TNFα, provide tumor-promoting-inflammation and consequently promote tumor survival and proliferation. Presentation of tumor-associated antigens such as carcinoembryonic antigen (CEA) to the immune system accompanied by anti-cancer agents could decrease tumor-promoting inflammation and elevate anti-tumor immune responses. Tumor lysates as the tumor cells fragments contain damage-associated molecular patterns (DAMPs) and tumor antigens. In addition, selenium as an essential trace element plays anti-tumor roles in tumor and immune cells. This review discusses mechanisms of tumor lysate and selenium on the immune system in colorectal cancer.

Methods: We searched relevant articles between 2000–2020, and only English published articles in EMBASE, Cochrane, and MEDLINE were included in this study.

Results: Evidence recommended that tumor lysate and selenium increase anti-tumor immune responses through multiple mechanisms. Membranous fragments of tumor cells introduce DAMPs and tumor antigens such as CEA to the monocytes and lymphocytes. DAMPs could interact with toll-like receptors (TLRs)1/2/4/6 on the macrophages and dendritic cells which activate intracellular TLR signaling pathways and transcription factors, NFKβ. TLR activation in antigen-presenting cells increases phagocytosis and tumor antigens (CEA) presentation to CD8+T lymphocytes. In addition, DAMPs -TLR1/2/4/6 interaction on the T lymphocytes, might stimulate CD4+/CD8+ T lymphocytes and increase recognition of CEA+ cells which eventually lead to elevated expression and secretion of IFNγ, IL2, and perforin/granzyme. On the other hand, selenium in the selenoproteins (glutathione peroxidase) suppresses TNFα expression and tumor-promoting inflammation by 1) reduction of reactive oxygen species, hydrogen peroxide, and nitric oxide, 2) stimulation of IL2 / IL2R expression and 3) augmentation of antitumor activated CD8+T/NK cells.

Conclusion: The combination of CRC tumor-lysates with selenium might augment the effects of tumor-lysate and selenium on the phagocytosis, tumor antigens presentation/recognition, and cytotoxicity which may play a role in the improvement of CRC.

Keywords: Colorectal cancer, Selenium, Tumor lysate, Immune Response.

P-207

An investigation into the effects of 6-gingerol on glutamine metabolism in breast cancer cell lines

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Background: One of the most significant signs of cancer is reprogramming of energy metabolism, especially enhancement of glutaminolysis to support the speedy growth of tumor cells. In this study, we investigated the effects of 6-gingerol on the glutamine metabolism of MCF-7 and SKBR3 breast cancer cells.

Methods: MCF-7 and SKBR3 cells were cultured with different concentrations of 6-gingerol (0, 5, 10, 25, 50 μM) for 24 h. Effects of 6-gingerol on cell viability were assessed. The expression of SLC1A5 (ASCT2) transporter and glutamate dehydrogenase genes was examined by real-time polymerase chain reaction. Glutamate dehydrogenase (GDH) activity was determined using spectrophotometry.

Results: Treatment with 6-gingerol demonstrated a dose dependent decrease in cell viability and resulted in reduced GDH specific activity. Our data also showed a significant down-regulation of GDH, SLC1A5 gene expression after 24 h treatment of MCF-7 and SKBR3 cells with 6-gingerol;

Conclusion: These findings suggested that 6-gingerol inhibited the proliferation of breast cancer cells and any reduction in cell viability was associated with reduced GDH enzyme activity and down-regulation of SLC1A5 gene expression, so this could indicate that 6-gingerol is beneficial for the treatment of breast cancer.

Keywords: Breast cancer, 6-Gingerol, GDH, SLC1A5.

P-208

The effect of Genistein on skeletal muscle inflammation of C57BL/6L mice fed a high-fat diet

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Background: Genistein (GEN), a well-known isoflavone mainly extracted from soybeans, has been demonstrated to exert beneficial effects on inflammatory responses, but the role of this polyphenol in obesity-induced skeletal muscle inflammation has not yet been well studied. This study aimed to investigate the effects of GEN skeletal muscle inflammation in high-fat diet (HFD) fed mice.

Methods: Thirty C57BL/6 male mice were categorized into two dietary groups for 10 weeks; normal chow diet (n=10) and a high fat diet (n=20). After this period, half of the HFD-fed mice were fed with HFD-supplemented with 0.2% Genistein [HFD+GEN] for 16 weeks. At the end of the study, the gastrocnemius muscle was excised and real-time PCR was performed to evaluate the expression levels of pro-inflammatory cytokines. A portion of the skeletal muscle was fixed in 10% (v/v) neutral buffered formalin for hematoxylin and eosin (H&E) staining.

Results: The GEN groups had a lower level of fasting blood glucose (FBG), and body weights compared with HFD. While HFD feeding led to the polarization of macrophages to the M1 direction, as well as increasing the expression of a number of M1 pro-inflammatory cytokines [tumor necrosis factor α (TNF-α), interleukin 1 β (IL-1β) and interleukin 6 (IL-6)], GEN could ameliorate inflammation by switching macrophages into M2 phenotype and decreasing the expression of pro-inflammatory cytokines. GEN treatment was also able to increase IL-10 and decrease Chemokine MCP-1 in mice fed HFD.

Conclusion: These results suggest that the GEN ameliorates inflammation in skeletal muscle of an HFD - induced model of obesity.

Keywords: Genistein, HFD, IL-6, TNF-α, IL-1β.

P-209

Anticancer potentiality and mode of action of naturally occurring non-glycosylated proteins from mushrooms

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Background: Mushroom-derived proteins are among the naturally occurring compounds that have been the subject of a body of research on their potentiality in cancer therapy. In this regard, the greatest attention (both in research and review articles) has been paid to well-known mushroom-derived glycoproteins (such as lectins, PSK, PSP) containing 50-90% carbohydrates. However, a number of other studies have been conducted on mushroom-derived anticancer proteins containing low or no amounts of carbohydrates. So far, no review has addressed the achievements made in this regard. In addition, findings on specific mechanisms of action and pathways related to these protein types have not been summarized.

Methods: In order to collect the latest information on the mushroom species containing anticancer proteins with low or no carbohydrate; we searched important databases such as Medline, THPdb, and Scopus, using keywords “low carbohydrate proteins”, "anti-cancer", and "mechanism of action".

Results: A significant number of naturally occurring proteins with or without carbohydrate were found in various mushroom species, which have been reported to exert their activities through different pathways than glycoproteins. For the first time, we termed these mushroom-derived proteins as “non-glycosylated proteins” (NGPs), and divided them into four groups: fungal immunomodulatory proteins (FIPs), ubiquitin-like proteins, enzymes, and unclassified proteins. Specific pathways of NGPs included DNase activity, endoplasmic reticulum stress, PI3K/Akt/mTOR signaling pathway, and ubiquitin-mediated pathway, which were illustrated as a diagram.

Conclusion: The most promising NGPs include natural FIPs (from various mushroom species), RBUP (from Ramaria botrytis), AAD (from Agrocybe aegerita), marmorin (from Hypsizigus marmoreus), PSULP (from Pleurotus sajorcaju), CULP (from Calvatia caelata), and an unnamed ubiquitin-like peptide (from Agrocybe cylindracea). Purity, specific cytotoxicity against cancer cells, having a well-defined structure, full-length (or N-terminal) amino acid sequences, and distinct pathways have made mushroom NGPs among the best scoring candidates for further clinical settings in cancer research.

Keywords: Anticancer, Mode of action, Mushroom, Non-glycosylated protein.

P-210

P53 gene expression correlates with AgNOR features in patients with liver cirrhosis

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Background: P53 is a tumor suppressor that controls tumorigenesis in different tissues including the liver. Nucleolar organizer regions (NORs) are active portions of chromosomes that are present in the nucleoli and contribute to ribosomal synthesis. An alteration in the AgNORs features indicates changes in proliferation rates and differentiation processes. The present study aimed to investigate the p53 gene expression and its correlation with Total AgNOR Number, Total AgNOR Area, and Total AgNOR Length in cirrhotic patients.

Methods: P53 gene expression and NORs were analyzed in 38 cirrhotic liver tissue samples with different etiologies including nonalcoholic steatohepatitis (NASH), autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC), alcoholic hepatitis (ALH), and viral hepatitis (HBV and/or HCV) (n=8/group, ALH: n=6) and in 10 control liver samples by the quantitative Real-Time polymerase chain reaction (qRT-PCR) and the AgNOR methods, respectively.

Results: Our results showed that the p53 gene expression was significantly higher in AIH-, NASH-, and PSC-related cirrhosis than in control ($p<0.01$). Also, it was observed that the p53 gene expression had significantly positive correlation with Total AgNOR Number ($r=0.374$; $p=0.009$), Total AgNOR Area ($r=0.444$; $p=0.002$), and Total AgNOR Length ($r=0.443$; $p=0.002$).

Conclusion: Considering the findings of the present study along with the results of the previous studies, it can be concluded that increased hepatic p53 gene expression in patients with AIH-, NASH-, and PSC-related cirrhosis and its positive correlation with AgNOR features may be associated with proliferation potential in cirrhotic livers. Therefore, they can be used as potential prognostic markers for cirrhotic patients, especially in patients with AIH-, NASH-, and PSC-related cirrhosis.

Keywords: P53, Nonalcoholic fatty liver disease, Cirrhosis.

P-211

A single amino acid replacement (D226N) influences macromolecular association of retinal IMPDH1 isoforms: a possible clue to RP disease

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Background: Inosine monophosphate dehydrogenase (IMPDH), plays important roles in purine metabolism and cell homeostasis. The enzyme catalyzes the rate-limiting step in the GTP biosynthetic pathway, through NAD^+-dependent oxidation of IMP to XMP. Two mouse IMPDH1 isoforms are produced by alternative splicing, including 514 and 603. Mutations inside the IMPDH1 coding region are linked to adRP10 disease, a sort of eye blindness. One of the adRP10-causing mutations is D226N replacement. This mutation in IMPDH1 isoforms changes purine nucleotide-dependent allosteric regulation and fibrillation of the enzyme.

Methods: After overlap extension PCR mutagenesis, cloning, expression and affinity purification of mouse retinal isoforms, we evaluated the structure of the mutated and wild types of enzymes in the presence or absence of purine nucleotides by Sephadex G-200 size exclusion chromatography. We incubated identical concentrations of the enzymes in the assembly buffer (50mM Tris, pH 7.4, 100mM KCl, 1mM DTT) plus 3mM IMP, 5mM NAD for 15 min at room temperature in the presence of 0.1mM of either ATP or GTP.

Results: Our chromatography results indicated that the purified mutant retinal isoforms form macroaggregates relative to the wild type enzyme. In addition, multifold activity enhancement of the retinal isoforms under the influence of ATP was parallel to the formation of macromolecular associates of homotetramers. Unlike the wild type isoforms, the macromolecular associates of the mutant enzymes did not lose their catalytic activities upon exposure to GDP/GTP, the common inhibitors of IMPDHs.

Conclusion: Based on our data, it could be concluded that D226N replacement in the mutated IMPDH affects macromolecular assembly of the enzyme with final impacts on binding sites of some of the activity-related modulators.

Keywords: IMPDH, Retina, GTP

P-212

Recent advances on the biosensing and bioimaging based on polymer dots as advanced nanomaterial: Analytical approaches

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Background: Polymer dots (Pdots) have emerged as a great class of fluorescence probe in biosensing, and bioimaging. As an advanced nanomaterial, Pdots generally have high fluorescence brightness and high photo stability. This article aims to describe various features of the Pdots currently used for biosensing and bioimaging. Also, the structure, synthesis, advantages, and disadvantages of Pdots were discussed.

Methods: All relevant information has been collected by reading the relevant articles in this field until 2020.

Results and conclusion: In this paper, the recent research progress of polymer dots-based nanomaterials, focusing on their optical biosensing and bioimaging applications were discussed. The reason for Pdots use in biosensors is high brightness and efficient modulation of their energy transfer. Other uses of Pdots include easy to use and chemically controlled surfaces for great applications in biomedical imaging and measurement. Consequently, we discuss several outstanding properties of the optical methods, research opportunities and the development potential and prospects. Bioimaging is an important method to get insights into biological processes. In the past decades, various techniques including magnetic resonance imaging (MRI), computerized tomography (CT), ultrasound imaging, and positron emission tomography (PET) were expanded to create images of organs, veins, and cells. Bioimaging is an important method to get insights into biological processes.

Keywords: Polymer dots, Nanotechnology, Optical biosensing, Bioimaging, Biomedical analysis, Biotechnology, Advanced nanomaterial

P-213

Effects of sample storage conditions on HbA1c levels

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Background: Glycated hemoglobin (HbA1c) is an important indicator of blood glucose level and is used as a gold standard for measuring the long-term glycemic control and quality of care in diabetic patients. It should be noted that different sample storage conditions may affect HbA1c concentrations and thus, clinician’s diagnosis. In the present study, we evaluated the effects of different temperatures of storage over time on HbA1c results.

Methods: This present study was conducted in the laboratory of the Diabetes Research Center, affiliated to the Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, during the year 2018. The study protocol was approved by the Institutional Review Board at the Endocrinology and Metabolism Research Institute. In In this study, a total of 40 fresh whole blood samples with different levels of HbA1c were selected for separate HbA1c assessments at 3 various temperatures (-20°C, 4°C, and 25°C) on subsequent days (0, 7, 14, and 21 days after sample collection) with COBAS INTEGRA 400 assays (Roche Diagnostics, Mannheim Germany).

Results: The mean value of HbA1c at initial measurement (7.05 ± 1.45) was insignificantly higher than results of HbA1c stored at -20°C (6.89% ± 1.48% after 7 days, 6.85% ± 1.47% after 14 day, 6.89% ± 1.46% after 21 days) and 4°C (6.94 % ± 1.46% after 7 days, 6.91% ± 1.49% after 14 days, 6.92% ± 1.48% after 21 days). Nevertheless, when it was compared to the concentrations of HbA1c at the temperature of 25°C (6.08% ± 0.86% after 7 days, 5.52% ± 0.8% after 14 days, 4.81% ± 0.66% after 21 days), we observed significant changes.

Conclusion: It seems that that the refrigerator or freezer storage temperature is appropriate for the assessment of HbA1c by COBAS INTEGRA 400 in clinical trials and large-scale epidemiological studies.

Keywords: Diabetes, HbA1c, COBAS INTEGRA 400

P-214

An ultrasensitive sandwich immunosensor for detection of breast cancer specific carbohydrate (CA 15-3)

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Background: Breast cancer is the most common threat in women worldwide. Increasing death rate of diagnosed cases is the main leading cause of designing specific immunoassay for tumor marker in breast cancer. Cancer antigen 15-3 (CA 15-3) is a tumor protein for many types of cancer, most notably breast cancer. Herein, we report a sandwich-type electrochemical immunosensor based on signal amplification strategy of multiple nanocomposites for detection of CA 15-3 biomarker.

Methods: The proposed immunosensor was fabricated by reduced graphene oxide (ERGO), poly-dopamine (P(DA)) and amino functionalized mesoporous silica (MCM-41-NH2) doped by gold nanoparticles composite on the glass carbon electrode (GCE) with a large surface area in a new platform for immobilization of primary antibodies (Ab1) and provision of excellent conductivity. To further amplify the electrochemical signal, the trace tag on the foundation of gold nanoparticles (AuNPs) was coated with MCM-41-NH2 nanocomposite through thionine linking, to provide more amino groups to capture more horseradish peroxidase-labeled antibodies (HPR-Ab2) and enhance the conductivity.

Results: Under optimal conditions, the developed immunosensor exhibited excellent analytical performance for the determination of CA 15-3 with a wide linear range from 0.002 to 125 U/mL and a low limit of quantification (LLOQ) of 0.002 U/mL. Furthermore, satisfactory results were obtained for the determination of CA 15-3 in human plasma samples, indicating the potential of the immunoassay to be applied in clinical analysis.

Conclusion: In summary, an effective and ultrasensitive sandwich-type immunosensor was successfully fabricated to detect CA 15-3 in human plasma samples. Functionalized AuNPs/Thi/MSNP-NH2 nanocomposite was used as the signal amplifying probe to fix HRP-anti-CA 15-3 and unique nanocomposite ERGO-P(DA) as the supporting substrate. All these features demonstrated that the proposed immunosensor can be used as a versatile platform for other biomarkers and may have application potential in biological and clinical diagnoses.

Keywords: Tumor protein 15-3, Electrochemical immunoassay, Biomedical analysis, Sandwich-type immunosensor, Gold nanoparticle

P-215

A novel electrochemical magnetic immunosensor based on Fe3O4 magnetic nanoparticle and cysteamine functionalized AuNPs for detection of prostate specific antigen (PSA)

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Background: Prostate cancer is the most common threat in men worldwide. Increasing death rate of diagnosed cases is the main leading cause of designing specific immunoassay for tumor marker in prostate cancer. Prostate specific antigen (PSA) is a serine protein, which is one of the common proteins in semen and it can be transmitted to the blood stream

Methods: In this study, we report a magnetic sandwich- type immunoassay based on signal amplification strategy toward ultrasensitive detection of PSA biomarker, for this purpose, iron oxide magnetic nanoparticles (Fe_3O_4) and drop-casted on the surface of glassy carbon electrode (GCE) after adding PSA antigen. Also, secondary antibody (HRP-Ab2) encapsulated on gold nanoparticles caped by cysteamine was immobilized on the surface of GCE modified electrode.

Results: A transmission electron microscopy images (FE-SEM) showed that the gold nanoparticles were successfully capsulated to the surface of magnetic nanoparticles through sandwich immunoreaction events. The parameters of immunoassay, including the loading of magnetic nanoparticles, the amount of gold nanoparticle conjugate, and the immunoreaction time, were optimized. Under the optimized operating conditions, the prepared immunosensor presented a suitable linear range from 0.001 to1 µg. L^{-1} (by SWV) with a low limit of quantification (LLOQ) of 0.001 µg/L^{-1}.

Conclusion: It is found that such magneto-bioassay could be readily used for simultaneous parallel detection of multiple proteins by using multiple inorganic metal nanoparticle tracers. The good performance might be attributed to the enhanced surface area, excellent electrical conductivity, fast electron transfer, good catalysis properties and desirable biocompatibility of the substrate. All of these features demonstrate that the proposed immunosensor can be used as a versatile platform for other biomarkers and has application potential in biological and clinical diagnoses.

Keywords: Antibody binding, Magnetic nanoparticles, Conductive interface, Gold nanostructure, Encapsulation

P-216

Molecular identification of ophtalmomyiasis flies and its incidence rate in referred patients to Khalili Ophthalmology Clinic, Shiraz, Iran 2019

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Background: Myiasis is the infestation of fly larvae in living or dead tissues of the human body or animal. The ophtalmomyiasis is divided into internal and external types. The external type includes eyelid and conjunctiva disorders, and larvae penetrate into the eye in the internal type. The aim of this study was morphomolecular identification of ophtalmomyiasis flies and determining the ophtalmomyiasis incidence rate in referred patients to Shiraz ophthalmology clinics in 2019.

Methods: During one year, all the isolated larvae form the eyes of patients were identified by the morphological method according to the Zumpt, 1965 diagnostic key in the first step and then molecular confirmation was performed using a pair of specific primers for the cytochrome oxidase I (COI) gene. The expected amplicons were sequenced and their results were aligned and analyzed using the nucleotide BLAST. In this study, 224 larvae were collected.

Results: According to the morphological analysis, all larvae belonged to the genus and species of Oestrus ovis, and bioinformatics analysis confirmed the morphological results. Patients who presented the symptoms of acute conjunctivitis had a mean age of 34 years and those were 4 females (11%) and 32 males (89%). The highest incidence was related to autumn (55.8%). On examination of their conjunctiva and cornea, some larvae were found, and the symptoms were resolved within a few hours after removal. According to the conducted studies in Iran and neighboring countries, it is expected that the cases of ophtalmomyiasis are higher than reported in the published articles. It might be due to the absence of a regular monitoring program to care for the ophtalmomyiasis cases in the public health system.

Conclusion: Therefore, due to the importance and potential incidence of this disease in Fars province, the establishment a disease care program is necessary in the health surveillance system.

Keywords: Morphomolecular, Oestrus ovis, ophtalmomyiasis, Shiraz, Iran

P-217

Recent advances on aptamer-based biosensors to detection of circulating tumor cells (CTCs)

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Metastasis is the leading cause of cancer deaths. Circulating tumor cells (CTCs) are cells that are secreted from the primary tumor into the bloodstream and are known to be the main stimuli of metastasis. These cells are very important for identifying cancer cells and managing their growth and have received a lot of attention from researchers in recent years. Lately, biosensors have become a vital technology in laboratory medicine, especially in the field of care testing. Biosensors are analytical devices that use biological/biochemical reactions to detect target analyzes and consist of a biological element and a transducer. Aptamers, as biocatalysts, are single-stranded DNA or RNA molecules that can bind to small molecules, proteins, and cells with high specificity and affinity. The unique characteristics of aptamers over antibodies include durability, flexibility in labelling, denaturation resistance, stability, etc., and make aptamers excellent candidates for biological analysis and biosensors. In this review, studies related to aptamer-based biosensors for CTCs detection have been summarized, and we attempt to describe and compare the performances of recently developed CTCs aptasensors with different detection methods. To the best of our information, there are a few review articles about aptamer-based biosensors (aptasensors) for detection of CTCs have been published, in this review, we reported the most recent studies in this field.

Keywords: Aptasensors-Biosensors-CTCs

P-218

Microfluidic devices for clinical diagnostic

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Diagnosis is the first step in treating a disease that helps identify the cause and condition of a patient. Therefore, due to the need for rapid results in clinical diagnosis, the development of Point of care (POC) diagnostic tools has made significant progress in recent decades. POC diagnosis is used in the early detection of some diseases such as cardiovascular disease, diabetes, cancer and monitoring of health conditions. Microfluidic technology is utilized to fabricate cost-effective portable POC devices that can be employed to perform laboratory analysis in a short time with a small volume of the specimen. This reduction in time is very valuable in patients with traumatic conditions such as preoperative bleeding, critical illness, and severe blood loss due to coagulation abnormalities. Microfluidics is palm-sized devices or chips that contain micro-scale channels and circuits that are connected by small tubings and fluid samples circulate inside them. Depending on the purpose and application of the device, various materials such as polymers, glass and silicone are used to make the system that micro-channels are etched or moulded into the material. These systems allow quick and easy detection in remote areas with non-existent or limited-resource healthcare settings. This can be done by integrating POC devices with other technologies such as the Internet, Wi-Fi or Bluetooth. In fact, the merger of these portable tools with mobile phone connectivity for computing and analyzing huge data leads to remote monitoring of diseases and their control, as well as improving communication between doctor and patient. Due to the exceptional quality and efficiency, cheapness, portability and effective results with an insignificant error rate, the future of microfluidic devices in clinical diagnosis is extremely promising.

Keywords: Microfluidic, Point of care, Clinical diagnosis, Portable device

P-219

An innovative thermostable enzyme-based biosensor toward detection of L-proline

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Background: Proline as a non-essential amino acid plays an exceptional role in metabolic regulation. Proline metabolism is very important in cancer reprogramming. Therefore, due to the importance of proline detection, an innovative enzyme-based electrochemical biosensor was developed to selectively detect it.

Methods: The proline dehydrogenase (PRODH) enzyme was first entrapped onto the magnetic mesoporous silica nanoparticles (M-MSNPs) and then immobilized on the surface of glassy carbon electrode modified with poly-cysteine supported beta-cyclodextrin. The prepared electrode was utilized as an electrochemical biosensor for electrooxidation and specific detection of L-proline in phosphate buffer solution at physiological pH. In order to prove the technology, engineered biosensor was also employed to detect L-proline in biological specimens. Due to the good performance of the biosensor, it was also used to detect L-proline in normal cell-L929 and some cancer cells such as colon cancer cell-SW, colon cancer cell-HCT breast, breast cancer cell-MCF7, and gastric cancer cell-CAT3.

Results: The results revealed that L-proline oxidation is irreversible and was controlled by diffusion. The outcomes of studying the thermal stability of the prepared enzyme electrode displayed that the electrocatalytic activity of PRODH loaded in the M-MSNPs was preserved towards the oxidation of L-proline in the analytical solution up to 70 °C. Under optimized conditions, the concentration range of L-proline was 1 to 100 mM and 1 µM to 1 nM and the detection limit was 1 nM.

Conclusion: The proposed approach resulted in an increase in the resistance of the enzyme loaded in the M-MSNPs against temperature compared to the free enzyme. The developed biosensor can be used as a rapid, sensitive and selective method for the determination of L-proline in biological specimens.

Keywords: Proline dehydrogenase (PRODH), L-proline, Enzyme-based biosensor, Magnetic mesoporous silica nanoparticles (M-MSNPs)

P-220

Interaction of doxorubicin with human hemoglobin in presence of glucose: UV-visible and protein stability studies

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Background: Doxorubicin (DOX) is an anticancer drug with toxic side effects that may result from its interaction with the hemoglobin (Hb). Interaction of DOX with Hb alters DOX pharmacokinetics.

Method: In this study, structural changes imposed by DOX on Hb, in the absence and presence of glucose (Glc) were investigated, using UV-vis spectroscopy and protein thermal stability studies. Hb was purified from fresh human blood. The concentration of Hb fractions was determined spectrophotometrically at 541 nm. Appropriative concentrations of DOX were incubated with Hb for 2 hours at 37°C in phosphate buffer. Different concentrations of Glc were mixed with Hb and DOX. Absorbance spectra of samples were recorded in the range of 250-500 nm against free drug and Glc. Also, Thermal stability of Hb was investigated in the presence of Glc in different situations.

Result: There were two distinct peaks at absorption spectrum of DOX in the range of 200–600 nm, a sharp peak at 230 nm and another wide peak at 480 nm. Absorption spectrum of Hb under the same condition shows two peaks at 280 nm and 410 nm corresponding to the tryptophan and heme group, respectively. The results showed that Glc increases the absorbance of DOX and the hyperchromic effect. Thermal stability studies showed that melting temperature of Hb increased in the presence of Glc concentrations alone (300, 500 mg/dl) from 68.47°C to 69.47°C and 70.47°C. This effect was also observed in the presence of DOX (alone). But thermal stability of Hb decreased in presence of Glc/DOX (combined). Melting temperature of Hb decreased from 69.47°C to 67.47°C and 66.47°C.

Conclusion: Therefore, it seemed that DOX induces protein unfolding and relaxation that leads to hyperchromicity. Glc molecules may reduce the interaction between DOX and Hb through allosteric effects.

Keywords: Doxorubicin, Hemoglobin, Glucose, Uv-vis Spectroscopy, Protein Stability

P-221

The effect of Moringa Olifera leaf extract on antioxidants and free radicals in diabetic rats

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Background: Diabetes is a metabolic disorder of the endocrine system that disrupts the secretion of the hormone insulin which causes high blood sugar in patients. The basic treatment for this disease is the administration of insulin and some chemical drugs to lower blood sugar. Due to the side effects of chemical drugs, the use of herbal medicines is recommended.

Methods: Thirty adult male Wistar rats weighing 200 ± 50 were distributed semi-randomly to 6 groups of five. Group 1 (control), group 2 (diabetic), group 3 (diabetic + extract at a dose of 100 mg / kg WB), group 4 (diabetic + extract at a dose of 200), group 5 (diabetic + extract at a dose of 400), group 6 (diabetic + metformin at a dose of 100). Diabetes in animals was induced by a single intraperitoneal injection of streptozotocin at a dose of 55 mg/kg body weight in rats. Diabetic rats received three doses (100, 200, 400 mg/kg body weight) of the extract and a group of diabetic rats was treated with 100 mg/kg body weight of metformin; the control and diabetic groups without treatment received distilled water by gavage for 21 days. Cardiac blood samples were taken from all rats and in addition to blood, rat liver tissue was used to evaluate FRAP and MDA. Data obtained from blood and tissue factors were compared using statistical analysis.

Results: Comparing the groups treated with the extract with the diabetic group without treatment, there was a significant decrease in the amount of MDA (serum) and MDA (hepatic) while a significant increase was noted in the amount of FRAP (serum) and FRAP (hepatic).

Conclusion: The results showed that Moringa Olifera extract can be effective in preventing and reducing the complications of diabetes, reducing free radicals and increasing antioxidants.

Keywords: Diabetes, Moringa olifera, Extract, Rat, Streptozotocin

P-222

Significant role of oncogenic mutant phenotype of KRAS G12D and tissue-specific expression patterns of miR-21 and miR-148a as well as CR-1 in sporadic CRC transformation

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Background: Colorectal cancer (CRC) is classified as a most prevalent member of the idiopathic colorectal mucosal disorders group. Various genetic and epigenetic lesions, as well as protein diversity, can influence the tumor transformation process in sporadic or nonhereditary CRC.

Methods: From a total 85 tissue specimen, including 15 precancerous adenomatous polyps/adenomas and 35 CRC with matched normal tissue, total RNA was extracted for miRNA qPCR analysis. Furthermore, KRAS somatic mutations and pattern expression of Cripto-1 (CR-1) protein were also detected by DNA Sanger sequence and immunohistochemistry (IHC) assays, respectively.

Results: According to our findings, spanning of KRAS somatic mutations were only attributed to codon12 (p. G12D), respectively, 13.3% in adenomas and 36% in CRC. On the other hand, miR-21 epigenetic alterations were manifested strongly as a significantly increased expression status in colorectal cancerous mucosa versus precancerous adenomas or matched normal mucosa ($p < 0.001$). Conversely, miR-148a expression changes were characterized by significantly decreased expressions between colorectal adenomas and colorectal adenocarcinoma mucosa against normal matched mucosa ($p<0.001$). IHC expression analysis of CR-1 protein showed a higher staining quantity in CRC than adenomatous polyps and normal control ($p < 0.001$); besides, there were different expression properties in the polyp group compared with the paired normal group ($p < 0.05$).

Conclusion: The results suggested that oncogenic mutant phenotype of KRAS G12D and tissue-specific expression patterns of miR-21 and miR-148a along with CR-1 could be significant in sporadic CRC transformation.

Keywords: CRC, KRAS G12D, miR-21, miR-148a, CR-1.

P-223

The effect of glucose on doxorubicin and human hemoglobin interaction: Characterization with fluorescence and CD spectroscopies

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Background: Doxorubicin (DOX) is one of the most effective antibiotics in cancer chemotherapy. But its use is limited due to its cardiac toxicity. It has been found that iron facilitates the development of DOX toxicity. More than 50% of the total iron content in the normal adults is estimated to associate with Hb. Therefore, understanding the role of Hb interaction with DOX may have important clinical implication in the presence of Glc as a main blood sugar. So probable Glc influence on Hb and DOX interaction may be different in normal and diabetic subjects.

Method: We investigated Hb structural changes, caused by interaction with DOX, in the presence of different concentrations of Glc. After purification of Hb, the CD measurements in the absence and presence of DOX were made in the range of 200–250 nm at 0.2 nm intervals. The samples for CD were prepared with the fixed concentration of Hb and various drug concentrations. Fluorescence spectra were recorded in the range of 300–500 nm upon excitation at 280 nm.

Result: Incubation of Hb with DOX induced hypochromicity in the emission spectrum of intrinsic fluorescence. It can be seen that higher concentrations of DOX lead to more effective quenching of the chromophore molecule fluorescence. In the presence of Glc, the reduction effect of DOX in the intrinsic fluorescence of Hb is reduced and also the emission peak is displaced to higher wavelengths. CD results showed that DOX reduces α-helix structure of Hb. The level of the α-helix significantly decreased in combination with DOX/Glc.

Conclusion: Based on the results, Glc influences the Hb/DOX interaction and suppresses the DOX effects on Hb structure by exposing tryptophan residues. The quenching of the Hb fluorescence clearly indicated that the binding of the drug to Hb changes the microenvironment of fluorophores.

Keywords: Doxorubicin, Hemoglobin, Glucose, CD Spectroscopy, Fluorescence spectroscopy

P-224

Preparation and evaluation of laccase-loaded organic-inorganic hybrid nanoflowers as a biocatalyst

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Background: Growing demand for efficient biocatalysts has led to innovations in designing supports for enzyme immobilization. Flower-shaped nanostructures, organic-inorganic hybrid nanoflowers (HNFs), are constructed by coordination of nitrogen atoms in enzyme structure with bivalent metallic atoms. Large surface area of HNFs enhances enzymatic activity by overcoming mass transfer limitations. In this regard, laccase-loaded HNF was prepared by sono-chemical technique and the activity and reusability of HNFs were determined.

Methods: Laccase-loaded HNFs were prepared by addition of copper (II) sulfate to laccase solution (PBS, 1X) in a liquid ultrasonic bath. Blue precipitates were washed three times and centrifuged at 5500 rpm followed by drying under vacuum at ambient temperature. Cupric phosphate powder was also prepared by the same procedure without incorporation of the enzyme. Formation of Flower-like structures was evaluated by scanning electron microscope (SEM) images. Activity and reusability of HNFs were assessed by measuring the resultant absorbance at 420 nm after sequential incubation with 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) as the enzyme substrate. The fabricated HNFs is a potential laccase-based biocatalyst for bio-synthesis of valuable substances and/or bio-removal of organic pollutants.

Results: Formation of flower-like nanostructures was confirmed by SEM images. Contrary to some immobilization techniques, incorporation of laccase inside HNFs enhanced the enzyme activity. It is presumed that copper ions are responsible for the increased enzyme activity in comparison with the free enzyme. Also, HNFs have shown excellent reusability.

Conclusion: Organic-inorganic hybrid nanoflowers are becoming more popular due to their unique characteristics. Incorporation of laccase into HNFs induced the enzyme activity and reusability. Fabricated laccase-loaded HNFs could be used as promising biocatalyst for bio-synthesis of valuable compounds and/or bio-removal of organic pollutants.

Keywords: Biocatalyst, Nanoflower, Laccase, Pollution, Enzyme immobilization

P-225

Curcumin and cinnamaldehyde ameliorate the harmful effects of the nicotine, in the endometrial stromal cells

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Background: Nicotine plays an important role in disrupting the secretion of the estrogen and progesterone, the important hormones in the female reproductive system. The effects of nicotine on estrogen and progesterone receptors are still unclear. Cigarette smoking include nicotine, may cause infertility and endometriosis. Considering the importance of fertility, and the fact that, quitting smoking is a very time-consuming and difficult task, it seems necessary to confront and reduce the effect of this substance as much as possible. In this study we investigated the ameliorated effects of curcumin and cinnamaldehyde on the stromal endometrial cells, treated with nicotine.

Methods: Stromal endometrial cells were extracted from endometrial tissues of healthy volunteers. The stromal cells confirm with Anti Vimentin Ab, with immunocytochemistry assay. The cells were treated with nicotine (5-10 M) and divided into the groups of: treated with curcumin 5-10 M, treated with cinnamaldehyde 5-10 M, treated with curcumin and cinnamaldehyde. The expression of the ERa, PR, and VEGF genes in each group were evaluated with real time PCR.

Results: The real time PCR results indicated that the nicotine alone, significantly decreased the expression of Era, PR genes and increased expression of VEGF genes. There was a significant decrease in the expression of the VEGF genes in the stromal cells treated with curcumin and cinnamaldehyde. Curcumin and cinnamaldehyde significantly increased the expression of ERa, PR genes respectively. The combination of the curcumin and cinnamaldehyde synergically decreased the expression of the VEGF and increased the expression of the ER α and PR, respectively.

Conclusion: Curcumin and cinnamaldehyde ameliorated the harmful effects of the nicotine, in the endometrial stromal cells. More studies are needed to confirm the protective effects of curcumin and cinnamaldehyde against the cigarette smoking and infertility in women.

Keywords: Curcumin, Cinnamaldehyde, Nicotine

P-226

Investigating the relationship between infertility and its markers with stress and depression in women

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Background: Nowadays, about 50 to 80 million people in the world suffer from infertility. Stress and anxiety and depression have clear and undisputed role in infertility. What is not clear is whether infertility causes stress and anxiety or vice versa. In this study, we investigated stress and anxiety status and the fertility hormones levels in the infertile women.

Methods: One hundred and fifty women were participated in this study. The volunteers was asked to fill out a stress and anxiety and beck's questionnaires. The serum levels of cortisol, AMH, LH, FSH and inhibin B markers were measured with the electro-quantitative luminescence and ELISA techniques. Data analysis were done with ChiSquare, independent t-test, Pearson correlation coefficient, one-way analysis of variance and linear regression at the significance level of alpha coefficient < 0.05, with the SPSS software version 24.

Results: One hundred infertile women (age 34.4± 7.1) and fifty fertile control (age 34.2± 6.2) participated in this study. We found a significant difference between LH, FSH and AMH serum levels of the case and control groups (p> 0.05). Surprisingly, no significant difference was observed between cortisol and inhibin B concentrations between groups (p> 0.05). Also, there was a significant difference in the depression status, between two groups (p = 0.003).

Conclusion: According to the results, depression is a major problem that is closely related to infertility and its markers. Depression seems to act like a double-edged sword for female fertility, and we still need more studies to be able to definitively state the role of depression as the cause of infertility. It is very critical to pay attention to different aspects of infertility and especially to women's psychological condition to achieve successful fertility.

Keywords: Cortisol, AMH, LH, FSH, Depression

P-227

Investigation of insulin-like growth factors/insulin-like growth factor binding proteins regulation in metabolic syndrome patients

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Background: The insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) are thought to play a significant role in metabolic pathways and glucose metabolism. Unregulated levels of IGFs/IGFBPs have been associated with the development of glucose intolerance and metabolic syndrome X (MSx). We hypothesized that change of IGFs/IGFBPs levels could increase the risk of MSx; thus, this study aimed to evaluate the serostatus of IGFs/IGFBPs in individuals with MSx.

Results: After adjustment for metabolic parameters, MSx patients had a lower level of IGF-1, IGFBP-1, and IGFBP-2 compared with subjects in the control group. Further analysis revealed a positive correlation between serum levels of IGF-1 and IGF-2 ($p < 0.05$), as well as serum IGFBP-3 and IGF-2 ($p < 0.05$). Also, the statistical analysis showed a negative association of serum IGF-1 with plasma glucose and total cholesterol levels ($p < 0.05$). Besides, a negative relationship was found between serum concentrations of IGF-1/IGF-2 and the risk of developing MSx. These data indicated that some components of IGFs/IGFBPs are linked with the pathogenesis of MSx.

Conclusion: These inverse associations showed a possible linkage between the IGF/IGFBP signaling pathway and the development of MSx. The decreased concentrations of IGFs may be regarded as a potential biomarker for early diagnosis or even prognosis of MSx but more systematic studies are needed.

Keywords: Metabolic syndrome X, Insulin-like growth factor, Insulin-like growth factor receptor

P-228

Caffeic acid phenyl ester modulates the effect of nicotine on the endometrium receptivity markers

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Background: Nicotine adversely affects the female reproductive system and changes the methylation pattern of some genes in the placenta. In contrast, caffeic acid phenylethyl ester (CAPE) a potent antioxidant, has protective effects against the harmful effects of oxygen free radical molecules, methotrexate, and pesticides on the reproductive system. To find the effect of nicotine on the endometrium, we investigated three markers of endometrium receptivity including fibroblast growth factor 2, vascular endothelial growth factors A, and C-X-C motif chemokine ligand 12. In addition, we evaluated the protective effect of CAPE against nicotine.

Methods: The appropriate treatment dose was selected based on literature, the endometrial stromal cells were divided into 4 groups, including control, treated with nicotine, CAPE, and nicotine followed by CAPE. Finally, the quantitative polymerase chain reaction and methylation-specific PCR were carried out.

Results: The results showed that treatment of endometrial stromal cells with nicotine (10-6 µM) for 24 h significantly reduced expression of CXCL-12, FGF, and VEGFA genes. However, a decrease in CXCL-12 expression was not associated with increased methylation levels in the studied promoter region. In contrast, endometrial stromal cells treated with CAPE (4 µg/ml, 24 h) reversed nicotine-induced reduction of CXCL-12, FGF, and VEGFA genes expression.

Conclusion: Exposure to nicotine has negative effects on uterine receptivity, implantation, and fertility, via reducing the expression of VEGFA, CXCL-12, and FGF2 genes. In contrast, CAPE has a protective effects and improves these genes expression.

Keywords: Caffeic acid phenyl ester, CXCL-12, FGF, VEGFA

P-229

Casein-coated cobalt ferrite nanoparticles as the support for immobilization of laccase

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Background: Enzyme immobilization is one of the most attractive approaches for production of efficient biocatalysts. Incorporation of nanotechnology in fabrication of support for enzyme immobilization has been increasing during past decades. Howsoever, immobilization procedure may well enhance enzymatic activity, stability, and reusability. In this study, we prepared magnetic biodegradable and feasible support for enzyme immobilization. Magnetic casein particles (MCPs) were fabricated by renin-assisted encapsulation of magnetic cobalt ferrite nanoparticles.

Methods: An adequate amount of iron (III) chloride and cobalt (II) chloride were mixed and heated for half an hour. The mixture was added to a boiling NaOH solution and stirred for 1 h. Finally, the obtained nanoparticles were separated by an external magnet and dried at 40 °C. Casein powder was dispersed in double-distilled water and stirred overnight at 10 °C. The hydrated casein was mixed with renin and then added to cobalt ferrite nanoparticles. The mixture was introduced to cold sunflower oil and stirred at 500 rpm for 5 min. MCPs were obtained by increasing the temperature to 40 °C. Finally, MCPs separated with centrifugation and washed several times. After adsorption of laccase on the surface of magnetic casein particles, the enzyme activity was evaluated after incubation with 2,2chr('39')-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The surface of the MCPs was assessed by SEM. Stability of the immobilized enzyme was calculated by measuring the enzyme activity after storage at 4 °C.

Results: Casein-coated magnetic cobalt ferrite nanoparticles were easily separated by an external magnetic field. Stability and activity of the immobilized laccase was higher than the free enzyme. Also, the immobilized enzyme showed a remarkable reusability.

Conclusion: Renin-assisted assembling of MCPs could be used as a promising support for immobilization of different enzymes.

Keywords: Biocatalyst, Enzyme immobilization, Laccase, Magnetic nanoparticle, Casein

P-230

Design and synthesis of targeted nanoparticles for non-viral gene transfer to breast cancer cells

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Background: Gene therapy is a powerful tool for the treatment of gene defect diseases such as diabetes, hypertension and cancer, which involves using DNA that encodes a functional, therapeutic gene to replace a modified gene.

Methods: In the present study FA (folic acid)-PEG (polyethylene glycol)-PEI (polyethyleneimine)-PEG-FA was first synthesized and then confirmed using HNMR and FTIR spectroscopy, respectively. The FA-PEG-PEI-PEG-FA/DNA nanoparticles were prepared by combining different concentration of FA-PEG-PEI-PEG-FA and DNA and incubated for 30 minutes at room temperature. Transmission electron microscope (TEM) was used to examine morphology and dispersion of the nanoparticles. MTT assay and fluorescence microscopy were used to evaluate the toxicity as well as the ability of nanoparticles to deliver DNA into MCF-7 cells, respectively.

Results: The results of H-NMR and FTIR spectroscopy showed that the PEG-FA was properly attached to the PEI. The TEM figures of FA-PEG-PEI-PEG-FA/DNA nanoparticles showed that the nanoparticles had a spherical shape. Moreover, no aggregation was observed among the nanoparticles. The particle size and zeta potential of FA-PEG-PEI-PEG-FA/DNA nanoparticles were in the range of 100 to 200 nm and -17.41 to 3.24 mV respectively. In general, the size and zeta potential of the nanoparticles were influenced by the concentration of FA-PEG-PEI-PEG-FA. So that, particle size and zeta potential of the nanoparticles increased with increasing the concentration of FA-PEG-PEI-PEG-FA. MTT results showed that the toxicity of PEI/DNA was significantly lower than that of the FA-PEG-PEI-PEG-FA/DNA nanoparticles. The results of fluorescence microscopy showed that FA-PEG-PEI-PEG-FA copolymer has a high ability to deliver genes into MCF-7 cells compared to PEI/DNA complex.

Conclusion: The results of this study show that PEG-coated PEI significantly reduces the toxicity of PEI. The results also showed that the folic acid-functionalized nanoparticles significantly increase gene transfer efficiency.

Keywords: Folic acid, FA-PEG-PEI-PEG-FA copolymer, Gene delivery, MCF-7

P-231

Association of hyperleptinemia and low zinc concentration with insulin resistance and overweight in women with PCOS

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Background: To investigate the possibility of zinc deficiency and increase in leptin concentration and its relationship with insulin resistance and overweight in women with polycystic ovary syndrome (PCOS).

Methods: In this case-control study, 104 PCOS cases and 99 controls were included. Concentrations of zinc, leptin, lipid profiles, insulin and glucose and androgens were measured in fasting blood samples.

Results: Zinc level was 11.32 ± 87.20 in the case group and 13.68 ± 8.31 in the control group (p <0.001). Leptin (23.06 ± 3.33 ng/mL versus19.37 ± 3.34 ng/mL in control group, p = 0.000), insulin (11.41 ± 3.84 µU/ml versus 7.02 ± 3.29 µU/ml in control group, p = 0.000), insulin resistance (2.37 ± 0.83 versus 1.45 ± 0.74 in control group, p = 0.000) levels were significantly different. Zinc had a significant inverse relationship with leptin, insulin and insulin resistance index (p = 0.000), but no correlation was found between zinc and insulin resistance in the control group (p = 0.5). The effect of hyperleptinemia and zinc reduction in PCOS was significant considering waist circumference and body mass index as confounding factors, but with the elimination of confounding factors, only zinc reduction was associated with PCOS (OR:0.782, p=0.000).

Conclusion: The present findings show that zinc reduction is more effective on PCOS while the association of hyperleptinemia with PCOS depends on waist circumference and BMI.

Keywords: Zinc, Leptin, PCOS, Insulin

P-232

Design and synthesis of novel glucose targeted copolymer and evaluation of its DNA binding ability

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Background: In recent years, targeted nanoparticles have increased the efficiency of drug and gene delivery to specific cells and tissues in addition to decreasing its side effects.

Methods: In the present study, glucose (Glc)-polyethylene glycol (PEG)-polyethylenimine (PEI)-polyethylene glycol -polyethyleneimine-glucose (Glc-PEG-PEI-PEG-PEI-Glc) copolymer was synthesized. Then, H-NMR and FTIR spectroscopy were used to confirm the successful synthesis of Glc-PEG-PEI-PEG-Glc. The DNA/Glc-PEG-PEI-PEG-Glc nanoparticles were formed by gently mixing the DNA solution with the Glc-PEG-PEI-PEG-Glc co-polymer and incubated for 30 min at room temperature. The particle size, zeta potential and morphology of the DNA/Glc-PEG-PEI-PEG-Glc nanoparticles were evaluated by DLS and TEM, respectively. Agarose gel electrophoresis was also used to confirm the ability of Glc-PEG-PEI-PEG-Glc to interact with DNA.

Results: The results of H-NMR and FTIR spectroscopy showed that the Glc-PEG-PEI-PEG-PEI-Glc copolymer was successfully synthesized. The results of DLS and TEM revealed that most of the nanoparticles had spherical morphology with sizes ranging from 150 to 200 nm. The zeta potential of DNA/Glc-PEG-PEI-PEG-Glc nanoparticles was in the range of -5.6 to 3.6. These results also showed that the zeta potential of the nanoparticles increased by increasing the concentration of Glc-PEG-PEI-PEG-Glc copolymer in DNA/Glc-PEG-PEI-PEG-Glc nanoparticles. Moreover, the electrophoretic analysis confirmed that the Glc-PEG-PEI-PEG-Glc copolymer has a high ability to interact and neutralize the negative charges of DNA.

Conclusion; In this study, Glc-PEG-PEI-PEG-PEI-Glc were successfully synthesized for targeted gene delivery to cancer cells. Our results also shown that Glc-PEG-PEI-PEG-PEI-Glc have the capability to interact and neutralize the negative charge of DNA.

Keywords: DNA, Glc-PEG-PEI-PEG-Glc copolymer, Glucose, Gene delivery, Targeted nanoparticles

P-233

Evaluation of apoptosis in three-dimensional culture of MCF-7 breast cancer cell line in the presence of tamoxifen

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Background: Breast cancer is an important issue for global health. The type of breast cancer is generally characterized by the origin of the cancer cells, which almost always form in the lobes, lobules, or ducts. The MCF-7 cell line was first obtained in 1970, these cells have estrogen and progesterone receptors. Tamoxifen is used in the treatment of breast cancer through the inhibition of classical estrogen receptors.

Methods: In this study, hanging drop culture method was used, the cells were cultured in 2D form in flask, then following trypsinization the cells were transformed into cell suspension. After cell counting, hanging drop was performed in Petri dish, spheroids were treated with tamoxifen after 48 h in a 96-well plate covered with agarose, after 48 h they were prepared for MTT assay for apoptosis.

Results: The results of MTT in MCF-7 cell line showed that 106%, 97%, 88.8%, 40.8% and 9.9% of cells survived at concentrations of 40, 150, 200, 270 and 320 µg/ml of tamoxifen, respectively.

Conclusion: The findings showed that with increasing concentration of tamoxifen, mortality in MCF-7 cell line increased, which seems to be due to increased expression of apoptosis-related genes in this process.

Keywords: Breast Cancer, MCF-7, Tamoxifen, MTT

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Subacute renal toxicity study of bisphenol A in Wistar rats

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Background: Bisphenol A is a chemical substance that is used in polycarbonate plastics and epoxy resins because of its positive characteristics. BPA may be absorbed after oral administration during ingestion of foods packaged in plastic containers. BPA is conjugated with glucuronic acid in the colon and liver and excreted by the kidneys as BPA-glucuronide.

Methods: A total of 36 two-month female Wistar rats were divided into six groups: control, and BPA receiving groups 0.1, 1, 10, 50, 100 mg/kg/BW. After one month of gavage and 24-hour collection of urine on the 30th day, rats were anesthetized and blood samples from the heart were taken. Finally, biochemical parameters including urea, creatinine and total protein in serum and urine and serum albumin were calculated by calorimetry.

Results: In the body weight comparison, at a dosage of 100 mg/kg, the final weight was lower than 1mg/kg. Urine creatinine increased significantly at a dosage of 50 mg/kg compared to 1mg/kg. Serum urea increased dramatically at a dose of 10 relative to dose 1. Urinary urea levels increased significantly at dose 10 relative to dose 1. Serum albumin had a substantial difference in the treatment groups relative to the control group and the dose was decreased by 100 relatives to dose 10. Serum protein decreased significantly at a dose of 100 compared to the control group. Finally, urinary protein was substantially increased at dose 50 relative to lower doses.

Conclusions: The findings of our research at various doses of BPA have shown that this agent can have a damaging effect on kidney tissue at high doses and, over time, causing failure of this important organ in the body.

Keywords: Bisphenol A, Subacute toxicity, Biochemical parameters

P-235

Evaluation of serum zinc, copper, oxo-nitrosative stress and lipid profiles due to exposure to organophosphorus-based pesticides

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Background: Pesticide sprayers are commonly exposed to organophosphorus-based pesticides because of their wide usage in agriculture, medicine and industry. The aim of this study was to determine whether exposure to organophosphorus (OP) pesticides was associated with adverse effects on serum biochemical parameters.

Methods: Forty professional sprayers and 40 control subjects were recruited. Oxidative stress and inflammatory biomarkers, lipid peroxidation, lipid profile, zinc and copper were measured in serum.

Results: Copper was significantly increased in the exposed group compared with controls (1.27±4.84 mg/dL vs. 1.01±2.06 mg/dL, p=0.013). Significantly higher MDA and TNF-α were found among sprayers compared to controls [(4.21±1.33 nmol/ml vs. 2.23±0.83 nmol/ml, p=0.028) and (1.97±1.33 µg/L vs. 1.77±1.75 µg/L, p=0.026), respectively]. However, TAC levels were significantly lower in sprayer group than in controls (4.23±3.86 µmol vs.16.65±1.39 µmol, p=0.001).

Conclusion: Our results highlight the critical role of ROS-mediated injury in OP-intoxicated patients and identified copper as a predictable marker of exposure to OP-based pesticides.

Keywords: Zinc, Copper, Oxo-Nitrosative ptress, Lipid profiles, Organophosphorus pesticides

P-236

The association between vitamin D deficiency and homocysteine levels in the cardiovascular disease patients undergone angiographic technique

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Background: Cardiovascular diseases account for more than 50% of mortality worldwide. High homocysteine levels have been correlated with an increased risk of atherosclerosis coronary heart disease. Recently, one of the emerging risk factors for these diseases is 25-hydroxyvitamin D (vitamin D) deficiency. The aim of this study was to assess relationship between plasma vitamin D status and homocysteine levels in patients with acute myocardial infarction and the control group.

Methods: In this case-control investigation, we selected 87 patients (including 52 males and 35 females) with an acute myocardial infarction diagnosed by angiography method, at Bushehr Heart Center. The level of serum vitamin D and homocysteine levels were measured in the case and the control groups. Different variables including sex, age, ethnicity, family history of myocardial infarction, body mass index, total cholesterol, triglycerides, C-reactive protein, diet, and daily vitamin D intake were matched between the two groups. Plasma homocysteine was assayed using the ELISA technique. Vitamin D level was measured using the HPLC method. The data was considered as the mean ± SD. Data analysis was performed using SPSS version 18. A statistical significance level was considered as $p < 0.05$.

Results: The mean value of vitamin D in the control and the case group were 65±43 and 45±35 ng/mL, respectively. Fasting serum homocysteine levels in CAD patients were significantly higher than the patients without coronary artery disease ($p < 0.001$). Also, homocysteine levels correlated significantly with increasing severity of CAD ($p < 0.001$). Statistical analysis showed no significant difference between the two groups, with regard to the level of vitamin D and homocysteine. Finally, there was no association between vitamin D level and homocysteine between the two groups (p-value > 05).

Conclusion: There was no significant association between vitamin D concentrations and plasma homocysteine levels in healthy subjects and patients with cardiovascular disease.

Keywords: Vitamin D deficiency, Homocysteine, Cardiovascular disease

P-237

Interaction effect of cisplatin and capecitabine as gastric cancer drugs on DNA

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Background: As is well known, the goal of anticancer drugs is to completely stop DNA synthesis which inhibits tumor growth. In this study, the interactions effect of gastric cancer drugs (cisplatin and capecitabine) on calf thymus DNA has been measured by various test.

Methods: Different spectrometric approaches such as diffraction resonance beam spectroscopy, viscometry, circular dichroism and molecular modeling have been used to determine the type of interaction forces at the target binding site with DNA.

Results & Conclusion: According to the results of Stern Wallmer and Venthoff diagrams, the extinction process was obtained with considerable accuracy and was a factor responsible for the cisplatin arbitration of van der Waals and hydrogen forces and was determined for hydrophobic kappa-sitabine. Also, the thermodynamic values (enthalpy and entropy) are negative for cisplatin and these values are positive for capecitabine. According to the resonance beam spectroscopy test, the results in both complexes show that the resonance beam diffraction has increased and the sample size has increased. This indicates changes in DNA; but as these changes in the cisplatin interaction diagram are greater than that of capecitabine, the drug therefore has a stronger interaction with DNA than capecitabine. The use of circular dichroism spectroscopy to evaluate drug-DNA interactions has resulted in theta-positives in both negative graphs indicating drug-type interactions between DNA strands. The viscosity observation test and molecular modeling confirm the spectroscopic approaches and certify the results.

Keywords: Cisplatin, Capecitabine, Calf thymus DNA, Absorption spectroscopy, Molecular pattern

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Application of polymer dots for quantification of methotrexate in biological fluids using fluoreimetric method

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Background: Methotrexate (MTX) is an immune suppressing drug which has been used as a chemotherapeutic agent for cancer therapy and also applied as the safest anti-inflammatory agent for different types of arthritis. Similar to other drugs, MTX have some adverse side-effects at high concentrations. Thus, it is important to check and monitor the blood levels of MTX to control and prescribe the appropriate dosage of MTX. Various analytical approaches have been introduced for the detection of MTX including electrochemical, separation based and spectroscopic techniques. In this study, a spectrofluorimetric method was developed for the detection of MTX in plasma samples.

Methods: Amine-functionalized polymer dots as fluorescent materials were used as probe for tracing of MTX. Upon addition of the MTX to the probe, the fluorescence of the polymer dots is changed which the changed values were regarded as analytical signal for MTX recognition. It is worthy to note that synthesis and functionalization of polymer dots were characterized using various techniques including TEM, SEM, FTIR, etc.

Results: To increase the sensitivity of the method, effects of various parameters were optimized such as time, temperature, concentration of polymer dots and pH. From obtained results, it is obvious that the optimized conditions for the determination of MTX are 2 min, 25 ˚C, 10 mM, and 6.0, respectively. Calibration curve revealed that the developed method is linear from 50 ng/mL to 10 µg/mL with low limit of detection of 50 ng/mL.

Conclusion: A simple and sensitive approach was developed for the detection of MTX in plasma samples. Results revealed a wide dynamic range and high sensitivity of the developed method for the detection of MTX. After optimization and validation of the method, its application was tested for the determination of MTX in real patient samples which confirmed the specific detection of MTX in real samples.

Keywords: MTX, Polymer dots, Fluorimetry, Real samples

P-239

Association between serum estradiol levels and insulin resistance in women with polycystic ovary syndrome

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Background: Polycystic ovarian syndrome (PCOS) is a complex endocrine and metabolic disorder which is characterized by ovulatory dysfunction and hyperandrogenism. This study was done to evaluate the insulin and estradiol in women with polycystic ovary syndrome (PCOS) and its relationship with BMI.

Methods: This case-control study included, 100 women with polycystic ovary syndrome as case group and 100 women without polycystic ovary syndrome, (other causes were referred to the above medical centers), as a control group. The concentrations of insulin, glucose and estradiol were measured in fasting blood samples.

Results: Insulin level was 7.02 ± 3.29 in control group, 11.41 ± 3.84 in case group. FBS level was 82.75 ± 7.18 in control group versus 84.03 ± 5.82 in case group. Estradiol level was 70.74 ± 53.03 in control group and 60.21 ± 40.58 in case group. Insulin resistance level was 1.45 ± 0.74 in control group versus 2.37 ± 0.83 in case group. There was a significant difference between serum insulin levels and insulin resistance between PCOS patients and healthy individuals ($p = 0.000$) but no correlation was found on estradiol levels between PCOS patients and healthy individuals ($p = 0.245$).

Conclusion: Present data showed that estradiol levels did not differ in PCOS and non PCOS patients, but insulin level in PCOS were significantly increased and PCOS women have significant insulin resistance which is dependent on BMI.

Keywords: PCOS, Insulin, Estradiol

P-240

Investigating the variation of porcine pancreatic elastase (PPE) under the influence of CuO nanoparticles by various techniques: Spectroscopic, Spectrofluorometric and circular dichroism methods

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Background: Nowadays, with the increasing use of nanoparticles, the need to study their interaction with biological macromolecules also increases. The aim of this study was to investigate and compare the structure and activity of only elastase with elastase-CuO-NP complex.

Methods: All stages of this study were performed at three temperatures of 303, 313 and 323 K in Tris buffer at pH 8.5. For this purpose, we used the experimental method and the theoretical method. Experimental studies were performed using UV-vis spectrophotometry, spectrofluorometry and circular dichroism and docking was used for theoretical results.

Results and conclusion: The change in the tryptophan environment can be seen by increasing the absorption in the UV-vis spectrophotometry. Emission intensity of elastase decreased to a large extent, and the static quenching was proven. The entropy and enthalpy values indicate that van der Waals forces or hydrogen bonds play the most important role in the formation of elastase-CuO-NP complex. The copper nanoparticles decreased the Vmax of the elastase but did not change Km, so it can be said that this nanoparticle acts as a non-competitive inhibitor for elastase. We saw a very little alteration in the secondary structure of elastase (11.80 % increase for α-helix and 1.85 % for β-sheet) and Tm change was from 333.0 for native elastase to 332.1 for elastase-CuO-NP.

Keywords: CuO nanoparticles, Kinetics, Elastase, Protein Stability, Quenching, Thermal stability

P-241

Protein hydrogel/rod-like hydroxyapatite nanocomposites for bone regeneration applications

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Background: With the intention of mimicking the structure of natural bone, some studies have emphasized on the incorporation of hydroxyapatite (HA) into the biopolymers. On the other hand, the ability to encapsulate cells in three-dimensional (3D) hydrogels is of potential benefit for bone tissue engineering applications. Although, gelatin hydrogels have recently been shown to be promising candidates for tissue engineering constructs, little is known about the gelatin networks incorporated with HA nanoparticles for application in regenerative medicine.

Methods: Herein, rod-like HA nanoparticles were synthesized using a chemical precipitation followed by hydrothermal treatment. The as-synthesized HA were then incorporated into a type-A porcine skin gelatin that was modified with methacrylic anhydride. The final protein hydrogel/rod-like HA nanocomposites were formed by the UV in the presence of photo-initiator molecules, while, at the same time, NIH-3T3 fibroblasts were encapsulated in the resulting construct.

Results: Rod-like HA nanoparticles increased the strength of protein hydrogels, while they maintained their high water-retention ability. According to the results, while the bare protein cannot show a significant bioactivity, bone mineralization occurs inside the protein nanocomposites after incubation in simulated body fluid. In addition, encapsulated fibroblasts readily proliferated and formed a 3D interconnected pattern in the construct, showing the suitability of the protein hydrogel/HA nanocomposites for cellular functions. It was also found that there is an optimal concentration for HA nanoparticles to achieve the highest mechanical strength. In addition, the encapsulation of fibroblasts revealed that the process of nanocomposite fabrication was compatible with living cells.

Conclusion: This work represented a successful attempt to fabricate hydrogel nanocomposites for application in low load-bearing orthopedic constructs. This study may also be a significant step toward the development of new generation of nanocomposites suitable for bone scaffolds.

Keywords: Nanocomposite, Orthopedic construct, Bone, Protein hydrogel

P-242

Molecular dynamics and docking study of several medicinal plant compounds to predict potential inhibitors of novel coronavirus main protease

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Background: The incidence of the 2019 novel coronavirus (SARS-CoV-2) has caused a major public health problem throughout the world. Lack of a specific drug or vaccine against the coronavirus disease 2019 (COVID-19) makes it more destructive. Thus, there is a pressing need to boost up research for the discovery of therapeutic targets and novel potential drugs. Recently, COVID- 19 main protease (Mpro) has been considered as a promising target against coronavirus infection due to its vital role in the reproduction of the virus. Therefore, the present in silico study was designed to evaluate the effects of several medicinal plant compounds including carvacrol, catechin, cinnamaldehyde, coumarin, cyanidin, delphinidin, fisetin, genistein, glabridin, isorhamnetin, liquiritigenin, p-cymene, piperine, pterostilbene, silibinin, and thymoquinone on Mpro by molecular dynamics and docking study.

Methods: After downloading the PDB files of COVID-19 Mpro and phytochemical molecules from the Protein Data Bank (http://www.rcsb.org) and Pubchem server, the simulation and molecular docking studies were performed by Autodock v4.2. After docking analysis, COVID- 19 Mpro was simulated in the presence of phytochemical molecules.

Results: Among the studied compounds, glabridin, catechin, and fisetin showed the highest affinity for interaction with COVID-19 Mpro through hydrophobic and hydrogen bonds. Interestingly, a significant reduction in the root mean square fluctuation (RMSF), rise in the radius of gyration (Rg), and induced change in the secondary structure of Mpro was observed following the docking of the above-mentioned phytochemicals to the COVID-19 Mpro.

Conclusion: The high binding affinity of glabridin, catechin, and fisetin to COVID-19 Mpro caused conformational change in the secondary structure of the enzyme which resulted in enzyme inhibition. The present in silico study demonstrated the potent inhibitory effects of glabridin, catechin, and fisetin against COVID-19 Mpro. However, more in vivo and in vitro studies are needed to confirm their potential therapeutic applications.

Keywords: Molecular docking, COVID-19 main proteas, Phytochemical compounds, Inhibitors

P-243

The inhibitory effect of Crocus sativus on cholinesterase

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Background: Dementia is a progressive diseases in understanding and cognitive functions, which can be a result of any illness or injury. According to the "cholinergic theory", in advanced type, the levels of acetylcholinesterase (AChE) and butyrylcholinesterase (BchE) are increased in hippocampal neurons and areas of the brain that are associated with memory and learning. Therefore, inhibitors of these two enzymes are the main therapeutic medicine in the treatment of Alzheimer's disease. However, most of the drugs such as galantamine and donepezil are intended to control the disease and prevent its progression, but without any perpetual effect. Despite the benefits of common drugs, side effects such as hepatotoxicity and gastrointestinal disorders have also been reported. So, different investigations should be done to find new drugs. Plants with biologically active constituents are good resources for this. According to the many researches, saffron has been used historically against Alzheimer diseases.

Results: The methanolic extracts of the stamen of saffron had high anticholinergic activities at the final concentration of 100 µg/ml with IC50 values 17 and 15 against AChE and BchE, respectively, whereas the butanolic extract shows inhibitory effects with IC50 values 22 and 15 against Ache and BchE, respectively. Butanolic sub-fractions (40% ethyl acetate with 60% hexane and 50% water with methanol 50%) had the highest ability to inhibit butyrylcholinesterase. A methanolic sub-fraction obtained from 100% methanol has the same positive effect on butyrylcholinesterase. Name of Compound was identified and the enzymatic inhibitory activity was approved.

Conclusion: Methanolic subfraction was obtained by VLC chromatography and liquid-liquid extraction was used for butanolic subfraction. Peak 3 of 100% methanolic subfraction by HPLC has the most inhibitory effect on butyrylcholinesterase. Butanolic subfraction (40% ethyl acetate and 60% hexane) with 3 peaks has been diagnosed with NMR method (DEPT, HSQC, COSY, H-NMR and C-NMR) and according to the enzymatic inhibitory effect, the lower IC50, the greater inhibitory ability. The IC50 recorded for the selected compounds was very close to the IC50 of donepezil. Both butanolic and methanolic subfractions of stamen show a great antioxidant capacity

Keywords: Butyrylcholinesterase, Acetylcholinesterase, Crocus sativus, Galantamine, Donepezil, Saffron, Alzheimer, Molecular Docking.

P-244

Effect of 24-hydroxycholesterol on gene expression and protein level of ABCA1 with or without β-amyloid in astrocytes isolated from C57BL/6J mice

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Background: Alzheimer's disease (AD) is a neurodegenerative disease where β-amyloid plays an important role in its pathogenesis. Cell cholesterol accumulation is reportedly involved in pathophysiological features of AD. So, regulation of cholesterol in brain, as the most cholesterol-rich organ in the body, is very important. Production of 24-hydroxy cholesterol (24s-OHC) by neurons is one of the mechanisms that prevent cholesterol accumulation in the brain. Here, we investigate the effect of 24s-OHC on ABCA1 protein level which is involved in cholesterol efflux in the presence of β-amyloid in astrocytes.

Methods: Mouse astrocytes were isolated and cultured in DMEM/ FBS. Cells were treated with 24s-OHC in the presence or absence of β-amyloid. After harvesting the cells, SDS-PAGE was done and ABCA1 was visualized with Western-Blotting. Cholesterol efflux was determined by cholesterol quantitation kit. Also, Real-time PCR was carried out to investigate the ABCA1 expression.

Results: 24s-OHC was able to enhance the protein level of ABCA1 and also the release of cholesterol in condition media. Surprisingly, cholesterol efflux was reduced by β-amyloid despite a remarkable increase of ABCA1 protein level in mice astrocytes. Also, 24s-OHC didn't have a significant effect on ABCA1 expression in the presence of β-amyloid.

Conclusion: As an excreted metabolite of cholesterol in the brain, 24s-OHC has a critical role in preventing cholesterol accumulation by stabilizing of ABCA1 which is a key player in cholesterol efflux. It has been shown that cholesterol is increased in Alzheimer's disease and 24s-OHC can probably invert β-amyloid effect by increasing the cholesterol efflux.

Keywords: β-Amyloid, 24-Hydroxy cholesterol, ABCA1, Cholesterol metabolism, Brain

P-245

Association between gender and age with esophageal cancer

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Background: Esophageal cancer is the sixth leading cause of death from cancer in the world. In this study the finding for new cancer-related biomarkers, including esophageal cancer, may be a step towards early detection. Research into the relationship between age and gender is a subject in cancer research today. M

Methods: The study involved 47 esophageal cancer patients who were identified by endoscopic examination and confirmed by histopathological studies and 50 patients admitted to esophageal cancer hospital who underwent endoscopy that showed negative results in the community test. After sampling the case and control individuals and collecting their information, the samples were examined in terms of input and output criteria and the study of community was matched based on gender and age.

Results: Age was significantly associated with incidence of esophageal cancer, the correlation coefficient was negative for age parameter ($R=-0.049$) in fact with increasing age, the incidence of esophageal cancer increases in the community. Gender was significantly associated with cancer and a more significant factor in women than men ($p>0.05$, $R=-0.01$)

Conclusion: In this study, we investigated and controlled individuals based on age and gender of all patients with esophageal cancer. In summary, we have shown that age and gender can be a factor in the evaluation of esophageal cancer. There is a significant relationship between age and gender and the incidence of esophageal cancer.

Keywords: Esophageal cancer, ESCC, EAC, Age, Gender, cDNA, RNA, Real Time PCR

P-246

Evaluation of papaverine-iron oxide nanoparticles for anticancer drug delivery

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Background: Breast cancer has been recognized as the most prevalent cancer and the primary cause of cancer-related death among females. Many studies have focused on Fe_3O_4 superparamagnetic nanoparticles (SPIONs) as prominent drug delivery vehicles due to their good biocompatibility, easy synthesis and surface modification for multimodality imaging, and drug delivery. This study was conducted to deliver papaverine (an opium alkaloid) to metastatic breast cancer cells by the aid of SPIONs.

Methods: SPIONs were first synthesized using the co-precipitation method. The surface of SPION was coated with chitosan and papaverine via ionotropic gelation method. The drug system was characterized by XRD, FTIR, DLS, TEM, and spectrophotometry. To study the effect of the drugs on the viability of 4T1 triple-negative breast cancer cells, MTT assay was applied.

Result: The complex with an average particle size of 11 nm and a loading rate of about 96% was considered for further evaluation. The XRD pattern was in agreement with standard magnetite. Our result showed that papaverine and papaverine-magnetic nanoparticles (MNP) attenuated 4T1 proliferation dose-dependently with an IC50 value of 62/4 and 11/5 µg/mL respectively, demonstrating its potentiality to increase cell mortality. Whereas, SPION and SPION-chitosan were found to have no cytotoxicity.

Conclusion: Considering that natural compounds and their derivatives constitute more than 60% of anticancer drugs, our results propose papaverine-MNP as the therapeutic option to surmount invasive breast cancers.

Keywords: Magnetic nanoparticles, Papaverine, Breast cancer, Drug delivery

P-247

Chemical composition analysis of Cyrtopodion scabrum extract: A novel natural source of anticancer compounds

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Background: Cancer is one of the major threatening factors of human health worldwide. Unfortunately, chemotherapy, the powerful arm of cancer therapy, is accompanied with many side effects, therefore alternative treatments with greater specificities and fewer side effects are highly required. The extract of a kind of lizard, named Cyrtopodion scabrum, has been studied by our research group for its potential anti-tumor properties in-vitro and in-vivo. According to our previous studies, the Cyrtopodion scabrum extract (CSE) selectively suppress some gastrointestinal cancer cell lines and successfully treat the tumor-bearing mice, with no significant harmful effect on the normal cells. In the current project, we partially characterized the composition of CSE using some chemical and biochemical methods.

Methods: In this study, we investigated the protein content by Bradford method and carbohydrate content by phenol-sulfuric acid method as well as gas chromatography/mass spectrometry (GC/MS) analysis of CSE.

Results: Data showed that CSE contains 7.41% protein and 2.4% carbohydrate. The results of GC/MS revealed that 82% of the entire GC-MS chromatogram, were successfully characterized including 24 compounds among them, tricosanoic acid, 2-hydroxy-methyl ester (17.86%), thymol (8.19%), carvacrol (6.143%) and pyrido[3,4-d] pyrimidine-2,4(1H,3H)-dione 1,3-dimethyl- (5.23%) were four major compounds. Out of the 24 compounds identified, 11 of them exhibited antitumor potential.

Conclusion: The bioactive compounds present in CSE crude extract and their corresponding biological activities are supporting our previous findings obtained by in vitro and in vivo studies that showed anticancer properties of CSE. Thus, CSE could be used as an alternative/complementary natural therapeutic anticancer agent, in support of its traditional medicinal uses.

Keywords: Natural anticancer product, Cyrtopodion scabrum extract, GC-MS analysis

P-248

Probing of the interaction between EGCG with HSA and ct-DNA by different spectroscopy techniques

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Background: The interaction between EGCG with HSA protein and calf thymus DNA were examined separately under physiological conditions by different spectroscopy methods including emission spectroscopy, attractive spectroscopy, heat transfer study, convergent beam electron diffraction (CBED), and viscometry. The results showed that increasing the amount of ligand in fluorescence spectroscopy lets the protein diffractive spectrum break up and lead to change in protein structure and complex formation.

Methods: Variables such as binding constant, available fluorophore level, and the number of binding sites on the protein were calculated; the breakup mechanism was static. The use of fluorescence resulted in changes in the surrounding environment providing amino acids. In the CBED method, increasing the Rayleigh light indicates an increase in protein size due to the addition of a ligand, which is due to complex formation. Following the study process, the results of the EGCG interaction with the calf thymus DNA was examined. In the emission spectroscopy method, the increase in the ligand concentration caused a breakup of the DNA emission spectrum, which indicated a complex formation. Thermodynamic quantities in the ligand interaction with DNA indicated the role of hydrophobicity in the interaction.

Results: The data from Stern-Welmer diagrams at several temperatures showed that the breakup mechanism was dynamic. In the spectrum of competitive emission spectrum with ethidium bromide and acridine orange, as an intercalator marker, by adding EGCG to the DNA-ethidium bromide and DNA-acridine orange complexes, the change in emission spectrum indicated that there was a competition. In the viscometrical method, the viscosity of DNA solution increased by adding a ligand to the DNA site which resulted from EGCG binds intercalary.

Conclusion: In heat transfer studies, ligand stabilized the DNA structure by getting in intercalated. Relevant to the intercalator combinations such as potassium iodide ions and sodium chloride, which compete with a ligand on the binding site.

Keywords: Fluorescence spectroscopy, Spectroscopy methods, EGCG, HSA, Intercalator combinations, Stern-Welmer diagrams, Potassium iodide ions, Sodium chloride, CBED method, Ethidium bromide, Acridine orange

P-249

Preparation and characterization of a novel cancer targeted nanoparticles for drug delivery into MCF-7 cells

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Background: Efficient delivery of functional nucleic acids and drugs into specific cells or tissues is still a challenge for clinical therapy using nanoparticles. The folate receptor (FR) is known to be over-expressed extracellularly on a variety of human cancers. Therefore, this molecule is ideal candidate for targeting nanoparticles to drug delivery using the nanoparticles to cancer cells or cancer tissues.

Methods: In this study, we designed an amphiphilic nanoparticle, comprising biocompatible and hydrophilic poly (ethylene glycol (PEG), biodegradable and hydrophobic poly lactic acid (PLA) segments, and also folic acid (FA) as targeting group, as a highly efficient drug carrier to folate and glucose receptors highly expressed on therapeutic resistant MCF-7 cancer cells. FT-IR and NMR spectroscopy were used to confirm the structure of the nanoparticles.

Results: The results showed that the synthesis of the nanoparticles have been performed successfully. Transmission electron microscopy (TEM) showed the nanoparticles were spherical in shape. Moreover, biocompatibility assay of the blank nanoparticles was performed using MTT test. Also, the ability of the nanoparticles to paclitaxel delivery into MCF-7 cells was analyzed using MTT test.

Conclusion: Our results showed that the nanoparticles exhibited low cytotoxicity and excellent drug delivery efficiency, due to its FA induced targeting ability to MCF-7 cells. The results of this study show that folic acid-coated nanoparticles significantly increase drug deliver efficiency.

Keywords: Drug delivery, Folic acid, Nanoparticle targeting.

P-250

The relation between polymorphisms in exon 5 and exon 6 of GSTP1 gene and the risk of lung cancer in Iranian people

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Background: The GSTP1 gene, which is located on chromosome 11q13, consists of 7 exons and 6 introns. There are two polymorphisms in GSTP1 that have been exposed to a transposition for codon 105 (Ile/Val) and 114 (Ala/Val) in exons 5 and 6, which have been studied previously in relation to lung cancer. Since the level of GSTP1 expression in lung tissues and other human epithelial tissues is high, GSTP1Val-105 polymorphism is recognized as a sensitive factor for tobacco-related cancers, especially lung cancer.

Methods: One hundred and twenty tissue block samples of patients with lung cancers and 120 peripheral blood samples of the control group were obtained from two referral cancer centers in Tehran, Iran, from 2011 to 2016. Genomic DNA was extracted from tissue blocks and buffy coat of study cases to detect SNP of the GSTP1 gene using Tetra-primer ARMS-PCR.

Results: There was a notable correlation between the incidence of lung cancer and variant Val105 (P-value=0.001; OR=2/6; 95% CI=1.49-4.53) and Ile105 (p=0.003; OR=0.41; 95% CI=0.23-0.73). The odds ratio for lung cancer in the homozygous Ile105/Ile105 genotype was 3.56 times higher than that of individuals with heterozygous Ile105/Val105 (p<0.001; OR=3/56; 95% CI=1.826-6.934) genotype, that was statistically significant. Furthermore, the results showed that there was no significant correlation between Ala114/Val114 genotypes and lung cancer. The BC (p=0.007; OR=0.16; 95% CI=0.04-0.61) and AA (p=0.001) genotypes were statistically significant (p <0.05); and for those who had AA genotype, the odds ratio was almost six times higher than those with BC genotype.

Conclusion: The study of GSTP1 polymorphisms indicated that unlike the polymorphism in exon 5, the GSTP1 exon-6 polymorphism correlated with the lung cancer risk in the select group of Iranian people. Likewise, the potential use of this genetic polymorphism as a lung cancer predictor is confirmed.

Keywords: Glutathione s-transferase pi, Lung neoplasms, Single-nucleotide polymorphism (SNP), ARMS-PCR

P-251

Evaluation of different doses of nickel on calcium and phosphorus of female Wistar rat

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Background: Nickel (Ni) and its compounds are frequently used in industrial and commercial products such as stainless steel, alloys and batteries. This heavy metal is widely present in air, water, soil and food products. Therefore, humans could be exposed to nickel throughout the environment. Nickel exposure can put human health at high risk zone and cause multiple severe abnormalities including respiratory cancer, diverse toxicity effects on respiratory system, genotoxicity, and allergies. Various investigations have been carried out in order to demonstrate these results.

Methods: 32 female Wistar rats were separated into 4 groups. The first group obtained water without treatment whilst three other groups received nickel by doses of 10, 15 and 25mg/kg, sequentially. All items were injected intraperitoneally and carried out thrice on days 8, 12 and 16. After 20 days, in order to evaluate biochemical values of calcium and phosphorus, blood samples were taken from every rat. Ultimately, rats were euthanized under general anesthesia by Ketamine and Xylazine.

Results: Although a dose-dependent increase in levels of calcium and phosphorus has been shown, the alterations between groups were not observable. Nevertheless, calcium and phosphorus of control group were at minimum levels. As nickel dose rises, calcium and phosphorus levels get higher. Thus, the highest levels belonged to nickel dose 25mg/kg treated rats.

Conclusion: Through this research, it can be manifested by elevated levels of calcium and phosphorus that nickel can cause destructive effects on rats and kidney lesions should be considered as subsequences of nickel intakes.

Keywords: Nickel, Wistar rat, Calcium, Phosphorus, Biochemistry

P-252

Effect of different doses of nickel on albumin and total protein values of female Wistar rat

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Background: Nickel is one of the main heavy metals in the environment, which is present in crust and core of the Earth. Although nickel is essential to many microorganisms and plants, if humans get exposed to high amount of nickel, they might reveal different anomalies. As extensive quantities of studies have been implemented during recent years, numerous teratogenicity, carcinogenicity, respiratory diseases and allergies that have been reported are attributed to nickel effects which people can be exposed through either gastrointestinal tract or inhalation.

Methods: 32 female Wistar rats were classified into 4 groups evenly. Control group only received water via intraperitoneal injection, whilst three other groups received nickel intraperitoneally as treat, respectively, by the doses of 10, 15 and 25mg/kg on days 8, 12 and 16 of the experiment. After 20 days, to obtain blood samples from every rat, they were anesthetized by Ketamine and Xylazine prior to euthanasia. Eventually, albumin and total protein values of taken samples were assessed.

Results: Biochemical evaluations demonstrated that there is a dose-dependent relation between elevated nickel dose and albumin and total protein values. In other words, by increasing the dose of nickel, mentioned factors rise. In addition, dose 25mg/kg of nickel causes highest levels, whilst lowest levels were linked to control group.

Conclusion: According to mounted levels of albumin and total protein, it ought to be reckoned that nickel has negative impacts on human and animal health and toxicity effect of nickel could be considered as one of them.

Keywords: Nickel, Wistar rat, Albumin, Total protein, Biochemistry

P-253

In silico investigation of signal peptide sequences to enhance secretion of CD44 nanobodies expressed in Escherichia coli

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Background: The selection of a suitable signal peptide that can direct recombinant proteins from the cytoplasm to the extracellular space is an important criterion affecting the production of recombinant proteins in Escherichia coli, a widely used host. Nanobodies are currently attracting the attention of scientists as an antibody alternative due to their specific properties and feasibility of production in E. coli. CD44 nanobodies constitute a potent therapeutic agent that can block CD44/HA interaction in cancer and inflammatory diseases. This molecule may also function as a drug target against cancer cells and has been produced previously in E. coli without a signal peptide sequence. The goal of this project was to find a suitable signal peptide to direct CD44 nanobodies extracellular secretion in E. coli that extensively leads to optimization of experimental methods and facilitates downstream steps such as purification.

Methods: We analyzed 40 different E. coli signal peptides retrieved from Signal Peptide database and selected the best candidate signal peptides according to relevant criteria including signal peptide probability, stability, and physicochemical features which were evaluated using signalP software version 4.1 and the ProtParam tool, respectively.

Results: In this in silico study, suitable candidate signal peptide(s) for CD44 nanobody secretory expression were identified. CSGA, TRBC, YTFQ, NIKA, and DGAL were selected as appropriate signal peptides with acceptable D score, appropriate physicochemical and structural properties. Following further analysis, TRBC was selected as the best signal peptide to direct CD44 nanobody expression at the extracellular space of E. coli.

Conclusion: The selected signal peptide(s) are the most suitable to promote high level secretory production of the CD44 nanobodies in E. coli and probably will be useful for scaling up CD44 nanobody production in experimental research as well as in other CD44 nanobody applications. However, experimental work is needed to confirm the data.

Keywords: CD44 nanobody, E. coli, In silico cloning, Physicochemical properties, Secretory production, SignalP software

P-254

In silico approach to predict the candidate miRNA derived SARS-CoV2 as potential antiviral therapy

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Background: The present pandemic of the corona virus disease 2019 (COVID-19) is a contagious disease originated by severe acute respiratory syndrome corona virus 2 (SARS-CoV2). MicroRNA (miRNA) is small, single-stranded RNA which exist in viruses as well as in animals. Experimental works through viral infection, demonstrate that viral miRNAs have important functions in pathogen-host interaction, immune escape, host cell death, and tumorigenesis. So these molecules can play pivotal role in viral pathogenesis. Since no drugs or vaccines currently exist for this virus and its pathogenic mechanism is not known exactly, we are exploring and proposing potential antiviral therapeutic agents against COVID-19 by targeting miRNAs platforms which may result in down regulation of viral gene expression to suppress viral proliferation.

Methods: In this work, in order to attain insight into the potential role of SARS-CoV2 derived miRNA in the viral infection background, we used a set of computational methods to scan SARS-CoV2 genome that finally led to 13 computationally predicted potential viral microRNAs candidates in the genome. Furthermore, we predicted the potential targets of the candidate vmiRNAs in human host following infection by applying mirPath3 software.

Results: Our work proposes a curious theory that these predicted viral miRNAs, may have plausible role on the alteration of target human genes expression that mainly contributed to infectious state, inflammation and immune system escape. These vmiRNAs have therapeutic trends as antiviral agents against covid-19 infection.

Conclusion: This finding offers a reference idea for supplementary study on miRNA identification and increase the understanding of genome structure in SARS-CoV2.

Keywords: SARS-CoV2, MicroRNA precursor, In silico screening, Target prediction, VMir software

P-255

Association of overweight with high levels of testosterone and ferritin in women with PCOS

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Background: To investigate the determination of testosterone and ferritin levels in women with polycystic ovary syndrome (PCOS) and its relationship with body mass index (BMI)

Methods: This case- control study, included 100 PCOS cases and 100 controls. Concentration of testosterone, ferritin, lipid profiles, insulin and glucose and androgen were measured in fasting blood sample.

Results: Testosterone level was 1.08 ± 0.50 in case group and 0.90 ± 0.66 in control group (P< 0.001). Ferritin (64.69 ± 52.25ng/ml versus 47.84 ± 56.82 ng/ml in control group, p< 0.001), insulin (11.41 ± 3.84 µU/ml versus 7.02 ± 3.29 µU/ml in control group, p= 0.000), insulin resistance (11.41 ± 3.84 versus 7.02 ± 3.29 in control group, p= 0.000). There was a significant relationship between serum testosterone concentration and BMI) p=0.001(and in contrast no correlation was observed between serum ferritin concentration and BMI (p=0.987).

Conclusion: The present findings show that the levels of testosterone, endogenous and ferritin in patients with PCOS increase and a significant relationship between waist circumference and BMI with testosterone has been seen.

Keywords: Testosterone, Ferritin, PCOS, Insulin

P-256

FBS as a BBB basement membrane in vitro BBB models

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Background: Nowadays, studies on different aspects of blood brain barrier (BBB), drug delivery, or its alternation during CNS disease has risen. In this regard, *in vitro* BBB models (In- BBB-M) have been developed to make comparable models to *in vivo* conditions. One of the challenges for the In-BBB-M to reach the real condition is providing the BBB basement membrane (BM) simply. Given that serum proteome contains different amounts and types of collagens, laminins, and fibronectin, and proteins are considered the major part of the BBB BM, they could be employed as coating agents in the In- BBB-M. However, other FBS components could influence BBB characterization and its integrity in addition to these three BM components. Accordingly, in this study, we tried to optimize the coating condition using FBS to develop a monoculture transwell system as an In- BBB-M.

Methods: The hCMEC/D3 cell line was cultured in RPMI-1640 with 5% FBS. Different inserts of a transwell plate were coated with different concentrations of FBS at different times, and then 50000 cells/well were seeded on the coated and uncoated inserts. The integrity of In- BBB-M was measured through TEER assay. TEER values were calculated from the submission of R-total from R-blank and multiplied by surface area (0.33 cm).

Results: In all groups, TEER values of the coated groups at different time of incubation were significantly higher than uncoated inserts. Time of incubation also showed effect on quality of our final BBB models.

Conclusion: Generally, TEER values in the groups coated by FBS were significantly higher than control, which showed the influence of FBS on improving the critical parameter of BBB, impermeability. Given that the proteins, including collagen type I, IV, and fibronectin, are usually employed to coat the inserts, here, based on the results, FBS has been suggested as an alternative substance.

Key words: Blood brain barrier, TEER, Fetal bovine serum

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In vitrocytotoxicity assay of D-limonene niosomes: an efficient nano-carrier for enhancing solubility of plant-extracted agents

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Background: The low solubility of the plant-extracted agent like D-limonene in cancer therapy is a critical problem. In this study, we prepared D-limonene loaded niosomes (Dlimonene /Nio) for cancer therapy through in vitro cytotoxicity assay of HepG2, MCF-7, and A549 cell lines.

Methods: The niosomal formulation was prepared by film hydration technique with Span ®40: Tween®40: cholesterol (35:35:30 molar ratio) and characterized for vesicle distribution size, morphology, entrapment efficiency (EE %), and in vitro release behaviour.

Results: The obtained niosomes showed a nanometric size and spherical morphology with EE% about 87 ± 1.8%. Remarkably prolonged release of D-limonene from niosomes compared to free D-limonene observed. The loaded formulation showed significantly enhanced cytotoxic activity with all three cancer cell lines (HepG2, Macf-7 and A549) at the concentration of 20 µM.

Conclusion: These results indicated that niosome loaded with phytochemicals can be a promising nano-carrier for cancer therapy applications. Keywords: Cancer therapy, D-limonene, Niosome, Solubility.

Keywords: Cancer therapy, D-limonene, Niosome, Solubility

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Design and synthesis of magnetic nanoparticles for targeted delivery of drug to breast cancer cells

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Background: Efficient delivery of nanoparticles to cancer cells is hopeful method in the treatment of many diseases including cancer, which increases the possibility of treatment by reducing side effects and increasing the efficiency of drug delivery to target cells.

Methods: In this study, biocompatible and biodegradable compounds such as PLA (Polylactic acid), PEG (Polyethylene glycol) were used for synthesis of PLA-PEG copolymer and also, folic acid (FA) was used to modify the surface of nanoparticles for targeted delivery of nanoparticles to breast cancer cells (MCF-7). H-NMR spectroscopy and FTIR were used to confirm the synthesis of PLA-PEG-FA copolymer. PLA-PEG-FA synthesized copolymer was used to transfer paclitaxel (PTX) to breast cancer cells. Solvent release echnique was used to simultaneously load paclitaxel drug and also, iron oxide magnetic nanoparticles coated by oleic acid. The features of the nanoparticles were characterized by transmission electron microscopy (TEM) and DLS device. MTT test was used to evaluate the efficiency of PLA-PEG-FA copolymer in drug delivery to MCF-7 cells.

Results: The results of H-NMR and FTIR showed that the synthesis of PLA-PEG-FA copolymer was successful. The results of electron microscopy and also DLS showed that the resulting nanoparticles had spherical structure and a size of about 100-200 nm. Also, the results of MTT test showed proper biocompatibility of this copolymer and increased the efficacy of PTX against MCF-7 cells.

Conclusion: In this study, nanoparticles not only presented as a high efficiency in drug efficacy, also due to their magnetic properties and their ability in targeted drug delivery to cancer cells, can be useful in future therapeutic and imaging applications.

Keywords: PLA-PEG-FA copolymer ،Folic acid (FA) ،Paclitaxel (PTX) ،MCF-7

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Evaluation the effect of alkaloid Berberine on dopamine and prolactin hormone and superoxide dismutase in schizophrenia patient

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Background: Schizophrenia is one of the most severe psychiatric disorders, affecting 1% of the population worldwide. Little is known about the biological mechanisms underlying the disease pathology in spite of overwhelming research in the field. In this study, we evaluated the effect of berberine alkaloid as an antioxidant with neuroprotective effect on dopamine and prolactin neurotransmitters and superoxide dismutase. We investigated whether Berberine could be used as a supplement to help to treat schizophrenia.

Methods: At first, serum samples were separated from patients' blood and all of patients were treated with Risperidone and Berberine supplementation for 45 days. We monitored the condition of the patients during this period. Plasma dopamine level was measured using a sensitive technique, based on high-performance liquid chromatography in first episode schizophrenia.

Result & Conclusion: These results were consistent with an involvement of these markers in the pathogenesis of schizophrenia as well as in the responses to treatment, and the usefulness of Berberine indices as supplement for schizophrenia.

Keywords: Berberine, prolactin, dopamine, super oxide dismutase, schizophrenia

P-260

The synergistic effects of cisplatin and Piperine combination on the induction of apoptosis in breast cancer MCF-7 cell line

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Background: Piperine, the bioactive compound of Piper nigrum, exhibits anti-proliferative and anti-cancer activity and decreases tumor progression. This research evaluated the cytotoxic effect of piperine and cisplatin combination in the human MCF-7 breast cancer cell line and the underlying mechanism.

Methods: In the present study, MCF-7 cells were cultured and divided into four groups: 1) an untreated group, 2) Cisplatin, 3) Piperine, and 4) Cisplatin+ Piperine. Cell viability was assessed using the MTT assay. Flow cytometric analysis was performed for apoptosis. The mRNA expression of caspase 3 and caspase 9, and the expression of apoptosis-related proteins p53, Bcl-2 and Bax were investigated by quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting analysis.

Results: Our results demonstrated that Piperine, combined with Cisplatin for 24 hours, synergistically decreased MCF-7 cell viability more than Piperine or Cisplatin used alone. Synergistic anti-tumor effects of Cisplatin and Piperine were via apoptosis induction. Our results also showed that Piperine and Cisplatin for 24 hours induces apoptosis strongly through Bcl-2 reduction and p53, Bax, caspase 3, and caspase 9 induction.

Conclusions: This results strongly demonstrated that Piperine combined with Cisplatin could induce p53-mediated apoptosis more effective than Cisplatin or Piperine alone in the MCF-7 cell line, and this may reduce the toxic dose of cisplatin in breast cancer chemotherapy

Keywords: Apoptosis, Breast cancer, Cisplatin, Piperine

P-261

Systematic Bioinformatics Approach Represents the Neuroprotective Effects of Neobaicalein

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Background: Parkinson disease (PD) is the second common neurodegenerative disorder, which has no certain cure. However, recent studies have indicated that small phytochemical molecules, especially flavonoids, could have neuroprotective potential. Therefore, we investigated the neuroprotective effects of a flavonoid, neobaicalein, one of the main constituent of an Iranian species of the Scutellaria genus, Scutellaria pinnatifida (S. pinnatifida).

Methods: In this study, we applied the systematic bioinformatics approach to predict the mechanism of neuroprotective activities of Neobaicalein. First, we determined the structural properties of Neobaicalein and specified that it could be used as a drug. To this end, we used MolSoft and the PreADMET server to assessed Lipinski RO5 and toxicity. The Network and enrichment analysis were used to identify the key proteins and pathways in PD. The DisGeNET database was applied to determine PD-related genes and the STRING app in Cytoscape to illustrate the PD network. Enrichment analyses were studied by DAVID, based on the PD network hubs proteins. Finally, Molecular docking and dynamics simulation was performed to investigate the interaction of Neobaicalein with the key proteins in the most significant pathways derived from enrichment analysis.

Results and Conclusion: Our analysis demonstrated that Neobaicalein is a non-mutagen compound and obeys Lipinski RO5. PD network hub proteins are significantly associated with three pathways, including apoptosis, PD, and Ras signaling pathways. Among these proteins, MAPK14, CASP3, MAPK8, and TP53 were selected, as their inhibition can block downstream signaling cascades with the aim of neuroprotectivity. Our simulation demonstrated that Neobaicalein has significant negative binding energy against MAPK14, CASP3, and MAPK8. Also, the stability of MAPK14 and CASP3 were shown by MD simulation. Our results suggest that Neobaicalein could have been a neuroprotective compound by an inhibitory effect on crucial proteins in the pathways leading to PD, such as apoptosis and the Ras signaling pathway.

Keywords: Neobaicalein, neuroprotective, Network analysis, Docking simulation, molecular dynamics (MD) simulation

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Recommendation for optimizing the function of nanoniosmes with doxorubicin to inhibit the proliferation of BCSCs

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Background: In recent years, the structures of pharmaceutical lipid nanocarriers have attracted much attention. Nanoniosomes are a suitable and targeted drug delivery system that has a higher capability than conventional drug therapies. Breast cancer stem cells (BCSCs) are a heterogeneous subset of cells in the breast. These cells survive even after chemotherapy and are responsible for starting a new tumor. Targeting BCSCs is essential for achieving a radical treatment in breast cancer. The use of lipid nanocarriers can target BCSCs. The fabrication of nanoniosmes containing breast cancer drugs such as doxorubicin is one such case. Doxorubicin interacts with DNA by interfering with and inhibiting macromolecular biosynthesis, which inhibits the development of the enzymatic topoisomerase II. This stops the reproduction process. **Methods:** In this review study, searches in electronic and scientific databases of PubMed, Medline, Google Scholar, Scopus, and ISI were performed and valid articles related to the subject were studied using the keywords Niosome, Nano, Doxorubicin, and BCSCs Were searched.

Results: Certainly, by making such nanoniosmes, a new step can be taken in the discussion of drug delivery and effective treatment. Also, research in this area could be more accurate in treating other diseases and cancers.

Conclusion: By designing and developing new drug delivery systems such as nanoniosmes, those could be a great help in treating many diseases since it not only increases the activity of the drug in the target tissue but also greatly reduces the toxicity of the drug, and releases correctly at the operation site. Certainly, this new drug delivery system can replace the current drugs in the not too distant future.

Keywords: Keywords: Noisome, Nano, Doxorubicin, BCSCs

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Emerging role of Non-Coding RNAs in the Pathogenesis of Neurodegenerative Diseases

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Background: Neurodegenerative diseases are a heterogeneous group of disorders that affect the central nervous system (CNS) and cause progressive degeneration of neuronal cells. Today, new evidence drove our attention to the notable role of non-coding RNAs (ncRNAs) as mediators of gene expression in these debilitating conditions. These molecules, including microRNAs (miRNAs), long ncRNAs (lncRNAs), and competing endogenous RNAs (ceRNAs) confer neuronal cells with the potential to exert wide control over the neurobiological mechanisms.

Methods: We searched the PubMed, google scholar, and scopes database to explore the role of ncRNAs and the development of neurodegenerative disorders, such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's diseases (PD), Huntington's disease (HD), multiple sclerosis (MS), and epilepsy between September 2010 and September 2020. After performing the literature search and review, 100 eligible studies were identified.

Results: There is a close relationship between these novel transcripts and the development of neurodegenerative disorders. Interestingly, unveiling the underlying pathways helps better understand their physiological and pathological interactions with ncRNAs. Therefore, this approach is a decisive step to open new windows for efficient therapies in this field of research. It should be considered that the role of miRNAs, such as miR-146a or miR-155 and lncRNAs, such as UCA1 or H19 in the development of neurodegenerative diseases has been more studied rather than the new concept of ceRNAs.

Conclusion: What is particularly interesting in the light of our study is a comprehensive look at the role of all the related ncRNAs in this research area, including ceRNAs (lncRNAs MALAT1 and TUG1 acting as ceRNAs for miR-101 and miR-9-5p, respectively). Hence, our narrative review may provide additional insight into the expansion of clinical interventions related to neurodegenerative diseases.

Keywords: Neurodegenerative diseases, Non-coding RNAs (ncRNAs), Inflammation

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Investigation of the mechanism of the effect of doxorubicin and methotrexate loaded on nanoparticles on cytotoxicity and apoptosis: A systematic review

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Background Doxorubicin and Methotrexate are highly effective approved agents against Osteosarcoma cancer cells. Nanosystems are designed to deliver drugs with more efficiency, so enhance apoptosis and cytotoxicity in cancer cells. To determine the state-of-the-art related with effects of nanoparticles loaded with DOX/or MTX on osteosarcoma. It is very important to explore and find the best concentration of the Drug/nanosystem to apply to the research.

Methods: Therefore, we systematically review original studies that investigated the apoptosis and cytotoxic effect of nanoparticles loaded with DOX/or MTX via in vitro models. Articles were systematically collected by screening the literature published online in the following databases; PUBMED and SCOPUS and Web of Science and EMBASE.

Results: Most of the studies were carried out on the MG-63 cancer cell line and common types of nanosystems preparation were Nanoparticle-based systems in all works. DOX and MTX were applied in 32 and 12 articles respectively. Two articles examined the synergistic effect of DOX with Cisplatin and Gemcitabine. Time and/or concentration-related-cytotoxicity of Drug/ Drug-nanosystem explained in studies. The usage of nanosystem significantly enhanced DOX/MTX-induced mortality rates. Most of the nanosystems (27 articles) showed low cytotoxicity to osteosarcoma cell lines. (Ten studies reported no cytotoxic data.) Synthesized drug-nanosystems differ in shape, and a size ranging from <10 nm to >300 nm had a wide range of cytotoxicity potency, with IC50 values between 0.5-20μg/ml. The percent of apoptosis stated in the rage of 21% to 99.85%.

Conclusions: Delivery of DOX/or MTX to tumors with carriers is necessary because this strategy will omit a lack of specificity and selectivity and prevent the use of high dosages in the treatment[15]. This effect will be a rise in a time/or dose-dependent manner. The studies which assayed cytotoxicity and apoptosis of DOX or MTX nanosystems by using osteosarcoma cell lines, exposure time, drug dose, drug/NP dose, NP size, and morphology in in-vitro models, analyzed in this review. Also, an overview of IC50 drug and drug/NP in various osteosarcoma cancer cells. Further studies are required to address the mechanisms of cytotoxicity and apoptosis, with a vital need for in vivo studies.

Keywords: osteosarcoma, nanopaticle, doxorubicin, Methotrexate, cytotoxicity, apoptosis

P-265

Design and evaluation of Real-Time PCR for rapid detection of V. cholerae genome in water and stool samples.

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Background: Vibrio Cholerae O1 and O139 have been identified for their capacity to cause epidemics. Surviving organisms are colonized in the small intestine and produce cholera toxin, a major virulence factor for V. Cholerae pathogenic strains. “viable but non-culturable” (VBNC) cells of V. Cholerae must also be identified as to origin diarrhea. The aim of this study was to develop a rapid, sensitive, and specific assay for the detection all of V. Cholerae pathogenic strains.

Methods: A Real-Time PCR assay was designed targeting ctxA gene of V. Cholerae pathogenic strains followed by a collection of the extracted DNAs of the standard strains ATCC 14035 and spiked in water and stool samples. The assay was developed using SYBR Green, and designed primers for the detection, and the products were differentiated based on melting temperature (Tm) analysis. The results were compared with the PCR method.

Results: Analysis of 44 specimens showed successful detection of V. Cholerae. The LOD of these cases was 10^3 CFU for Real-Time PCR and 25 CFU/PCR reaction for PCR method. The addition of a DNA purification step prior to the assay increased the sensitivity 10-fold to 10^3 CFU.

Conclusion: These results indicated that Real-Time PCR can be used for rapid detection of V. cholerae from various environmental water samples and stool specimens. This method has a strong potential for detecting toxigenic strains of V.cholerae by using the ctxA marker. The development and marketing of an academic research-led potential Real-Time PCR for pathogen detection help generate health for policy and wealth both for the institution and the country. Considering the disease, bacterial pathogenic potential, cholera killing as bioterrorism, the diagnosis of V.cholerae is significant and not commonly performed in medical diagnostic laboratories. Methods for practical, rapid, and sensitive detection of V. cholerae are in great demand.

Keywords: V. cholerae, SYBR green Real-Time PCR, ctxA

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Lipid accumulation and SIRT1 gene expression in HepG2 cells and their viability in response to trans-palmitoleic acid

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Background: The relation between consumption of trans fatty acids and metabolic diseases is still controversial. In the present study we investigated the in vitro effects of trans-palmitoleic acid (tPA) in comparison to palmitic acid(PA) on lipid accumulation and cell viability in hepatocytes, focusing on the gene expression of sirtuin 1 (SIRT1) as well as the transcriptional activity of peroxisome proliferator-activated receptors alpha (PPARα).

Methods: Human hepatoma (HepG2) cells were cultured and treated with various concentrations of tPA(C 16:1),PA (C16:0), and cis-palmitoleic acid (cPA). Cell viability was assessed by MTT assay after treatment of the cells with 0.25 – 2.5 mM concentrations of the fatty acids. The accumulation of triglyceride (TG) in the cells were measured by enzymatic method. Gene expression was evaluated by real-time PCR. The activity of PPARα was assessed by luciferase reporter assay after transfection of HEK293T cells by a vector containing the PPAR response element.

Results: tPA revealed a positive effect on cell viability and increased cell survival. On the contrary, concentrations more than 1mM for PA and cis-palmitoleic acid reduced the viability of hepatocytes. Additionally, tPA at high concentration (1.5 mM) significantly augmented the expression of SIRT1 while its physiological concentration had no effect on the expression of SIRT1. Low concentration of tPA (0.1 mM) modestly increased PPARα transcriptional activity. Both tPA and palmitic acid caused lipid accumulation in HepG2 cells; however, TG content of cells that received tPA was considerably lower compared to PA-treated cells and tPA could also attenuate the steatotic effect of PA.

Conclusion: tPA causes less lipid accumulation in hepatocytes, increases cell viability and might be beneficial by increasing the PPARα activity. Further human and animal studies might provide evidence for the possible benefit of consumption of this fatty acid in non-alcoholic fatty liver disease.

Keywords: Trans fatty acid (TFA), trans-Palmitoleic acid, Sirtuin 1, Peroxisome proliferator –activated receptor (PPAR), Liver, Gene expression

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In vitro permeation of nanoliposome containing bevacizumab (Avastin) across the blood-retinal barrier model

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Background: Bevacizumab (Avastin), an anti-VEGF antibody, is a humanized monoclonal antibody that inhibits VEGF-dependent angiogenesis. It is one of the most effective drugs widely used for the treatment of ocular angiogenesis diseases. Due to limitations for the permeation of large protein therapeutics, including the anti-Vascular Endothelial Growth Factors, nanoliposome was employed as an acceptable ophthalmic drug delivery system.

Methods: Nanoliposome containing Avastin (NLP-BVZ) was prepared using a thin-film evaporation method followed by hydration. The retinal pigment epithelial (RPE-19) cell monolayer was established on the permeable membrane inserts as a blood-retinal barrier model. Barrier integrity was measured via transepithelial resistance (TER). To determine the permeation of BVZ encapsulated in NLP through the cell barrier, BVZ was fluorescently labeled using fluorescein isothiocyanate (FITC). Moreover, the toxicity of NLP-BVZ was evaluated by MTT assays.

Results: TER values were obtained in the range of 40 – 45 Ω cm 2. The amount of fluorescence appearing in the basolateral compartment of the transwell showed the transport of the formulation through the cell layer. MTT assay indicated that NLP-BVZ did not have a considerable influence on the mitochondrial metabolism of RPEs.

Conclusion: Our prepared formulation can across the retinal barriers model, and it may offer improvements in the treatment of ocular neovascular disease.

Keywords: Bevacizumab (Avastin), nanoliposome, Blood–retinal barrier model, ocular drug delivery

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Evaluation of the impact of purine nucleotides and mycophenolic acid inhibitor on the mouse retinal IMPDH1 in response to structural regulation

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Background: Inosine monophosphate dehydrogenase (IMPDH) controls the biosynthetic pathway of guanine nucleotides. The IMPDH-catalyzed conversion of IMP to XMP is the rate-limiting step in guanine nucleotide biosynthesis. It is nowadays accepted that binding of purine nucleotides to the allosteric regulatory domains leads to overall conformational changes of the enzyme. In this report, we are aimed to evaluate the mode of allosteric regulation of IMPDH1 (514) and IMPDH1 (546), via their structural variation, using mainly chromatography techniques.

Methods: Following cloning, expression and affinity purification of the mouse retinal enzymes, we added identical concentrations of each enzyme to the assembly buffer (50mM Tris, 100mM KCl, 1mM DTT, pH 7.4 containing 3mM IMP, 5mM NAD and 0.1mM of either ATP, GTP and/or MPA), incubated for 15 min at room temperature, followed by chromatography/spectroscopy overall structural evaluations.

Results: Our results clearly indicated that ATP augmented the association of the monomeric subunits of both IMPDH1 isoforms to octameric format with activity enhancement. However, MPA treatments led to macromolecular aggregates devoid of catalytic activity. Despite ATP, GTP treatments led to dispersion of the macromolecular associates and activity quenching.

Conclusion: Based on our data, it can be concluded that modulation of IMPDH1 (514,546 variants) activities by the allosteric modulators (ATP or GTP) and the MPA inhibitor mainly occur via subunit association/disassociation of the enzyme subunits. Different responses of the 514 and 546 isoforms to these treatments were seen.

Keywords: IMPDH1, retinal isoforms, association

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Quantitative evaluation of SGCA gene expression in testicular biopsies of infertile men

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Background: Spermatogenesis is a highly dynamic process that is controlled by many factors including genetics. Sarcoglycan α, 50 kDa dystrophin-associated glycoprotein (Sgca), as an integral plasma membrane protein, specifically expresses in cytoplasm of primary and secondary spermatocytes. Recently, researches implicated the important role of SGCA protein in structure/motility of sperm in mice. This study was aimed to evaluate the expression profile of the SGCA gene in the testicular tissues of infertile men under testicular epididymal sperm extraction (TESE) operation.

Methods: Twenty-five biopsy samples were obtained from infertile men under TESE procedure, including eight samples from patients with hypospermatogenesis (positive control), eight samples from patients with Sertoli cell only syndrome, and nine of them from patients with complete maturation arrest at second spermatocyte level. After cDNA synthesis, the mRNA level of SGCA was evaluated by quantitative real-time polymerase chain reaction and GAPDH gene was used as the internal normalization control.

Results: Our collective data revealed significantly lower expression of SGCA transcripts in groups with complete maturation arrest at second spermatocyte and Sertoli cell only syndrome, in comparison with positive control group.

Conclusion: It seems that decreased expression of SGCA have destructive effects on sperm motility and function, which consequently cause to male infertility/subfertility.

Keywords: Infertility, Spermatogenesis, SGCA, qRT-PCR

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Is there a relationship between MPO-129G/A polymorphism and atherosclerosis in west of Iran?

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Background: Coronary artery diseases (CAD) are identified as the major cause of mortality in developed and developing societies. One of the leading causes of CAD is atherosclerosis. Different risk factors, including environmental and genetic factors, can affect the progression of atherosclerosis. Myeloperoxidase polymorphism (MPO-129G/A) may consider as a genetic risk factor for atherosclerosis. The aim of the present study is to investigate the relationship between MPO-129G/A polymorphism and atherosclerosis in west of Iran.

Methods: A total of 255 subjects containing 125 controls and 130 cases were included in this study. Blood samples were collected from all participated subjects in two tubes with and without EDTA for assessment of MPO-129G/A polymorphism and biochemical factors, respectively. Lipid profile and FBS were evaluated using an auto-analyzer. Furthermore, the MPO-129G/A was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: There was a significant difference between total cholesterol, LDL and FBS in the control and patient groups ($P<0.05$). Moreover, no significant relationship was observed regarding MPO-129G/A polymorphism in both groups ($P>0.05$).

Conclusion: 129G/A polymorphism was not related to atherosclerosis. While, the incremented LDL level and total cholesterol were recognized as risk factors for atherosclerosis.

Keywords: Atherosclerosis, Myeloperoxidase, MPO-129G/A polymorphism

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Oxidant/antioxidant status in type-2 diabetes mellitus patients with metabolic syndrome

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Background: Diabetes mellitus (DM) and metabolic syndrome (MS) co-occurrence is increasing worldwide. These chronic diseases cause life-threatening complications in long term. Oxidative stress is related to pathogenesis of many diseases. To realize pathophysiological mechanisms of Type-2 diabetes mellitus (T2DM) and its related complications, we investigated the oxidant/antioxidant status and Na+-K+ATPase activity in T2DM with concurrent MS subjects.

Methods: Ninety individuals including fifty patients diagnosed with T2DM and MS, but without overt diabetes complications, and forty individuals without T2DM or MS as control group participated in this study. Plasma malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) activities, total antioxidant capacity (TAC), and Na+-K+ ATPase activity was assessed by standard laboratory methods.

Results: Serum MDA in patient group was significantly higher than control group ($P<0.05$). Whereas, Na+-K+ ATPase activity was statistically significantly lower in the patient group ($P<0.05$). TAC, SOD, and GPx enzyme activities were not statistically significantly different between two groups ($P>0.05$). Results from the patient group showed positive correlations between GPx activity and weight, body mass index, and waist circumference. In addition, there was a positive correlation between MDA results with high-density lipoprotein-cholesterol and total cholesterol and a negative correlation with TAC, BMI, and weight ($P<0.05$) in the control group. There were correlations between Na+/K+-ATPase activity with some of the components of MS.

Conclusion: The lack of significant differences showed in the present study in plasma antioxidant markers levels between the two groups may be due to the lack of progression of diabetes complications in the patient group. These results emphasize the necessity of initiation and sustainable assessment of cardiovascular disease risks in diabetic subjects. Implementation of early interventions could improve diabetes management and prevent the progression of diabetes complications.

Keywords: Metabolic syndrome, Oxidative Stress, Na+-K+ATPase, Type 2 diabetes

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Investigation of biochemical parameters and antioxidant markers in diabetic patient with metabolic syndrome

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Background: Metabolic syndrome (MetS) is a clustering of risk factors that occur together, increasing risk of type 2 diabetes, heart disease and stroke. An oxidant/antioxidant disequilibrium has an important role in MetS and type2 diabetes. In this study, we aimed to evaluate the levels of biochemical parameters and oxidative stress markers in plasma of healthy and type 2 diabetic men with MetS.

Method: In this case-control study, 40 men with type 2 diabetes and MetS and 40 apparently healthy men between 40 and 60 years of age were recruited. Subjects were not associated with any diabetic micro and macrovascular complications. To evaluate the indicators of oxidative stress and lipid oxidation, the activity of catalase (CAT) as a marker of oxidative stress and malondialdehyde (MDA), as an indicator of lipid peroxidation, were measured.

Result: MDA level was significantly elevated in T2DM with MetS patients ($p<0.05$). In contrast, there was no important difference in CAT activity levels between the two groups ($p>0.05$). CAT activity correlates positively with the triacylglycerol levels in the patient group and whole subjects. MDA positively correlates with HDL-C and total cholesterol and negatively correlates with waist circumference and BMI in the control group.

Conclusion: No significant difference was observed in the CAT activity levels between the two groups. Possibly, since the subjects had no diabetic micro and macrovascular complications. Complications of type 2 diabetes are potentially associated with impaired antioxidant defense and changes in oxidative stress markers. These points ultimately indicate the importance of early detection of this disease.

Keywords: Antioxidants, Metabolic syndrome, Type 2 diabetes

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Comparison of Creatinine and Cystatin c for Evaluation of Renal Function

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Background: To evaluate the renal function, the glomerular filtration rate (GFR) is measured using endogenous and exogenous material clearance. So far, an ideal marker for determining GFR has not been discovered. However, inulin is the gold standard but methodological limitations are associated with the use of this method. In clinical practice, GFR determination is based on creatinine. However, its application is limited by a number of patient-dependent and patient-independent factors. Recently, cystatin C protein has been introduced to be as an alternative endogenous glomerular filtration marker. It is produced by nuclear cells, filtered out of glomeruli, degraded by the proximal tubular epithelial cells of the kidney and not affected by external factors.

Methods: This review provides an overview of several articles on websites such as PubMed about renal function.

Results: According to one study, 117 patients with kidney transplantation was selected and their GFR was estimated based on cystatin C and creatinine. For control, GFR was measured using an exogenous biomaterial called diethylenetriaminepentaacetic acid (DTPA) and compared accuracy, precision and bias. The mean 99mTc-DTPA GFR was 58 ml/min per 1.73 m2. GFR based on cystatin C with Fischer equation had the lowest bias (1.7 ml /min per 1.73 m2), the highest precision (11.4 ml/min per 1.73 m2) and the highest accuracy (89%).

Conclusion: Cystatin C is more sensitive but less specific than creatinine for measurement. The sensitivity of cystatin C and creatinine were 86%and 78%, respectively, and the specificity were 70% and 73%, respectively, so the estimated GFR based on cystatin C is more accurate than creatinine.

Keywords: Creatinine, Cystatin C, GFR, 99mTc-DTPA

P-274

A Comparative Study of 25 (OH) Vitamin D Serum Levels in Patients with metabolic syndrome and healthy individuals

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Background: The incidence of metabolic syndrome has been rising in the population of Iran. In parallel, vitamin D deficiency has also been increasing in Iran. This study aims to explore the association of vitamin D serum concentrations with metabolic syndrome and its components in the population of Iran.

Methods: A case-control study was managed. We enrolled 110 metabolic syndrome patients, according to the National Cholesterol Education Program Adult Treatment Panel III (ATP III) criteria as a case group and 130 healthy individuals as a control group. The serum level of 25-hydroxy vitamin D (25 (OH) D), lipid profile, and FBS status were determined using a commercially available ELISA method. Enzymatic methods determined total cholesterol (Chol), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) and triglyceride (TG) levels.

Results: In case group, the prevalence rate of 25 (OH) D deficiency (<10 ng/mL) was 10%, 25 (OH) D insufficiency (10–29 ng/mL) was 71.0%, 25 (OH) D sufficiency (>30 ng/mL) was 19.0%. A non-significant association between Chol level and age was noted ($p=0.46$, $p=0.124$). The levels of FBS (0.000) and TG (0.000) were significantly higher, and the levels of 25 (OH) D (0.01), LDL (0.002), and HDL (0.000) were significantly lower in case of the group compared to the control group.

Conclusion: We found that the serum level of 25 (OH) D in patients with metabolic syndrome is lower than the healthy group, and a low level of 25 (OH) D is related to increased risk of metabolic syndrome and its components.

Keywords: vitamin D, metabolic syndrome

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LncRNA; Application in Treatment and Diagnosis of Neurodegenerative Diseases

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Background: Long non-coding RNAs (lncRNAs), described as transcripts with more than 200 nucleotides lengths, generally lack the protein-coding potential. However, there are some exceptions. As a regulatory RNA, they have a significant role in the regulation of gene expression in various levels, such as transcription, translation, and epigenetic via cis or trans manner. They also are under the control of more complicated and precise factors such as Swr1 chromatin remodeling complex and oncogenic factors like Myc. They also play a crucial role in the development and differentiation of the brain and CNS. This means that their mutation or abnormal expression may contribute to neurodegenerative diseases, characterized by progressive loss of neurons in specific regions of the brain.

Methods: To explore the role of lncRNAs and the development of neurodegenerative diseases, such as Alzheimer, Parkinson, Huntington, and Amyotrophic Lateral Sclerosis and to find the latest information in this field, we searched in various citation databases, including Science Direct, PubMed, and Google Scholar between September 2010 and September 2020 and reviewed them.

Results: Numerous studies have revealed a strong association between different lncRNA expression patterns and neurodegenerative diseases. For instance, a growing body of evidence has suggested that the BACE1-antisense transcripts are considerably up-regulated in the brain samples of Alzheimer's disease patients. Furthermore, the up-regulation of Na-pink1 disturbs the mitophagy mechanism which leads to cell apoptosis and neuronal loss in Parkinson’s disease.

Conclusion: Since the aberrant expression of lncRNAs causes the development and advancement of neurodegenerative disorders, lncRNAs could be regarded as suitable biomarkers and therapeutic targets for early diagnosis and treatment. Subsequently, this issue is due to the specific expression of these molecules in the progressive development of the disease and their relatively constant and measurable levels in the body fluids as well.

Keywords: Long Non-Coding RNA ،lncRNA ،Neurodegenerative Disease ،Alzheimer ،Parkinson ، Huntington ،Amyotrophic Lateral Sclerosis (ALS).

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Cucurbitacin I from Ecballium elaterium (L.) A. Rich induces LC-3 gene upregulation in human gastric cancer cell line AGS

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Background: Plants either directly or indirectly have been a component of human healthcare system. As the research continues, it is becoming clear that some medicinal plants can be utilized as a treatment of cancers. Ecballium elaterium, commonly known as squirting cucumber is an herb that grows abundantly in the Mediterranean region and produces cucurbitacins. Cucurbitacins target several signalling pathways and display a range of anti-cancer functions. In this study, we examined the effects of Cucurbitacin I purified from E. elaterium fruits on LC-3 gene expression in AGS cell line.

Methods: Using quantitative reverse transcription polymerase chain reaction (qRT-PCR), the expression of LC-3 gene was quantified in AGS cells 24 hours after treatment with cucurbitacin I.

Results: Purified cucurbitacin I upregulated LC-3 in AGS cells (p-value <0.05).

Conclusion: Cucurbitacin I purified from E. elaterium fruits upregulates LC-3 gene in human gastric cancer cell line AGS. The present study provides new insights into the molecular mechanisms underlying cucurbitacin-mediated cell death in gastric cancer.

Keywords: Cucurbitacin I, Gastric cancer, LC-3 gene

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Evaluation of anticholinesterase activity of propolis samples from three different regions of Kerman Province

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Background: The administration of acetylcholinesterase inhibitors is very common for the symptomatic treatment of Alzheimer’s disease (AD) and the other forms of dementia and CNS disorders. Nowadays, developing the new anticholinesterase drugs with higher pharmaceutical properties and less side effects from natural sources has been attracted much attention. Propolis is one of the honey bee products which is known for its many biological activities such as anticancer, anti-inflammatory, antimicrobial and antioxidant. Recently, the scientific reports indicating the anticholinesterase activity of several propolis samples from Korea and Turkey have been published. In this study, the anticholinesterase activity of three propolis samples collected from different regions of Kerman Province (Lalehzar, Rayen and Kerman) has been evaluated.

Methods: Ellman method was applied to investigate the anticholinesterase activity of the ethanolic extracts of propolis samples from three different regions of Kerman Province (Lalehzar, Rayen and Kerman). For this purpose, the ability of six different concentrations of the extracts to inhibit acetylcholinesterase enzyme (AChE) activity was measured and the IC50 value for each sample was obtained using the plotted dose-response curve. Neostigmine was used as a reference compound.

Results: Comparison of IC50 values corresponding to the extracts with neostigmine IC50 (22.26 ng/ml) demonstrated that all three propolis samples possess a good ability to inhibit AChE, but the anticholinesterase activity of Lalehzar propolis (IC50: 14/37 µg/ml) is almost 5 and 5.5 times higher than Rayen (IC50: 69/53 µg/ml) and Kerman samples (IC50: 77/73 µg/ml), respectively.

Conclusion: The results indicate that the ethanolic extract of Lalehzar propolis has a great potential to find new anticholinesterase drugs. However, this requires to identify and thoroughly study the anticholinesterase components of the extract.

Keywords: Propolis, Alzheimer's disease (AD), Acetylcholinesterase inhibitors, Anticholinesterase activity, Ellman method.

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Investigation of antioxidant activity of propolis samples from three different regions of Kerman Province

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Background: Nowadays, the replacement of synthetic antioxidants with antioxidants derived from natural sources has been widely considered in the food industry. In addition, based on the scientific reports, consumption of natural antioxidants not only reduces the risk of several diseases such as cancer, cardiovascular and Alzheimer's disease, but also helps the treatment process. Propolis is one of the most important honey bee products that has many biological properties such as antioxidant, antibacterial and antifungal. In this study, the antioxidant capacity of three different propolis samples from Kerman Province has been investigated.

Methods: DPPH method was used to evaluate the antioxidant activity of ethanolic extracts of propolis samples from three different regions of Kerman Province (Lalehzar, Rayen and Kerman). For this purpose, the ability of six different concentrations of the extracts to inhibit DPPH free radical activity was measured and the IC50 value for each sample was calculated using the plotted dose-response curve. Ascorbic acid was used as a reference compound.

Results: The comparison of the IC50 values corresponding to the propolis extracts with ascorbic acid IC50 showed that all three propolis samples have a good ability to scavenge free radical DPPH, but the antioxidant activity of Lalehzar propolis (IC50 = 5.64 µg/ml) is almost 7.1 and 8 times higher than Kerman (IC50 = 39.97 µg/ml) and Rayen (IC50 = 44.47 µg/ml) samples, respectively.

Conclusion: The results demonstrated that the ethanolic extract of Lalehzar propolis is a rich source of natural antioxidants and have a great potential to identify and isolate this group of antioxidants.

Keywords: Natural antioxidants, Propolis, DPPH method, Ethanolic extract, Antioxidant activity.

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Cross Reacting Material 197 (CRM197) Structure and Anticancer Effect

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Background: Genetic heterogeneity accompanied by metastasis is the most important factor which has faced cancer treatment with the challenge. Recent studies have introduced cross-reacting materials (CRM197) as a promising new biological anticancer drug to improve cancer therapy in patients who have previously resistant to chemotherapy. The weak toxicity of CRM197 accounts for the stimulation of cell apoptosis and antitumor effect. Increasing evidence has indicated that the expression of Heparin-binding epidermal growth factor-like (HB-EGF) growth factor enhanced in most of the cancer cells and in CRM197 is the specific inhibitor of HB-EGF.

Methods: The current study has focused on the structure, properties, and anticancer activity of CRM197.

Results: This review provides strong evidence for CRM197 binding with HB-EGF and also soluble HB-EGF which inhibits the mitogen-activated protein kinase (MAPK) and protein kinase B (Akt) signaling pathways, thereby suppress the cell proliferation, survival, and tumorigenicity. CRM197 inhibits angiogenesis, invasion, and metastasis through protein tyrosine kinase 2 (PTK2), vascular endothelial growth factor (VEGF), and interleukin 8 (IL- 8) genes silencing. The toxicity of CRM197 depends on the activation of p53 pathway in cancer cells carrying a wild type elongation factor 2 (EF2) gene. The combination of chemotherapeutic drugs with CRM197 enhances the effectiveness of cancer treatment. CRM197 as an ideal carrier protein induces the highest production of antibodies with specific and selective functions against cancer cells.

Conclusion: The weak or lack of enzymatic activity of CRM197 in comparison to diphtheria toxin, decreases the survival of cancer cells that over-expressed the heparin-binding EGF-like growth factor (HB-EGF) either by inhibition of HB-EGF interaction with epidermal growth factor receptor (EGFR) or by residual enzymatic activity in cells transfected with CRM197.

Keywords: cross-reacting materials (CRM197), diphtheria toxin (DT), heparin-binding EGF-like growth factor (HB-EGF), epidermal growth factor receptor (EGFR), cancer

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Evaluation of TaqMan Real-Time PCR for detection of Mycobacterium tuberculosis genome in sputum specimen of patients with tuberculosis

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Background: Tuberculosis (TB) is an infectious disease caused by the Mycobacterium tuberculosis complex. That is one of the most serious health problems worldwide, causing high morbidity and mortality rate. Sputum smear microscopy and culture on the Lowenstein-Jensen medium use for TB detection. However, these methods have low sensitivity and specificity. In this study, we use the TaqMan real-time PCR IS6110 to identify Mycobacterium tuberculosis in sputum samples and comparison with PCR results.

Methods: In this study, we designed IS6110 specific primers and TaqMan probe to detect Mycobacterium tuberculosis. DNA was extracted from 20 sputum samples of patients with tuberculosis. Finally, TaqMan Real-time PCR method was compared with PCR results.

Results: Among the 20 samples studied, every 20 samples were positive by TaqMan real-time PCR and 18 samples were positive by PCR method. According to these results, PCR was not able to detect Tuberculosis in 2 positive clinical samples, as compared to TaqMan real-time PCR.

Conclusion: According to the obtained results, the sensitivity of TaqMan real-time PCR has been more than PCR. We showed the effectiveness of using IS6110-TaqMan real-time PCR in sputum specimens. That is a useful diagnostic method for sensitive detection of mycobacterium tuberculosis in sputum samples.

Keywords: Mycobacterium Tuberculosis, IS6110, TaqMan Real-Time PCR

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The monocyte PLAU gene expression is controlled by miR-193b-3p in in-stent restenosis patients

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Background: Neointimal hyperplasia is known as the main contributing factor of in-stent restenosis. Since white blood cells (WBCs), especially monocytes, may play a central role in restenosis process after stent implantation thus the aim of this study was to investigate comparatively the relationships between the urokinase-type plasminogen activator (PLAU) gene expression levels and miR-193b-3P in PBMC samples isolated from patients with coronary artery restenosis.

Methods: A total of sixty-three subjects undergoing coronary artery angiography (Controls, n=21 and stenosis<0.05%; In-stent without restenosis, n=21, stenosis<70%; In-stent with restenosis, n=21, stenosis>70%) were studied. The PBMC samples were isolated from whole blood using ficoll solution. The gene and microRNA expression levels were measured using RT-qPCR technique. The microRNA and gene predictions were performed on the bioinformatics databases and datasets.

Results: Based on the bioinformatics databases and datasets, PLAU gene was suppressed by the miR-193b-3p. The amount of PLAU and miR-193b-3p and their ratio in patient with in-stent restenosis was statistically more than control and stent non-restenosis groups ($P < 0.05$). However, there were not markedly correlation between the level of molecular parameters and other studied variables.

Conclusion: In this study, we suggest that the positive relationship between monocyte PLAU gene expression and miR-193b-3p. However, other studies are needed to support the results.

Keywords: Restenosis ،Prediction ،PLAU ،miR-193b-3p

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A case report of fibromatous epulis tumor in a West Highland white terrier dog and biochemical and immunohistochemical evaluation of this tumor

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Background: Congenital epulis is an unusual benign oral mucosal lesion in dogs with no tendency to recur after excision. Epulis is a non-specific, clinical designation for a localized, exophytic growth on the gingiva. Epulis include two types of fibromatus and acanthomatus and their incidence increases with age in dogs. Oral involvement can cause nutritional problems and impair digestion and general health. The oncological examination may suggest the malignant disease (some changes in the history and physical examination of the patient) and the laboratory analysis (markers of tumor) may bring additional information regarding the presence of the neoplastic process. In this case report, we examined the biochemical and immunohistochemical factors of this tumor.

Case presentation: A West Highland white terrier dog aged about 5.5 years and weighing 9 kg with a history of bad breath, bloody discharge from the mouth, and loss of appetite and weight were referred to a veterinary clinic. On oral examinations and pathological examinations, lesions of epulis masses around canine, incisive, and pre-molar teeth in the maxilla and mandible were observed. Tissues fiex on formalin and Immunohistochemical reactions were carried out on formalin-fixed tissue by the avidin-biotin. Immunohistochemical studies revealed intense staining for vimentin, STRO-1, and CD44, suggesting that it was derived from mesenchymal cells. The positivity for vimentin can be explained by the large amount of collagen and collagen precursors that are present in tumor microenvironment.

Conclusion: In this study, we attempted to define the histogenesis of congenital epulis by using neural histiocytic, epithelial, myogenic, fibroblastic, and glial antigens as cell-type markers. Our case showed in CGGT the presence of vimentin, currently regarded as a marker of intermediate filaments in cells derived from mesenchymal origin. The presence of vimentin and the lack of desmin suggest a mesenchymal non-muscular, non-neural, and non-glial nature of granular cells.

Keywords: case report, congenital epulis, oral lesion, laboratory analysis, tumor markers

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Effects of Curcumin on cell proliferation in A-375 cell line

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Background: The aim of this study was to investigate the lethal effect of curcumin nanomicell on malignant melanoma cancer cell line. Curcumin is a plant-derived polyphenol that has been shown to have antitumor activity, but due to its low aqueous solubility and low bioavailability, its clinical use is limited. Encapsulation of hydrophobic drugs in nanoparticles or nanoparticles such as micelles is one way. It is effective in improving its pharmacological activity.

Methods: After treating the cell line with different concentrations 0.281, 0.141, 0.070, 0.0352, 0.0176 ng / ml of curcumin nanoparticles using MTT method, the corresponding 50% lethal dose (IC50) was calculated.

Results: MTT test data after 24 hours showed a concentration of 0.637 pg / ml as a 50% lethal dose for curcumin nanomicels.

Conclusion: Based on the data of this study, it can be concluded that cell line treatment with a combination of curcumin nanoparticles has effective results in reducing cell survival and reducing the proliferation of melanoma cancer cells.

Keywords: Curcumin nanomicelles, Melanoma, MTT, Apoptosis

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Investigation of the effects of acetylation of human insulin on their structural properties by Molecular dynamics simulation

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Background: Modification and environmental conditions can cause formation of unfolded protein forms that exhibit enhanced tendency to aggregation into amyloids and amorphous precipitates. Protein aggregation is a major concern during purification, formulation, and manufacture of therapeutic protein products.

Method: In this study, molecular dynamics simulation experiments, have been performed on native and acetylated forms of human insulin. Actually, we have investigated the effects of acetylation of lysin and N termini residues on the structure and aggregation properties of human insulin and compare both native and acetylated forms of insulin with each other. Acetyl groups were added to PheB1 and LysB29 of B chain of insulin. Molecular dynamics simulation experiments were done under two different temperatures (37°C and 50°C) and acidic pH environment in YASARA (17.3.30) for 3x-50ns MD simulations in NPT ensemble and using Amber 14 force field.

Results: The results have indicated that acetylated form of insulin undergoes more structural changes and tends to aggregation at higher temperatures and acidic pH conditions which can be the main suspected reasons for protein aggregation. The acetylation of lysin residue of insulin on surface increase the participation of polar residues (particularly acetylated lysin) in protein-protein interfaces. Other results have exhibited that native form of insulin tend to be excluded from interfaces because of its positive change and not because of a loss in conformational entropy while acetylated insulin has less constrained geometrically and more openly packed than native insulin. According to the data of RMSD, acetylated forms of insulin in PheB1 and LysB29 of B chain indicated most changes in structure. Determine the second structure of different forms of insulin were the evidence of these changes.

Conclusion: Therefore, it could provide interesting insights in to an insulin formulation for diabetes to guide development of more effective therapy.

Keywords: Insulin, Molecular dynamic Simulation, lysin acetylation, Protein aggregation.

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Epigenetics and COVID-19 infection: A literature review

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Background: The COVID-19 pandemic is a viral respiratory and contagious disease caused by a new coronavirus named SARS-COV-2. Some molecular mechanisms regulate COVID-19 pathogenicity include virus-host interactions related cell entry, replication and viral infectivity.

Methods: Literatures were searched by following keywords: COVID-19, coronavirus, epigenetics, DNA methylation, histone modifications, non-coding RNA. Electronic databases (PubMed/Medline, Scopus and Web of Science) were screened.

Results: Epigenetic investigations such as DNA methylation, histone modifications, non-coding RNAs and chromatin remodeling survey the genetic and nongenetic agents that regulate external and environmental factors that change host gene expression patterns without any alteration in the underlying genotype. Structural analyses clarified hot spots in viral binding domains and some specific proteins in the host such as receptor angiotensin-converting enzyme 2 (ACE2) and the transmembrane protease serine 2 (TMPRSS2) that involve in cell entry and viral infectivity. In this review article our goal is to provide an update of the main investigations at the interface of epigenetics and coronavirus infection. Specially, we emphasize the epigenetic agents that interfere in viral replication and infection of COVID-19.

Conclusion: Recent epigenetic studies have revealed that global DNA methylation in ACE2 gene and post-translational histone modification may have an effect on host tissues, biological age- and sex-biased patterns in viral entry and infection.

Keywords: COVID-19, Epigenetics, DNA methylation, virus infection.

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Designing and characterization of an antiangiogenic and antitumor KDR binding peptide

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Background: VEGFs family potently promote angiogenesis through VEGFR2. Recent studies aimed at inhibiting angiogenesis have focused specifically on inhibiting the VEGFR-2 receptor, and significant advances have been made in clinical trials of antagonists designed as antiangiogenic agents.

Methods: In the present study, based on the VEGF-A/VEGFR-2 structure, a cyclic peptide (VGB-A1) was designed from the L1 loop region containing residues 33-51 of VEGF-A. The peptide sequence was VGB-A1 2HN-CVDIFQEYPDEIEYIFKPSC-COOH. The ability of VGB-A1 to bind to both VEGFR-1 and VEGFR-2 receptors was evaluated and the bioactivity and efficacy of the peptide were evaluated in vitro and in vivo.

Results: Unexpectedly, VGB-A1 bind to VEGFR-1 as well as bind to VEGFR-2. VGB-A1 inhibited cell proliferation in human umbilical vein endothelial cells (HUVEC) and 4T1 mammary carcinoma tumor cells. Remarkably, based on immunocytochemical studies, VGB-A1 bounded to both VEGFR1 and VEGFR2 and blocked their homo- and heterodimerization in human umbilical vein endothelial cells (HUVECs) as well as 4T1 mammary carcinoma tumor cells. Also, VGB-A1 was able to bind to VEGFR-1 and VEGFR-2 receptors instead of bt-VEGF in the displacement assay. Peptide blocked cell proliferation, cell migration and metastasis by inhibiting phosphorylation and thus inhibiting the activation of ERK1/2, AKT and further inhibiting the FAK / Paxillin, MMP and E-Cad and NF-KB signaling pathways in both cell lines HUVEC and 4T1. In a murine 4T1 MCT model, VGB strongly inhibited tumor growth and metastasis without causing weight loss.

Conclusion: VGB-A1 can be a strong candidate for therapeutic applications in various angiogenesis therapies, especially cancer.

Keywords: VEGF-A , KDR , Angiogenesis , Peptide Design

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Harmful effects of micro / nanoplastics on human health

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Background: In fact, microplastics actually describes very small particles and plastic fibers (particles less than 5 mm). The uptake of most MPs in the gut is mediated by gut-associated lymphoid tissue (GALT), especially by (M) and other cells. Nanoparticles are transported from the intestine through M cells and from there through the lymphatic system and the liver and bladder into the blood. The particles are then re-released in the intestine along with bile before defecation and urine. Respiratory symptoms associated with airway obstruction and interstitial lung disease Occupational exposure to airborne microplastics is seen in workers in the synthetic textile industry, birds, and polyvinyl chloride or polyvinyl chloride, whose lesions are successfully replicated in vivo. Exposure of macrophages and culture of lung epithelial cells to PS (60 µm) causes ROS and stress on the endoplasmic reticulum (due to accumulation of incorrect proteins) and leads to autophagy cell death. Therefore, toxicity and oxidative stress may be important mechanisms of microplastic toxicity. Circulating microplastics can cause inflammation, pulmonary hypertension, clogged arteries, increased coagulation, and blood cytotoxicity. Indeed, in vitro, PS (3243 nm) led to endothelial accumulation and adhesion of erythrocytes. After exposure to microplastics, they may cause a local or systemic immune reaction, depending on their release. It may also lead to neurotoxicity.

Methods: To investigate the effects of different dimensions of micro / nanoplastics on human health to review and collect information on this topic, a review of related articles in the databases of PubMed, Scopus, Web of Science, ScienceDirect, etc. has been done between 2010 and 2020.

Result & Conclusion: Due to the increasing consumption of plastics (micro / nano), increasing the prevalence of neurological diseases, immune disorders and cancer may be related to increased exposure to environmental pollutants, including microplastics.

Keywords: Keywords: Microplastic- Nanoplastic- Polystyrene- PS- MPs

P-288

Serum serotonin, ceruloplasmin and copper levels as markers of response to chemotherapy in breast cancer compared to CEA and CA15-3

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Background: After lung cancer, breast cancer is the most common cancer in all developed countries. Finding effective remedies has always been a concern of the human mind so, tumor markers can be helpful on this way. The purpose of this study was to compare the levels of serotonin, copper and ceruloplasmin beside the routine breast cancer markers such as CEA and CA15-3 in patients with invasive ductal breast cancer, before and after chemotherapy.

Materials and methods: This study was performed on 30 patients with breast cancer before and during chemotherapy. Venous blood samples were taken from patients. Necessary data including age, tumor grade and status of Her-2, ER, PR receptors were obtained from patient records. Serotonin, CEA and CA15-3 levels were measured by ELISA, ceruloplasmin was measured by nephelometry and calorimetric copper was measured.

Results: Results showed mean decrease in serotonin, ceruloplasmin, copper, CEA and CA15-3 after treatment. Whereas the decrease in serotonin and ceruloplasmin was monotone. No significant relationship was observed between Tumor grade and ER-PR, Her-2 receptors.

Conclusion: This study showed that unlike CA15-3 and CEA that did not change uniformly during chemotherapy, serotonin and ceruloplasmin had a relatively uniform decreasing trend. Therefore, , they can be considered as a viable alternative to routine markers in the treatment process as long as the current results be confirmed by further researches.

Keywords: Keywords: Serotonin, Ceruloplasmin, Copper, CEA, CA15-3, Breast Cancer

P-289

Production of polyclonal antibodies against caspase 3 and 7

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Background: Caspases are homologous cysteine proteases that cleave after Aspartic acid residues. The executioner caspases of 3 and 7 play key roles in the execution stage of apoptosis including the initiation of DNA fragmentation, inactivation of DNA repair processes and reorganization of cytoskeletal proteins. These caspases display 57% sequence identity. Therefore, special care is needed to produce specific polyclonal antibodies that specifically recognize these antigens. Polyclonal antibodies (PAb) are widely in research and diagnostics. They specifically interact with antigens, mostly protein antigens, and are used in western blots and several different immunoassays. The objective of this study was to produce polyclonal antibodies against executioner Caspases- using recombinant protein antigens.

Methods: Caspases -3 and -7 were expressed in Escherichia coli system (E. coli BL21) and then purified using the Ni-NTA column. The expressed proteins were analyzed with SDS-PAGE and their activity were measured. Pure recombinant proteins were then used to immunize white New Zealand rabbits for the production of the antibodies. Rabbits' bloods were collected and the sera were separated. Antibodies were further purified and tested for western blotting.

Results: The antibodies were analyzed for its specificity to Caspases without any cross reactivity to each other antigen.

Conclusion: In this study, to generate pAb, immunogens are intentionally inoculated into the host, similar to the process of vaccination. The purified antibodies were capable of recognizing Caspase antigens specifically. Despite the high similarity of caspases -3 and -7, they did not cross react suggesting that the exposed epitopes are similar. These antibodies also recognized large and the small subunits of endogenous caspases. Keywords: Polyclonal antibodies; Caspase-7; Caspase-3

Keywords: Polyclonal antibodies, Caspase-7, Caspase-3

P-290

Polyclonal human caspase-9 Antibody production in rabbit for research application

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Background: Caspase-9 is a cysteine protease expressed in many cells, and is responsible for the initiation of apoptotic by cleavage of caspase-3 and its activation. Therefore, investigation of the intrinsic apoptosis pathway requires specific antibodies against this enzyme. Polyclonal antibodies are secreted from the B cells, which can bind to different epitopes of the antigens. Polyclonal antibodies are used in many laboratory techniques such as Western blotting (WB). Immunoprecipitation and immunofluorescence assays. WB of caspase-9 is an important technique in biological research involving apoptosis and differentiation. In this method, the protein of interest is detected through specific antigen-antibody interaction. On a blot. Detection of cleaved caspase-9 in a Western Blot can be a sign of cellular apoptosis.

Method: Antigen production was done by the expression of the recombinant human caspase-9 in E. coli bacteria and purification by Ni-NTA affinity resin. Following SDS-PAGE analysis of the protein, White New Zealand female rabbit was selected as a host animal for polyclonal antibody production. Antigen mixed with Freund's complete adjuvant and injected into the rabbit for immunization. The injection was repeated four times with Antigen mixed with incomplete Freund's adjuvant every 2 weeks to amplify the immune response. After the injection series process, blood serum was separated from the rabbit's blood and the antibodies were further purified by Ammonium sulfate.

Result: The antibody could recognize both the recombinant and endogenous caspase-9 protein, in WB. Because of its polyclonal characteristic, all epitopes of caspase-9 protein including procaspase-9 (46kDa), large (35kDa) and small subunits (10kDa) were detected.

Conclusion: The produced polyclonal antibody was successfully tested in WB and it can be used in other biological research application techniques such as ELISA an immuno precipitation.

Keywords: Polyclonal antibody, caspase-9, western blotting

P-291

Two Simple Methods for Optimizing the Production of “Difficult-toExpress” GnRH-DFF40 Chimeric Protein

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Background: GnRH-DFF40 (gonadotropin releasing hormone - DNA fragmentation factor 40) is a humanized recombinant immunotoxin and serves as a prospective candidate for targeted therapy of gonadotropin releasing hormone receptor (GnRHR) overexpressing malignancies. However, its production in Escherichia coli in a soluble and functional form still remains a challenge. Here we introduce two successful and reproducible conditions for production and purification of “difficult-to-express” GnRH-DFF40 protein.

Methods: A synthetic codon optimized GnRH-DFF40 fusion gene was cloned in pET28a plasmid. Two methods including high cell density IPTG induction (HCDI) and autoinduction method (AIM) with a focus on obtaining high cell density have been investigated to enhance the protein production in (E. coli). Moreover, to obtain higher protein production several factors in the AIM method including carbon sources, incubation time and temperature, plasmid stability and double colony selection, were optimized**.**

Results: Remarkable amounts of soluble GnRH-DFF40 protein were achieved by both methods. Cell density and protein yields in AIM was about 1.5-fold higher than that what obtained using HCDI. Initial screening showed that 25ºC is better to achieve higher protein production in both methods. pH alterations in AIM were maintained in a more constant level at 25ºC and 37ºC temperatures without any detrimental effects on cell growth during protein production phase up to 21 hours after incubation. Plasmid stability during growth and expression induction phase was maintained at a high level of 98% and 96% for AIM and HCDI methods, respectively. After parameter optimization and double colony selection in AIM, a very high yield of recombinant protein was achieved (528.3 mg/L).

Conclusion: With the optimization of these high cell density expression methods, reproducible manifold enhancement of soluble protein yields can be achieved for “difficult-to-express” GnRH-DFF40 compared to conventional expression methods.

Keywords: • Humanized recombinant immunotoxin • GnRH-DFF40 chimeric protein • Autoinduction method (AIM) • High cell density IPTG induction (HCDI) • Targeted therapy

P-292

Induction of apoptosis by Cyrtopodion scabrum extract in colon cancer cells: A preliminary study on targeting P53 signaling pathway

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Background: Cancer is one of the major serious health challenges worldwide. Since current treatments are non-specific and expensive, finding a safer and inexpensive way of treatment is highly required. Our research group has been studied potential anti-tumor properties of the extract of a kind of lizard, named Cyrtopodion scabrum. Our previous studies showed that the Cyrtopodion scabrum extract (CSE) suppressed some human cancer cell lines selectively while it has no toxic effect on normal cells and successfully treat the tumor-bearing mice. In the current project, we investigated the potential role of P53 signaling pathway in anti-cancer effects of CSE.

Methods: In this study, we investigated the mechanism of action of CSE in SW742 (colon cancer cell line), using western blotting, real-time PCR, and PI staining/flow cytometry analysis.

Results: QRT-PCR results revealed that CSE did not increase p53 mRNA while western blot analysis showed that CSE up-regulated P53 protein expression significantly in SW742 cancer cell line proposed that P53 protein may play its role as a tumor suppressor through the protein stability mechanism and not through the increase in the gene expression. Moreover, QRT-PCR results revealed that the mRNA expression of two of P53 target genes, p21 and mdm2 were significantly increased by CSE suggesting that the induction of G2 cell cycle arrest, which we have previously reported, may happen through P53 and P21over-expression. The blocking of P53 transcriptional activity by PFT-α in SW742 cells was designed to investigate whether apoptosis occurs through P53 transcription pathway or not. No significant decrease in apoptosis of SW742 cells co-treated with CSE and PFT-α suggesting that the induction of apoptosis by CSE does not occur through P53-dependent transcriptional activity.

Conclusion: The results showed that the observed anti-cancer effect of CSE may occur through TP53 up-regulation, but with P53-independent transcriptional activity.

Keywords: Natural anticancer product, Cyrtopodion scabrum extract, Western blot analysis, Real-time PCR, Flow cytometry, P53 signaling pathway

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Effect of long-term administration of oral magnesium sulfate on hepatic and serum irisin in type 2 diabetic male rats

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Background: Type 2 diabetes mellitus is the most prevalent metabolic disorder in humans. Irisin, a recently identified myokine, is thought to play a role in energy metabolism, glucose/lipid homeostasis. Our aims were to examine the effects of magnesium supplement on metabolic parameters and a metabolic regulator, irisin, in high fat- type 2 diabetic rats.

Methods: A total of 18 rats were randomly divided into magnesium supplement, diabetic and control groups. Control or non-diabetic group received normal diet and the rest of the rats fed with a high-fat diet (for 3 months) and low-dose streptozotocin injection to induce type 2 diabetes. Diabetic rats were divided into ٢ groups; diabetic and magnesium supplement groups ($MgSO4$ 10g/lit of tap water). After 2 month of treatment, hepatic and serum irisin levels were measured by irisin ELIZA kits.

Results: Our study showed that there was no statistically significant difference between hepatic and serum irisin levels in the diabetic group compared to controls. Also, there was no significant difference in the levels of irisin in the magnesium-treated group compared to the control and diabetic groups. However, in rats treated with magnesium supplement, the levels of serum irisin was higher than the diabetic group.

Conclusion: These findings suggest that magnesium might be assistance to improve the status of type 2 diabetes by increasing serum irisin levels.

Key words: Irisin, Magnesium Sulfate, Type 2 Diabetes, Rat

P-294

Correlation of TG to HDL-C Ratio with Insulin Resistance in Women with Polycystic Ovarian Syndrome

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Background: Dyslipidemia and insulin resistance (IR) are frequently observed in most polycystic ovarian syndrome (PCOS) patients. It has recently been reported that triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio can be used as a simple clinical indicator of IR. Hence, the present study was performed to investigate the relationships between TG/HDL-C ratio and IR in PCOS women.

Methods: This case-control study was conducted in 300 PCOS patients. PCOS patients were diagnosed according to the Rotterdam criteria. Follicle stimulating hormone (FSH), luteinizing hormone (LH), insulin, and free testosterone were assessed during the early follicular period in PCOS patients using ELISA technique. Fasting serum glucose and lipid profile levels (triglyceride, total cholesterol, LDL-C and HDL-C) were analyzed using autoanalyzer. The homeostasis model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and TG/HDL-C were calculated in PCOS patients. Total patients were divided into two groups according to the HOMA-IR and QUICKI.

Results: Insulin levels and TG/HDL-C were significantly higher in HOMA-IR positive PCOS patients. While, only insulin level was higher in QUICKI positive PCOS. HOMA-IR significantly correlated with increase of TG/HDL-C according to Pearson correlation ($r=0.209$, $p<0.001$), regression analysis (OR=1.295, 95% CI=1.065-1.575, $p=0.009$) and ROC curve. However, our results did not show a significant association with QUICKI index.

Conclusion: TG to HDL-C ratio was directly correlated with HOMA-IR and may be used as a simple, reliable and economic marker of IR in PCOS patients.

Keywords: Polycystic ovarian syndrome, TG/HDL-C ratio, HOMA-IR, QUICKI

P-295

Deamidation is not the main process in chondroitinase ABC I thermoinactivation

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Background: Chondroitinase ABC I (cABCI) is a bacterial lyase that specifically digests chondroitin sulfate and/or dermatan sulfate glycosaminoglycans. Medical application of this enzyme has been limited by its thermal instability as reported in some studies. A variety of chemical modifications, including deamidation of Gln and Asn, are known to cause irreversible thermal inactivation of enzymes at high temperatures. Non-enzymatic deamidation is a hydrolytic reaction resulting in a change in the primary structure, which in turn may affect secondary and tertiary structures of proteins. In the present study, to elucidate the mechanism of irreversible thermal inactivation of cABC I, the extent of deamidation of amino acid residues in the proteins was determined.

Methods: Ammonia liberated in the deamidation reactions was determined using glutamate dehydrogenase. Production of ammonia during thermal inactivation of cABC I was assessed by incubating samples of the enzyme in 50 mM phosphate buffer, pH 6.8 in sealed tubes, at 40 °C for 15 min. The tubes were then cooled, opened and the amount of dissolved ammonia was determined enzymatically using glutamate dehydrogenase.

Results: Our results indicated that the enzyme completely lost its catalytic activity after 15 min incubation at 40 °C. However, the amount of ammonia during thermal inactivation of cABC I was minor.

Conclusion: Based on the results, it can be concluded that deamidation is not the major contributor to the thermal inactivation process of cABC I.

Keywords: Chondroitinase ABC I, Deamidation, Thermal inactivation

P-296

Design of vaccine candidate against SARS-CoV-2: an immunoinformatics study

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Background: COVID-19 has spread quickly to worldwide to date. COVID-19 is caused by SARS-CoV-2. No vaccine or specific drug has been proven to be successful against SARS-CoV-2 infection. Thus, we employed in silico approach to design a proficient vaccine against SARS-CoV-2.

Method: Several methods by a combination of the comparative genomics and immunoinformatics studies were employed to determine the potential T-cell peptides for designing the peptide vaccine using the S1 subunit of Spike and Nucleocapsid of SARS-CoV-2 as a target.

Results: The designed vaccine construct promote humoral, cellular, and Interferon-gamma immunity responses. Also, these epitopes have strong antigenicity and no allergenicity probility. To improve the vaccine immunogenicity, we employed N-terminal Flagellin, as a potent adjuvant, a driven protein from Pseudomonas aeruginosa. The immunological and physicochemical properties of the vaccine construct were assessed. The 3D structure of the vaccine construct was forecasted and improved by I-Tasser and Galaxi refine servers and confirmed using Rampage and ERRAT servers. Results of Ellipro showed many residues from the vaccine could be discontinuous B cell epitopes. Docking of vaccine with Toll-Like Receptor 5 confirmed a proper interaction between the vaccine and TLR5. In silico cloning showed that the construct can be professionally expressed in E. coli.

Conclusion: The peptide vaccine was designed for SARS-CoV-2utilzing the S1 subunit of Spike and Nucleocapsid proteins as immunogenic targets along with the proper adjuvant. Nevertheless, the results of the in silico evaluations showed the designed peptide vaccine can be a suitable anti-COVID-19 candidate. However, in the future, in vitro, and in vivo immunological tests should be done to ensure its immunogenic efficacy and safety.

Keywords: SARS-CoV-2, In silico, S1 subunit of Spike and Nucleocapsid, Flagellin

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Effects of the aqueous extract of the leaves of Nerium oleander on glucose level and lipid profile of the serum and liver enzymes of streptozotocin induced diabetic male rats

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Background: The use of non-chemical therapies (herbs) is a new approach to controlling diabetes. Diabetes mellitus is associated with biochemical and pathophysiological changes in the body.

Methods: In this study, 80 rats randomly selected in healthy groups receiving Nerium oleander extract (50, 100 and 200 GW/BW), Diabetic group (streptozotocin 60 mg/BW), And diabetic groups + aqueous extract of Nerium oleander leaves (50, 100, and 200 mg/kg) were categorized. Streptozocin and extract of Nerium oleander leaves were administered intraperitoneally and by gavage, respectively. Rat serum samples after 28 days tested for blood glucose, ALP, AST, ALT and albumin, total protein, total bilirubin, cholesterol, triglyceride, LDL, and HDL. Data were analyzed using a one-way analysis of variance and LSD test.

Results: The results of the study showed a significant increase in blood sugar, the activity of ALP, AST, ALT, cholesterol, triglycerides, LDL, and decreased concentrations of albumin, total protein, and HDL in the diabetic group compared to the healthy control group. In the diabetic group receiving an aqueous extract of Nerium oleander leaves showed a significant decrease in the mean blood sugar, cholesterol, triglyceride, LDL, ALT, AST, ALP enzymes, and a significant increase in the concentration of albumin, total protein, and HDL compared to the diabetic control group. In Addition, the total bilirubin concentration and direct bilirubin concentration did not show significant changes between the study groups.

Conclusion: The results of the study show that the aqueous extract of Nerium oleander leaves reduces the effects of diabetes on blood sugar, liver damage indices, and lipid profiles.

Keywords: Diabetes, Nerium oleander leaves, Rat, Albumin concentration

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Nephroprotective effects of Zataria multiflora Boiss ethanolic extract, carvacrol and thymol on kidney toxicity induced by Cisplatin in rats

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Background: Cisplatin (Cis) is an anti-cancer drug; however, it has dose-dependent renal toxicity. The current study aims to investigate the protective effects of Zataria multiflora Boiss ethanolic extract (Z.M.B), carvacrol and thymol on cisplatin-induced nephrotoxicity in rats.

Materials and Methods: The Wistar male rats were randomly allocated into six groups (n = 7). Group I received normal saline; group II received Cis (7 mg/kg. i.p); group III received the Z.M.B extract only (500 mg/kg/d, p.o); group IV received Z.M.B extract (500 mg/kg/d, p.o) + Cis; group V received carvacrol (50 mg/kg/d, p.o) + Cis; and group VI received thymol (50 mg/kg/d, p.o) + Cis. The levels of biochemical markers, oxidative stress parameters, and histopathological staining were determined in serum and renal tissue. Also, the chemical compositions (carvacrol and thymol) of Z.M.B extract were assayed by HPLC.

Result: The results revealed that Z.M.B extract, carvacrol and thymol markedly decreased serum creatinine and blood urea nitrogen levels as compared to the Cis only group. Furthermore, Z.M.B extract, carvacrol and thymol significantly attenuated Cis-induced increase in malondialdehyde, nitric oxide metabolite and ferric reducing antioxidant power in serum and renal tissue homogenates. Additionally, histopathological examination showed that Z.M.B extract, carvacrol and thymol markedly ameliorated Cis-induced renal tubular necrosis.

Conclusion: The results showed renoprotective effects of Z.M.B extract, carvacrol and thymol in Cis-induced nephrotoxicity in rats. Therefore, Z.M.B extract can be considered a potential candidate for protection of nephrotoxicity induced by Cis.

Keywords: Cisplatin, Nephrotoxicity, Zataria multiflora Boiss, Carvacrol, Thymol

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Fluvoxamine ameliorates liver injury induced by bile-duct ligation in male

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Background: Cholestasis is a condition in which the bile ducts become narrowed or blocked by a variety of reasons and bile acids are not released easily. Bile acids-induced liver injury is facilitated by oxidative stress and inflammation. The present study aimed to investigate the protective effects of fluvoxamine (Flu) on inflammatory cytokines and oxidant-antioxidant balance in the bile duct ligated (BDL) rats.

Methods: Thirty-two rats were randomly divided into four groups; sham-control (SC), bile duct ligation (BDL), SC+ 150 mg/kg Flu (SCF), and BDL+ 150 mg/kg Flu (BDLF). The animals received distilled water and Flu orally for 7 days. Hematoxylin and eosin staining, biochemical analysis and oxidant/antioxidant status were evaluated. Also, the gene expression of TNF-α, IL-1, TGF-β1, and α-SMA were measured.

Results: The results showed serum levels of alanine transaminase, alkaline phosphatase and total bilirubin slightly reduced in the BDL+ Flu group in comparison to the BDL merely group. Renal ferric reducing antioxidant power, total thiol level and catalase activity were significantly reduced in the BDL+ Flu group as compared to the BDL group ($P\leq0.05$). Administration of Flu in the BDL rats significantly decreased the levels of liver and kidney malondialdehyde, liver nitric oxide metabolite, plasma protein carbonyl, and TNF-α mRNA level ($P\leq0.05$). Histological changes were ameliorated in the BDL+ Flu group as compared to the BDL alone rats.

Conclusion: We conclude that Flu reduced oxidative stress possibly by suppressing certain enzymes responsible for protein oxidation, lipid peroxidation, and nitric oxide production. Also, it increases antioxidant capacity by increasing catalase activity and plasma total antioxidant capacity.

Keywords: Key words: Oxidative stress, Cholestasis, Fluvoxamine, Antioxidant, Inflammation

P-300

Probing of the interaction between Fisetin with HSA and ct-DNA by different spectroscopy techniques

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Background: The interaction between Fisetin with human serum albumin protein and calf thymus DNA under physiological conditions was studied.

Methods: Spectroscopic methods such as emission spectroscopy, absorption spectroscopy, temperature transfer study, and resonance beam diffraction, and viscometry were used

Results and conclusion: The results of emission spectroscopy and resonance beam diffraction indicate a change in protein structure and complex formation. Quantities such as fixed constant, available fluorophore level, and the number of binding sites on the protein were calculated, as well as blackout mechanism was static. Simultaneous fluorescence has presented results on changes in the environment around amino acids. Then, the results of the interaction of Fisetin with calf thymus DNA were examined. In emission spectroscopy, increasing the ligand concentration caused the DNA emission spectrum to be a blackout, indicating a complex formation. The increase in resonance beam diffraction is another reason for its confirmation. Thermodynamic quantities indicate the role of van der Waals forces and hydrogen bonds in the interaction. The data from the Stern–Volmer plot also indicate a static blackout. In the competitive emission spectrum with ethidium bromide and acridine orange as intercalator markers, changes in the emission spectrum indicate competition. In the viscometric method, its viscosity was increased by adding a ligand to the DNA solution. In temperature transfer studies of ligand, intercalation between pairs of bases stabilized the DNA structure. The intercalator compounds such as potassium iodide and sodium chloride ions also competed with the binding site's ligand. The amount of KSV in the ligand's interaction with single-stranded and double-stranded DNA also shows an increase in the ligand's affinity for double-stranded DNA. The experiments' results showed that the type of binding of Fisetin to DNA is of the type of binding between two strands or intercalate.

Keywords: Human Serum Albumin, Calf Thymus DNA, Fisetin, Fluorescence Spectroscopy, Quenching, Acridine Orange, Ethidium Bromide, Potassium Iodide, Sodium Chloride, Intercalate Binding, Groove Binding

P-301

Nesiritide Secretory Production in E. coli using in Silico study of Signal Peptides

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Background The secretory production of recombinant proteins into the extracellular space of E. coli significantly make easy their downstream processing and reduce the production costs, time savings, and dues to a reduction in endotoxin contamination. Signal sequences have a major task in proteins targeting for translocation to the extracellular space during the secretion process. Nesiritide (Natrecor) is a recombinant form of brain natriuretic peptide that causes arterial vasodilatation and venous. Nesiritide is the only FDA approved drug for the cure of heart failure. Production of recombinant Nesiritide protein, utilizing a prokaryotic system needs a proper signal peptide (sp) to prevent of misfolding and simplified downstream processing.

Methods: In this study, to predict the finest signal peptide for secretory expression of Nesiritide in E. coli, different 42 signal peptides from bacterial were elected and the most crucial features of them investigated.

Results: Signal peptide probability of them and their physicochemical features were assessed by signalP “version 4”, Portparam and PROSO II tools severally. Also, in-silico cloning in a pET28a expression plasmid estimated the of best sp + Nesiritide expression in E.Coli. Cytochrome c-type biogenesis protein (cmmH) evaluated as the best option for the secretory production of Nesiritide in E. coli (with D scores 0.869**).**

Conclusion: Cytochrome c-type biogenesis protein (cmmH) can be used as a proper candidate for proficient extracellular secretory of Nesiritide protein in E. coli.

Keywords: E. coli, Signal peptides, Secretory production, in-silico

P-302

Sargassum boveanum ethanolic extract represses hTERT gene expression in human colon cancer cell line SW742

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Background: Measureless proliferation of cells is one of the hallmarks of cancer and earned by preventing telomere in the 3' end of the chromosome. Telomerase considered as a main factor in telomere maintenance; Telomerase activity is dependent on the expression level of hTERT as a catalytic subunit of telomerase. The aim of the present study was to investigate the effect of Sargassum boveanum ethanolic extract on hTERT gene expression in human colon cancer cell line SW742.

Methods: In this study, the cytotoxic effect of the extract was measured 72 hours after treatment by MTT assay. The expression of hTERT gene expression was quantified by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) for the treated cells and the control.

Results: The 50% inhibitory concentration of Sargassum boveanum ethanolic extract was 23.25 µg/ml in SW742 cells. This extract significantly suppressed hTERT gene expression at treatment times of 24,48 and 72 hours compared with untreated cells ($p.Value < 0.0001$).

Conclusion: Our results propose that the ethanolic extract of Sargassum boveanum may be a promising treatment option for cancer via repression of hTERT gene expression.

Keywords: Telomerase, hTERT, Sargassum boveanum, SW742

P-303

Fluvoxamine ameliorates liver injury induced by bile-duct ligation in male rats

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Background: Cholestasis is a condition in which the bile ducts become narrowed or blocked by a variety of reasons and bile acids are not released easily. Bile acids-induced liver injury is facilitated by oxidative stress and inflammation. The present study aimed to investigate the protective effects of fluvoxamine (Flu) on inflammatory cytokines and oxidant-antioxidant balance in the bile duct ligated (BDL) rats.

Methods: Thirty-two rats were randomly divided into four groups; sham-control (SC), bile duct ligation (BDL), SC+ 150 mg/kg Flu (SCF), and BDL+ 150 mg/kg Flu (BDLF). The animals received distilled water and Flu orally for 7 days. Hematoxylin and eosin staining, biochemical analysis and oxidant/antioxidant status were evaluated. Also, the gene expression of TNF-α, IL-1, TGF-β1, and α-SMA were measured.

Results: The results showed serum levels of ALT, ALP and total bilirubin slightly reduced in the BDL+ Flu group in comparison to BDL merely group. The renal ferric reducing antioxidant power, total thiol level and catalase activity were significantly reduced in the BDL+ Flu group as compared to BDL group ($P \leq 0.05$). Administration of Flu in BDL rats significantly decreased the levels of liver and kidney malondialdehyde, liver nitric oxide metabolite, plasma protein carbonyl, and TNF-α mRNA level ($P \leq 0.05$). Histological changes were ameliorated in the BDL+ Flu group as compared to BDL alone rats.

Conclusion: We concluded that Flu reduced oxidative stress possibly by suppressing certain enzymes responsible for protein oxidation, lipid peroxidation, and nitric oxide production. Also, it increased antioxidant capacity by increasing catalase activity and plasma total antioxidant capacity.

Keywords: Oxidative stress, Cholestasis, Fluvoxamine, Antioxidant, Inflammation

P-304

The methanolic extract of Sargassum boveanum reduces hTR gene expression in human colon cancer cell line SW742

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Background: Telomerase shows a main role in cellular immortalization, and suppression of telomerase activity potentially demonstrates a selective goal for the cure of cancer. Sargassum boveanum is a brown macroalga. It has antioxidant anticarcinogenic actions. Therefore, we evaluated the effect of Sargassum boveanum methanolic extract on the human telomerase RNA (hTR) mRNA level in colon cancer cell line SW742.

Methods: Sw742 cell line was treated with various concentrations (10-200 µg/ml) of Sargassum boveanum methanol extract for 72 hours to evaluate its cell viability by MTT assay. Cells were treated to the 50% inhibitory concentration (IC50) of extract for different time periods (0, 24, 48, and 72 h). The expression of hTR gene was quantified by using a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) method**.**

Results: The findings of our study demonstrated that the IC50 of Sargassum boveanum methanolic extract was 140.1 µg/ml within 72 hours. The IC50 concentration of extract significantly decreased hTR gene expression at treatment times of 24, 48, and 72 h when compared with untreated SW742 cells ($p.\ Value < 0.0001$) in colon cancer cells.

Conclusion: Our data suggest that methanol extract of Sargassum boveanum may potentially apply its anti-cancer effects through suppression of hTR gene expression.

Keywords: Telomerase, hTR, sargassum boveanum, SW742

P-305

Antioxidant Effects of Streptococcus Thermophilus on the Induction of Oxidative Stress by MCF-7 Cells

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Background Probiotics are live microbial food supplements. Various beneficial effects have been attributed to probiotics such as anticancer effects. The present study investigated the effects of Streptococcus Thermophilus oxidative activity on the MCF-7 carcinoma cells.

Methods: Different dairy products from Fars Province were collected and isolated. Biochemical and molecular tests were used to identify the bacteria. MCF-7 breast cancer cell lines were treated by the isolated metabolites of Streptococcus Thermophilus and the induced oxidative stress was evaluated using Griess test, Catalase and LDH assays.

Results: Different levels of NO, LDH and Catalase were measured from the isolated Streptococcus Thermophilus bacteria and it was shown that different diaries affect the induction of oxidative stress by MCF-7 cancer cell lines in different ways.

Conclusion: In this study, some strains of Streptococcus Thermophilus samples from dairy products showed positive results against MCF-7 cancer cells, indicating that certain strains of Streptococcus Thermophilus may affect the cancer cells.

Keywords: Dairy, Streptococcus Thermophilus, MCF-7, Oxidative Stress

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P-306

The increased level of 15-Lox-2 is associated with tumor size and invasion in patients with hormone-secreting Pituitary Adenoma

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Background: Pituitary adenomas impose burden of morbidity on patients and characterizing the molecular mechanisms underlying its pathogenesis received remarkable attentions. The bioactive lipid mediators received attentions toward their contribution in cancer cell proliferation, progression and death. Amongst, 15-Lipoxygenase-2 enzyme and its related products display appealing role in cancer pathogenesis which their possible effect in pituitary adenoma tumor genesis is perused in the current study.

Methods: In this case-control study, 50 patients with Functional hormone secreting pituitary adenomas who were referred to the Firouzgar Hospital in Tehran were participated. Tumor tissues were used to extract mRNA and cDNA, and to determine the gene expression of 15-Lox-2, the Real-Time PCR-based SYBR Green method was used. The correlation of 15-Lox-2 with patient’s pathophysiology features were evaluated. Finally, statistical analysis was performed using version 6 of GraphPad Prism software and independent t-test.

Results: Measurement of 15-Lox-2 expression level in tumor tissues of patients with Functional hormone secreting pituitary adenomas revealed that the level of this gene was significantly increased in patients compared to normal tissues. Also, the increased level of this gene was associated with the elevated level of tumor size and invasion. Based on our results, in tumors with more than 10 mm in size also tumors with invasive grade, the level of 15-Lox-2 was more elevated.

Conclusion: The results of the current study have shown that the 15-Lox-2 gene can account as a local cancer marker in patients with functional hormone secreting pituitary adenomas and can be noticed as a possible biomarker follow-up the disease.

Keywords: 15-Lox-2, functional pituitary adenoma, tumor size, tumor invasion, cancer

P-307

The effectiveness spermidine on the structure and kinetics of lysozyme

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Background: The lysozyme protein is an enzyme [EC 3.2.1.17] commonly found in tears, egg white and other secretions, which hydrolyses polysaccharides found in many bacterial cell walls. Polyamine spermidine is cationic molecules present in all cells and is essential for cellular function.

Methods: To comprehend the influence of spermidine on conformation, stabilization and function of lysozyme, we used fluorescence spectroscopy, UV- Vis spectroscopy and kinetic assay. Structural variability of lysozyme was investigated at different concentrations of spermidine.

Results: Results show that with the rising temperature, K_{sv} values decreased; K_q is also more than 2.0×10^{10}mol-1s^{-1}. So, static mode is the quenching system with some form of binding interaction, static quenching is usually indicated. Thermodynamic parameters analysis proposed that van der Waals and hydrogen bond forces play a basic role in complex stability. Different kinetic parameters (V_{max} decreases and K_m increases) with rising concentrations of spermidine to Lysozyme were evaluated. Therefore, it can be stated that spermidine acts as a Mix inhibitor. UV- Vis measurement also showing the tertiary structure change of lysozyme as an action of concentrated spermidine.

Conclusion: UV- Vis spectra, fluorescence spectroscopy and kinetic display structural alteration in the enzyme and also show an interaction between spermidine and lysozyme. In addition, kinetic studies revealed that spermidine reduced the enzyme activity of lysozyme in a concentration-dependent manner.

Keywords: Lysozyme, spermidine, UV- Vis spectroscopy, fluorescence spectroscopy, kinetic

P-308

Biochemical comparison of biodegradable polymers of chitosan and calcium alginate to loading Nanobody against Pseudomonas aeruginosa

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Background: Pseudomonas aeruginosa is a Gram-negative bacillus without spores which have motility that are found everywhere in decomposing soil and organic matter in plants and water. Due to the importance of hospital infections caused by Pseudomonas aeruginosa and also increasing resistance of this bacteria against antibiotics, it seems necessary to try different ways of prevention and treatment. Nanobody antibodies do not have a light chain, which has many properties due to its small size**.**

Methods: In the present study, after the preparation of nanobody against Pseudomonas aeruginosa, the nanoparticle purification conditions were determined by ion exchange chromatography by using DEAE Cellulose and CM-Sephadex C-50 Optimized. The purified nanoparticles were then loaded on the chitosan and calcium alginate to confirm and compare the loading, fluorescence spectroscopy, particle diameter measurement, and zeta potential, as well as scanning electron microscopy were done.

Results: The results of this study showed that using of CM-Sephadex C-50 is more suitable for Nanobody purification. Also, the results of fluorescence spectroscopy showed that the emission intensity of Nanobodies loaded on chitosan reduced more than alginate-loaded Nanobodies, and the zeta potential in chitosan-loaded Nanobodies increased more than alginate-loaded Nanobodies. Moreover, measurement of the average size of particle also confirms this result. The average size of particle in chitosan-loaded Nanobodies decreased and in alginate-loaded Nanobodies increased. Furthermore, in electron microscopy experiments, increasing in particle size was observed after Nanobody loading.

Conclusion: Comparison of the experimental results shows that the loading of Nanobodies on chitosan has a better response than the loading of Nanobodies on alginate.

Keywords: chitosan, calcium alginate, Pseudomonas aeruginosa, Nanobody

P-309

Efficacy of fortified low-fat yogurt by nano-encapsulated vitamin D on serum antibody titers to heat shock protein 27 and dyslipidemia in abdominal obese adults; a randomized clinical trial

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Background: Vitamin D plays an important role in general health. Also, obesity is common world problem which is related to the vitamin D deficiency and chronic low-grade inflammatory disorders. Dyslipidemia is one of the major complications of obesity and recent studies have suggested a possible relationship between deficiency of 25-hydroxyvitamin D and dyslipidemia. Previous reports demonstrated that heat shock protein 27 antibody (anti-Hsp27) considered as an indicator of inflammation and data showed vitamin D has anti-inflammatory properties. However, the results have been conflicting and need further investigation. The purpose of the present study was to evaluate the effects of fortified low-fat yogurt by nano encapsulated vitamin D on serum anti-Hsp27 levels in obese and overweight adults.

Methods: The study conducted as a randomized control total blind trail that participated were recruited from the students and staff of Mashhad University of Medical Sciences (MUMS). One hundred and thirty-five middle age cases divided into two groups; 94 normal weight and 41 overweight and obese subjects. The intervention group received nano-encapsulated vitamin D fortified yogurt at a dose of 1500 IU daily for 10 weeks and the control group consumed plain yogurt at the same duration. Before and after the intervention, the serum levels of 25-hydroxy vitamin D (25(OH) D) and anti-Hsp27 concentrations, were measured and compared.

Results: Based on the current study, consumption of low-fat yogurt fortified with 1500 IU nano-encapsulated vitamin D for ten weeks, reduced the concentration of anti-Hsp27 and dyslipidemia (LDL and Triglyceride) by increasing the serum levels of vitamin D in both overweight and obese people. However, longer-term follow-up studies with larger numbers of subjects are required.

Conclusion: Based on the current study, consumption of low-fat yogurt fortified with 1500 IU nano-encapsulated vitamin D for ten weeks, reduced the concentration of anti-Hsp27 and dyslipidemia (LDL and Triglyceride) by increasing the serum levels of vitamin D in both overweight and obese people. However, longer-term follow-up studies with larger numbers of subjects are required.

Keywords: Vitamin D, Fortification, Dairy products, Anti-Hsp27, Obesity

P-310

The Effects of consuming low-fat milk fortified with nano-encapsulated vitamin D on serum pro-oxidant-antioxidant balance (PAB) in adults with hypertension; a randomized control trial

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Background: Hypertension (HTN) is a major cardiovascular disease which is considered to be a worldwide silent killer. In addition, vascular inflammation is a part of the pathophysiology of hypertension. Evidence showed that there is an associated between vascular inflammation and oxidative stress. On the other hand, prevalence of vitamin D deficiency is also a health problem in cardiovascular patients, and data showed that low levels of vitamin D are associated with a higher risk of developing hypertension. However, the results have been conflicting and need further investigation. The purpose of the current study was to investigate the effects of fortified milk with nano-encapsulated vitamin D on serum pro-oxidant anti-oxidant balance (PAB) in adults with HTN**.**

Methods: This study design as a randomized total blind controlled trial. Cases were recruited from the students and staff of Mashhad University of Medical Sciences (MUMS). One hundred and thirty adults (aged 30 to 50 years) divided into two groups; 24 HTN and 106 healthy subjects. The intervention group received nano-encapsulated vitamin D fortified milk at a dose of 1500 IU daily for 10 weeks. The control group was given plain milk for the same duration. Before and after the intervention, the serum levels of 25-hydroxy vitamin D (25(OH) D) and PAB, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured and compared.

Results: The findings of the present study indicated that consumption of low-fat milk fortified with 1500 IU nano-encapsulated vitamin D for ten weeks, leads to a significant reduction in both systolic and diastolic blood pressures and decreased serum PAB concentrations highlighted the probable anti-oxidative effect of vitamin D in HTN subjects. However, longer-term follow-up studies with larger numbers of subjects are required.

Conclusion: The findings of the present study indicated that consumption of low-fat milk fortified with 1500 IU nano-encapsulated vitamin D for ten weeks, leads to a significant reduction in both systolic and diastolic blood pressures and decreased serum PAB concentrations highlighted the probable anti-oxidative effect of vitamin D in HTN subjects. However, longer-term follow-up studies with larger numbers of subjects are required.

Keywords: Vitamin D, Fortification, Dairy products, Prooxidant-antioxidant balance, Hypertension

P-311

Production of antibody against alpha-synuclein in rabbit

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Background: Alpha synuclein (AS) is one of the small soluble proteins that is made of 140 amino acids. Members of this family are α–synuclein, β-synuclein, γ-synuclein and synoretin. AS is found in brain, muscle, heart, and other tissues. Although the roles of AS are known in neurodegenerative disorders such as Parkinson's and Alzheimer's diseases, but researchers suggest that this protein has potential roles in differentiation of neurons, inhibition of lipid oxidation, and folding of synaptic proteins. According to important roles of this protein incidence of neurodegenerative disorders, we investigated the structural studies of protein by production of antibody**.**

Method: Comparative expression of this protein in 2 different temperatures including 30˚C and 37˚C was done in TB medium and concentration of 0.1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) for 5h and AS was purified by affinity chromatography.

Result: In this study, the best expression temperature was 37˚c and purification of protein was confirmed by sodium dodecyl sulfate poly acril amaide gel electrophoresis (SDS-PAGE). Then intradermal injection was performed with suitable concentration of antigen to rabbit. After three steps, antibody was produced.

Conclusion: The protein was successfully purified and antibody was produced that was confirmed with dot blot and western blot. Further studies needed for characterization of this antibody.

Keywords: Alpha synuclein (AS), antibody, Parkinson's disease

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Association between zinc and copper with hypertension in subjects with short sleep

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Background: Zinc and copper play an important role in the central nervous system. Moreover, they have an important role in the regulation of blood pressure. As respects, insomnia increases the risk of high blood pressure. So, the aim of the current study was to investigate changes in the levels of zinc and copper in people with low sleep, which increases the chances of developing high blood pressure.

Methods: A total of 9184 subjects were recruited in MASHAD cohort study and divided into four groups. Then, subjects were categorized into two groups, HTN negative and HTN positive. Logistic regression was used to assess Odds Ratios of zinc and copper according to HTN in subjects with nightly sleep < 5h.

Results: Our findings showed that the rate of long night sleep in women is higher than men, while short night sleep in men was higher than women ($p< 0.05$). The mean zinc level in subjects with very short nightly sleep was lower than others ($p< 0.05$). Also the mean copper level was significantly higher in this group ($p< 0.05$). Prevalence of hypertension in males was 29.6% and in females was 32.7%; females more than males suffered from HTN ($p< 0.05$). Prevalence of very short and short nightly sleep in hypertensive subjects was significantly higher than others ($p< 0.05$). There was positive significant correlation between copper and SBP and DBP ($p< 0.05$). We found that the participants who had nightly sleep < 5h and serum copper more than normal range (> 140 µg/dl in men and > 155 µg/dl in women) had 2.293-fold higher risk of hypertension than normal subjects.

Conclusion: Based on the current study increased serum copper levels in people who have a very short nightly sleep increases the risk of developing hypertension. There is a negative correlation between SBP, DBP and copper with nightly sleep.

Keywords: Zinc, Copper, Hypertension, Nightly sleep

P-313

Biochemical comparison of biodegradable polymers of chitosan and calcium alginate for nanobody loading against Staphylococcus aureus

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Background: Due to the risk of Staphylococcus aureus bacteria for human health and the development of antibiotic-resistant infections, the fight against this pathogen is of great importance. Nanobodies (single-domain antibodies) are heavy-chain, non-light-chain antibodies found in camels. Their unique properties, including their subminiature size, make them more distinctive and more effective than conventional antibodies. During the micro-coating process, a compound is coated with one or two materials to prevent the reaction between the active substance and the environment and to prevent side effects. Chitosan and alginate are inexpensive, biocompatible, biodegradable, non-toxic, and degradable polymers that can use as carriers.

Methods: Nanoparticles against Staphylococcus aureus were prepared using camel immunization. Nanoparticles were optimized by ion-exchange chromatographic experiments using DEAE cellulose and Sephadex C-50**.** The purified nanoparticles were load onto chitosan and calcium alginate. Zeta potential, particle size, scanning electron microscopy, and spectroscopy tests were used to measure and compare the charge.

Results: The results of this study showed that the use of Sephadex C-50 for nanobody purification has a better outcome. Also, the results of fluorescence spectroscopy showed that the emission intensity of nanobodies loaded on chitosan decreased more. Also, the zeta potential of nanoparticles loaded on chitosan was higher than nanomaterials loaded on alginate. The average particle size also confirms the obtained result.

Conclusion: Observations and results showed that nanomaterials loaded on chitosan show a better response than nanomaterials loaded on alginate.

Keywords: chitosan, calcium alginate, Pseudomonas aeruginosa, Nanobody

P-314

Elevation of Total Cholesterol to HDL-Cholesterol Ratio as an Indicator for Insulin Resistance in Women with Polycystic Ovarian Syndrome

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Background: Polycystic ovarian syndrome (PCOS) is a highly prevalent endocrine-metabolic disorder, which is related to hyperinsulinemia, hyperlipidemia, diabetes, and cardiovascular disease. The majority of available approaches to assess insulin resistance are costly and time consuming. The aim of this study was to investigate the association between the total cholesterol to HDL-cholesterol (TC/HDL-C) ratio and insulin resistance in women with PCOS to provide new ideas for evaluation and treatment of PCOS with insulin resistance.

Methods: Three hundred infertile women with PCOS were selected based on the 2003 Rotterdam Criteria in this case-control study. After a fasting night, fasting serum glucose (Glu), insulin, triglycerides (TGs), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) were measured using commercial kits. FSH, LH, free testosterone and insulin levels were analyzed using ELISA technique. Also, TC/HDL-C, homeostasis model assessment (HOMA)-insulin resistance and fasting glucose/ insulin ratio (FGIR) were calculated. All PCOS subjects were sub-grouped into two groups according to the HOMA-IR and FGIR.

Results: Insulin levels and TC/HDL-C were significantly different in HOMA-IR and FGIR positive PCOS patients. HOMA-IR index was significantly correlated with increase of TC/HDL-C which was analyzed by Pearson's correlation ($r=0.249$, $p<0.001$), regression analysis (OR=1.551, 95% CI=1.134-2.123, $p=0.006$) and ROC curve. Also, FGIR index was negatively correlated with TC/HDL-C ($r=-0.175$, $p=0.002$) and after adjustment by age, BMI and lipid profile remained correlated (OR=1.429 95% CI=1.04-1.961).

Conclusion: The results of this study showed that TC/HDL-C ratio can be used as a simple and reliable clinical indicator of insulin resistance in PCOS women.

Keywords: Polycystic ovarian syndrome, TC/HDL-C ratio, HOMA-IR, FGIR

P-315

Apoptotic and anti-proliferative effects of combination treatment with all-trans retinoic acid (ATRA) plus γ-secretase inhibitor (DAPT) on gastric cancer cells

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Background: Available therapeutic options for gastric cancer have been accompanied by a low rate of success and resistance in many cases. It has been pointed out that NOTCH/γ-secretase inhibitors may be considered as the effective drugs in controlling tumors. Moreover, the chemo-prevention impacts of all-trans retinoic acid (ATRA) has been proved in many malignancies. The effects of ATRA in combination with N-[N-(3,5-difluorophenacetyl-l-alanyl)]-S-phenylglycine t-butyl ester (DAPT) as a γ-secretase inhibitor on viability and apoptosis of the AGS and MKN-45 derived from human gastric cancer is the main crux of the matter in this study.

Methods: Both cell lines were treated with different concentrations of ATRA or DAPT alone or ATRA combined with DAPT, and then viability, death, and apoptosis of cells were examined by MTT assay and ethidium bromide/acridine orange staining. Flow cytometry was utilized to analyze the gastric cancer cell cycle distribution. Besides, caspase 3/7 activity and the expression of caspase-3 and bcl-2 were examined.

Results: The gastric cancer cell viability was declined following ATRA and DAPT treatment in a concentration-dependent manner; however, a combination of these agents demonstrated considerable synergistic inhibitory effects on these cells. The results showed that a striking proportion of combination-treated gastric cancer cells were accumulated in the G0/G1 phase of the cell cycle. Furthermore, combination-treatment of gastric cancer cells demonstrated a remarkable increase in the percentage of apoptotic cells and the level of caspase 3/7 activities in comparison with the single treatment which were associated with caspase-3 up-regulation and bcl-2 down-regulation.

Conclusion: The combined treatment of DAPT/ATRA led to notable decreases in cell viability along with apoptosis induction in the investigated cell lines compared to single treatment with DAPT or ATRA

Keywords: Gastric cancer, ATRA, DAPT, Combination therapy

P-316

Resveratrol attenuates insulin resistance in skeletal muscle tissue of HFD-fed mice via promotion of antioxidant capacity and activating the Nrf2-Keap1 pathway

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Background: Oxidative stress plays a vital role in mediating obesity-induced insulin resistance. Previous studies have shown that high-fat diet leads to excess production of ROS in several tissues and triggers insulin resistance (IR). Skeletal muscle (SM) tissue is the major organ of insulin-stimulated glucose uptake. There is increasing evidence that resveratrol (a natural polyphenol) has strong antioxidant properties. The purpose of this study was to examine the beneficial effects of resveratrol supplementation on oxidative status and stress-sensitive signaling pathway in SM of HFD feeding C57/bl6 mice.

Methods:30 Six-week old mice were divided into three groups, each group with 10 animals. Mice were fed with a normal chow diet, and high-fat diet (60%). Resveratrol was added to high fat diet (400 mg/kg diet) for 16 weeks. At the end of this period, food intake, body weight and blood glucose levels were measured. Skeletal muscle samples (quadriceps) were examined for assessment total antioxidant capacity (TAC), malondialdehyde (MDA), and activity of antioxidant enzymes by colorimetric assay. Nrf2-Keap1 signaling pathway-related genes (Nrf2, Keap1, NQO1, HO, SOD, CAT, and GPX) were analyzed by qRT-PCR. In addition, the protein levels of Nrf2, AKT and p-AKT were also assessed by Western blot analysis.

Results: Resveratrol treatment decreased body weight and blood glucose levels in the mice fed with high-fat diet. Significant increase in MDA, and significant decrease in TAC and antioxidant enzymes activities were seen after HFD feeding. However, resveratrol controlled oxidative stress by decreasing the MDA level and increasing the antioxidant enzymes activity and their mRNA expression in SM tissue. TAC was significantly improved in resveratrol group, compared with the HFD group. Furthermore, resveratrol upregulated the mRNA and protein expression of Nrf2, compared to the HFD-treated group. Similarly, resveratrol enhanced the phosphorylation of Akt in the high-fat-fed mice.

Conclusions: We concluded that resveratrol is effective in ameliorating antioxidant defense system and IR.

Keywords: Resveratrol, high-fat diet, obesity, oxidative stress, skeletal muscle tissue

P-317

Quercetin attenuates oxidative stress and inflammatory cytokines in Lipopolysaccharide -stimulated human peripheral blood mononuclear cells

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Background: Oxidative stress and inflammation have been found to be involved in the development of a large number of human diseases. The flavonoid quercetin displays various biological effects, especially anti-inflammatory and antioxidant activities. Nevertheless, there are few studies that examined the effects of quercetin on human PBMCs. Here, we aimed to investigate whether quercetin could alleviate inflammation and oxidative stress, stimulated by LPS in PBMCs.

Methods: Using quantitative-PCR and ELISA, the protein and gene expression levels of inflammatory cytokines were determined. Western blot was utilized to investigate the phosphorylation of NF-κB-P65. Colorimetric procedures were used to examine oxidant-antioxidant markers.

Results: The results indicated that quercetin significantly suppressed the effect of LPS on the phosphorylation of NF-κB-P65, which was accompanied by significant decreased inflammatory mediators including IL-1β, IL-6, and TNF-α. Moreover, quercetin alleviated oxidative stress in LPS-stimulated PBMCs by enhancing TAC, GPX and MnSOD activities.

Conclusion: Collectively, these results indicate that quercetin has the potential to prevent inflammation and oxidative stress in the LPS-stimulated PBMCs.

Keywords: Quercetin, Inflammation, Oxidative stress, Peripheral Blood Mononuclear Cells

P-318

Investigation of Antitumor Effect and Cytotoxic Properties of New Hybrid Beta Lactam-Anthraquinone on Human Cancer Cell Lines

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Background Nowadays, one of the principal causes of death in the world is cancer. One of the major essential side effects of cancer result from chemotherapy drugs. Hence it is necessary to study the biological properties of compounds with biological activities. Therefore, this research investigated the cytotoxic, and antitumor activities of a novel β-lactam-anthraquinone hybrid, which can lead to a new horizon in anticancer research.

Methods: Firstly, in this project, MTT method was performed to evaluate the toxicity of the compound and doxorubicin on MCF7, HCT116, and PC3 cancer cell lines, Apoptotic genes Bcl-xl, Bax and TPX2, KI-67 proliferative genes compared to TBP (housekeeping gene) in HU02, MCF7, HCT116 and PC3 cell lines were assayed by Real-time PCR.

Results: The results of MTT test and IC50 measurement showed that the β-lactam-anthraquinone and doxorubicin have high toxicity effects on the studied cancer cells, but by consideration of the best results on human normal fibroblast cell line (HU02), revealed toxic effect of doxorubicin on normal human fibroblasts is more than β-lactam-anthraquinone. gene expression results indicated that the expression of Bcl-xl, KI-67, TPX2 and BAX genes were significantly increased or decreased in the cancer cell lines studied by treatment with doxorubicin and β-lactam-anthraquinone.

Conclusion: The results of gene expression in normal human cell lines by β-lactam-anthraquinone show a slight change in the expression of the corresponding genes compared to doxorubicin. As a result, higher concentrations of β-lactam-anthraquinone compound can be used for treatment of cell lines due to lower toxicity to normal cells and greater effect on cancer cells. Also, according to the change of gene expression and their known role in cancer cells, their possible activity is confirmed in apoptosis pathway in the studied cell lines.

Keywords: Beta Lactam-Anthraquinone, Antitumor and Cytotoxic activity, MTT, Real-time PCR, Doxorubicin

P-319

Blood Coagulation Parameters in Patients with Severe COVID-19 from Kermanshah Province of Iran

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Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is responsible for coronavirus disease 2019 (COVID-19) resulted in systemic inflammatory response and imbalance between homeostatic mechanisms of procoagulant and anticoagulant and is complicated with thrombotic complications. The main aim of the present study was to find the coagulation profile of intensive care unit (ICU)-admitted patients with COVID-19 from Kermanshah, Western Iran.

Methods: Coagulation parameters were analyzed using appropriate methods in 74 patients (24 aged <60 years and 50 ≥60 years) and were compared between 35 survivors and 39 non-survivors severe COVID-19 patients, admitted to the ICU.

Results: Twenty-six out of 74 patients (35.1%) required tracheal intubation (64.1% non-survivors and 2.9% survivors, $p<0.001$). Fifty-one out of 74 patients (around 69%) had comorbidities (hypertension, diabetes mellitus, coronary artery disease, cancer, renal transplantation, chronic obstructive pulmonary disease, and osteomyelitis). Thrombocytopenia was detected in around 30% of mostly older patients with comorbidities and in non-survivors. About 42% of patients had abnormal prothrombin time and international normalized ratio. The rates of mortality and comorbidity in patients ≥ 60 years were 73.7 and 78.4% compared to 26.3 and 21.6%, respectively in patients <60 years.

Conclusions: We detected a high rate of coagulopathy (around 42%) in severely affected patients with COVID-19. Furthermore, severe COVID-19 patients had low levels of platelets, high prothrombin time and international normalized ratio that were associated with poor prognosis. The abnormal pattern of coagulation parameters was highly associated with comorbidities and mortality. We found abnormal pattern of coagulation parameters and association of advanced age and comorbidities with high rate of mortality in severe COVID-19 patients which should be considered in management of these patients.

Keywords: COVID-19, coagulation, prothrombin time, international normalized ratio, mortality

P-320

Leukocytosis and Alteration of Hemoglobin Level in Patients with Severe COVID-19: Association of Leukocytosis with Mortality

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Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for coronavirus disease 2019 (COVID-19). In severely affected patients with COVID-19 leukocytosis was more prevalent.

Methods: The white blood cells (WBCs) count and hemoglobin (Hb) levels were studied in 74 patients with severe COVID-19 and were compared between 35 survivors and 39 non-survivors severe COVID-19 patients admitted to the intensive care unit (ICU) of Farabi hospital of Kermanshah University of Medical Sciences.

Results: Higher WBCs count was detected in patients with comorbidities (hypertension, diabetes mellitus, coronary artery disease, cancer, renal transplantation, chronic obstructive pulmonary disease, and osteomyelitis) than those without comorbidities. Comparing survivors with non-survivors indicated that 41% of non-survivors had WBCs count upper normal range. The mean Hb level in survived patients was 139.3±22.9, and in non-survived patients was 141.1±25.8 g/L (p=0.75).

Conclusions: Our study indicated a significant association between leukocytosis and the rate of mortality in patients with COVID-19. Also, our findings indicated association between mortality rate with hemoglobin level among COVID-19 patients.

Keywords: COVID-19, SARS-CoV-2, Leukocytosis, Mortality, Hemoglobin

P-321

Sirtuin 1 C allele (rs375391) is associated with the risk of type 2 diabetes mellitus, diabetic neuropathy and diabetic retinopathy

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Background: Sirtuin 1 (SIRT1) is downregulated in patients with type 2 diabetes mellitus (T2DM) and is associated with oxidative stress. On the other hand, the TT genotype of SIRT1 (rs375391) is associated with higher mRNA expression.

Methods: We studied 300 patients with T2DM with and without complications (mean age 56.8 years) including 100 patients without complications, 100 patients with diabetic neuropathy and 100 diabetic retinopathy patients along with 98 healthy individuals (mean age 52.9 years) for SIRT1 polymorphism and oxidative stress parameters. The SIRT1 T>C variants (rs3758391) were detected using PCR-RFLP method. The oxidative stress parameters including glutathione, glutathione peroxidase, total oxidative status, total antioxidant capacity and malondialdehyde were measured using chemical methods.

Results: The frequencies of SIRT1 genotypes and alleles were significantly different comparing all diabetic patients with controls ($p<0.001$) and also comparing diabetic patients without complications, diabetic patients with neuropathy and also diabetic patients with retinopathy with controls ($p<0.001$). The frequencies of SIRT1 CC genotype were 18.7% in all diabetic patients compared to the absence of this genotype in controls. However, the levels of oxidative stress parameters were not significantly different comparing three genotypes of SIRT1 in each group.

Conclusion: Our findings indicated association of SIRT1 CC genotype with the risk of T2DM and its complications including diabetic neuropathy and diabetic retinopathy.

Keywords: Type 2 diabetes, diabetic neuropathy, diabetic retinopathy, oxidative stress, sirtuin 1 polymorphism

P-322

Nobiletin potently up-regulates SIRT1-AMPK signaling pathway in HepG2 liver cells

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Background: Nobiletin (NOB) is one of the major citrus flavonoids with beneficial effects in liver metabolism. Many metabolic processes in liver are regulated by SIRT1. Aim: This study was carried out to investigate the effects of NOB on the activation of SIRT1-AMPK signaling pathway in HepG2 cells and compare with resveratrol (RSV) as a well-known SIRT1 activator.

Methods: HepG2 cells were incubated with NOB at different concentrations and SIRT1 gene expression was measured using quantitative real-time PCR. Fluorometric assay was applied to assess SIRT1 enzyme activity. SIRT1 protein and AMPK phosphorylation levels were determined by Western blotting. RSV and EX-527 were used as SIRT1 activator and inhibitor, respectively.

Results: Our findings showed that NOB markedly induced hepatic SIRT1 activity and expression. In addition, it significantly increased the phosphorylation of AMP-activated protein kinase (AMPK). EX-527 significantly reduced SIRT1 expression and AMPK phosphorylation.

Conclusion: NOB can induce SIRT1-AMPK signaling pathway which might be a new therapeutic approach for high fat-mediated liver disorders.

Keywords: Nobiletin, SIRT1, AMPK, HepG2

P-323

The effect of crocin on apoptosis induction and prevention of metastatic factors in the 4T1-induced breast cancer in mice

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Background: Breast cancer is one of the most popular neoplasms in human and the second cancerleading cause of death in women, worldwide. We previously showed the anticancer effect of saffron components, especially crocin, in cancer cells and chemical-induced rat model of breast cancer. Here, we investigated the anticancer effect of crocin in the mice model of metastatic breast cancer.

Methods: The mice model of breast cancer was developed by injection of 4T1 breast cancer cell line. Then, the tumor-bearing mice were divided into two groups, a control group and a group treated with crocin. All mice were weekly weighed and the size of their tumors was determined by palpation and using a caliper. After tumor appearance, crocin (150 mg/kg) was weekly injected intraperitoneally to the mice, up to four weeks. The control group was injected by vehicle. Finally, the mice were sacrificed under anesthesia, tumors were separated, weighed, their volumes were determined and saved. Then, the expression of caspase9 and CXCR4 were determined in thetumor lysate and analyzed by Western blotting.

Results The data showed that tumors volume and weight declined in the treatment group comparing with the control group. Western blot analysis showed that cleaved caspase 9 was significantly higher in the crocin-treated group than the control. It indicates the activation of caspase 9 and apoptotic pathway due to the crocin treatment. Western blot data also showed a significant decrease in the expression of CXCR4 in the crocin-treated group in comparison with the control.

Conclusion: Crocin inhibited the growth of 4T1-induced breast cancer in mice. It also activated the apoptotic caspase 9 and reduced the expression of a metastatic protein, CXCR4. These effects confirm the anticancer and antimetastatic effects of crocin.

Keywords: Crocin, Chemokine Receptor 4 (CXCR4), Caspase 9.

P-324

Serum C1q/TNF-Related Protein-2 (CTRP2) Levels are Associated with Coronary Artery Disease (CAD) in Men

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Background: Recent studies have shown that complement C1q tumor necrosis factor-related proteins (CTRPs) have different regulatory roles in the cardiovascular system. CTRP2 is the most similar to adiponectin and one of the best characterized beneficial adipokines, important in the regulation of whole body metabolism. However, there were no studies about the relationship between CTRP2 and Coronary artery disease (CAD). This study aimed to evaluate the serum CTRP2 levels in patient with coronary artery disease.

Methods: In this study, a total of 82 participants who underwent vascular angiography were included. All of subjects were male. According to their coronary angiography results, all participants were divided into CAD group (n = 42) and control group (n = 40). Serum CTRP2 levels were determined quantitatively with enzyme-linked immunosorbent assay (ELISA).

Results: Our study for the first time showed that the CTRP2 levels were higher in CAD patients (1.79 ± 1.46 ng/mL) compared to control subjects (1.08 ± 0.78 ng/mL; p = 0.001). The levels of CTRP2 also were positively correlated with the severity of CAD (r = 0.356, p = 0.001). In addition, logistic regression analysis indicated that CTRP2 had an independent association with the risk of CAD (OR [CI] = 3.366 [1.605 - 7.060]; p = 0.001).

Conclusion: Increased levels of CTRP2 in CAD patients were independently associated with the progression of the CAD; however, more study is required in this regard.

Keywords: Complement C1q Tumor Necrosis Factor Related Protein, CTRP2, Coronary Artery Disease, Adiponectin, Adipokine

P-325

Circulating Tumor DNA in the Liquid Biopsy as an early diagnostic tool

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Background: Current tumor diagnosis is affected by a variety of pathological experiments, among them, a tissue biopsy is recognized to be the gold standard. Despite, tissue biopsy-based tumor diagnosis holds many limitations. The detection of early-stage tumors or remaining lesions is unacceptable, and its use in the evaluation of treatment efficiency and prognosis is also inadequate. Researches have noticed that tumor-relevant protein molecules as well as circulating tumor cells (CTC) and circulating tumor DNA (ctDNA) are all proper tumor biomarkers in the liquid biopsy of cancer. Liquid biopsy-based on ctDNA is better for previous plasma biomarkers. Circulating tumor DNA held many cancer-associated molecular characteristics, like methylation changes, single-nucleotide mutations, and cancer-derived viral sequences. The advent of next-generation sequencing (NGS) technology in combination with the early findings of the cancer genome project (CGP), have significantly improved the sensitivity and specificity of ctDNA detection.

Methods: By systematic review from 2016 to 2019, including Medline, PubMed, Scopus, and google scholar, 33 articles were reviewed.

Result: ctDNA from the same patients at different stages can be used to dynamically control the genetic mutations through cancer progression. The half-life of protein markers is several weeks in plasma while the half-life of ctDNA is fewer than 2 hours. Examination of ctDNA represents the genetic mutations of the entire tumor tissue.

Conclusion: Use of ctDNA as a liquid biopsy can increase cancer diagnosis and treatment through genotyping, disease monitoring, and treatment evaluation. It gives a novel possibility for functional study and more extensive information including DNA, RNA, and protein-based molecular profiling.

Keywords: liquid biopsy, circulating tumor DNA, biomarker

P-326

Association of the -420 (C/G) Resistin Gene Polymorphism with Serum Lipoprotein (a) Concentrations in Rheumatoid Arthritis

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Background: Lately the rs1862513 (-420C>G SNP) situated in the resistin gene (RETN) promoter has been suggested to participate a possible character in proinflammatory conditions. We investigated whether-420 (C/G) resistin gene polymorphism is associated with serum Lipoprotein (a) [LP (a)] concentrations in arthritis disease in Iranian population.

Methods: 100 patients (75 women, 25 men) with rheumatoid arthritis (RA) and 100 (75 women, 25 men) healthy individuals were studied. -420(C/G) promoter polymorphism were determined using PCR-RFLP technique. Concentrations of LP (a), RF and CRP were measured using immunoturbidometric method.

Results: The genotypes frequencies of rs1862513 polymorphism were CC (1%), GG (2%), CG(97%) in the case group and , CC(9%), GG(29%), CG(62%) in the control group. There was significant difference between the genotypes of CC, GG and CG in two groups (P< 0.001). Serum Lp (a) levels in individuals with rheumatoid arthritis were significantly higher than control groups (P =0.001). But, there was no association between this polymorphism and serum Lp (a) levels in the two groups (p= 0.5). There was no significant difference in the age of case and control groups (46.14±12.11 vs. 45.48±11.8).

Conclusions: The present findings suggest that while resistin may play a role in the pathogenesis of RA, there is no association between this polymorphism and serum Lp (a) levels in two groups. In addition, serum lipoprotein (a) levels in individuals with rheumatoid arthritis were significantly higher than control groups.

Keywords: Lipoprotein (a), rs1862513 (-420C>, G SNP), Resistin

P-327

Determination of plasma malondialdehyde levels as lipid peroxidation laboratory factor in multiple sclerosis patients and comparison with normal subjects

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Background: Lipid peroxidation reaction and plasma molondialdehyde production are important predictors of diseases formation and progression. In this survey, multiple sclerosis in multiple sclerosis and normal subjects plasma were measured.

Methods: 70 multiple sclerosis patients and 70 normal subjects were selected. Their venous blood sample collected in buffer and separated the plasma fraction. Then, the plasma molondialdehyde levels were measured by thio-barbituric acid (TBA) method.

Results: Results showed the plasma molondialdehyde levels in multiple sclerosis patients and normal subjects were 1.49 ± 0/46 µmol/L and 0/58 ± 0/15 µmol/L.

Conclusion: In thisstudy, it became known that the plasma molondialdehyde levels in multiple sclerosis patients is higher than normal subjects.

Keywords: malondialdehyde, multiple sclerosis

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Development of biocompatible Chitosan-based nanoemulsion for controlled release of Zataria essential oil in breast cancer cells and deciphering its binding mode with gDNA

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Background: Efficacy of chemotherapy is limited by the resistance of cancer cells. Phytochemicals especially Essential Oils (EOs) provide an alternative mode of cancer therapy. However, EOs utilization is restricted because of low bioavailability, and high degradation. Nanoemulsification is a method developed to overcome these obstacles. Accordingly, Chitosan (CS) nanoemulsion of Zataria Essential Oil (CS/ZEONE) was prepared to evaluate the anticancer activity and the mechanisms responsible for the caused cytotoxicity.

Methods: ZEO was extracted by hydrodistillation and analyzed by GC-MS. ZEO loaded into CS nanoparticles was prepared in aqueous solution by mild emulsification into nanometric particles. Physical properties and FTIR spectra of CS/ZEONE were characterized. To evaluate the cytotoxic effect of CS/ZEONE on MDA-MB-231, T47D and MCF-7 breast cancer and L929 normal cells, MTT assay was used. EB/AO staining, electrophoresis, various flow cytometry techniques (Annexin V-FITC/PI, TUNEL assay, ROS production, Rhodamine123 staining, cell cycle phase distribution) and comet assay were performed to understand the anti-cancer mechanisms of CS/ZEONE and determine the death mode in treated MDA-MB-231 cells. Moreover, the binding interaction of CS/ZEONE to gDNA was also investigated by applying multiple spectroscopic techniques.

Results: GC/MS analysis revealed that carvacrol is the major ingredient of the ZEO. FTIR spectroscopy exhibited no covalent interaction between active groups of ZEO and functional groups of CS. CS/ZEONE increasingly improved the proliferation inhibition rate of breast cancer cells without harming normal cells. CS/ZEONE exposure induced apoptosis through ROS generation, loss of $\Delta\Psi m$ and DNA damage which consequently caused G2/M cell cycle arrest. To find out the mechanism more precisely, the interaction of CS/ZEONE with gDNA was elucidated and Intercalative binding with strong stabilization of the DNA helix has been proposed.

Conclusion: our data shed light on the apoptotic mechanisms related to ZEO and introduced ZEO-loaded CS Nano-particles as a promising antiproliferative and therapeutic candidate against breast cancer.

Keywords: Breast cancer, Zataria Essential Oil, CS/ZEONE, DNA oxidation, Apoptosis, DNA interaction

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Effects of Curcuma longa and Cinnamon aqueous extracts on Serum Carbohydrates and Lipids metabolism and oxidative status in high Fructose-fed Rats

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Background: This study was designed to assess the effect of Curcuma longa (turmeric) and cinnamon extract on the blood glucose, insulin, lipid profile levels and oxidative status in high fructose-fed rats.

Methods: Forty rats of 5-8 weeks old were divided into five groups with 8 rats in each group. Each group was fed with different diets as follows Group 1: common diet (Cont); Group 2: 21% fructose (Fru); Group 3: 10% turmeric (0.3ml/day/rat) with 21% Fru (Fru + Tur-1); Group 4: 10% turmeric (3ml/day/rat) with 21% Fru (Fru+ Tur-2); and Group 5: healthy rats consuming 10% turmeric (3ml/day/rat) (Cont + Tur-2). Cinnamon also followed the same grouping (Cont, Fru, Fru + Cin-1, Fru + Cin-2 and Cont + Cin-2). The experimental feeding was continued for 10 weeks. Treatment with cinnamon and curcuma (turmeric) was administered orally from the 21th day of fructose feeding. The animals were sacrificed. Then abdominal incision was given and blood was collected from the jugular vein and serum was separated and stored at −80°C. The serum glucose, ALT, AST, Cholesterol, Triglyceride, HDL-C and LDL-C were determined. Also, insulin, NO, total antioxidant capacity (TAC), and maolnedialdehyde (MDA) as a marker for lipid peroxidation were measured. Total oxidant status (TOS) in serum samples was determined using the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange. Feeding rats with high fructose diet leads to increase in glucose and insulin.

Results: There was an increase in cholesterol, triglyceride and LDL-C in fructose fed rats as compared to controls. Treatment with turmeric and cinnamon extract significantly reduced these parameters.

Conclusion: These findings indicate the improvement of blood glucose, insulin resistance, lipid profiles and antioxidant activity by Curcuma longa (Turmeric) and Cinnamon in high fructose-fed rats.

Keywords: Cinnamon, turmeric, diabetes, fructose

P-330

Assessing the Possible Association between Polymorphism of C677T MTHFR (rs1801133) with Preeclampsia Risk: A Systematic Review and Bayesian Hierarchical Meta-Analysis

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Background: Preeclampsia (PE) is a pregnancy-related disorder with an incidence of 2–5% which causes death of 40000 women each year worldwide. Among different accepted etiologies, it has been proved that hyperhomocysteinemia is a key player in the progression of PE. Considering the solid role of methylenetetrahydrofolate reductase (MTHFR) in metabolism of homocysteine, genetic polymorphism of MTHFR which could affect its activity may trigger the risk of PE. This hierarchical Bayesian meta-analysis aimed to assess the possible association between C677T MTHFR polymorphism (rs1801133) and risk of PE.

Methods: In this study, PubMed, Scopus, and Web of Science databases were searched from 2000 until 2019 for evaluating the association of MTHFR C677T polymorphism with the risk of PE in relevant case–control studies. The relevant studies were included regardless of population ethnicity and geographical limitation. The extracted data were statistically analyzed using hierarchical Bayesian method and the association strength was estimated by log(OR) with 95% credible interval.

Results: Thirty-three studies with 3930 cases and 5236 controls met our inclusion criteria. The pooled results indicated no significant effect of MTHFR C677T (C>T) on PE risk under allelic (log(OR) = 0.09, 95% CI = -0.02, 0.204), homozygous (log(OR) = 0.173, 95% CI = -0.027, 0.378), heterozygous (log(OR) = -0.009, 95% CI = -0.123, 0.104), dominant (log(OR)= 0.009, 95% CI = -0.109, 0.133) and recessive (log(OR)=0.173,95%CI=-0.012,0.366) models.

Conclusion: MTHFR C677T polymorphism had no significant effect on the risk of PE.

Keywords: Preeclampsia, Polymorphism, Methylenetetrahydrofolate reductase, Bayesian hierarchical meta-analysis

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Variants of CXCL12 chemokine is associated with the risk of chronic lymphocytic leukemia

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Background: Chronic lymphoblastic leukemia (CLL) is one of types of blood cancer with the origin of B lymphocytes. Some genetic factors affect the diagnosis and prognosis of the disease. Regarding the role of chemokine CXCL12 in proliferation, differentiation and transfer of hematopoietic stem cells such as B lymphocytes, CXCL12 variants might be involved in the incidence and prognosis of CLL. The aim of present study was to find an association between the CXCL12 variants and haplotypes with the risk of CLL in CLL patients from Kermanshah.

Methods: Blood samples were collected from 100 CLL patients from the Taleghani hospital and Mahdieh Clinic of Kermanshah University of Medical Sciences and 100 healthy individuals. Using specific designed primers and PCR-RFLP method, the variants and haplotypes of CXCL12 were detected and statistically analyzed.

Results: The frequency of SNP rs1029153 CC was 10.1% in controls compared to 2% in patients (P=0.048). Also, the frequency of SNP rs266093 CC was 10.1% in controls and 6% in patients that was not associated with the risk of CLL (P=0.074). The SNP rs1801157 AA was detected in 11% of controls compared to 8% of patients that did not reach to a statistical significance (P=0.252).

Conclusion: Our study indicated the SNP rs1029153 was significantly associated with the risk of CLL. However, the rs266093 and the rs1801157 were not associated with the risk of CLL.

Keywords: CLL, PCR, SNP, CXCL12

P-332

Lipid profile, vitamin D level and vitamin D receptor and transporter gene variants in sickle cell disease patients form Kurdistan of Iraq

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Background: Alteration of lipid profile has been reported in sickle cell disease (SCD) patients. Also, vitamin D deficiency is associated with increased respiratory infections and muscle weakness in these patients.

Methods: In the present study 104 patients carrying sickle gene including 63 sickle cell anemia, 36 sickle/beta thalassemia, 4 sickle cell trait individuals and 1 sickle/D patient along with 110 healthy individuals from Kurdistan of Iraq – Duhok- Jin center for pediatric hemato-oncology and Qaladze Public Hospital were studied for lipid profile, 25 (OH) - vitamin D and vitamin D receptor (VDR) variants and also group-specific component (GC) variants. The mean age of controls was 15.2 years and the mean age of patients was 15.9 years. Polymorphisms were detected by PCR-RFLP method. Vitamin D concentration was determined by ELISA method, and lipid profile was determined by colorimetric method. Statistical analyses were performed by SPSS 16.0.

Results: The mean level of 25 (OH)-D in sickle patients was significantly lower (11.05 ± 6.6ng/mL) than controls (13.5± 8.37ng/mL, $p=0.018$). Total cholesterol, HDL-C and LDL-C in patients were significantly lower than controls. The frequency of VDR FOK1 C allele was significantly higher in case group compared to controls. However, the frequencies of TaqI and GC variants were not significantly different comparing both groups**.**

Conclusion: Our study indicated the presence of hypocholesterolemia, reduced LDL-C and HDL-C and vitamin D deficiency among SCD patients from Kurdistan of Iraq and different frequency of VDR FOK1 polymorphism in these individuals compared to healthy controls.

Keywords: Sickle cell anemia, sickle/beta-thalassemia, vitamin D, lipid, VDR, GC variants

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Evaluation of microRNA-145 expression in patients with Type 2 Diabetes

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Background: In light of emerging global epidemics of type 2 diabetes mellitus (T2D), the discovery of novel biomarkers is important for early detection of the disease. MiRNAs may implicate in both the initiation and progress of pathologic conditions such as diabetes. This study aimed to assess the expression of miR-145-5p in plasma from diabetic and pre-diabetic patients in comparison with the control group and subsequently evaluate its diagnostic potential for T2D.

Methods: The plasma level of miR-145-5p was assessed in three groups including 20 prediabetic patients, 20 T2D patients, and 20 healthy controls using RT-qPCR. Biochemical parameters were also measured by the auto-analyzer. Bioinformatics analyses were performed for target prediction of miR-145-5p by TargetScan and miRDB databases.

Results: The expression level of miR-145-5p was down-regulated in the prediabetics and the T2D patients compared to the controls. In the control group, miR-145-5p showed a borderline correlation with FBS (p= 0.06), while in the prediabetic group miR-145 showed a significant negative correlation with FBS and finally in the T2D patients. MiR-145 was negatively correlated with HbA1c and TC and showed a negative borderline correlation with FBS. The ROC analysis indicated a significant ability for miR-145-5p in discriminating between the diabetics and pre-diabetics from the healthy subjects. The bioinformatics analysis demonstrates that target genes of miR-145-5p such as IRS, AKTB, and IGF1R are involved in the diabetes pathway.

Conclusion: Our results have demonstrated the deregulated expression of plasma miR-145-5p in the diabetics and the prediabetics. Furthermore, miR-145-5p displayed a significant ability to discriminate the diabetics from healthy subjects. These results suggest that miR-145-5p may be a useful biomarker for the diagnosis of T2DM.

Keywords: MiR-145-5p, Prediabetes, Type 2 diabetes, Bioinformatics analyses.

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Fabrication of magnetic nanoparticles containing papain enzyme to increase enzyme stability

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Background: Immobilization of enzymes on different supports has many advantages such as increasing stability and also their ability to recover from the reaction medium. Nanoparticles with properties such as large surface to volume ratio and easy dispersion in the reaction medium, are suitable supports for enzymes immobilization. Among the various nanoparticles, magnetic nanoparticles have received more attention today due to their easy recyclability from the enzymatic reaction media. Papain is a protease which has industrial and medical importance.

Methods: In this study, after synthesis of Fe_3O_4 magnetic nanoparticles and coating it with chitosan biopolymer, papain enzyme was immobilized on the surface of nanoparticles covalently, using glutaraldehyde. The resulting nanoparticles were investigated using FTIR and SEM and then its enzymatic properties were studied.

Results: FTIR data showed that synthesized Fe_3O_4 nanoparticles contain chitosan and papain was attached to the chitosan surface via glutaraldehyde. The SEM results showed that the enzyme-containing magnetic nanoparticles had a diameter of about 50 nm. The results of enzyme characterization showed that immobilization caused an increase in Km from 2.02 mg/ml to 5.35 mg/ml and decreased the enzyme Vmax from 21.03 µmol $mg^{-1}min^{-1}$ to 10.39 µmol $mg^{-1}min^{-1}$. Stability results showed that the immobilization increased the enzyme stability.

Conclusion: Finally, it can be said that the immobilization of papain enzyme on magnetic nanoparticles containing chitosan, can be a suitable way to use the enzyme in industrial and medical applications.

Keywords: Immobilization, Magnetic Nanoparticles, Chitosan, Papain.

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The Effect of Oral Co-Administration of Zinc Sulfate and Copper sulfate on some Biochemical Parameters and serum enzymes in healthy female rats

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Background: Consumption and absorption of minerals are required for various metabolic functions including immune responses to pathogens, reproduction and growth. Copper and zinc play an essential role in the structural and catalytic components of metalloenzymes and are essential nutrients needed for growth and development. Accordingly, in the present work we decided to study the effect of combined 0.6 gm/liter of zinc sulfate and of 0.3 gm/liter copper sulfate in drinking water in a period of 3 months treatment on serum enzymes and lipids in rats.

Methods: Forty Rats with 160-180gr of body weight were selected and divided into four groups of ten, including control group, group treated with zinc sulfate, group treated with copper sulfate, group treated with a combination of zinc sulfate and copper sulfate. For a period of three months, zinc sulfate and copper sulfate dissolved in tap water was used to drink by the treated group while the control group drank tap water. Three month after treatment, animals were euthanized and serum activities of, alkaline phosphates and lactate dehydrogenase and lipids were evaluated. At the end, the data were analyzed by SPSS software, using ANOVA.

Results: The results of this study showed that treatment with copper sulfate and combination of copper sulfate and zinc sulfate caused significant increase in cholesterol, triglyceride, LDL and alkaline phosphates and decrease in HDL and lactate dehydrogenase compared to the control group. Treatment with zinc sulfate caused significant decrease in total cholesterol, triglyceride, LDL and alkaline phosphates and increase in HDL and lactate dehydrogenase compared to the control group.

Conclusion: Copper and zinc are as one of the rare essential metals in the body, but excessive amounts in the environment and the body can exert damage in the body.

Keywords: Keywords: copper sulfate, Zinc sulfate, Lipid profile, serum enzyme

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Inositol Triphosphate Receptor3 (IP3R3) as a biomarker in cancers

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Background Ca^{2+} is a controlling factor in many intracellular processes, including cell proliferation and apoptosis. Inositol triphosphate receptors (IP3Rs), located on the endoplasmic reticulum membrane, regulate releasing intracellular calcium reserves into the cytoplasm and establish calcium homeostasis. Alternations in the expression level of Type3 IP3R (IP3R3) have been shown in different types of tumors.

Methods: The literature search was performed on databases including “Science Direct”, “google scholar” and “PubMed” between 2008 to 2020 by the combination of terms: “IP3R3”, “Inositol triphosphate receptor3” and “cancer” as the keywords. Of 94 articles, 8 articles were included in the review.

Results: It was shown that IP3R1 and IP3R2 were expressed in both normal mucosal cell types and colorectal cancer cells, but IP3R3 was expressed only in the cancer cells and it was associated with the depth of tumor invasion, metastasis, and reduction in 5-year survival. The expression of IP3R3 was significantly high in glioblastoma cells, and inhibition of IP3R3 by caffeine led to inhibition of invasion cell migration. IP3R3 was shown to be involved in the migration of breast cancer cells. Turning off the gene led to rounding the shape of the cells and decreased the amount of adhesion in the invasive cells of the breast carcinoma. It was revealed that IP3R3 expression was increased in hepatocellular carcinoma cells while it was absent or expressed in low amounts in hepatocytes from normal liver, and this increased expression was associated with disease prognosis. It was shown that the elevated expression of IP3R3 is associated with the migration capacity of human breast cancer cells. An increase in IP3R3 expression in head and neck squamous cell carcinoma (HNSCC) and kidney cancer has been shown.

Conclusion: These results suggest that the study of changes in IP3R3 expression can be used as a diagnostic and therapeutic purpose.

Keywords: IP3R3, Inositol triphosphate receptor3, cancer

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Circulating levels of CircHIPK-3 circular RNA in type 2 diabetes patients

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Background: Recent evidence indicates that Circular RNAs (circRNAs) levels seem to be deregulated in type 2 diabetes mellitus (T2DM). The aim of this study was to investigate the expression of CircHIPK-3 circRNAs and its association with biochemical and anthropometric parameters in peripheral blood of patients with T2DM, pre-diabetes, and control subjects.

Methods: The study population consisted of 196 subjects including 70 patients with T2DM, 60 prediabetes, and 69 age and sex-matched healthy controls. The expression level of CircHIPK-3 in peripheral blood samples was measured by the RT-PCR method.

Results: Our findings revealed that the CircHIPK-3 expression level was higher in the peripheral blood of T2DM patients than pre-diabetics ($p=0.016$). Moreover, a positive correlation was found between the expression of CircHIPK-3 and BMI, systolic, and diastolic blood pressure, HbA1c, and FBS levels($P<0.05$).

Conclusion: These findings provided evidence that increased expression of CircHIPK-3 could be associated with the pathogenesis of T2DM.

Keywords: Circular RNA, Type 2 Diabetes Mellitus, CircHIPK-3

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The Nrf2 and Keap1 variants and oxidative stress parameters are associated with Diabetic Neuropathy

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Background: Diabetic neuropathy (DN) affects at least 50% of diabetic patients and is an important factor in disease-related disabilities. Chronic hyperglycemia activates the inflammatory pathways and oxidative stress mechanisms with consequent damage to nerve tissue. The Keap1-Nrf2 pathway acts as one of the most important antioxidant pathways of the organism.

Methods: In the present study, 100 patients with DN and 100 healthy individuals were investigated. The activity of glutathione peroxidase (GPX) was measured by a Randox kit, glutathione (GSH) was measured using a fluorescent reagent (o-Phthalaldehyde), the plasma level of malondialdehyde (MDA) was detected by thiobarbituric acid method, plasma total antioxidant capacity (TAC) was measured using FRAP reagent, and total oxidative status (TOS) was measured by a chemical method. Using PCR-RFLP method, the Nrf2 (rs6721961) and Keap1 (rs11085735) variants were identified.

Results: A significant difference was observed between DN patients with controls in terms of reduced activity of GPX and glutathione level, decreased TAC, and increased MDA and TOS levels in DN patients. A higher frequency of mutant allele of Nrf2 (41%) in DR patients compared to controls was observed (27%, $p<0.001$). However, the frequency of mutant allele of Keap1 in DR patients was non-significantly lower than controls.

Conclusions: Our study indicated association between oxidative stress and DN that is reflected in the imbalance between oxidant and antioxidant system. The results also showed the decreased frequency of Keap1 mutant allele and the increased frequency of mutant allele of Nrf2 that decreases the Nrf2 expression.

Keywords: Type 2 diabetes, diabetic neuropathy, oxidative stress, Nrf2, Keap1 variants

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Association between Nrf2 and Keap1 variants and oxidative stress parameters with diabetic retinopathy

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Background: Diabetic retinopathy (DR), as one of the most life-threatening complications of diabetes, is a microvascular disorder that threatens vision. Oxidative stress might be involved in the pathogenesis of DR. The Keap1-Nrf2 pathway is one of the most important antioxidant pathways of the organism. During oxidative stress, Nrf2 is cleaved from the Keap1-Nrf2 complex and transported to the nucleus to induce the expression of antioxidant genes.

Methods: We studied 100 healthy individuals and 200 patients with type 2 diabetes mellitus (T2DM) including 100 T2DM patients without complications and 100 T2DM patients with retinopathy. Duration of diabetes was at least five years. Glutathione peroxidase (GPX) activity was measured by a Randox kit, glutathione was measured using a fluorescent reagent (o-Phthalaldehyde) and the plasma level of malondialdehyde (MDA) was measured by thiobarbituric acid method. PCR-RFLP method was used to determine the Nrf2 (rs6721961) and Keap1 (rs11085735) variants.

Results: Comparing plasma oxidative stress parameters between diabetic patients without complications and controls indicated a significant difference in GPX activity ($p < 0.001$), and the level of MDA ($p < 0.001$). In addition, in DR patients compared with controls, reduced activity of GPX and glutathione level, and increased MDA level were detected. A higher frequency of mutant allele of Nrf2 (60.5%) in DR patients compared to controls was observed (27%, $p < 0.001$). However, the frequency of mutant allele of Keap1 in DR patients was significantly lower than controls.

Conclusions: Our study indicated increased oxidative stress in diabetes, both in patients without complication and those with DR compared to controls. Additionally, the frequency of mutant alleles of Nrf2 and Keap1 was increased and decreased, respectively in DR patients compared to controls.

Keywords: Type 2 diabetes, diabetic retinopathy, oxidative stress, Nrf2, Keap1, genetic variants

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Autophagy protects peripheral blood mononuclear cells from high glucose-induced inflammation

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Background: Previous works have linked high concentration of glucose to inflammation by autophagy modulation. However, the ways in which high glucose (HG) regulates inflammation in peripheral blood mononuclear cells (PBMCs), has not been well characterized. In the present study, we therefore aimed to investigate the role of autophagy in inflammatory responses of PBMCs exposed to HG.

Methods: PBMCs were treated with 33 mM glucose (HG) for various times. The expression of the genes was evaluated by real-time PCR. Protein levels were measured using western blotting.

Results: HG increased the level of LC3-II at 12h, 24h and 48h. NH4Cl, a lysosome inhibitor that can block the autophagic flux could further promote LC3-II accumulation in HG-treated cells at 12h, 24h and 48h. The protein level of p62 was significantly decreased from 12h to 48h in HG-treated cells, suggesting an induction of autophagic flux in HG-treated PBMCs. Inhibiting autophagy with chloroquine (CQ) significantly augmented HG-induced PBMCs inflammation as demonstrated by increased expression of TNF-α, IL-6 and IL-1β.

Conclusions: These data revealed that the autophagy system could be activated in HG-treated PBMCs. Our results also indicated that induction of autophagy might play an adaptive and protective role in HG-induced inflammation of the PBMCs.

Keywords: High glucose, PBMC, autophagy, inflammation, LC3-II

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Growth hormone deficiency in children with acute lymphoblastic leukemia

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Background: The most common cancer in children is acute lymphoblastic leukemia (ALL) and the patients often undergo a combination of different treatments. Endocrinopathy (e.g. pituitary) may occur as a late effect of treatments. The growth hormone (GH) produced by the pituitary gland plays an important role in human development. Survivors of childhood ALL who received intensive combination chemotherapy and radiation, are at risk of GH deficiency and impaired growth and short stature as adults. So GH tests in childhood ALL can help to provide an appropriate solution for these problems.

Methods: In this review, searching on SID, Google Scholar, PubMed, and Science Direct were done between 2010-2020 using “growth hormone”, “acute lymphoblastic leukemia “, and “children” as keywords to find relevant articles. 107 articles were found. After examining the titles and abstracts, 10 articles were selected.

Results: In reviewed studies, unfavorable height and endocrine side effects in ALL patients were shown. Also, it was demonstrated that GH level in ALL patients After achieving remission with 6 weeks of therapy was highly decreased while it was not significantly higher compared with healthy controls before starting treatment. Researchers have shown that Cranial Radiotherapy (CRT) often leads to dysfunction of the hypothalamic-pituitary axis with a spectrum of hormone deficiencies particularly growth hormone deficiency (GHD). In a recent report from the St. Jude Lifetime (SJLIFE) Cohort, the most common endocrinopathy in ALL patients was GHD, while 78% of those survivors with GHD had no other endocrinopathies.

Conclusion These results suggest that the choice of GH test is crucial for the diagnosis of GHD in childhood ALL especially in cases who treated with CRT, to control and prevent subsequent complications.

Keywords: growth hormone, acute lymphoblastic leukemia, children

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Decreased Plasma Level of TRIB3 is Associated with Circulating miR-124a in Patients with Type 2 Diabetes

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Background: Recent evidence indicates that TRIB3 and miR-124 levels have been deregulated in type 2 diabetes (T2D); however, the simultaneous evaluation of these markers in diabetic patients has not been investigated to date.

Methods: This case-control study included 50 T2D patients and 40 age/gender matched controls. The circulating level of miR-124a was assessed by real-time PCR. TRIB3 plasma level was measured using the enzyme-linked immunosorbent assay.

Results: Our findings revealed that the TRIB3 plasma level was significantly increased ($p = 0.025$), while miR-124a plasma levels were significantly reduced ($p = 0.028$) in diabetic patients compared to healthy subjects. ROC analysis showed that TRIB3 and miR-124a levels could discriminate control subjects and diabetic patients. Interestingly, a significant negative correlation was found between the TRIB3 and miR-124a plasma levels. Furthermore, there was a significant positive correlation between the TRIB3 plasma level with fasting blood glucose and insulin resistance.

Conclusions: In this study, we showed deregulation of TRIB3 level in diabetic patients and its association with miR-124a circulating level and clinical parameters. These findings suggest that miR-124a may affect T2D incidence and progression by modulating the expression of TRIB3 protein level.

Keywords: Type 2 diabetes, TRIB3, miR-124a

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Characterization and application of pyridine-3,5-dicarbonitriles as efficient inhibitors for the docking simulation of ligand bond with cyclin-dependent kinase

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Background: The cyclin-dependent kinase (CDK) show a role in apoptosis and in the control of transcription. Among the computational methods, quantitative structure–activity relationships (QSAR) have found distinct applications for predicting the properties of compounds, including biological activity prediction, physical property prediction and toxicity prediction.

Methods: In the present study, the AutoDock 4.0 package was employed for docking synthetic compounds into a protein, cyclin-dependent kinase 2 (PDB ID: 1GIH). A residue ARG36 in active site was also chosen due to its possible specific hydrogen bonds. This residue (ARG36) was set as flexible residue, while the others residues were kept as rigid residues.

Results: In this results 2,6-Diamino-4-(4-nitrophenyl) pyridine-3,5-dicarbonitrile was found to be the best selective known inhibitor of cyclin-dependent kinase2 because it showed lowest docked energy.

Conclusion: The phosphonic acid moiety is considered to bind to the affected protein more strongly than the corresponding carboxylic acid because of its dianionic character. Analysis of the number of hydrogen bonds between inhibitors and CDK2 shows that the CDK2–2, 6-Diamino-4-(4-nitrophenyl) pyridine-3, 5-dicarbonitrile complex has a higher number of intermolecular hydrogen bonds, which indicates that it has higher affinity for CDK2 than others. Further inhibition experiments can confirm this prediction.

Keywords: pyridine-3, 5-dicarbonitriles, docking, simulation, inhibitor

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In-silico analysis of the role of hsa-miR-212-3p in human cancers

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Background: Non-coding RNAs are among the most important epigenetic changes. MicroRNAs (miRNAs) are small regulatory non-coding RNAs that have been reported to play a critical role in the tumorigenesis of many cancers. For this study, we have selected the hsa-miR-212-3p that can inhibit tumor suppressor and oncogene genes in several cancers.

Methods: In this study, after reviewing target genes of hsa-miR-212-3p, by using bioinformatics databases such as Targetscan, microRNA.org, miRWalk, miRDB, we analyzed target genes in DAVID database. At the end, we analyzed this microRNA by using mirPath v.3.

Results: Our results confirmed that hsa-miR-212-3p has important roles in breast cancer by targeting BRCA1, colorectal cancer by targeting SMAD2, SMAD5, and DCC, prostate cancer by targeting SOS1 and PIK3CA, and Pancreatic cancer by targeting RB1 and CDK6. This molecule can be interfere in HIF-1, cGMP-PKG, MAPK, PI3K-Akt, VEGF, TGF-beta, and Wnt signaling pathways.

Conclusion: In general, sufficient evidences show that hsa-miR-212-3p can interact with different targets and participate in several pathways, as well as immune responses, in cancer. Dysregulated hsa-miR-212-3p may function as a promoter or suppressor in different aspects of tumorigenesis including cell proliferation, invasion, metastasis, and apoptosis.

Keywords: Computational Molecular Biology, microRNA, prostate cancer, breast cancer, colorectal cancer, pancreatic cancer

P-345

A Promising effect of Zerumbone with miR- 34a in Colorectal Cancer Cell Lines

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Background*:* Cross-talk among inflammation and colorectal cell types is predominantly through a network of cytokines, chemokines, and other growth factors. MiRNAs regulate the expression of many genes. miR34-a is a tumor suppressor and its expression results in cell cycle arrest and inhibition of growth. Combined application of different miRNA-based agents and chemotherapeutic drugs have been used to augment drug sensitivity and may reinforce the antitumor effect. Combination therapies are of considerable interest for patients who fail to respond adequately to monotherapy. Combined application of different miRNA-based agents and chemotherapeutic drugs have been used to augment drug sensitivity and may reinforce the antitumor effect. Herbs, plants and plant-based compounds that are commonly referred as safe compounds have been demonstrated to exert chemo preventive and mediate anticancer functions in different cells. One such herbal compound is Zerumbone. The anti-inflammatory activity of Zerumbone (ZER) was investigated in many cancers.

Methods*:* In this study, the level of the inflammatory cytokines including CXCL-12 (SDF-1), CCL-2 (MCP-1), TGF-β and IL-33 was measured in pmiR-34a-5p transfected and pmiR-34a-5p+ZER treated CRC cell lines (HCT-116 and SW48) by QRT-PCR and ELISA methods.

Results: Generally, we found that miR-34a could considerably decrease the expression of inflammatory cytokines and the combination of Both ZER+miR-34 could boost this effect on CRC cell lines.

Conclusion: Although in most cases combination of miR-34a and ZER did not induce a significant synergistic effect on expression of cytokines, compared with the use of miR-34a and ZER alone, the important thing is that these therapeutics targets can modulate effect of each other when one is not presenting its inhibitory effect.

Keywords: Zerumbone (ZER), pmiR-34a-5p, CXCL-12 (SDF-1), CCL-2 (MCP-1), TGF-β cytokines

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Candidate miRNAs as human prostate cancer biomarkers: a systematic review

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Background: Prostate cancer is the most prevalent cancer and the main cause of cancer deaths among males around the world. For the early diagnosis of the prostate, there would be an immediate and essential requirement to search for sensitive biomarkers. MiRNAs are a large family of small 20-25 nt single-stranded non-coding RNAs, which can interfere with the expression of ~60% protein-coding genes through post-transcriptional suppression, target mRNA degradation, or translational inhibition.

Methods: To identify candidate miRNA biomarkers for prostate cancer, we performed a general systematic review regarding the published miRNA profiling researches comparing miRNA expression level between prostate cancer and normal tissues. A systematic review was performed by completely searching the PubMed database (last updated search being September 11, 2020) for articles in English using a combination of the following terms: microRNA, MicroRNA, miRNA, cancer, prostate cancer, and predictive marker.

Results: We determined that two miRNAs (miR-25-3p, and miR-191) were upregulated consistently and seven miRNAs (miR-129, miR-185, miR-342-3p, miR-124, miR-224, and, miR-222, miR-205) were downregulated consistently in at least three studies.

Conclusion: Although these miRNAs need to be validated and further investigated, they could be potential candidates for prostate cancer miRNA biomarkers and used for early prognosis or diagnosis.

Keywords: miRNA, prostate cancer, microRNA

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Study of nitric oxide concentration, gene expression of nitric oxide synthase isozymes and iNOS gene 2087A>G polymorphism in women with polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is one of the most common hormonal disorders, which leads to menstrual disorders, hirsutism, acne, obesity, and in some cases, infertility and miscarriage. Disease complications decrease the quality of life. The cause of this syndrome is unclear. Oxidative stress pathways are involved in the pathogenesis of PCOS, but the status of nitric oxide (NO) is still unknown. Nitric oxide can play a pivotal role in many of the physiological activities that are compromised in PCOS. The aim of this study was the investigation of NOS gene expression, iNOS gene 2087 G to A (rs2297518) polymorphism and NO levels in PCOS patients.

Methods: Peripheral blood samples were obtained from PCOS women (N=100) and normal menstruating women as controls (N=100). The expression of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) mRNA was performed using RT-qPCR (N=30). Plasma levels of NO were measured using Griess spectrophotometric assay. Also, we analyzed the polymorphic site in the iNOS (rs2297518), by the design of a new ARMS protocol.

Results: A significant decrease in mRNA expression of iNOS was observed in PCOS patients. No significant change in eNOS gene expression was detected. In this way, PCOS women showed significantly reduced plasma NO compared to controls. Subjects carrying the G/A (rs2297518) variation had a low risk of PCOS.

Conclusion: Our study shows that PCOS women have lowered NO due to reduced iNOS expression. An in-depth analysis of the redox biology of PCOS to open up potential therapeutic strategies is highly recommended.

Keywords: Polycystic ovary syndrome, Inducible nitric oxide synthase, Nitric oxide, endothelial dysfunction, Genetic polymorphism

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The effect of SP/NK1R on the expression and activity of Catalase and Superoxide dismutase enzymes in Glioblastoma cancer cell

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Background: Glioblastoma is one of the most malignant brain tumor and its mortality rate depends on the different stages of this cancer. Various methods are used to treat glioblastoma, including surgery, radiotherapy and chemotherapy. Substance P (SP), as one of the peptides released by sensory nerves, increases cellular excitability by activating the neurokinin-1 receptor (NK1R) in several human tumor cells. Aprepitant, is a specific powerful and long-lasting NK1 receptor antagonist, considered as a new agent for inhibiting proliferation and induction of apoptosis in malignant cells. The aim of this study was to evaluate the effects of SP / NK1R system on the expression and activity of enzymes catalase and superoxide dismutase in glioblastoma U87 cancer cell line.

Methods: Glioblastoma cancer cells were grown at 37 °C and at appropriate humidity. Cytotoxicity was measured by Resazurin test, 24 hours after treatment with increasing concentrations of aprepitant. The production of reactive oxygen species was measured 24 hours after treatment with SP and aprepitant. Enzymes activity of catalase and superoxide dismutase was measured by the corresponding assay kits. The expression of CAT and SOD enzymes was measured by Real-Time PCR.

Results: Aprepitant significantly reduced the life of U87 cells in a time-dependent and concentration-dependent manner. The production of reactive oxygen species (ROS) was significantly reduced in 24 hours. The activity of CAT and SOD enzymes increased significantly in 24 hours after treatment with aprepitant. Real-Time PCR data showed that the expression of CAT and SOD enzymes increased significantly in the presence of aprepitant.

Conclusion: The present study showed that aprepitant, as a specific NK1R, can inhibit the induction of SP effects by inducing the antioxidant effects of CAT and SOD in the U87 cell line. Therefore, this drug can be introduced as a potential candidate for controlling glioblastoma cancer in animal models and clinical trials.

Keywords: Aprepitant, Glioblastoma cancer, U87, Catalase, Superoxide dismutase

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Determination of total protein in poultry by-product

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Background: The Kjeldahl method determines protein concentration by estimating the total nitrogen content, which is not a precise method and have errors. Therefore, the protein concentration in poultry by-product meal (PBM) was estimated using the Biuret assay, which shows only protein peptide bonds. In this study, the sample was prepared using a new method to be suitable for Biuret assay.

Methods: To determine the total protein content of the crude powder, one gram of sample was added with 0.6 M NaOH and placed in a boiling water bath. After cooling and adding the solution to the sample, 500 µl of the aqueous layer sample was centrifuged and then mixed with 2.5 ml of the Biuret reagent. Subsequently, absorbance was measured at 540 nm a spectrophotometer.

Results: The total protein content in poultry by-product meal was determined using the Kjeldahl (N× 6.25) method (492.452 mg/g). The amount of total protein found in raw poultry by-product meal (measured by Biuret assay) was calculated (457.9 ± 14.5 mg/g). The difference between the two results was about 7.017%. The difference between the results obtained from the Kjeldahl method and the Biuret assay can be due to the difference in the measurement of the full amount of the existing elastin content and the non-protein nitrogen molecules (NPN) in the sample.

Conclusion: The results showed that protein concentration measurement using the Kjeldahl method because of the nature of this method is not a precise method for calculating the actual protein content. Therefore, the total protein content of the poultry by-product meal was calculated by using Biuret reagent without the presence of nitrogenous non-protein molecules. This method can be used as a substitute for the Kjeldahl method to determine the total protein content of red meat, fish, and poultry.

Keywords: Poultry by-product meal, Biuret assay, Kjeldahl method, Total protein

P-350

In Silico Design and Evaluation of scFv-RTX-A as a Novel Immunotoxin for Breast Cancer Treatment

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Background: Breast cancer (BC) is regarded as the most prevalent form of cancer among women. Human epidermal growth factor receptor 2 (HER2) is overexpressed in many BC patients. Hence, immunotherapy is a proper treatment option for HER2-positive BC patients. There is accumulating evidence indicating that immunotoxin therapy is a new strategy to improve the potency of targeted therapy. Immunotoxins are antibodies or antibody fragments coupled with a toxin.

Methods: We designed a novel immunotoxin. The physicochemical properties were assessed using ProtParam servers and secondary structure was detected by PROSO II and GORV. By using I-TASSER, a 3D model was built and refined by GalaxyRefine. The model was validated using PROCHECK and RAMPAGE. To predict immunotoxin allergenicity and mRNA stability, AlgPred server and RNAfold were used. Furthermore, the immunotoxin and HER2 were docked by ZDOCK.

Results: The scFv +RTX-A could be a non-allergenic and stable chimeric protein, and the secondary structure of its components did not alter. This protein had a proper 3D structure that might have stable mRNA structure which could bind to HER2.

Conclusion: it seems that the designed immunotoxin was a non-allergenic and stable chimeric protein and that it could bind with high affinity to HER2 receptors, we suggested that this chimeric protein could be a helpful candidate for HER-2 positive BC patients.

Keywords: Immunotoxin, Breast Cancer, Immunotherapy

P-351

Circulating levels of Angiopoietin-like protein 3 in polycystic ovary syndrome woman: a case-control study

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Background: There is strong link between the serum levels of adiponectin with the clinical and hormonal indices of PCOS. ANGPTL3 is recognized as a regulator of lipid metabolism. There has not been enough data to explain association between the levels of angiopoietin-like protein 3 (ANGPTL3) with insulin resistance (IR)-related polycystic ovary syndrome (PCOS). The aim of this study was to investigate the changes of serum ANGPTL3 and adiponectin in PCOS women with normal body mass index (BMI).

Methods: In this case control study, a total of 160 premenopausal women (100 with PCOS and 60 without PCOS) were enrolled. Serum concentrations of adiponectin, ANGPTL3, insulin and other hormonal variables related with PCOS were measured by ELISA method and biochemical parameters were measured by an autoanalyzer.

Results: There were significant variations in the amount of ANGPTL3, adiponectin, high-density lipoprotein (HDL-C), insulin, Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), follicle-stimulating hormone (FSH), between the two groups. In addition, we observed significant differences in serum levels of adiponectin, hs-CRP, and ANGPTL3 in recurrent pregnancy loss and infertile subgroups of PCOS compared to the control group ($p<0.001$).

Conclusion: Our results suggest that increased serum ANGPTL3 and decreased adiponectin levels are significantly and independently associated with risk of PCOS.

Keywords: Polycystic ovary syndrome, insulin resistance, angiopoietin-like protein 3, adiponectin

P-352

The anti-depressant and anti-anxiety effects of Crocin and Crocetin in the rat model of depression

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Background: Depression is a debilitating condition that can have profound effects on both the mind and body of individuals. The therapeutic effect of saffron on depression has long been discussed. In this study, the effect of saffron carotenoids, Crocin and Crocetin, was investigated on improving behavioral symptoms in the rat model of depression.

Methods: Adult male Wistar rats (weighing 250–300 g) were randomly divided into 8 groups (# 6 per each). The chronic stress model (CMS) was used to induce depression, and establishment of the model was confirmed by behavioral tests, including the forced swimming test (FST), sucrose preference test (SPT), open filed test (OFT) and elevated plus maze (EPM). After induction of depression, Crocin (30 mg/kg)/ Crocetin (10 mg/kg)/ Fluoxetine (10 mg/kg) were daily injected intraperitoneally, up to 21 days, to the rat groups; the control group received Normal Saline. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a post hoc Tuky's test.

Results: In FST, the immobility time was significantly ($p=0.000$) reduced in the depressed groups treated with Crocin in comparison with the Fluoxetine and control groups. In SPT, the sucrose intake was significantly ($p=0.000$) increased in the depressed groups treated with Crocin/ Crocetin/ Fluoxetine in comparison with the control group. In EPM, the open arm time/ total time ratio was increased significantly ($p=0.00$) in the depressed group treated with Crocetin compared to the control group, and the depressed group treated with Fluoxetine. In OFT, the distance of movement was significantly ($p=0.000$) increased in the depressed groups treated with Crocin compared to the control group.

Conclusion: This study showed the anti-depressant and anti-anxiety effects of both Crocin and Crocetin. However, Crocin was a more effective anti-depressant than Fluoxetine. Crocetin showed the anti-depressant and anti-anxiety effects similar to Fluoxetine.

Keywords: Crocin, Crocetin, Fluoxetine, FST, SPT, OFT, EPM

P-353

Neuroprotective effect of Crocin on the Alzheimer-like model of PC12 cells

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Background: Alzheimer's disease (AD) as an irreversible, progressive degeneration of brain cells, is associated with the abnormal accumulation of the amyloid-β (Aβ) peptides. Here, we studied the neuroprotective effect of crocin on AD-like-dPC12 cells induced by Aβ1-42.

Methods: The PC12 cells (suspend, CRL-1721, and adherent, CRL-1721.1) were grown in PDL-coated 24-wells plates. After 24 h, the growth medium was switched to differentiation medium, containing 10-100 ng/ml of NGF-2.5 S for 14 days. The data on adherent cells were not reproducible, so the study was continued with the suspend cells. The best protocol for oligomerization of Aβ was resuspension of Aβ1-42 powder in phosphate buffer (50 mM, pH=7.4) and incubation at 37°C for 180 min. The aggregation process was examined by Thioflavin T (ThT) assay and the oligomer structure was confirmed by Atomic Force Microscopy (AFM) and Western blot. Then, we exposed the dPC12 cells to different doses of AβOs (0.1-10 µM) for 24 hours to obtain the toxic dose of AβOs, using the MTT assay. After that, the dPC12 cells that were treated with the toxic dose of AβOs, were treated with crocin (0-100 µM) for 24 h and assessed by MTT assay.

Results: For PC12 (CRL-1721) cells, we achieved the best neuronal characteristics (length and density of neurites) after 14 days of incubation with 75 ng/mL NGF. The data from ThT assay and AFM analysis confirmed that Aβ solution in Lag phase has oligomeric structure and in plateau phase has fibrillar structure. The cell viability assay showed that both of the IC50 value of AβOs and the neuroprotective dose of crocin in these cells was 10 µM of each.

Conclusion: These results indicated that 10 µM of AβOs at 24 h was toxic as a neurodegenerative agent for dPC12 cells, and crocin could reverse this toxic effect.

Keywords: Alzheimer's disaese, Amyloid β Oligomers, PC12, Crocin.

P-354

Effects of cinnamon extract on liver histopathological features and oxidative stress in STZ-induced diabetic rats

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Background: Due to the antioxidant properties of cinnamon extract and the role of antioxidants in improving diabetes, this study was conducted to evaluate the effects of cinnamon extract on liver histopathological features and oxidative stress in diabetic rats.

Methods: Diabetes was induced in male Sprague -Dawley rats by one dose of streptozotocin (50 mg/kg body weight, ip). The rats were divided into four groups: healthy control, diabetic control, diabetic+cinnamon and healthy control+cinnamon groups. In this study, the cinnamon extract was administrated orally by a dose of 40 mg / kg body weight. The contents of reduced glutathione (GSH) and malondialdehyde (MDA) in the liver of rats were determined after eight weeks. Also the effect of cinnamon extract on liver histopathological changes and blood glucose level in healthy and diabetic rats were investigated.

Results: The results of this study showed that cinnamon extract could significantly decrease the blood glucose level in diabetic rats ($p<0.001$). Also, cinnamon extract reduced the amount of MDA in the liver of diabetic rats at the end of the experiment time ($p<0.001$). But cinnamon administration for diabetic rats had no significant effect on GSH level compared to diabetic control rats. In addition, induction of diabetes resulted in mild hypertrophic degeneration and lymphocyte infiltration in hepatocytes and interestingly, administration of cinnamon extract could not restore these abnormalities. The liver tissue of the healthy control rats and healthy control rats that received cinnamon extract had normal structure and hepatocytes. **Conclusion:** The results of this study confirm the anti-diabetic and antioxidative effects of cinnamon in diabetic rats. However, cinnamon administration could not change the histopathological abnormality in the liver of diabetic rats.

Keywords: Diabetes, Liver, Oxidative stress, Cinnamon

P-355

Effect of Substance P on the redox status of glioblastoma multiforme cells and its regulation by neurokinin-1receptor (NK1R) antagonist, aprepitant

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Background: Alterations in the redox homeostasis is one of the main contributing factors in developing glioblastoma multiforme (GBM), a highly aggressive grade IV brain tumor. Neuropeptide substance p (SP) plays a critical role in modifying the cellular redox status, through activation of neurokinin-1receptor (NK1R). In this study, we aimed to explore the redox-modulating properties of SP, and a commercially available NK-1R antagonist, aprepitant in GBM cells.

Methods: In order to detect the effect of aprepitant on the viability of U87 glioblastoma cells, resazurin assay was applied. The level of intracellular ROS was evaluated using 2-dichlorodihydrofluorescein diacetate (H2DCFDA) assay. The expression of glutaredoxin and the proteins of the thioredoxin system were measured by quantitative real-time polymerase chain reaction (qRT-PCR). Concurrently, the enzymatic activity of the proteins of the thioredoxin system as well as glutaredoxin were also analyzed by a commercial kit (ZellBio GmbH).

Results: We found that SP elevated the intracellular levels of reactive oxygen species (ROS) in U87 GBM cells and aprepitant significantly reduced this effect. We also explored the effects of SP / NK1R signaling on the glutaredoxin and thioredoxin system as the main cellular redox buffers in GBM cells. SP reduced both expression and enzymatic activity of glutaredoxin, and the proteins of the thioredoxin system (thioredoxin (Trx) and thioredoxin reductase (TrxR)) and these effects were significantly decreased by aprepitant.

Conclusion: In conclusion, our results suggest the possible involvement of SP/NK1R signaling in GBM pathogenesis through affecting the cellular redox status and offer new insight for the application of aprepitant as a redox modulating strategy in stress-related cancers including GBM.

Keywords: Substance P, aprepitant, glioblastoma multiforme, glutaredoxin, thioredoxin, thioredoxin reductase

P-356

Antioxidant activity of biosynthesized gold nanoparticles (AuNPs) from herbal plant Anethum graveolens

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Background: Due to the extensive applications of AuNPs in biotechnology, optoelectronic devices, medicine, biosensors, information storage, catalysts, etc. they are currently under wide investigation. While a wide range of physio-chemical methods has been developed for synthesis of AuNPs, these methods have some drawbacks including environmental pollution, requirement of high energy and high process cost.

Methods: Through an eco-friendly, facile, cost-effective and fast process, AuNPs were synthesized by the reaction of Gold (III) chloride trihydrate with Anethum graveolens leaf extract. Then, their antioxidant activity against ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrzyl) free radicals was investigated.

Results: Dynamic light scattering (DLS) results determined that the as-obtained Anethum graveolens-AuNPs had an average particle size of 86.40 ± 29.51 nm and were negatively charged. Transmission electron microscopy (TEM) analysis illustrated that AuNPs were spherical, quasi-spherical and triangular. The face centered cubic (FCC) structure of Anethum graveolens-AuNPs was detected by XRD spectrum. Furthermore, Fourier transform infrared spectroscopy (FTIR) approved the existence of numerous functional groups in the structure of Anethum graveolens-AuNPs. The ABTS and DPPH assays confirmed that with increasing concentration of Anethum graveolens-AuNPs, the Anethum graveolens-AuNPschr('39') ability for scavenging of free radicals increased.

Conclusion: The biosynthesis of AuNPs throughout green chemistry processes (plant extracts) has become a major focus of researchers whereas they are cost-effective and suitable for large-scale production. Inasmuch as antioxidant property of Anethum graveolens-AuNPs was approved, we suggest that in the future, their other potential biomedical applications in drug delivery, biomedicine, cancer therapy and biosensors should be studied.

Keywords: Antioxidant activity, Gold nanoparticles, Anethum graveolens, Eco-friendly, Green chemistry

P-357

The evaluation of progesterone drug derivations for providing optimum conditions

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Background: This study was designed to examine the interaction of different complexes of progesterone using molecular modeling techniques under gas, polar and non-polar environments at three temperatures (25, 37, and 40 degree centigrade). The potential energy (kcal/mol) and overall charges of binding energy via force fields during Molecular Dynamic, Monte Carlo and Langevin Dynamic simulations was calculated.

Methods: Semi-empirical, molecular mechanics and molecular dynamics methods were utilized at arriving at the proposed structures. A 100 ps molecular dynamics simulation was performed using the molecular mechanics AMBER force field under constant temperature conditions after placing the progesterone derivations in test conditions. A bath relaxation time of 0.1 ps and a step size of 0.001 ps were used for the simulation. Molecules were prepared using Chem Draw 8.0. The geometry was optimized through QM/MM procedure.

Conclusion: In results it was found that temperature and environment can influence interaction of different derivations and introduced interference between them. We are grateful to Taha Sanat Company that is a trading Co. which seeks to provide new and used equipment and machineries in the field of scrapping and recycling area in country from all over the world by utilizing technicians, engineers and strong commercial team since 2015 for his support of this work.

Keywords: QM/MM procedure, progesterone, Taha Sanat Company

P-358

Bee venom (BV) exerts anti-inflammatory and anti-cancer effects through modulating the cyclooxygenase-2 (COX-2) in a mouse colorectal cancer model

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Background: Cyclooxygenase-2 (COX-2), a pro-inflammatory enzyme involved in prostaglandin synthesis, has been found to be overexpressed in colorectal cancer (CRC) and plays critical roles in CRC cell proliferation, and metastasis as well as CRC -related inflammatory processes. Hence, COX-2 must be precisely regulated; otherwise, it would contribute to more aggressive behavior of CRC. Recently, the anticancer and anti-inflammatory effects of Bee venom (BV) have been investigated in different tumor types. Here, we explored the modulatory effect of bee venom on COX-2 as well as other inflammatory mediators in a CRC mouse model.

Methods: Herein, we injected subcutaneously 4×10^5 CT-26 cells to female BALB/c mice, and then randomly divided into 3 groups. The control group (n=6) received no treatment, while the 5-FU group (n=6) received 3 mg/kg 5-FU once a day for 2 weeks, and BV group (n=6) received 3 mg/kg BV once a day for 2 weeks. Subsequently, mice were sacrificed and the mRNA expression levels of COX-2 and pro-inflammatory cytokines (TNF-α and IL-1β) in the tumor tissue were evaluated by quantitative real-time PCR (qRT-PCR).

Results: The results showed that BV reduced the Cox-2, TNF-α, and IL-1β mRNA expression and significantly suppressed tumor growth in tumor-bearing mice.

Conclusion: Our findings support the anti-inflammatory and anti-cancer effects of Bee venom in CRC, however, further in vivo and clinical studies are required to determine the exact molecular mechanism as well as the efficacy and safety of BV in CRC patients.

Keywords: Bee venom, COX-2, Colorectal cancer

P-359

Microalgae: A green and cost-effective alternative for removal of micro- and nanoplastic contamination

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Background: Due to the mass production and widespread use of plastics, these compounds are found in large quantities on a macro, micro, and nanoscale in freshwater, oceans, and sediments. Plastics pollution has become one of the biggest environmental problems in recent decades. To deal with these problem, extensive studies are being conducted to investigate the interactions between micro/nanoplastics and organisms in aquatic environments.

Methods: Two databases including Scopus and Web of Science were used to search for finding the suitable literature. The main focus of this study was on case report and review articles from 2016 to 2020.

Results: Up to now, most studies have been performed on microalgae as a model with characteristics including rapid growth, environmentally friendly, easy to use and high biomass production. In these studies, the effect of factors including concentration, type, size, surface chemistry and charge of micro/nanoplastics on different microalgae was investigated. Micro/nanoplastics cause growth inhibition, chlorophyll and photosynthesis reduction, induction of oxidative stress and morphological changes in microalgae. Some reports suggest that microalgae are capable of adapting to these conditions. Some studies also suggest that extracellular polymeric substances (EPSs) produced by microalgae are able to adsorb and aggregate micro/nanoplastics from aquatic environments. These results were confirmed by analyzing the SEM and TEM images.

Conclusion: In the light of the results of various studies, scientists claim that microalgae can be a promising bio-solution to remove micro/nanoplastics from aquatic environments and wastewater. Obviously, to achieve the goal, more studies are needed, including the study of interactions between micro/nanoplastics and microalgae metabolism in order to increase and improve the activity of microalgae in the bio-solution optimization process.

Keywords: Microplastics, Nanoplastics, Microalgae, Extracellular Polymeric Substances (EPSs)

P-360

Evaluation of inhibitory properties of EBR-derived chimeric peptides from human and rat in the presence of 17-beta-estradiol on ZR-75-1 cancer cells on in vitro

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Background: Breast cancer is one of the leading causes of cancer death in women. Many breast cancer cells including Zr-75-1 contain estradiol receptors (ER +) and proliferate in the presence of this hormone. HMI chimeric peptides of human and rat estradiol binding regions (EBR) have the ability of binding to 17 beta-estradiol hormone and can be a good candidate for inhibition of their growth. Chimeric peptides HMI, HMJ, HAFP and RFP were tested in different concentrations on the Zr-75-1 cells in vitro using MTT assay.

Methods: ZR-75-1 cells were cultured in RPMI medium containing 10% FBS, 1% penicillin and streptomycin, and incubated at 37 ° C with 95% humidity, and 5% CO_2. The cell medium was changed every 48 hours using 1 ml of 0.25 % trypsin. After obtaining appropriate cells, MTT test was performed on 96-well microplates, each well containing 5000 cells and 180 µL of culture medium. After 24 h of incubation, 8 replicates of 20 µL of different concentrations of peptides with 1 nM estradiol were added to each well. On the 7th day 10 µL of MTT (tetrazolium) solution was added and incubated for 4 h at 37 °C and 100 µL DMSO was added and shaken for 30 minutes and the results were read by microplate spectroscopy.

Results: The HMI mutant in the concentration of 10-8 M bound to 17 beta estradiol and inhibited growth of ZR-75-1 CELS.

Conclusion: Recombinant HM-I chimeric peptide, which includes human and rat EBR, binds to estradiol more strongly than human EBR and inhibits the growth of ZR-75-1 cells at a concentration of 1 nM estradiol. It can be concluded that HM-I inhibits the growth of estradiol-dependent ZR-75-1 breast cancer cells in vitro.

Keywords: Breast cancer, Zr-75-1 cells, HMI chimeric peptides, Estradiol

P-361

An overview of the diagnostic applications of nanostructures

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Background: Diagnostic tests play an important role in consumer health. Early diagnosis and rapid monitoring can have a significant impact on patient recovery and reduce health care costs. For example, despite recent advances in cancer treatment, many cancers are diagnosed only after they have metastasized in the body. In addition, in the case of infectious diseases, rapid identification of the infectious agent prevents the spread of the disease and helps the person to be treated properly. Therefore, to enhance the performance of diagnostic techniques and to develop innovative strategies to meet the challenges, a continuous effort is required.

Methods: In this paper, an overview of the diagnostic applications of nanoparticles is provided. In addition, challenges and future developments that may lead to diagnostic tools and techniques are also discussed. For this purpose, the articles that published between 2000 and 2020 from various databases such as PubMed, Google Scholar, Elsevier, Science Direct were investigated.

Results: Nanotechnology is one of the areas that is being developed and offers new materials with unique physical and chemical properties that can be used in diagnostic and therapeutic applications. Quantum dots (QDs), gold nanoparticles (AuNPs), magnetic nanoparticles, nanotubes, nanowires, and multi-purpose nanomaterials are the most promising nanostructures for laboratory diagnostic applications. The use of nanotechnology in diagnostics has several advantages such as the need for low sample size, harmlessness, high speed, extraordinary sensitivity and point-of-care use.

Conclusion Nano-diagnostics is an important component of nanomedicine. They provide an opportunity for diagnosis and treatment. Further research is needed to optimize the use of nanotechnology products for clinical diagnosis and to address some concerns about the potential health and environmental hazards. Recent advances in nanotechnology will open new frontiers for the diagnosis and treatment of diseases.

Keywords: Nanotechnology, nano-diagnostics, nanomedicine, nanomaterials, imaging applications, Molecular diagnostics, Clinical laboratory, point-of-care

P-362

Designing a secretory form of recombinant Granulocyte colony-stimulating factor: an in silico approach

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Background: Recombinant Granulocyte colony-stimulating (G-CSF) factor which is produced in Escherichia coli can be applied for improvement of neutropenia after chemotherapy. Inclusion bodies formation is a bottleneck in producing active proteins and recovery process increases the costs. The best solution is producing secretory soluble proteins. Choosing suitable signal peptides (SPs) is the main factor in producing secretory proteins. In this work, we evaluated many signal peptides via bioinformatics to find the most appropriate signal peptides for secretory production of G-CSF.

Methods: The amino acid sequences of SPs and G-CSF were collected from Signal peptide website and UniProt. Presence of SPs and their cleavage site were predicted by SignalP. ProtParam and Protein-sol were applied to evaluate physicochemical features and solubility. Sub-cellular localization of SPs-protein chimeras was investigated by ProtCompB.

Results: SignalP rejected PET, ELBH, HST3, AIDA, TSH, LPP, and ELBP. THIB, PPA, TORT, and OMPC had most aliphatic index. It is found that THIB, PSPE, BLAT, TORT, ELAP, and OMPC had the highest GRAVYs. Moreover, all chimeric proteins which consist of SPs and G-CSF were soluble except three of them. Lastly, it is indicated that OMPT, OMPF, PHOE, LAMB, SAT, and OMPP could help to secret G-CSF from inside of bacteria.

Conclusion: Bioinformatics can open a new horizon in designing novel chimeric proteins with desirable features. In this study, it is indicated that six SPs could be appropriate for secretory production of G-CSF which can be evaluated in experimental researches to find the best.

Keywords: Signal peptide, Escherichia coli, Bioinformatics

P-363

Spectroscopic analysis of the interaction between Co3O4 nanoparticles and acid phosphatase

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Background: In this study, the structure and stability of acid phosphatase in its interaction with Co3O4 nanoparticles were evaluated.

Methods: Absorbance, enzyme activity assay, fluorescence, thermal stability, and circular dichroism spectroscopy were used for the purpose of the study.

Results and Conclusion: It was found that with the enhancement of Co3O4 nanoparticles concentration, the intensity of the enzyme's ultraviolet spectrum was increasing. In fact, under these conditions (pH=4.8, T=310 K), the activity of acid phosphatase was raised too. Also, it was revealed that by enhancing the concentration of the nanoparticles, the enzyme thermal stability increased from 342.0 to 346.0 K. Also, Far-UV CD investigations illustrated that the Co3O4 nanoparticles could alter the secondary structure of acid phosphatase through an increase in the value of the α-helix structure (from %10.8 to %13.9) and a decrease in the β-sheet (from %30.2 to %28.0). By raising the temperature from 298 to 308 K, the Stern-Volmer constant decreased from 5.72*10^4 to 4.39*10^4 M^-1. Also, it was found that Co3O4 nanoparticles quenched the intrinsic fluorescence of acid phosphatase by the static quenching mechanism. Therefore, the thermodynamic parameters showed that the binding process was spontaneous because the value of ΔG was negative. Also, van der Waals forces and hydrogen bonding interactions had the main effects on the interaction of Co3O4 nanoparticles with acid phosphatase because the values of ΔH and ΔS were negative. So, Co3O4 nanoparticles increased the stability and activity of acid phosphatase.

Keywords: Acid phosphatase, Nanostructures, Circular dichroism, Fluorescence analysis, Thermal stability, Ultraviolet spectra

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P-364

Severe hyperhomocysteinemia in MTHFR defect, clinical deterioration despite biochemical control

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Background: Methylenetetrahydrofolate reductase (MTHFR) and molybdenum-containing enzyme sulfite oxidase are both enzymes involved in sulfur amino acid metabolism, which defects causes epileptic encephalopathy, progressive psychomotor retardation and severe microcephaly.

Case Study*:* This is a descriptive study on clinical features, treatment follow up, molecular data and outcome of a neonate affected of hyperhomocysteinemia. Homocystein level was measured by highly performance liquid chromatography (HPLC).Genetic was done with next generation sequencing (NGS) and confirmed by Sanger sequencing.

Result*:* A normal term girl newborn, body weight: 3700 grams, head circumference: 35 centimeters, second child of a consanguineous couple whose first deceased at 23rd days of age without diagnosis was admitted electively to neonatal intensive care unit for supervision. Breast feeding was started, discharged at 7th days, asymptomatic, normal first laboratory tests but readmitted at 9th days for somnolence and poor feeding when neonatal metabolic screening test results showed homocystein and methionine :251 (NL:5-10)and 5.9(NL:8-100)µmol/L respectively. Targeted NGS panel for homocyseinemia detected homozygote mutation c.547C>T in exon 4 of MTHFR gene .During follow up after standard homocysteinemia treatment ,severe hydrocephaly leading to bilateral ptosis occurred at 3 months, subsequently skull suture closed at 6 months ,intractable seizure started at7 months despite homocystein level :16 µmol/L .She unfortunately died at 8.5 months body weight:8500 grams, head circumference:39 centimeters ,severely retarded. Whole exome sequencing result, ready later, revealed homozygote mutation c.1064C>T in exon8 of MOCS1 gene known to cause molybdenum cofactor deficiency besides previously mutation found in MTHFR gene.

Conclusion*:* Severe hyperhomocysteinemia can be due to molybdenum cofactor deficiency and whole exome sequencing is a better NGS method for genetic confirmation.

Keywords: homocysteinemia, seizure, MTHFD, MOSC1

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IL-6 gene expression in diabetes-related depression in patient with type 2 diabetes

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Background: Type 2 diabetes is a metabolic disorder which causes hyperglycemia. Increased inflammatory cytokines such as IL-6 can be associated with incidence of depression and type -2 diabetes. Therefore, this study investigated the expression of IL-6 gene in diabetes-related depression in patients with type 2 diabetes.

Methods: This study was performed on 50 patients with type 2 diabetes mellitus with depression and 50 patients with type 2 diabetes but without depression as control group. RNA extraction from blood was performed by Yekta tajhiz kit and finally gene expression assay was performed quantitatively using real-time RT-PCR.

Results: Interleukin 6 gene expression was decreased in patients with type 2 diabetes with depression compared to those with type 2 diabetes without depression, which was not statistically significant.

Conclusion: The present study showed that decreased levels of interleukin-6 gene expression are not associated with post-diabetes depression. Therefore, more research is needed in this Field.

Keywords: Gene Expression, IL-6, Diabetes Type 2, Depression

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Evaluation of the Effect of Nanoparticles Containing Naringin on the Anti-apoptotic BCL2 Protein Expression in 4T1 Cell line

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Background: Flavanone naringenin and the disaccharide Neohesperidose make the formation of naringin. One of the most important secondary metabolites and sources of bioactive compounds in plants are flavonoids, which have antioxidant, anti-inflammatory, anti-ulcer, anti-osteoporosis, and anti-cancer properties. Low aqueous solubility is the major problem encountered in the formulation development of naringin. Various techniques are used for the enhancement of the solubility of poorly soluble compounds which includes the physical and chemical modifications of the compounds. Solid Lipid Nanoparticles (SLNs) is one of the new lipid carriers for targeted pharmacy, for the treatment of breast cancer. The aim of the present study was to prepare and optimize naringin -loaded SLNs and explore the enhanced anti-cancer activity against 4T1 breast cancer cell line.

Methods: Naringin -loaded SLNs were prepared by hot homogenization technique. Cytotoxicity of naringin and naringin -loaded SLNs on breast cancer cell line (4T1) was evaluated by MTT assay. The cells were treated with IC50 concentrations of naringin and equivalent doses of naringin -loaded SLNs for 24 hours. The expression of anti-apoptotic Bcl-2, which is involved in apoptosis, were evaluated by Western blotting.

Results: The nanoparticles with appropriate characteristics (particle size of 84 nm and Encapsulation Efficiency of 91%) were prepared. Cytotoxicity evaluations demonstrated that naringin -loaded SLNs prevented breast cancer cells growth in a low IC50 value compared to the naringin. The data showed that the treatment of 4T1 cells with 2 µM naringin and 7 µg/ml naringin -loaded SLNs caused 0.2 and 0. 48 fold decreases in the expression level of anti-apoptotic Bcl-2, respectively.

Conclusion: SLN has the potential to inhibit the growth and proliferation of 4T1 breast cancer cell lines and in turn, induce apoptosis by reducing the expression of the anti-apoptotic protein BCL2.

Keywords: Naringin, 4T1 Cell line, Breast Cancer, SLN, Bcl-2

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Different expression patterns of p27 and Ep-CAM in Cancer Primary Epithelial Cells Isolated from Iranian Women Breast tumors after treatment with crocin: An experimental evidence for crocin application in personalized medicine

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Background: Crocin, as the main carotenoid of saffron, is useful for cancer prevention and therapy. DNA mutation changes can be used as a basis for personalized treatment.

Methods: Here we isolated normal and cancerous human epithelial cells from the mammoplasty and human breast tumors respectively; and characterized by flow cytometry using antibodies against ESA, CD44, CD24 and CD49f cell surface receptors. Then, the cells were exposed to different concentrations of crocin at various time intervals and some parameters were determined.

Results: The half inhibitory concentrations (IC50) of crocin in five isolated breast cancer cells were between 2.56 to 4.6 mM, as determined by MTT assay, while there was no effect on normal breast epithelial cells. Flow cytometric analysis and caspase-9 expression indicated crocin treatment induced apoptosis in the cancerous epithelial cells. Crocin increased the levels of spliced XBP-1; accumulation of LC3-II and ungeranylgeranylated-Rap-1α and down regulated CXCR-4 and Lamin B protein in all cancer cells, but in different degrees. Crocin also changed p27 expression in both mRNA and protein levels. Anti-proliferative action of crocin might be affected by HER-2 overexpression that decreases stability of p27 in cancer cells. These results are compatible with our previous data about the changes in the cyclin D1-p21-p53 signaling pathway in the NMU-induced breast cancer in rat under the effect of crocin treatment. Furthermore, according to the pathologic features, higher EpCAM expression in response to crocin showed significant associations with low grade (II) of specimens that cancer cells isolated from.

Conclusion: It seems that the effect of crocin specifically depends on the proteins that are involved in proliferation, differentiation and grade of the tumor. This finding can be used as a novel therapeutic approach of crocin application in clinical trials and provides personalized therapeutic options for individual patients where standard clinical options have exhausted.

Keywords: Breast Cancer, Crocin, Saffron, Personalized therapy, p27, EpCAM.

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Flavonoid-based compounds as potential inhibitors against the major protease of coronavirus

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Background: Severe Acute Respiratory Syndrome Coronavirus-2 has evolved from unknown hostility to global anxiety for several months. Virologists are focusing on making a vaccine for the virus, and others are looking for a drug to treat the disease. Although there is no approved drug with effective efficacy for the treatment of coronavirus, there are potential targets for the production of new drugs. The requirements of protease inhibitors as drugs in terms of potency, pharmacokinetics, and toxicity will vary depending on the nature of the infection and the goals of therapy. 3CL protease as a main protease (Mpro) of coronavirus can be a potential target for drug design.

Methods: In this study, antiviral design focused on inhibitory effect of flavonoid based compounds on active site of Mpro enzyme using Molecular Docking Simulation. The docking analyses were performed using Molegro Virtial Docker.

Results: Some antiviral drugs inhibit the viral replication through interaction with enzymes. Conformation of docked flavonoid-based compounds and FDA-approved drugs with Mpro enzyme was analyzed in terms of Energy and MolDock Score. The values of MolDock Score were -150.607, -101.836, -79.8993, -78.0685, -97.7445, -79.5275, -107.852 and -127.153 for Rutin, Acteoside, Apigenin, Daidzein, Kaempferol, Naringenin, Quercetin and Quercetin-3-O docked to Mpro enzyme, respectively. MolDock Score for Camostate, Cobicistat, Darunavir, Favipiravir, Lopinavir, 2-Iodoadenosine-5′-O-[(phosphonomethyl)phosphonic Acid] and Ritonavir interacted with Mpro enzyme was -109.258, 468.445, -101, -79.3217, 274.189, -143.772 and 180.589 respectively.

Conclusion: The computational studies on 16 flavonoid-based compounds and FDA-approved drugs exhibited that herbal compounds have a higher binding affinity than chemical compounds as anti-protease agents. The calculated ligand energy and MolDock Score of investigated structures exhibited that three herbal-based and two chemical-based compounds showed more reactivity on protease.

Keywords: Molecular Docking, COVID-19, Coronavirus, Flavonoids, Protease Inhibitor.

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The increased level of COX-2 was associated with tumor size and invasion in patients with hormone-secreting Pituitary Adenoma

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Background: Pituitary adenomas as multifactorial intracranial neoplasms impose a massive burden of morbidity on patients and characterizing the molecular mechanism underlying their pathogenesis has received considerable attention. Despite the appealing role of cyclooxygenase enzymes and their bioactive lipid products in cancer pathogenesis, their relevance to pituitary adenoma pathogenesis is debated and yet to be determined. Thus, the current study perused this relevance.

Methods: In this case-control study, 50 patients with Functional hormone secreting pituitary adenomas who were referred to the Firouzgar Hospital in Tehran were participated. Tumor tissues were used to extract mRNA and cDNA, and to determine the gene expression of COX-2, the Real-Time PCR-based SYBR Green method was used. The correlation of COX-2 with patient clinical and pathophysiologyical features were evaluated. Finally, statistical analysis was performed using version 6 of GraphPad Prism software and independent t-test.

Results: The results of the current study demonstrated that COX-2 expression level was increased in pituitary tumors including acromegaly, Cushing’s disease and prolactinoma compared with normal pituitary tissues. Furthermore, the COX-2 expression level was significantly increased in macroadenoma and invasive tumors. The diagnostic value of COX-2 isoform was considerable between patients and healthy groups. COX-2 revealed more value in distinguishing endocrinologically active and non-active pituitary tumors.

Conclusion: Data from the current study provides the expression pattern of COX-2 in prevalent pituitary tumors and their association with patients’ clinical features which may open up new molecular targets for early diagnosis/follow up of pituitary tumor growth.

Keywords: Cox-2, functional pituitary adenoma, tumor size, tumor invasion, cancer.

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Evaluation of salivary enzymatic and non-enzymatic antioxidant markers in chronic periodontitis.

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Background: Periodontal disease is a chronic inflammatory disease of the oral cavity affecting the supporting structures of the dentition. Periodontal tissue and gingival sulcus which are responsible for high level of lysosomal enzymes generation of superoxide and reactive oxygen species (ROS) in periodontitis. The imbalance between ROS production and antioxidant substances can directly cause tissue damage. Saliva contains enzymatic and non-enzymatic antioxidant such as superoxide dismutase (SOD) and uric acid (UA). Purpose of this study was to investigate the association between periodontitis and the levels of biochemical markers as well as enzyme activity.

Methods: Unstimulated whole saliva samples were obtained from 30 patients with periodontitis and 30 healthy subjects. Biochemical factors including the levels of uric acid (UA) and superoxide dismutase (SOD) activity were measured.

Results: SOD levels was higher in the cases (median = 46.25 µM) compared to the controls (median = 32.60 µM) ($P < 0.0001$). Uric acid concentrations showed significantly lower levels in patients (median = 2.5 and 3.8 µM, respectively; $P = 0.0003$).

Conclusion: Antioxidant are locally release at sites of inflammation by inflammatory cells, and protect tissues against ROS. Higher SOD activity was found in the saliva of patients with periodontal disease with the progression of inflammation. Uric acid, as a non-enzymatic antioxidant, was decreased in the saliva of periodontitis patients. Since altered levels of salivary antioxidant substances might contribute in the systemic and local complications in the patients, these informative antioxidants can be used as a promising factor for the early diagnosis of the disease.

Keywords: Periodontal disease, salivary biomarkers, Oxidative stress, Antioxidant

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Evaluation of salivary protein β-HEX and lactoferrin in chronic periodontitis.

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Background: Periodontitis is a multifactorial chronic inflammatory disease, affecting the supporting structures of dentition. Innate immune cells such as neutrophils are stimulated by infection in an inflammatory chronic periodontitis (CP) which release various types of salivary protein markers. Enhanced levels of oxidative stress and defensive enzymes contribute to the pathogenesis of periodontitis. Enzymatic (B-hexosaminidase) and nonenzymatic (lactoferrin) salivary protein markers have been identified in periodontitis. Aim of this study was to investigate the association between periodontitis and levels of salivary protein markers.

Methods: Unstimulated whole saliva samples were obtained from 30 patients with chronic periodontitis and 30 healthy individuals (30-60 years of age) and were included in this study case-control. Biochemical factors including the levels of lactoferrin and β- hexosaminidase (β-HEX) activity were measured by ELISA.

Results: Concentrations of lactoferrin (median = 202 vs. 122 ng/ml) (P = 0.0006) as well as β-HEX activity (median = 202 vs. 122 IU/L) (P = 0.0001) were remarkably higher in the case group, compared to the control group.

Conclusions: Our observations revealed that lactoferrin and β-HEX levels in saliva samples of patients with CP were statistically higher than healthy controls and these findings corresponded with previous results. β-HEX is the most active exoglycosidase that degrades gingival tissue glycoconjugates and various studies have shown a reduction in the activity of this enzyme following periodontitis treatment. Lactoferrin, a promising non-enzymatic marker for periodontal diseases, is an iron-binding glycoprotein in saliva which has an important defense role against periodontopathic bacteria.

Keywords: Periodontal disease, salivary protein, Oxidative stress, Antioxidant

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Comparison of Salivary MDA and NO in Patients with Chronic Periodontitis and Healthy Subjects.

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Background: Periodontal disease is initiated by a complex interaction between pathogenic periodontal microorganisms and inappropriate host immune to the destruction of teeth supporting tissues and ends up with tooth loss. Oxidative stress has been implicated as a major contributor in chronic periodontitis (CP) and therefore, there is a linkage between periodontal disease and elevated levels of oxidant biomarkers including malondialdehyde (MDA) and nitric oxide (NO). This study was designed to examine the levels of MDA and NO in Iranian patients with periodontal disease compared with healthy individuals.

Methods: The study population included 30 healthy individuals and 30 patients with CP (30-60 years). Unstimulated saliva samples were collected based on Navazesh method. The levels of MDA and NO were measured by ELISA.

Results: MDA was significantly higher in patients (median = 3.6 µM) as compared with healthy controls (median = 1.3 µM) ($P = 0.006$). The levels of NO were also elevated in periodontitis patients than controls (median = 39 and 33 µM, respectively) ($P = 0.023$).

Conclusions: The results of our study indicated that the markers of oxidative stress such as MDA and NO concentrations were significantly higher in the saliva of patients with periodontitis than healthy individuals. Our results are inconsistent with results of previous studies in Iranian and non-Iranian population. Although a significant increase has been shown in the levels of serum MDA in periodontitis. Increased level of lipid peroxidation status likely plays an important role in the pathogenesis of CP and is closely associated with the clinical status of patients with the disease.

Keywords: Periodontal disease, MDA, NO, Oxidative stress, Antioxidant

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Designing a new immunotoxin against breast cancer: a computational approach

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Background: Breast cancer is the most prevalent cancer among women worldwide and nearly 15-20% of breast cancer patients are HER2 positive. Traditional cancer therapy methods such as surgery, radiation, and chemotherapy are not effective enough to decrease mortality rates. Immunotherapy is a new approach which is more efficient and has less side effects. We designed a new immunotoxin which includes anti-HER2 “trastuzumab” derived scFv connecting to active domain of Campylobacter jejuni cytolethal distending toxin (Cj-CdtB).

Methods: ProtParam, PROSO II and GORV were utilized to analyze physico-chemical properties, solubility and secondary structure of the immunotoxin, respectively. I-TASSER and GalaxyRefine were employed for building and refinement of 3D model. The structures of primary and refined models were assessed by PROCHECK and RAMPAGE. The allergenicity of immunotoxin was predicted by AlgPred server. The free energy of related mRNA was analyzed via RNAfold webserver. Finally, the 3D model of immunotoxin was docked to Her2 receptor.

Results: The designed immunotoxin was a stable and soluble protein which had appropriate secondary and tertiary structures. The protein was not allergen for human body and had a stable mRNA. The immunotoxin can successfully bind to the receptor**.**

Conclusion: The computational designed immunotoxin showed appropriate features in silico but it should be produced and analyzed in experimental research.

Keywords: Bioinformatics, Immunotoxin, Breast cancer

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Human amniotic fluid mesenchymal stem cell condition medium inhibits VEGF as angiogenesis factor in breast cancer cell line (MCF-7)

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Background: Breast cancer is one of the most common cancers in women and the leading mortality in the world. Stem cell-based therapeutic strategies exhibit therapeutic potential for the treatment of numerous human disease, such as various cancer types. Moreover, using stem cell-derived conditioned medium instead of stem cells provides highly advantages including simple to prepare, easy to store for a long term and deliver for the patients. Angiogenesis is a process in which the new blood vessels are formed from existing vessels in both physiological and pathological conditions. The inhibition of tumor angiogenesis may suppress tumor development. Vascular endothelial growth factor (VEGF) has been found as a vital modulator of the angiogenesis. Therefore, inhibition of the VEGF is a key approach in tumor therapy.

Methods: In this study condition medium (CM) was provided from human mesenchymal stem cell derived from amniotic fluid stem cells and cultured in DMEM-L without FBS. Human breast cancer cell line (MCF-7) was treated with CM for 24 hours and cells' viability was measured using MTT assay. The expression of VEGF was evaluated by Real Time PCR.

Results: After 24 hours' exposure to CM, viability of MCF-7 cells were significantly suppressed at dose of 80% compared with control. In addition, mRNA expression of VEGF in CM-treated MCF-7 cells was decreased significantly (P value < 0.05) after 24 hours.

Conclusion: Human amniotic fluid stem cell-derived conditioned medium inhibits the growth of breast cancer by reducing VEGF production and angiogenesis, which could be a promising candidate for anti-angiogenesis treatment in breast cancer.

Keywords: Stem cell, Condition media, Breast cancer, Vascular endothelial growth factor (VEGF)

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Comparison of blood glucose measurement with glucometer and standard laboratory method

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Background: One of the simplest and available sugar blood measuring devices, after diagnosis of diabetes for prevention of its side effects is measuring sugar blood at home. In this research, the sugar blood sampled from vein and tip of the finger measured by ysi device (which is capable of measuring capillary blood) and a reference out analyzer (biolis 24) were compared; due to the difference between oxygen blood pressure of vein and tip of the finger.

Methods: This research was done on 180 patients who were fasting, at Endocrine and Metabolism institute. At first their hands were washed for preventing false results. Then their hands were held down for better blood flow. The blood sample of the tip of the finger was measured by glucard. Capillary blood sample was taken simultaneous and sugar blood was measured by ysi device. Also glucose of vein blood sample was measured by a reference auto analyzer (biolis 24) and glucard. The results were analyzed by spss software.

Results: Glucard device used at home has to be used temporary. It is suggested to measure blood glucose every month at laboratory.

Conclusion: There were 180 patients including 48 males and 132 females. The mean and standard deviation of patient’s age were 17.2±59.1. The difference between mean glucose of vein blood measured by biolis and glucard was significant at $p<0.01$. The difference between glucose of top of the finger measured by glucard and ysi was not significant $p< 0.6$. Tip of the finger blood glucose measured by biolis was $p<0.5$ and by ysi was $p<0.6$. vein blood glucose measured by biolis was $p<0.01$.

Keywords: blood glucose, glucometer,laboratory method

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Comparison of six formulas for estimating LDL-C in Iranian individuals

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Background: High cholesterol and lipids, have caused heart diseases in the recent years. Direct measurement of LDL-C is appropriate but costly. In most of the laboratories, computational methods are being used but they are affected by many important factors like high triglyceride, fasting and being affected by coefficient of variation of other factors. This study wass done for comparison of LDL-C level measured by enzymatic method and computational methods like Fridewald, Chen, Anandaraja, Hattori, Cordova, Ahmadi.

Methods: In this study there were 108 fasting serum samples, including 51 males and 57 females; for measuring LDL-C level with both enzymatic and computational methods. The studying society were all diabetics whose triglyceride level was less than 400 mg/dl. The LDL-C levels were obtained by computational methods and were compared to results of enzymatic method by SPSS software.

Results: The comparison between LDL-C level of enzymatic and computational method showed that Cordova method had more correlation compared to other methods ($p< 0.781$). Computational methods caused pre-estimation of LDL-C level ($p<0.001$)

Conclusion: According to importance of LDL-C level in vascular diseases, it is suggested to use enzymatic method for preventing interference and due to Cordova formula, it is suggested to do this study on more samples.

Keywords: LDL-C,Fridewald, Chen, Anandaraja, Hattori, Cordova, Ahmadi.

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Genetic Association of PNPLA3 rs738409 C>G Polymorphism with Non-alcoholic Fatty Liver Disease in Iranian Population: A Case-Control Study

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Background: The prevalence of non-alcoholic fatty liver disease (NAFLD) is widely increasing all over the world. Lots of efforts have been shaped in previous studies focusing on the different population rates related to the single nucleotide polymorphism that was associated with liver fat content, known as Patatin-like phospholipase domain-containing protein 3 (PNPLA3 rs738409). The present study investigates the association between PNPLA3 rs738409 polymorphism and NAFLD among the Iranian population.

Methods: Genomic DNA was extracted from the blood of 53 NAFLD patients and 107 healthy subjects with normal liver ultrasound. PNPLA3 rs738409 was genotyped by PCR-RFLP method. During laboratory experiments, fasting blood sugar (FBS), triglyceride, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured. The Chi-square ($\chi 2$), one-way Analysis of Variance (ANOVA), Kruskal-Wallis, and Mann-Whitney U tests were implemented to analyze the obtained data by using SPSS software version 18.0.

Results: The frequencies of G allele were 56% and 36%, and for the genotypes were obtained 35.8 and 47.7 % (CC), 17 and 31.8% (CG), 47.2, and 20.6% (GG) in patients and control groups, respectively. The ORs for PNPLA3 rs738409 C>G were 3.11 (P= 0.004) and 0.7(P = 0.52) for GG and CG genotypes, respectively. The levels of triglyceride were significantly higher in subjects carrying the G allele compared to CC genotype (P=0.001). Moreover, there were significant differences between the serum AST and ALT levels between three genotypes (P=0.006 and 0.005, respectively). No significant differences in serum levels of LDL, HDL, FBS, and BMI were observed between CG, GG genotypes, and those with the CC genotype.

Conclusion: The study findings suggest that the PNPLA3 rs738409 single nucleotide polymorphism can be utilized as a diagnostic and prognostic factor for early detection and prevention of the high-risk individuals suffering from the NAFLD.

Keywords: Nonalcoholic Fatty Liver, Adiponutrin, PNPLA3 protein

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The role of TRIM21 in the breakdown of target proteins

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Background: TRIM21 is a member of the tripartite motif Trim family. The TRIM motif includes three zinc-binding domains, a RING finger domain, a B-box type 1 and a B-box type 2 zinc finger, and a coiled-coil region. Due to the complex nature of TRIM proteins, they are involved in a variety of cellular functions and biological processes, including regulation of cell proliferation, cell division and growth processes, cancer transformation, regulation of cell metabolism, chromatin status modification, genotyping regulation, post-translational changes, and interactions with pathogens. TRIM21 can also be used to kill specific proteins with the corresponding antibodies, a method known as Trim-Away. In this method, a specific antibody binds to TRIM21 on one hand with the FC domain, and on the other hand, the target protein is attached to the antibody inside the cell by the fab region, then the whole set goes to a protein mill called Proteasome. This protein will be broken down into amino acid blocks.

Methods: In this review study, searches were conducted in the electronic and scientific databases of PubMed, Medline, Google Scholar, Scopus, and ISI and valid articles related to the subject were searched using the keywords TRIM21 and antibodies.

Results: Certainly, using TRIM21 in treatment and research, an important revolution in the field of medical science and cellular and molecular biology can be achieved.

Conclusion: Further knowledge of the morphology and physiology of the function of this protein leads to an important step in creating new and more effective experiments. Also, due to the role of TRIM21 in the identification of other target proteins by antibodies, it can be used in research and treatment of other emerging infectious diseases.

Keywords: Keywords: TRIM21, Trim-Away, antibody, Proteasome

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Type2 diabetes mellitus prediction based on the long non-coding RNAs expression

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Background: About 90% of diabetic patients suffer from T2DM. Many research revealed the significant role of lncRNAs in diagnosis of T2DM. Machine learning and Data Mining techniques are tools that can improve the analysis and interpretation or extraction of knowledge from the data. These techniques may improve the prognosis and diagnosis associated with reducing diseases such as T2DM. We applied three classification models, including K-nearest-neighbor (KNN), support-vector-machine (SVM), logistic regression for detecting T2DM. The algorithms were performed on six LncRNA variables.

Methods: The variables were used for predicting T2DM and comparing the performance of the various data mining techniques. Because our samples were more than 100, we used the stratified 10-fold cross-validation approach. Consequently, the results are reliable and more credible. We applied three data mining approaches on the lncRNA variables (Regression, KNN, and SVM).

Results: To select the best performance, we considered the AUC, sensitivity, specificity, plotted the ROC curve, and showed the average curve and range. The mean AUC for the KNN algorithm was 91% with 0.09 standard deviation (SD); sensitivity=96% and specificity=85%. After applying the SVM algorithm, the mean AUC obtained 95% after stratified 10-fold cross-validation, and the SD obtained 0.05; sensitivity=95% and specificity=86%. At last, for the logistic regression algorithm, results showed 95% of mean AUC, and the SD of 0.05; sensitivity=92% and specificity=85%. According to the ROCs, the Logistic Regression and SVM had a better area under the curve compared to the others.

Conclusion: According to the finding, the maximum AUC dedicated to SVM and logistic regression, among others, KNN also had the high mean AUC and small standard deviations of AUC scores among the approaches, KNN had the highest mean sensitivity and the highest specificity belonged to SVM. The results of this study could improve our knowledge about the early detection and diagnosis of T2DM using the lncRNAs as biomarkers.

Keywords: Data mining, Gene expression, Machine learning algorithms, Type 2 diabetes mellitus

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Enzymatic activity, structural study and molecular docking of Catalase after Natural Orange 6 derivatives exposure

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Background: Natural Orange 6, as a naphthoquinone derivative, has been reported to have a wide range of biological and pharmacological applications, containing anti-cancer and anti-bacterial property. On the other hand, conformational and functional alterations of bovine liver Catalase (BLC), as a major protecting enzyme under the effect of different compounds have been received considerable attention.

Methods: In the present study, the interaction between two different derivatives of Natural Orange 6 (BNQ and ANQ) and catalase were evaluated utilizing ultraviolet-visible (UV-vis) absorption, ATR-FTIR spectroscopy and fluorescence as a combined experimental method and molecular docking studies as a theoretical technique.

Results: The reaction kinetics evaluations demonstrated that additional concentrations of BNQ and ANQ resulted in remarkable decrease in catalase activity through mixed and competitive inhibition mechanisms, respectively. Furthermore, UV–vis, FTIR and fluorescence spectroscopic data represented notable change in secondary structure of the enzyme in the presence of both BNQ and ANQ. Also, the fluorescence quenching measurements at two different temperatures illustrated a gradual reduction in the intrinsic fluorescence emission of BLC by a static quenching mechanism. Molecular docking data in agreement with experimental results, verified that both BNQ and ANQ have only one binding site on the BLC conformation.

Conclusion: Study on the effect of different compounds on the activity and structure of the catalase as a principal biological enzyme could be important. Excessive utilization of these compounds in various drugs can cause side effects and may threaten human health.

Keywords: Bovine liver catalase, Natural Orange 6, mixed inhibition, competitive inhibition

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Intensification of the inhibitory effect of Orange Yellow S on catalase activity in the presence of curcumin: An experimental and MLSD simulation approach

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Background: Over the years, food dyes have been widely used to enhance the aesthetic appeal of food products. Orange Yellow S (OYS), as a synthetic food colorant, is one of the commonly used food additives due to its high stability. Also Curcumin (CUR), as an extensively utilized flavor have been received considerable attention as an important antioxidant compound.

Methods: This work, were investigated the combination efficacies of mixed food additives OYS and CUR through spectroscopic studies and MLSD simulation. Therefore, the stability and reversibility of SNY- catalase complex were evaluated under the effect of CUR through equilibrium dialysis system using UV-spectroscopy method. To investigate the binding site of SNY and CUR on catalase structure and the effects of CUR on the affinity of SNY-catalase complex the MLSD simulation were used.

Results: It is previously introduced that OYS and CUR decrease and enhances the free catalase activity, respectively. The equilibrium dialysis method and releasing rate of SNY from the complex in the absence and presence of CUR indicate that additional concentrations of CUR boost the stability and reduce the reversibility of SNY- catalase complex. The kinetics study of SNY-catalase complex illustrated that CUR could enhance the inhibitory effect of SNY on catalase activity so the catalase activity is completely inhibited via co-administration of SNY-CUR. The MLSD simulation outcomes demonstrated that the binding energy of SNY-catalase complex was diminished in the presence of CUR so the affinity of SNY-enzyme complex enhances.

Conclusion: Although the consumption of food additives at their optimized concentrations is considered safe but their combinatorial effects may be changed. Since these two food dyes are often used together, it seems important to investigate the synergistic or antagonistic effects of CUR on SNY-catalase complex and control of their usage is an important human health concern.

Keywords: Bovine liver catalase, combination effect, Orange Yellow S, curcumin, reversibility

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Expression and function of engineered red Opto-mGluR6 as an optogenetic neural inducer in HEK-GIRK cell line

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Background: Hereditary retinal degenerative disorders such as retinitis pigmentosa (RP) are among the leading causes of blindness worldwide. Optogenetic gene therapy, introduces light-sensitive proteins into surviving retinal cells. Opto-mGluR6 is a chimeric protein consisting of the intracellular domain of the ON-bipolar cell specific receptor, mGluR6, and the light-sensing domain of melanopsin. Melanopsin, is a blue-light-sensitive retinal photopigment (mouse λ = 467 nm). Stimulation with the blue light carries high risk of photochemical damage in the sensory retina. This research aims to design an engineered red Opto-mGluR6 that have a broader, red-shifted action spectrum compared to Opto-mGluR6.

Methods: 3 red shifted Opto-mGluR6 were designed by bioinformatics studies. The genes were synthesized and cloned into pAAV-MCS-IRES-EGFP plasmid. To check the accuracy of cloning, the resultant constructs were subjected to digestion and sequencing experiments. HEK-GIRK stable cells were transfected with the desired constructs using calcium phosphate precipitation method. After 48 hours, expression of Opto-mGluR6 in HEK-GIRK stable cell line was assessed by RT-PCR and immunocytochemistry. Whole-cell voltage clamp on transfected HEK293-GIRK cells were performed to pursuit for functional designed genes.

Results: 3 red shifted constructs, (Red Opto-mGluR6) ROM 19 (λmax = 557-730 nm), ROM18 (λmax = 540-557 nm) and ROM17 (λmax = 520-540 nm), were designed by bioinformatics studies and tools. Opto-mgluR6, ROM19, ROM18 and ROM17 genes were successfully cloned into the designated AAV-MCS-IRES-EGFP vector. Large scale DNA plasmids of the interested genes were prepared. HEK-GIRK stable cell line was generated and transfection and expression of optogenetic constructs in engineered HEK293-GIRK cells were confirmed. Patch clamp study represented that, red sifted optogenetic constructs activated GIRK channels when induced by the desired wavelengths of the light.

Conclusion: This research represented that, red sifted Opto-mGluR6 were light sensitive and directly coupled light stimuli to G-protein signaling.

Keywords: Optogenetic, Red Opto-mGluR6, Patch clamp, Retina retinal degenerative disorders

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Evaluation of anti-inflammatory and cholesterol lowering effects of atorvastatin

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Background: Fats are easily stored in body tissues to be used as an energy source, Cholesterol is a type of fat that is made in the liver from the fats in your diet. Hypercholesterolemia occurs when the amount of cholesterol in your blood increases. High cholesterol builds up in the blood vessels and hardens the walls of the arteries over time. This complication narrows the arteries, reduces blood flow, and increases the risk of cardiovascular disease, including heart attacks and strokes. Atorvastatin is a statin drug that lowers blood cholesterol by inhibiting cholesterol-making enzymes. Lowering cholesterol reduces the risk of cardiovascular disease. Atorvastatin also reduces the risk of cardiovascular disease in people with the disease) Even with normal blood cholesterol (Statins have been shown to have anti-inflammatory activities independent of the effects of lowering blood cholesterol.

Methods: In a study, carrageenan-induced edema in the sole of the rat foot and the Air Patch model in mice were used as acute and local inflammatory models. Animals take the above drug in amounts of 1; 5; And 10 mg / kg orally 20; 12; 6; And 1 hour before injecting carrageenan into the sole of the foot into the patch.

Results::Atorvastatin reduced the maximal inflammatory response during the 4 hour after induction of inflammation and neutrophil infiltration into the inflammatory site. During this time, statins did not alter serum cholesterol and triglyceride levels. Atorvastatin (10 mg/kg) the strongest anti-inflammatory effect 70% reduction; $p < 0.0001$ and anti-leukocyte accumulation 80% reduction; showed $p < 0.0001$. The anti-inflammatory effect of atorvastatin was comparable to the anti-inflammatory effect of indomethacin as a standard anti-inflammatory drug.

Conclusion: This study shows the strong anti-inflammatory effects of statins according to the potency of their inhibitory effect on the enzyme hydroxymethylglutaryl coenzyme A reductase and independently of their effect on bloodlipids.

Keywords: Atorvastatin, Cholesterol, anti-inflammatory

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Spectroscopic and molecular docking studies on the interaction of a natural prenylatedcoumarin with calf thymus DNA

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Background: DNA is a well-known pharmaceutical target for many anticancer drugs. Hence, there has been notable interest in the drug- DNA interaction studies. Understanding the mode of drug molecules interaction with DNA is a significant feature in pharmacology and plays an essential role in designing new drugs with enhanced selectivity and reduced side effects.

Methods: In this study, the interaction of diversin (as a natural prenylatedcoumarin) with calf thymus DNA (ct- DNA) was investigated using UV–Vis absorption spectroscopy and molecular docking studies.

Results: The UV-Vis absorption studies revealed that the absorption intensity of DNA increased with increasing diversin concentration and a sligth red shift was observed These results indicated that diversin can cause damage in double helix structure of DNA. Spectroscopic studies indicated that diversin binds to ctDNA with a binding constant of 1.5 × 104 M-1 which is comparable to that of groove binding drugs. The results of molecular docking study accompanied by those from experimental results propose that DNA binding by diversin may occur at minor groove of DNA.

Conclussion: The obtained results would provide useful information about drug–DNA interactions, which can be helpful for designing new anti-cancer agents that target the DNA molecules.

Keywords: Calf thymus DNA, molecular docking, groove binding, diversin

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Serum levels of IL-32 in patients with type 2 diabetes mellitus and its relationship with TNF-α and IL-6

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Background: Type 2 diabetes mellitus (T2DM) is an important public health worldwide. The main underlying mechanism of T2DM is insulin resistance which is associated with chronic inflammation. Interleukin-32 (IL-32) is a pro-inflammatory cytokine which has been implicated in pro-inflammatory responses of several human diseases. Previous studies have reported higher levels of IL-32 in inflammatory disease and obesity. the present study aimed to evaluate the serum concentrations of IL-32 in patients with T2DM and its association with cardio-metabolic parameters.

Methods: This study was undertaken on 93 patients with TDM and 74 healthy controls. T2DM was diagnosed based on ADA criteria. Serum levels of IL-32, adiponectin, TNF-α, and IL-6 were measured by ELISA technique.

Results: Our findings revealed independent elevated levels of IL-32 in T2DM group compared to the control. Furthermore, it was associated with increased risk of T2DM incidence. IL-32 indicated a positive correlation with BMI, FBG, TNF-α, and IL-6 in patients with T2DM. Furthermore, linear regression showed independent association between IL-32 and IL-6 plus TNF-α in patients' group.

Conclusion: The results of the present study revealed higher levels of IL-32 in T2DM patients which have been associated with inflammatory markers.

These results suggest the possible role of IL-32 in chronic inflammation in patients with T2DM.

Keywords: Diabetes, Insulin resistance, Interleukin, Inflammation, IL-32

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Triglyceride-glucose (TyG) index as a risk marker of insulin resistance in Iranian polycystic ovary syndrome woman

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Background: Polycystic ovary syndrome (PCOS), as a complex hormonal disorder commonly occurring in the women of reproductive age. A growing body of evidence shows that insulin resistance (IR) has a central role in the pathophysiology of the PCOS and is associated with great risk of metabolic disorders including type 2 diabetes mellitus (T2DM), dyslipidemia, nonalcoholic fatty liver disease (NAFLD), and cardiovascular disease (CVD). Previous investigations have shown that triglyceride-glucose (TyG) index can be used as a simple clinical indicator of IR in some disorders and ethnicities. Therefore, the present study was conducted to evaluate the correlation between TyG with IR (as measured by homeostasis model assessment of IR [HOMA-IR], quantitative insulin sensitivity check index [QUICKI] and fasting glucose to insulin ratio [FGIR]), in the Iranian women with PCOS.

Methods: Totally, 305 women with PCOS were evaluated in this study. TyG index were calculated using the related formula. Fasting insulin level was measured using ELISA technique. IR was defined as a HOMA-IR value of ≥2.63, FG-IR value of <8.25, and QUICKI value of <0.33.

Results: The insulin-resistant and insulin-sensitive groups, established by the HOMA-IR, FG-IR, and QUICKI values, were different in terms of TyG indix. This index was associated with IR after adjusting for age and BMI. The area under ROC curves (AUC) of TyG was obtained as 0.639, 0.623 for predicting the HOMA-IR index, which was significant, with a P-value of 0.027.

Conclusion: Therefore, TyG index could be utilized as an indicator of IR among the Iranian women with PCOS. Keywords: PCOS; IR; TyG index; HOMA-IR; Dyslipidemia

Keywords: PCOS, IR, TyG index, HOMA-IR, Dyslipidemia

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Design, development and evaluate an application In order to increase user knowledge for identification of laboratories which providing services in the state of Tehran

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Background: Medical diagnostic laboratories are one of the most important centers in the treatment cycle of patients. Most of the patients refer to these centers in the process of diagnosis and treatment. The conscious choice of medical diagnostics laboratories is one of the challenges patients face in the treatment process.

Methods: This is a descriptive-developmental research with an applied approach. The steps involved include desk research, needs assessment, gathering knowledge and demographic data, drawing through UML diagrams, implementing using Java programming language in the Android studio programming environment, and app evaluation.

Results: A comprehensive system of laboratory information and experiments were developed in all Tehran laboratories, which are actually nationally representative, to increase userschr ('39') awareness. And based on factors such as location access, types of laboratories, types of tests, laboratory tariffs, types of insurance coverage, etc., a system was designed that allows users to access the most appropriate lab services with their needs. Evaluation was done by a researcher-made questionnaire whose validity and reliability were confirmed. The target population consisted of one expert and five normal users. According to the Likert criterion, the answer of all respondents to the questionnaire were higher than 4.05

Conclusion: The above application showed that the priority factors in need assessment significantly increased user satisfaction as well as ease of use of laboratory services according to userschr ('39') needs.

Keywords: laboratory finds Application, smart phone, Android.

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Vitamin D deficiency and infection of COVID-19

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Background: Decreased vitamin D is associated with server acute respiratory syndrome caused by SARS-CoV-2. In fact, Vitamin D due to antiviral effect can interfere with viral replication and promote both innate and adaptive immune system response. On the other hand, Vitamin D can enhance autophagy to prevent viral entrance.

Methods: This review was limited to original papers in the English language from 2019 to 2020 using different database, including Web of science, PubMed, Scopus and Google Scholar was done for evaluating SARS-CoV-2 and Vitamin D. The keywords used to search are as follows: "SARS-CoV-2" OR "COVID-19" OR "Coronavirus" AND "Vitamin D".

Results:This systematic review is based on 8 articles. We found 40 papers, 2 of which were duplicates, 4 of which were irrelevant, and 26 of which were review articles, with a total of 32 articles omitted.

Conclusion: Indigenous people who receive low ultraviolet radiation to produce vitamin D are more expose to infection with COVID-19 because vitamin D deficiency can contribute to the acute respiratory syndrome. It is believed that the active form of vitamin D (cholesterol) can protect the body against COVID-19 infection and can also improve treatment outcomes. One study reported that taking vitamin D as a safe, over-the-counter supplement by greatly altering viral gene expression greatly reduced infection-related mortality, especially in the elderly.

Keywords: SARS-CoV-2, Vitamin D deficiency, viral infection

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Elevated Ang II in COVID-19 determined the severity of disease progression: Ang (1-7), Ang (1-9) along with AT1R blockers are suitable candidate in treatment of COVID-19

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The most commonly symptom by SARS-COV-2 which affected patients include cough, fever, muscle pain, fatigue and shortness of breath. However, some complains such as high blood pressure, thrombosis, kidney diseases, neurological disorders, skin pigmentation, and diabetes mellitus are diagnosed. It seems that the imbalance between some cellular metabolites by this virus initiates these perturbances. To gradually follow up the virus disturbance pathways, it observed when the virus attached to angiotensin-converting enzyme 2(ACE2) in addition to shedding it by ADAM17, leads to ACE2 downregulation. Moreover, the main role of ACE2 is to convert angiotensin I and angiotensin II to angiotensin and angiotensin respectively. This ACE2 products have a key role in vasodilation, ati-inflammation, anti-oxidant, anti-fibrosis and lung protection which decreased in this scenario instead of angiotensin II enhanced. As well known, angiotensin II develops some viral pathogenicity cascades such as vasoconstriction, hyperplasia, hypertrophy, apoptosis, inflammation, cytokine storms, fibrosis, altered redox balance, lung injury, skin pigmentation disorders and hypoxia/acidosis. To the best of our knowledge, these findings based on viral infectivity virulence explicitly state that the imbalance between Ang II, Ang and Ang determined the severity of COVID-19. Moreover, with regarding to low diagnostic accuracy PCR test, angiotensin II has appropriate diagnostic indicators in evaluating of COVID-19 and disease progression. Importantly, according to numerous evidences Ang and Ang along with AT1R blockers are suitable candidate in the treatment of COVID-19.

Keywords: SARS-COV-2, angiotensin-converting enzyme 2, angiotensin angiotensin), angiotensin II.

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Multi-spectroscopic studies of interaction between β-carotene with Lysozyme

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Background: Studies on the conformation and activity changes of enzymes in co-solvents are essential in biological, pharmaceutical and industrial applications.

Methods: Therefore, the effect of β-carotene on Lysozyme function and structure was investigated in this study using various techniques such as UV−Vis absorption, kinetic experiments and fluorescence spectroscopy, UV–Vis absorption curve increased with increasing β-carotene concentration, presented the structural changes of enzyme in various concentration of enzyme.

Results: The results of fluorescence spectroscopy also indicating that van der Waals and hydrogen bond forces interaction between β-carotene and Lysozyme. The Stern−Volmer quenching constants (Ksv) for the Lysozyme- β-carotene complex were obtained at two temperatures, revealing that β-carotene quenched the emission of Lysozyme through the static mode of the quenching mechanism. The more polar environment for Trp, Tyr residues was recommended by the fluorescence quenching. In addition, kinetic studies exposed that with an increase in β-carotene concentration, the maximum rate of Lysozyme (Vmax) increase and Km are decreased.

Conclusion: The results show that β-carotene is an inhibitor for Lysozyme and advance in the stability of Lysozyme using β-carotene.

Keywords: Keywords: Lysozyme, β-carotene, Function, Structure.

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Comparison of the results of gene sequencing amplified by three types of DNA polymerase enzymes under same conditions

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Background: Today, the production of recombinant drugs has attracted the attention of many researchers around the worldFor the production of recombinant drugs, the choice of the type of DNA polymerase enzyme in the replication of the desired gene is of great importance. In some cases, an error can affect the conformation and activity of the protein in question. In this paper, the aimed to compare three types of DNA polymerase enzymes, Taq polymerase, Tsg DNA Polymerase, and Pfu DNA polymerase in amplification of 1200bp fragments of L. sericata collagenase gene.

Methods: Using the PCR method and using specific primers of L. sericata collagenase gene, 1200 bass fragment was performed with three types of enzymes and then the PCR product was purified DNA, and the DNA was cloned in PTG-19 vector and then It was transformed into E. coli bacteria competent (DH5α) and finally delivered to Pishgam company of Iran with Universal M13 primers for sequencing in two readings.

Results: After assembling the fragments the results of sequencing the amplified l.sericata collagenase gene with the three types of enzymes were aligned using Clustal Omega website.

Conclusion: This study showed that the mistake rate is zero in all three types of enzymes in the amplification of the 1200 fragment. Considering doing 5 repetitions for each enzyme, it is suggested that the mentioned enzymes can be used to amplify parts with a length of 1200 bp in research works.

Keywords: DNA polymerase, enzymes, amplification

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Determination of causative species of human cutaneous leishmaniasis using PCR in Hamadan province during 2017 and 2018

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Background: Hamedan province is one of the endemic centers of cutaneous leishmaniasis. The aim of this study was to evaluate the specific PCR (polymerase chain reaction) method on stained patient slides in order to diagnose the disease and identify different species of Leishmania in patients referred to the laboratory of Hamadan Health Center.

Methods: This descriptive cross-sectional study was performed on people positive for cutaneous leishmaniasis who referred to the laboratory of health centers in Hamadan province from 1396-1397. Diagnosis was made by preparing a direct spread of skin lesions and then staining with Giemsa. Initially, microscopic examination was performed to correctly identify the pre-prepared samples and also to determine the parasite species, species-specific PCR was used.

Results: In this study, 120 samples of Leishmania positive smears were examined, of which 101 (84.2) were male and 19 (15.8) were female. The highest frequency was in the age group of 30 to 37 years and the lowest was in the age group of 38 to 45 years. n this study, the species of Leishmania parasites isolated from patients using species-specific PCR were Leishmania tropica 6 people (5.0%) percent Leishmania Major 89 people (74.2%) percent.

Conclusion: Since in many cases direct smear is reported negatively and this method has a low sensitivity and on the other hand PCR has a high sensitivity, so it is recommended if the disease is suspected, especially in endemic areas ،In order to more accurately diagnose or rule it out, PCR should be used.

Keywords: Keywords: Cutaneous leishmaniasis, Specific PCR, KDNA, Leishmania major

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MiR-340-5p expression in Endometriotic Mesenchymal Stem Cells of endometriosis patients

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Background: Endometriosis is a chronic, estrogen-dependent disease with high prevalence among women in reproductive age, which causes infertility and pelvic pain. Although this gynecological disease has been negatively affected the woman quality of life, the exact etiology of it did not discover yet. According to Sampson's theory, retrograde menstruation is a causative factor in the pathogenesis of endometriosis. Previous studies showed the genetic and epigenetic alterations in endometriotic mesenchymal stem cells (MSCs) play a significant role in the incidence of endometriosis. As a vital part of epigenetic regulation, miRNAs can determine the proliferation and differentiation/decidualization rate of the MSCs. Therefore, the expression pattern of miRNAs has become a fascinating topic for the pathogenesis of endometriosis. We aimed to explore the MSCs levels of miRNA 340-5p in endometriosis patients compared to healthy women.

Methods: Endometrial tissues were collected from six women, including three endometriosis and three non-endometriosis patients during laparoscopy or hysterectomy. MSCs were isolated from tissue, and flow cytometry assessment confirmed the accuracy of isolated stem cells. We also analyzed the differentiation potential of MSCs. Total RNAs were isolated and used to synthesize complementary DNA (cDNA). Then, the expression pattern of miR-340-5p was analyzed by RT-qPCR. We normalized the pattern expression of the miRNA to the miR-16-5p.

Results: We found the up-regulation of the miR-340-5p ($P < 0.05$) in endometriotic mesenchymal stem cells of endometriosis patients compared to healthy women.

Conclusion: Our study demonstrated the differential expression of miR-340-5p in endometriosis patients, which could propose the miRNAs possible role in determining the stem-cell fate by disrupting the equilibrium between proliferation/differentiation as an important biological process. However, it is necessary to investigate how miRNAs work in this context in different communities and independent patient groups.

Key words: Endometriosis, miRNA, RT-qPCR, Stem cell

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Molecular identification of a new Chlorella sp. as Omega3 Fatty acids source

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Background: Omega3 is one of the most important nutrients in the diet that is not normally produced by the human body. Omega3s play a vital role in the body physiological activities due to their presence in the cell membrane. It is useful for eye health, brain development in infants, heart disease, and cancer. Finding new sources of omega3 is essential. Chlorella is a major source for omega3 production.

Methods: Microalgae were isolated from the north of Iran. Primary culture was performed in the nutrient medium of microalgae,and then was transferred to a specific Chlorella medium. Single cell green algae were cultured on solid medium for purification. Purified samples were screened for omega3. The microalgae showed the highest amount of omega3 was selected by gas chromatographic results. After morphological examination, PCR was performed for molecular identification. After sequencing, the sequence was submitted in the gene bank. Phylogenetic analysis did by the neighbor-joining (NJ) method with MEGA 10 software.

Results: Based on geographical location, most single-celled species were isolated from rice fields. The samples were purified in a specific culture medium. The percentage of fatty acids in the selected sample was calculated to be 38%, which according to the standard was 18% omega3. Based on morphological study had a spherical structure and was light green. Molecular identification was performed by 18 srRNA. This species showed 99% similarity with the sequence of Chlorella sp. TGA2, and 98% similarity with the sequence of Chlorella vulgaris isolate SB2-3 using BLAST analysis.

Conclusion: Omega3 is essential for human health. Finding new sources of Omega3 is important. In this study single cell green algae were isolated from north of Iran. The best species for omega3 was screened. This species was identified Chlorella sp. by morphological and molecular methods.

Keywords: Molecular Identification, Chlorella sp, Omega3, Fatty acids.

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Application of cold atmospheric plasma in virus inactivation

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Viruses as submicroscopic infectious agents can cause serious pathogenic contamination in all cell-based organisms including bacteria, human, animals and plants. Each of the available methods used decontamination has great drawbacks such as production of waste and toxic byproducts. Cold atmospheric plasma (CAP) is a potential alternate to eliminate pathogenic viruses. In this review study, the articles were searched in electronic databases including pubmed/medline, scopus and web of science by following keywords: cold atmospheric plasma and virus inactivation CAP is outcome of electrical discharges within a neutral gas under atmospheric conditions. This technology as a novel, effective and clean solution has been used for a wide range in virus inactivation due to its components including reactive oxygen species (ROS), reactive nitrogen species (RNS), charged particles, free radicals, electromagnetic fields, eletrons, photons and physical foeces. These plasma-generated reactive species efficiently inactivate different kinds of viruses by damaging both nuceic acid and proteins involvement viral replication and pathogenicity. In this review article we present recent progresses in this promising field of virus inactivation.CAP in combination with another available technologies can help to ameliorate virus inactivation via synergistic effects, so providing an efficient decontamination tool.

Keywords: Cold atmospheric plasma, virus inactivation, reactive oxygen species

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Primary microglial cells represent a severe inflammatory response to cotreatment with docosahexaenoic acid and ferrous sulfate in high glucose conditions

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Background: Increased receptor for advanced glycation end products (RAGE) expression that occurs due to diabetes leads to RAGE-mediated microglial activation, which is involved in neurodegenerative disease progression due to induced production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α). The anti-inflammatory benefits of n-3 polyunsaturated fatty acids (n-3 PUFA) supplements have been proven on microglia cells. Metal-catalyzed oxidation of PUFA induces in the presence of glucose. The present study investigated the effects of co-treatment with iron and docosahexaenoic acid (DHA) on inflammatory markers in rat primary microglial cells in high glucose conditions.

Methods: For this purpose, primary microglial cultures were prepared from the brain of newborn Wistar rats and were confirmed by immunocytochemistry. Groups were designed with normal and high glucose conditions. MTT assay, RT-PCR for TNF-α and RAGE mRNA assessing, and ELISA for protein detection was performed.

Results: The results of this study showed that mRNA expression and secretion of TNF-α and RAGE proteins in microglia cell media were strongly affected by ferrous sulfate and DHA co-supplementation, in high glucose conditions compared with normal control even though the concentration of iron used was very low. These inflammatory conditions eventually led to reduced cell viability.

Conclusion: According to the results, co-treatment with Fe^{2+} and DHA especially in uncontrolled diabetic patients (high glucose conditions) can easily activate microglia chronically to produce TNF-α and RAGE which facilitates the development of neurodegenerative diseases. Conditions associated with these two supplements companionship, along with control of hyperglycemia in diabetic patients, are essential to prevent neurodegenerative diseases progression.

Keywords: Docosahexaenoic acid, Ferrous sulfate, N-3 polyunsaturated fatty acids, Neurodegenerative diseases, Receptor for advanced glycation end products, Tumor necrosis factor-alpha

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Structural study in adult and fetal human hemoglobin due to mobile frequency electromagnetic field by ANS fluorescence

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Background: In today world, the use of mobile phones has become an inevitable thing, and the effect of mobile phone electromagnetic field (EMF) on the health and function of the body has been considered. In this study, structural changes of adult human hemoglobin (HbA) and fetal human hemoglobin (HbF) that have been exposed to an EMF with a mobile phone frequency (940 MHz) have been investigated using ANS fluorescence.

Methods: The samples were exposed to the 940 MHz EMF in a waveguide for 40 minutes at a temperature of 37 ◦C. Fluorescence spectroscopy is one of the most sensitive and practical optical methods for studying the structure of proteins. The emission spectrum of the ANS-hemoglobin complex was recorded using the Agilent Cary Eclipse Fluorescence Spectrophotometer at 37 ° C between wavelengths of 380 to 700 nm and excitation wavelength of 355 nm.

Results: The intensity of ANS-HbF fluorescence emission was increased after EMF exposure. Connection of ANS to the detected hydrophobic patches at the protein surface is accompanied by a significant increase in its fluorescence. This indicates exposed HbF has more hydrophobic patches adjacent to the solvent. The intensity of ANS- HbA fluorescence emission was reduced after 940 MHz EMF exposure. This indicates that exposed HbA has less hydrophobic patches adjacent to the solvent.

Conclusion: EMF with Mobile phone frequency changes the HbA and HbF structure. Exposure to the EMF causes the structure of HbA to be more compact and the structure of HbF to be partially unfolded. The results of this study confirm the results of intrinsic fluorescence of exposed HbA and HbF in our previous reports.

Keywords: ANS fluorescence, 940 MHz electromagnetic field, adult hemoglobin, fetal hemoglobin, structural changes.

P-398

Protective effects of crocin and Crocetin against intracerebroventricular streptozotocin-induced spatial learning and memory deficit in rat

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Background: Alzheimer's disease (AD) is a progressive age-related neurodegenerative disorder that heavily affects the hippocampus and the cerebral cortex. In this study, the effect of saffron carotenoids, Crocin and Crocetin, was investigated on improving behavioral symptoms in the rat model of streptozotocin (STZ)-induced AD.

Methods: Adult male Wistar rats were randomly divided into 6 groups (# 6 per each). Rats were anesthetized under ketamine/xylazine administration). The head was positioned in a stereotactic frame and a midline saggital incision was made in the scalp. Burr holes were drilled in the skull on both sides over the lateral ventricles using suitable coordinates. STZ (3 mg/kg) was injected ICV bilaterally on days 1 and 3 of the experiment. After 15 days, Crocin (30 mg/kg)/ Crocetin (10 mg/kg) were daily injected intraperitoneally, up to 21 days; control group received vehicle. Spatial learning and memory abilities of rats were tested using MWM task. Statistical analysis (ANOVA) was performed using SPSS.

Results: In the course of MWM training, the STZ-treated animals represented a higher latency time than the sham group, showing a poorer learning performance. Treatment with Crocin/ Crocetin significantly improved the learning performance. The results of probe trial were evaluated based on two parameters, the time spent (%) and crossing numbers (s) in the target quadrant. The results of the first factor showed that, STZ-treated animals failed to remember the location of platform, spending less time in target quadrant than other groups. However, the time spending in target quadrant was significantly increased via the administration of Crocin/ Crocetin. A remarkable difference of the crossing numbers in the target zone was observed between STZ- treated and other groups.

Conclusion: This study showed that Crocin/ Crocetin effectively attenuated spatial memory deficit caused by intracerebro ventricular STZ treatment in rats.

Keywords: Crocin, Crocetin, streptozotocin, learning and memory deficit, rat

P-399

Determination of Plasma and Erythrocyte Level of Copper and Magnesium by Atomic Absorption Spectrometry in Type-2 Diabetes Mellitus Patients with Metabolic Syndrome

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Background: An alteration in trace elements content of the blood is a risk factor for metabolic syndrome (MetS) and type 2 diabetes mellitus (DM), as well as diabetic complications. This study aims to investigate plasma and erythrocyte levels of Copper and Magnesium, and to assess their correlations with the biochemical components of the metabolic syndrome in type 2 diabetes mellitus patients with metabolic syndrome and in healthy controls.

Methods: 40 men recently diagnosed DM with MetS without complications, and 30 sex and age-matched apparently healthy control subjects were recruited for this cross-sectional study. Trace elements concentrations in the plasma and erythrocyte were measured by graphite furnace atomic absorption spectroscopy (GFAAS).

Results: The results of the present study showed significantly lower plasma levels of copper and magnesium and lower erythrocytes copper, in the patients group compared to controls ($p<0.05$). There were significant negative correlations between plasma levels of copper with waist circumference, hip circumference, waist to hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood glucose, and glycated hemoglobin levels in all subjects; while erythrocyte copper levels showed significant negative correlations with triglyceride ($p<0.05$).

Conclusion: These alterations can lead to insulin resistance through a variety of mechanisms, and alteration in metabolic enzymes activity.

Keywords: Atomic Absorption Spectrometry, Copper, Diabetes mellitus, Magnesium, Metabolic syndrome

P-400

Association between adherence to a Western dietary pattern and insomnia among young women

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Background: Diet has been shown as one factor that can affect sleep patterns. The aim of this research was to measure the correlations among major dietary patterns and, insomnia in young females.

Methods: The study participants included 159 healthy young females. Neuropsychological evaluation was performed applying standard questionnaires, that contained: a cognitive ability questionnaire (CAQ), depression and anxiety stress scales (DASS-21), insomnia severity index (ISI), Epworth sleep scale (ESS), and quality of life questionnaire (QLQ). A validated food frequency (FFQ) questionnaire that included 65 food items, was used and, this was applied to assess major dietary patterns in this group, with using principal component analysis (PCA).

Results: Two major dietary patterns were found: “Traditional” and و “Western”. The Western pattern was determined with high use of snacks, nuts, dairy products, tea, fast foods, chicken and, vegetable oils. persons with moderate/severe insomnia have lesser scores of total cognitive ability task, nocturnal sleep hours, physical and, mental health, but higher scores for depression, anxiety, stress, and daytime sleepiness contrasted to those without insomnia ($p<0.05$). Adherence to Western dietary (WD) pattern was connected with higher odds of insomnia (OR=5.9; 95% confidence intervals: 1.9-18.7; P-value = 0.003) after adjustment for potential confounders.

Conclusion: Our results demonstrated adherence to WD pattern incremented the odds of insomnia.

Keywords: Keywords: western dietary, insomnia, depression, stress, quality of life

P-401

Investigation of structural stability in laccase enzyme in different pH by fluorescence method

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Background: Laccase is a practical enzyme that is used in various industries. It belongs to a group of polyphenol oxidases with copper atoms in the catalytic center. Low substrate specificity is one of the most important properties that makes this enzyme potentially useful for biotechnological applications. Structural stability is very important in different pH for industrial enzymes in varying environmental conditions. Fluorescence is a method that uses an emission and absorbed wavelength to study structural changes.

Methods: Laccase was purified from fungal species. For investigation of structural stability in different pH was used fluorescence method. Intrinsic fluorescence is investigated due to the availability of tryptophan. The experiments were performed using an Aviv 215 Spectropolarimeter of IBB of the University of Tehran. The excitation wavelength was 295 nm for tryptophan and 330 nm for the copper atom in the laccase structure. Fluorimetric studies of purified laccase enzyme were performed at pH 3, 5, 7 and 9.

Results: The emission of intrinsic fluorescence results from its intrinsic fluorophores, such as the tryptophan, which is related to the spatial shape of the protein, which may be on the surface or buried in the protein. The results of fluorescence spectroscopy were evaluated at different pH conditions. At alkaline pH the fluorescence emission increased, indicating the presence of tryptophan in the hydrophobic location, while the fluorescence emission decreased in acidic conditions. PH 5 and 7 did not show much difference. Structural stability was better in acidic conditions and the enzyme was less stable in alkaline conditions.

Conclusion: Laccase is an important industrial enzyme. The study of its structural stability in different pH is essential for application in acidic and alkaline conditions. Intrinsic fluorescence results showed the structural stability of this laccase was better in acidic conditions than in alkaline conditions.

Keywords: Structural study, laccase enzyme, fluorescence.

P-402

The Effects of Massage on Lactate Dehydrogenase (LDH) and Creatine kinase (CK) After Exercise-Induced Muscle Damage

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Background: A heavy training session may cause transient increases in inflammation markers of muscles. The activity of CK and LDH in blood plasma is commonly used as an indicator of skeletal muscle damage. Massage therapy promotes recovery from injury. Therefore, the aim of this study was to evaluate the effects of Massage therapy on muscle damage following severe exercise.

Methods: Twenty healthy sedentary men (age 25.26 ± 2.56 years and BMI 23.61±1.18 kg/m2) randomly divided into two groups including Exercise training with massage (EM, n=10) and without message (EC, n=10) or control. The exercise protocol consisted of 15 minutes of running on a variable treadmill slope at a speed of 8 km / h until exhaustion. A circular method (squeezing, slip, and kneading) were used for massage therapy. Blood sampling was drawn in five stages (1, 2: before and after training, 3, 4, 5: immediately, 24 and 48 hours after massage).

Results: Present study findings indicated that After one session of exhausting training significantly increased the levels of LDH and CK; however, remained above immediately after exercise and massage in both groups, but CK decreased significantly after 24 hours in the EM group after message, after 48 hours both groups were equal. LDH after 24 and 48 hours decreased and two groups didn't observe significant difference.

Conclusion: According to the comparison of the results of this study, massage had beneficial effects on muscle injury indices, so it is recommended to use the massage to reduce muscle damage.

Keywords: LDH, CPK, exercise training, massage damage

P-403

Insights into Controlled Cells' Shear Stress by a Composite Hydrogel

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Background: In recent decades, the 3D bio-printing technology has found widespread use in tissue engineering applications. Hydrogels are a group of biomaterials with printing characteristic like processability, which made them highly desirable in 3D-Bioprinting applications.

Methods: In this study, a hydrogel with the most suitable viscosity was prepared and $CaCl_2$ as a crosslinker used to print along with cells. Cell viability and cell *proliferation were investigated* at room temperature. *Rheology analysis was performed on different hydrogels (two samples) made with different amount of alginate, collagen, and gelatin. For investigation of thermal behaviors, physical properties of two samples were obtained. After printing, simulation was done. Finally, Morphology, degradation, diffusion coefficient, mechanical properties and florescent staining were analyzed.*

Results: The results led to the combination of percentages collagen:Alginate:Gelatin (1:4:8)% as the best conditions for printing. In addition, the highest diffusion rate for a cross-linked sample with 1.5% $CaCl_2$ during 1 hour was obtained 0.5×10^{-5} cm^2/s. A sufficient mechanical strength of scaffolds to be used in physiological conditions was achieved. The results of this test reported a tensile modulus value of 1.86±0.49 MPa. The highest stress occurred where the maximum elongation was 53±4% of the initial length, and the maximum stress for the sample was 1.02±0.27 Mpa. In *florescent staining,* cells showed cell viability 98.41%±1.26% in the first day. This cell viability was retained on days 7 and 14 with constant values of 98.62%±1.43% and 98.51%±1.02%, respectively. During this period, the high cell viability rate was maintained at a constant level.

Conclusion: Based on the mechanical strength and biodegradation and favorable biocompatibility, the new hydrogel with printing ability at ambient temperature can be a good choice in tissue engineering applications.

Keywords: Shear stress, Alginate, Gelatin, 3D-bioprinting, Collagen, Hydrogel

P-404

Rapid and simple detection of E.coli O157 using Loop-Mediated Isothermal Amplification (LAMP)

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Background: Bacterial food poisonings are global problems. Escherichia coli O157: H7is one of the most important bacteria that cause food contamination and poisoning.There are several methods to identify these bacterium. Although PCR-based methods are accurate and sensitive, they have some limitations, such as high thermal cycles that take up to three hours and using an expensive thermocycler. Today isothermal methods(such asLAMP)are used as an alternative of PCR-based methods.LAMP is a simple and fast method, and since it does not require a thermocycler to perform this method, it is also considered a cost-effective .

Methods: In the present study, two pairs of primers were prepared for 16SrRNA and rfbE genes in order to identified E. coli O157 based on PCR method. Also, two pairs of primers were designed for the eae gene to launch the LAMP diagnostic method for this bacterium. In the following, monoplex PCR, multiplex PCR and LAMP methods were set up to detection of this bacterium.

Results: The results of the sensitivity assay for multiplex PCR reactions were estimated about 0.38 pg of the genome. Then LAMP method was set up to detect E.coli O157:H7 and in order to improve and accelerate the diagnostic process of the factors affecting the LAMP reaction, it was optimized using Taguchi reaction design software. The results of sensitivity assay for LAMP reactions were estimated about 0.38 fg of E. coli O157 genome as a model for replication.In addition, it was found that the best replication rate was observed in 8 mM MgSO4 and the best incubation time was 90 minutes and the optimum temperature was 60 ° C.

Conclusion: As a result, showed that this method can be used to design detect kits for this bacterium.

Keywords: Food poisoning, Escherichia coli O157:H7, detection, LAMP, PCR.

P-405

The Role of PEGylation in Changing Chaotropic and Kosmotropic Properties of Magnetic Nanoparticles on Adsorbed Protein

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Background: nanoparticles (NPs) has vital roles in various fields of study. Since concerns on NP-biological molecule interaction have been raised, several procedures have been employed to increase the biocompatibility of NPs.

Methods: In this study, the effect of magnetic nanoparticles (MNPs) which are Fe3O4 and Polyethylene glycol (PEG)-coated Fe3O4 (Fe3O4@PEG) on the structure and function of egg white lysozyme (LYZ) of hen was investigated. The synthesized NPs were characterized using Fourier transform infrared (FT-IR), X-ray diffraction (XRD), vibrating sample magnetometer (VSM), scanning electron microscopy (SEM) and dynamic light scattering (DLS). Structural changes of LYZ as a result of interaction with bare and PEGylated MNPs was monitored using fluorescence and circular dichroism (CD) spectroscopies. Moreover, remaining activity of LYZ was tested against M.luteus with the help of UV-visible absorption spectroscopy.

Results: The study showed kosmotropic and chaotropic behaviors of both Fe3O4 and Fe3O4@PEG under and above a concentration threshold, respectively. Destabilization of LYZ within chaotropic concentrations of MNPs was associated with disruption of its active site and secondary structure which was always alleviated upon PEGylation. Although, higher kosmotropic potential of Fe3O4 was only observed below a preference shift point (PSP). Additionally, magnitude of kosmotropic potential is inversely proportional with breadth of the concentration threshold as it was identified to be 0.0067 mg mL-1 and 0.0091 mg mL-1 respectively for Fe3O4 and Fe3O4@PEG.

Conclusion: Therefore, since PEG weakens both structure-making and structure-breaking abilities of MNPs, MNP/LYZ ratio is the critical factor regarding selection of MNP (bare or PEGylated) for any application.

Keywords: Protein-nanoparticle interaction, Fe3O4 magnetic nanoparticles, PEG-coated nanoparticles, Kosmotropic, Chaotropic

P-406

Evaluation the effects of resveratrol on cyto-toxicity and reactive oxygen species production comparing with cisplatin effects in a prostate cancer cell line (LNCap)

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Background: Prostate cancer is often caused by a mutant or by a disorder in DNA modification. Cisplatinis a potent chemical anticancer drug that causes cellular resistance; moreover, it shows side effects in normal tissues. Resveratrol is a polyphenol compound present in many plants species, including grapes, peanuts, and berries, as an antioxidant that prevents the transformation of normal cells into cancer cells, as well as the anti-cancer action in multiplying cancer cells. The aim of this study was to evaluate and compare the effects of resveratrol with cisplatin on cytotoxicity and production of reactive oxygen species in a prostate cancer cell line (LNCaP).

Methods: Prostate cancer cell line (LNCaP) was purchased from the Pasteur Institute. The cultured cells were treated with concentrations of 1, 10, 100, 1000 µg /ml of Res and cisplatin separately. The survival rate of cancer cells was examined using MTT assay and spectrophotometry. In addition, the production of reactive oxygen species in treated prostate cancer cell line was evaluated using flow cytometry.

Results: IC50 for cisplatin was 12.54 µg /ml and for Res it was 205.6 µg/ ml. The results of MTT assay showed that Res has cytotoxic effect in all examined concentration, and this effect is dose dependent. However, cisplatin showed higher cytotoxic effect comparing to Res. Moreover, Res showed strong antioxidant activity.

Conclusion: Res as a plant derivative has anti-cancer effects and antioxidant properties. Regarding to the side effect of cis-platin, it can be a good candidate for using in chemotherapy. The studies on other cell lines or animal model of cancer is required to reveal more details.

Keywords: cancer, cisplatin, resveratrol, reactive oxygen species.

P-407

Cloning of mmu-miR-96 into pAAV-MCS vector to pursue for its role in diabetic retinopathy

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Background: Diabetic Retinopathy is the most common microvascular complication of diabetes mellitus and the most common reason of blindness in the elderly population. MiRNAs are among the most related causes of the disease onset. MiR183 cluster, which contains miR-183, miR-96 and miR-182, is highly expressed in the retina and plays an important role in retinal development through targeting the cognate genes ‘expression. Many recently published studies have shown that miR-183 cluster content is increased in diabetic retinopathy. So, we aimed to investigate its role in DIABETIC RETINOPATHY. We searched for miRNA-96 component of the miR-183 in progression of DIABETIC RETINOPATHY.

Methods: pri-mmu-miR-96 codding gene was taken from NCBI. Then, 150 bp upstream and downstream of the gene sequences was selected for synthesis. The cleavage sites of KpnI and XhoI enzymes were located in the upstream and downstream area of the sequence, respectively. The gene was ordered and the synthesized gene was sub-cloned from BSK vector to pAAV-MCS vector by using the related enzymes.

Results: Recombinant vector named “AAV-MCS-GFP-intron-mmu-miR-96” was constructed and accuracy of the cloning was assayed by quick check, restriction enzyme digestion, PCR and sequencing.

Conclusion: We proceeded to clone miR-96 gene in Adeno Associated Virus plasmid to study its role in diabetic retinopathy. Adeno associated virus carrying mmu-miR-96 gene will be recruited in future In Vitro and In Vivo experiments soon.

Keywords: Diabetic Retinopathy, microvascular complication, mmu-miR-96, Adeno Associated virus

P-408

Plasma microRNA 199b-3p as a diagnostic biomarker for endometriosis

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Background: Endometriosis is a chronic inflammatory disease that involves about 10% of reproductive-aged women all over the world. Laparoscopy is the gold standard diagnostic method of the disease. Despite the high prevalence and disruptive effect on women quality of life, a non-invasive diagnostic method for endometriosis is not available yet. Previous research has established that miRNAs are differentially expressed in several gynecological diseases and suggest their role in endometriosis pathogenesis. Hence, their expression pattern as a potential biomarker has become an interesting subject for the diagnosis of endometriosis. We aimed to explore the miRNA 199b-3p expression in plasma of endometriosis patients to estimate its potential as a diagnostic biomarker for the disease.

Methods: Fifty women, including twenty-five patients with histologically confirmed endometriosis and twenty-five healthy subjects free of endometriosis, participated in the current study. Peripheral blood samples were collected before laparoscopy, and plasma was separated. Isolated total RNA was used to synthesize complementary DNA (cDNA). Next, real-time PCR (RT-PCR) was performed for analyzing the gene expression of miR-199b-3p. All data were normalized to mir-16-5p expression levels, and all the measurements were performed duplicate. We performed ROC curves analysis to assess the sensitivity and specificity of the miR-199b-3p as a diagnostic biomarker.

Results: The up-regulation of miRNA 199b-3p ($P < 0.001$) was shown in women with endometriosis compared to healthy subjects, and the AUC curve was 0.843 with 96% sensitivity and 80% specificity.
Conclusion: The present study showed that the transcript levels of miRNA 199b-3p in plasma are an appropriate candidate for usage as a non-invasive diagnostic biomarker for endometriosis.

Keywords: miRNA, RT-qPCR, non-invasive, diagnostic method, endometriosis

P-409

The effects of rosmarinic acid on oxidative stress parameters and inflammatory cytokines in lipopolysaccharide-induced peripheral blood mononuclear cells

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Background: Rosmarinic acid (RA) is a potential herbal medicine and has received considerable attention due to its strong antioxidant properties. The aim of this study is to investigate the impact of RA on inflammation and oxidative stress induced by lipopolysaccharide (LPS) in peripheral blood mononuclear cells (PBMCs).

Methods: PBMCs were pre-treated with various contents of RA (20, 40, 80 μM) for 24 h, then, stimulated with LPS (10 ng/ml) for more 6 h. ELISA and Real-time PCR were done to detect the levels of IL-6, TNF-α, COX-2, IL-1β and IL-10. Western blot was done to investigate the phosphorylated amounts of P65-NF-κB and JNK. Inflammatory cytokines and oxidant-antioxidant parameters were determined by colorimetric and ELISA methods.

Results: The results indicated that LPS augmented the protein levels of IL-6, TNF-α, and IL-1β cytokines as well as the mRNA levels of IL-6, TNF-α, IL-1β, COX-2, and IL-10 cytokines in in PBMCs. However, pretreatment with RA could reduce the impact of LPS on inflammatory markers. In addition, RA inhibited P65-NF-κB and JNK phosphorylation. LPS also caused a decrease in antioxidant enzymes, total thiol, and total antioxidant capacity as well as an increment in malondialdehyde and nitric oxide metabolite contents that RA abrogated them.

Conclusion: Collectively, our finding demonstrated that RA ameliorates LPS induced inflammation in PBMCs. RA reduces oxidative stress by preventing lipid peroxidation and nitric oxide production as well as restarting the activity of the GPx and SOD enzymes. Furthermore, our findings indicated that RA was able to protect PBMCs from inflammation via inhibiting the NF-κB and JNK MAPK pathways. This evidence shows a promising therapeutic role for RA in inflammatory status.

Keywords: Rosmarinic acid, Inflammation , Peripheral blood mononuclear cells , Inflammatory cytokines, Oxidative stress

P-410

A metabonomics study serum of patients with recurrent miscarriage using 1HNMR spectroscopy

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Background: Recurrent miscarriage refers to having three or more recurrent miscarriages prior to the twentieth week of pregnancy. Since the primary reasons of 50 percent of miscarriages have been reported to be unknown, more research studies were needed to investigate the reasons why recurrent miscarriages occur. This primary objective of this study was to investigate the exploitation of H1NMR comparatively between the serum samples of women with recurrent miscarriages and those who had no record of miscarriages and had children. Nuclear Magnetic Resonance (NMR) is used to obtain information about metabolic profiles based on chemical structures of molecules for early detection of diseases.

Methods: Two groups of 30 women, the experimental group with recurrent miscarriages and control group from 30 women with no record of miscarriages and having two children. The serum samples were sent for 1HNMR spectroscopy with 450 Hz Burker NMR machine and the spectra analyzed by chemometrics analysis on Matlab software. The Pro-metab program was used in MATLAB platform on raw spectra and converted them to Excel files after binning to 1600 bins and using Partial Least Squares Discriminate Analysis (PLS-DA) to classify the samples and obtain the differentiating metabolites which were identified using the Human metabolome database (HMDB) in both the experimental and control groups. The pathway analysis option of the Metaboanalyst.ca website was used to identify the changed metabolic pathways using the identified metabolites.

Results: The results of the study revealed that the most changes occurred in Biotin, glutathione, Tryptophan, lysine, threonine and tyrosine metabolites.

Conclusion: Biotin participates in glucose, amino acid metabolism and fatty acid synthesis and is essential for maintaining reproductive function. There is a correlation between spontaneous miscarriage and low plasma an intracellular activity of glutathione peroxidase enzyme. The aforementioned metabolic pathways of amino acids are significantly important in preventing recurrent miscarriage.

Keywords: recurrent miscarriage, NMR, metabonomics, metabolic pathways, metabolic profiles.

P-411

Evaluation of interleukin 3 and 11 levels in Iranian Gaucher disease patients

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Background: Gaucher disease is the most common autosomal recessive disorder in the category of lysosomal storage diseases (LSDs). The disease is caused by mutations in the glucosurberosidase (GBA) gene, which causes a deficiency in the GBA enzyme. In this disease some complications such as anemia, hepatomegaly, splenomegaly, and bone disease occurs. In addition, the expression and secretion of cytokines such as interleukin-3 (IL-3) and interleukin-11 (IL-11) affect the production, differentiation, and metabolism of blood and bone cells. In this study, plasma levels of IL-3 and IL-11 were investigated.

Methods: Enzyme activity of GBA and Chitotriosidase was determined by the fluorimetric method in 33 patients from different parts of Iran and 55 healthy individuals who were age-sex adjusted. Peripheral blood mononuclear cells(PBMCs)of patients (n = 10) and healthy individuals (n = 10) were cultured in RPMI medium. Interleukin 3 and 11 concentrations in plasma and supernatant of patients and healthy individuals were measured by ELISA technique.

Results: The plasma concentration of IL-3 in patients was significantly higher than the concentration of interleukin in healthy individuals ($p = 0.033$), But the plasma concentrations of IL-11 and both interleukins in the supernatant of PBMCs were not different in the two groups.

Conclusion: the present study suggests the determination of plasma IL-3 concentration as an adjunctive diagnostic test for Gaucher disease.

Keywords: IL-3, IL-11, PBMCs, Gaucher disease

P-412

Malvidin prevents lipopolysaccharide-induced oxidative stress and inflammation in human peripheral blood mononuclear cells

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Background: It is indicated that malvidin has anti-inflammatory and antioxidant effects on various cells, although the function of malvidin in preventing inflammatory reactions caused by lipopolysaccharide (LPS) in peripheral blood mononuclear cells (PBMCs) is still not known. The objective of this study was to examine the impact of malvidin on inflammatory responses and oxidative stress in PBMCs as caused by LPS.

Methods: PBMCs were pre-treated with various contents of malvidin (10, 25, 50, 100 µM) for 22 h, then, stimulated with LPS (10 ng/ml) for more 6 h. The anti/proinflammatory cytokines were evaluated by real-time polymerase chain reaction and enzyme-linked immunosorbent assay. Total protein levels/phosphorylation of c-Jun N-terminal kinase (JNK), P65-NF-κB, and IKKα/IKKβ were evaluated by western blot analysis. Inflammatory cytokines and oxidant-antioxidant parameters were determined by colorimetric and ELISA methods.

Results: The present findings showed that LPS significantly increased the expression of IL-6, TNF-α, IL-1β, and COX-2 mRNA and protein release from PBMCs 22 hr after treatments. It was also revealed that increased levels in cytokine expression coincided with increased phosphorylation of JNK, P65-NF-κB, and IKKα/IKKβ. Also, the expression of IL-6, TNF-α, IL-1β, and COX-2 mRNA induced by LPS as well as secretion of protein in PBMC has been significantly decreased by pretreatment of malvidin. Importantly, pretreatment of the cells with malvidin completely abrogated the phosphorylation of P65-NF-κB, JNK, and IKKα/IKKβ in LPS treated cells. Malvidin protection against LPS-induced inflammation was coupled with a decline in the levels of nitric oxide metabolite and malondialdehyde, along with an increase in the ferric reducing antioxidant power, total thiol activity, and also superoxide dismutase and glutathione peroxidase activity.

Conclusion: In accordance with this finding, malvidin may represent a promising therapeutic agent for the prevention of inflammation in PBMCs.

Keywords: inflammation, inflammatory cytokines, malvidin, oxidative stress, peripheral blood mononuclear cells

P-413

The Effects of Massage Therapy on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) After Strenuous Exercise

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Background: A strenuous exercise session may cause a transient increase in serum ALT and AST concentrations, which are used as an indicator of skeletal muscle damage in the bloodstream. Massage therapy heals the injury. Therefore, the aim of this study was to evaluate the effects of massage therapy on muscle injury following strenuous exercise.

Methods: Twenty healthy sedentary men (age 25.3±2.6years and BMI 23.6±1.2 kg/m2) randomly divided into two groups including Exercise training with massage (EM, n=10) and without massage (EC, n=10) or control. The exercise protocol consisted of 15 minutes of running on a variable treadmill slope at a speed of 8 km/h until exhaustion. A circular method (squeezing, slip and kneading) were used for massage therapy. Blood sampling was drawn in five stages (1,2: before and after training, 3,4,5: immediately, 24 and 48 hours after massage).

Results: Compared with the baseline, a significant increase in AST and ALT was observed in both groups immediately after exercise and massage ($p < 0.05$). According to the findings, massage did not significantly change AST and ALT levels in blood samples taken at 24 and 48 hours ($p < 0.05$).

Conclusion: According to the present findings, it was concluded that massage after exhaustive exercise did not affect the liver enzyme activity in untrained individuals.

Keywords: AST, ALT, exercise training, massage therapy

P-414

Evaluation of changes in the inflammatory process on the osteogenesis of mesenchymal stem cells (MSCs) under the influence of apigenin as an anti-inflammatory flavonoid

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Background: The effects of inflammation on the process of osteogenesis have had contradictory results. Accordingly, in the present study, we investigated the effect of inflammation on osteogenesis. We also examined the effect of apigenin as an anti-inflammatory flavonoid compound on this process under inflammatory conditions.

Methods: MSCs were isolated from adipose tissue, and then osteogenesis was induced. Then, along with the differentiation, they were treated by LPS/PA. To evaluate the effect of apigenin, another group was treated simultaneously with apigenin. In the end, the degree of cell differentiation was assessed by alizarin red staining. Also, the expression of the NF-KB gene was examined by Real-time PCR along with the protein expression of IL-6 by the ELISA method.

Results: Induction of inflammation by LPS/PA in MSCs was observed as an increase in the expression of the NF-KB gene and IL-6 protein. This inflammation also suppressed osteogenesis observed by alizarin red staining. Apigenin was able to inhibit inflammation, which was significantly observed by reducing the expression of the NF-KB and IL-6 ($p<0.001$). Apigenin was also able to improve the osteogenesis process of cells affected by inflammation, which was observed in Alizarin Red S staining.

Conclusion: The results indicate the inhibitory effect of inflammation on the osteogenesis of MSCs. Moreover, using the anti-inflammatory effects of compounds such as apigenin, the inhibitory effect of inflammation on the differentiation pathway can be limited.

Keywords: Osteogenesis, Inflammation, Mesenchymal stem cell

P-415

Effect of Vitamin D3 on Indicators of oxidative stress and expression of the nlrp3 inflammatory gene in type 2 diabetes mellitus

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Background: In recent years, serious research is conducted on the role of NLRP3 inflammasome in the development of insulin resistance and type 2 diabetes mellitus (T2DM)‹That the reactive oxygen species (ROS) appears to be the most common cellular response to NLRP3 activation among a variety of available signals. These findings provide new contexts for T2DM research and treatment. In this regard, vitamin D supplementation has taken into consideration as a potential intervention to reduce the risk of diabetes. In this article, we investigate the effect of vitamin D3(VitD3) on oxidative stress indices and nlrp3 gene expression in patients with type 2 diabetes.

Methods: The study was designed as a randomized controlled trial. Participants included 64 people with T2DM [hemoglobin A1C (HbA1c) > 6.5(, and 25-hydroxyvitamin D levels ≤30 ng / ml]. They were randomly divided into two groups: placebo (n= 32), and intervention (n= 30). The intervention group received a VitD3 supplementation (50,000 IU per week) for 8 weeks. Using the kit, the level of oxidative stress indices was assessed by spectrophotometry/fluorometry and gene expression by real-time PCR.

Results: In the intervention group, levels of 25 hydroxyvitamin D significantly increased compared to the placebo group (P <0.001). But Significant differences in the level of oxidative stress indices included TAC (p=0.96) Thiol groups (p = 0.90) and malondialdehyde (MDA) (p = 0.89) were not observed between the two groups. In addition, the comparison of gene expression between the two groups was not significant (p = 0.346).

Conclusions: This study demonstrated that VitD3 Vitamin D3 has no effect on reducing MDA and nlrp3 gene expression and increasing TAC and Thiol Group. It is possible that with increasing intervention time and increasing sample size, we will witness the effect of VitD3 in improving oxidative stress status and reducing gene expression levels in diabetic patients.

Keywords: VitD3 supplementation, ROS, NLRP3

P-416

Determination of some Folate Derivatives by HPLC after preconcentration by Ultrasound-assisted emulsification microextraction method in serum

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Background: The determination of some folate derivatives such as folic acid (FA) and 5-methyl THF (5-Me-THF) are great of clinical importance in various patients. Several chromatographic apparatus have been reported for folates analysis in human serum, including radioimmunoassay, capillary electrophoresis, and liquid chromatography. The goal of this work was to develop a simple, accurate, and sensitive method to determine some folate derivatives, including FA and 5-MeTHF, by HPLC after ultrasound-assisted emulsification microextraction (USAEME).

Methods: At first 30 µL of 100 mM DTE was added to 100 µL of the serum sample, and was mixed with 100 µL n-hexane and methanol (1:10) as solvent. The mixture sample solution shaken manually for 2 min and centrifuge the vial at 12000 rpm for 3 min. Finaaly 50 µL of the prepared sample were injected in to HPLC. The study was financially supported by Abadan Faculty of Medical Sciences (ID: 98u-573; Ethics Code: IR. ABADANUMS. REC.1398.026).

Results: A rapid and efficient microextraction method was proposed for the determination of trace levels of FA and 5-MeTHF in serum samples. In this study, mean relative standard deviations (RSD) less than 6% and detection limit were obtained 0.21 and 0.08 ng mL-1 for FA and 5-MeTHF respectively. Mean recoveries FA and 5-MeTHF in serum samples were in the ranges of 86-107% and 84-96%, respectively.

Conclusion: The advantages of this method were low detection limit, fast preparation, and high recovery. The obtained results shown that USAEME- HPLC is an applied method for measurement of FA and 5-MeTHF in serum samples.

Keywords: Ultrasound-assisted emulsification microextraction, HPLC-DAD, Hybrid Box–Behnken design, Folic Acid, 5-methyltetrahydrofolate

P-417

Is the effect of Decitabine limited to hypomethylation and DNMTs?

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Background: Histone modifications play a crucial role in chromatin structure. Among enzymes that regulate these processes, histone deacetylases (HDACs) can remove acetyl groups from histone tails, thus increasing their interaction with DNA and leading to chromatin condensation. 5-Aza-2-deoxycytidine (AZad) or Decitabine is a potent hypomethylating agent that incorporates into DNA and traps DNA methyltransferase in the form of a covalent protein–DNA adduct. Azad, not only change the gene expression through demethylation of the gene promoter, but it also can change gene expression independently from DNA demethylation. So, the present study was to distinguish whether AZad in addition to inhibitory effects on DNA methyltransferase, can change HDAC3 and HDAC7 mRNA expression in NALM-6, HL-60 cancer cell lines.

Methods: HL-60, NALM-6, and normal cells were cultured, and the treatment dose of the AZad was obtained (1µM) by the MTT test. Finally, HDAC3 and HDAC7 mRNA expression were measured by Real-Time PCR in HL-60 and NALM-6 cancerous cells before and after treatment. And HDAC3 and HDAC7 mRNA expression in un-treated HL-60 and NALM-6 cancerous cells were compared to the normal cells.

Results: Our result revealed that expression of HDAC3 and HDAC7, in HL-60 and NALM-6 cells increases as compared to normal cells. After treatment of HL-60 and NALM-6 cells with AZad, HDAC3 and HDAC7 mRNA expression were decreased significantly.

Conclusion: Our data showed, the effects of AZad are not limited to direct hypomethylation of DNMTs but it can indirectly affect other epigenetic factors, such as HDACs activity, through converging pathways.

Keywords: HDAC3, HDAC7, HL-60, NALM-6, Decitabine, AZad

P-418

Decoding the interaction between Putrescine and human serum albumin and the elucidation of binding sites: a multi-spectroscopic and molecular docking study

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Background: In a living system, biogenic polyamines, namely Spermidine, Spermine, and Putrescine, are basic amines. They engage in multiple cell functions such as differentiation, cell growth, modulation of gene expression, activation of DNA synthesis, and facilitation of protein-DNA interactions, enzyme activities. Also, research demonstrated that they have a substantial role in cancer cell growth and differentiation. Drug attachment to serum proteins in the blood has a substantial effect on drug absorption, distribution of metabolism, and excretion.

Methods: used Diverse techniques such as UV-spectrophotometry, spectrofluorometry, and molecular simulation, and also molecular docking were used in this inestigation. HSA stock solution in phosphate buffer (pH 7.2) prepared.

Results: Putrescine has been able to efficiently quench this protein intrinsic fluorescence by static quenching mode. With rising Putrescine concentration, the UV–Vis absorption curve increased, suggesting a non-covalent interaction between Putrescine and HSA. The binding constant (Ka) for Putrescin interplay with HSA at two 25° and 35° C temperatures considered. In linking Putrescine to HSA, hydrophobic forces were instrumental. Putrescine capable of altering the fluorophore of protein in the micro-environment near the Trp and Tyr. Putrescine had changed the protein secondary structure. Using simulation studies of molecular dynamics (MD) and molecular docking, the putative binding sites and residues within the respective protein matrix surrounding the Putrescine molecule were determined, demonstrating that HSA amino acid residues organize at drug sites I interact. The molecular dynamics simulation showed that at attendance Putrescine, leaves HSA more stable. As well the conformational flexibility of complex putrescine-protein was evident from the MD simulation studies. The RG result shows that during the simulation, the structural density of the protein has increased. ASA values increased for Trp and some Tyr.

Conclusion: we researched Putrescine interactions with Human Serum Albumin, to comprehend their mechanism molecular.

Keywords: HSA, Putrescine, fluorescence quenching, molecular docking, molecular dynamics simulation

P-419

Physicochemical characterization of MiRGD peptide and sFLT01 gene nano-carrier

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Background: Success in gene therapy depends on the ability of drug delivery systems to reach to the target sites. Viral vectors are the most efficient gene carriers but immunogenicity and toxicity are the major known disadvantages of these type of vectors. Non-viral vectors with low toxicity and immunogenicity have been developed to overcome the aforesaid problems. MiRGD peptide carrier, cell penetrating peptide, is composed of several functional motifs to overcome barriers in gene transduction pathway. We aimed to study MiRGD peptide carrier physicochemical properties to efficiently transfer sFLT01, anti-angiogenesis gene, into mammalian cells.

Methods: The gene encoding MiRGD carrier was designed. The recombinant peptide was purified by Ni-NTA affinity chromatography. The purity of the carrier was determined by 15% SDS-PAGE. sFLT01 gene was cloned into pAAV-IRES-GFP vector and transformed to the desired bacterial cells. Nanoparticles were produced by MiRGD nanocarrier and sFLT01 plasmid at different N/P Ratios. The DNA binding of MiRGD carrier was examined by gel retardation assay. The stability of the MiRGD/pDNA nanoparticles in the presence of serum was examined. Size and zeta potential measurements of nanoparticles were performed using DLS.

Results: MiRGD peptide effectively condensed pDNA and neutralized its negative charges, through increasing N: P ratio (2-20). Nanoparticle were stable in the presence of serum and effectively protected pDNA from degradation by the serum nucleases. Particle size and zeta potential assay showed that the surface charge of the MiRGD/pDNA nanoparticles remained positive (+19mV) at higher N: P ratios (4-20). Size of nanoparticle decreased with increasing N: P ratios.

Conclusion: DNA Binding motif of 16 mer histone H1 of MiRGD condensed DNA and protected it from serum nucleases. Particle size and zeta potential adequately controlled the level of cellular uptake. With increasing N: P ratios, the particles less than 250 nm were observed.

Keywords: MiRGD peptide, sFLT01 gene, nano-carrier

P-420

Elucidation of the human serum albumin (HSA) and spermine interaction: multiple simulation spectroscopy and dynamics approach

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Background: Polyamines are aliphatic polycations which exist in nearly every living organism. In living systems, the primary amines, defined as biogenic, entail Putrescine (Put), Spermine (Spm), Spermidine (Spd). Spermine found in mammalian cells. Spermine association with other substances such as proteins, ATP, and DNA indicates its role in physiological functions. Possible particular Spermine functions include prevention of ROS-induced damage, enabling the suitable current to cross through inwardly rectifying K+ channels, influencing memory and learning-related brain glutamate receptor activity, stress defense, and adjustment of growth responses.

Methods: Structural changes were explored in this study and the binding of human serum albumin (HSA) in the attendance of Spermine. For this reason, we used various techniques such as UV-spectrophotometry, spectrofluorometry, and molecular simulation as well as and molecular docking. This work done at a pH of 7.2 (phosphate buffer) at two temperatures of 298 and 303 K.

Results: Increased UV absorption, the results of spectrophotometry, provided a change in the tryptophan environment. The emission rate of HSA was reduced significantly, and static quenching was reported. The values of entropy and enthalpy suggest that the HSA-Spermine bond consisted of van der Waals forces or bonding with hydrogen. The thermodynamic assessment shows that the complex structure is spontaneous ($\Delta G° < 0$). Simulation dynamics and docking corroborated the experimental data by including information about binding sites and their respective microenvironment. These results illustrated that spermine enhances HSA stability and reduces its flexibility. According to the results, Spermine increases the amount of residue ASA in Trp and most of the Tyr residues.

Conclusion The potential use of HSA in spermine delivery was assessed here. It is of great importance to research protein carriers for their transition to target molecules and to enhance their pharmacokinetic properties.

Keywords: HSA, Spermine, fluorescence quenching, molecular docking, molecular dynamics simulation

P-421

Exploring the interactions of a natural gammariza with human serum albumin: Surface plasmon resonance and molecular modeling studies

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Background: Gammariza is the vital bioactive compound, which is a mixture of ferulic acid ester and plant sterols. Binding of various drugs and therapeutic agents to serum albumin, as the most abundant and carrier protein, provides some main information in the drug development.

Methods: In this work, the interaction of gammarizawith human serum albumin (HSA) was investigated using surface plasmon resonance (SPR) and molecular modeling studies.

Results: The real-time monitoring of the binding of ORY to HSA was carried out using SPR technique. The equilibrium constant value (KD) was obtained by using SPR data analysis to be 1.23 × 10-6 M. The small KD value calculated by SPR analysis indicated high affinity of gammarizatoward HSA.The molecular modeling studies confirmed that gammarizahas only one binding site on HSA and binds HSA in a cavity between subdomain IIA and IIIA.

Conclusion: This study can some main information about the pharmacological and pharmacokinetic properties of gammariza and serum albumin role in the transportation of gammariza in the systemic circulation.

Keywords: Human serum albumin, Gammariza, Surface plasmon resonance, Molecular docking

P-422

The effect of clozapine and risperidone antipsychotic drugs on expression of CACNA1C in rat' model of schizophrenia

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Background: Schizophrenia is one of the most debilitating psychiatric illnesses.One way to treat schizophrenia is to prescribe antipsychotic drugs. This study aimed to investigate the effect of neuroprotective effects of risperidone and clozapine on behavioral disorders in an animal model and examine the effect of drugs on CACNA1C gene expression.

Methods: In this study, 45 male Wistar rats were divided into 5 groups. Schizophrenia, risperidone and clozapine groups received ketamine intraperitoneally for 10 days at a dose of 30 mg / kg. The solvent group received normal saline for 10 days. Fifteen days after the last injection of ketamine and normal saline, social interactions test performed. One month after the last injection of ketamine, we started injecting clozapine at a dose of 7.5 mg/kg, risperidone at a dose of 1mg/kg, and normal saline into three groups of rats for 28 days. Twenty-four hours after the last injection, social interactions test was performed. Rats were killed and gene expression levels of beta-actin and CACNA1C in hippocampus, were analyzed by using of real-time quantitative PCR.

Results: The results of social interaction test revealed a significant decreased in cumulative time with KET 30 mg/kg ($p < 0.001$), compared to VEH. Compared to KET, increased with CLOZ at 7.5 mg/kg ($p < 0.001$) and RISP at 1 mg/kg ($p < 0.001$). Our results showed that chronic administration of Risperidone and clozapine improves anxiety-like behaviors. There were no significant differences in CACNA1C mRNA expression between groups in rat hippocampus, but mRNA fold change in risperidone group is higher than others.

Conclusion: The results of our study indicate that Ketamine-induced hippocampal damage and treatment with clozapine and risperidone is not related to CACNA1C mRNA expression.

Keywords: Schizophrenia, Ketamine, Clozapine, Risperidone, Social interaction test, Real-time PCR, Beta-actine, CACNA1C

P-423

The evaluation of Covid-19 virus hot spot areas

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Background: Coronavirus 2019 or Covid-19, which causes acute respiratory syndrome and it is affecting 213 countries and territories around the world. Although ,according to worldomete website the number of confirmed cases to the date 5 Sep 2020 have been reported over 29 million , of which 879866 were killed and over 100,000 viral genomic sequences of nCOV-19 shared via GISAID website .Considering the mortality statistics related infected people around the world and the consequent mutagenicity of this virus, we also decided to investigate the mutagenic points in this virus because to make a diagnostic kit and vaccine to treat and prevent this disease, we need a stable part of the virus sequence.

Methods: In this study, 145 mutations were studied from 27/1/2020 till 30/5/2020 for a population of 82 consisting of 53 males and 39 females from 9 Asian countries. In this study GIASAID, Worldometers, Gene, Nucleotide databases were used.

Results: Our study showed that out of this number, 39 males and 22 females had mutant sequences. Most mutations were observed in E-ORF7b - ORF7a - ORF1ab - M - N - ORF3a - ORF10 - S - ORF8 - ORF6 sequences, respectively, and the most nucleotide changes occurred in the cytosine and guanine bases, respectively, that were converted to thymine. In some sequences such as 29461 to 29860, more mutations occurred in men, and in some sequences such as 7741 to 8401, the most mutations were observed in women and no mutation was observed in the sequence of E gene. Also more mutations were present in guanine and cytosine.

Conclusion: Based on studies, the sequence of E gene is recommended as the appropriate sequence for studying of diagnostic kit and vaccine.

Keywords: Mapping, covid-19 virus, hot spot area, causes acute respiratory syndrome

P-424

The effect of omega-3 polyunsaturated fatty acids supplementation on NRF2 and Sestrin2 gene expression and antioxidant status in patients with type 2 diabetes: a randomized placebo-controlled double-blind clinical trial

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Background: NRF2 is a transcription factor that induces the expression of several proteins with antioxidant properties such as Sestrin2 and is therefore considered as the major regulator of anti-oxidative defense systemThe aim of this research was to study the effect of supplementation with n-3 PUFAs on the antioxidant status and the gene expression of NRF2 and Sestrin2 in patients with type 2 diabetes mellitus. Participants: Sixty patients with type 2 diabetes mellitus were enrolled in a placebo-controlled, double-blind, randomized trial.

Methods: The participants were randomly allocated to two intervention groups receiving either n-3 PUFAs (2700mg/day)(n=30) or placebo soft gels containig 900 mg of edible paraffin (n=30). The main outcome measures were the expression of Sestrin2 and NRF2 gene which were assessed in peripheral blood mononuclear cell. After RNA extraction and cDNA synthesis by realtime PCR. Total antioxidant status in plasma samples was also measured based on the ferric reducing ability of plasma.

Results: NRF2 gene expression was significantly increased in n-3 PUFAs supplemented subjects, compared with the placebo group. Plasma total antioxidant status was also significantly augmented in n-3 PUFA-supplemented subjects. Sestrin2 gene expression was not significantly affected by n-3 PUFA supplementation although a slight up-regulation was observed.

Conclusion: Supplementation with n-3 PUFAs enhanced NRF2 gene expression and improved overall antioxidant capacity and thus might be considered beneficial in the amelioration of oxidative stress and prevention of type 2 diabetes mellitus complications.

Keywords: NRF2, SESTRIN2, ROS, diabetes, antioxidant defense, n-3 PUFA ,omega-3

P-425

Clinical applications of comet assay inside the forensic investigation

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Background: A tremendously huge breakthrough has emerged recently in the applying of laboratory and clinical methods in order to determine and detect crimes committed based on the undergone toll and damages across the world. Two Swedish Scientists known as Ostling and Johanson (1984) manipulated a new manner which contributes to evaluate the DNA damage and its degradation as time goes by, called comet assay that is actually said that the comet-like degraded DNA is substantially the origin which this name is emitted from.

Methods: Through this technique the degradation associated with the DNA structure could be qualitatively and quantitatively measured and visualized as well, following the required steps, including sample preparation, alkaline lysis, DNA unwinding, alkaline electrophoresis and the subsequent process of neutralization and staining. Initially the cells are mixed with low melting agarose (LMA) and then immobilized on the comet slide, treated with lysis and alkaline buffer to unwind DNA and undergo alkaline electrophoresis as well as visualization across using specific intercalating dye respectively. Additionally, Comet-assay in combination with PNA-FISH could reliably determine mutagen-induced DNA damage as well as specific repeat sequences within the damaged DNA of the particular cells being investigated.

Results: This occurred DNA degradation will function as a molecular clock which particularly aids to reach an estimation of postmortem interval (PMI), time since death (TSD) or the precise time crime committed using single-cell gel electrophoresis (SCGE) or comet assay. Alongside the detection of an indispensably robust correlation between DNA fragmentation and PMI or the people involved in crime, this versatile method is also capable of assessing exerted DNA damages in myriad clinical disorders.

Conclusion: Although the typical comet assay is only able to reveal strand breaks and alkali-labile sites and accompanying other shortcomings as well, with the judicious application, its utility surpasses its potential drawbacks incessantly.

Keywords: DNA degradation, Comet assay, PMI, SCGE, PNA-FISH

P-426

Fluorescence Spectrometry Studies on the Interaction of CuO Nanoparticles with Bovine Liver Catalase

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Background: Different nanoparticles such as CuO nanoparticle (CuO NPs) led to generate oxidative stress and deregulate normal cellular activities, which subsequently leads to cellular toxicity. Bovine liver Catalase (BLC) plays an important role in defending the cell against oxidative. That is used in medicine for prevent oxidative damages associated with some pathophysiological situations, including inflammatory diseases, aging, and cancer. Therefore, this research study aims to investigate the interaction between BLC and nanoparticles.

Methods: The structural change and interaction between CuO NPs and BLC are investigated under the physiological condition using fluorescence spectroscopy at 298 and 310 K. The quenching constants, binding constant as well as thermodynamic parameters are calculated and the interaction mechanism is also suggested.

Results: Fluorescence data represented the decreasing in intrinsic emission of enzyme with increase in CuO NPs concentrations, which indicates that changes have been done at three dimensional environments around the enzyme chromophore. These results demonstrated that fluorescence quenching of CuO NPs occur through a static mechanism. Negative values of ΔG is obtained suggests that the interaction of the CuONPs with BLC is spontaneous. The negative thermodynamic parameters of enthalpy (ΔH) and entropy (ΔS), suggest that the binding reaction is mainly mediated by van der Waals forces and hydrogen bonds. The binding constants and the number of binding sites at different temperatures are also calculated, and it is found that a single binding site exists.

Conclusion: The analysis of fluorescence data indicated the presence of static quenching mechanism in the binding. The negative values of enthalpy (ΔH) and entropy (ΔS) of CuONPs- BLC complexation indicate that the binding is mainly enthalpy stabilized and the entropy destabilized.

Keywords: Catalase, Spectrofluorimeter, CuO nanoparticle

P-427

NiO Nanoparticles Change the Structure of Bovine Liver Catalase

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Background: Enzymes are bio-macromolecules which attract a lot of attention because of their catalytic activity in biochemical and chemical reactions. Therefore, structural studies of enzymes in the present of different cosolvents such as inorganic nanoparticles are necessary it can help better design nanocomposites fused in diagnostics, drug delivery and cell monitoring. Bovine liver catalase is a component of the anti-oxidative defense system acting in tissues against hydrogen peroxide, which is a deleterious reactive species that oxidizes cellular molecules.

Methods: In this work, we used fluorescence spectroscopic methods for investigating the changes in tertiary structure and binding properties of enzyme at different temperatures based on the changes in intrinsic emission in the present of NiO nanoparticles. Fluorescence techniques are great aids in this study because of their high sensitivity, rapidity, and ease of implementation. The quenching constants and thermodynamic parameters are calculated, and the interaction mechanism is also suggested.

Results: The fluorescence intensity of enzyme decreases regularly with the addition of NiO nanoparticles, which indicates that an interaction between the enzyme and nanoparticles occurs. The results showed that the catalase fluorescence was quenched by nanoparticles through the static quenching mechanism. The negative values of enthalpy (ΔH), entropy (ΔS) and (ΔG) of NiO nanoparticles and BLC complexation are obtained. The binding constants and the number of binding sites are calculated, and it is found that a single binding site exists.

Conclusion: The results show that the Stern–Volmer quenching constant (KSV) in all cases is inversely proportional to temperature, indicating that the possible quenching mechanism is initiated by static quenching. The fact that all negative values of thermodynamic parameters suggests that the van der Waal’s interactions and hydrogen bonds play major role in the protein ligand interaction.

Keywords: NiO nanoparticle, Bovine Liver Catalase, structural changes

P-428

Detection of a new Potato Virus Y strain in Iran

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Background: Potato virus Y (PVY) is the type member of the Potyvirus genus in Potyviridae family and causes economic losses in potato. PVY genome consists of a single-stranded positive-sense RNA about 9700 nt in length. PVY isolates from potato consist of several strains with various pathogenesis properties. Three non-recombinant PVY strains (PVYo, PVYn, and PVYc) have been reported from potato and other crop fields of Iran. In this study, we detected a new PVY strain by molecular typing using RT-PCR.

Methods: Nineteen PVY isolates were collected from potato fields and the surrounding weeds in Markazi and Hamadan provinces (Center and West Iran), during 2018-2020. All isolates were propagated on Nicotiana tabacum cv. Samsun. The strain type of each PVY isolated was determined using two approaches; first, by serological profiling with M.Ab specific to PVYo, PVYn, and PVYc (Neogen, UK) and second, using RT-PCR with two differentiating primer sets.

Results: One PVY isolate (PVY-G16) was identified as a new strain. In RT-PCR typing assay using the primers and protocol described by Lorenzen et al. 2006 a single about 260 bp fragment was amplified for PVY-G16 showing a possible PVYo strain, however, it did not produce any amplicon in RT-PCR typing assay by primers described by Chick-Ali et al., 2013, showing a novel undescribed PVY genotype. PVY-G16 was isolated from Physalis floridana, a common weed in the visited regions. PVY-G16 produced mosaic symptoms in Nicotiana tabacum cv. Samsun after mechanical inoculation. Nucleotide sequence data of the CI gene region of PVY-G16 showed a small fragment with 100% nucleotide identity to PVYo strain.

Conclusion: Both serological and molecular RT-PCR typing assays show that PVY-G16 is a new undescribed strain of PVY in Iran. In further studies, it is necessary to determine its full genome sequence and pathogenesis properties.

Keywords: Potyvirus, Molecular Typing, PVYo, PVYn, PVYc

P-429

Preparation and in vitro evaluation of photodynamic therapy of anti EGFR targeted curcumin loaded PLGA nanoparticles

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Background: One Approach to increase the therapeutic efficacy is drug encapsulation in nanoparticles (NPs). One of the biodegradable polymeric NPs is poly (D, L-lactic-co-glycolic acid) (PLGA) NPs. Epidermal growth factor receptor variant III (EGFRvIII) is tumor specific and highly immunogenic, thus, it can be used as a target for targeted drug delivery toward tumor cells. The major aim of this study was to develop anti-EGFRvIII monoclonal antibody (MAb) conjugated to CUR loaded PLGA NPs to enhance the targeting specificity and photootoxic effect of CUR on EGFRvIII-overexpressing cell line.

Methods: In this study, CUR was encapsulated in PLGA NPs using the nanopercipitation method. The carboxylic groups on the surface of the PLGA NPs were covalently conjugated to the amino groups of a monoclonal antibody against EGFRvIII (A-EGFRvIII-f). The prepared NPs were fully characterized by Zetasizer, scanning electron microscope (SEM), differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR), and then entrapment efficiency (EE), drug loading efficiency (DLE), CUR release, cell internalization, intrinsic cytotoxicity, and phototoxicity were evaluated.

Results: Non-targeted and targeted CUR-PLGA NPs showed particle size of 280 ± 16 nm and 251 ± 3 nm, polydispersity index of 0.1 and 0.1, entrapment efficiency of 66% and 27% and, drug loading efficiency of 11% and 4.5% respectively. Targeted CUR-PLGA NPs showed higher cytotoxicity in comparison to the non-targeted CUR-PLGA NPs on DKMGvIII cells (EGFRvIII overexpressed human glioma cell line). The selective cellular internalization of targeted PLGA NPs by DKMGvIII cells in comparison with DKMG low (low expressed EGFRvIII human glioma cell line) was also confirmed. The targeted CUR-PLGA NPs were able to show 2.4-fold more effective photodynamic effect on the cancer cells as compared to non-targeted CUR-PLGA NPs.

Conclusion: These results suggest anti-EGFRvIII MAb-targeted NPs as a potential drug delivery system for PDT treatments in overexpressed EGFRvIII tumor cells.

Keywords: Curcumin, PLGA nanoparticles, EGFRvIII monoclonal antibody, DKMGvIII cell line, PDT

P-430

Modified Methodology of Amniotic Membrane Fixation in Tissue Engineering

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Background: Many variables can be substituted to increase the fixation quality of the specimens to ensure that the texture images are realistic. The only way is to compare the results of living cell studies. Considering the wide range of fixators and also more attention to the use of Amniotic Membrane (AM) in various fields, it seems that introduction of the best and most suitable fixative for AM is necessary by investigating the effects of the most common fixators used in pathology of AM.

Methods: The Amniotic Membrane was obtained from maternity ward of Gha'em Hospital which was attached to placenta. After being prepared, it has been divided into six parts to be fixed in different fixatives as following with most recommended time managing in articles; methanol -20°c in 10 minutes, methanol in 20 minutes, acetone in 5 minutes, paraformaldehyde 4% in 10 minutes, paraformaldehyde 4% in 15 minutes, formalin in 10 minutes. Then the effects of fixators were examined using Hematoxylin and Eosin staining.

Results: The images showed that employed fixators had different effects on the texture of AM due to their chemical features. Acetone had the most destructive effect and methanol represented the most realistic images of AM.

Conclusion: Although the type of selective fixator may vary depending on the purpose of the study on a tissue, from the point of view of preserving tissue morphology, it has been shown that methanol as a coagulative fixator at -20 °c for 10 minutes is the most suitable fixator for the AM.

Keywords: Amniotic Membrane, fixator, histopathology

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Exosomes Derived from Human Wharton' jelly of Mesenchymal Stem Cells Alleviate Liver Fibrosis

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Background: Liver fibrosis is a wound healing complication that is responsible for various chronic liver diseases characterized by the accumulation of extracellular matrix. Hepatic stellate cells (HSCs) are the main target cells in liver fibrosis. Transforming growth factor beta-1 (TGFβ1) has been identified as a major profibrogenic mediator and plays an important role in HSCs activation and subsequent liver fibrosis. Recently, Mesenchymal Stem Cells (MSCs) have been proposed as an effective treatment for liver disease. Therefore, in this study, the effect of exosomes isolated from Wharton' jelly of the MSCs (WJ-MSCs) was used in the treatment of liver fibrosis.

Methods: In this study, we used human TGF-β1 to treat LX-2 cells, an immortalized human HSCs line (gifted by Professor Scott Friedman). Then, we examined the expression level of genes involved in the activation of HSCs, including collagen I, E-cadherin, α-SMA and GAPDH. After isolation of WJ-MSCs, differentiation into osteogenic and adipogenic as well as flowcytometry were used to confirm. Then, the exosomes of WJ-MSCs were purified, and after determination of protein concentration, they were used to treat LX-2 cells.

Results: LX2 cells were treated with 10 ng/ml TGF-β1 for 72h. TGF-β1 increased α-SMA and collagen I expression and decreased E-cadherin expression. Then, treatment with exosomes isolated from WJ-MSCs at a concentration of 40 μg/ml was performed. After 24 h, E-cadherin expression increased and α-SMA and collagen I expression decreased compared to the control group ($p<0.05$).

Conclusion: The paracrine effects of MSCs are very effective on tissue repair. Exosomes of cells can reduce hepatic fibrosis by acting on the TGFβ1 signaling pathway and participate in liver regeneration.

Keywords: WJ-MSCs, LX-2 cells, Exosomes, E-cadherin, α-SMA and collagen I

P-432

The relationship between TTV and HPV!

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Background: Torque teno virus or transfusion-transmitted virus (TTV) is a non-envelopedvirus with single strand circular DNA genome that currently is classified in the Alphatorquevirus genus and the family of Anelloviridae. Unlike other DNA viruses, TTV has an extremely wide genomic diversity, so that five main cladesand more than 35 genotypes have already been detected. This virus, based on previous studies, infects both healthy people, and those who have HCV and HPV. In this study we tried to examine the virus incidence in 150 cervical specimen, which containes 50 high risk HPV, 50 low risk HPV, and 50 samples without HPV.

Methods: Cervical specimen were collected from 150 women referred to Dena laboratory in Tehran. Viral DNA was extracted from samples by High Pure Viral Nucleic Acid kit according to manufacturer’s protocol. Then, nested polymerase chain reaction (Nested PCR) was performed using specific primers, which replicated 5’-UTR region of TTV genome. Observation of an amplified fragment equal 220 bp in the second step of PCR was considered as the positive result. Nested PCR products purified and sequenced using a DNA sequencing system.

Results: From 50 cervical specimen without HPV, 14 specimen were TTV positive (%28), and from 50 Low Risk HPV cervical specimen 23 were TTV positive (%46), and from 50 Highrisk HPV cervical specimen 48 were TTV positive (%96).

Conclusion: The number of virus in high risk HPV cases is more than low risk HPV cases, and in low risk cases it is more than healthy ones. Furthur studies need to consider TTV role in high risk HPV sarcoma

Keywords: TTV, HPV, Cervical, PCR

P-433

Association Between cardiometabolic risk factor and responsiveness to vitamin D supplementation: A New Approach using Artificial Neural Network analysis

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Background: Accumulating data have highlighted the prominence of supplementation as an effective approach for vitamin D deficiency. But individuals vary in their response to vitamin D supplementation. In this study, the effect of cardiometabolic risk factors were evaluate on magnitude of response to vitamin D supplementation by using novel statistical analysis, artificial neural networks(ANNs).

Methods: 608 participants aged between 12 to 19 years old were assed in this prospective interventional study. Nine vitamin D capsules containing 50000IU vitamin D/weekly were given to all participants over the 9 week period. The change in serum 25(OH)D level was calculated as the difference between post-supplementation and basal levels. Suitable ANNs model were selected between different algorithms in the hidden and output layers and different numbers of neurons in the hidden layer. Then, the major determinants in predicting response to vitamin D supplementations were identified (Trial registration: IRCT201509047117N7; 2015-11-25; retrospectively registered)

Results: Sigmoid in both hidden and output layers with 4 hidden neurons had acceptable sensitivity, specificity and accuracy area under the ROC curve in our study. Baseline serum vitamin D (30.4%), waist to hip ratio (10.5%), BMI (10.5%), systolic blood pressure (8%), heart rate (6.4%), and waist circumference (6.1%) were the greatest importance in predicting the response in serum vitamin D levels.

Conclusion: We provide the first attempt to relate anthropometric specific recommendations to attain serum vitamin D targets. With the exception of cardiometabolic risk factor, the relative importance of other factors and the mechanisms by which these factors may affect the response requires further analysis in future studies.

Keywords: waist to hip ratio, adolescent girls, Artificial Neural Network, waist circumference

P-424

Jaw bone changes in panoramic radiography in patients with hyperparathyroidism in Babol

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Background: One of the important functions of the kidney is the regulatory effect on bone metabolism by inducing the active vitamin D. Hypocalcemia occurs as a result of phosphate retention and loss of calcium in the kidneys in chronic renal failure. This condition stimulates the secretion of parathyroid hormone and leads to secondary hyperparathyroidism.

Methods: This descriptive cross-sectional study was performed on 17 healthy individuals and 17 patients with Hyperparathyroidism in the dialysis center of Shahid Beheshti Hospital in Babol in 1397 by the census. If patients need and have 2 or more decayed teeth, the dentist was referred to a specialized maxillofacial clinic for free for panoramic imaging. MI, MCI, LD, Bone change, Manaliblar cortical resorption, and General cortical them indices were examined.

Results: The results showed that there was a statistically significant difference between MI, the cortical bone thickness of the ion region, and visibility of the lower dental canal in the hyperparathyroidism group and the control group ($P <0.05$). On the other hand, in the hyperparathyroidism group and the control group, changes in bone density and LD vision did not show a significant difference ($P> 0.05$).

Conclusion: In this study, in Hyperparathyroidism compared to healthy individuals, a decrease in the thickness of cortical bone in the guinea pig, mental region, and lack of dental canal was seen. Therefore, the dentist should be aware of the radiographic symptoms of these patients so that in case of any of the mentioned indices, it will be useful and effective in diagnosing general health and treating patients.

Keywords: Jaw bone, panoramic radiography, hyperparathyroidism

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P-435

MicroRNA as Novel Diagnosis Biomarkers in Endometriosis Patients

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Background: Endometriosis is the most prevalent gynecological disease among women in reproductive age. The disease is one of the main causes of infertility and has a tremendous negative effect on women life quality. Despite all its destructive economic effects on the health care system, a non-invasive method to diagnose affected women properly and efficiently has not been identified yet. Laparoscopy remains the only definite way to diagnose and treatment of endometriosis, which results in several years of delay and intensification symptoms of the disease. Therefore, it is inevitable to find and validate a non-invasive diagnostic method with sufficient specificity and sensitivity. Increasing attention toward miRNAs as a regulator of gene expression with high stability in various tissues turns them into an attractive possible biomarker.

Methods: This study conducted to assess miRNAs diagnostic utility to distinguish endometriosis patients from non-endometriosis ones. Three selected databases: PubMed, Embase and, ProQuest, were searched comprehensively on 7 Jan 2020. We included original articles of any study design except in-vivo and in-vitro studies. The evidence quality of the included studies was assessed using QUADAS-2. Data collection was conducted by four authors separately.

Results: Databases search resulted in 5,492 articles; after screening the articles title and abstract, 181 articles were identified as eligible. According to the full-text screening, 62 articles were included in our systematic review. Over 100 differentially expressed miRNAs in endometriosis patients were found, of which the applicability of only 19 miRNAs was re-assessed in further studies to confirm them as a biomarker.

Conclusions: miRNAs appear to be appropriated candidates for utilized as a biomarker for non-invasive diagnostic of endometriosis; however, it is necessary to conduct more research to find a miRNA or panel of them with acceptable sensitivity and specificity before they can be suggested in diagnostic approach.

Keywords: Endometriosis, miRNA, non-invasive, diagnostic method

P-436

Effects of thymus honey on intestinal barrier leakiness and intestinal tight junction proteins of claudin-1 and ZO-1 in rats exposed to chronic mild unpredictable stress

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Background: A growing body of evidence has highlighted a clear link between chronic stress and enhanced intestinal permeability in the pathogenesis of systemic and neural diseases. Under stress conditions, a perturbed intestinal barrier results in enhanced bacteria translocation from intestinal lumen into blood stream influencing peripheral tissue.

Methods: Here, we investigated the therapeutic effects of honey on ileal tight junction (TJ) protein complex, claudin-1 and ZO-1 and serum LPS following chronic unpredictable mild stress (CUMS) by Western blotting and ELISA kit. Male rats were subjected to CUMS for 28 consecutive days. Honey (0.2 and 2 g/kg/day, by gavage) was administered pretreatment (10 day) and during stress.

Results: Honey reduced stress-induced LPS elevation by preventing reduction in the intestinal TJ proteins of claudin-1 and ZO-1.

Conclusion: Together, our study indicated that honey protects against stress-induced intestinal damage by modulating intestinal barrier protection via TJ protein complex expression.

Keywords: Honey, Intestinal permeability, Intestinal TJ proteins

P-437

Study the effect of resveratrol on cell proliferation rate comparing with cisplatin effects in a prostate cancer cell line (LNCaP)

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Background: Resveratrol (3, 4', 5 trihyroxy-trans-stilben) is dietary polyphenol derived from grape, berries, peanuts and other plant sources. Resveratrol is increasing in prominence because it has preventive and anti-cancer properties. It has also been shown that resveratrol can reverse drug resistance in a variety of tumor cell by sensitizing them to chemotherapeutic agent. Cisplatin is also used to treat cancer. The aim of this study was to investigate the anti-cancer effect of resveratrol comparing with cisplatin.

Methods: Prostate cancer cells were cultured in DMEM medium containing FBS. Then the cancer cells were exposed to different concentration of cisplatin and resveratrol for 24 hours separately. After treatment with FBS the cancer cells were washed and trypsinized and the cells suspension was prepared. Finally, the cells were stained with trypan blue and the dead and alive cells were counted. In addition, MDA as an index of lipid peroxidation was measured in different culture media of treated cells.

Results: Resveratrol showed cytotoxic effect in cell proliferation assay in different concentrations; however, it was less than those of cisplatin. MDA was also reduced in treated cancer cells with cisplatin. Both effects of resveratrol were increased by increasing its concentration.

Conclusion: The results of this study showed that resveratrol reduced the survival rate of prostate cancer cells and as a plant antioxidant, reduced the lipid peroxidation in the cancer cell line. To reveal more details study on other cancer cell lines and determination of other related factors are required.

Keywords: Resveratrol, Cisplatin, Prostate cancer, Lipid peroxidation

P-438

A duplex RT-PCR assay for simultaneous detection of BNYVV and BBSV

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Background: Soilborne viruses cause serious diseases and yield reduction in sugar beet crop. This is the case of Beet necrotic yellow vein virus (BNYVV) and Beet black scorch virus (BBSV), which both are transmitted by soilborne plasmodiophorid Polymyxa betae and Olpidium brassicae, respectively. In fields with infested soils by BNYVV and/or BBSV, only resistant sugar beet cultivars can be surviving and susceptible cultivars suffer from serious crop losses. It is necessary to use rapid and economically cost effective diagnostic methods like Duplex RT-PCR for the detection of BNYVV and/or BBSV in soil samples.

Methods: BNYVV and BBSV infested soils were obtained from our previous works which stored in the Iranian Research Institute of Plant Protection. Susceptible sugar beet cultivar (Jolgeh) was cultivated in each soil sample and after 45 days, the lateral roots of these bait plants were used for RNA extraction using TriReagent. Primers BN-F and BN-R were designed to amplify a 550 bp fragment for BNYVV and primers BB-F and BB-R were designed according to the published BBSV sequences which amplify a 300 bp DNA fragment. RT-PCR parameters were optimized by adjusting PCR mix and program.

Results: Our optimized PCR protocol amplified DNA fragments with the expected size 550 and 300 bp for BNYVV and BBSV, respectively. In comparison to uniplex RT-PCR method, our duplex assay showed similar detection sensitivity. It could detect both BNYVV and BBSV in naturally infected sugar beets.

Conclusion: The duplex RT-PCR assay optimized in this study presents a rapid and sensitive method for the simultaneous detection of BNYVV and BBSV, and it will reduce the time and cost of the routine detection tests in sugar beet breeding and production programs.

Keywords: Beet necrotic yellow vein virus, Beet black scorch virus

P-439

Isolation and identification of glutathione peroxidase (GPX) gene in grass pea (Lathyrus Sativus)

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Background: Glutathione peroxidase is an antioxidant enzyme family with peroxidase activity that protecting the organism from oxidative damage and development and stress tolerance are its main biological role.

Methods: In this study, the GPX gene was isolated and identified in Grass pea (Lathyrus sativus L.), a pulse crop, for the first time. Therefore, total RNA was extracted from leaves using the Ribospin Plant kit, cDNA of interest gene was synthesized and used as a template in a polymerase chain reaction (PCR). Primers were designed according to the conserved regions of this gene in other plants. Then, the GPX gene was amplified by PCR. The PCR product was analyzed by electrophoresis for insert size, amplification quality, and quantity. Consequently, the gene was sequenced. Multiple sequence alignment and sequence homology analyses were carried out with software from NCBI. A neighbor-joining phylogenetic tree was constructed using MEGA6 software.

Results: PCR results indicated the existence of this gene and amplification of 490 nucleotides fragment belonging to the glutathione peroxidase gene. This gene was recorded in NCBI with GenBank accession MT210155. Glutathione peroxidase homolog was 99 identical to Cicer arietinum and 90% identical to Medicago truncatula. Phylogenetic analysis showed that GPX was grouped into a branch with Cicer arietinum.

Conclusion: Our study provides the first data regarding GPx gene identification in Lathyrus sativus.

Keywords: Grass pea, Lathyrus sativus, Glutathione peroxidase (GPX), Homology

P-440

The role of DREB transcription factor in drought stress in Lathyrus sativus

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Background: In plants, abiotic stresses such as drought adversely influence not only survival, biomass production but also crop yield. Some transcription factors have been identified that regulate the expression of several genes related to stress. One class of the transcription factors is DREB/CBF that binds to drought-responsive cis-acting elements.

Methods: To characterize the role of this type of transcription factor (DREB) in drought stress conditions, we analyzed the relative expression of this gene in Grass pea (Lathyrus sativus L.) as the hardiest crops for adaptation to abiotic stress. Therefore, grass pea (Lathyrus sativus L.) seeds were grown in polyethylene pots in three different drought treatments (100, 50, and 25% of field capacity – FC). Real-time quantitative PCR was conducted with Real-time PCR System. Relative expression levels were calculated using the using 2-ΔΔCT.

Results: The relative expression level of the DREB gene surged with continuing stress treatment (mild and severe stresses) compared to normal conditions (100% FC).

Conclusion: This result indicates that DREB as a stress-responsive transcription factor, can be used for the production of stress-tolerant transgenic crops.

Keywords: Lathyrus sativus, DREB transcription factor, gene expression, Real-Time PCR

P-441

Survey on effects of gum, soft skin and nuts of Akbari pistachio ethyl acetate extract on some enzymes and biochemical factors arising from ''Cisplatin'' in serum of male Wistar rats

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Background: Cisplatin is an anti-cancer drug that may be used in cancers to apply its effects by harming the DNA of cells by disrupting anti-oxidant in cells. Cisplatin has some hazard side effects on non-cancerous cells, such as disorder on the activity of SOD and GPX and concentration of MDA. Akbari pistachio plant has lots of anti-oxidant materials in its essential oil which can help cells recover themselves. This study presents the effects of gum, soft skin and nuts of Akbari pistachio ethyl acetate extract in male Wistar rats taken a single dose of Cisplatin, to comparison their recovery after that.

Methods: In this research study, 72 male rats were randomly divided into 12 groups and treated for 15 days by this arrangement: 1: Control group 2: one unit of nuts extract 3: two units of nuts extract 4: one unit of soft skin extract 5: two units of soft skin extract 6: one unit of gum extract 7: a single dose of Cisplatin in 10th day 8: one unit of nuts extract + a single dose of Cisplatin 9: two units of nuts extract + a single dose of Cisplatin 10: one unit of soft skin extract + a single dose of Cisplatin 11: two units of soft skin extract + a single dose of Cisplatin 12: one unit of gum extract + a single dose of Cisplatin. On the 16th day, serum of the rats was sampled and the activity of SOD and GPX and concentration of MDA was measured and compared with the Chi-squared test by SPSS.

Results: Gum extract could reduce the amounts of MDA and rise the activity of SOD and GPX in comparison to group 7.

Conclusion: In conclusion, pistachio gum, nuts and soft skin extracts are considerable herbal medicines as a supportive factor helping someone who wants to get better after treatment by Cisplatin.

Keywords: cancer ،Cicplatin ،Akbari pistachio ،SOD ،MDA ،GPX.

P-442

Investigation of cytotoxic effect and biological activity of a novel derivative of cisplatin on hepato cellular carcinoma cell line (HepG2)

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Background: Liver cancer is a malignancy which either initially originates from the liver itself or is a secondary metastasized cancer originating from other. Chemotherapy with cisplatin is one of the available treatments against liver cancer, however, numerous side effects limit its therapeutic use. Therefore, in recent years, many efforts have been made to synthesize new platinum-based complexes to increase their anticancer effects, reduce their side effects and avoid drug-resistance. In the present study, the biological activities of a novel platinum-based complex [Pt(ppy)(PPh2py-k1P)(SR)] with potent antitumor effects were investigated on HepG2 liver cancer cell line compared with cisplatin as a standard drug. MTT assay, flow cytometric-based apoptosis and cell cycle assays were employed.

Results: MTT assay results showed that compound **2a** (IC50 = 12.70 ± 4.10 µM) could inhibit HepG2 cells proliferation in lower concentrations compared to cisplatin (IC50 = 23.00 ± 1.98 µM). Apoptosis assay also showed that with an increase in the concentration of **2a**, the percentage of cells in the early-stages of apoptosis gradually increased from 7.24% in untreated cells to 18.68%, 23.63% in cells treated with 24 and 48 µM doses. Different concentrations of compound **2a** also induced an arrest in HepG2 cells at the G1-stage of the cell cycle.

Conclusion: Based on the structure, comparing **2a** compound with cisplatin indicates that the presence of two phosphorus nuclei may provide stronger binding to DNA and enhance the antitumor cytotoxicity effects of platinum. This inhibition is partly through inducing programmed cell death in cancer cells as well as arresting cells at the G1 stage of the cell cycle though determining the exact mechanism requires further in-vitro and in-vitro studies. The results also suggested **2a** as a candidate for further studies in the field of cancer treatment.

Keywords: Liver cancer, Anti-cancer drugs, Platinum-based complex, Cytotoxicity, Apoptosis, Cell cycle

P-443

Evaluation of anti-diabetic effects of L-lysine and Lysulin in streptozotocin-induced diabetic rats

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Background: Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia. Chronic hyperglycemia increases the non-enzymatic glycation of various biomacromolecules, which cause an alteration in the structure and function of proteins and increases oxidative stress. We previously showed the L-lysine beneficial effects, as a chemical chaperone, on reducing the diabetic complications in different diabetic rat models and *in vitro*. Considering the successful results of using L-lysine, as well as vitamin C and zinc antioxidant activities, it was decided to evaluate the beneficial effects of a combination of these three compounds, which was named Lysulin, and compared with L-lysine alone, *in vivo*.

Methods: The streptozotocin (50 mg/kg body weight) was injected intraperitoneally into adult rats to induce diabetes. Then, six groups of normal or diabetic rats (# 6 in each group) were treated with 0.1% of L-lysine or equal amount of L-lysine in Lysulin, dissolved into the drinking water, or vehicle. Body weight, serum glucose, advanced glycation end product (AGEs), hemoglobin A1C (HbA1c), insulin, lipid profile, and some other parameters were determined in the serum/ plasma samples.

Results: The study showed that both Lysulin and Lysine improved body weight of the rats. They also refined hyperglycemia by reducing glucose and HbA1c, lowered the elevated AGEs and improved lipoprotein abnormalities.

Conclusions: The combination of vitamin C and zinc with lysine in the form of lysulin lowered the diabetic complications better than L-lysine alone, in diabetic rats. It is not only as a hyperglycemia modifier and glycation inhibitor but also as an enhancer of antioxidant capacity impairment.

Keywords: Streptozotocin, Adult Rats, Diabetic Complications, AGEs

P-444

Optimization of different methods of RNA extraction in tea plant under drought stress

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Background: Achieving high quality results in molecular studies of plants such as Real-Time PCR, RNA Sequencing, and other popular methods, depends on the quantity and quality of extracted RNA. There are several standard methods for extracting RNA from plant tissues, However, RNA extraction in plants with a high content of secondary metabolites such as phenolic compounds is a challenging task, because of the disruption and deposition of these compounds with RNA. In tea plant (Camellia sinensis), due to its very high polyphenolic content (25-35% dry weight), RNA extraction is not performed well using conventional methods. Therefore, finding a suitable RNA extraction method will be the first step in advancing the molecular study of tea.

Methods: In this research work, we used five methods including two types of extraction kits, two methods of modified extraction kits, and the modified method of Jaakola et al. (2001) for extracting RNA from tea leaves under drought stress.

Results: The achieved results showed that the RNA, extracted by the modified method of Jaakola et al. (2001) with a few changes of high quantity and quality (with a wavelength of 260/280 nm) is in the acceptable range of 1.8-2. Besides, the highest amount of RNA with good quality was obtained from 100 mg of fresh leaf tissue at 861 ng /µl, which has been obtained using the Jaakola et al. technique, which was superior to the other four methods.

Conclusion: The results of the experiments showed that the method of Jaakola et al. with a few changes could be the right solution for RNA extraction in tea plants.

Keywords: RNA extraction, plant tissue, poly phenolic compounds, tea plant, Camellia sinensis, optimization, drought stress

P-445

Solvent effect on antioxidant properties of citrus peels

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Background: The growing interest in the replacement of synthetic antioxidants with natural ones has directed many research interests toward the plant-derived raw materials. Special attention is focused on inexpensive or residual sources from food agricultural industries. Fruit peels are valuable wastes obtained from domestic and industrial sources. The potential of fruit wastes as sources of natural antioxidants was explored in the present research. The aim of this study was to determine the antioxidant activity of citrus peels after their extraction using various solvents.

Methods: The dried citrus peels were ground into a fine powder and extracted using different solvents, including methanol, acetone and methanol-acetone. The antioxidant capacity of the different extracts was evaluated through their free radical scavenging activity on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

Results: According to the results, various extracts of citrus peels showed strongly scavenging activity on DPPH radical. The IC50 value for methanol, acetone and acetone-methanol extracts were 29.35, 17.44 and 6.46 mg/ml, respectively.

Conclusion: This study demonstrated that citrus peels could serve as potential sources of antioxidants in the food and pharmaceutical industries.

Keywords: Citrus peels, Natural antioxidant, DPPH scavenging activity, Solvent effects

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In silico and *in vivo* investigation of the effect of zinc oxide nanoparticle on the paraoxonase1 activity and structure

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Background: The paraoxonase1 (PON1) enzyme prevents oxidation of LDL and is able to delay of atherosclerosis progression. One of the most widely used nanoparticles in food industries and medical products is zinc oxide nanoparticles (ZnO NP). In the present study, through *in vivo* and *in silico* methods the effect of ZnO NP on PON1 activity and structure has been investigated.

Methods: The molecular dynamics (MD) of the PON1 in the presence of ZnO NP were simulated at 50 nanoseconds (ns) and compared in the Free State by Gromacs v.5.1.2 software package to obtain RMSD, RMSF, and Rg parameters. For *in vivo* study, three groups of male Wistar rats were selected (each group=10) including the two groups treated (Subcutaneous injection) with 150 and 200 mg/kg doses of ZnO NP alongside a control group. Serum enzyme activities were measured through spectrophotometric assay with phenylacetate as a substrate.

Results: The analysis results of MD simulation have indicated that the values of RMSD and RMSF analysis of PON1 in the presence of ZnO NP increased in comparison to the Free State while Rg value decreased. *In silico* results indicated that ZnO NP has caused alteration and instability in the protein structure of the PON1. Spectrophotometric assay for measurement of arylesterase activity of the enzyme indicated the activity of the serum PON1 in rats that treated with 150 & 200 mg/kg doses of ZnO NP has reduced significantly ($p<0.05$) compared to the control group.

Conclusion: Alteration in the protein structure of PON1 and reduction in its enzyme activity (based on the results) demonstrate that ZnO NP could be a reason to reduce the protective effects of PON1. Therefore, the people who are continuously exposed to ZnO NP may be at risk of rapid progression of atherosclerosis and cardiovascular diseases.

Keywords: Paraoxonase1, Atherosclerosis, Nanoparticle, Molecular dynamic simulation

P-447

Biochemical analysis of taxus baccata extracts

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Background: Oxidative stress is resulted from an imbalance between pro-oxidant and antioxidant defense systems, leading to the formation of toxic forms of oxygen and other free radicals. Different types of oxidative stress can cause the development of various chronic disorders, including atherosclerosis, cancer, aging, neurodegenerative disorders (Alzheimer and Parkinson's disease) and type 2 diabetes. Natural antioxidants in medicinal plants are potential candidates to prevent oxidative stress and reduce its harmful effects. This study was devoted to the determination of antioxidant activity of Taxus baccata using various extraction solvents.

Methods: The air-dried and grounded leaves Taxus baccata were extracted using three solvents: methanol, acetone and acetone-methanol. The antioxidant activity of the extracts was measured by investigating their DPPH radical scavenging potential.

Results: The results showed that various extracts of Taxus baccata have significant potential in DPPH free radical scavenging. The IC50 value for methanol, acetone and acetone-methanol extracts were 26.22, 9.98 and 2.28 mg/ml, respectively.

Conclusion: Based on the results, it is suggested that Taxus baccata can be considered as a promising source of natural antioxidant agents for the management of oxidative damage and pharmaceutical and food purposes.

Keywords: Taxus Baccata, Reactive oxygen species, Antioxidant activity

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The effect of eryngium billardieri extract on oxidative stress in Wistar rats liver

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Background: Eryngium Billardieri, a native plant in Iran with a long history of being used as an antidiabetic agent. However, its mechanism of action has not yet been fully investigated. This study examines the effects of hydroalcoholic eryngium billardieri (EEB) extract in the Wistar rat model of type 2 diabetes.

Methods: Forty-two male Wistar rats (215±15 g) were divided randomly into two groups: normal control group (NCG; n=6) and Test group (TG; n=36). The control group was fed on a normal diet whilst the Test group was fed on a high fat diet (HFD) for two weeks before the administration of streptozotocin 30mg/kg IP. Diabetes mellitus (FBG ≥ 150 mg/dl) was confirmed in TG but not NCG a week later. Animals were then randomly divided into six groups, each containing 6 animals as outlined below: diabetic control group (DCG), diabetic+100 mg/kg extract (EEB-L), diabetic+300 mg/kg extract (EEB-M), diabetic+500 mg/kg extract (EEB-H), diabetic +2.5 mg/kg glibenclamide (Glib), diabetic+200 mg/kg metformin (MET). All animals were treated daily for 10 weeks by oral gavage with their selected investigational products (dosing volume not exceeding 1ml). At the end of the study, biochemical assessments including total oxidant and total antioxidant status, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels were measured.

Results: At the end of the study, SOD, CAT levels significantly decreased in DCG compared with NCG, whereas MDA levels were increased ($p<0.01$). There were significant ($p<0.05$) increases in SOD, CAT levels in EEB-H compared but not in EEB-L or EEB-M groups. The increase in SOD and CAT levels in EEB-H groups were significantly higher than those seen in MET and Glib groups.

Conclusion: This study indicated that a high dose (500mg/kg) of eryngium billardiari extract markedly improved oxidative stress in an animal model of diabetes to a greater extent than metformin or glibenclamide.

Keywords: Diabetes mellitus, Eryngium Billardieri, Oxidative stress, Metformin, Glibenclamide

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Autophagic activity in peripheral blood mononuclear cells of type 2 diabetic patients compared with non-diabetic patients

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Background: Defective autophagic activity in specific peripheral tissues is involved in the pathogenesis of T2D. The purpose of the present study was to evaluate autophagy in PBMCs of T2D patients compared with non-diabetic (ND) subjects and its relationship with their biochemical characteristics.

Methods: Autophagic genes expression and protein markers, p-mTOR, and p-AMPK levels in PBMCs were measured by real-time PCR and/or western blot.

Results: Our results showed that the mRNA expression of BECN1 and LAMP2 were significantly lower in PBMCs of patients with T2D compared to controls. The MAP1LC3B, ATG5 expression, and p-AMPK level did not differ between the two groups. The protein level of LC3B-II (± NH_4Cl) was significantly decreased in PBMCs of T2D patients in comparison to the ND group. The levels of the p62/SQSTM1 and p-mTOR were significantly increased in PBMCs of T2D patients. Insulin levels were significantly correlated with BECN1 transcription levels (r=0.509, p=0.044), LC3B-II ± NH_4Cl (r=-0.545, p=0.029 and r=-0.682, p=0.004, respectively), p62 protein levels (r=0.758, p=0.001), the p-mTOR level (r=0.724, p=0.002), and p-AMPK level (r=-0.558, p=0.025) in PBMCs of the total study population. Moreover, HbA1c levels were negatively correlated with BECN1 gene expression (r=0.523, p=0.038) and positively correlated with p62 protein levels (r=0.824, p<0.001) in PBMCs.

Conclusion: Our data revealed that autophagic activity was reduced in PBMCs of T2D patients and was associated with long-term hyperglycemia, and/or hyperinsulinemia and the activation of the mTOR signaling.

Keywords: Autophagy, Type 2 diabetes, Peripheral blood mononuclear cells, Insulin.

P-450

Enhancing Cellular Uptake of Oligoarginine Peptides by p-sulfonatocalix [4] arene

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Background: Unlike proteins and enzymes, peptides are highly dynamic molecules and do not possess a defined three-dimensional structure. Cell-penetrating peptides (CPPs) are capable of wrapping around a cargo-molecule and transport it through the cell membrane. Oligoarginine peptides with the length of 6-8 residues are well known CPPs. However, synthesis of them is with low yield and difficult to purify.

Methods: In this study, we synthesized a shorter sequence of oligoarginine by only four residues. The tetra-arginine peptide was fluorescently labeled. We enhanced its poor cellular uptake by incubating it with p-sulfonatocalix [4] arene (CX4). CX4 molecules bind to arginine side chains and drive the entire peptide into live cells. The peptides J-RRRR (1) and J-DDDD (2) were synthesized with a solid phase peptide synthesis method using Fmoc strategy. J stands for (5/6)-carboxyfluorescein and is a fluorescent probe. We have measured the binding strength of 1 with CX4 by outlining its isothermal binding curve previously explained. 1, 2 and 1/CX4 were incubated with MCF-7 cells for entering them. The Cellular uptake was assayed by fluorescence microscopy and flow cytometry.

Results: The binding constant of 1/CX4 is Ka = 1.07x106 M-1; while, 2 did not bind to CX4. Fluorescent microscopy images confirmed that only 1/CX4 entered the cells while 1 itself is incapable of passing through the cell membrane. The 2 remained neutral; it did not enter the cells nor it affected the cells adversely.

Conclusion: Calixarenes enter cells by passive diffusion and improve cellular internalization of its cargo, peptide 1. CX4 eliminates necrosis effect of 1 on the cells. Cellular uptake of CX4 cannot be explained by its negative electrostatic charges as 2 is an anionic peptide but did not internalize.

Keywords: Cell-penetrating peptides, Oligoarginine, electric charge, p-sulfonatocalix[4]arene

P-451

Synthesis of iron nanoparticles from aqueous extract of Saponaria officinalis and evaluation of its antioxidant activity

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Background: Oxidative stress is a significant problem for centuries and the origin of various diseases from cataracts to cancer. Free radicals are atoms or molecules with a single electron that is remarkably reactive and can be irreversible damage to vital molecules in organisms. Antioxidants are capable to protect biological systems against these elements. Iron nanoparticle (Fe-NPs) has antimicrobial, anticancer, and antioxidant properties. This paper describes the antioxidant activities of green synthesis Fe-NPs.

Methods: The nanoparticle of Fe-NPs was synthesis through employing the aqueous leaves extract of Saponaria officinalis. Antioxidant activity of iron nanoparticles in the range of 0-250 µg/ml was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Ascorbic acid (vitamin C) was considered for positive control. Finally, the absorption of samples was measured at 517 nm.

Results: Antioxidant activity of Fe3O4 nanoparticles was measured by DPPH. The DPPH radical scavenging effect of 250 µg/mL Fe3O4-NPs was about 56.25 %, while standard samples at the same concentration showed 87.5 % radical scavenging effect. By increasing the concentration of nanoparticles, the percentage of radical scavenging increased.

Conclusion: The present study demonstrated that the aquatic extract of Saponaria officinalis has a natural antioxidant source and might be exploited for functional foods and nutraceutical applications.

Keywords: Iron nanoparticle, Saponaria officinalis, Antioxidant activity, DPPH

P-452

Astaxanthin attenuates Methotrexate-induced nephrotoxicity in rats

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Background: Methotrexate (MTX) is an anti-metabolite used widely for a variety of tumors and autoimmune conditions. Clinical uses of MTX were severely limited by its concomitant renal intoxication. Astaxanthin is a powerful antioxidant and possesses several beneficial effects against various human diseases and physiological disorders. This experimental study aimed to evaluate the effect of Astaxanthin on methotrexate-induced renal toxicity in rat's kidneys.

Methods: Thirty-six male Wistar rats weighing 230 ± 8.5 g were selected and randomly divided into six groups. The intoxication was done with methotrexate (MTX) on three different days 6, 8, and 10 after the start of the course. In the treatment groups, they were treated with astaxanthin (ASX) for ten days from the beginning of the course (oral gavage). They include; normal control, ASX control (75 mg/kg), MTX control (10 mg/kg), MTX (10 mg/kg)+ASX (25 mg/kg), MTX (10 mg/kg)+ASX (50 mg/kg), and MTX (10 mg/kg)+ASX (75 mg/kg). At the end of the experiment, the activities of superoxide dismutase (SOD) and catalase (CAT) and the levels of malondialdehyde (MDA) as well as serum urea and creatinine, were measured.

Results: Methotrexate significantly increased tissue MDA levels similar to serum urea and creatinine, while significantly decreases SOD and catalase activity compared with controls ($p < 0.05$). Astaxanthin administration was associated with a significant decrease in the level of MDA as like as serum urea and creatinine and an increase in SOD and CAT activity compared to MTX-treated rats ($p < 0.05$).

Conclusion: This study indicated that astaxanthin, through its antioxidant action, alleviates methotrexate-induced oxidative damage, therefore co-administration of astaxanthin with MTX is a reasonable therapeutic strategy for attenuation of MTX -induced nephrotoxicity.

Keywords: Nephrotoxicity, Astaxanthin, Methotrexate

P-453

Isolation, purification and biochemical characterization of a new microbial transglutaminase (MTGase) from Streptomyces fradiae

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Background: Transglutaminase (TGase) belongs to a large family of intracellular and extracellular enzymes that catalyze cross-linking between protein molecules. In this study; isolation, purification, biochemical characterization of a new microbial transglutaminase from Streptomyces fradiae was studied.

Methods: The colonies were firstly screened qualitatively using filter paper disc (FPD), and then the enzyme activity was evaluated by quantitative assay.

Results: One factor of culture medium optimization showed that the best medium for bacterial growth S. fradiae is ISP NO.4 medium which is supplemented by peptone 1%, casein 1%, glycerol 1%, and yeast extract 1%. Furthermore, the maximum microbial TGase (MTGase) production in the culture medium was achieved at 27 ºC and pH 7. SDS-PAGE analysis revealed a molecular mass of 35 kDa for the purified enzyme. Likewise, MTGase obtained from S. fradiae an optimal activity at 50 ºC and pH 8.5.

Conclusion: Based on the features of the purified enzyme, it may be used in various industrial processes to improve the strength, viscosity, elasticity and water holding capacity of products in the food and pharmaceutical industries. MTGase can also be used as a blood clot and meat glue.

Keywords: Transglutaminase, Streptomyces fradiae, purification, characterization

P-454

The effect of exogenous urokinase plasminogen activator and its inhibitor on pulpal derived mesenchymal stem cell proliferation

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Background: Mesenchymal stem cells (MSC) are well known for their role in tissue regeneration and tissue engineering. Their capability of self-renew and infinite proliferation properties are critical for tissue repair processes. Urokinase plasminogen activator (uPA) is a serine protease secreted from a variety of cell types. By binding to its cell surface receptor elicits intracellular pathways, leading to cell proliferation and migration. However, it is yet to be elucidated the effect of exogenous uPA and its inhibitor on growth parameter of dental pulp stem cells.

Methods: Human dental pulps were isolated from 3 intact molar teeth followed by culturing in the alfa-MEM medium containing 10% FBS. Cells were characterized for stem cell surface markers of CD 90, CD105, CD73 and lack of CD45 using flow cytometry analysis. Also, cells were examined for their capacity to differentiate towards different lineage (chondrogenic, adipogenic and osteogenic). MTT assay were used to study cell proliferation for which cells were seeded in 96 well plates and after 24h were incubated with different concentrations of uPA (0.5, 1, 2, 4, 8 nM) and amiloride (0.05, 0.1, 0.2, 0.4, 0.8mM) for 3 days.

Results: Cells were positive for CD 90, CD105, CD73 and negative for CD45. They were capable of differentiating to osteoblast, chondrocyte and adipocytes. uPAat 2nM induced stem cell proliferation while exposure to its inhibitor, amiloride highly affected cell growths at low concentration and reduce cell proliferation to 80% at 0.05 nM.

Conclusion: Our results indicated the critical role of endogenous urokinase plasminogen activator and the proliferative effect of exogenous uPA on mesenchymal stem cells. The data might suggest the potential utility of this protease in maintaining cell proliferation and stem cell self-renewal in tissue engineering application.

Keywords: Plasminogen Activator Inhibitor, plasminogen activator urokinase, plasminogen activator urokinase receptor

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Determination of serum selenium levels in multiple sclerosis patients and comparison with normal subjects

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Background: Multiple Sclerosis is an autoimmune disease, resulting in chronic inflammatory lesions of nerve in the central neuron system. In this survey, serum selenium levels in multiple sclerosis and normal subjects were measured.

Methods: 70 multiple sclerosis patients and 70 normal subjects were selected. Their venous blood sample collected and separated the serum fraction. Then, the serum selenium levels were measured.

Results: Our results showed that the serum selenium levels in multiple sclerosis patients and normal subjects were 85 ± 40 µmol/L and 130 ± 30 µg/L, respectively.

Conclusion: In this study, it became known that the serum selenium levels in multiple sclerosis patients is lower than normal subjects.

Keywords: Multiple sclerosis, Selenium

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P-456

A Systematic Review: Comparison of loop mediated isothermal amplification (LAMP) and real-time polymerase chain reaction (PCR) assays for the detection of SARS-COV-2 (COVID19)

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Background: Today, with the global prevalence of COVID19 and the importance of its clinical diagnosis, the research for the best and reliable method of diagnosis has increased in various ways. The most commonly used technique is Real Time PCR. However, to perform this procedure, specialists and advanced equipment are required. Our purpose is to provide an alternative and advantageous method instead of the widely-used PCR technique to increase economic benefits, reduce laboratory diagnosis time and ultimately prevent the spread of this murderous disease and save many lives.

Methods: To study the desired subject and find the latest information in this field, we searched various citation databases, including Scopus, PubMed and Google Scholar, and through this, we derived a number of articles and reviewed them.

Results: A novel coronavirus pandemic is the crisis that has plagued almost all countries today, and has forced scientists to detect the most efficient way for accurately screening patients from other individuals. By studying 20 articles in different citation databases, we reached the conclusion that lesser-known LAMP technique has more advantages than Real Time PCR. For instance, LAMP technique, as its name implies, is an isothermal process in which the temperature remains constant. It has a single protocol, so it is faster. It is also cheaper, smaller, simpler and has a higher diagnostic sensitivity. Therefore, it reduces costs and by early diagnosis of patients, it can help break the transmission chain of this infectious disease.

Conclusion: Based on the data obtained from the present study, it can be concluded that LAMP is a much more appropriate technique instead of Real Time PCR because in areas with low facilities and also in a short time, we are easily able to perform it without trained specialists. It is suggested that more attention be paid to this technique.

Keywords: COVID-19, PCR, LAMP, coronavirus pandemic, diagnostic, SARS-CoV-2

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The interaction of ascorbic acid with pepsin: Insights from spectroscopic to molecular dynamics studies

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Background: In recent years, greater attention has been paid to the use of natural antioxidant sources to prevent different diseases. This paper focuses on the interactions between Vitamin C (ascorbic acid), which is one of the most prevalent antioxidant nutrients, with pepsin as a digestive enzyme in the stomach.

Methods: The impact of these interactions will be described by using the spectroscopic technique, circular dichroism technique (CD), molecular docking, and molecular dynamics (MD) simulation methods.

Results: Increased absorption in the UV–Vis spectrophotometry results suggested that ascorbic acid–pepsin complexes were formed. The fluorescence analyses explained that emission intensity decreases significantly with a small red-shift to 342 nm in the emission maximum of pepsin. Negative enthalpy ($\Delta H°$) and entropy ($\Delta S°$) values of the interaction of the ascorbic acid–pepsin show that the binding is mainly for the van der Waals interactions and hydrogen forces. The CD result shows that the α-helix, β-turn, and random coil content decreased, followed by an increase in β-sheet. The ascorbic acid–pepsin complex showed an average RMSD lower than that of the free pepsin system. The result of RMSF shows that the residues involved in the complex contacting are less flexible. The gyration radius (Rg) decreases, which means a more compact.

Conclusion: These studies are beneficial for understanding the safety and biological action of foodstuffs in the body. These findings, give essential results of the binding mechanism of ascorbic acid in living organisms, which are useful in determining the therapeutic effectiveness and biological activity potential such as antioxidant and stomach inhibitory behaviors.

Keywords: Ascorbic acid, Pepsin, Fluorescence quenching, Molecular dynamics (MD) simulation

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A Molecular Simulation and Spectroscopic Approach to the Binding Affinity between Pepsin with Naringenin

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Background: Naringenin is a flavonoid, with a flavanone structure, found especially in citrus fruits, grapefruit, tomatoes, oranges, etc. Naringenin has anti-microbial, anti-oxidant, anti-amnesic, and anti-viral properties. Pepsin is a digestive enzyme in the stomach. This paper proposes the interactions between naringenin and pepsin.

Methods: The binding potential was measured using the spectroscopic and circular dichroism techniques (CD), molecular docking, and molecular dynamics (MD) simulation methods.

Results: It was found that the increase in the pepsin, UV–Vis absorption could be due to the formation of the pepsin-naringenin complexes. The results of fluorescence spectroscopic measurements at the temperatures of 298, 308 and 318 K also demonstrated that naringenin could quench the intrinsic fluorescence of pepsin with the quenching mechanism was static. From the thermodynamic parameters calculated according to the van't Hoff equation, positive enthalpy ($\Delta H°$) and entropy ($\Delta S°$) values of the interaction of naringenin-pepsin show that the binding is mainly for the hydrophobic forces. The stability of pepsin in the absence and presence of multiple concentrations of naringenin was decreased. CD spectra results revealed that naringenin had a partial effect on pepsin structure, which leads to changes in its secondary structure. The naringenin-pepsin complex showed an average RMSD (1.34nm) more than of the free pepsin system (1.33nm). The result of RMSF shows that the residues involved in the complex contacting are less flexible. These observations are compatible with the binding experimental results obtained from spectroscopy methods.

Conclusion: These studies give essential findings of the binding mechanism of naringenin. The findings of this research may be a valuable way to enhance the quality of human life in clinical trials that will be conducted in the future.

Keywords: Naringenin, Pepsin, Fluorescence quenching, Molecular dynamics (MD) simulation

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Bioinformatic Analysis of Bacteria with Protease Activity

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Background: Nowadays, enzymes are widely used as alternatives to chemical catalysts in various industries. Proteolytic enzymes are consumed as models to interpret what relationship is between protein structure and enzyme function. Proteases are one of the three most major industrial enzymes, and include the 60 percent of the worldwide sales.

Methods: In this study, 16srRNA gene-sequencing method was used to identify the isolated species. In the next step, in order to detect the species, the BLASTn database was applied. And, to study the second and third structures of protease enzyme, from the databases https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page = / NPSA / npsa_sopma.html SOPMA and http://www.sbg.bio.ic.ac.uk/phyre PHYRE2 was used. BIBI DATABASE server was used to draw a phylogenetic tree.

Results: After sequencing, the strain was identified, and then by using blasting in the NCBI database and following identifying the bacterial species, the species was recorded on the NCBI site with the access number MT358627. By drawing the phylogenetic tree via BIBI database and examining the phylogenetic tree, it was observed that this strain has the highest overlap with Staphylococcus sciuri T URS0000610641 strain. The evaluation of SOPMA database revealed that the enzyme protease has 201 alpha helices (35.09%), beta turn (7.60%) and random coils (33.33%). According to PHYRE2 software, the percentage of Alpha Helix, beta-strand and the predicted turbulence have been forecasted 40, 26 and 3, respectively. **Conclusion**: Most of the commercial proteases, mainly neutral and alkaline, are produced by organisms belonging to the genus Bacillus. Proteases have a wide range of applications in various industries to make changes in the taste, texture and appearance of the product and waste recycling. In addition, they have a wide range of applications in the food, laundry detergent, leather, bio-environmental processes and pharmaceutical industries.

Keywords: Protease enzyme, Peptidase enzyme, 16srRNA gene, Phylogenetic shrub, BIBI Database, Bioinformatics analysis

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Identification and Screening of Protease-Producing Bacteria

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Background: Protease enzymes (peptidases) are classified in the group of hydrolase (EC 3.4.21-24 and 99) and convert proteins to smaller polypeptides. Their action mechanism occurs by breaking down water molecules. Proteases are involved in many biological functions, including breaking folded proteins, protein catabolism, and cell signaling. Proteases have a catalytic triad in their active site, which includes the three amino acids aspartate, serine, and histidine. The enzymes possess myriad applications in food, laundry detergents, leather making, environmental processes and pharmaceutical industries.

Methods: In this study, 20 bacterial samples were isolated from livestock soils of Kerman province. Protease-producing bacteria were screened on specific solid medium (SMA) based on halo diameter, and 9 bacterial species were identified. The best bacterial species were detected by srDNA16 method, and were delivered to Bionier Company of South Korea and Takapo Zist Company for sequencing. The amount of protease activity at various temperatures and acidity was evaluated.

Results: The results of sequencing and phylogenetic tree matching showed that the similarity of species to the strain Bacillus toyonensis is 97%, and that was registered with access number MT358634 in the gene bank. Enzyme studies expressed that this enzyme has a protease activity in the temperature range of 25-45 °C, most of which at 37 ° C with the average halo diameter 3.75 during 48 hours.

Conclusion: The created halo indicates the activity of protease enzyme and consuming casein as a substrate and producing tyrosine, and that these proteases are of great importance for usage in various industries.

Keywords: Protease enzyme, Bacillus bacteria, Peptidase

P-461

Quercetin Reduces Hepatic Fibrogenesis by Inhibiting TGF-B/Smad3 Signaling Pathway in LX2 Cell Line

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Background: Hepatic fibrosis is a very important stage in the development of liver diseases from the onset of fatty liver to cirrhosis and liver failure. Activation of hepatic stellate cells (HSCs) is a significant phase in hepatic fibrogenesis. Quercetin, a flavonoid found in fruits and vegetables, which has strong anti-inflammatory capacities, but its ability to prevent liver fibrosis is not yet known. In this study, we demonstrated that Quercetin reduces the expression of αSMA and collagen-1 in activated HSCs using TGF-β-induced human HSCs LX2 cell line.

Methods: Human hepatic stellate cells were seeded into well plates for 24 hours. Then the medium was changed to different concentrations of Quercetin, 25, 50, 75, and 100 µM for 24h. For TGF-β stimulations, LX2 cells were activated with 2 ng/ml TGF-β in DMEM supplemented with 1% FBS for 24h results. We used 25, 50, 75, and 100 µM concentrations for further experiments to verify the effects of Quercetin on HSC activation.

Results: The real-time-PCR analysis showed that TGF-β markedly increased the mRNA levels of α-smooth muscle actin (α-SMA), and collagen-1 (COL1A1) which are representative markers of HSC activation that almost completely reversed by Quercetin treatment

Conclusion: In this study, we observed that Quercetin reduced the expression of genes involved in the progression of liver fibrosis including α-SMA, and COL1A1 and disrupted the SMAD3 signaling pathway in activated human HSCs LX2. Inhibition of TGF-β signaling in many studies that have been done so far suggested as a potential mechanism to reduce fibrogenesis.

Keywords: liver fibrosis, hepatic stellate cells, Quercetin, transforming growth factor-beta, Smad3

P-462

The effect of vitamin C intake on BDNF serum levels and consequence effect of BDNF levels on depression in people with depressive disorders

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Background: The aim of this review study was to evaluate the effect of vitamin C on BDNF and the effect of BDNF serum levels on depression in people with depressive disorder.

Methods: Several groups with depressive disorder were randomly selected and divided into two groups of supplement group and control group. The supplement group received 500 grams of vitamin C daily for 4 weeks and the control group received a placebo in the same way. Before and after consumption, BDI_II depression test was taken from the two groups and on the other hand, serum levels of BDNF in them before and after taking vitamin C and placebo were examined by Elisa.

Results: The analysis of the results by R software did not show a significant increase in BDNF serum levels in the supplement group comparing to the control group, on the other hand, the rate of depression in these samples was significantly reduced.

Conclusion: The results of this study showed that vitamin C intake increases serum levels of BDNF and on the other hand showed the effectiveness of increasing BDNF serum levels in reducing depression of these people. The results of this study can be used by relevant physicians as an effective treatment solution for people with depressive disorders.

Keywords: BDNF, Vitamin C, Depression

P-463

The effect of resistance training on BDNF level and the effect of BDNF levels on memory function of the elderly in Guilan province

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Background: BDNF is a member of the neurotrophin family. Evidence suggests that exercise has a significant effect on neurotrophin-induced neuronal growth. BDNF is formed by CNF and peripheral tissues. The aim of this study was to investigate the effect of continuous resistance training sessions on changes in BDNF levels and its performance on the memory of the elderly with short-term memory impairment.

Methods: Fifty elderly people over 55 years old in Guilan province participated in this study, who were divided into two groups of control and experimental (N=25). Wechsler test was used to find the elderly with memory problems. In this study, the experimental group performed resistance training for 30 minutes on alternate days for 4 weeks. Before and after these 4 weeks, BDNF serum levels were determined by Elisa. Finally, the Wechsler test was taken from both groups and the data were analyzed by R data mining software.

Results: The results of this study by R software showed a significant increase in BDNF serum levels in the study group, but no change was observed in the control group. On the other hand, the results of Wechsler test showed a significant improvement in the experimental group.

Conclusion: This study showed that resistance training has an increasing effect on BDNF serum levels in the elderly with short-term memory impairment, and this increase subsequently improves their short term memory performance. The results of this study can be used by relevant physicians to reduce the risk of memory impairment in the elderly.

Keywords: BDNF, Memory function, Resistance training

P-464

An Overview of the Chemicals and Biological Materials Can Be Used to Lead to Damage and Repair of Heart

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Background: Cardiovascular diseases are major causes of mortality in developing countries. One of the challenges during cardiovascular diseases is the poor regenerative ability of the cardiac cells. In this regard, many studies have investigated different ways in order to induce cardiac damages and then regenerate it. Therefore, in this review we collected information about some chemicals used to create damaged models both *in vitro* and *in vivo* as well as explained some methods for repairing the damage of the heart.

Methods: A comprehensive published original articles about cardiovascular diseases were searched in PubMed between the years 2000 and 2020 with the following keywords: “cardiovascular diseases, heart repair, heart damage, chemicals, extracellular vesicles, exosomes, animal model, cardiovascular regeneration, ischemia and ischemic model” in combination or alone.

Results: In this review we reported different approaches of inducing ischemic damage, *in vivo* and *in vitro*, which uses as models to study heart regeneration. Subsequently, we reported different chemicals and methods effective in accelerating cardiovascular regeneration by focusing on signaling pathways. Finally, we mentioned briefly the role of extracellular vesicles in the repair of heart damage.

Conclusion: Because cardiovascular disease is one of the leading causes of death in the world, increasing knowledge about the mechanisms of damage and repair of heart cells is one of the priorities of scientists in the medical sciences. In this regard, damage can be done mechanically or chemically, and although progress has been made today, we must also look for new ways to induce ischemic damages. Similarly, different chemicals and drugs can be used to accelerate cardiac regeneration. Nevertheless, more studies are needed to ensure the performance and safety of the drugs and chemicals produced.

Keywords: Cardiovascular diseases, Heart damage, Ischemia, Extracellular vesicles

P-465

Molecular Detection of Prunus Necrotic Ringspot Virus in Pollen of Apricot

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Background: Prunus necrotic ringspot virus (PNRSV) (genus Ilarvirus, Bromoviridae family) infects a large number of stone fruit and pome fruit trees. PNRSV causes fruit yield losses up to 56% and decreases fruit quality. In nature, RNRSV is transmitted by infected pollen and seed. PNRSV has been previously reported from Iran, however, in this study PNRSV in apricot (Prunus armeniaca) pollen was detected using RT-PCR.

Methods: PNRSV infected apricot young trees were stored in big pots in the greenhouse condition. Pollens were collected from the trees stored in a greenhouse as well as apricot trees in open gardens (in Tabriz county) and their possible surface contaminations were eliminated by washing them five times using sterile PBS-T with shaking. Total RNA was isolated from pollen using TriReagent and their PNRSV infection was detected using RT-PCR. PN-F and PN-R specific primers were designed to amplify a 400 bp DNA fragment of the PNRSV-CP gene.

Results: In this study, apricot pollen samples from four young trees in the greenhouse and 11 trees in open gardens were tested for their PNRSV infection. In RT-PCR tests, an amplicon with the expected size 400 bp was amplified in two out of four greenhouse-samples (50%) and 7 out of 11 garden-samples (64%). **Conclusion**: Our results showed a considerable PNRSV infection rate of pollens produced by infected apricot trees. Also, the presence of PNRSV inside pollens of apricot was confirmed. Natural transmission of PNRSV by pollens from infected trees to healthy ones should be considered in establishing healthy nurseries and new gardens.

Keywords: Ilarvirus, Transmission, Prunus armeniaca

P-466

Study of the use of medicinal plants in the treatment of pet cancer

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Background: Cancer, the uncontrolled growth of cells due to genetic or environmental factors, is one of the most serious pet health problems. In methods such as radiation therapy, the target cancer cells are killed using radiation from radioactive materials and chemical compounds. These treatments also destroy normal cells, so they cause many and severe side effects. Control and treatment of cancer with minimal side effects is one of the concerns of veterinarians, among which herbal compounds have been considered.

Methods: In this study, while searching for valid scientific databases and valid case reports, plant compounds effective in controlling and treating cancer in pets have been studied. The effect and performance of 20 medicinal plants including Pennyroyal, Black cohosh, Chrysanthemum, Aloe vera and Isodon rubescens were studied. The use and function of some medicinal plants in the treatment of Lung carcinoma, malignant cervix carcinoma, Ovary adenocarcinoma and a number of other cancers have been proven. The increasing importance of medicinal plants, the vegetation of Iran and fewer side effects than synthetic drugs, requires extensive studies on medicinal plants.

Results: The mechanism of action of most of these substances is related to their antioxidant properties and inhibition of the growth of their tumor cells. The results of the study show that the use of herbal medicines with anti-cancer properties can be used as a substitute or supplement to chemical drugs effective in the treatment of cancer.

Conclusion: Today, laboratory studies have shown that many plants used in traditional medicine contain antioxidants and in animal studies have anti-cancer effects on cell lines. Since the anti-cancer effects of many plants have not yet been carefully studied in the laboratory, and due to the potential of these plants for use in the treatment of various cancers, more laboratory and clinical research is needed in this field

Keywords: Cancer, Herbal medicines, Antioxidant, Chemical drugs, Traditional medicine

P-467

Long non-Coding RNA Based Cancer Assessment

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Background: Cancer diagnosis have mainly relied on the incorporation of molecular biomarkers as part of routine diagnostic tool. The molecular alteration ranges from those involving the DNA, RNA, non-coding RNAs (miRNAs, LncRNAs,) and proteins. The long non-coding RNAs (LncRNAs) are recently discovered non-coding endogenous RNAs that critically regulate the development, invasion and metastasis of cancers. They are altered in cancers and have the potential to serve as diagnostic markers for cancer.

Methods: Comprehensive published original articles in the most common types of cancer were searched in PubMed between the years 2000 and 2018 with the following keywords, “long non-coding RNA”, diagnosis, prognosis, detection, biomarker, “biomarker discovery, cancer, “body fluids such as blood, plasma, serum, urine and saliva” and circulating in combination or alone.

Results: In this review article, we reported the current biomarker-based cancer detection in most 8 common cancers and also the lncRNAs which can be used in cancer detection. LncRNAs are release outside of the cells in the body fluids which makes them to use as a non-invasive method in order to cancer detection or prognosis for example PCA3 in urine, Lnc-PCDH9-13:1 in plasma and saliva, MALAT-1 in plasma of prostate cancer (PCa), Hepatocellular carcinoma (HCC) and Lung cancer (LCs). Besides, some of them have specific tissue expression pattern are more valuable for cancer detection for example PCA3, UCA-1. **Conclusion**: In the field of malignancies we face with two major problems, 1-lack of proper methods or biomarker for early detection and 2-the drug resistance of cancer cells. The current biomarkers for cancer diagnosis do not have proper sensitivity and specificity and may increase the rate of false positives and false negatives.

Keywords: Cancer, Biomarker

P-468

Evaluation of the protective potential of hydroalcoholic extract of Thymus daenensis on acetaminophen-induced nephrotoxicity in rats

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Background: Acetaminophen (APAP) is an antinociceptive and antipyretic drug that can be useful in therapeutic doses, although it can cause serious damage to the kidney if used overdose. The current study aimed to evaluate the protective effect of Thymus daenensis (TD) extract on APAP-induced kidney damage in rats.

Methods: Thirty female Wistar rats were randomly divided into 5 groups: control, APAP (3 g/kg), TD (500 mg/kg), APAPþTD (500 mg/kg), and APAPþN- acetylcysteine (140 mg/kg). The APAP groups received APAP on the 6th day and the rats were sacrificed on the 7th day. Plasma levels of creatinine (Cr) and urea were measured. Ferric reducing antioxidant power (FRAP), nitric oxide (NO) metabolite, total thiol (T-SH), tumor necrosis factor-α (TNF-α) and antioxidant enzymes activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase(CAT) were measured in kidney tissue. The gene expression of TNF-α was also measured by real-time PCR. The histological examination of kidney tissue was also performed.

Results: Results showed that urea, Cr and FRAP markers markedly elevated in the APAP rats compared with the control group. There was a significant decrease in T-SH levels in the APAP animals in comparison with the control group. CAT activity also augmented in the APAP group compared to the control group. Urea and Cr levels were significantly decreased in the APAPþTD group in comparison with the APAP group. The administration of TD extract significantly increased the SOD enzyme activity. Histological findings were improved in the group treated with TD extract.

Conclusion: In general, the results indicate that TD extract can protect against APAP-induced nephrotoxicity by improving biochemical, histological and antioxidant effects. However, more studies are required to determine the mechanism of this extract.

Keywords: Thymus daenensis, Acetaminophen, Nephrotoxicity, Oxidative stress, Nephroprotective

P-469

A Novel Rye Beverage with High Flavonoids and Essential Amino Acids

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Background: Currently the use of functional foods is increasing worldwide. Among others, Rye due to having a considerable amount of nutrients has got much attention scientifically. Hence, in this study, we aimed to produce a grain-based beverage (rye) and determine its amino acids and polyphenols concentrations. The beverage has been produced with two sweeteners of sucrose (B) and apple concentrate (C) and compared with a non-alcoholic malt beverage (A).

Methods: Beverage production was done according to the protocol of the National Standard Organization of Iran. Phenolic compounds and free amino acids (AA) of beverages were analyzed. Freshly prepared rye-based beverages (B and C) along with control malt beverage (A) were subjected to HPLC analysis of amino acids (AA) as described previously by the Bieleski method with minor modifications. The total phenol content of the beverages (B and C) along with control malt beverage (A) was also measured by the Folin-Ciocalteu method.

Results: The phenol content of all beverages (A: 5.3 ± 0.11, B: 16.6 ± 1.09, and C: 14.1± 0.35 mg GAE/ml) was determined. It was found that drink A had the lowest and drink B contained the highest concentration of total phenol. Amino acid analysis showed that both rye-based beverages (B and C) had significantly higher amounts of AA than the malt-based beverage (A). In this study, five amino acids, which are considered essential substances, were screened and all were found to be higher than those in the control beverage. The highest levels of alanine and histidine were found in beverages introduced as beverages B and C, while the lowest levels of lysine were found in beverages A.

Conclusion: Our data suggest that the new rye-based beverage may be beneficial and useful due to higher phenolic content, richer amino acid profile and might be considered for certain health deficiencies.

Keywords: Amino Acids, Beverage, Phenolic compounds, Rye

P-470

Autophagy and Ubiquitination as Two Major Players in Colorectal Cancer: a Systematic Review

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Background: As one of the most commonly diagnosed cancers among men and women, colorectal cancer (CRC) leads to high rates of morbidity and mortality across the globe. Recent anti-CRC therapies are now targeting specific signaling pathways involved in colorectal carcinogenesis. Ubiquitin proteasome system (UPS) and autophagy are two main protein quality control systems, which play major roles in carcinogenesis of colorectal cancer. Balanced function of these two pathways is necessary for the regulation of cell proliferation and cell death. In this systematic review, we will discuss the available evidence regarding the roles of autophagy and ubiquitination in progression and inhibition of CRC.

Methods: The search terms "colorectal cancer" or "colon cancer" or "colorectal carcinoma" or "colon carcinoma" in combination with "ubiquitin proteasome" and "autophagy" were searched in PubMed, Web of Science, and Scopus databases, and also Google Patents (https://patents.google.com) from January 2000 to Feb 2020.

Results: The most important factors involved in UPS and autophagy have been investigated. There are many important factors involved in UPS and autophagy but this systematic review shows the most studies have focused on the role of ATG, 20s proteasome and mTOR in CRC, and the more important factors such as ATG8, FIP200, and TIGAR factors that are effective in the regulation of autophagy in CRC cells have not yet investigated.

Conclusion: Focus on the most important factors involved in UPS and autophagy such as ATG, 20s proteasome and mTOR, ATG8, FIP200, and TIGAR can be considered in drug therapy for controlling or activating autophagy.

Keywords: Autophagy, Ubiquitination, Colorectal Cancer

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FABP1 gene variant is associated with risk of metabolic syndrome

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Background: Metabolic Syndrome (MetS) is defined by a clustering of metabolic abnormalities associated with an increased risk of cardiovascular disease and type 2 diabetes mellitus. There has been an increasing interest in the associations of genetic variants involved in diabetes and obesity in the FABP1 pathway. The relationship between the rs2241883 polymorphism of FABP1 and the risk of MetS remains unclear. We aimed to examine the association between this genetic polymorphism and the presence of MetS and its constituent factors.

Methods: A total of 942 participants were recruited as part of the Mashhad Stroke and Heart Atherosclerosis Disorders (MASHAD study) Cohort. Patients with MetS were identified using the International Diabetes Federation (IDF) criteria (n=406) and those without MetS (n=536) were also recruited. DNA was extracted from peripheral blood samples that was used for genotyping for the FABP1 rs2241883T/C polymorphism using Tetra-Amplification Refractory Mutation System Polymerase Chain Reaction (Tetra-ARMS PCR). Genetic analysis was confirmed by gel electrophoresis and DNA sequencing.

Results: The clinical characteristics of the individuals in the MASHAD study have been analyzed. Using both univariate and multivariate analyses after adjusting for age, sex and physical activity, carriers of C allele (CT/CC genotypes) in FABP1 variant had an increased risk of MetS, compared to non-carriers (OR: 1.38, 95%CI: 1.04,1.82, p=0.026).

Conclusion: The present study shows that C allele in FABP1 variant can be associated with an increased risk of MetS. The evaluation of these factors in a larger population may help further confirm these findings.

Keywords: FABP1, Metabolic syndrome, Genetic variants

P-472

Metformin Attenuates Lipid Accumulation in Hepg2 Cells through Sterol Regulatory Element-Binding Protein 1

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Background: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease that is associated with obesity, type 2 diabetes, hyperlipidemia, hypertension, and metabolic syndrome. While currently, there is no standardized approved treatment has been established and pharmacological treatment of NAFLD is still limited, also, sterol regulatory element binding protein 1(SREBP1) is known to be the master regulator of lipid homeostasis in mammals, we aimed to evaluate whether the effect of metformin on lipid accumulation in HepG2 cells treated with high glucose is related to SREBP1.

Methods: HepG2 cells were treated with high glucose with or without metformin. The expression of the FAS, SREBP-1C and ACC and protein levels CPT-1 and CROT were evaluated using real-time PCR and western blotting, respectively.

Results: Our results showed that metformin (2 mM) significantly decreased lipid content (35%) in HepG2 cells through inhibiting the expression of de novo lipogenesis genes, FAS (50%), SREBP-1C (44%) and ACC (40%) and increased the expression of genes involved in β-oxidation CPT-1 (60%), and CROT (70%) in comparison to the control group.

Conclusion: We in the present study demonstrated that metformin attenuated high glucose-induced lipid accumulation in HepG2 cells by downregulating the expression of SREBP-1C and subsequent increasing fatty acid β-oxidation.

Keywords: Lipogenesis, Metformin, HepG2, High Glucose, SREBP-1C

P-473

Proteomic Profile of 4T1 Murine Breast Cancer Cell Line Before and After Treatment with Saffron Extract and Its Carotenoids, Crocin and Crocetin

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Background: Paying attention to the results of the studies on different medicinal properties of Saffron (Crocus Sativus L.), it is well known that Saffron and its carotenoids, Crocin and Crocetin, show anticancer effects *in vivo* and *in vitro*. There are some mechanisms suggested for this effect such as, cell cycle arrest, induction of apoptosis, etc. But, there still remains the lack of precise information on probable protein expression changes in those cells which are exposed to Saffron active ingredients.

Methods: The 4T1 mouse mammary breast cancer cells were cultured in RPMI medium in proper conditions. The MTT assay was used to measure the 24-hours inhibitory concentration (IC50) of Saffron aqueous extract (SAE), Crocin and Crocetin for 4T1 cells. Then, the cells (cultured in 9 cm plates with approximate %70 confluency) were treated with SAE, Crocin and Crocetin in concentrations equal to their IC50 doses, for 24 hours. Finally, cells were harvested for the purpose of two other proceedings, a) Flow cytometry technique to find the type of cell death (Apoptosis or Necrosis) and measuring the total DNA content for cell cycle analysis, b) Cell lysis and protein extraction for 2-dimentional Electrophoresis (2DE).

Results: The IC50 of SEA, Crocin and Crocetin were obtained 3000, 4500 and 1000 µg/ml, respectively. The data obtained from Flow cytometry technique demonstrated high percentage of Apoptosis induction and the occurrence of cell cycle arrest in all cases. The 2DE also indicated significant changes in the protein expression profiles after the mentioned treatments in comparison with the control, untreated 4T1 cells. Bioinformatic analysis of the 2DE data are doing to find the changed proteins.

Conclusion: SAE, Crocin and Crocetin induced some changes in the protein expression profile of 4T1 mouse mammary cells. The study is continuing to detect the changes proteins.

Keywords: proteomics, 4T1, Crocin, Crocetin, Saffron

P-474

MicroRNA-103 Dysregulation in Type 2 Diabetes Mellitus: Is It a Glucose Homeostasis Riboregulator?

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Background: The regulating role of circulating microRNAs (miRNAs/miRs) has been considered in many types of disease, recently. It has been proposed that some miRs are involved in regulating insulin sensitivity leading to type 2 diabetes mellitus (T2DM) as the most prevalent chronic metabolic disease. We aimed to identify the potential role of miR-103 as a regulator of glucose homeostasis in T2DM and whether it could yield a better prediction, added to clinical and biochemical markers.

Methods: In this case-control study, we evaluated serum miR-103 expression in 33 T2DM participants along with 38 healthy subjects with a quantitative polymerase chain reaction. Fasting plasma glucose, fasting insulin, hemoglobin A1c, cholesterol, and triglyceride levels were measured. Homeostasis model assessment insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were used to investigate basal insulin resistance and sensitivity, respectively. All statistical analyses were performed using SPSS version 24.00 (Mann–Whitney U test was employed to evaluate demographic and clinical differences between groups and receiver operating characteristic (ROC) analysis as well as backward multiple linear regression models to evaluate the potential of miR plasma levels as a predictive biomarker).

Results: In the present study of 71 participants with no significant different BMI, we found that miR-103 levels were slightly lower in T2DM in comparison with healthy ones ($p<0.002$). The miR-103 levels showed a significant association with age, fasting glucose, HbA1c, triglyceride, and cholesterol levels, as well as HOMA-IR and QUICKI indexes.

Conclusion: The results suggest that the miR-103 was downregulated in T2DM patients, indicating that it may be considered as a novel biomarker for the early diagnosis of T2DM. It seems that the evaluation of miR-103 along with the other diagnostic techniques can be used to better understand the pathological sequence for T2DM.

Keywords: MicroRNAs, miR-103, Type 2 Diabetes Mellitus, Insulin Resistance

P-475

Determining the Optimum Length of Homologous Arms (Has) for Lambda Red Recombination in Asaia Spp.

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Background: Annually at least 17 percent of infectious diseases belonged to the vector-borne diseases. Paratransgenesis is a new method to control and fight against pathogen's vectors. In this method, the symbiont agents of vector are recruited for impacting on vector or parasite. The Asaia spp. is a well characterized candidate to use in this method and isolated from different vectors such as Aedes, Anopheles and culex. The Lambda Red Recombination (LRR) system is a potent approach for performing chromosomal genetic manipulation. Using this method, the target sequence is inserted into the host genome by Homologous Arms (HAs). In this study, different lengths of homologous arms was evaluated for performing recombination in the Asaia spp. To find the optimum and shortest arm.

Methods: The functional cassette was contained two homologous arms, kanamycin marker gene, constant promoter and terminator were amplified by SOE-PCR. The 50 bp, 100 bp, 200 bp, 300 bp, 400 bp and 500 bp of homologous arms were created and then dsDNAs with different length were used to perform recombination processes.

Results: The functional cassette with deferent in the length of homologous arms were obtained successfully by SOE-PCR. The recombination was performed for all constructs and observed that the 500 bp length of homologous arm has better efficacy in comparison to others. The 50 bp length has the lowest efficacy. **Conclusion**: we showed that the LRR system is highly efficient in recombination in Asaia spp. Also, this is the first report that the length of humongous arms and its efficacy was evaluated in Asaia.

Keywords: Asaia, paratransgenesis, Lambda Red Recombination (LRR), SOE-PCR

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Transfer the T7 RNA Polymerase Gene into Wild Strain of Asaia Spp. to Use in Paratransgenesis Method for Fight Against Malaria

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Background: Malaria is one of the most important vector-borne diseases. There are different strategies to control the disease and its vector. Paratransgenesis is a new method to control vectors. Asaia spp. is a symbiont of Anopheles that have attractive characteristics to use as a paratransgenesis candidate. There is no T7 RNA polymerase coding sequence in the Asaia genome that leads to some limitations in using of this strain in inquiries. We use Lambda Red Recombination (LRR) system to integrate the T7 polymerase coding sequence in Asaia spp chromosome.

Methods: The functional cassette of our target gene was assembled using the SOE-PCR (Overlap PCR). There were in the designed construct two homologous arms, Kanamycin marker gene, T7 polymerase gene, constant promoter and terminator.

Results: The functional cassette was successfully amplified using specific primers and then assembled by SOE-PCR. The 4975 bp amplicon was used in the recombination process. The recombination was done in the Dead-box protein A (dbpA) loci of Asaia spp. chromosome. The kanamycin screening was performed to confirm the recombination. SDS-PAGE and Western Blot assays confirmed the expression of the T7 sequence.

Conclusion: In this study, the T7 polymerase coding sequence was inserted in the Asaia spp. genome for the first time in the world. This recombinant strain could be used for highly efficient expression of different target genes in the Asaia spp.

Keywords: T7 polymerase, Lambda red recombination, paratransgenesis, Malaria, Asaia

P-477

Diabetic remission after bariatric surgery

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Background: Bariatric surgery improves glucose homeostasis. Levels of plasma insulin concentration can be affected by changing in insulin secretion or insulin clearance after bariatric surgery. Expression of many genes and molecular pathways changes after bariatric surgery. In this study, we mentioned the changes of other gene expressions in obese subjects with type 2 diabetes after bariatric surgery.

Methods: We used PubMed, Google Scholar, Science Direct, Scopus and Springer databases to find our documentation. Based on keywords including bariatric, type 2 diabetes, glp-1, IDE and gene expression, we came across 275 related articles from 2005 to 2020. Finally, 100 articles were selected and studied, a summary of which is given below.

Results: GLP-1 regulates the secretion of insulin. The GLP-1 level increases after bariatric surgery. The two most common bariatric techniques, RYGB and VSG, are associated with elevated bile acids and upregulation of GLP-1. Lymphatic GLP-1 and the GLP-1 receptor (GLP-1r) mediate bile acid-induced glucoregulatory effects via an intestinal Fxr-Glp-1 axis. Many studies proved the use of GLP-1 agonists to glucose homeostasis. However, there is evidence that reduced insulin clearance is likely the primary factor in obesity-induced hyperinsulinemia. Insulin clearance occurs mainly in the liver by the action of insulin-degrading enzyme (IDE), which degrades approximately 50% of insulin in its first passage through the hepatic portal system. The taurine conjugated bile acid tauroursodeoxycholic (TUDCA) has emerged as a possible candidate due to its beneficial effect upon glucose homeostasis.

Conclusion: TUDCA increased IDE expression in human hepatic cell line HepG2. Therapeutic strategies focusing on increased IDE expression and insulin clearance could be helpful in the prevention and/or treatment of T2D, especially when hyperinsulinemia precedes the development of this pathology. So that it is proposed assaying of IDE expression after bariatric surgery.

Keywords: Bariatric surgery, IDE, GLP-1, Gene expression, Diabetic remission

P-478

MiR-181b and miR-204 prevent VSMC proliferation and migration by targeting HCK

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Background: VSMC proliferation and migration, which are affected by different factors, promote plaque formation, resulting in stenosis and restenosis. MicroRNAs, as gene expression regulators, play an important role in controlling the cell proliferation and migration rates. In this study, we aim to investigate the effects of miR-181b, miR-204, and miR-599 on the gene and protein expression levels of hematopoietic cell kinase (HCK) as well cell proliferation and migration rates in VSMCs.

Methods: The VSMCs were transfected by PEI containing the selected microRNAs. Then the HCK gene and protein expression levels were evaluated using RT-qPCR and western blotting techniques, respectively. Moreover, the cellular proliferation and migration were evaluated by MTT method and *in vitro* scratch assay, respectively.

Results: Although miR-599 didn't significantly effect on neither HCK gene nor protein levels of HCK, miR-181b and miR-204 significantly reduced HCK gene and protein (both total and phosphorylated values) expression levels. Data analysis demonstrated that proliferation and migration rates of VSMCs were suppressed by miR-204, miR-181b as well as miR-599.

Conclusion: In conclusion, unlike miR-599, miR-204 and miR-181b inhibit VSMC proliferation and migration by downregulation the gene and protein expression levels of HCK.

Keywords: VSMC, HCK, miR-204, miR-181b, miR-599

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Molecular Detection of Potato Virus Y in Myzus Persicae

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Background: Potato virus Y (PVY) (Potyviridae family, Potyvirus genus) is an economically important virus causing serious yield losses in potato. PVY is transmitted non-persistently by several aphid species. There is a need for a rapid and specific diagnosis method for the detection of PVY transmitting aphids. Current methods are time-consuming. In this study, a simple RT-PCR method was developed to detect PVY in a single aphid.

Methods: Individual aphids (Myzus persicae) were inoculated by PVY during their feeding on infected potato plants. A single aphid was pressed onto the nitrocellulose membrane (1X0.5 cm2) and let to dry. This membrane can be stored at room temperature for at least one week and posted between laboratories. Each membrane was washed in 20 ul DDW, incubated for 2 min at 95C, and centrifuged. The resulting supernatant was used as an RNA template in the RT-PCR test using PVY specific primers designed to amplify a 650 bp DNA fragment.

Results: In 18 out of 20 RT-PCR tests on individual aphids an amplicon with the expected size of 650 bp was amplified. This method was able to detect about 400 to 200 ng of PVY RNA. Storage of aphids in 70% ethanol for 30 days cause no problem in PVY detection.

Conclusion: Here we present a simple method for extracting RNA templates from individual aphids which easily can be used in RT-PCR assays to detect PVY. This method is suitable for testing a high number of aphid samples for monitoring PVY transmission and spread.

Keywords: RT-PCR, Aphid vector, Epidemiology, Virus transmission

P-480

Effect of Methanolic Extract of Spirulina Plathensis on Htr Gene Expression in Human Colorectal Cancer Cell Line

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Background: The shortening of telomere from the 3′ ends of linear chromosomes is described as one of the tumor-suppressor mechanisms and controls the proliferation of eukaryotes cells. In the most cancers reactivate telomerase enzyme. Telomerase is a reverse transcriptase that contains a catalytic protein subunit entitled telomerase reverse transcriptase (TERT) and an essential RNA component recognized as human telomerase RNA (hTR). In this study, we aimed to evaluate the effect of Spirulina methanolic extract on the gene expression of (hTR) in human colorectal cancer cell line SW-742.

Methods: MTT assay was used to determine half maximal inhibitory concentration (IC50) value of the cells treated to different concentrations of methanolic extract of Spirulina plathensis for 72 h. SW742 cells were treated with IC50 concentration of extract for 24, 48, and 72 h. The gene expression of hTR was measured by qRT-PCR.

Results: The IC50 value for extract was 87.63±1.02 μg/ml. Incubation of the cells with methanolic extract of Spirulina plathensis for 24, 48 and 72 h showed a significant decrease in the gene expression of hTR ($p\text{-value} < 0.0001$).

Conclusion: According to the findings of the current study, hTR gene expression in colorectal cell line SW742 inhibits by spirulina methanolic extract.

Keywords: Spirulina plathensis, hTR, Telomerase, SW742, Colorectal cancer.

P-481

Evaluation of serum trace element (Zn, Cu, Se) levels, antioxidant (PON-1, SOD, MDA, TAC) status and their relationship in Hashimoto's thyroiditis patients

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Background: Hashimoto's thyroiditis (HT) is the most prevalent autoimmune thyroid disorder. Thyroid hormones have a crucial physiological role to maintain the balance of metabolism of body. Also, oxidative stress has been implicated in the pathogenesis of several inflammatory and immune-mediated disorders including Hashimoto thyroiditis. Therefore, the present study was aimed to find the changes in serum Zinc, Copper and Selenium levels and also evaluate the effect of HT on body antioxidant status.

Methods: The study people consisted of 86 subjects divided into two groups, 43 people with Hashimoto thyroiditis (HT) and 43 age-matched healthy individuals. This study evaluated total triiodothyronine (T3), total thyroxine (T4), thyroid stimulating hormone (TSH) by ELISA method, Anti-TPO titer using chemiluminescence method, trace element (Se, Cu, Zn) status by atomic absorption spectroscopy and some antioxidant (PON-1, SOD, MDA, TAC) status parameters by specific methods.

Results: The mean TSH and SOD and MDA levels were significantly increased in HT patients (1.56±0.73) compared to the control group (1.09± 0.62). The levels of T4, Se and TAC were significantly lower in HT patients compared to control group ($P<0.05$). However, there was no significant difference in the mean of T3, Zn, Cu and PON-1 between hypothyroidism and control ($P<0.05$).

Conclusion: These outcomes establish the hypothesis that people with HT have elevated oxidative stress and decreased trace elements levels. Therefore, monitoring antioxidant levels and trace element status in HT patients before treatment is important and it can help to the treatment process.

Keywords: Hashimoto's thyroiditis, Zn, Cu, Se

P-482

Evaluation of cytotoxic effect of platinum (IV) and cisplatin on proliferation of EJ138 and main population of HT1080 cells and their Cancer Stem Like Cells

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Background: Cancer Stem Cell (CSC) are the subpopulation of cancer cells which attribute resistance and recurrence of tumors. These cells have self-renewal, differentiation and tumor-initiating properties. Consequently, targeting CSCs seems to be crucial in tumor eradication. In spite of great potency, cisplatin as a broad-spectrum antitumor agent is restricted due to its severe side effects and resistance. Some studies have suggested that platinum (IV) have shown promising selectivity, and could be utilized as an alternative for cisplatin. Here, we evaluated the cytotoxicity effects of a synthesized platinum (IV) agent in comparison with cisplatin in (EJ138) (HT1080) cell lines and Cancer Stem-Like Cells (CSC-LCs) originated from parenteral HT1080.

Methods: In this study, we have investigated the toxicity effects of [Pt(dpyam)Cl4] where dpyam is 2,2'-dipyridylamine, as a platinum (IV) agent. MTT assay was performed to evaluate the cytotoxicity. Moreover, apoptosis and cell cycle flow cytometry were carried out to evaluate the mechanism of drugs toxicity. Finally, to assess the efficacy of cisplatin and Pt (IV) complex on stemness characteristics in CSC-LCs, sphere and colony formation assays were conducted.

Results: IC50 value of cisplatin was considerably lower than Pt (IV) complex. As well as, cisplatin induced apoptosis effectively. Cisplatin induced cell cycle arrest in G2/M in EJ138 and S phase in HT1080 and CSC-LCs. Additionally, Pt (IV) complex induced cell cycle arrest in G2/M, G1 and S phase in EJ138, HT1080 and CSC-LCs, respectively. Then, cisplatin inhibited sphere growth (size) at the IC50 concentration. Also cisplatin was more decreased colony formation in CSC-LC than Pt (IV) complex.

Conclusion: Our results demonstrated that cisplatin displayed the most potent activity in comparison with Pt (IV) complex, but the toxicity of Pt (IV) complex was also reported considerable. Nevertheless, regarding other studies that proposed high selective toxicity of Platinum (IV), they could be a novel candidate for chemotherapy regimens.

Keywords: CSC, Drug resistance, Cisplatin, Platinum (IV)

P-483

Gene Expression Changing in Improving Diabetes after Bariatric Surgery in Patients with Type 2 Diabetes Mellitus

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Background: Bariatric surgery is the most effective therapeutic strategy for obesity and diabetes. Several factors including changes in gene expression affecting the diabetes remission after bariatric surgery. In this study, we review gene expression changes in improving type 2 diabetes after bariatric surgery.

Methods: A literature search was conducted in PubMed, Scopus and Cochrane Library from 2000 to 2020 by using Medical Subject Headings (MeSH): bariatric surgery, diabetes and gene expression for English-language reports.

Results: After bariatric surgery, the expression of many genes changes. Studies show that bariatric surgery increases the expression of ghrelin, transforming growth factor-β (TGF-β), Peptide YY (PYY), Regenerating islets derived (REG) genes and decreases the expression of tumor protein p53 (TP53), gamma-glutamyltransferase 1 (GGT1), toll-like receptors (TLRs), cathelicidin antimicrobial peptide (CAMP), growth differentiation factor 8 (GDF8), growth factor receptor-bound protein 14 (GRB14) genes in obese diabetic patients.

Conclusion: According to the findings of the collected studies, bariatric surgery can affect lipid metabolism, insulin resistance, intestinal hormones, inflammation pathway and body weight by altering the expression of various genes, and thus can be effective in improving patients with type 2 diabetes. However, some of these changes in gene expression have been reported to be dependent and some to be independent of weight loss in patients, and it is unclear to what extent the change in gene expression has directly affected the improvement of glycemic factors in patients.

Keywords: Bariatric surgery, Diabetes, Gene expression

P-484

Cytotoxic effect of Spirulina plathensis ethanolic extract in colorectal cancer cell line through suppression of hTERT

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Background: Telomerase is a ribonucleoprotein complex that consists of human telomerase RNA (hTR) and human telomerase reverse transcriptase (hTERT). Telomerase reactivation in the cell results in carcinogenesis and tumor immortalization. Spirulina platensis (Blue-green alga) is a well-known microalga due to its high nutritional and therapeutic properties. In this study, we investigated the inhibitory effect of Spirulina ethanolic extract on the gene expression of hTERT in colorectal cancer cell line SW742.

Methods: The cytotoxic activity of the extract was measured by means of the methyl thiazolyldiphenyl-tetrazolium bromide (MTT) assay after 72 h treatment. The gene expression of hTERT was quantitatively evaluated by real-time RT–PCR method.

Results: The half-maximal inhibitory concentration (IC50) of Spirulina plathensis ethanolic extract was 13.26±1.5 µg/ml in SW742 cells. This extract significantly attenuated hTERT gene expression at treatment times of 24, 48 and 72h in comparison to untreated control (p-value < 0.0001).

Conclusion: The consequence of our data recommend that Spirulina plathensis ethanolic extract can have an anticancer effect via downregulation of hTERT gene expression.

Keywords: Spirulina plathensis, hTERT, Telomerase, SW742, Colorectal cancer

P-485

Lupeol Reduced the Plasma Level of LDL, Cholesterol and Insulin Resistance Index in Mice with Non-Alcoholic Fatty Liver Disease

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Background: Nonalcoholic fatty liver disease (NAFLD) is one of the major health concerns worldwide. NAFLD is a complex metabolic condition and recognized as the main cause of liver-related mortality. In this study, the therapeutic effects of lupeol as a dietary triterpene was investigated on high fat/high fructose-induced NAFLD in mice.

Methods: NAFLD was induced in mice using a high fat/high fructose diet for 12 weeks. Following NAFLD induction in test groups, the animals were divided into 3 groups (n=10) as Sham, metformin (MET) and Lupeol (LPL). In control group animals received no extra treatment than a normal diet, while sham group received the test compound vehicle. The other two groups of MET and LPL were treated with Metformin (200 mg/kg/day) and Lupeol (40 mg/kg/day) for the next 2 weeks. Twenty-four h after the last treatment, animals were anesthetized and the blood samples were collected. The plasma level of Insulin was determined by using Mouse INS ELISA Kit (USA). Other biochemical biomarkers including the concentration of hepatic enzymes (ALT and AST), and lipid profile (LDL, HDL, total cholesterol and TG) were assessed. Ultimately, the insulin resistance index was calculated according to the conventional formula: IRI = (FBG x FBI)/22.5.

Results: Biochemical analyses revealed that NAFLD induction resulted in a significant ($p<0.05$) elevation of LDL, total cholesterol, FBG, FBI and IRI and non-significant ($p>0.05$) increase of ALT, when compared with the control group. Treatment with MET and LPL for two weeks reduced the NAFLD-increased plasma level of LDL, total Cholesterol, FBG, FBI and IRI significantly. The test compounds were not able to change remarkably in the concentration of HDL, TG and AST.

Conclusion: Results of the current study suggest that LPL might be considered as a potential therapeutic agent in the treatment of NAFLD as it was able to improve the experimentally-induced biochemical alterations.

Keywords: Biochemical Analyses, Hepatic Functional Enzymes, Lipid Profile, Insulin Resistance Index

P-486

Crocetin Supplementation Can Improve Anti-inflammatory Function of HDL in Atherosclerotic Patients.

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Background: Cell, animal and our recent human studies have proven that crocetin causes the improvement of atherosclerosis via multiple mechanisms. Thus, in this study, for the first time, we investigated the effect of crocetin consumption on the HDL proteome profile.

Methods: 15 clinically diagnosed coronary artery diseases (CAD) patients who referred to Shahid Madani Hospital were randomly selected and received 60 capsules containing 10 mg crocetin at the beginning of the study, for oral consumption one capsule per day (IRCT: IRCT20161102030649N1). The patients were followed by weekly calling to perusing the daily consumption of the capsules. The high-density lipoprotein (HDL) was isolated from the plasma of the samples before and after a two-month crocetin supplementation. Then, the proteome profile of the isolated HDLs was determined and compared using 2-D gel electrophoresis followed by bioinformatics analysis and MALDI-TOF mass spectrometry.

Results: Our results indicate that crocetin supplementation resulted in significant anti-inflammatory changes in the HDL proteome profile. Also, paraoxonase 1 and Apo A-IV content of HDL increased (+ 14.93 ± 1.08 and +12.08 ± 1.26; percentages of changes, respectively) after supplementation, however, serum amyloid A decreased (-11.89 ± 1.93 percentage of change) in the isolated HDL fraction of the patient's plasma.

Conclusion: In the present study, based on the observed proteomic changes in HDL profile or lipoproteomics analysis, after a two-month crocetin supplementation (10 mg per day), we can conclude that crocetin improved the anti-inflammatory properties of the HDL in atherosclerotic patients. Therefore, crocetin can be considered as a promising nature-inspired nutraceutical in the management of the CVD risk factors especially HDL functionality and inflammation.

Keywords: Crocetin, Coronary Artery Disease, Lipoproteomics, Paraoxonase 1, Apo A-IV, Serum Amyloid A.

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IL8 mRNA as a potential blood-based biomarker in ulcerative colitis patients

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Background: Early and timely diagnosis of inflammatory bowel disease (IBD) is still a major public health challenge globally. However, lack of sensitivity and imprecision of the currently available biomarkers have impaired the ability to implement potentially effective therapies in a timely manner. The present study aimed to evaluate the clinical utility of expression of IL-8 genes in diagnosis as well as assessment of IBD. **Methods**: This case-control study was performed on 45 patients with IBD (25 ulcerative colitis and 20 crohn's disease) and 45 controls. For this purpose, the RNA of patients and control subjects was extracted and after cDNA synthesis, the quantitative real time PCR (qRT-PCR) method was used to evaluate and express the target gene.

Results: The results of this study showed that IL-8 expression in UC patients was significantly higher than control subjects (p-value: 0.026). While the comparison of IL-8 gene expression in CD subjects did not show a significant increase compared to controls.

Conclusion: IL-8 gene can be used as a biomarker to distinguish between celiac, CD, and UC, as well as UC diagnosis from healthy subjects.

Keywords: IL8, Biomarker, Ulcerative colitis, IBD

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Evaluation of liver enzyme disorders in patients with coronavirus disease 2019 (COVID-19) infection

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Background: There are conflicting results regarding liver enzyme disorders in patients with coronavirus disease 2019 (COVID-19) infection, and these disorders have not yet been clearly investigated. The aim of our study was to describe the clinical characteristics and evaluation of liver enzyme disorders in patients with COVID-19.

Methods: In a retrospective analytical study 598 patients with confirmed COVID-19 pneumonia were divided into two groups, patients with underlying liver diseases and patients without liver diseases. The admission period was from April 5th to Jul 4th. Here in this study, we report the prevalence of underlying conditions and biochemical test results (ALT, AST) in the study participants.

Results: 81 (8%) of patients had underlying liver disease. The frequency of diarrhea and vomiting of disease was significantly higher among patients with liver disease g. (P <0.05). ALT enzyme level was significantly higher among patients with liver diseases (54.5±45.6 vs 37.1±28.4), P=0.013. Moreover, the AST enzyme level was significantly higher among patients with liver diseases (41.4±27.2 vs 29.2±24.3), P=0.028. A statistically significant difference for the level bilirubin was not present in either group.

Conclusion: The presence of underlying liver disease should be considered as one of the poor prognostic factors for worse outcomes in the COVID-19 infected population. Liver enzymes were significantly impaired in liver patients with COVID 19 compared with none liver patients.

Keywords: Liver enzyme disorders, Liver diseases, Coronavirus disease 2019

P-489

Effects of human amniotic fluid mesenchymal stem cells condition medium on secretion of enzymes involved in Amyloid β protein degradation in SH-SY5Y human neuroblastoma cell lines

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Background: Alzheimer's disease (AD) is one of the most common neurological disorders, with clinical signs of selective cognition, especially those associated with memory decline. One cause of AD is the deposition of beta amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) in the brain, which results in synaptic dysfunction and neurodegeneration.

Methods: The amniotic fluid mesenchymal stem cell conditioned medium (AF-MSCs-CM) sample was collected from the placenta with the conscious consent of the pregnant mothers. Condition media were then used to treat 5 to 12 passages of cells. We treated the sh-sy5y cells and then the levels of ACE and MMP9 enzymes were evaluated.

Results: When cells SH-SY5Y were treated with AF-MSC, the activity of ACE and MMP9 enzymes increased compared to the control group.

Conclusion: According to various studies on the therapeutic potential of AF-MSCs-CM secretions on various diseases and due to the fact that to date no study has been conducted on the potential therapeutic effects of AF-MSCs-CM secretory factors on AD, so in this project aims to determine the effects of these factors on Aβ degrading enzymes and calpain1 protein in the cellular model of AD, which can be used in the future treatment of AD if the desired result is achieved.

Keywords: Alzheimer's disease, Beta amyloid (Aβ), Neurofibrillary tangles, Amniotic fluid mesenchymal stem cell conditioned medium

P-490

Effects of cerium oxide and graphene oxide nanoparticles on oxidative stress in streptozotocin-induced diabetic mice

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Background: Diabetes is a metabolic disease rapidly growing. Oxidative stress is a contributing factor in the pathogenesis of diabetes that its prevention has health benefits. Recently, cerium oxide nanoparticles gained much attention worldwide due to its antioxidant and self-regeneration properties therefore it worth investigating more. The effect of graphene oxide is controversial.

Methods: Thirty rats divided into five groups: control group, diabetic group, the diabetic group treated with 60 mg/kg daily, the diabetic group treated with 10 mg/kg every four days, and the diabetic group treated with 60 mg/kg cerium oxide nanoparticles+10 mg/kg graphene oxide nanoparticles. Nanoparticles were administered orally. Before the induction of diabetes, all rats weighted then diabetes induction performed with a single intraperitoneal injection of 50 mg/kg of streptozotocin. Diabetes induction was confirmed for FBS>300 mg/kg with a glucometer. After 5 weeks, all rats weighted again. Blood samples were collected for biochemical analysis and the liver isolated for oxidative stress parameters measurements.

Results: Cerium oxide and graphene oxide nanoparticles significantly reduced blood glucose, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase(ALP). Total thiol groups (TTM) were reversed by cerium oxide nanoparticles and also the reduction of lipid peroxidation in treatment groups was seen.

Conclusion: Our study showed that cerium oxide nanoparticles are powerful antioxidant and capable candidates for the development of antioxidant strategies, although more animal studies are required. Graphene oxide nanoparticles are controversial.

Keywords: Cerium oxide nanoparticles, Graphene oxide nanoparticles, Diabetes, Oxidative stress

P-491

Palmitic acid and glucose induces hepatic fibrogenesis in activated hepatic stellate cell line

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Background: Hepatic fibrosis is considered integral to the progression of chronic liver diseases. Activation of hepatic stellate cells (HSCs) is the dominant event in hepatic fibrogenesis. We investigated the accumulation of palmitic acid and glucose in HSCs, which accelerates experimental liver fibrosis. TGF-β is a major profibrotic cytokine in the liver that activates HSCs and converts them to myofibroblasts, which increases αSMA and collagen1α expression and excessive accumulation of extracellular matrix and providing conditions for the development and progression of hepatic fibrosis. We investigated whether the accumulation of palmitic acid and glucose could lead to increased TGF-β, followed by activation of stellate cells and increased expression of α-SMA, collagen1α genes.

Methods: Human hepatic stellate cells were seeded into well plates for 24 hours. Then the medium was changed to different concentrations of palmitic acid and glucose, 75 and 150 µM for 24h.

Results: After treated with 75 µM palmitic acid and glucose, there was no significant increase in collagen1α, α-SMA, and TGF-β genes compared to the control group, but the expressions of these genes were significantly increased with 150 µM palmitic acid and glucose ($P< 0.05$).

Conclusion: Palmitic acid and glucose are known to be an important factor in elevating blood lipids and causing inflammation and liver fibrosis disease but its precise mechanism is not known. In this experiment, it was shown that increased palmitic acid and glucose in HSCs can increase markers of hepatic fibrosis onset and provide the context for the onset and progression of liver fibrosis.

Keywords: Liver fibrosis, Palmitic acid, Glucose, TGF-β, collagen-1, HSCs

P-492

Preparation of nano drug carrier for delivery of eosin B as anti malarial drug

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Background: Malaria is one of the serious infectious disease. Eosin B is a potent inhibitor of a variety of drug resistant malarial strains. One possible solution to the problem of antimalarial drugs is to use the encapsulated drugs in liposomes with obvious application in the treatment of infectious disease. This study aimed to develop a novel liposome for delivery of Eosin B as an antimalarial reagent.

Methods: The total amount of lipid (DSPC) and cholesterol were dissolved in chloroform. The organic solvent was evaporated using a rotary evaporator at 150 rpm to form a thin dry film of the components. For hydration, 6 ml of 120 mM calcium acetate solution was added to the contents of the thin layer inside the balloon and the lipid hydration was performed by rotary evaporator. To remove excess calcium acetate by dialysis membrane, the formulations were stirred in MES-HBSS dialysis medium. The resulting suspensions were mixed with 1 ml of Eosin solution and incubated for 60 min in a rotary incubator. Morphology and size of the synthesized liposomes were evaluated by scanning electron microscopy and dynamic light scattering (DLS). Encapsulation efficiency of eosin B evaluated by the centrifuge method. Eosin B release from liposomes was calculated with the dialysis method.

Results: The synthesized liposome showed uniformed size distribution and high efficacy of loading into the liposomal formulation. The optimal particle size, polydispersity index (PDI) and entrapment efficiency (EE) were determined to be 163.3 nm, 0.250 and 69.94%, respectively. Scanning electron microscopy image showed that the liposome acquired spherical morphology with econometric size. The release profile of Eosin B from the liposomal formulation exhibited a sustained manner.

Conclusion: The results of this study highlighted the potential of EosinB loaded liposome as an antimalarial reagent.

Keywords: Nano drug, Liposome, Malaria, Eosin B

P-493

FAK Gene Overexpression in Colorectal Cancer Patients

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Background: Focal adhesion kinase (FAK) protein is a major integrin-dependent tyrosine phosphorylated protein. FAK known as protein tyrosine kinase 2 interacts with other protein kinases Src kinase in particular. The interaction of the structural features of FAK with various kinases can be associated with cancer growth, survival, and metastasis. The role of FAK in cancer progression and metastasis suggests that an increase in FAK expression can lead to the development of colorectal cancer. Because of FAK overexpression levels in human colorectal cancer cells, it is hypothesized that targeting FAK could be a prognostic marker and an anticancer candidate for cancer treatment. Our aim was to evaluate FAK gene expression levels in tumor tissue samples and adjacent normal tissue samples from colorectal cancer patients.

Methods: The expression of FAK was determined by qRT-PCR.

Results: FAK expression in CRC tissues was observably higher than that in adjacent colorectal tissues.

Conclusion: FAK biomarkers may serve as prognostic biomarkers in CRC and should be evaluated in a larger clinical study.

Keywords: Colorectal cancer, Focal adhesion kinase, Gene expression

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P-494

Effects of betanin on gene expression of SIRT1 and AMPK in patients with coronary artery disease: an *ex vivo* study

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Background: Activation of Sirtuin-1 (SIRT1) is a potent approach to the prevention of cardiovascular diseases. There is a dynamic interaction between SIRT1 and adenosine monophosphate protein kinase (AMPK) activation. Betanin, the active component of beetroot, has antioxidant and anti-inflammatory effects. This study evaluated the potential impacts of betanin on some of the atherosclerosis-related gene expression in patients with coronary artery disease (CAD).

Methods: Participants of this trial included 10 CAD patients and 10 healthy subjects. Whole blood was collected in tubes containing EDTA in order to the isolation of peripheral blood mononuclear cells (PBMC) using Ficoll. Then the PBMCs were treated with betanin at doses of 10 and 20 µM for 24-hr. After isolation of RNA, gene expression of SIRT1 and AMPK in PBMCs were assessed by real-time PCR.

Results: A notable elevation in AMPK gene expression level was observed in both healthy and CAD patients after high dose treatment of betanin ($P = 0.008$ and $P < 0.0001$). Betanin in 20 µM could increase the gene expression of SIRT1, 2.40 fold and 4.02 fold in both healthy and CAD patients compared to untreated. There was no significant difference between CAD and the healthy group in the expression of SIRT1 and AMPK genes ($P > 0.05$).

Conclusion: In conclusion, it appears that betanin could be considered as the new candidate in CAD patients by increasing the gene expression of SIRT1 and AMPK.

Keywords: CAD, Betanin, Atherosclerosis

P-495

Association of angiotensin-converting enzyme 2 gene polymorphisms with COVID-19 disease

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Background: The new respiratory disease that caused by SARS-COV2, uses human angiotensin-converting enzyme 2 as receptor for entry the cell. ACE2 native function is regulating blood pressure. Human genetic studies suggest that ACE2 genomic polymorphisms may play important role in susceptibilities to COVID-19.

Methods: We have used PubMed, Google Scholar, Science Direct, and Springer databases to find our documentation, based on keywords.

Results: The previous study showed that some regions or neighboring amino acids in human ACE2 would be involved in the interaction with SARS-COV2. The three variants (rs200180615 and rs140473595 with the AF < 0.01 and rs22285666 with 0.556) were identified in Han Chinese in the Beijing population. An Iranian study predicted that ACE2 variant S331F can increase the susceptibility to viral infection, while V485L may be a natural resistance mutation among the Iranian population. The ACE2 variant rs4646116 that caused K26R mutation more frequently among Caucasians. According to the Al-Mulla study, N720D (rs41303171) and K26R (rs4646116) were the most frequent in the global datasets, and R708W with MAF 0.105%, may indicate a protective role in the Kuwaitis. In Spain, the variants rs41303171 and rs35803318 were observed without any association with SARS-COV2 infection. In Italian study, three missense mutations p. (Asn720Asp), p.(Lys26Arg), and p.(Gly211Arg) identified, another study in 131 Italian patients identified two missense (Asn720Asp and Asp630His) in coding region and c.439+4A>G (rs2285666) in intronic region. Only Asp630His show a statistically different frequency compared to the ethnically population.

Conclusion: Since the outbreak of COVID-19, various studies have been conducted in different populations to identify variants related to COVID-19. These studies are performed to identify susceptible variants and to take preventive measures and therapeutic options. Further studies should be performed in different regions, given the ethnic characteristics of these variants.

Keywords: Angiotensin-converting enzyme 2, polymorphism, COVID-19, Coronavirus

P-496

Betanin could improve the gene expression of PGC1 and NF-kB in patients with coronary artery disease: an ex vivo study

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Background: PGC1a and NF-kB are two important genes, which play critical roles in regulating oxidative stress and inflammation processes. Betanin; the active component of beet root has an antioxidant and anti-inflammatory effects. We purpose to assess the effects of betanin on NF-kB and PGC1a gene expression in coronary artery disease (CAD) patients.

Methods: Participants of this trial included 10 CAD patients and 10 healthy subjects. Whole blood was collected in tubes containing EDTA in order to isolation of peripheral blood mononuclear cells (PBMC) using Ficoll. Then the PBMCs were treated with betanin at doses of 10 and 20 µM for 24-hr. After isolation of RNA, gene expression of PGC1a and NF-kB in PBMCs were assessed by real-time PCR.

Results: A significant elevation in PGC1a level ($P < 0.001$) and a considerable decrease in the expression levels of NF-kB ($P = 0.007$) were observed after treatment with high dose of betanin (20 µM). There was no significant difference between CAD and healthy group in the expression of PGC1a ($P > 0.05$).

Conclusion: Betanin may have beneficial effects on CAD patients by increasing the gene expression of PGC1a and decreasing NF-kB.

Keywords: Betanin, PGC1α, NF-kB, peripheral blood mononuclear cells, coronary artery disease

P-497

Association between miRNAs expression and signaling pathways of oxidative stress in diabetic retinopathy

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Background: Diabetic retinopathy (DR) is a disease that is a major cause of vision reduction in diabetic patients. Hyperglycemia is the major instigator of the development of DR although the role of oxidative stress pathways in the pathogenesis of DR is established. The studies indicate that microRNAs (miRNAs) play an important role in the etiology of DR, thus changes in miRNAs expression levels may be associated with onset and progression of DR. The aim of this article is the investigation of the function and expression levels of target miRNAs in oxidative stress pathway and pathogenesis of DR.

Methods: We systematically searched the PubMed, google scholar, and Scopus database to explore the oxidative stress-related miRNAs and DR between March 2005 and February 2020. After performing the literature search and review, 112 eligible studies were identified.

Results: DR is a multifactorial disease that epigenetics factor could be one of the main contributing participants in its development and progression. Based on evidence, microRNAs are important mediators that regulate gene expression and their dysregulation can be involved in a widespread range of processes such as cell differentiation, proliferation, metabolism, inflammation, angiogenesis and apoptosis. Moreover, it is discriminated miRNAs that have essential roles in both development, survival retinal cells and in their normal functioning.

Conclusion: miRNAs play a critical role in the pathogenesis of diabetes. Circulating miRNAs have emerged as a useful disease marker for DR detection because of their availability and stability. miRNAs may also be therapeutic targets or even therapeutic agents (as anti-miRNAs) that additional studies will help in identifying and assessing their therapeutic potential for the treatment of DR

Keywords: Diabetic retinopathy, miRNAs, oxidative stress

P-498

Analysis of C100T polymorphism of CYP2D6 in predicting the risk of premature coronary artery disease

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Background: The genetic background of premature coronary artery disease (pCAD) is revealed to be multifaceted concerning numerous genes responsible for the metabolism of both endogenous and exogenous molecules. Cytochrome P450 2D6 (CYP2D6) enzyme plays a key role in the inactivation of drugs and reactive species. Therefore, polymorphisms in CYP2D6 may lead to an increased susceptibility to pCAD. Here, we analyzed the association between C100T polymorphism of CYP2D6 gene with pCAD in the Iranian population.

Methods: In the present case-control study, a total of 335 subjects (men under 45 and women under 55 years old) enrolled as 168 pCAD patients and 167 controls. Samples were collected at Shahid Mohammadi teaching hospital, Bandar Abbas, Iran. Prior to the study, informed written consents were provided from contributors, and the study was approved by the ethics committee of Hormozgan University of Medical Science. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for C100T polymorphism.

Results: Genotype frequencies related to C100T polymorphism were determined as 67.7%, 32.3%, and 0% for CC, CT, and TT, respectively in the control group. Furthermore, it was determined as 77.4%, 22%, and 0.6%, respectively in the patient group. Allele frequencies were also calculated in the study population. The C allele in the control and patient groups was 83.8% and 88.3%, respectively, and the T allele was 16.2% in the control group and 11.7% in the patient group. We observed that allelic frequencies for C100T polymorphism of CYP2D6 were not different between case and control groups. Additionally, it was not associated with the risk of pCAD development in the study population.

Conclusion: The present study showed no significant association of C100T polymorphism of CYP2D6 with pCAD in the Iranian population. The probable explanation may be attributed to the differences in ethnic, genetic, environmental backgrounds, and also dissimilar sample sizes.

Keywords: Premature coronary artery disease (pCAD), polymorphism, HbA1c, Cytochrome P450 2D6 (CYP2D6).

P-499

Identification of bacteria with transovarial transfer in Anopheles stephensi (mysorensis strain) to identify new candidates for paratransgenesis method

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Background: Malaria is one of the most important parasitic diseases throughout the world. The Anopheles stephensi is the main vector of malaria in Iran. WHO suggested different strategies for combatting against malaria. Paratransgenesis is one of the effective solutions for developing the Global Malaria Eradication Programme. In paratransgenesis, symbiont microorganisms are manipulating genetically for blocking the parasite life-cycle, affecting the fitness and longevity of vectors. In this study, by sterilizing eggs of Anopheles stephensi and removing surface bacteria, vertically transmitted bacteria were identified in Anopheles stephensi mysorensis strain using the culture-dependent method.

Methods: An. stephensi eggs were collected from the insectary in Pasteur Institute of Iran where they were raised. Sterilization was performed by the Limsopatham method for obtaining the sterile seven generations. The suspension was prepared from the sterile eggs and it was inoculated into the enrichment mediums. Then, dissimilar morphological colonies were selected for performing the differential biochemical tests. Next, PCR was performed by 16s rRNA gene specific primers on isolated clones and finally sequenced.

Results: The results of this study demonstrated that the eggs of microbiota of An. stephensi mosquito were gram negative and gram positive bacteria which include: Serrratia marcescens and Asaia

Conclusion: According to the fact that the paratransgenic technique is an effective method for eradication malaria so, introducing the suitable candidates is essential for developing this technique. Therefore, in this study, the best bacteria candidates in An. stephensi mosquito eggs that have specific features like simple transformation capacity, and transovarial transmission were identified and provided the fundamental data for developing paratransgenic technique.

Keywords: Paratransgenesis, Malaria, Anopheles stephensi

P-500

Molecular evidence of increase in BNYVV titer during mixed infection with BBSV

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Background: Beet necrotic yellow vein virus (BNYVV) infection has been reported from several sugar beet production regions of Iran and it causes Rhizomania disease in this plant. There are little studies about BNYVV titer within host plants in single and mixed infections with other sugar beet infecting viruses, particularly, Beet black scorch virus (BBSV). Both BNYVV and BBSV are soilborne viruses (transmitted by soilborne fungi) and they infect sugar beets cultivated in the infested soils, which cause serious yield losses up to 80%.

Methods: Soil samples in which their single infection by BBSV and BNYVV have been confirmed in our previous works were chosen. Four soil treatments including BNYVV, BBSV, BNYVV+BBSV, and soil without BNYVV and BBSV (negative control) were prepared. Sugar beet cv. Jolgeh, a susceptible cultivar, was sown in the soil treatments. After five weeks, total RNA was extracted from roots, and BNYVV and BBSV virus titers were assayed using semi-quantitative RT-PCR. For this end, specific primers for the coat protein (CP) gene of BNYVV and about one-third of the genomic 3’ end of BBSV were used.

Results: Results of the agarose gel electrophoresis of RT-PCR products showed that the intensity of the DNA amplicon band for BNYVV increases at least two times in mixed infection with BBSV in comparison to single infection. These data were also confirmed by ELISA (data not shown).

Conclusion: BNYVV is an important sugar beet infecting virus causing economic yield losses. Resistant sugar beet cultivars against BNYVV is the main tool for controlling this virus. In this study, we showed that BNYVV titer increases during mixed infection with BBSV. As confirmed for other plant infecting viruses, BNYVV titer increase would be considered a cautious problem particularly in sugar beet breeding programs, because this phenomenon can break resistance in valuable resistant sugar beet cultivars.

Keywords: Sugar beet, RT-PCR, virus, Rhizomania

P-501

Folic acid ameliorates palmitate-induced inflammation through decreasing homocysteine and inhibiting NF-κB pathway in HepG2 cells

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Background: Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver illness world-wide. Studies have reported that the extra lipid accumulation is associated with alteration in one-carbon metabolism during hepatic steatosis. Prevention of inflammation is one of the possible remedy procedure for steatohepatitis during NAFLD. Currently investigations have shown that folic acid (FA) can reduce inflammation in some inflammatory disorders. FA is a B vitamin that acts as a crucial agent in pathways of one-carbon metabolism and provides one-carbon units for the synthesis of nucleic acid, sulfur-containing amino acid, and other methylation processes. In this study, we investigated the FA potency to attenuate the inflammation of palmitate (PA) -treated HepG2 cells and the related signaling pathways.

Methods: The HepG2 cell line was treated with 0.5mM PA as a lipid accumulation agent, and with three concentrations of FA. The mRNA level of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin 1β (IL-1β) genes were evaluated via real-time PCR. The concentration of TNF-α, and IL-6 in cell culture media were determined by ELISA. Homocysteine (Hcy) was assayed via flourimetry. The level of nuclear transcription factor-kappa B (NF-κB) p65 protein was assessed via western blot analyzing. Reactive oxygen species (ROS) amounts were assessed via flow cytometry.

Results: The results indicated that while PA enhances the expression and secretion of TNF-α, IL-6, and IL-1β, and also intracellular ROS level, FA at concentrations of 25, 50, and 75 μg/mL significantly reversed these effects in HepG2 cells. In addition, FA could ameliorate inflammation and decreases ROS production induced by Hcy. Furthermore, FA pretreatment suppress PA-induced (NF-κB) p65 level in PA or Hcy stimulated cells.

Conclusion: Overall, these results suggest that FA reduces inflammation in HepG2 cells through decreasing ROS and Hcy concentration level resulting in inhibiting the NF-κB pathway.

Keywords: Keywords: Nonalcoholic fatty liver disease, Inflammation, Folic acid, Homocysteine

P-502

Curcumin (a constituent of turmeric): New treatment option against COVID-19

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Background: Coronavirus disease 2019 (COVID-19) is a viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Several medications are prescribed to patients, but no definitive treatment is for COVID-19 yet. Various clinical trials are underway to find a specific drug against this virus. **Methods**: We considered all articles of curcumin—human and animal studies—that could be effective to treat or rescue COVID-19-infected patients. PubMed and Web of Science were used as databases. The keywords used for the search were as follows: coronavirus-19, COVID-19, SARS-CoV-2, curcumin, Curcuma longa, turmeric, curcumin and antiviral, curcumin and anti-inflammatory, curcumin and antipyretic, curcumin and lung, curcumin and acute lung injury, curcumin and fatigue, curcumin and antioxidant, curcumin and ARDS, curcumin and bradykinin, curcumin and fibrosis, curcumin and Interleukin-6, curcumin and TNF-α, curcumin and NF-κB, curcumin and Toll-like receptors, curcumin and antiapoptotic.

Results: Curcumin has some useful clinical effects such as antiviral, antinociceptive, anti-inflammatory, antipyretic, and antifatigue effects that could be effective to manage the symptoms of the infected patient with COVID-19. It has several molecular mechanisms including antioxidant, antiapoptotic, and antifibrotic properties with inhibitory effects on Toll-like receptors, NF-κB, inflammatory cytokines and chemokines, and bradykinin.

Conclusion: In this article, we summarized clinical and molecular mechanisms that curcumin might be effective to treat COVID-19. Research evidence suggests that curcumin will be useful to treat patients especially in ARDS cases with high mortality risk. Curcumin has several therapeutic effects including antiviral, antinociceptive, anti-inflammatory, antipyretic, and antifatigue effects with several molecular mechanisms such as antioxidant, antiapoptotic, antifibrotic effects, and inhibitory effects on NF-κB, inflammatory cytokines and chemokines, Toll-like receptors, and bradykinin. The importance of this review is that curcumin as a nutraceutical could be a new treatment option to combat the COVID-19 pandemic. Further clinical studies should focus on curcumin against COVID-19 infection.

Keywords: antiapoptotic, antifatigue, antifibrotic, anti-inflammatory, antiviral, Coronavirus-19, curcumin

P-503

Review of the effects of vitexin in oxidative stress-related diseases

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Background: Vitexin is a chemical compound found in many plants, such as buckwheat, hawthorn, Echinodorus, and bamboo. Vitexin has a variety of pharmacological effects, including antioxidant, anti-inflammatory, anticancer, anticholinesterase, antibacterial, antiviral, antinociceptive, hepatoprotective, cardioprotective, and neuroprotective effects. This study thus summarizes the antioxidant effects of vitexin and its derivatives on oxidative stress-related diseases.

Methods: All the major in vivo or in vitro studies conducted over the past decade about the effects of vitexin as an antioxidant on oxidative stress were selected for this review study. All the studies related to herbal medicines in which vitexin plays a major role as an antioxidant were also selected. Scopus, PubMed, and Web of Science were used as the databases, and the search was focused on the effect of vitexin on oxidative markers.

Results: This study showed that vitexin has protective effects as an antioxidant against reactive oxygen species, lipid peroxidation, and other oxidative damages in a variety of oxidative stress-related diseases, including seizure, memory impairment, cerebral ischemia, neurotoxicity, myocardial and respiratory injury, and metabolic dysfunction, with possible molecular and cellular mechanisms.

Conclusion: Vitexin is found in food sources and is used as an active component with herbal supplements. Vitexin has protective effects as an antioxidant against reactive oxygen species, lipid peroxidation, and other oxidative damages with changes in oxidative and defense biomarkers in the nervous system, heart, and respiratory systems with possible cellular signaling including activation of AMPK, Nrf-2, and mTOR or inhibition of JNK and BACE1. The diversity of the mechanisms of effect of vitexin against different oxidative stress models is one of the most important points to consider regarding vitexin.

Keywords: antioxidant, lipid peroxidation, oxidative stress, reactive oxygen species, vitexin

P-504

Evaluation of antibiotic effects of Artemisia sieberi essential oil in Iran

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Background: Today, due to the adverse effects of chemical agents and the increase of multidrug-resistant pathogens (MDR), the use of safe antimicrobial compounds is essential. Artemisia (Artemisia) as a plant genus in the family Asteraceae has applications such as antispasmodics, worms, blood thinners and laxatives in traditional Iranian medicine. The remarkable diversity of Artemisia species in Iran (34 species) and the extent of its habitats in our country have made its different species the subject of numerous studies, including the study of antimicrobial effects. Artemisia Siberi is a famous species of this genus. Extensive studies have been performed in view of the possible antimicrobial effects in them. The present study will review the scientific reports obtained from these studies and discuss them.

Materials and Methods: The article is a systematic review. 170 articles were reviewed using internal databases including Iran Dock (Irandoc), SID and external databases including Science Direct, Scopus, Google Scholar, PubMed, Elsevier, Directory of Open Access.

Results: It is rich in secondary metabolites including terpenoids, phenolic compounds, sterols and acetylene derivatives. Extensive studies have been performed on the antimicrobial effects of extracts and volatile oils of these plants. The effect of antibiotics on gram-positive bacteria was more than gram-negative. In addition, it has been proven to have antiparasitic and viral effects.

Conclusion: The antimicrobial properties of this plant depend on the composition of essential oils, including flavonoids. In some cases, it has been aimed at purifying the effective components of plant compounds. Analysis of essential oil compounds depends on the geographical diversity of the region and cultivation conditions. Proper purification and optimization of extracts of these plants can play an important role.

Keywords: essential oil, antimicrobial, Artemisia sieberi, antifungal, antioxidant

P-505

Correlation between the frequency of Blactam resistance pattern in Klebsiella pneumoniae strains isolated from hospitalized patients

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Background: Pneumonia is one of the most common pathogens of gram negative bacteria, which is caused by important infections of the hospital such as urinary tract infection, pneumonia, sepsis, and burn wounds. Today, multidrug-resistant is global challenge in world that the isolates of Klebsiella pneumoniae resistant to quinolones is rapidly widening. The aim of this study was to determine of quinolone antibiotics-resistant in Klebsiella pneumoniae strains isolated from wounds infection in Imam Khomini hospital of Kermanshah, west of Iran. 2017.

Methods: This was a descriptive cross sectional study performed on 126 samples of Klebsiella pneumoniae strains isolated from hospitalized patients in the Imam khomini Hospital, Kermanshah 2018. Identification of specimens were conducted from biochemical tests. An antibiotic susceptibility test was detected by D-zone test. After DNA extraction, the presence of QNR and aac genes was performed by polymerase chain reaction (PCR).

Results and conclusion: Overall, from126 isolates of Klebsiella, it was isolated from 53 (42.06%) male and 73 (57.94%) female patients. The highest antibiotic resistance was against ceftodoxime (68.6%) and ciprofloxacin (64) and the lowest resistance was against Norofloxacin (18.6%) and nitrofurantine (34.3%). Also, the highest prevalence was related to the aa-Ib-cr gene with prevalence rate of 64%. The frequencies of other genes were 54.7%, 5.8% and 0 for qnrB, qnrS and qnrA, respectively.

Keywords: Antibiotic, resistance pattern, betalactam, multidrug-resistant,

P-506

Application of Proteochemometric Modeling to Development of Novel pjDHFR Inhibitors

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Background: Nowadays, using computational modeling has provided a powerful approach for the discovery of new potential therapeutics. This attractive approach can reduce cost and time for the complex processes including design, synthesis, preclinical research, and clinical trial studies of a new candidate. Proteochemometric (PCM) modeling, as an extended QSAR model, correlates ligand-receptor interactions with their inhibitory activity in a single model. In the present study, a unified PCM model was constructed using a combination of ligand/receptor descriptors, to explore the chemical interaction space governed by Pneumocystis jirovecii/human dihydrofolate reductases (DHFR) and their inhibitors to identify selective inhibitors for pneumocystis pneumonia infection.

Methods: Three PCM models were constructed applying the partial least square (PLS) regression method. Biological activities were corrected with Grid-Independent descriptors (Almond/Amanda/VolSurf) and sequence-based z-scale descriptors. The quality and robustness of the models were evaluated by different internal/external validation parameters. Based on the validation parameters, the model constructed by the Amanda algorithm was selected for further investigations.

Results: Acceptable values of statistical validation parameters were acquired. Furthermore, PLS regressions analysis has revealed valuable structural information that can lead to the design of the selective inhibitors for the Pneumocystis jirovecii DHFR. According to these finding, virtual ligands were designed and their inhibitory activity were predicted by the PCM model. Novel hits with better selectivity for Pneumocystis jirovecii DHFR were then retrieved. Finally, in silico ADMET properties of new hits were predicted and evaluated.

Conclusion: For a specific receptor, PCM modeling can be applied to discover selective compounds with higher potencies and fewer side effects.

Keywords: DHFR, GRIND-based PCM, PLS regression method, ADMET

P-507

The assessment of serum levels and gene expression of Vaspin and Omentin-1in PBMCs from NAFLD patients with and without diabetic type 2

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Background: Vaspin and Omentin-1are new kinds of adipokines that are related to insulin resistance, inflammation, obesity, NAFLD, and metabolic syndrome. This study investigated serum levels and gene expression of Vaspin and Omentin-1in PBMCs cells of diabetic male patients with NAFLD + T2D, NAFLD and healthy control.

Materials and Methods: This study was performed on 26 men with NAFLD + T2D, 26 men with NAFLD and 26 healthy men. Fatty liver was diagnosed by a specialist physician using ultrasonography and laboratory tests.

Results: We found serum concentration and gene expression of Vaspin and Omentin-1 in both groups of patients were significantly higher and lower respectively.

Conclusion: These adipokines may be considered as candidates of the potential targets in the treatment of NAFLD and diabetes. It may be helpful to measure these adipokine levels to evaluate the course of diseases such as fatty liver and diabetes.

Keywords: Vaspin, Omentin-1, Adipokine, NAFLD, T2D

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Evaluation of the effect of methanolic extract of garlic, turmeric and tomato on the elimination of lead-induced side effects in all neonates of Wistar rats

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Background: The use of lead as a natural element has various effects on hematopoiesis, nervous system, kidney, reproduction and bone. In this study, the effect of garlic, dandelion and tomato extracts in the treatment of lead-induced kidney poisoning in rat neonates was investigated.

Methods: In this experimental study, immature rats were divided into 6 groups. Control group (without drug solvent), control (daily gavage 0.6 g / l per kg body weight only lead acetate), treatments (daily gavage 0.6 g / l lead acetate and 25% plant extracts individually and in combination) . At the end of the period, the animals were weighed and anesthetized by ether. Blood samples were taken from the heart by blood sampling method. Then all the animals were removed and weighed. Plasma concentrations of uric acid, urea, creatinine, sodium and potassium were measured.

Results: Body weight and kidney weight in mice treated with all three extracts together showed a significant decrease compared to other groups. The amount of creatinine, uric acid, potassium, urea in the group treated with all three extracts compared to the other groups decreased significantly.

Conclusion: Consumption of the drug produced from tomato, turmeric and garlic extracts due to its strong antioxidant properties has a significant effect on the elimination of lead poisoning in rat neonates. It can also significantly reduce the negative effects of lead on the kidneys compared to the group that was affected by lead but did not take the drug.

Keywords: Lead, Kidney, Garlic, Turmeric, Tomato, Rat

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Evaluation of knowledge, attitude and nutritional performance of female high school students in Farzanegan, Tehran

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Background: Awareness of proper nutrition during adolescence can prevent many diseases of adulthood. In addition, adolescent girls are future mothers and the concepts of nutrition learned by them are transmitted to adulthood. They play an important role in the health of the family and children. The aim of this study was to evaluate the knowledge, attitude and nutritional performance of female middle school students in Tehran

Methods: In this descriptive study, 102 female students in Farzanegan schools in the age range of 13-14 years in Tehran were selected and examined by simple random sampling method to evaluate the knowledge, attitude and nutritional performance of the people from the questionnaire. Awareness, attitude and performance were estimated using good, moderate and poor criteria. People who scored more than 76% were rated as good, people who scored in the range of 51-76% were rated as average out of the total, and those who scored less than 51% were rated as poor. In this study, the height and weight of each student were measured and the BMI ratio was calculated as well.

Results: Students 'awareness is 91% good and 8% average, students' attitude is 58% average and 41% good, and performance rate is 36% average and 62% good. Overall, students' scores were assessed on the status of knowledge of good performance attitude.

Conclusion: The results of this study showed that the nutritional awareness of students aged 13-14 years is at a high level but their performance is at a lower level. Therefore, it is very important to improve the nutritional performance of students who will be future mothers.

Keywords: Nutrition, Female students, Knowledge, Attitude, Performance

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The effect of noise pollution on muscle relaxation and motor activity in male Wistar rats

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Background: One of the human problems is noise pollution that is heard every day. Noise pollution is one of the most important environmental pollutants that endanger human health in various dimensions. Noise pollution causes many problems, including hearing loss and effects on the nerves and psyche.

Methods: The study was conducted on male Wistar rats. Mice were divided into treatment and control groups. The control group received noise pollution in an environment without noise pollution, but the treatment group received noise pollution daily seven to fourteen hours for ten days. Barfex test or no rod grip was performed.

Results: The results showed that the strength of mice to run the horizontal bar and not to let go of the bar and the amount of standing up and fast movement in the treatment group increased sharply compared to the control group. Also, the rate of slow motion index in the mice of the treatment group had a significant decrease compared to the control.

Conclusion: Noise pollution, in addition to affecting the mice moving faster and faster, also had a significant effect on their nerves and violent behaviors. Because the mice in the treatment group were more prone to escape, bite, and attack, their movement was also significantly faster and more violent than in the control group during the experimental period. Therefore, in addition to affecting the anatomy of mice, noise pollution has a significant effect on their nerves.

Keywords: Noise pollution, Muscle relaxation, Motor activity, Rats

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The effect of fermented milk with kefir mushrooms (*Kefir Probiotic Dough*) on *Cicilad Macro* fish growth indices

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Background: The ornamental fish trade is one of the most important issues in terms of economics and profitability. The success of the ornamental fish industry necessitates the widespread use of antibiotics and drug therapies to improve health and nutrition, which in turn leads to the development of drug-resistant damage and pathogenic microorganisms. Be. On the other hand, these chemicals inhibit the growth of bacterial flora in the gastrointestinal tract of fish, which in turn have beneficial effects on existing health. The origin of *kefir* dates back to more than two thousand years ago. *Kefir Probiotic Dough* contains high levels of thiamine (B12), calcium, folate and vitamin K and has benefits for the immune system. In this study, the effect of fermented milk with *kefir* mushrooms on the growth status and health of *Cicilad Macro* fish has been investigated.

Methods: 60 g/l of *kefir* mushroom was poured into milk and placed under fermentation. After 18 to 24 hours, the probiotic *kefir* dough was filtered and consumed. *Kefir* feeding was performed every other day for two months with a concentration of 0.005% aquarium water. Fish were weighed four times every 15 days during the treatment period to evaluate the characteristics of the growth index and at the end of the period; the data were analyzed by Minitab software.

Results: Studies showed that the group treated with fermented milk with *kefir* in terms of special growth index is in a significant difference in the period of 30 days (87%) and 60 days (93%).

Conclusion: *Kefir* probiotic buttermilk probably due to its effect on intestinal cells and the length of the villi causing more and better absorption of nutrients, and better functioning of the digestive system of fish.

Keywords: Kefir Probiotic, Cicilad Macro, Growth indices

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The effect of a combination ointment of honey and *Curcuma longa* extract and Aloe vera gel to heal skin wounds in Wistar rats

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Background: Surgical wounds as the main and most important problem in the past has been of particular interest to medical researchers and physiology and they have always been looking for the best medicine to repair and reduce the recovery period.

Methods: In this study, the effect of the ointment were studied. The ointment was obtained from a combination of honey (20%), Curcuma longa extract (1%) and Aloe vera gel (30%) based on Osrine ointment. Wistar rats were randomly divided into 8 groups, including ointment group (individual and combination compounds), the positive control group (Phenytoin), and the negative control group (Osrine). Then, on day 0, surgery was performed on the lumbar region and the entire skin. The treatment was performed. Wound dimensions were measured every 3 days with a caliper and analyzed by Minitab software at the end of the course.

Results: The effect of the combined ointment of all three substances is greater than their individual use and the active ingredient (effective material) of these compounds has a synergistic effect on each other. The combined ointment had significant changes in terms of recovery percentage, scar rate, duration of treatment compared to positive and negative control ($p \leq 0.05$).

Conclusion: The results of this study show that the honey due to its antibacterial properties, Aloe vera with antioxidant properties, and Curcuma longa with anti-inflammatory and antioxidant properties accelerate wound healing, reduce the duration of inflammation and thus increase the rate of formation.

Keywords: Honey, Curcuma longa, Aloe vera, Wound

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Evaluation of hypoglycemic effect of methanolic extract of llium ampeloprasum and Morus nigra tree leaves in Streptozotocin-induced diabetic rats and its complications

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Background: Currently, the tendency to use alternative and traditional therapies to control and reduce blood sugar levels in diabetic patients is increasing. According to the research evidence on the similarity of *llium ampeloprasum* and *Morus nigra* tree leaves to garlic in terms of some effective compounds and anti-diabetic effect of garlic, in this study, the effect of oral consumption of them on lowering blood sugar and diabetes complications in mice small Diabetic Laboratory was examined.

Methods: In this study, the effect of methanolic extracts individually and in combination with two factors of hypoglycemia and symptoms of hyperglycemia at concentrations of 400 mg/kg was investigated. Laboratory mice were randomly divided into 5 groups including treatment groups (individual and combined compounds), positive and negative control groups. Diabetes was induced intraperitoneally (175mg/kg). Treatment was performed by gavage of extracts and normal saline in the negative control group for 5 weeks in the presence and absence of extracts. Fasting blood glucose was measured every 3 days and at the end of the period, the data were analyzed by Minitab software.

Results: The results showed that the greatest effect of hypoglycemia was related to the composition of extracts and then *llium ampeloprasum* extract. In addition, diabetes-related complications such as visual impairment (Impaired vision) and clinical symptoms were not observed in mice treated with the combination of extracts, unlike the negative control group, which developed blindness.

Conclusion: The combination of *llium ampeloprasum* and *Morus nigra* tree leaves extract has a better effect on lowering blood sugar and as a result reduces the visual effects. Its effect is probably due to the presence of its antioxidant compounds. Therefore, these extracts can play the role in lowering blood sugar and ocular complications caused by high blood sugar.

Keywords: *llium ampeloprasum, Morus nigra tree*, Mice small Laboratory, Diabetes

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Cytotoxic T cell markers and cytokines in human papilloma virus16

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Background: Cervical cancer is the fourth main cause of mortality among the women and annually about five million new cases are detected in developed countries. Papillomavirus types 16 and 18 are considered the most dangerous types in this cancer.

Methods: In this review study, more than 200 articles which is related to human papilloma virus and the function of the immune system against this virus are reviewed from 2015 to 2020. Thirty-four articles which is related to the markers and cytokines in cervical cancer was chosen from Google Scholar, Scopus, and PubMed.

Results: Identification of markers has been done by exposing cytotoxic T cells with HPV in vitro. One of the methods in identification of markers and cytokines related to cervical cancer such as MHC, PD, PDL, CD, FASL, TLR, IFN, IL is infection of DCs with vector that leads to antigen expression, markers presentation and maturation of the native T-cells.

Conclusion: IL8 and IL6 are one of the most important and main cytokines that are expressed by exposing virus and A2 is the most important markers which has high potency in activating HPV specific CTL. Since this marker is expressed when exposed to CTL, it can be used as an agonist for making a vaccine against human papilloma virus.

Keywords: Human Papilloma Virus, Cervical cancer, Cytotoxic T-Cell

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The Effect of Curcumin on Serum Copper and Zinc and Zn/Cu Ratio in Individuals with Metabolic Syndrome: A Double-Blind Clinical Trial

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Background: Metabolic syndrome is a complex disorder with high socioeconomic costs and a high global prevalence. The serum concentrations of some trace elements are higher in people with metabolic syndrome compared to normal individuals. Curcumin may therefore have a potential role in the management of cardiovascular risk. The aim of this study was to investigate the effects of curcumin on serum copper (Cu), zinc (Zn), and Zn/Cu ratio levels in patients with metabolic syndrome.

Methods: A double-blind clinical trial was designed in which 120 individuals with metabolic syndrome were randomly assigned to one of three groups: curcumin 1gr/day, phospholipidated curcumin 1gr/day, or a placebo, each taken for 6 weeks. Serum copper and zinc were measured before and after intervention. At baseline, in addition to obtaining the anthropometric characteristics of participants, a fasting blood sample was taken from each participant, and the concentrations of serum Cu and Zn were measured by atomic absorption (Varian AA 240 FS model).

Results: Serum Zn concentrations rose significantly in the phospholipidated curcumin and curcumin groups, being significantly higher ($p < .001$) in the phospholipidated curcumin group than in the curcumin group ($p < .05$). Serum Zn concentration fell in the control group ($p < .05$). Changes in serum Zn level from baseline to the levels after six weeks' intervention were significantly different between the groups, but changes in serum Cu from between baseline until after intervention were not significantly different. The serum Zn/Cu level in phospholipidated curcumin and curcumin groups after intervention was higher than the control group, but it was more significant in the group taking phospholipidated curcumin ($p < .001$).

Conclusion: Curcumin and phospholipidated curcumin complex, given at a dose of 1 g per day for six weeks, were associated with an increase in serum zinc and consequently zinc-to-copper ratio.

Keywords: Curcumin, Metabolic syndrome, Copper, Zinc

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Assessment of Mir-29a expression profile in serum and tissue of colorectal cancer patients

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Background: Colorectal cancer (CRC) is a fast becoming serious public health concern because of high mortality and late diagnosis. Development of this disease is influenced by genetic and environmental factors. MicroRNAs (miRNAs) are small non-coding RNAs that possess a crucial role in many human malignancies especially in CRC. Recently, circulating microRNAs as non-invasive biomarkers are introduced as potential diagnostic and prognostic factors. To this end, comprehensive application of their expression profile is demanded. Therefore, this study aims to determine the miRNA signature in serum and tumor specimens of patients harboring CRC.

Methods: Serum, fresh frozen tumor and adjacent normal tissue specimens of 50 CRC patients who were underwent surgery without chemo adjuvant treatments from 2017 and 2018 were collected. Also, sera specimens of 50 healthy people were used as control group. The expression levels of miR-29a were evaluated using quantitative reverse transcription Polymerase chain reaction (qRT-PCR). In order to investigate the relationship among mir-29a expression and clinicopathological features, the Mann-Whitney U test was applied.

Results: Our data indicated that the differential expression of mir-29a was significantly up-regulated in tumor tissue compared with normal adjacent colorectal tissues. The increased expression of circulating mir-29a was observed in CRC patients versus healthy controls. Besides, this enhancement was closely linked to the clinical stage of cancer.

Conclusion: Here we report the differences in the expression of mi-29a between sera and tumor in CRC patients. These results suggest that circulating miR-29a could be introduced as a potential prognostic marker along with its regulatory role in CRC development. Thus, further studies are required to identify the possible oncogenic feature of this miRNA.

Keywords: Colorectal cancer, mir-29a, biomarker

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The effect of cinnamon on lipid profile in individuals with type 2 diabetes mellitus: A systematic review and meta-analysis

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Background: Cinnamon is one of the most widely used spices with diverse biological properties, including anti-diabetic, antioxidant, anti-inflammatory, and anti-tumor activities. Although a number of studies have investigated the effect of cinnamon supplementation on the blood lipid profile, the available results are inconclusive and conflicting. Therefore, the present systematic review and meta-analysis aimed to investigate the effect of cinnamon supplementation on the blood lipid profiles in patients with type 2 diabetes mellitus.

Methods: A systematic search using electronic databases, including Scopus, PubMed, Web of Science, Embase and Cochrane Library was performed for articles published up to 8th March 2020. Data were pooled using the random-effect model and presented as weighted mean difference (WMD) with 95% confidence interval (CI).

Results: Sixteen randomized controlled trials (including 1025 participants) were included in the present study. Our results demonstrated that cinnamon supplementation significantly decreased serum triglyceride (WMD: -26.27 mg/dl, 95% CI: [-38.93, -13.61], P <0.001), total cholesterol (WMD: -13.93 mg/dl, 95% CI: [-25.64, -2.22], P = 0.020), and low-density lipoprotein cholesterol (LDL-C) (WMD: -6.13 mg/dl, 95% CI: [-10.72, -1.53], P = 0.009) levels in the patients with type 2 diabetes as compared with the placebo group. However, there was no significant change in the high-density lipoprotein cholesterol (HDL-C) (WMD: 0.64 mg/dl, 95% CI: [-0.18, 1.46], P = 0.128) level of type 2 diabetic patients.

Conclusion: The results of present systematic review and meta-analysis suggested that cinnamon supplementation can improve serum triglyceride, total cholesterol and LDL-C levels in patients with type 2 diabetes. However, more RCTs are required to investigate the effectiveness of cinnamon as a complementary therapy for the treatment of hyperlipidemia in type 2 diabetic patients.

Keywords: Cinnamon, Lipid profile, Diabetes, Systematic review, Meta-analysis

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Prevalence of Escherichia coli causing urinary tract infections and their antibiotic profile in Tehran hospitals

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Abstract

Backgrounds: One of the most common infections in the world is urinary tract infection and one of the most important bacterial agents that because it is *Escherichia coli*, which due to the high antibiotic resistance that is observed in its isolates today, treats patients with problems and annual costs. Introduces a high level to medical and health centers. The aim of this study was to investigate the prevalence of *Escherichia coli* causing urinary tract infections and their antibiotic profile in Tehran.

Methods: The present study is a retrospective study and the records of patients with urinary tract infections in terms of bacterial agent and their antibiotic profile have been statistically reviewed. Finally, the obtained data were analyzed using SPSS software.

Results: The results showed that the prevalence of *Escherichia coli* in urinary tract infections was 67% and the highest resistance of the studied isolates to ceftazidime, cotrimoxazole, cefotaxime, cefazolin and amoxicillin and the lowest resistance to imipenem and nitrofurantoin was observed.

Conclusion: The present study indicates that the most common cause of urinary tract infection is *Escherichia coli* and some antibiotics have little effect on this bacterium. It is suggested that in antibiogram by disk diffusion method, the resistance of this bacterium to fosfomycin is also reviewed.

Keywords: *Escherichia coli*, UTI, Fosfomycin, Tehran

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Prevalence of clonal groups in urinary *Escherichia coli* in Iran in a review study

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Backgrounds: Urinary Escherichia coli clonal groups contain virulence factors and specific antibiotic resistance genes. Therefore, studying the prevalence of these clonal groups can be very helpful in treating urinary tract infections. Therefore, in the present study, the prevalence of these clonal groups has been investigated.

Methods: The present study is a review study that has searched more than 76 articles published in reputable journals by searching for reputable databases such as PubMed, Scopus, and Google Scholar. The results of this study were analyzed.

Results: The highest prevalence of clonal groups was related to ST131, ST69, and CGA groups, which also contain different antibiotic resistance genes, respectively.

Conclusion: The results of the present study indicate that the ST131 clonal group is the most common clonal group and in turn is the most dangerous one. It is suggested that extensive studies be conducted in this field in our country.

Keywords: Clonal groups, UPEC, ST131, ST69, CGA, Escherichia coli

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Prevalence of *CTX-M1* gene in clinical isolates of Enterobacteriaceae family in a review study

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Backgrounds: In recent years, a new group of broad-spectrum beta-lactamases has been identified, called the *CTX-M1* group, which can preferably hydrolyze cefotaxime but are usually sensitive to ceftazidime. The present study investigates the prevalence of this gene in bacteria of the Enterobacteriaceae family.

Methods: For this study, more than 120 articles published in reputable citation databases such as Scopus, Elsevier, PubMed and research gate were reviewed and the data were finally analyzed.

Results: The prevalence of this *CTX-M1* gene resistant to beta-lactam antibiotics in Enterobacteriaceae bacteria is on average 43% and *Klebsiella pneumoniae* is the most common bacterium that has this gene.

Conclusion: It can be concluded that the prevalence of *CTX-M1* in the world has increased significantly in recent years and treatment of various infections can be difficult. Antibiogram should be performed by disk diffusion method with high accuracy.

Keywords: *CTX-M1*, Enterobacteriaceae, beta-lactam, *Klebsiella pneumoniae*

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Evaluation of *fosA3* gene distribution in clinical isolates of *Escherichia coli* with carbapenemase enzyme

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Backgrounds: *Escherichia coli* is a gram-negative, spore-free bacillus and the most common cause of urinary tract infections in the world. The last line of treatment for urinary tract infections caused by Escherichia coli is the antibiotic fosfomycin. In case of resistance to the antibiotic fosfomycin, treatment of patients will be difficult. The aim of this study was to investigate the prevalence of fosfomycin antibiotic resistance gene in clinical isolates of *Escherichia coli* with carbapenemase antibiotic resistance.

Methods: Among 136 *Escherichia coli* isolates, isolates with carbapenemase resistance were identified and examined for the presence of *fosA3* gene.

Results: Forty-eight samples were resistant to the antibiotic carbapenem, and among these antibiotic-resistant samples, one sample had the *fosA3* gene.

Conclusion: The present study indicates that the presence of the antibiotic resistance gene fosfomycin in *Escherichia coli* isolates could be a warning sign for the treatment of this infection. It is recommended to examine the fosfomycin disk in antibiogram by disk diffusion method.

Keywords: *Escherichia coli*, Carbapenemase, Fosfomycin, *fosA3*

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Prevalence of *mcr-1* gene in *Escherichia coli* isolates with carbapenemase resistance

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Backgrounds: *Escherichia coli* is one of the leading causes of intestinal and extraintestinal infections in the world, and recently, isolates resistant to various antibiotics, especially colistin, have caused many problems in the treatment of patients. One of the genes for resistance to this antibiotic is the *mcr-1* gene. A plasmid-encoded colistin resistance gene *mcr-1* encoding phosphoethanolamine transferase has been recently described in Enterobacteriaceae. In the present study, the aim was to evaluate the prevalence of *mcr-1* gene in Escherichia coli isolates with beta-lactamase resistance.

Methods: In the present study, among 136 *Escherichia coli* samples, samples with beta-lactamase antibiotic resistance were identified and among these samples, the prevalence of colistin resistance gene (*mcr-1*) was investigated.

Results: Among the present samples, 76 samples had beta-lactamase, of which 2 samples had the colistin resistance gene (*mcr-1*).

Conclusion: The presence of *mcr-1* gene in clinical isolates will cause many problems in the treatment of patients and therefore more attention should be paid to the antibiotic resistance of *Escherichia coli* in clinical isolates. In addition, further studies to study the genotyping of the present isolates to identify the ancestor are needed. In the present study, among 136 *Escherichia coli* samples, samples with beta-lactamase antibiotic resistance were identified and among these samples the prevalence of colistin resistance gene (*mcr-1*) was investigated.

Keywords: *Escherichia coli*, beta-lactamase resistance, *mcr-1*, colictin

P-523

The first report and early diagnosis in an Iranian child affected by Cerebrotendinous Xanthomatosis

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Background: Cerebrotendinous Xanthomatosis (CTX) is a rare autosomal recessive disease due to mutation in CYP27A1 gene results in inactive mitochondrial enzyme, sterol 27 – hydroxylase, which is participated in the degradation of cholesterol to bile acids. The lack of this enzyme prevents cholesterol from being converted into a bile acid called chenodeoxycholic acid. Deposits of cholesterol and a related compound called cholestanol accumulate in the nerve cells and membranes potentially causing damage to the brain, spinal cord, tendons, lens of the eye and arteries. Chenodeoxycholic acid has been effective in treating affected individuals. Some studies in adults emphasizes the importance of early diagnosis of CTX. It is characterized by infantile diarrhea, childhood onset cataract which can be the first manifestation in 75% of cases. Xanthomas in the second or third decade appear on Achilles tendon, extensor tendons of the elbows and hands, lung, bones and CNS. Some individuals show mental impairment in infancy but majority have normal or subnormal intellectual function until puberty. Neuropsychiatric symptoms, pyramidal or cerebellar signs almost invariably manifest between ages 20 and 30.

Case report: A 9.5 years old girl was referred because of weakness, activity intolerance, demonstrated mild delayed motor development, normal speech, axial and proximal hypotonia.

Results: CPK:218, EMG myopathic, echocardiogram, normal muscle biopsy, mild vacuolar myopathy, normal alpha glucosidase activity, increased urinary abnormal bile acid: 10 micro M/L (N; 1.0- 5.4). Molecular genetic analysis revealed P.Pro 384 leu due to Cerebrotendino Xanthomatosis on gene CYP27A1. Chenodeoxycholic acid was started mainly in prevention of further deterioration and demonstrated good results in 2.5 year follow up.

Conclusion: This is the first report of CTX in childhood from Iran, in a girl with normal intelligence but muscular weakness due to vacuolar myopathy (fat deposit). This case has demonstrated abnormal high urinary bile acid whose diagnosis was confirmed by genomic sequencing (P.Pro348 Leu, C.1151 C > A) and responded well to chenodeoxycolic acid treatment.

Keywords: Cerebrotendinous Xanthomatosis, Echocardiogram, rare autosomal recessive

P-524

The possible association of hypertension and other indices in pCAD patients

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Background: Premature coronary disease (pCAD) is identified as the existence of a myocardial ischemia with an obstructive coronary artery disease in young population. It has a notable association with various environmental factors. Among these, hypertension is known to be the most significant changeable risk factor. Therefore, this study investigated the anthropometric and clinical characteristics of pCAD patients compared to healthy individuals.

Methods: This study investigated a total of 335 subjects who referred to Shahid Mohammadi teaching hospital, Bandar Abbas, Iran. The study population included men ≤ 45 years and women ≤ 55 years. All contributors underwent a coronary angiography procedure under interventional cardiologist and participants with at least a stenosis more than 50% in one of the arteries were classified as pCAD patients. The anthropometric and clinical characteristics of all participants, including weight, height, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), family history, and smoking status were evaluated thorough clinical examination. BMI was calculated based on the ratio of weight in kg divided by height in m^2.

Results: There was a significant difference in the hypertension (p value = 0.05), SBP (p value = 0.03), and cigarette smoking status (p value < 0.01) between the patient and control groups. Nevertheless, there was no significant difference between the case and control groups regarding family history (p > .05). Moreover, no significant difference was reported concerning BMI between the patient and control groups (p > .05).

Conclusion: Regarding this study along with others, pCAD is greatly associated with the status of hypertension and smoking. In this manner, the effect of hypertension and smoking on the risk for pCAD and their effect on therapeutic decisions should be under great attention in clinical practice.

Keywords: Premature coronary disease (pCAD), Hypertension, Smoking, BMI

P-525

Silymarin induces apoptotic genes in colorectal cancer cell lines

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Background: Silybum Marianum is one of the medicinal plants that has many healing properties. One of the important compounds of this plant is Silymarin. The aim of this study was to investigate the effect of Silymarin on the expression of genes affecting apoptosis.

Methods: First, human colorectal cancer cell lines (LS-180 and HCT-116) were treated with different concentrations of Silymarin for 24 hours. Then, genes expression of Bax, Bcl2, Caspase3, P53, and PPARγ were evaluated by Real time-PCR method.

Results: The results showed that gene expression of Bax, Bcl2, Caspase3, Bax / Bcl2 ratio, P53, and PPARγ increased in both colorectal cancer cell lines, but these increases in LS-180 cell line were higher than HCT-116 cell line.

Conclusion: The results showed that silymarin increased the expression of genes effective in apoptosis. It seems that these effects appear to be through P53, and PPARγ-dependent pathways.

Keywords: Silymarin**,** Bax, Bcl2, Caspase3, P53, PPARγ, colorectal cancer

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Evaluation of glutathione concentration affected by protective effect of basil extract against lead acetate toxicity-induced in Wistar rats

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Background: Lead, as an environmental pollutant in the vicinity of body tissues, causes oxidative stress disorders. Basil as a natural precursor of herbal medicines has strong antioxidant effects. Glutathione is considered as an oxidative stress marker in the face of oxidizing agents. The aim of this study was to investigate the concentration of GSH obtained from the effect of basil extract on Wistar rats against the toxicity induced of lead acetate.

Methods: In this study, 48 male Wistar rats were divided into three groups of negative control receiving only physiological serum, positive control (lead-treated) received no extract, and groups (lead-treated) received aqueous extract of basil. After one month of daily intraperitoneal injection of basil aqueous extract, their GSH concentration was evaluated.

Results: The results of statistical analysis showed that the mean GSH concentration of gamma glutamyl transferase (GGT) activity at concentrations of 100 and 200 (mg/kg) of basil aqueous extract in rats poisoned with lead acetate was significantly lower than the control group.

Conclusion: Since the GGT enzyme is involved in glutathione metabolism, following the induction of oxidative stress, the activity of this enzyme increases in the use of GSH. Therefore, changes in circulating glutathione concentration indicated the protective effect of basil aqueous extract on liver detoxification performance against lead acetate toxicity in treated rats.

Keywords: Glutathione, Antioxidant, Gamma glutamyl transferase (GGT), Basil extract

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The effect of capsaicin on antioxidant genes with H2O2-induced oxidative stress in BE (2)-C cells

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Background: Capsaicin, the active ingredient in hot peppers, has various properties such as anti-cancer, anti-inflammatory and antioxidant. The antioxidant properties of this substance can protect the body's cells from free radical damage. Since oxidative stress plays an important role in the development of neurodegenerative diseases, the aim of this study was to investigate the effect of capsaicin on the expression of antioxidant enzyme genes after induction of oxidative stress with hydrogen peroxide (H2O2) in human neuroblastoma cells.

Methods: BE (2) -C neuroblastoma cells were treated with different concentrations of capsaicin for 24 hours, then the cells were exposed to 400 µM H2O2 for 2 hours. After cell collection and RNA extraction and cDNA synthesis, the expression level of Nrf2 genes and superoxide dismutase, catalase, and glutathione peroxidase were determined by real time-PCR

Results: The results showed that the expression of Nrf2, superoxide dismutase, catalase, and glutathione peroxidase genes decreased after induction of oxidative stress, while cell treatment with capsaicin increased gene expression of catalase and glutathione peroxidase but decreased gene expression of superoxide dismutase.

Conclusion: According to the results, it seems that reducing the expression of superoxide dismutase in capsaicin-treated groups prevents the increase of H2O2 production, while increasing the expression of catalase and glutathione peroxidase leads to increased production of these enzymes and the breakdown of H2O2 and reduce its toxicity in the environment. These changes may be appropriate to counteract the oxidative stress created and protect cells against oxidative stress induced by H2O2.

Keywords: Capsaicin, Nrf2, superoxide dismutase, catalase and glutathione peroxidase, BE (2) –C cell line

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The effect of Silymarin on the expression of apoptotic genes with H2O2-induced oxidative stress in BE (2)-C human neuroblastoma cell line

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Background: Silymarin is a flavonolignan complex derived from Milk thistle, which has anti-inflammatory, anti-cancer and antioxidant properties. The aim of this study was to evaluate the effect of Silymarin on the expression of apoptotic genes against hydrogen peroxide (H2O2)-induced oxidative stress.

Methods: In this interventional study, BE (2) -C cell line of human neuroblastoma was treated with different concentrations of Silymarin for 24 hours. Then, cells were exposed to H2O2 at 400 µM for 2 hours to induce oxidative stress. The expression level of Bax, Bcl2, and Caspase 3 genes were examined by Real time-PCR.

Results: The results showed that oxidative stress induced by H2O2 increased genes expression of Bax and caspase 3 but treatment with Silymarin decreased the expression of both of them.

Conclusion: According to these results, it seems that Silymarin may have a protective role against H2O2-induced oxidative stress by reducing the expression of genes effective in apoptosis.

Keywords: Silymarin, Apoptotic genes, BE (2)-C cell line

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The effect of Hydroxytyrosol on Apoptotic genes in HCT-116 cell line

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Background: Hydroxytyrosol is one of the major and effective compounds in olive oil that has various properties such as inhibiting cell proliferation and inducing apoptosis. The aim of this study was to determine the effect of Hydroxytyrosol on the expression of genes effective in apoptosis such as Bax, Bcl2, Caspase3, P53, and PPARγ, in HCT-116 cell line of human colorectal cancer.

Methods: First, the cells were treated with different concentrations of Hydroxytyrosol for 24 hours**.** Then, cell survival was assessed by MTT method and genes expression of Bax, Bcl2, Caspase 3, P53, and PPARγ were investigated by real-time PCR**.**

Results: The results showed that Hydroxytyrosol decreased cell survival and induced apoptosis by increasing expression of Bax, caspase3, and Bax/Bcl2 ratio but had no effect on the expression of P53 and PPARγ.

Conclusion: According to the results, it seems that the effect of Hydroxytyrosol on the inhibition of proliferation and induction of apoptosis in HCT-116 cells is without the intervention of P53 and PPARγ.

Keywords: Hydroxytyrosol, Apoptotic genes, HCT-116

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The effect of Capsaicin on Nrf2 expression and antioxidant enzymes activity in colorectal cancer

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Background: Capsaicin is one of the main and active components of hot peppers that has antioxidant properties. The aim of this study was to evaluate the effect of Capsaicin on the expression of Nrf2 and the activity of the antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) in human colorectal cancer cells.

Methods: In this study, human colorectal cancer cell lines (LS-180 and HCT-116) were treated with different concentrations of Capsaicin for 24 hours. Then, the expression level of Nrf2 was determined by Real time-PCR method and the activity of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) were determined by calorimetric methods.

Results: The results showed that Capsaicin significantly increased Nrf2 and the activity of superoxide dismutase, catalase, and glutathione peroxidase enzymes in both cell lines compared to the control group.

Conclusion: According to the results of the study, it seems that Capsaicin by increasing the expression of Nrf2 gene, increases the expression and activity of antioxidant enzymes.

Keywords: Capsaicin**,** Nrf2**,** Antioxidant enzymes**,** Colorectal cancer

P-531

The effect of Hydroxytyrosol on inhibition of oxidative stress induced by H_2O_2 in BE(2)-C human neuroblastoma cell line

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Background: Hydroxytyrosol is a natural polyphenol mixture in olive oil and has strong antioxidant activity against all types of ROS. The purpose of this study was to investigate the effect of Hydroxytyrosol on the activity of the antioxidant enzymes SOD, CAT, and GPx in human BE(2)-C neuroblastoma cell line.

Methods: BE(2)-C neuroblastoma cells were treated with different concentrations of Hydroxytyrosol for 24 hours. Then, to induce oxidative stress, cells were treated with 400µM of H_2O_2 for 2 hours. The level of malondialdehyde and activity of antioxidant enzymes were measured by calorimetric methods.

Results: The results showed that Hydroxytyrosol reduced the level of malondialdehyde and increased the activity of SOD, CAT, and GPx antioxidant enzymes compared to the control group.

Conclusion: According to results, it seems that Hydroxytyrosol may protect BE(2)-C cells against oxidative damage by increasing activity of antioxidant enzymes.

Keywords: Oxidative Stress, Hydroxytyrosol, Antioxidant Enzymes, BE(2)-C cell line

P-532

The role of microRNA 155 expression in cyclophosphamide-induced immunotoxicity

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Background: Cyclophosphamide (CTX) is a cytotoxic drug that can suppress both humoral and cellular immunity. The aim of this study was to investigate the role of miR-155 and its protein targets after repeated intraperitoneal administration of CTX for 28 days.

Methods: Mice were randomly divided into 2 groups treated as follows: (1) IP injection of 20 mg CTX/kg for 5 days as the positive control; (2) IP injection of normal saline for 5 days as the negative control. At the next step, T cells were isolated from spleens of BALB/c mice and expression of microRNA-155 (miR-155) were measured by real-time quantitative PCR. Finally, Western blot was done for various miR-155 targets including phosphatidylinositol-3, 4, 5-trisphosphate 5-phosphatase 1 (Ship1), suppressor of cytokine signaling 1 (SOCS1) and macrophage activating factor (c-MAF).

Results: Exposure to CTX resulted in significantly lesser expression of miR-155 in mice. Western blot results revealed that CTX increased the Ship1, Socs1, but not c-MAF.

Conclusion: In conclusion, intraperitoneal administration of CTX suppressed innate and acquired immunity. These results suggest that miR-155 and targeted proteins might be involved in immunotoxicity observed in mice exposed to CTX.

Keywords: microRNA 155, cyclophosphamide, immunotoxicity

P-533

The effect of personalized nutritional interventions on PPARα gene expression and signaling pathways of lipid metabolism and ketogenesis

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Background: New discoveries have shown that personalized nutrition and biochemistry can manage diseases. Since the role of micronutrients and macronutrients has been proven in human health, a large part of the nutritional adaptation systems of these compounds depends on the biochemical and molecular processes of the target tissues. The aim of this study is to investigate the metabolism of obese people. Today, one of the most important strategies to prevent obesity is the ketogenic diet (KD), which is low-carbohydrate and rich of protein and fat. This diet stimulates ketogenesis and increases the ketone bodies (KB) production in liver cells. KB regulates adipose tissue intracellular processes such as signaling pathways and fatty acid metabolism by the PPARα gene.

Method: In this systematic review, related articles were collected from PubMed and Scopus databases. Finally, nutritional interventions were evaluated on fatty acid oxidation and ketogenesis processes.

Results: Studies have shown that ketogenic diet was associated with increased adiponectin in obese individuals, and KB was considered as a high-energy tissue fuel. These metabolites were the major regulators of signaling networks, and regulate β-oxidation and ketogenesis by activating nutritional signals. Feeding people with a KD increases the levels of KB, free fatty acids in the liver, and activates PPARα gene expression.

Conclusion: The overall goal of personalized nutrition is to maintain or enhance the individual's health, because the type of nutrition depends on our specific genetic characteristics that can modulate gene expression, protein synthesis and cellular metabolic processes. In this study, the importance of the effect of ketogenic diet on stimulation of β oxidation pathways and hepatic ketogenesis by increasing the gene expression of PPARα was identified.

Keywords: Nutrition, PPARα, Ketogenesis, Ketone bodies, Lipid metabolism, Ketogenic diet

P-534

The microbiome and cancer therapy: a systematic review

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Background: The condition in which the diversity of gut microbial species decreases or shifts to the increase of a particular species is dysbiosis. Several diseases such as cancer are associated with dysbiosis, for example, gastrointestinal, clone, liver, bile ducts, and breast cancers. The aim of this article is to investigate the ways in which the microbiome could be regulated and the mechanisms by which these microorganisms might influence the treatment of cancer.

Methods: We systematically searched PubMed, Google Scholar, and Scopus database to explore the microbiome and cancer therapy between March 2010 and February 2020. After performing the literature search and review, 60 eligible studies were identified.

Results: There is a bidirectional relationship between cancer treatments and the microbial system. As many cancer therapies induce dysbiosis and the other hand, the microbiome affects the response to a variety of therapies including radiotherapy, chemotherapy, immunotherapy, and stem cell transplantation through various mechanisms. Therefore, the goal is to increase the response to cancer treatments or reduce their complications. There are several methods for regulating the microbial population, including fecal microbiome transplantation, probiotics, diet, and tumor microbiome regulation.

Conclusion: Given the extent of the microbial ecosystem and its modulatory effect on the immune system and cancer treatment, as well as different ways to regulate this ecosystem, this approach may be useful in cancer therapy to increase the impact of different treatments while reducing their side effects.

Key words: Gut microbiome, microbiota, cancer therapy, microbiome manipulation, dysbiosis.

P-535

Effect of osmoprotectants on lactoproxidase Enzyme

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Background: lactoperoxidase Enzyme (LPO) is the special Enzyme exists at mammals, secretion like milk, tear, saliva and etc. An oxidoreductase Enzyme with glycoprotein composition has a defensive protein that is not counted as part of Immunoglobulins group. It is one of the important parts of the immune system against bacterial factors. The osmoprotectant is an organic molecule Amphother, and water binding very compatible with the cell metabolism which can act as Enzyme protector toward the denature factors. The osmoprotectant is being used as a compatibilizer factor and the stabilizer of lactoproxidase Enzyme in this study.

Method: Lactoperoxidase Enzymes together with hydroxyectoine which is provided through a buffer in solution from stabilizal during 48 hours in 25c temperature. The stabled and free lactoperoxidase Enzymes were analyzed to measure the stability of Enzyme in different pH, temperature, time, different concentration Enzyme and different concentration stabilizer. Finally, the free and the stabled Enzymes level of activity and stability were measured by the (UV-visible absorption spectroscopy). In order to make sure of the Enzymes stabilization procedure it analyzed through the FTIR spectra and fluorescence spectroscopy method.

Results: The reminder activities of the stabilized Enzyme with osmoprotectants after measuring stabled at different pH and temperatures are as follows (89.17, 70.45, 70.33, 58.29, 44.12, 39.75) respectively and (91.44, 70.49, 77.38, 89.25). While the free Enzyme without stabilizer of the level of the reminder activities are as follows respectively (20.32, 12.77, 29.75, 18.16, 25.44, 19.59) and (41.72, 23.41, 18.43, 9.31). In addition, the stabilized lactoperoxidase enzyme have the best stability effect and activity in during 48 hours stabilization and in presence of 0.8M concentration of stabilizer and 75µgr/ml of Enzyme.

Conclusion: Results of this research revealed that stabilization with osmoprotectants like hydroxyectoine causes the Enzymes to be stable at intolerable circumstances

Keywords: osmoprotectant ، hydroxyectoineb ،Lactoperoxidase

P-536

Serum LDL, HDL, and Cholesterol status and its effects on Cardiovascular disease in rats under gamma radiation

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Background: Radiation therapy is one of the treatments for cancer that produces free radicals and active oxygen species, leading to damage to cellular proteins, membrane lipids, nucleic acids, and ultimately cell death. The results of this study showed that high-dose radiation therapy can disrupt the lipid profile of blood lipids and lead to cardiovascular disease. As a result, cholesterol levels, LDL increased significantly, and HDL levels decreased significantly compared to the control group (without radiation therapy), indicating that the rats under study had cardiovascular disease.

Methods: 60 male WISTAR rats (160 ± 5 gr), were selected and then randomly divided into 4 main groups, 1 group without radiation and 3 groups respectively received 2,4 and 8 doses of gamma radiation (each group 15 number). The radiation groups were irradiated for 2 days a week, each time, 20 min and 2, 4 and 8 gray doses respectively gamma radiation 8.4, 16.8, and 33.6 minutes animals were killed by Ketamine Zailazin administration, and blood samples were taken directly from the heart. Amount Cholesterol, LDL, and HDL were measured in blood serum.

Result: The results of the present study showed that following radiation therapy in doses 4 and 8, the levels of cholesterol and LDL in the blood serum increased significantly, and HDL values in toxic doses of 4 and 8 were observed with minor changes and radiation therapy prevented its increase in My head is bleeding.

Conclusion: Radiotherapy is one of the most effective treatments for some cancers. Radiation therapy in toxic doses reduces HDL and increases LDL and CL, which in effect increases the risk of cardiovascular disease by producing free radicals.

Key words: cholesterol, LDL, HDL, Radiation therapy

P-537

Evaluation of the Therapeutic Effect of Doxorubicin and Rosmarinic acid, Co-loaded with Zein Nanoparticle on Breast Cancer

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Background: Breast cancer is the second leading cause of death in the world. Numerous treatments are being used based on the type of cancer and the rate of progression. One of the problems is the non-specific destruction of cells. The present study investigates the therapeutic effect of Doxorabicin and Rosmarinic acid with Zein nanoparticles as a specific drug delivery system.

Methods: The present study is a systematic review in which we collected and analyzed data by searching for keywords in databases such as Pubmed and google scholar.

Results: The method of definitive diagnosis of breast tumors is pathological evaluation, but when the drug reaches the target tissue, we have to increase the dose of the drug due to its low concentration to increase its effect, which also increases the toxicity of the drug. The specific drug delivery system in the target tissue can increase the effectiveness of the drug at low doses and reduce its toxicity. Doxorubicin is one of the most effective drugs for cancer. In this system, the drug is coated by special carriers such as Zein nanoparticles due to their high solubility and stability and then ready to be delivered. Rosmarinic acid is also a compound that has an anti-cancer effect, but due to the non-specificity of the drug, a small concentration of it reached the target tissue. As a dedicated delivery system, slow drug release can be adjusted over a period of time.

Conclusion: In the traditional method of cancer treatment, due to the increased toxicity of the drug, the need for a dedicated delivery system is felt more than before. Using this system, the performance of the drug can be purposefully adjusted. It is hoped that the results of the present study will improve the treatment approach of patients.

Keywords: Breast cancer, Zein, Doxorubicin, Rosmarinic acid, specific drug delivery system

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The local and circulating SOX9 as a potential biomarker for the diagnosis of primary bone cancer

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Background: The status of the local and circulating SOX9, a master regulator of the tumor fate, and its relevance to tumor types, severity, invasion feature, response to therapy, and chemotherapy treatment were surveyed in bone cancer in the current study.

Methods: The SOX9 expression level was evaluated in tissue and peripheral blood mononuclear cells from patients with different types of malignant and benign bone tumors also tumor margin tissues using Real-Time PCR. The protein level of SOX9 was assessed using immunohistochemistry and western blot analysis. Also, the correlations of the SOX9 expression level with the patient's clinical and pathological features were considered.

Results: The remarkable overexpression of SOX9 was detected in bone tumors compared to tumor margin tissues ($P < 0.0001$). Malignant bone tumors revealed a higher expression of SOX9 compared to benign tumors ($P < 0.0001$) while osteosarcoma tumors showed higher expression levels compared to Ewing sarcoma, and chondrosarcoma. Overexpression of SOX9 was observed in high grade, metastatic, recurrent tumors also tumors with poor response to therapy. Besides, the patients under the chemotherapy treatment demonstrated higher levels of SOX9 compared to the rest of malignant tumors ($P = 0.02$). The simultaneous up-regulation of circulating SOX9 in the patients with bone cancer was observed compared to healthy individuals ($P < 0.0001$) accompanying with overexpression of SOX9 in malignant tumors compared to benign tumors ($P < 0.0001$). The circulating SOX9 expression was up-regulated in the patients with malignant bone tumors who receive chemotherapy treatment also patients with high grade, metastatic, recurrent tumors. The protein level of SOX9 was in line with our data on the SOX9 gene expression.

Conclusion: The simultaneous overexpression of local and circulating SOX9 in bone cancer besides its positive correlation with tumor severity, malignancy, size, and chemotherapy may deserve receiving more attention in bone cancer diagnosis and therapy.

Keywords: Bone cancer, SOX9, CSC marker, malignant bone tumors, Benign bone tumors

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Autophagy induction in the brain of NMRI mice culminates in enhanced longevity of mice challenged with the street rabies virus

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Background: Rabies is a public health threat and one of the oldest known infectious diseases. It still propounds a major challenge. Autophagy is a programmed cell death controlled and functions as the mechanism for the removal of unwanted cellular structures by the degradation of excess or injured organelles to maintain homeostasis. Aims: This study aims to explore the effect of in vivo autophagy induction through overexpression of Beclin1 (autophagy initiator), on the longevity of mice challenged with the street rabies virus.

Methods: Exogenous Beclin1 was overexpressed by the pIRES2-EGFP-Beclin1 vector in the cortex of NMRI mice. The expression of autophagy-related genes (Map1lc3b, Beclin1, and Atg5) was examined at the mRNA level by the RT-PCR technique. To evaluate the formation of autophagosome, immunohistochemical analysis of LC3 expression was done. TUNEL assay and virus titration was indagated in the brain tissues of the rabid mice.

Results: Results showed significant induction of autophagy-related genes in the vector receiving group compared to normal tissues. TUNEL assay indicated that RABV and vector did not cause apoptosis. Kaplan-Meier analysis revealed higher survival in mice receiving exogenous Beclin1 vector compared to controls ($P < 0.05$). Titration analysis of RABVdisplayed significantly lower titer of virus in the vector receiving group. Histological studies TUNEL assay showed that RABV and vector did not cause apoptosis.

Conclusion: It can be concluded that overexpression of exogenous Beclin1 could induce autophagy, and autophagy played a positive role in invading RABV, leading to amelioration of mice challenged with the rabies virus. It seems that overexpression of Beclin1 played an important role in the maintenance of autophagy formation and function.

Keywords: Belin1 overexpression, Autophagy induction, Street rabies virus, Longevity

P-540

Anti-aging Properties of Saffron Carotenoids in Primary Human Fibroblasts

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Background: Reactive oxygen species (ROS) and UV irradiation are two potent factors involved in collagen disruption which is known as the main cause of skin aging. Saffron is composed of the carotenoids that are considered as the potent natural antioxidants that have UV-protection properties. Thus, in the present study, we aimed to investigate the effects of saffron active ingredients individually on the proteome pattern and age-related factors in the primary skin cells.

Methods: Human dermal fibroblasts (HDFs) were obtained with the biopsy of a healthy young male donor (Royan Institute, Tehran, Iran). The cells were plated in T-75 flasks and cultured in DMEM supplemented with 15% heat-inactivated FBS and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere containing 5% CO2. All experiments were performed using only cells between passages 3 and 4. The MTT assay was performed in the presence of saffron aqua extract (SAE), crocin, crocetin, and picrocrocin in culture media. Finally, HDFs were treated with 50 µM of SAE, crocin, crocetin, and picrocrocin for 24 hours. The proteome pattern of HDF cell lysates before and after treatment were determined using 2-D gel electrophoresis followed by bioinformatics analysis of the profile.

Results: We observed no toxicity after 24 h incubation of fibroblast cells at doses of 1 to 3 mM for all treatments. Also, it appears that saffron and its active ingredients can affect the proteome profile of HDF cells, decreasing the matrix metalloproteinase (MMP) levels and increasing other antioxidant proteins.

Conclusion: Reduction in the expression levels of MMPs can prevent the disruption of extracellular matrix proteins including collagen. In summary, saffron components have the potential to limit the collagen disruption mechanisms via lowering the MMPs in fibroblasts, but with various degrees. Therefore, they can be served as anti-aging agents in skincare formulations.

Keywords: Saffron, Aging, Fibroblast, Proteome Profile

P-541

Prostatic acid phosphatase and selenium as Dietary Supplement

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Background : Antioxidants deactivate the activated oxygen species from the macromolecules oxidation through producing aldehyde, ketones, and ester compounds, and other beneficial compounds involved in the biological system. These compounds reduce the incidence of various diseases such as cancer through reducing the number of free radicals. Therefore, to achieve optimal cell function and prevent the disorders, it is necessary to control the oxidative stress process by consuming antioxidant compounds and consequently reducing free radicals. Selenium research has attracted tremendous interest because of its important role in antioxidant enzymes and participates in various functions.
Methods : Men were stratified based on their baseline serum selenium levels ≥200 µg/L or <200 µg/L. Only those with serum levels of <200 µg/L were supplemented with 200µg/L selenium for three months (200 men over the age of 50) and 100 men were also included in the study as controls. Selenium and Prostatic acid phosphatase (PAP) levels were measured in serum at pre- and post- supplementation. Changes in selenium and PAP levels after supplementation were assessed. Statistical calculations were performed by T test method.

Results : The Prostatic acid phosphatase of the study group was measured before and after selenium consumption. The results showed that the amount of Prostatic acid phosphatase increased despite selenium supplementation (6/68±0.11 to 6/79±0/11). This difference is statistically significant (p-value=0.002). In the control group that did not take selenium supplements, the Prostatic acid phosphatase level was 5/ 69±0.07 ng/dl at the beginning of the study and increased to 5/89±0.08 ng/dl at the end of the study that difference is statistically significant (p-value = 0.000).

Conclusion: The studies indicate that selenium decreases the growth rate of prostate cancer cells hence these results highlights the importance of selenium levels on prostate health.

Key words: selenium, oxidative stress, prostate

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Evaluation of autoantibodies against vimentin and α-enolase in the Rheumatoid arthritis patients

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Objective: Rheumatoid arthritis (RA) is categorized as the auto immune disease with the frequency of 0.2-1% all around the world. It is reported that various autoantibodies are produced in the RA population, particularly against citrullinated peptides. Among discrepant candidate markers for RA diagnostic, the citrullinated proteins have the most specificity and sensitivity for both diagnosis and prognosis of RA. Anti-mutated citrullinated vimentin and α-enolase are the new class of autoantibodies for early detection of RA.

Materials and Methods: Here, 45 serum samples and 19 Synovial Fluid (SF) specimens were collected from RA cases patients were considered for American College of Rheumatology (ACR) criteria and 20 serum samples and 10 SF specimens were provided from healthy subjects as control group. To assess the quantity of anti–MCV and anti- α-enolase in the serum and SF of RA patients, were determined by enzyme-linked immunosorbent assay (ELISA) method. For the evaluation of disease activity and joint destruction, used the Disease Activity Score of 28 joint based on ESR(DAS28-ESR). Furthermore, to measure the molecular weight of vimentin and α-enolase, electrophoresis on 10% SDS-PAGE as described before.

Results: The anti- α-enolase level among serum sample from patients was significantly higher than healthy subjects (4.49 ± 0.20 ng/ml vs 0.76 ± 0.12 ng/ml) ($p<0.001$). There was direct relation between α-enolase quantity and RF and CRP levels. The mean of ESR in positive and negative anti-CCP patients was 38.2±22.6 and 9.2±5.8 respectively ($P<0/0001$). The mean of DAS28-ESR was 3.3. The level of anti-CMV in the serum of RA patients (244.6 ± 53.3 U/ml) was higher than serum of healthy groups (148.73 ± 71.8) ($P<0.0001$). The level of anti-MCV in the Synovial Fluid of patients was 687.5±148.4 (U/ml).

Conclusions: both autoantibodies against MCV and α-enolase are the two important markers that increase in serum and SF of RA patients.

Key words: Rheumatoid Arthritis (RA), autoantibodies, vimentin, α-enolase, Synovial Fluid (SF).

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Adenosine deaminase (ADA) and antigen 125 (CA-125) level in patients with colon cance

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Aim: Colorectal cancer is the third common cancer in the world. This cancer develops slowly and silently so it is usually detected very late. Finding some simple methods for early diagnosis can lead to timely prevention and treatment of the cancer. Molecular biomarkers are factors that assessing their roles in diagnosis, prognosis and follow-up different diseases especially cancers have got a lot of attention in recent years. CA-125 and ADA are two proteins that their increase has been observed in various cancers. In this study we want to compare ADA and CA-125 level in colorectal cancer patients and healthy controls.

Methods: 50 blood samples from patients and 50 samples from healthy control were obtained and ADA and CA-125 were assessed in both groups. ADA level was assessed using enzymatic method by autoanalyzer (BT3000). CA-125 was measured by Elecsys based on Chemiluminescence. Data was analyzed by SPSS 16 software.

Results: The mean level of ADA in patients' group was 36.57 ± 1.5 U/L. Its level was 12.83±5.7 U/L in control group. The average value of CA-125 in patients and healthy control were 63.54 U/ml and 15.67 U/ml, respectively.

Conclusion: Based on obtained results, ADA level was increased significantly compared to control group ($P<0.05$) Although serum CA-125 level showed no notable differences.

Key words: Colorectal cancer, Colon cancer, Cancer antigen 125(CA-125), Adenosine deaminase (ADA).

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Endothelin-1 stimulates phosphorylation of Smad2 in Bovine Aortic Endothelial Cells via transactivation pathways

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Background: The carboxy terminal of Smad2 can be phosphorylated by the TGF-β receptor. Also, the linker region of Smad2 phosphorylated through numerous serine/threonine kinases. Recent studies have shown that, G protein coupled receptors (GPCRs) and their ligands can lead to phosphorylation of Smad2. The aim of this study was to investigate the role of Endothelin (ET-1) induced transactivation pathways on phosphorylation of Smad2 in Bovine Aortic Endothelial Cells (BAECs).

Materials and methods: In this study, phosphorylated Smad2 level was measured by western blot using phospho-Smad2 residues antibody.

Results: Our data revealed that the exposure of BAECs to ET-1(100nM) induces time-dependent phosphorylation of Smad2 in BAECs. AG1478 (EGFR antagonist) and SB431542 (TGF-β antagonist) inhibited ET-1 mediated Smad2 phosphorylation.

Conclusion: This study revealed that ET-1 stimulates phosphorylation of Smad2 through a mechanism involving the transactivation pathway of EGF and TGF-β receptors.

Keywords: Endothelin-1, Smad2L, transactivation, ET-1

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