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Aim and Scope

The International Journal of Molecular and Cellular Medicine (IJMCM) is a peer-reviewed, quarterly publication of Cellular and Molecular Biology Research Center (CMBRC), Babol University of Medical Sciences, Babol, Iran. The journal covers all Cellular & Molecular biology and medicine disciplines such as the genetic basis of disease, biomarker discovery in diagnosis and treatment, genomics and proteomics, bioinformatics, computer applications in human biology, stem cells and tissue engineering, medical biotechnology, nanomedicine, cellular processes related to growth, death and survival, clinical biochemistry, molecular & cellular immunology, molecular and cellular aspects of infectious disease and cancer research.

The following types of papers are considered for publication: original research works, reviews, mini-reviews, case reports, short communications and letters to the editor in the above mentioned fields. IJMCM is a **free access** journal.

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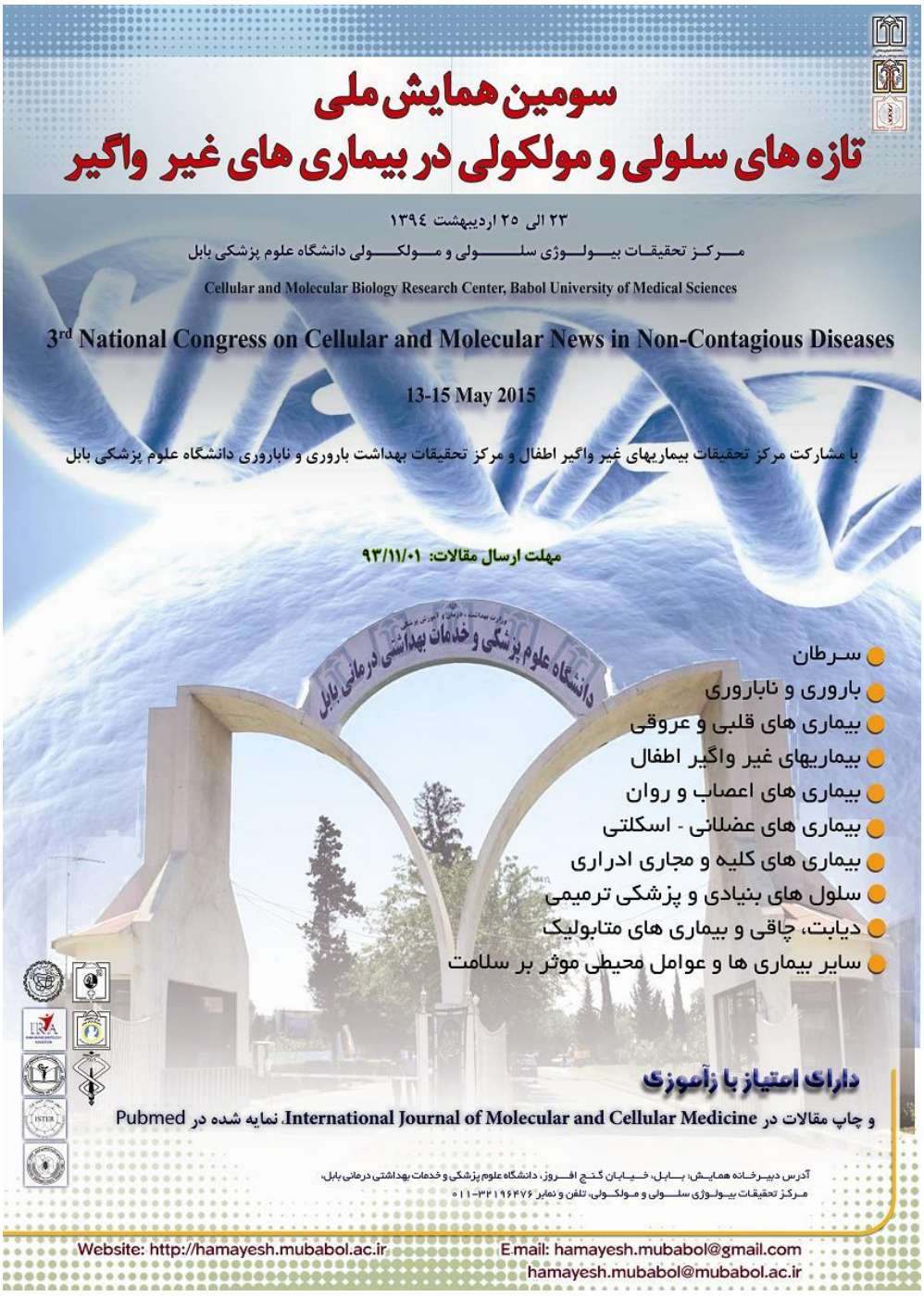

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Dear Colleagues

By promoting the public health level and using more efficient ways to prevent infectious diseases, all the healthcare centers have shifted their focus to deal with another unresolved issue, non-contagious diseases. Education and scientific researches on these diseases can strongly influence on their diagnosis and treatment. In the past decade, Babol University of Medical Sciences (BUMS) has held two international and national congresses in this field. Many researchers from all around the country have been working with BUMS research centers in the field of cellular and molecular research correlated to noncontagious diseases. We are extremely hopeful that the"**3rd National Congress on Cellular and Molecular News in Non-contagious diseases**" provides an opportunity for researchers and officials to have an update on the latest achievements of their colleagues. We are going to make it feasible to all researchers to meet in an intimate atmosphere in favor of the national health-care development in our country. We hope that the green Ordibehesht (May) of Babol would be more magnificent in 1394 (2015) decorated by blossoms of research and new scientific achievements.

Dr Seyed Mozafar Rabie
The Chairman of Congress
President of Babol University of Medical Sciences

Dear Colleagues

It seems that in the next upcoming decades, by promoting health care levels and changing Iran's age pyramid, noncontagious diseases would have the better share of health challenges and hence the focus of medical researchs. With continuous and ever growing cellular and molecular technologies, billions of dollars funds spent annually on medical laboratory researches fields like: stem cells, tissue engineering, DNA and RNA manipulation etc. in noncontagious diseases including cancer, diabetes, infertility, and children genetic diseases. Moreover, access to new molecular technologies help fast and more accurate diagnosis needed for improving the citizens' healthcare. One of the main needs of investigators in the field of cellular and molecular research is being aware of the findings of other universities in our country, to avoid parallel works and maximum gain from domestic knowledge. We hope that the honorable guests of this congress enjoy the spring of north of Iran while taking full advantage of this three days event.
Hope to see you on the congress days.

Dr Ebrahim Zabihi
Scientific Secretary

Oral Presentations

O-1 DNA Damage, Genome Instability and Cancer Predisposition

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Recent developments in genetics and molecular biology haveallowed detailed characterization of genetic alterations which occurring in normal human tissues. Following exposure to chemicals or physical agents, a wide variety of DNA lesions are induced eitherdirectly or indirectly via reactive oxygen species (ROS) formation, several of which may be converted into stable genetic alterations. The formation of ROS produces not only DNA strand breakages, but also may act as a signaling event leading to the release of cytokines or epigenetic changes, or trigger DNA repair machinery which in turn may lead to genetic alterations contributing in reproductive failure, miscarriage, genetic malformations and cancer induction. Accumulating evidence points to the unrepaired double strand breaks (DSBs) as the major lesion in the cellular, chromosomal, mutagenic and oncogenic effects of physical and chemical agents. All primary lesions induced in the DNA are subjected to cellular repair processes; the main key events in carcinogenesis are involvement of DNA repair genes, p53, cell cycle checkpoint genes, chromosomal rearrangements, apoptosis, contact inhibition and specific genes for each type of cancer. Unrepaired DNA (DSBs) double- strand breaks have been shown to contribute in genome instability of cells which may later be manifested as chromosomal alterations (CA), micronuclei, cell transformation, gene amplification, apoptosis, sister chromatid exchange, and so on. It has been known for a long time that there is an association between genome instability and tumor formation but various cellular phenomena operating after induction of genome instability in cells might change the faith of damaged cell. There are some known heritable genetic disorders such as *ataxia telangiectasia (*AT), Nijmegen breakage syndrome, Fanconi anemia, Xeroderma pigmentasum, Bloom syndrome and others suffering from genomic and chromosomal instability. Association of genome instability with cellular responses involved in carcinogenesis will be discussed.

Key words: Carcinogenesis, genome instability, DNA damage, cancer predisposition

O-2 Clinical Applications of MolecularMarkers

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Diagnostic methods are important decision- making clinical tools for at every stage of care-risk assessment, screening, diagnosis, staging, prognosis and selection of therapy. Biological marker (biomarker) is a biological, biochemical or molecular substance thatcan be measured either quantitatively or qualitatively. The biomarkers should be relevant to the pathogenesis of a disease or severity of disease activity. The use of biomarkers in medical diagnosis has progressed significantly over the past decade. Today, biomarkers are used in various diseases including prenatal diagnosis, cancer, infectious diseases, cardiovascular diseases, and pharmacogenetics.

Key words: Biomarker, diagnosis

O-3 Major Histocompatibility Complex (MHC) Plays a Central Role in Allogeneic Hemato-poietic Cell Transplantation (HCT)

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Early attempts in the1950s to transplant living cells from one individual to another had been carried out, but were not successful for ten years. Immunology plays a central role in allogeneic hematopoietic cell transplantation (HCT). By the late 1960s, much was known about the histocompatibility antigens (HLA) system. Immune reactions provoked by grafting tissue from one individual to another are caused by transplantation or HLA. The human MHC is located on the short arm of chromosome six. An HLA-matched sibling can be readily identified by an informative family study, especially if family members are HLA heterozygous. For a given patient, there is a 25% probability of a sibling havinginherited the same parental haplotypes. HLA class I antigens encoded by three loci termed HLA-A, HLA-B and HLA-C, and HLA class II antigens are encoded using three clusters of loci (HLA-DR, DQ DR and DP). In our BMT center, when patients were selected for hematopoietic stem cell transplantation, HLA class I antigens were

identified serologically. If patients and donors were HLA-matched in class I antigens, HLA class II antigens could be identified by PCR methods. In our BMT center, more than 500 HLA typing were requested from 6 September 2010 to March 2014. Seventy-two BMTs were done in our center. More than 50 HLA matched-donors were identified. In our center, 47 allogenic BMTs were done. HLA-matched related donors were the patient's brother, sister, father, mother and aunt. Immunology has an important role in allogeneic hematopoietic cell transplantation. HLA-matched related donor can be readily identified. We can pursue HLA-matched donor in brothers, sisters, and other family members. As a result of increasing national and international cooperation, large panels of volunteer marrow donors can be identified in our center in the future.

Key words: Immunology, major histocompatibility complex, bone marrow transplantation, human leukocyte antigens

O-4 Recent Findings about Radiation Modifiers in Treatment of Cancer

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Ionizing radiations may induce biological responses that depend on many factors such as physical specifications of ionizing radiation as well as biological system factors. It is shown that patients with same type of cancer and treatment regimen may show different radiosensitivity, which may be due to different specifications of their biological systems. It is revealed that many biological factors including age, sex, smoking and existence of genetic background disease affect radiosen-sitivity. However, by eliminating these factors and alsowith similar doses of radiation, patients may still show different biological responses such as acute and low responses. A review on recent findings about radiation modifiers in treatment of cancer has been the subject of the present article.

Keywords: Radiation modifiers, cancer, ionizing radiations

O-5 Molecular- Genetic Evaluation in Childhood Leukemia

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Leukemia is the most common childhood malignancy and occurs more than 30 percent of all malignancy in childhood. Acute lymphoblastic leukemia (ALL) is about 80 percent, acute myeloblastic leukemia (AML) about 15 percent and chronic myelogenous leukemia (CML) less than 5 percent of leukemia. Diagnosis and prognosis and treatment of leukemia are based on morphologic and cytochemistry and immunologic markers and molecular genetics study. More than 10 years ago, molecular and genetic studies had important role in treatment and prognosis, so recently in Amirkola hematology and oncology ward, we have investigated molecular genetic studies by sending bone marrow or blood sample of patients to specific centers for this study. The most common molecular genetics study for leukemia include:

<u>For ALL</u>
1. t (9;22) BCR-ABL (m-bcr) RT-PCR (Poor prognosis ALL)
2. t (4;11) MLL-AF4 RT-PCR (Pro- B Cell ALL, infantile)
3. t (12;21) TEL-AML1 RT-PCR (good prognosis ALL)

<u>For AML</u>
1. t (15;17) PML-PARα RT-PCR (Promyelocytic AML, good prognosis)
2. inv (16) CBFB-MYH11 RT-PCR (good prognosis)
3. T (8;21) AML1-ETO RT-PCR
4. FLT3 RT-PCR
5. t (1;19) E2A- PBX1 RT-PCR

<u>For CML</u>
1. t (9;22) BCR- ABL (M-bcr) RT- PCR

Keywords: Molecular genetic, leukemia, prognosis

O-6 Molecular Mechanism of Nicotine-induced Chemotherapeutic Resistance in Breast Cancer Cell Line MCF-7

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Nicotine, the main addictive compound in tobacco smoke, has been linked to promotion and progression of not only lung, but also head, neck, pancreatic, and breast cancers. In addition, it can induce drug resistance in chemotherapy which its underlying mechanism (s) remains elusive. Here, we show that nicotine induces the changes in alpha-7 and 9 nicotinic receptor subunits expression in breast cancer cell line. Nicotine-induced up regulation of anti-apoptotic (Bcl-2) and down regulation of pro-apoptotic (Bax) parameter were alsoobserved. The increase in mitochondrial integrity and decrease in cytochrome C release were demonstrated in nicotine-induced drug resistance

MCF-7 cells. In nicotine-treated cells, the levels of executer apoptotic enzyme (capase-3) were greater than those in control cells. In addition, cyclin D1 as a marker of cell proliferation signi-ficantly increased following nicotine treatment. Besides over data, it has been shown that nicotine can induce enrichment of side population cells with cancer stem cell-like properties. Although, drug resistance is a clinical problem in the treatment of cancer, it may also be a useful approach by transplanting cells expressing the drug resistance feature to protect the bone marrow during high-dose chemotherapy. Therefore, it is probable that the ability of nicotine to produce cancer cells with innate resistance to apoptosis and higher proliferating rate can be considered to that why chemotherapeutic drugs cannot injure such cells.

Keywords: cancer, chemotherapy-resistance, nicot-ine, MCF

O-7 Scrophularia Megalantha induces apoptosis and G2/M cell cycle arrest in Jurkat (Pre-T cell) human leukemia cell line

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Scrophularia megalantha Boiss (Scrophulariaceae) is a medicinal plant and isbeing used as a traditional herb for various inflammatory disorders. This study was designed to investigate the cytotoxic effects of *Scrophularia megalantha (S. megalantha)* extracton Jurkat human leukemia cell line (Pre-T cell leukemia). Phytochemical assay by thin layer chromatography (TLC) and the 2, 2 diphenyl-1-picryl-hydrazyl (DPPH) were used to evaluate the main compounds and the antioxidant capacity of the plant extract, respectively. The inhibitory effect of the extract on the Jurkat cells was evaluated by MTT assay. In addition, cell cycle distribution and apoptotic cell death were evaluated by PI (propi-dum iodide) and Annexin V-FITC/PI staining, respectively. The results showed that the main components; including flavonoids, phenolic compounds and phenyl propanoids were presented in the *S. megalantha extract*. The treatment with extract significantly showed significant cytotoxicity effect on tumor cell line. In addition, flow cytometry analysis indicated that *S. megalantha extract* induced cell cycle arrest in G2/M phase and apoptosis on tumor cell. The findings in this study indicated that *S. megalantha extract* could inhibit leukemia cell growth through inducing G2/M phase arrest and cell apoptosis however, future studies are necessary.

Key words: Leukemia cell, G2/M phase arrest, *S. megalantha extract,* apoptosis

O-8 *In Vitro* Spermatogenesis: Past, Present, Future

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Men with azoospermiaprefer genetic parenthood instead of using donated gametes. Considering self-renew and differentiation ability of pluripotent stem cells, some studies have pointed out on the possibility of stem cell derived sperm production. Most studies in this context have been conducted on rodents and some results are promising but studies on human face with some ethical issues and are progressing slowly. However, recently some expression specific markers of human mature germ cells have been reported. Previously, sperm-like cells with fertilizing ability were produced from mouse embryonic stem cells. The resulting embryos from these cells lead to live offspring, although the offspring died prematurely due to DNA methylation abnormalities. Some new methods for differentia-tion of stem cells such as embryoid body, co-culturing and various feeder cells, have also been used. These techniques may prepare niche more similar to *in vivo* condition and solve DNA methy-lation abnormalities. Although, still a gonadal-like three-dimensional structure is required for producing germ cells with correct imprinting. Also, due to the unavailability of embryonic cells in adults, future research should move towards the use of adult stem cells residing in bone marrow and peripheral blood. Since *in vitro* spermatogenesis can give hope to male without sperms who are untreatable now and can be a useful system to study the precise mechanism of spermatogenesis, more research techniques are required. In this review, we have described recent studies of *in vitro* spermato-genesis and its related techniques. We also discuss the possible cell surface markers and cultured conditions, which can improve *in vitro* spermato-genesis.

Keywords: Spermatogenesis, gametogenesis, infertility

O-9 Effect of Synovial Fluid on Mesenchymal Stem Cell Proliferation *In Vitro* Situation

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Nowadays, stem cell therapy presents a promising technique for tissue repair and regeneration. The plasticity of the adult stem cells such as mesenchymal stem cells enabled them as a treatment tool for a broad spectrum of diseases. Mesenchymal stem cells (MSCs) are attractive cell sources for application in regenerative medicine due to their excellent proliferation and differentiation capacities. MSCs present in various tissues like bone marrow (BMSC) and capable of differentiating in to multiple cells Types. In this study, we examined the effect of synovial fluid on proliferation of mesenchymal stem cells under invitro culture. Mesenchymal stem cells were isolated from rat femurs and tibias and cultured in DMEM high glucose medium supplemented with fetal bovin serum. Definition of MSCs has been confirmed using antibodies against CD71 and CD90. Although synovial fluid was centrifuged at 2000 r/min then supernatant was filtered (0/22 µm). The first and the second passages of cellswere harvested and subjected to examination of the synovial fluid effect on proliferation of these cells. The MSCs exhibited high capacity of proliferation in the presence of synovial fluid and making more colony forming units to compare cultured MSCs without synovial fluid. According to the results, proliferation of MSCs has been accelerated in presence of synovial fluid therefore, synovial fluid can be a valuablesupplement for culture of MSCs.

Keywords: Bone marrow mesenchymal stem cells, proliferation, synovial fluid.

O-10 Cellular Response of Limbal Stem Cells on Polycaprolactone Nanofibrous Scaffolds for Ocular Epithelial Regeneration

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The aim of this study was to evaluate the development of nanofibrouspolycaprolactone (PCL) substrate for limbal stem cell (LSC) expansion that could serve as a potential alternative substrate to replace human amniotic membrane (AM). The human limbus stem cell was used to evaluate the biocompatibility of substrates (nanofibrous scaffold and human AM) based on their phenotypic profile, viability, proliferation and attachment ability. Biocompatibility results indicated that all substrates were highly biocompatible, as LSCs could favorably attach and proliferate on the nanofibrous surface. Microscopic figures showed that the human LSCs were firmly anchored to the substrates and were able to retain a normal corneal stem cell phenotype. Microscopic analysis illustrated that cells infiltrated the nanofibers and successfully formed a three-dimensional corneal epithelium, which was viable for two weeks. Immunocytochemistry (ICC) and real time-PCR results revealed no change in the expression profile of LECs grown on nanofibrous substrate when compared to those grown on human AM. Not only electrospunnanofibrous PCL substrate provides a milieu supporting LSCs expansion, but also serve as a useful alternative carrier for ocular surface tissue engineering and can be used as an alternative substrate to AM.

Keywords: Cellular analysis, cornea regeneration, limbal stem cells, nanofibrous scaffold, polycaprolactone

O-11 The Effect of Direct Contact with Mesenchymal Stromal Cells on MRNA Expression and DNA Methylation Status of HOXB4 and GATA2 Genes in the *Ex Vivo* Expanded Cord Blood Hematopoietic Cells

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Ex vivo expansion of cord blood hematopoietic stem cells (CB-HSCs) have important applications in cell-based therapy. Direct cell to cell contact between CB-HSCs and mesenchymal stromal cells (MSCs) considerably affects the efficacy of CB-HSCs expansion. HOX B4 and GATA2 genes are closely related to the self- renewal potential of hematopoietic stem cells. So, in this study we expanded CB-HSCs in co-culture with MSCs and compared the adherent and non- adherent fractions of hematopoietic cells in terms of differentiation potential, clonogenic potential, mRNA expression and DNA methylation status of HOXB4 and GATA2 genes. CB-HSCs were cultured with MSCs in stem span medium supplemented with three cytokines (SCF, TPO, FL). After 7 days culture, the adherent and non-adherent fractions of hematopoietic cells were isolated and analyzed. Clonogenic potential were assessed in MethoCult

medium. Differentiation status was evaluated by flowcytometry. Moreover, mRNA expression of HOX B4 and GATA2 genes in expanded hematopoietic cells were analyzed by real time RT-PCR and DNA methylation status of these genes were determined by methylation specific PCR. The clonogenic potential was higher in adherent than non-adherent hematopoietic cells ($p<0.05$). Adherent hematopoietic cells were less differentiated in comparison to non-adherent hematopoietic cells and the proportion of $CD34^+$ cells and $CD34^+/CD38^-$ cells was higher in adherent cells than non-adherent cells ($p<0.05$). The mRNA expression levels of HOXB4 and GATA2 genes were significantly higher in adherent cells than non-adherent cells ($p<0.05$). DNA methylation status of HOX B4 and GATA2 genes did not differ significantly between adherent and non- adherent cells. Direct contact between cord blood hematopoietic cells and MSCs in co-culture condition significantly increased the clonogenic potential and mRNA expression of HOXB4 and GATA2 genes in adherent hematopoietic cells.

Key words: *Ex vivo* expansion, cord blood, mesenchymal stromal cells, HOXB4, GATA2

O-12 Pluripotency in Mouse Spermatogonial Stem Cells *In Vitro*

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Although testis-derived embryonic stemcell-like (ES-like) cells have been obtained in several studies, the time window for the shift to pluripotency is not clear yet. Here we describe, that only during a special time window (41 until 125 days) after initiation of germ line stem cell (GSCs) cultures from neonate and adult promoter-reporter Oct4-GFP transgenic mouse the spontaneous appearance of germline-derived pluripotent stem (gPS) cells from both neonate and adult GSCs occurred. The isolated and long-term cultured (more than one year) GSCs which were isolated by a morphology based selection procedure expressed germ-cell markers and exhibited a similar morphology with a high nucleus/cytoplasm ratio in comparison to undifferentiated SSCs (spermatogonial stem cells) *in vivo*. The generated gPS cells expressed pluripotency marker, in-vitro differentiated into all three germ lineages, formed complex teratoma after transplantation in SCID mice and produced chimeric mice. Although the exact mechanism of the development of gPS cells from GSCs is still unclear, this new information can provide an ideal strategy for scheduling natural conversion mechanisms of ES-like cells from mouse testis.

Key words: Spermatogonial stem cells, pluripotency, embryonic stem-like cells

O-13 The Assessment of Alginate Scaffold Ability in Chondrogenic Differentiation of Human Adipose-Derived Mesenchymal Stem cells

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Cartilage ability to repair damages is limited due to lack of blood vessels and low cell density. Recently, tissue engineering as a way to solve this problem considerably preferred to other treatments. Regardless of cell sources, one of the crucial factors in tissue engineering is to select an appropriate scaffold which is essential for healing and renewal procedure of tissues *in vivo* and *in vitro*. MSCs were isolated from adipose tissue in liposuction surgeries by use of collagenase enzyme. These cells were embedded into the alginate scaffold and then they were cultured in chondrogenic medium for 3 weeks. The ability of alginate scaffold was assessed by theuse of MTT assay and histological analysis. Also, analysis of chondrogenic genes expression by Real time PCR was done. The obtained data were analyzed statistically by means of SPSS software. There was no significant difference between alginate and control group in maintaining cells viable but about chondrogenic differentiation analyzed by use of real time PCR, statistical analysis showed a significant difference in the expression of aggrecan (as a cartilage specific gene) and collagen I (as an osteogenic specific gene) between cell/alginate and MSCs ($p<0.05$). Chondrocyte differentiation of cells was verified by histological analysis. alginate scaffold can provide a suitable environment for chondogenic differentiation of adipose derived mesenchymal stem cells.

Key words: Alginate, Chondrogenic differentiation, Mesenchymal stem cell, Tissue engineering

O-14 Gene Expression of SPATA19 and PAWP during Mouse Spermatogenesis *In Vivo* and *In Vitro*

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The understanding of the regulation of genes that are specifically expressed during spermatogenesis will contribute to a better appreciation of mechanisms underlying male infertility. Thus, in this study, we have investigated the expression of spermatogenesis-associated-19 (SPATA19) and Postacrosomal sheath WW domain-binding protein (PAWP) genes during testis development *in vivo*, in parallel with mouse embryonic stem cells differentiation into male germ-like cells *in vitro*, as well as Sertoli cell (TM4), mouse embryonic fibroblasts (MEF), and NIH3T3 cancerous cell line. Mouse ESC line C57BL/6J expressing the Stra8-EGFP was differentiated into male germ-like cells by retinoic acid and separated using Fluorescence-activated cell sorting (FACS). Then, PAWP and SPATA19 gene expressions were evaluated in these cells 5, 11, 19 and 27 days after differentiation, as well as 5, 15 and 25 days old Balb/c mouse testis, TM4, MEF and NIH3T3 cancerous cell lines using Real-time PCR technique. The expressions of both SPATA19 and PAWP increased gradually over time during mouse testis development *in vivo* and it is strongly up regulated in 25- day -old -mouse testis. During male germ-like cell derivation from mouse ESC *in vitro*, no SPATA19 gene expression was detected until 11 days of mouse ESC differentiation and it was expressed at post meiotic phase of spermatogenesis. Nevertheless, we observed PAWP expression at all phases of spermatogenesis and it was overexpressed in 30d after differentiation. Both SPATA19 and PAWP were overexpressed in NIH3T3 cell line, but there were no or negligible expressions of the genes in TM4 and MEF cell lines. These data suggested PAWP and SPATA19 as markers that could be looked in ESC studies as a confirmed testis-specific genes. Also, the results revealed additional possible roles for PAWP and SPATA19 in proliferation of cancerous cells and in the tumorigenic process in general.

Keywords: Spermatogenesis, PAWP, SPATA19, embryonic stem cell

O-15 The Study of Omega 3 and Omega 3, 6, 9 Fatty Acids on *In vitro* Maturation (IVM) of Immature Mouse Oocytes

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In vitro maturation can provide oocyte for infertile women and be useful for them, but has only been achieved with limited degree of success. Given the fact that omega-3 and omega 3, 6, 9 known as fatty acids and the fatty acids are a source of energy and activity during maturation, it is possible to improve the *in vitro* maturationof oocytes. Therefore, this study was deviced to evaluate the effect of Omega-3 and Omega 3, 6, 9 fatty acids on maturation of immature oocyte. Immature oocyte from the ovaries of NMRI female mouse has been collected at the ages of 6 to 8 weeks in a sterilized situation. Oocytesweredivided into three groups: Maturation medium for control group was α -MEM medium with 10 percent of FBS; the experimented groups1 and 2, having control group medium, with the addition of Omega 3, 6, 9 and Omega-3 fatty acids. For maturation, the oocytes in each group were cultured in incubator, for 24 hours and maturation has been recorded under an invert microscope. Analysis of data was done with the help of SPSS software. Omega 3 and Omega 3, 6, 9 fatty acids support the progressandresumption of meiosis, and increase ($P<0.05$) the oocyte maturation as compared tothe control group. When there were no Omega-3, and Omega 3, 6, 9 in culture medium IVM, statistically, less maturation was observed; while with 10 % V/V Omega-3 or Omega 3, 6, 9 in maturation culture medium, a better result and more maturation have been achieved than with control group. The results show that anaddition of 10 % V/V Omega-3 or Omega 3, 6, 9 into culture medium IVM during *in vitro* maturation of mouse oocyte will increase oocytes maturation.

Keywords: Omega 3, and Omega 3, 6, 9 fatty acids, *In vitro* maturation, mouse, immature oocyte

O-16 Reconstruction of Testis Germinal Epithelium using Mesenchymal Stem Cells, is it possible?

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Evaluation of the possibility of differentiation of bone marrow-derived mesenchymal stem cells (BM-MSCs) into the main cell types of the spermatogenesis process- germ and sertoli cells- have been performed in two separate research programs, one on the rat and the second on the sheep model. Methods and Materials: In the first study, after isolation and characterization of MSCs from male rats bone marrow samples, and confirmation of their stemness, the isolated MSCs were labeled with PKH26 and transplanted into the testes of infertile male rats (with busulfan injection). Transplanted testes were assessed 4, 6 and 8 weeks after transplantation to see the differentiation of transplanted BM-MSCs into the tubular cells (germ and sertoli cells). In the second study, ram BM-MSCs after characterization, were labeled with PKH26 and transplanted into the tests of ram lambs. The fate of transplanted cells in the testes was evaluated after 8 weeks. Evaluations showed that, BM-MSCs were alive in the testes of all three groups. Moreover, a number of donor cells were located in the germinal epithelium and expressed spermatogonia specific markers (Stella and Dazl). No cell colony with the origin of donor cells and also no further differentiation of generated germ-like cells were observed. Furthermore, donor cells were notable to differentiate into sertoli cells. In the second study, although, a number of transplanted cells were alive in the testis and even a very small ratio of them were homed at the germinal epithelium, differentiation into the germ cells was very poor. Totally, from the results of these two studies, it can be concluded that although MSCs have the capacity of differentiation into the germ cells, could not perform fully reconstruction of testicular germinal epithelium and establishment of spermatogenesis in an infertile male.
Key words: Mesenchymal stem cells, Testis, Germinal epithelium, Germ cells

O-17 Relationship Between Meiotic Spindles Visualization and Intracytoplasmic Sperm Injection (ICSI) Outcomes in Human Oocytes

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In assisted reproductive technology (ART), theuser attempts to select morphologically best embryos to predict embryo viability and proper implantation. Development of a polarized light microscope that evaluates the oocytes spindles according to birefringence of living cells had been helpful in oocyte selection. In this study, 264 oocytes from twenty-four patients with an average age of 32.5 years, and a duration of infertility between 1 and 10 years were collected. The oocytes were randomly allocated to the control injection group (n=126) and oocyteimaging group (n=138). In spindle-aligned group, the meiotic spindle was identified by the use of polarized light (CRi's PolScope technology) to align the spindle at 6 or 12 o'clock. Then the spindle-aligned group was divided into three sub-groups based on spindle morphology: fine, average and bad. After ICSI, embryos were checked every 24 hours and scored. 72 hours later, high-grade embryos were transferred intra-vaginal to uterus. This study showed that fertilization rate in spindle-aligned group was higher than control group ($p<0.05$). After cleavage, a positive correlation was observed between spindle morphology and embryo morphology. Among the sub-groups of spindle-aligned group, the embryos' morphology from the fine group was better than other sub-groups and embryos from bad group had lower quality and experienced more fragmentation. Conclusion: The results revealed that the selection of embryos based on meiotic spindle imaging could significantly improve the rate of implantation and pregnancy.
Key words: assisted reproduction, ICSI outcomes, meiotic spindle, embryo morphology

O-18 Recurrent Implantation Failure (RIF)

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Implantation is a complex process involving two main players: the mother as a host and the embryo as a guest. The absence of implantation after ≥3 embryo transfers with high quality embryos or after replacement of a total of 10 or more embryos in multiple transfers is considered as RIF. Inadequate uterine receptivity, is responsible for approximately two-thirds of implantation failures, whereas the embryo itself is responsible for only one-third of these failures. The changes in expression of growth factors such as FGF-1 are important maternal factors effectingimplantation. Variations in ovarian stimulation protocols have been suggested in some studies as a means of improving embryo development and quality. The use of GnRH antagonist protocols in controlled ovarian hyperstimulation (COH) has been shown to improve pregnancy outcome in patients with a history of RIF with

GnRH agonist protocols. Embryo transfer technique (ET) is important in achieving a successful pregnancy outcome. Avoidance of blood, mucus, bacterial contamination, trauma to the endometrium, touching the fundus, and excessive uterine contractions are all associated with better implantation rates after ET. Several techniques have been proposed to optimize the technique of ET (such as filled bladder, ultrasonographic guidance, and use of soft catheters. Chromosomal abnormalities both maternal and paternal play a key role in the etiology of repeated implantation failure in IVF. It showed significantly increased pregnancy and implantation rates for women who underwent blastocyst transfer, compared to those in which embryo transfer occurred on day 3. The use of ZIFT remains a powerful tool in the clinical management of selected patients with high-order RIF.

Key words: Recurrent Implantation, RIF ،EGF ، ZIFT

O-19 Applications of Nanotechnology in Medical Sciences

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Applications of nanotechnology for diagnosis, treatment, monitoring, and control of biological systems have recently been referred to as "nanomedicine". Nanomedicine is defined as the integration of nanotechnology in medicine for preserving and improving human health. Research into the rational delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents is at the forefront of projects in nanomedicine. Many diseases originate from alterations in biochemical processes at the molecular or nanoscale level. Oxidized biochemical compounds, misfolded proteins, mutated genes, and infections caused by viruses or bacteria can lead to cell malfunction or miscommunication, sometimes leading to life-threatening diseases. Nanomaterials have unique properties and applications, when they use in imaging or drug delivery, they have the potential to improve diagnostics and therapy of many human disorders including neurodegenerative disorders, by their ability to cross the blood brain barrier because they have very small sized particles. The metallic nanoparticles respond resonantly to the magnetic field, which varies with time so they transfer enough toxic thermal energy to the tumor cells as hyperthermic agents. Some of the most promising areas of nanomedicine are nanodiagnostics, nanobiosensors, nanopharmaceuticals, implanted nanopumps, nanocoated stents, nanorobotics (detection and destruction of cancer), nanosurgery such as nanolasers and nanosensors implanted in catheters. However, it is important to obtain information about the potential toxicity of the nanomaterials to discover and prevent serious unwanted human effects. The goal must be to realize the great opportunities and benefits of nanomaterials while at the same time minimizing the risk related to their applications. In the near future, nanomedicine can address many important medical problems by using nanomaterials and simple nanodevices that can be manufactured today, including the interaction of nanostructured materials with biological systems. In longer future, the earliest molecular machine systems and nanorobots may join the medical equipment, finally giving physicians the most potent tools imaginable to conquer human disease, ill health, and aging.

Key words: Nanomedicine, Nanobiotechnology, Nanopharmaceuticals, Nanorobotics, Nanobiosensors

O-20 Bioactive Glass Coatings For Bone Tissue Engineering: An *In Vitro* Study

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Metallic prostheses are used to treat skeletal injuries. However, metal alloy implants can sometimes fail due to complications of fibrous encapsulation and poor stress transfer between the bone and the implant. Bioactive glass (BG) coatings may promote the formation of a strong bond with living bone tissue thus decreasing the likelihood of fibrous encapsulation and have the added benefit that their dissolution ions stimulate cell activity (1, 2). Strontium (Sr) ranelate, a drug used to prevent osteoporosis, works via the action of Sr ions which stimulate the formation of new bone and prevent osteoclast-mediated resorption (3). We have previously shown that Sr-substituted BGs promote osteoblast activity *in vitro* (4) and explored the effect of altering phosphate content on the material structure of soda-lime-phosphosilicate glasses (5). The effect of increasing phosphate content in Sr-substituted BG on cultured osteo-blasts, however, remains unexplored. Here, we created Sr-substituted BG coatings with a range of phosphate contents and thermal expansion coefficients that matched that of Ti alloy, producing materials that combine the bone remodelling benefits of Sr and BG with phosphate to mediate pH changes which can affect cell viability. In the study presented here, we report the characterization of these multi-

component BG coatings in terms of their bioactivity and interaction with cells. Bioactive glasses in the system SiO_2-MgO-Na_2O-K_2O-ZnO-P_2O_5-CaO in which 10% of the Ca was replaced by Sr and the P_2O_5 content was increased from 1.07 to 6.42 mol% were produced by a melt quench route. Sufficient cations were added to ensure charge neutrality in the PO_4^{3-} complex formed. Simulated body fluid (SBF) was prepared according to Kokubo [6]. Glass particles (<38 micrometer) were immersed for up to 28 days and agitated at 60 rpm at 37°C. At indicated time points, the samples were filtered and dried for X-ray Diffraction (XRD) analysis. The human osteosarcoma cell line, Saos-2, was seeded in conditioned medium and cultured. On days 1, 14, 21 and 28, cell metabolic activity was measured using the tetrazole MTT as an indicator of cell proliferation. Glasses were coated on the surface of Ti6AL4V coupons with an enameling technique. Saos-2/cm^2 were seeded on BG coatings and viability was assessed after1, 7 and 14 days with a LIVE/DEAD stain. Some glass coating cultures were also fixed, gold coated and viewed on SEM. BG with high P_2O_5 content forms more apatite after immersion in SBF for 4 weeks than BG with low P_2O_5 content, as examined by XRD. MTT activity in Saos-2 cells treated with dissolution ions from BG increased in all samples with time in culture. MTT activity was also significantly greater (p<0.01) in cells treated with dissolution ions from 4.28 and 6.24 mol% P_2O_5 BGs as compared to controls at day 28. LIVE/DEAD staining indicated all coating materials were not cytotoxic. SEM imaging demonstrated that the BG coating encouraged cell attachment and that cells spread well over the surface. With increasing P_2O_5 content in the series of Sr-substituted BG, peaks in XRD traces associated with apatite crystallization increase suggesting the glass becomes more bioactive. Apatite formation on the coating surface is an essential factor for bone bonding as the more apatite that forms on the glass coating, the more bone bonding will be expected. Adding P_2O_5 to the glass composition in a controlled way prevents extreme pH rises, which can affect cell viability and proliferation.

Key words: Bioactive glass, strontium, bone tissue engineering

O-21 Effect of Bone Marrow on The Clinical and Mechanical Properties of Synthetic Bone Tissue Substitutes

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The aim of this study was to obtain the influence of bone marrow cells on clinical and mechanical properties of hydroxyapatite as a bone tissue substitute. Synthetic bone tissue substitute was fabricated by polyurethane foam and slurry containing hydroxyapatite (HA) powder, water and additives as a porous structure. The specimens were characterized with respect to their microstructure, morphology, phase composition and porosity. For *in vivo* tests, Synthetic Hydroxyapatite alone (HA) and Hydroxyapatite loaded with bone marrow (HA +BM) were evaluated into rabbits' femoral condyle bone defects for two periods of 1 and 3 months. Comparison of the osteoconductivity and mechanical compressive properties of implanted bone substitutes demonstrate the good biocompatibility and osteointegration of (HA +BM), with higher osteoconductive properties and earlier bioresorption, as compared to (HA). Compressive strengths of both substitutes are always significantly higher than the anatomic control. The elastic modulus of (HA +BM) became weaker and no significant difference was apparent between (HA +BM) and control 3 months after implantation. Considering the results; bone marrow cells improve mechanical properties and bone growth.

Key words: Bone tissue substitutes, bone marrow cells, hydroxyapatite

O-22 Motor Neuron-Like Cells Differentiation of Human Endometrial Stem Cells on Electrospining Nanofiber Scaffold

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Nerve tissue engineering (NTE) is one of the most promising methods to restore central nervous system in human health care. Three-dimensional (3D) distribution and growth of cells within the porous scaffold that composed of nano-fibres are of clinical significance for NTE. In this study, we investigated the potential of human endometrial stem cells, (hEnSCs) ability to differentiate into motor neuron-like cells on poly (d, l-lactide-*co*-glycolide) (PLGA) electrospun nanofibers. During the culture of hEnSCs on the PLGA scaffold (3D group) and tissue culture polystyrene (2D group), and differentiation of hEnSCs to motor neuron-like cells by induction media for 15days, the PLGA

nanofiber group was found better than 2D group, that a better growth of hEnSCs differentiated motor neuron-like cells was observed on the scaffold. Scanning electron microscopy imaging, real time-PCR and immunocytochemistry were used to analyze cultivated hEnSCs on scaffold and their expression of motor neuron-specific markers. Taken together, the results suggest that hEnSCs differentiation on PLGA can provide a suitable, three-dimensional situation for neuronal survival and outgrowth for regeneration of the central nervous system and these cells may be a potential candidate in cellular therapy for motor neuron disease.

Key words: Human endometrial stem cell, motor neuron differentiation, nanofibrous scaffolds

O-23 Designing and Construction of Specific Vector for Cancer Therapy

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The highest percentage of cancer-related death is related to lung cancer, because its diagnosis is at advanced stages and metastasis in the early stages and resistant to conventional treatments including surgery, radiotherapy and chemotherapy. New advances in molecular pathology of cancer offer new treatment strategies as gene therapy. One of the methods of gene therapy is the targeted gene expression in cancer cells. Gene expression is regulated at different levels, but transcription regulation is usually dominant. Expression of genes is controlled by the promoter. SLPI is a serine proteinase inhibitors, tissue-specific expression is tightly regulated at the transcriptional level. The promoter of this gene could be a good candidate for the targeting of gene expression in lung cancer. On the other hand, to optimize the expression of the promoter will be used of the Myc overexpression in lung cancer. C-Myc is a multi-functional phosphor-diesterase nuclear protein whichis involved in cell cycle progression and apoptosis. The purpose of this study was to establish specific expression vector for lung cancer cell. In this report, we studied the expression profile of lung cancer cell lines and selected promising candidates for designing of the promoter. Sequence of recombinant promoter was amplified by PCR using specific primers with appropriate restriction enzyme sites in their ends. It cloned in the pcDNA 3.1 (+) that had lost its promoter and GFP cloned in recombinant vector. Dot blot was done for the evaluation of specificity. Designed promoter was synthesized and inserted instead of the promoter in the plasmid and GFP was cloned in modified vector The construct was confirmed by colony PCR, restriction analysis and sequencing. Specificity of expression was evaluated by dot blot. Construction of specific expression vector is promising for gene therapy of lung cancer.

Keywords: Lung cancer, gene therapy, recombinant promoter,

O-24 CFTR Mutations in Iranian Cystic Fibrosis Children

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Cystic fibrosis is the most common monogenic disorder in the caucasians of Northern European heritage with a prevalence of 1 in 2500- 3300 live births. Over 1600 different mutations in the CF gene have been identified. It is an autosomal recessive disease. ΔF508 is the most common mutation causing classic CF in Caucasians. W 1282X is the most common gene causing classic CF in individuals of Ashkenazi Jewish heritage. To raise awareness of CF mutation in Iran, related researchstudies have been studied. Eligible articles were identified through electronic databases. It was included all studies related to the identification of cystic fibrosis mutation database (CFTR) mutation in Iran. Results: These studies resulted in the identification of 21.4% to 52% of all CF alleles. The most frequent mutation ΔF 508 represented only 10% to 21.7% of the expected mutated alleles. Discussion and conclusion: A heterogeneous mutation spectrum was observed at theCFTR in CF patients of Iran; but we have to study further about this subject in Iran.

Keywords: Cystic fibrosis, cystic fibrosis related diabetes, mutation

O-25 Association between Prolonged Juandice and TATA Box Dinucleotide Repeats in Gilbert's Syndrome

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Jaundice is a common condition during neonatal period. Prolonged jaundice occurs in a large

number of breastfed infants. Considering the impact of genetic factors on the incidence of jaundice, the aim of this study was to determine the association between prolonged jaundice and TATA box dinucleotide repeats in Gilbert's syndrome. The case group consisted of 51 patients with jaundice, aged more than 2 weeks with indirect bilirubin level higher than 10 mg/dL. Acute diseases and mother's use of phenobarbital and other medications were the exclusion criteria. The control group consisted of 54 newborns without jaundice, referring to Amirkola Hospital. The two groups were matched in terms of age and sex. TATA box polymorphisms in the promoter region of UGT1A1 genewere evaluated using polymerase chain reaction (PCR) to determine TATA box dinucleotide repeats. Overall, 64.7% and 50% of subjects in the case and control groups were males, respectively (P=0.168). The mean age of neonates in the case and control groups was 20.1±7.1 and 18.8±4.1 days, respectively. The distribution of Gilbert genome was not significantly different between the two groups. In the case group, 13.7% of the subjects were homozygous, 37.3% were heterozygous, and 49% were normal. In the control group, 7.4% of the participants were homozygous, 35.2% were heterozygous, and 57.4% were normal. The results of this study showed an association between TATA box polymorphism, and prolonged jaundice in neonates.

Keywords: Neonatal hyperbilirubinemia, Gilbert's syndrome, mutation

O-26 Overview of Gastrointestinal Diseasein Cystic Fibrosis

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Cystic fibrosis (CF) generally is thought of as a lung disease since much of the associated morbidity and mortality is related to pulmonary complica-tions. Gastrointestinal complications have become an increasingly important cause of morbidity in patients with CF. Pancreatic insufficiency, which is one of the most clinically important gastrointestinal issues, the underlying pathophysiology of cystic fibrosis is related to abnormal chloride transport caused by mutations in the cystic fibrosis trans-membrane conductance regulator gene. (CFTR) located on chromosome 7. The mutations cause the production of abnormally tenacious mucus and secretions in the lungs, gut, pancreas, and hepato-biliary system. As a result, the lumens of these organs become obstructed leading to the clinical findings associated with this disease process. The gastrointes-tinal manifestations of CF can be broken down into three categories: intestinal, pancreatic, and hepato-biliary. Intestinal abnormalities include gastroesophageal reflux disease (GERD), meconi-um ileus (MI), distal intestinal obstruction synd-rome (DIOS), intussusception, small intestine bacterial overgrowth, constipation, and rectal prolapsed and appendicitis. Pancreatic insufficiency leads to malabsorption of fat (with steatorrhea) and protein. Failure to thrive, the fat malabsorption can lead to deficiencies of the fat soluble vitamins chronic pancreatitis, dysfunction of the endocrine pancreas, leading to glucose intolerance and CF-related diabetes. Asymptomatic hepatomegaly, splenom-egaly, hepatosplenomegaly, Focal biliary cirrhosis, multilobular cirrhosis, and portal hypertension, hepatic steatosis, asymptomatic elevation in liver enzyme tests Cholelithiasis, cholecystitis and choledocholithiasis, neonatal cholestasis, , and micro-gallbladder, mimicking biliary atresia and end-stage liver disease.

Key words: Cystic fibrosis, intestinal, pancreatic, hepatobiliary

O-27 Endocrine Disorders in Cystic Fibrosis

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With improvement in controlling of CF patients and age increasing, endocrine complications are seen more and more. Cystic fibrosis related diabetes (CFRD) is one of them that exocrine pancreatic tissue is replaced by fibrosis and fat, resulting in islet cell injury and decreased insulin secretion. Also, due to chronic inflammation and use of steroids, insulin resistance worsens that condition, hence, CFRD is a condition between T1DM and T2DM. Females have higher risk of CFRD than males and incidence had increased tenfold between 2000-2008. In an investigation with OGTT test, indecency of CFRD was: <10Y: 2%, 11-17Y: 19%, 18-29Y: 40%, >30Y: 45-50%. CFRD is associated with worsening of lung function, nutritional status, and respiratory infections. The clinical presenta-tions are similar to T2DM and appear insidious but the occurrence of ketoacidosis is rare. Routine diabetes screening of all children with CF (starting at age 10 Yr) is recommended that OGTT is the best test for screening and diagnosis of diabetes. Treatment is similar to T1DM with insulin replacement. In CF patients, chronic pulmonary infection- pancreatic insufficiency-malabsortion and malnutrition contribute to decreased growth. The degree of growth retardation is related to severity and variability of pulmonary disease rather

than to pancreatic dysfunction. The GH- IGF axis assessment shows acquired GH insensitivity (lowered IGF1 and elevated GH level). GH treatment results in greater growth velocity and pulmonary function improvement that should be adjunctive to an aggressive nutritional program, appropriate pulmonary care without glucocorticoid administration.

Key words: Cystic fibrosis, growth hormone

O-28 Hyperglycemia as a Risk Factor for Retinopathy of Prematurity (ROP)

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Multiple risk factors are connected to the development of retinopathy of prematurity (ROP). This study was done to determine any association between the hyperglycemia and ROP in premature infants. In a retrospective case-control analysis, all infants with a gestational age (GA) <34 weeks and a birth weight (BW) <2000g admitted and treated in NICU at Amirkola Children's Hospital, Iran, during March, 2007-September, 2010 were included. Hyperglycemia was defined as a plasma glucose level of >150mg/dl during the hospital stay. The duration of being hyperglycemic was also recorded. All of these neonates were examined for ROP by a retinologist unaware of group assignment. The difference in the ROP incidence and alsothe severity of ROP compared between the hyperglycemic and non-hyperglycemic infants. Matching was done for GA, BW, and also Clinical Risk Index for Babies (CRIB) score. Data were analyzed by t-test, Chi-square test and logistic regression test and a P<0.05 was considered to be significant. Totally 155 neonates were examined. Seventy (45.2%) of them developed ROP, but 85 (54.8%) had no evidence of ROP. The frequency of hyperglycemia in patients with ROP was 33 (47.2%), but in patients without ROP, hyperglycemia occurred in 5 (5.9%) (P=0.0001). Severity of ROP showed no significant differences between two groups (P=0.35). Logistic regression for both GA and BW showed a significant correlation between hyperglycemia and ROP. (P=0.0001) According to our findings, both the presence and the duration of hyperglycemia are associated with an increased risk of ROP, although clinical trials need to determine if this association is causal.

Key words: hyperglycemia, retinopathy of prematurity, Infant, Premature

O-29 The IL-6 Concentration of Neonates in Vaginal and Cesarean Delivery

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Interleukin 6 (IL-6) has a major role in hemato-poiesis. Immune and acute phase cytokine response this cytokine in neonatal cord blood is a marker in defense against stress and infection. The aim of this study was to compare the levels of IL-6 in neonates between vaginal delivery and caesarean sec-tion. This cross-sectional study was done on 46 neonates in vaginal delivery and 35 neonates in cesarean section' delivery in 2012 in Rouhani Teaching Hospital in Babol. Inclusion criteria were appropriate-for-gestational age, apgar more than 8 (in the first and the fifth minutes). No clinical evidence or early neonatal infection and exclusion criteria was history or underlying disease. Cortico-steroid and other drug usage in mothers. IL-6 was evaluated by enzyme-immunoassays in umbilical cord. Other data include: mother age, sex, gravidity, parity. Live birth and gestational age were recorded. The mean (±SD) of mother age in vaginal delivery and cesarean section groups were 28±4.8 and 25.5±5.7 years oldrespectively (P=0.011). 20 neonates 43.5%) in vaginal delivery and 16 neonates (45.7%) in cesarean section were males (P= 0.841). The mean of neonatal IL-6 concen-tration in vaginal delivery (10.9) was higher than cesarean section (6.6). but this difference was not significant (P=0.86). Result shows that there was no significant difference between IL-6 level in neonates between normal vaginal delivery and cesarean section and according to high rate of cesarean section in our country, it needs more attention in this field.

Key words: Interleukin-6, vaginal delivery, cesa-rean section.

O-30 LimbalStem Cell Transplantation

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Corneal limbal stem cells continuosly replenish ocular surface. Limbal stem cell deficiency may be induced by different etiologies including chemical burns. Partial limbal stem cell deficiency mostly is treated by conservative measures. In the case of total unilateral stem cell deficiencythe surgical alternatives are cultivated limbal epithelial transplantation (CLET) and conjunctiv all imbal autograft (CLAU). In the case of bilateral total

limbal stem cell deficiency, the alternatives are keratolimbal allograft surgery (KLAL) and cultivated oral mucosal epithelial transplantation (COMET). In some cases, in addition to stem cell transplantation visual rehabilitation may need to penetrate or lamellar corneal graft.

Key words: Stem cells, ocular surface, stem cell transplantation

O-31 *In Vitro* Evaluation of Pulsed Electromagnetic Fields Stimulation Osteoblast Differentiation in Human Bone Marrow Mesenchymal Stem and Fatty Mesenchymal Cells as a Time-Dependent Factor

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This study was performed to investigate the effects of pulsed electromagnetic fields (PEMFs) on the proliferation and differentiation of human bone marrow mesenchymal stem cells and Fatty Mesenchymal Stem cells. Human mesenchymal stem cells (MSCs) are a promising candidate cell type for regenerative medicine and tissue engineering applications. Pulsed electromagnetic fields (PEMFs) play a regulatory role on osteoblast activity and are clinically beneficial during fracture healing. Human mesenchymal stem cells (MSCs) derived from different sources have been extensively used in bone tissue engineering. The hypothesis tested in this study was to evaluate whether PEMFs favor osteogenic differentiation in Bone Marrow MSCs and Fatty MSC to compare the role of PEMFs alone and in combination with the biochemical osteogenic factors in different time. Early and later osteogenic markers, such as alkaline phosphatase (ALP) activity, osteocalcin levels, and matrix mineralization, were analyzed at different times during osteogenic differentiation. The cells were exposed in the PEMF for 0 (control group), 1.0, 2.0, 3.0 and 4.0 h groups, respectively. After 24h, 48 h and 72h, cell proliferation was assayed by MTT method. Results showed that PEMFs induced osteogenic differentiation by increasing ALP activity, osteocalcin, Runx-2 and matrix mineralization in BMSCs and FMSCs, suggesting that PEMF activity is maintained during the whole differentiation period. We compared the cell viability, cell matrix distribution, and calcified matrix production in unstimulated and PEMF-stimulated (magnetic field: 1.3 mT, amplitude: 5mV) mesenchymal cell lineages. Contrast with control group, after PEMF exposure, in comparison with different time groups, BM-MSCs and FMSCs showed an increase in cell proliferation ($p< 0.05$), promoted maturation and an enhanced deposition of extracellular matrix components such as osteocalcin and Runex-2 ($p< 0.05$). Calcium deposition was 1.5-fold greater in FMSC and BM-MSC–derived osteoblasts ($p< 0.05$). The real- time polymerase chain reaction (RT-PCR) analysis revealed up-regulated transcription specific for osteocalcin, Runex-2 and ALP, but at a higher level for cells differentiated from MSCs. All together these results suggest that PEMFs could enhance early cell proliferation in MSCs-mediated osteogenesis and accelerate the osteogenesis and the no significant difference between two sources.

Keyword: Differentiation, bone marrow, osteoblast

O-32 Role of AMP-Activated Protein Kinase and Drugs in Diseases

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The AMP-activated protein kinase (AMPK) is a kinase type enzyme that plays role in several human diseases such as type II diabetes and cancer. Also AMPK, plays a role in biochemical metabolism. The Amp-activated protein kinase is a sensor and regulator of metabolism energy. The aim of the present research investigate the role of AMPK in diseases. AMPK level was measured by ELISA method, Casubio Biotech Co. Addison of Urtica dioica leaf extract increased the AMPK level. Increased AMPK is an important regulator of lipid biosynthesis. AMPK protects the cell against different biochemical condition. Activated AMPK is an important regulator of biochemical metbolism. Natural product extracts and drugs may work by activating AMPK. In future drugs that effect on AMPK activity or level may have potential for the development ofnovel natural products and drugs. AMPK inhibitors and stimulators may have a role as novel drug.

Keywords: AMP-activated protein kinase, drug, lipid, metabolism

O-33 The Therapeutic Effect of 8 Weeks Aerobic Exercise after Bone marrow Stem Cell

Transplantation on Behavioral Indicators in Parkinsonian Rats

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Parkinson's disease is a progressive neurodegene-rative in the central nervous system characterized by the loss of dopaminergic neurons in the substantianigra, resulting in loss of dopamine release in the striatum. Stem cell transplantation and exercise, including non-drug treatment options that have been considered for the treatment of parkinson's disease. The purpose of this study was to investigate the effect of 8 weeks of aerobic training on behavioral indicators in parkinsonian rats transplanted with bone marrow stem cells. 35 male rats were divided randomly into five groups: healthy control, Parkinson control, stem cells, exercise and stem cells + exercise. To create a model of Parkinson's, the striatum was destroyed by 6-hydroxy-dopamine injection into the striatum through stereotaxic apparatus. For the isolation of bone marrow stem cells, bone marrow of femur and tibia of male rats 6-8 weekswere used. After cultivation, approximately 105 cells in 5 microliter of medium was injected through the channel into the striatum of rats. Aerobic exercise was included 8 weeks of running on the treadmill with a speed of 15 meters per minute. To evaluate the behavioral of apomorphine rotation, balance and cylinders testswere used. In stem cells + exercise group decrease in the number of rotation in apomorphine rotation test, term of performance equilibrium test and asymmetry of movement in the cylinder test ($P \leq 0.05$) was observed significant. These results suggest a positive effect of stem cell transplantation with exercise on behavioral indicators. Bone marrow stem cell transplantation followed by exercise improved behavioral indicators of parkinsonian rats which can be considered as a non-drug therapy in Parkinson disease.
Keywords: Bone marrow stem cell, aerobic exercise, behavioral assessment, parkinson

O-34 Endogenous Expression of EGFR and ErbB2 mRNA and Protein in Some Commonly used Laboratory Cell Lines

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Epidermal growth factor receptors (ErbBs family) are essential for normal cell growth and development. Cell lines devoid of endogenous expression of ErbBs provide an ideal *in vitro* model for studying ErbBs function and biology. However, ErbBs are expressed differently among normal and tumor cell lines. The aim of this study was to investigate the endogenous expression of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2/ErbB2) in HeLa, HEK293, HepG2, CHO, BHK, VERO, COS-7, and NIH3T3 cells. Cell lines were cultured to subconfluent stage in the exponential phase of growth in culture media supplemented by 10% FBS and appropriate antibiotics. Then harvested cells were used for RNA extraction or cell lysate preparation. Expression of EGFR and ErB2 mRNAs and proteins were evaluated by using quantitative real-time PCR and western blotting, respectively. EGFR and ErbB2 expression levels were then presented relative to those of MDA-MB-468 and SKBR3 cells as positive controls, respectively. We found that ErbB2 was not endogenously expressed by VERO or BHK cells, while it was expressed by HeLa, HEK293, HepG2, CHO, COS-7, and NIH3T3 cells at much lower levels than SKBR3 cells. Likewise, EGFR was endogenously expressed only by HeLa and COS-7 cells but not by other cells. Our results indicate that VERO and BHK provided 'clean' experimental systems for EGFR and ErbB2-related studies.
Keywords: Endogenous expression, EGFR, ErbB2, cell line, Western blotting, qPCR

O-35 The Effect of LPS and LTA on Wnt5A Expression in Human OvarianCancer Cell line SKOV-3

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Wnt5A is a member of Wnt protein family implicated in inflammatory processes and is highly expressed by ovarian cancer cells. This study sought to assess the effect of inflammatory mediators and involved signaling pathways on Wnt5A expression in human ovarian cancer cell line SKOV-3. In this study, to induce inflammation

LPS (Lipopolysaccharide) and LTA (LipoteichoicAcid) were used. Assessment of inflammatory pathways was performed by using inhibitors of Stat-3 (S31-201) and NF-kB (BAY11-7082). SKOV-3 cells were treated with LPS (1ug/mL) or LTA (30 ug/mL) in the absence or presence of Stat-3 or NF-kB inhibitors for 8, 12, 24 and 48 hours. Then, Wnt5A gene and protein expression were assessed by real-time quantitative RT-PCR and western blot analysis for indicated times, respectively. In the presence of Stat-3 and NF-KB inhibitors, there was a significant decrease of Wnt5a expression in a time-dependent manner. Wnt5A expression was decreased from 12 to 48h with stat-3 inhibitor. While, with NF-kB inhibitor, Wnt5A reduced expression was observed from 8 to 24h. LPS and LTA treatment led to increased Wnt5A expression by 1.5- and 3- fold, respectively. Whereas, both LPS-and LTA-induced Wnt5A expression were abrogated in the presence of Stat-3 inhibitor. This study showed for the first time the key role of Stat-3 and NF-kB transcription factors on Wnt5A expression by ovarian cancer cells. In addition, Stat-3 pathway plays an important role in LPS- and LTA-induced Wnt5A expression. These data may suggest that Stat-3 inhibitor may be a useful therapeutic tool in ovarian cancer therapy.

Key Words: Wnt5A, Ovarian cancer, Inflammation, Stat-3, NF-kB

O-36 Evaluation of Inhibitory Effect of Melatonin on Gastric Adenocarcinoma AGS and MKN49 Cell Lines

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Melatonin is a neurohormone with important physiologic and pharmacologic role in human body especially in circadian rhythm. In recent years, some progress has been achieved to show its role in regulating in the prevention of cancer especially breast and colon cancer. According to this background and this point, there was not any precise cellular research about the role of melatonin in gastric cancer in which this study had been aimed. In this study, we used MTT assay procedure. Also, we have provided AGS and MKN-45 cell line from National Cell Bank of Iran, Institute of Iran and after the cells were cultured in RPMI medium in 5% CO2 370C in 96 wells culture plate. The cells were incubated with Melatonin and Cisplatin (as positive control) for 48 hr. in 5 different concentrations. Then, proliferation index as cell viability was achieved and compared with controls groups with ELISA concerning Formazan crystal color absorbance between 450-690 nm. Our results showed that melatonin in 12.5-200 µM has significant anti-proliferative effects in AGS cells and in 50-200 µM in MKN-450 compared with control and these results were in parallel with the effects of cisplatin. According to our data, we have shown that melatonin in a dose -dependent manner has antiproliferative effect in gastric adenocarcinoma cells and this effect in AGS cells was more potent than MKN-45 but more studies are needed to find these kind of receptors and the interacellular signaling pathways.

Keywords*:* Melatonin, Gastric adenocarcinoma, Proliferation, MTT assay, Cisplatin

O-37 The Impact of GST M1 Deletion (as an Antioxidant Enzyme) and Its Correlation with Expanded Disability Status Scale in Multiple Sclerosis Patients

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Multiple sclerosis (MS) is a chronic inflammatory demyelination disease of human central nervous system (CNS). There are growing evidences showing that oxidative stress plays a pivotal role in the pathogenesis processes of MS. On the other hand the glutathione S-transferases (GSTs) are a group of dimeric enzymes which catalyze the conjugation of reduced glutathione to a wide range of electrophilic compounds such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) to protect cells against oxidative stress. The aim of our study was to investigate the possible correlation between glutathione S-transferase M1polymorphism as a detoxification status and disability score measured by Kurtzke Expanded Disability Status Scale (EDSS) in a group of 69 RRMS patients and 74 healthy matched individuals as a control group. The genetic analyses were performed on blood samples of individuals by PCR. Results showed the incidence of null genotype was not significantly different for GSTM1 (P = 0.38, OR = 1.34, 95% CI = 0.69-2.36) between MS and control groups. Meanwhile a considerable higher frequency of GSTM1 null genotype was found in female subjects

compared with male subjects (P=0.0007, OR=6.2, 95% CI=2.034-18.9). Moreover, patients with GSTM1 null genotype had considerably lower age of onset in comparison with wild type carriers (25±1.76 vs. 30.79±1.68 years, respectively, P=0.039). Considering smoking, results indicated that smoker patients had higher EDSS score than non-smokers (2.97±0.37 vs. 2.14±0.2 respectively, P=0.04). Our result suggests a gender dependent manner of GSTM1 detoxification role and its possible use as a prognostic factor in MS patients.

Key words: Multiple sclerosis, GST M1, EDSS, smoking, oxidative stress

O-38 Role of Vitamin D in Type 2 Diabetes Mellitus

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Type 2 diabetes mellitus (T2DM), a chronic metabolic disorder, has become a significant global health care problem. Type 2 diabetes is associated with serious morbidity and increased mortality. The prevalence of T2DM is approaching epidemic proportions, and diabetes mellitus (DM) affects people of all ages. T2DM is a complex hetero geneous group of metabolic disorders including hyperglycemia and impaired insulin action and/or insulin secretion. T2DM causes dysfunctions in multiple organs or tissues. Current theories of T2DM include a defect in insulin-mediated glucose uptake in muscle, a dysfunction of the pancreatic β-cells, a disruption of secretory function of adipocytes, and an impaired insulin action in liver. The etiology of human T2DM is multifactorial, with genetic background and physical inactivity as two critical components. Recent studies have provided evidence that vitamin D may play a functional role in glucose tolerance through its effects on insulin secretion and insulin sensitivity. Animal studies have shown that vitamin D is a basic factor, necessary for normal insulin secretion. Vitamin D reduces insulin resistance probably through its effect on calcium and phosphorus metabolism and through up regulation of the insulin receptor gene. Seasonal variation in the control of glycemia in patients with type 2 diabetes, being worse in the winter when hypovitaminosis D is more prevalent. There is an inverse association between vitamin D status and prevalent hyperglycemia. There is a biological plausibility of an important role of vitamin D in type 2 diabetes, and lower vitamin D status and intake are associated with higher risk of incident type 2 diabetes in observational studies. Recent observational data have reported a beneficial effect of vitamin D on preventing the onset of diabetes. But the therapeutic role of vitamin D in glucose metabolism is still unclear. Experimental studies as well as large scale RCTs with good study design, optimal vitamin D supplementation and long-term follow up are needed on this topic.

Keywords: Type 2 diabetes mellitus, Vitamin D, hyperglycemia, Insulin resistance, Metabolic disorder

O-39 Acute Phase Protein Status in Obese Breast Cancer Patients vs. Non-Obese Ones

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It is hypothesized that chronic inflammation is associated with breast cancer development and to some extent disease recurrence. In this study, we determined the status of total protein, albumin and C-reactive protein (CRP) in obese breast cancer patients vs. to normal weight ones. In this cross-sectional study, among breast cancer patients (stage II-III) who were treated previously and referredto the Shahid Rajaee hospital (Babolsar, Mazandaran) for routine follow-up, 45obesepatients (body mass index (BMI) ≥25 kg/m^2) and30 normal weight ones (18.5≤ BMI <25 kg/m^2) wereselected. Serum levels of total protein and albuminwere measured with spectrophotometric method. CRP levels were determined by latex immunoturbidimetric assay. The resulted data were compared by independent T-test viaSPSS 18 software. Differences in the BMI status in two included patients were statistically significant (32.9 ± 5 vs. 22.9 ± 2 kg/m^2, P<0.05; respectively for obese and non-obese groups). Lower serum albumin and higher CRP concentrations were observed in obese patients in comparison to the non-obese ones (P<0.05). There were no significant differences in total protein levels between two groups (P>0.05). A positive correlation between CRP levels and BMI was found (r= 0.33, P= 0.003). Interestingly, a negative correlation between serum albumin levels with BMI was observed (r= -0.39, P= 0.000). As alterations ininflammatory markers were observed in included obese patients in comparison of non-obese one and such alterations synergize in obese patients, it is recommended that previously treated breast cancer patients, strictly control their BMI.

Key words: Breast cancer, obesity, body mass index, C-reactive protein, albumin

O-40 VDR *Cdx-2*-dependent Response of Central Obesity to Vitamin D Intake in Type-2 Diabetes: A Randomized Clinical Trial

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This study aimed to investigate the effects of daily intake of vitamin D-fortified yogurt drink on central obesity indicators and whether response of obesity indicators to vitamin D intake is modulated by vitamin D receptor (VDR) -*Cdx*-2 genotypes in subjects with type 2 diabetes (T2D). Sixty subjects with T2D were randomly allocated to two groups to receive either plain yogurt drink (PD; n=29, containing 170mg calcium and no vitamin D/250mL) or vitamin D3-fortified yogurt drink (FD; n=31, containing 500 IU/250mL) twice a day for 12 weeks. Serum 25 (OH) D, fasting serum glucose (FSG), glycated hemoglobin (HbA1c), QUICKI, percent of body fat mass (FM%), truncal fat mass (TF%), visceral adiposity tissue (VAT) and waist circumferences (WC) were assessed at the baseline and after intervention. VDR genotypes in extended number of T2D subjects in the FD group (n=60) were determined by *Cdx*-2 restriction enzyme. After 12 weeks, serum 25 (OH) D increased significantly in FD as compared to PD group (+35.4 nmol/L vs. -4.8 nmol/L, $p<0.001$). Mean changes of WC (-1.3 vs. +1.6 cm, $p=0.02$), FM (-5.1 vs. +0.60 %, $p<0.001$), TF (-1.1 vs. 0.13%, $p=0.003$) and VAT (-0.80 vs. +0.37 a. u., $p<0.001$) decreased significantly compared to PD group. Circulating 25 (OH) D was raised only in AA group (34.8 nmo/L in AA group vs. -6.4nmol/L in AG and -1.6nmol/L in GG groups, $p<0.001$). This difference was accompanied by a significant decrease in changes of WC ($p=0.004$), FM% ($p<0.001$) and TF% ($p<0.001$) in AA genotype. Daily intake of yogurt drink fortified with 1000 vitamin D, for 12 weeks improved the central obesity indices including trunk fat and visceral adiposity in the subjects with T2D which was more pronounced in those patients who were carrier of AA genotype of VDR-*Cdx*-2 polymorphism.

Key words: vitamin D, adiposity, visceral fat, type 2 diabetes, Polymorphism

O-41 Effect of Eight Weeks of Regular Swimming and Garlic Extract Intake on Levels of Apelin and its Receptor in Old Rat Kidney

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The present study aimed at determining the impact of eight weeks of regular swimming program on levels of apelin and its receptor in old rat kidney. In this study, 42 male old rats aged 40-50 weeks with an average initial weight of 250-300 g were randomly divided into 6 groups of 1-control, 2-saline, 3-sham, 4-exercise, 5-garlic, and 6-garlic-exercise. The exercise program consisted of 30-minute swimming sessions for 8 weeks, three days a week. The supplement group received 1 mL/kg/day garlic extract through gavage for 8 weeks. The rats were anesthetized 48 hours after the last exercise session and 10-12 hours of fasting and their kidney tissues were immediately removed. The tissues were frozen at -70 °C and were used to measure the levels of apelin and its receptor. The data were analyzed using one-way ANOVA and Tukey's post-hoc test. The results showed that swimming led to a significant increase in the levels of apelin and its receptor in old rats. However, exercise-garlic interaction was more efficient.

Keywords: Swimming, apelin and its receptor, old rat, garlic extract

O-42 Preventive Effect of *Daphne Mucronata* Extract on the Increase Serum lipids in Rats Fed High- Fat Diet

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Nonalcoholic fatty liver disease (NAFLD) is caused by the accumulation of fat in hepatocytes which ultimately leads to inflammation and damage to the cell. Free fatty acids plasma levels increase in this disease. Increase free fatty acid import and esterification and contemporary impaiered synthesis and secretion of lipoprotein can cause triacyl-glycerol to accumulate in the liver. Preventive effects of plant extracts contains anti-inflammatory compounds on fatty liver have been investigated. In this study, the effect of oral administration of *Daphne mucronata* on the prevention of fatty liver induced by high fat diet in rats was studied. Therefore, serum lipid profile changes in male Wistar rats with a high fat diet treated with different doses of plant extract was evaluated after 6 weeks. The results showed that high fat diet increases the

level of triglyceride, cholesterol, LDL and decrease HDL level. While, they are treated with 300 mg/Kg *D. mucronata* decreased the level of triglyceride, cholesterol and LDL by about 15, 20 and 15 percent, respectively. This study showed that *D. mucronata* extract has preventive effects on the development of fatty liver in rats with high fat diet.

Key word: Fed high- fat diet, *Daphne mucronata*, fattyliver, LDL.

O-43 Vitamin D Improved Learning and Memory Impairments in Streptozotocin-Induced Diabetic Mice

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Diabetes mellitus (DM) is associated with memory and learning deficits. It has provided evidence that Vitamin D is involved in brain function. The aim of the present study was to determine potential effect of vitamin D on the acquisition and retention of memory and learning in streptozotocin (STZ)-induced diabetic mice. Experiments was performed in four groups of mice (each group; n=7). Male mice were induced to diabetes by single dose (60 mg/kg i. p.) injection of freshly prepared STZ dissolved in normal saline. Treatment with vitamin D (5µg/kg daily i. p. dissolved in tween80) was begun at three days after diabetes induction. Passive avoidance (PA) learning method was used four weeks later. Retrieval test carried out 24 h after training. Our results demonstrate significant impairment in acquisition and retrieval processes of PA learning in STZ-induced diabetic mice. Treatment with vitamin D improved learning and memory compared to control group, both in acquisition and retrieval stages and reversed learning deficits in diabetic mice. In acquisition test, there were significant differences in the initial latency among the DM+ Vit. D treated and control groups ($p<0.05$). There was a significant difference in step-through latency between diabetic group treated with vitamin D compared to diabetic non-treated groups ($p<0.05$). It is possible that the effects of Vitamin D on cognitive deficits in STZ-induced diabetic mice could be mediated through calcium homeostasis modulation. These findings suggest a potential role for vitamin D in the treatment of diabetes-associated cognition deficits. The positive effect of vitamin D on the avoidance task may be contributed to its neuronal protective roles metabolic regulating roles of prolonged vitamin D administration.

Key Words: Streptozotocin, STZ, diabetes, memory, learning, Vitamin D

Poster Presentations

P-1 Up-regulation of Human Stem Cell Specific miR-371-373 Cluster in Esophageal Cancer

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Golestan province in the north of Iran has one of the highest rates of esophageal cancers in the world. Recently, several studies have been done to find the genetic and environmental factors for the disease. Micro-RNAs are a class of non-coding RNAs that found to be involved in different processes and could play a role in tumorigenesis and causing cancer. MiR-371, miR-372 and miR-373 are a gene cluster located in the region of human chromosome of 19q13.4. They are specifically expressed in human embryonic stem cells (ESCs) and involved in the maintenance of the stemness features through regulating the expression of certain key genes and signaling pathways. The present study investigated potential expression of miR-371-373 cluster in tumor and non-tumor tissue of esophageal cell carcinoma. The expression levels of miR-371-373 were analyzed in paraffin-embedded tissues of tumor and tumor margin of 36 patients with esophageal carcinoma. Total RNAs were isolated and the expression of miR-371-373 cluster was quantified by qRT-PCR expression analysis. CT analysis ($2^{-\Delta\Delta CT}$) and T -test were used to determine the relationship between the characteristics of the two groups tumor and non-tumor tissues. Statistically, P-values of <0.05 were considered significant. Data analysis was done using SPSS16. We provide evidence of miR-371, miR-372 and miR-373 up-regulation significantly with 14.36 fold, 26.9 fold, 21.1 fold in esophageal cancer cells compared with their adjacent normal cells (P<0.05), respectively. Also, investigation of grade in tumor did not show any significant difference between tumor and non-tumor cells. Our findings support the hypothesis that these micro-RNAs might play role in tumorigenesis in esophageal cancer. Also, according to expression of those miRNAs in the stem cells, they give novel point of view about the role of cancer stem cell in esophageal carcinoma.

Key words: Esophageal cancer, miR-371-373 cluster, embryonic stem cell

P-2 The effect of Arbutin and CCl4 on lipid peroxidation and antioxidant activity of liver, kidney and pancreas tissues in rats

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This study was designed to examine the protective effect of Arbutin on lipid peroxidation and antioxidant activity in animal model of hepatotoxicity induced by carbon tetrachloride (CCl4). This experimental study was carried out on 63 male Wistar rats, 7-11 weeks of age, and the body weight range of 150-200 g. They were divided into 9 groups. groups I, II were sham groups (received normal saline), group III the hepatotoxic group received CCl4, groups IV, VI, VIII received different doses of arbutin (50, 75 and 250 mg/kg) with CCl4 and at least groups V, VII, , IX received different doses of arbutin (50, 75 and 250 mg/kg). Antioxidant status and lipid peroxidation were assayed in liver, kidney and pancreas tissues. in liver tissue, lipid peroxidation (LP) increased significantly in the toxic group, (P=0.001) and it had a significant decrease in 250 mg/kg orall dose of arbutin (P<0.05). In addition, the antioxidant activity had a decrease in CCl4 treated group but it was not statistically significant and also had a significant increase in groups which received different doses of arbutin (P<0.05). In kidney tissues, LP had a significant increase in group which received CCl4 (P<0.05). The results of LP in pancreas tissue showed an increase in rats received CCl4 with arbutin (250 mg/kg) (P<0.001) also, it decreased in the group treated by arbutin (250 mg/kg) in comparison with the other doses of arbutin (P=0.014). The antioxidant activity in pancreas tissue showed a decrease in groups received CCl4 (alone or combined by different doses of arbutin) and also a significant increase in arbutin treated groups (50, 75 mg/kg) (P<0.05). The present observations suggested that the treatment with *Arbutin* enhance the recovery from CCl4 induced damage due to its antioxidant and protective property.

Key words: Antioxidant status, arbutin, lipid peroxidation, CCl4.

P-3 Epigenetic Gene BRCA2 in Epithelial Ovarian Cancer

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Ovarian cancer is the most common fatal gynecologic malignancy in women that its main symptoms are solid pelvic mass, constant and regular in physical examination. Methylation changes of BRCA2 may provide development of ovarian cancer. Our goal is to evaluate the association between ovarian cancer and BRCA2 gene methylation. In this study, methylation changes BRCA2 genes in 44 tissue samples from patients with ovarian cancer and 44 adjacent normal ovarian tissue samples were studied as a control group. After primer design and amplification of the BRCA2 gene sequence by PCR, gene methylation levels were evaluated using enzymatic digestion method (RFLP). According to the Survey methylation status of subjects, status changes were observed only in 3 cases. Studies did not show correlation between BRCA2 gene promoter methylation in ovarian cancer patients and healthy subjects. According to the results obtained in this study, this factor was at least seenin this sample, a risk factor. But in other cases, other factors or methylation changes in other suppressor genes or oncogenes have probably been effective in cancer development.
Keyword: Ovarian cancer, methylation, BRCA2 gene promoter, RFLP method

P-4 A Comparison between Serum Selenium Levels in Breast Cancer Patients before and after Radiotherapy

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Radiotherapy (RT) is one type of treatment used after surgery for many cancers. It can damage DNA, RNA, proteins and membrane in tumor cells through the production of various reactive oxygen species. RT at doses that currently used for treatment of cancers can affect the efficiency of the antioxidant system. Some parts of the antioxidant system are dependent to metal cofactors such selenium (Se). Se is part of glutathione peroxidase enzyme, which destroys peroxides. This study was designed to investigate the effect of RT on serum selenium levels in breast cancer patients. Eighty patients with breast cancer participated into this study. They received radiotherapy at a dose of 50 Gy with fraction size of 2 Gy for 5 weeks (five days weekly). The blood samples were obtained from all patients a day before and one day after the end of radiotherapy. Serum selenium concentration was determined by atomic absorption spectrometry (PG-990, china). Paired t-test was used for comparing pre and post radiotherapy data and a p-value <0.05 was considered significant. Results showed that patients in higher age group (>50 yrs old) had lower serum Se levels, while patients in higher BMI and clinical stage of the disease had higher serum Se levels in crude data (differences were not significant, $p>0.05$) before the treatment. Radiotherapy caused a significant decrease ($P = 0.018$) in serum Se levels from 51.4 ± 29.7 µg/l to 41.6 ± 19.6 µg/l. It seems that RT induces a significant decrease in serum Se. As Se plays an important role in antioxidant system, supplementation of this necessary mineral for patients that undergo RT is recommended.
Key word: Breast cancer, radiotherapy, selenium

P-5 The COX-2 Independent Effect of Celecoxib and Rofecoxib on Proliferation of HT-29, HCT-116 and A549 Cancer Cells

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The role of cyclooxygenase (COX) enzyme activity and its expression has been shown in the etiology of cancer. Clinical studies suggest that COX inhibitors can hinder tumor progression as treatment of epithelial carcinoma. We have evaluated the cytotoxic effect of various concentrations of celecoxib, rofecoxib as selective COX-2 inhibitors and Indomethacin as nonselective COX inhibitor on the viability of HT-29 and HCT-116 cells and A549 cell. Cell viability was measured using MTT assay and the expression of COX-2 and ERK1/2 was investigated using western blot analysis. Cells were

treated with celecoxib (10-200µM), rofecoxib (10-500µM) or indomethacin (10-500µM) for 24, 48 and 72 hrs. Our results indicate that celecoxib inhibits HT-29 proliferation in a dose and time dependent manners. Rofecoxib and indomethacin did not show any anti-proliferative effect in HT-29 cells. Moreover, in HCT-116 cells, celecoxib and rofecoxib inhibit cell viability in a dose dependent manner. Celecoxib and rofecoxib and to a weaker extent indomethacin exhibit cytotoxic effect in A549 cells. Furthermore, in A549 cells, selective COX-2 inhibitors induced COX-2 expression. These findings suggest a COX-2 independent anti proliferative effect. Changes in COX-2 expression, indicate a dual effect of COX-2 inhibitor as cytotoxic and inducer of COX-2 expression in A549 cells. In conclusion, our results indicate a COX-2 independent anti proliferative effect of celecoxib and rofecoxib in cancer cells.

Keywords: Celecoxib, rofecoxib, HT-29, HCT-116, A549

P-6 Identification of New Natural Inhibitors for K-Ras Using Structure-Based Virtual Screening Method

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Given the significant importance of K-Ras in intracellular signal transduction and its potential in several cancer inductions, this factor was selected as a subject for discovering of compounds with anticancer properties. Presence of mutants of this molecule in more than 75% of advanced colorectal cancer, 43% of advanced none-small-cell lung cancer and 49% of pancreatic ductal adenocarcinoma are reasons for the importanceof this protein in cancer induction. In this investigation, tridimensional structures of K-Ras and natural compounds (3658 structures) were taken from Protein Data Bank and ZINC databases, respecttively. Then, using molecular dynamic simulation and molecular docking, the structure of K-Ras protein was equilibrated and the necessary computations for the assessment of free binding energy content of each compound in different conformations, as well as theinteraction of compounds with binding site and ADMET index were performed. In conclusion, based on the predicted free-energyof binding, two compound ZINC35442801 and ZINC85546950with higher scores than the reference inhibitor were recommended as a possible inhibitor.

Key words: K-Ras, anticancer drug, molecular dynamic, docking, virtual screening

P-7 Cytotoxic Effect of Methanol Extract of Ganoderma lucidumon Hela Cancer Cell Lines

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Nowadays various methods are used to treat cancer. Unfortunately, in most cases, the response to treatment has been very poor and it is often associated with undesirable side effects. Lack of response to treatment and the rapid growth of the disease have made researchers try to achieve more effective drugs with fewer side effects. In this study, the effect of methanol extract of Ganoderma lucidumon on Hela cancer cell line was studied. The cell lines were cultured, 10000 cells were transferred to 96-cell plates. Then, the cells were exposed to different concentrations of 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.038 mg/ml that were dissolved in RPMI containing FBS. Cytotoxicity was investigated using MTT test after 24, 48 and 72 hours. According to the results of interaction between exposure time of cells and concentrations of ethanol extract of Ganoderma, it was found that the highest percentage of Hela cancer cell inhibition occurred after 72 hours in concentrations of 0.312 and 0.038 mg/ml. After 48 hours, the minimum inhibitory was observed activity at concentrations of 5 and 2.5 mg/ml. So by increasing the concentration of extract, growth inhibition has been decreased and best effect was seen in low concentrations and 72-hour incubation.

Keywords: Cytotoxicity, methanol extract of Ganoderma lucidum, Hela cell line

P-8 *In Silico* Analysis of MiR-584 Molecular Role as a Potential Prognostic Biomarker in Gastric Cancer Induced by *Helicobacter Pylori*

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Gastric cancer is the 4th commonly diagnosed cancer and the 2nd most common cause of death from cancer. *Helicobacter pylori* (*H. pylori*), the most common bacterial infection, is the major cause

of gastric cancer. Among the mediators induced in response to the infection, microRNAs (miRNAs) have the potential role to have a major impact on the outcomes of the bacteria-host interaction. miRNAs can play as either oncogenes or tumor suppressors. miRNA expression could be modified by *H. pylori* infection; therefore, this modification could be used as biomarkers for gastric cancer. miR-584 has over expressed in *H. pylori*-infected gastric tumors therefore, it could be a possible biomarker for gastric cancer diagnosis. MiRTarBase and miRWalk database were used to predict the target genes of the miR-584. The list of target genes was filtered by the data of UniGene database to identify the gastric cancer genes. Gastric expressed targetome of miR-584 was selected for enrichment analysis in DAVID database. DAVID database including KEGG signaling pathways showed that target genes were significantly involved in cancer pathways as well as Wnt signaling, TGF-beta signaling, adherence junction and VEGF signaling pathways. Comprehensive analysis of the coordinate expression of miRNAs and mRNAs reveals that miR-584 may play important role in the development of gastric cancer. These signaling pathways lead to insensitivity to anti-growth signals by irregularity in TGF-beta signaling pathway, evading of apoptosis, proliferation and also inhibiting the differentiation by irregulation in cell cycle and P53 signaling pathway. Despite the limited studies on the role of *H. pylori* eradication in the normalization of gene expression levels in gastric mucosa, such studies show genes with significant changes of expression. This may reveal molecular markers involved in inflammatory processes and mechanisms of progression from precancerous lesions to malignancy.

Key words: Gastric cancer, *H. pylori*, miR-584, cancer signaling pathway.

P-9 Compartive Study of Cytotoxic Effect of Fermented Wheat Germ Extract on Cancer Cell Line and Fibroblast for 72 hours

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Nowadays, although there is a wide range of cytotoxic agents used in the treatment of cervical cancer, but they have shown drawbacks in their use and are not as efficient as expected. Therefore, it is of great interest to find new therapeutic agents against cancer. Wheat germ is widely used in many fields. However, there is scarce information on its antitumor potential. The aim of this study was to evaluate the cytotoxic effects of fermented wheat germ extract on HeLa cervical cancer cell line *in vitro*. The cells were cultured in RPMI1640 liquid medium with 10٪ inactivated fetal bovine serum (FBS) and antibiotics; and were cultured with various concentrations of wheat germ (0.038, 0.078, 0.156, 0.312, 0.625, 1.25, 2. 5, 5 and 10 mg/ml) for 72 hours and were analyzed using the MTT test. The percentage of growth inhibition was calculated. The data were analyzed using SPSS software after 72 h incubation ($P<0.05$). Maximum inhibitory effects were found at 10 mg/ml (88.92%). Overall, results showed the fermented wheat germ extracts and time dependently suppressed the proliferation of HeLa cells. Therefore, fermented wheat germ extracts able to inhibit the growth HeLa cell line. In addition, the effect of fermented wheat germ extract on fibroblast cells showed that extract did nothave significant difference with control group. However, more studies are needed to elucidate the effects of fermented wheat germ extracts, with the aim of developing new strategies for the treatment of cancer and other illnesses.

Key words: Cytotoxic effect, fermented wheat germ extracts, HeLa cancer cell lines, MTT test, fibroblast

P-10 Expression Analysis of miR-205 in Gastric Cancer

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MicroRNAs (miRNAs) regulate gene expression post-transcriptionally and several reports showed that their altered aberrant expression is associated with cancer development and progression. Thus, miRNAs can be usedas novel diagnostic/prognostic biomarkers in various cancers. In this study, we compared miR-205 expression level in gastric tumor tissues with adjacent non-tumor tissues to assess the diagnostic value of this biomarker in gastric cancer. Total RNA was extracted from 37 pairs of gastric tumor and adjacent non-tumor tissues. The level of miR-205 expression was quantified by real time PCR and its relationship with the clinicopathological features of patients was studied. Statistical analysis was performed by Sigma-plot and GraphPad-Prism 5.0. The expression level of miR-205 significantly increased in gastric tumortissues compared to adjacent non-tumor tissues ($P<0.002$). The receiver operating charac-teristic (ROC) curve analysis on the miR-205 showed that the area under the ROC curve was high (0.80). No correlation was observed between

miR-205 expression level and clinicopathological features of patients. The over expression of miR-205 in gastric cancer indicates that it might act as an oncogene and can serve as a molecular diagnostic biomarker for gastric cancer.
Key words: miRNA, miR-205, gastric cancer, real time PCR, biomarker

P-11 Analysis of Tropomyosin Expression Pattern in the First Iranian Established Cell Line of Esophagus Cancer

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The actin cytoskeleton plays a significant role in the maintenance of normal cell phenotype. Any changes in cytoskeleton organization contributed in carcinogenesis processes. In this regard, tropomyosin family which is a family of actin binding proteins has an essential role in stabilizing the cytoskeleton structure. Tropomyosins are divided into two major groups: high molecular weight (HMW) and low molecular weight (LMW). Altered expression of tropomyosin isoforms has been shown in several cancers. Otherwise, squamous cell carcinoma of esophagus (SCCE), as a lethal malignancy, has the highest incidence rate in Iran. So, investigating the cellular and molecular features of esophagus cancer is essential for early detection and effective treatment. Since, there is no cell line of Iranian origin, studies on esophagus cancer have been limited to tissue samples in Iran. In this study, tissue samples of SCCE were applied for the preparation of primary cell culture and establishment of the first Iranian SCCE cell line. Subsequently, tropomyosin expression was investigated in the established cell line, relative to primary culture of normal esophagus cells by western blotting and real-time RT-PCR. Applying primary cell culture for tissue samples, resulted in the establishment of the first Iranian cell line of SCCE which provided the useful model for further investigation of esophagus cancer in Iran. Moreover, the expression of HMW tropomyosins was significantly downregulated in SCCE cell line, relative to esophagus normal cells. The mRNA level of tropomyosin genes noticeably decreased in SCCE cell line, as well. These data indicate that the newly established SCCE cell line could be a suitable tool for basic cellular and molecular researches of esophagus cancer. It is also useful for treatment and drug discovery approaches for esophagus cancer. Additionally, severe downregulation of HMW tropomyosins seems to play an important role in oesophageal carcinogenesis.
Key words: Esophagus cancer, tropomyosin, cell line, down regulation

P-12 Reducing the Growth of Prostate Cancer Cell Lines Treated with Androgen by Using Synthetic Oligonucleotides

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The androgen receptors possess ligand-binding region, which also bind to the hormone and then the hormone receptor complex transferred to the nucleus. The androgen receptors possess (DBD) DNA Binding Domain to bind to regulatory sequence elements upstream of responsive genes. Hormone receptor complex binds the hormone response element (HRE) and regulate transcription of some special genes. In this study, a 5' FITC conjugated single strand DNA oligonucleotid (Aptamer) was designed to compete HRE and bind to DBD of androgen receptor. The oligonucleotide forms double strand DNA (Hairpin) at 370C and mimics the sequence of HRE. HRE mimic aptamer were transfected to LNcap by polyfect reagent according to manufaturer's instruction. The transfected cells were washed with PBS, and the efficiency of transfection was evaluated by flow cytometery. Then transfected and untransfected LNcap were then cultured and treated with 10 and 100 nM of DHEA for 5 days. The viability of cells was assessed by MTT assay. The result of flow cytometery revealed that 73% of cells were transfected and there was a significant difference in viability of transfected and untransfected cells (P value=0.019). It seems that HRE mimic aptamer competed with native HRE on genomic DNA and neutralized the effect of androgen hormone complex to induce transcription of related gens.
Key words: Synthetic, LNCaP, DBD, polyfect

P-13 Assessing the Expression of BRAF Gene in Paraffin-Embedded Blocks of Patients with Colorectal Cancer

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Colorectal cancer is the second cancer related cause of death in the world. Understanding the molecular pathway of that can provide some useful information about therapeutic manners. Hyperactivity of BRAF gene has been reported in recent years and it can be proposed as a diagnostic molecular marker in many cancers. The purpose of this study was to assess the expression of BRAF gene in paraffin-embedded blocks of patients with colorectal cancer. In this study, five samples of paraffin-embedded blocks which were from a middle age patients with a sample of normal person were collected. After sectioning and removal of paraffin, RNA was extracted and then cDNA synthesis was performed by using MMULV enzyme, Oligo dt and random hexamer primers. BRAF specific primers and β-actin (as an internal control) were extracted from high-cited articles. RT-PCR reaction results indicated the expression of BRAF gene in carcinogenic cancer compared with normal sample. Conclusion: The results suggest the higher expression of BRAF gene in patient with colorectal cancer. Investigating the increased expression of BRAF gene in paraffin-embedded samples can be considered as an appropriate manner for research on old samples in hospitals and scientific institutes.

Keywords: Colorectal cancer, BRAF, RT PCR.

P-14 Identification of New Anticancer Compounds from Marine Living Resources Using Docking Based Virtual Screening in the Colchicine and Epothilone Binding Sites of Tubulin

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In the last decade using computational molecular modeling methods for discovering the cure of diseases specially cancer, has provided saving costs, time and quick access to new compounds with anticancer properties. The common characteristic of cancer cells is the uncontrolled division that microtubules play an essential role in this reproduction. Thus, one of the anticancer drugs targets is inhibition of inherent dynamic instability of microtubules. The main problem in the treatment of several tumor types is drug resistance after long-term exposure to them, which needs to discover new anticancer drugs. In this study, the structure of all of the compounds from marine living resources was collected from papers published till now. Then, for finding compounds with potent anticancer properties, the interaction of this library with the colchicine and epothilone binding sites of tubulin were analyzed, using molecular docking and molecular dynamics simulation methods. According to the calculated score, potent analogs were recognized. Given the respectable high-affinity binding of the compounds azaphilone and 5'-Hydroxy-chlorflavonin for tubulin, these inhibitors provide a new avenue for the development of anticancer agents, which possess tubulin-mediated antimitotic activity.

Keywords: Microtubule, Anticancer drugs, Molecularmodeling, Marine natural products

P-15 The Effect of Paclitaxel on Spheroid Culture Compared to Monolayer Culture of Human Ovarian Cancer Cell line SKOV-3

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Ovarian cancer is one of the most lethal malignancies with regard to lack of symptoms and primary diagnosis and high rate of recurrence because of chemoresistance of cancer cells. In Iran, the occurrence of ovarian cancer, death rate caused by it, and the percentage of people suffering from it are ranked: eighth, twelfth, and sixteenth respectively. In ovarian carcinoma, cancer cells detach from the surface of the tumor and move into the peritoneal cavity forming multicellular aggregates and by attachment to mesothelium they could metastasize. Compared to conventional monolayer cultures, multicellular spheroids are more similar to real tissues in terms of structural and functional properties. Cells in ovarian carcinoma spheroids exhibit changes in their position in the cell cycle and are protected from chemotherapeutic drugs compared to cells cultured as monolayers. The purpose of this study was to compare paclitaxel (PTX) cytotoxicity in spheroids with monolayer culture of human ovarian cancer cell line SKOV-3. Therefore, spheroids were formed by using hanging drops method and after 72 hours spheroids were transferred to agarose-coated 96 wells plates. Spheroids were treated with 0.1, 1, 5, 10, 15 and 20 µM PTX and monolayer cell culture was treated with 0.01, 0.05, 0.1, 0.25, 0.5 and 1 µM PTX for 48 hours. After indicated time, cytotoxicity was assessed by MTT assay. There was reduced spheroid compaction with 5, 10, 15 and 20 µM PTX compared to lower concentrations of PTX. The PTX IC50 was 0.1337 µM for monolayer cell culture, whereas PTX IC50 for spheroids was 13.82 µM. These results showed that spheroids could be used as a chemotherapeutic resistant model of ovarian cancer cells for further assessment of underlying molecular mechanism. This model would be very useful to study combination therapy

with different drugs targeting various important key pathways in ovarian cancer for better treatment.

Key words: Ovarian cancer, spheroid, adherent cells, paclitaxel

P-16 Assessing Interactions of Valproic Acid and Nitroglycerin Drugs in K562 Cancer Cells

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Leukemia is cancer of blood-forming tissues that derived from red and white blood progenitor cells. Red and white blood cells growth is regulated by growth factors usually in the body but in the leukemia the regulation is disrupted. Nitroglycerine realease nitric oxide (NO) to environment and NO increases cellular oxidative stress then induces cancer cell apoptosis. Valproic acid inhibits the histon deacetylases and induces differentiation and apoptosis in malignant cells, since it may be used to treat cancer. K562 cells were cultured and then different concentrations of nitroglycerine and valproic acid prepared and their antitumor properties at 24, 48 and 72 hours after treatment were measured by MTT assay. In the next stage, according to the IC50 of drugs, the combination drugs were prepared at different concentrations and its anticancer effect was measured by MTTassay. DNA electrophoresis and staining with Hoechst used for analyses of cell apoptosis. The results showed that the antitumor effects of nitroglycerin and valproic acid increase in a dose and time dependent manner. The IC50 of nitroglycerin and valproic acid was 79 and 80 (micromol/ml), respectively. Combination drugs significantly decreased cell viability and the synergism effect was observed only in concentrationof 100 µM. On the other hand, the apoptotic effects of these drugs showed by DNA electrophoresis. Based on the results, it was concluded that cytotoxic effect of nitroglycerine, valproic acid and also combination drugs on K562 cells is time and dose-dependent manner so the maximum inhibitory effect wasobserved at higher concentration and 72 hours after treatment. The results suggest that combination drugs perhaps may have effective role in cancer therapy. Therefore, combination drug is effective for the prevention and treatment of chronic myeloid leukemia.

Key words: Valproic acid, nitroglycerin, interactions, anti -cancer properties, K562 cell line

P-17 Exploration of Iranian Scientific Productions on Digestive System Neoplasms in the Medline 2002-2012

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Gastrointestinal neoplasm is one of the most dangerous cancers among people in Iran. It causes almost half of all cancerous deaths in Iran (44/4 percent). The high incidence of cancers in the world as well as in Iran makes negative consequences and undesirable influence on patients-life and their society; therefore, the scientific activities in this area seem to be very essential and compulsory. The current study is a cross-sectional analysis. Bibliometric indicators were recruited to analyze all data in the field of gastrointestinal cancer. Row data was extracted from the database of MEDLIN through 2012-2002. Extraction of data was based on Medical Subject Headings [MeSH]. In addition, the relevancy of data was checked by a specialist doctor in the field. Gathered data were transferred into a major checklist to be analyzed by Excel software. Analysis of obtained data showed a total number of 468 papers as a Medical Subject Heading of "*Digestive System Neoplasms*" that was indexed in MEDLINE through 2002-2012. Iran producing 0.3% of total publications in the field was ranked as 31st in the world، 10th in Asia and second in the Middle East. Regarding the Iranian institutes, ShahidBeheshti University of Medical Sciences was the most productive institute in the field of "Digestive System Neoplasms" sharing 15/17% of total papers originated from Iran. *The Asian Pacific journal of cancer prevention* was the most prolific journal that published Iranian papers in the field of Digestive System Neoplasms. The number of publications originated from Iran in the field of Digestive System Neoplasms has increased linear through the period of study. The most productive years were 2011 and 2012.

Key words: Scientific production, MEDLINE, scientometrics, digestive system neoplasms, Iran

P-18 Effects of Single and Gradual Doses of Doxorubicin on Cardiotoxicity Markers

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Doxorubicin is one of the most effectivechemo-therapy agents and is being used in many kind of cancers. The most serious drawback of doxorubicin is its dose dependent, obviously irreversible cardiotoxic effect. The aim of this research is to investigate the effects of single and gradual dose of doxorubicin on heart using cardiotoxicity markers such as cardiac troponin I (cTnI), total creatine kinase, CK-MB isoenzyme and lactate dehydrogenase (LDH). 54 Wistar rats were randomly divided into 9 equal groups of 6 (3 control saline and 6 test groups). The gradual test groups were injected with 2 mg/kg/week of doxorubicin until accumulative doses (8, 14 and 20 mg/kg), but single dose groups administered these dosages of doxorubicin once. Blood samples were taken from heart and after centrifugation; the sera were frozen at -180°C until assayed. Serum levels of troponin I was measured with ELISA method and other markers with colorimetric methods using UV-Vis Spectrophotometer. All data were analyzed with SPSS 18 software. Serum levels of troponin I, total creatine kinase, CK-MB and lactate dehydrogenase activities were increased proportional to dose of doxorubicin. There was a significant difference between all test groups with controls ($p<0.0001$). The levels of cardiotoxicity biomarkers in single dosage groups were significantly more than the gradual dosage groups ($p<0.005$). Doxorubicin should be administered gradually and in smallest dosage possible to reduce the risk of heart injury.

Key words: Doxorubicin, troponin I, cardiotoxicity, biomarker

P-19 Construction of an Expression Vector Containing Human Interleukin-2 Coding Sequence and Evaluation of the Recombinant Protein Expression in *E. coli*

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Interleukin-2 (IL-2) is a central regulator of immune responses and produced mainly by activated $CD4^+$ T cells. Human IL-2 (hIL-2) is a 15 kDa protein containing 153 amino acids and a disulfide bond between the residues cys58-cys105. The recombinant form of hIL-2 is used as a drug in variety of cancers especially renal cell cancer and melanoma. To produce active recombinant hIL-2, we employed a bacterial expression vector (pET32) and *Escherchia coli* Rosseta-gami (DE3) as the expression host. Recombinant hIL-2 was synthesized in the structure of a fusion protein that contained thioredoxin, poly-histidine tag and intein as a self cleaving tags. This structure helps correct folding of the recombinant protein and its purification. Also, the intein in structure of the fusion protein shows self-cutting effect through some parameters such as pH changes and results in the isolation of mature IL-2 protein without any additional amino acids. We used synthetic coding sequence of hIL-2 and cloned into pCYB2 vector by digestion with *Sal*I&*Nhe*I. The coding sequence of intein was also amplified by specific primers and after cloning into PTZ57R/T vector, sub-cloned in target vector (pCYB2) containing IL-2 fragment. To confirm the accuracy of cloning, positive colonies were selected by insert check PCR and digested with suitable restriction enzymes. In the next step, IL-2/Intein fragment was sub-cloned in expression vector pET32b (+) and finally the recombinant vector was transformed into the expression host *E. coli* Rosseta-gami (DE3) for expression of IL-2/Intein/Trx fusion protein. pET32b was used as a host vector for cloning of IL-2 and Intein fragments and recombinant protein expression in desired bacterial strain was analyzed by SDS-PAGE electrophoresis. The accuracy of recombinant expression vector was confirmed by restriction digestion and sequencing analysis. The size of recombinant hIL2 band (80 kDa) in SDS-PAGE showed that the fusion protein was expressed by *E. coli* after 6, 9 and 12 hr post-induction.

Key words: Human interleukin-2, self cleaving tag, *E. coli* Rosseta-gami (DE3), inclusion body.

P-20 Exploring the Association between the TAAAA Polymorphism in P53 Gene and Risk of Colorectal Cancer in Isfahan Population

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Colorectal cancer is the most common type of cancer of the gastrointestinal tract. Also, it is the third most common cancer in the world for which the couses are largely unknown up to the present day. Among females, this cancer is the third commonest cancer after lung and breast cancer. And regarding men, it ranks third after lung and prostate cancer. Colorectal cancer is the third and fourth common cancer among men and women with the rate of 8.3 and 7 persons in 100000 people respectively. This cancer often occurs due to a mutation in Wnt massage pathway where there are many genes involved such as APC, P53, and PTEN among which the P53 is the most important one.

The P53 gene takes a repressor role on tumor, though certain mutations could change it to an oncogene. The P53 is engaged in response to damage of cells in three different ways which are autophagy, apoptosis, and necrosis. The aim of this study was to examine the frequencies of polymerphisms of TAAAA in P53 and their relation with colorectal cancer in the population of Isfahan. The bioinformatics analyses of the P53 showed an area of sequencing repeats of TAAAA in introns 1 of this gene. The sequence of gene in certain location of the genome was received from the NCBI website and a pair of primer designed by oligo software. The location of a sequence in the genome was endorsed by BLAST. The sequencing repeats of TAAAA were multiplied by PCR method and the lengths of products were determined by polyacrylamide gel and direct sequencing. According to our experiments so far, we suggest that there is a possibility that 7/7 genotype and colorectal cancer to be correlated. Further experiments will be carried out by increasing the number of patients' blood samples and controls.
Key words: Colorectal cancer, P53 gene, sequencing repeats of TAAAA, polymorphism

P-21 STAT3-Induced Activity by Recombinant Human Galectin-3 in Human Ovarian Cancer Cell Line SKOV-3

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Epithelial ovarian cancer (EOC) is one of the most deadly cancer among women worldwide. Most of the cases are incurable because of lack of symptoms and late diagnosis. Thus, there is an urgent need for better understanding of key molecules and their related signaling events in EOC. Galectin-3 (Gal-3) is a carbohydrate-binding protein involved in growth, adhesion, migration, invasion and apoptosis of numerous cancerous cells. Our previous study showed that recombinant human Gal-3 (rhGal-3) can reduce apoptosis in SKOV-3 cells and the use of Gal-3 inhibitor in combination with Paclitaxel (PTX) may synergize cytotoxic effect of PTX in these cells. However, molecular mechanism of Gal-3 effect on proliferation and chemoresistance of ovarian cancer cells remains largely unknown. Here we sought to determine the influence of rhGal-3 on activity of JAK2/Stat3 signaling pathway as an important survival pathway in numerous cancer cells. To this order, sub-confluent SKOV-3 cells were treated with 30 uM rhGal-3 for 30 and 60 minutes and active Stat3 (pStat-Tyr705) was detected by western blot analysis. Stat-3 activity was induced by rhGal-3 after indicated time compared to untreated cells. Moreover, in the 3D culture, these cells formed spheroids which could mimic tumorogenesis *in vivo*. Interestingly, there was strong induced expression of Gal-3 and pStat in spheroids compared to monolayer cell culture. Our finding for the first time show the involvement of Gal-3 on Stat-3 activity and their increased expression and activity in SKOV-3 spheroids, respectively. These resultsmay suggest that it could be worth to use of Gal-3 or Stat-3 inhibitors as therapeutic tools in ovarian cancer treatment.
Key words: Ovarian cancer, Galectin-3, STAT3, Spheroids, SKOV-3

P-22 Modeling Traumatic Injury in Organotypic Spinal Cord Slice Culture Obtained from Adult Rat

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Currently there are various models that recreate mechanisms of spinal cord injury (SCI). Among them modeling injury in organotypic slice culture of spinal cord is a robust approach that confronts with less experimental and ethical challenges compared to animal models. Considering the fact that almost all ex-vivo models of injury are obtained from embryonic and postnatal ages of animals, these models can hardly mimic the features of adult human SCI. Therefore, this study was designed to develop contusion model in spinal cord slice culture of adult rats. The lumbar enlargement of adult rat was excised and cut transversely with vibratome and slices were cultured according to the standard interface method. During various time points of culturing *in vitro*, PI staining was performed to identify the number of dead cells. Seven days after culturing the slice *in vitro* (DIV: 7), a weight was dropped to stimulate injury. Afterwards PI staining was carried out to show cell death after injury and also hematoxylin and eosin (H&E) staining defined the general histological features. Moreover, immunostaining against β III Tubulin was doneto assess the neuronal integrity. PI staining of sections before injury revealed though the number of dead cells in the first days of culture since preparation method is the high, this number decreases by seven days culturing *in vitro*. Also, this staining after injury elucidated significant increase in number of dead cells comparing to undamaged counterparts.

Moreover, H&E staining and immunostaining clarified the changes of histological features and decreased neuronal integrity following the injury. In this study, for the first time contusive model of spinal cord injury was set up in cultured slices of adult rat. This study showed slices can adjust to culture environment after 7 days and also dropping 0.5 gram weight from 3 cm height at this time stimulates injury. Besides the fact that injury in slices of adult rat is more relevant to human SCI, obtained data of this experiment revealed the ability of this model to mimic both primary mechanical damage and secondary reactive damage of injury.

Key words: Spinal cord, Spinal cord injury, Organotypic culture

P-23 Efficacy of Mesenchymal Stem Cells Transplantation on Treatment of Multiple Sclerosis; a Systematic Review and Meta-Analysis of Clinical Trials

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Cell therapy is the future treatment of degenerative diseases. Multiple Sclerosis (MS) is one of them with autoimmune origin. Mesenchymal stem cells have a great potential to regenerate nervous system and regulate immune system. So they are a good choice for cell therapy of MS. There are some interventional studies which have been done in recent years, but there is not a systematic review in this issue. Therefore, we designed this systematic review to produce high level evidence. In August of 2014, internet search was performed on Medline, Pubmed Central, and Scopus databases and on clinical trials registry databases. Search protocol for each database was established and title and abstract screening was done by two reviewers separately. Articles matched with PICO was included in the study. Included articles were critically appraised and the required data were extracted. Meta-analysis was performed by CMA software. 1430 articles were evaluated in title and abstract screening and from these, 10 articles were included in the study. Out of these 10 articles, 4 of them were categorized as phase II clinical trial, 3 were phase I clinical trial, and the last 3 were quasi-experimental study. Two articles had good quality, 3 articles had moderate quality and the others had weak quality. Risk of bias in one article was low, in another one was moderate and the rest of them was high. Most patients suffering from progressive type of MS and article survey results indicated that intratechal or intravenous injection of mesenchymal stem cells prevent EDSS elevation and improve immunologic condition of most patients. Meta-analysis also showed MSCs transplantation could reduce progression incidence by 0.34 (95% CI: 0.26-0.44). Despite the low number of performed interventional studies in this issue and yet there was not any published phase III clinical trial, results of this systematic review revealed that mesenchymal stem cells have efficacy to treat multiple sclerosis.

Key words: Multiple sclerosis, mesenchymal stem cells, cell therapy, regenerative medicine, systematic review

P-24 Comparative Assessment of Pellet Culture System and PLGA Scaffold Ability in Order to Createa More Favorable Environmentforthe Proliferation and Differentiation of Adipose-Derived Stem Cells

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Cell therapy with stem cells has been a tremendous progress in the repair of damaged tissues and organs. Adult stem cells such as mesenchymal stem cells have been introduced as a promising candidate for cell therapy issue. Adipose derived mesenchymal stem cells have unique properties such broad potential to differentiate into different cell types, easy isolation and plentiful access that due to this properties, these cells have become one of the most attractive cell source for tissue engineering and regenerative medicine. The purpose of this study was to evaluate the ability of the pellet culture system and synthetic PLGA scaffold as suitable substrates for the growth and proliferation and differentiation of adipose-derived mesenchymal stem cells into chondrocyte. In this study, the PLGA scaffolds and pellet culture system was prepared and then mesenchymal stem cells was isolated from adipose tissue. Then, these cells were cultured and differentiated on the scaffold and pellet culture system, separately. After 2 weeks, viability and chondrogenic gene expression analysis was performed for each one by MTT and Real time PCR method. Also, the cartilage formation on the scaffolds was confirmed by histological analysis. The results of this study showed that the highest

potential of proliferation and differentiation of adipose-derived mesenchymal stem cells was in the PLGA scaffold compared than other pellet system culture. It was seen that synthetic scaffold such as PLGA had been a huge potential for appropriate growth and differentiation of cells.

Keywords: Differentiation into chondrocyte, PLGA, pellet culture system, adipose-derived mesenchymal stem cells

P-25 Investigation of Association between microRNA148a Polymorphism and Risk of Breast Cancer in Isfahan Population

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Breast cancer is the most common cancer and the major cause of death among women worldwide. Research shows that this cancer is one of the most common malignancy among women in Iran and the prevalence in Iranian women is 120 per 100000. This type of cancer is genetically complex but is strongly linked to steroid receptor signaling pathways. Most breast cancers are sporadic and occur in several genes and the exact number of genes that cause breast cancer risk is not clear. Because microRNAs act as expressional regulators of multiple genes, single nucleotid polymorphisms in microRNA genes can have influences on breast cancer development. The purpose of this study was to investigate the association between microRNA 148a polymorphism and the risk of breast cancer in Isfahan population using tetra primer ARMS-PCR for genotyping of 200 cases and 100 controls. To date, 70 samples including 50 controls and 20 cases were genotyped. Between the 50 controls, 39 samples show homozygous dominant genotype, 7 samples were heterozygous and 4 samples were homozygous recessive and between 20 cases, 5 samples were homozygous recessive, 14 samples were heterozygous and only 1 sample shows homozygous dominant genotype. Genotyping for the rest of samples will be performed in future.

Key words: Breast cancer, microRNA148a polymorphism

P-26 The effect of Cold Atmospheric Plasma Jets on Cervical Cancer (HeLa) Cells

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Cervical cancer is one of the most common cancers in women that chemotherapy and radiotherapy are the methods for its treatment. These methods have complications due to much drug doseand light radiation. Reduction of toxicity and complications related to radiation therapy is essential problem for the improvement of clinical results. Plasma is the fourth state of material that contains ions, energetic electrons. Free radicals produced by energetic electrons, react with the living cells. This feature of plasma is used to destroy cancer cells. Less non-invasive features is one of the advantages of plasma treatment than other treatment methods. In this paper, argon/air and oxygen plasmas have been used to apply to cancer cells of cervical. The atmospheric pressure plasma jet (AAPJ) consists of a tube of Pyrex glass. Powered electrode was set inside the Pyrex tube and grounded ring electrode was attached to the surface of the Pyrex nozzle. The APPJ was generated at the gas gap between two copper electrodes and exited into the surrounding air outside the nozzle. The parameters of applying plasma duration, gas flux, applied voltage and distance between nozzle orifice and surface of cells investigated in this paper. Cell culture was performed in 6 vials of 300, 000 cells and were cultured in the house. According to the obtained results, the percentage of destruction of cancer cells by Ar/air plasma is much more than oxygen plasma that in applying duration more than oneminute, almost up to 90 percent of cancer cells were destroyed.

Key words: Atmospheric plasma jet, cervical cancer cells (HeLa)

P-27 Serum Level of MicroRNA 10b in Breast Cancer Patients

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MicroRNAs (miRNAs) are a class of small RNA molecules that regulate gene expression at the posttranscriptional level. Moreover, microRNAs are present in a remarkably stable form in the blood. Several studies in recent years have shown that the alteration of miRNAs profile in tumor cells lead to the change of their circulating levels. MiR-10b is an oncogenic miRNA that induces metastases in breast

cancer cells by down regulationof homeobox D10 (HOXD10). In this study, we investigated the serum level of miR-10b in patients with breast cancer. For this purpose, serum level of miR-10b was investigated in 44 stage III breast cancer patients as well as 20 healthy woman. The serum samples were collected from four patients in three stages including: pre-operation, one month after tumor resection surgery and one month after chemotherapy and from 40 patients, one month after chemotherapy. We initially evaluated the appropriateness of our RNA extraction efficiency and microRNA assay by quantitative Real-time PCR and then compared the serum level of miR-10b in all samples with healthy group. We used endogenous miR-16 to normalize our quantitative RT-PCR data using the ΔΔCT method (Livak method). The results of this study showed that the serum level of miR-10b was higher in all four patients of the pilot group that decreased after tumor resection surgery, this was similar to normal group. Serum level of miR-10b in samples obtained one month after chemotherapy was significantly higher than the normal group. Collectively, our data indicate the increase of serum level of miR-10b in breast cancer.

Key words: miR-10b, breast cancer, serum

P-28 Bioinformatics Analysisof Potential Role of miR-520h in Patients with Gastric Cancer

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Cancer is a group of diseases characterized by the uncontrollable growth and spread of abnormal cells which can lead to death without timely intervention. Gastric cancer is more common in men than in women. These cancers develop from the cells that form the innermost lining of the stomach (known as the mucosa). When the term stomach cancer or gastric cancer is used, it almost refers to an adenocarcinoma. About 90% to 95% of cancers of the stomach are adenocarcinomas. Overexpression of hsa-miR-520h inhibits cell migration and invasion. Has-mir-520h is a tumor suppressor miRNA proposed as a biomarker in gastric cancer. Bioinformatically we propose a model which explains how loss of hsa-miR-520h expression and subsequent activation of ABCG2 expression have critical effect on the invasion and migration of human stomach cancer cells. The aim of our study was to expand current knowledge about molecular function of miR-520h and its SNP as a potential biomarker in gastric tumor cells by using bioinformatics tools. Validated and predicted targets of miR-520h were regained from miRtarbase and miRwalk databases, respectively. Expression of retrieved targetome in gastric cancer was evaluated in UniGene database. At last gastric cancer specific targetome was entered into DAVID database for molecular pathway enrichment analysis. Our data manifested KEGG signaling pathways “pathway in cancer” as the most statistical relevant pathway with miR-520h targetome. It revealed that miR-520h inhibits some important gastric cancer-related pathways such as ABCG2. According to our data, miR-520h may be related to gastric cancer through targeting oncogenes. It has been demonstrated that mir-520h targetome (such as ABCG2) leads to proliferation, invasion and migration in gastric cancer. Therefore, hsa-mir-520h is a potential tumor suppressor miRNA, whichcan be used as a worthwhile prognostic biomarker. This SNP could have prognostic value for gastric cancer centers due to its effect on mir-520h stability.

Key words: Gastric cancer, miR-520h, SNP, signaling pathway

P-29 Comparison of the Anti-Cancer Effect of Disulfiram and 5-Aza-CdR on Pancreatic Cancer Cell Line PANC-1

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Pancreatic cancer has poor prognosis by surgical and chemotherapy when it is diagnosed, so other anti-cancerous assistant therapeutic drugs are suggested e.g. epigenetic reversal of tumorsuppressor genes on promoter hypermethylation. 5-Aza-CdR is a nucleoside analog of DNMTi but it has long-term cytotoxicity effects. This study compared the anticancer effect of 5-Aza-CdR and disulfiram potencies on PANC-1 cell line and up-regulation of p21. PANC-1 cell line was cultured in DMEM high glucose and treated by 5-Aza-CdR with 10 µM concentration for four days and 13 µM DSF (disulfiram) for 24 hours. MS-PCR and Real-Time-PCR were carried out to detect the methylation pattern and estimate the mRNA expression of RASSF1A and p21 in PANC-1.

MS-PCR demonstrated partial unme-thylation after treatment with 5-Aza-CdR while there was no unmethylated band after DSF treatment. Real Time-PCR showed significant differences between re-expression of RASSF1A before and after treatment with 10 μM 5-Aza-CdR ($P < 0.01$) but not after treatment with 13 μM DSF ($P > 0.05$). The significant correlation of p21 upregulation was observed after treatment with both 5-Aza-CdR and DSF ($P < 0.01$). Our findings indicated that 5-Aza-CdR induces the reexpre-ssion of RASSF1A and p21 upregulation in PANC-1. DSF showed no epigenetic reversion while it affected p21 up-regulation.

Key words: 5-Aza-CdR, disulfiram, DNMT inhibitor, epigenetic, p21, PANC-1, RASSF1A

P-30 Impact of Measuring DNA Damage and Cytogenetic Alterations in Monitoring Cancer Patients or Cancer Prone Individuals

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About 10% of apparently normal individuals and more than 40% of cancer patients show elevated radio-sensitivity. These patients suffer from impaired DNA damage repair machinery especially DNA double starnd breaks. However, there is not yet an appropriate reliable method for the assessment of chromosomal radio-sensitivity when screening a large population. In this study, comet assay, γH2AX and Micronuclei assay was performed. Comet assay was performedto detect radiation-induced initial DNA damage on blood leukocytes of 30 breast cancer patients and similar number of normal individuals. γH2AX, an immu-nocytochemical assay is quite sensitive and is a specific indicator for the existence of a DSB. Residual DSB induced by radiation was assessed using γH2AX assay on breast cancer tissue and pair normal adjacent and control breast tissues after 24 hours incubation in 37˚C with 5% CO_2 atmosphere. Cytochalasin B cytokinesis–block micronucleus assay (CBMN) was done to assess the frequency of micronuclei in lymphocytes of breast cancer patients. Cells were irradiated at G0 phase and 1000 binuclei lymphocytes for the presence of MN were scored for each sample. Results obtained show that there is no difference in the DNA damage induced by ionizing radiation in the leukocytes of normal and cancer individuals when assessed by the neutral comet assay. However, there was a significant difference in the frequency of residual DSB in cancer cells compared to normal as assayed by γH2AX assay. Frequency of micronuclei was significantly higher in lymphocytes of breast cancer patients compared to control. From the results obtained, it can be concluded that processed DNA damage, which manifested as chromosomal aberrations or micronuclei could be used as biomarkers for the detection of cancer prone individuals. The difference in hypersensitivity seen in breast cancer patients and normal individuals might be due to the altered DNA repair capacity in these patients.

Key words: Breast cancer, DNA double-strand breaks, Micronuclei, Radiosensitivity.

P-31 Effect of Arbutin on the Survival of Prostate Cancer Cells (LNCaP)

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Arbutin is a chemical agent extracted from Telka (*PyrusbiossierianaBu-hse),* a plant with high antioxidant and radical scavenging abilities. In this study we investigated the role of arbutin in survival of prostate cancer cells. In our study arbutin at 4 different concentrations (50, 100, 200, 500 μg/ml) was added to the prostate cancer cells (LNCaP) and cell viability was measured after 24, 48, 72 hours using MTT assay. As the control group, cells were treated with cell culture medium without arbutin. After 24 hours of treating the cells with different concentrations of arbutin, we did not see any significant differences among arbutin-treated and control groups ($p>0.05$). Interestingly, after 48 hours of treating cells with arbutin, we noticed a significant increase in arbutin-treated compared to the control ($p<0.05$). At 72 hour time point, 50-200 μg/ml of arbutin increased the cell growth while at the concentration of 500 μg/ml arbutin decreased cell growth ($p<0.05$). Data obtained with other concentrations of arbutin at 48-72 hours showed that arbutin had less increase in cell growth. Our study revealed that high concentration of arbutin is time-dependent and could decrease cell growth in prostate cancer cells compared to the control group.

Key words: Arbutin, prostate cancer, LNCaP, antioxidant

P-32 Breast Cancer Derived Cell Line MCF-7 Growth Inhibition by Human Foreskin Fibroblasts Reversed via Serum Starvation

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Fibroblasts as heterogeneous cells in shape and function play a dual role in health and diseases. Cancer associated fibroblasts (CAF) can be recruited by some cancerous cells especially by breast cancer cells. Moreover, there is a severe interaction between tumor and stromal cells such as fibroblasts. In this study, we indicate that newborn foreskin isolated fibroblasts inhibit the proliferation of a breast cell line, MCF-7 (Michigan Cancer Foundation) and also we pointed out the most important event in tumor environment is starvation which can counteract inhibition effect and leads to tumor growth. Fibroblast cells were plated in 96-well plate (8×10^3 cells per well) upto reaching 100% confluence. Then, the culture medium were replaced by serum free medium and incubated for 6, 24, 48, 72, and 96h. Then, the medium was suctioned and 1×10^3 MCF-7 cells that suspended in RPMI-1640 were supplemented by 10% FBS and 1% penicillin-streptomycin was added to each well. After one week incubating the cells in each well was tripsinated, then they were counted by Neubauer slide and cultured in $25Cm^2$ flask for colony count. Generally, MCF7 cells growth enhanced co-culture with starved fibroblasts as compared to control group but interestingly, this process reached to its peak when fibroblasts starved for 16 hours. The data originated from this preliminary observation indicated that although fibroblasts slow down MCF-7 cells growth, however, serum starvation can reverse this inhibitory effect and lead to increase MCF-7 cells growth. Nevertheless, further stndies are needed to provide more support and understand the underlying molecular mechanisms resulting in such manifestations.

Keywords: Fibroblast, MCF-7, Serum starvation

P-33 The Study of Nitric Oxide Synthase 3 (*NOS3*) T-786C Gene Polymorphism in Iranian Infertile Men with Varicocele

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Varicocele is an abnormal dilation and tortuosity of veins of pampiniform plexus that drains the testis and causes an important change in semen. This abnormality is often one of the most common risk factors for male infertility. The aim of this study was to investigate the relationship between nitric oxide synthase 3 (*NOS3*) gene T-786C polymorphism, as a common genetic factor, with the risk of varicocele in Iranian infertile men. The relation of *NOS3* gene T-786C polymorphism was studied in 60 varicocele patients and 45 control subjects by Multiplex-ARMS PCR technique. In this study no genetic relationship was observed between *NOS3* gene T-786C polymorphism and varicocele. Therefore, this polymorphism has genetically nothing to do with Iranian infertile men suffering from varicocele.

Key words: *NOS3*, varicocele, polymorphism, Multiplex-ARMS PCR

P-34 Is It Time to Vitrify All Embryos in ICSI Cycles?

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The aim of this study is to evaluate the efficiency of vitrification in ICSI cycles. A retrospective study was conducted to assess the outcomes of clinical pregnancy and live birth from the vitrified embryo transfer (VET) in ICSI cycles. A total of 30 VET with negative result in previous fresh embryo transfer (group a) and 26 VET with all embryos vitrification because of ovarian hyper-stimulation syndrome or uterine asynchrony (group b) between 2013 and 2014 were included in the study. The women >40 years old and recipients' cycles were excluded. Only grade A vitrified embryo at cleavage stage was taken into account in the study. In groupA, clinical pregnancy and live birth rates were 23.3% and 20% respectively. In groupB these parameters were 23.1% and 19.2% in that order. No significant differences were apparent in measured parameters between two groups. Also, the cumulative rates of clinical pregnancy and live birth were 23.2% and 19.6%, respectively. In conclusion, VET could be an effective approach at first try in ICSI cycles. Cryopreservation of grade A embryos provides an opportunity to improve IVF outcome.

Key words: Vitrification, ICSI, cleavage, live birth

P-35 Effect of Mouse Embryo Hind Limb Bud Co-culture on *In vitro* Maturation of Immature Mouse Oocyte

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Ovarian hyper stimulation syndrome (OHSS), thrombosis, heart failure and even death is due to the usage of certain medications to stimulate ovulation in the treatment of infertility in-women. A new method to solve this problem is oocyte maturation *in vitro*. Various factors such as fibroblast growth factor have roles in the starting regulation of follicular grows and oocyte survival. Therefore, this study was deviced to evaluate the effect of co-culture of mouse embryo hindlimb bud containing FGF on maturation of immature mouse oocyte. Immature oocytes were collected from the ovaries of female mice from NMRI at the age of 6 to 8weeks in the sterilized conditions. Then the oocytes were placed in maturity medium including (Minimum Essential Medium: α- MEM) with FBS of 10% co-cultured with the hind limb bud belonging to 10.5-11.5 and 12.5-13.5 old mouse embryo and cultured in CO2 incubator with 5% CO2. Oocyte maturation was recorded under an inverted microscope after 24 hr. *In vitro* maturation rate was in control group, experimented groups 1 and 2 respectively 46, 67 and 52 percent that indicated a significant increase ($P<0.05$) in matured MII oocytes (in the presence of 10.5-11.5 days mouse embryo hind limb bud) than that control group and the second experimental group. The results show that the co-culturing of oocyte with 10.5-11.5 days mouse embryo hind limb budstimulated the resumption of meiosis and increased the rate of maturation of immature oocytes. Probably, it is due to the abundance of FGF8 and many other unknown developmental factors.

Key words: *In vitro* maturation, Hind limb bud, Co-culture, Mouse immature oocyte

P-36 Investigation of the Effect of Co-culture of the Heart Tissue of Mouse Embryo over the Maturity of Mouse Immature Oocytes Invitro

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Ovulation stimulation is a common method of treating female infertility in the process of ART. Ovarian hyper stimulation syndrome (OHSS), thrombosis, heart and kidney failure and even death are due to the use of certain medications for ovulation. A new method to solve this problem is oocyte maturation *in vitro*. Various factors such as fibroblast growth factor have roles in the starting regulation of follicular growth, oocyte survival and steroid and the developing heart secretes various factors such as fibroblast growth factor. Immature oocytes were collected from the ovaries of female mice from NMRI at the age of 6 to 8 weeks in the sterilized conditions and then were placed in maturity medium including (Minimum Essential Medium: MEM-α) with FBS of 10% co-cultured with the heart tissue belonging to 10 to 13-day old mouse embryo and cultured in CO2 incubator with 5% CO2. 24 hours later, oocytes were investigated with inverted microscope. Co-culturing the immature oocytes with heart tissue of mouse embryo stimulates the resumption of meiosis and increases the oocytes maturation in comparison to the control group. Ovule co-culturing with heart tissue of mouse embryo of 11 and 13-day old can increase maturity respectively to the degree of 50 and 57% in comparison to the control group (46%). The results show that the co-culturing of the heart tissue of mouse embryo of 11 to 13-day old stimulated the resumption of meiosis and increased the rate of maturation of immature oocytes. Probably it is due to the abundance of FGf2, FGF10, FGF1 and many other unknown developmental factors.

Key words: Co-culturing, Heart tissue, *In vitro* maturity, immature oocytes

P-37 Evaluating the Toxic Effects of Copper Sulfate on Testosterone Hormone, Sperm Quality and DNA Fragmentation in Male Wistar Rat

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This experiment was conducted to investigate the effects of different levels of oxidized of copper sulfate on male reproduction. The present study aimed to investigate changes in testis weight, Testosterone level, sperm parameters and DNA fragmentation in male rat following long term consumption. Animals were divided into three experimental groups. The first group received copper sulfate at a dose of 100 mg/ kg and the second group was given copper sulfate at a dose of 200 mg/ kg for 28 and 56 days. Control animals received normal saline using the same method. Testis weight was measured. Serum was used to evaluate testosterone level. Diameter of seminiferous tubules, spermatogonial and sperm cells parameters and level of sperm DNA

fragmentation were calculated. The mentionedmean values of sperm parameters in copper sulfate treated groups showed no significant decrease on 28 day compared to the control group. Also, in some parameters, further decreases were observed specially in the Cu200 group on the 56th day such as diameter of seminiferous tubules, level of testosterone hormone, spermatogonial and sperm cells parameters. The level of sperm DNA fragmentation showing maturation arrest also increased in treated animals. But testis weight decreased atthe dose of cu100 and higher doses in 28 and 56 days. ($p<0.05$). The results show that exposure to copper sulfate has the deleterious effects on spermatogenesis and DNA fragmentation of sperm which appeared as early as four weeks.

Key words: Copper sulfate, testosterone, toxicity, fragmentation

P-38 Polymorphism in CGA Affects the Function of miR-1302 and Increases the Risk of Men Infertility

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Infertility occurs in 10 to 15% of couples worldwide and close to half of it is caused by male factors. Despite decades of efforts to clarify mechanism of male infertility, most cases are still idiopathic. A lot of factors such as genetic and sexual problems can affect infertility. Among these problems, genetic disorders are the most common factors. A study has shown that one of genes that can affect male infertility is CGA. This gene is involved in miotic. CGA, α subunit of glycoprotein hormones, is the main part of thyrotropin glycoprotein hormone (pituitary TSH), lutropin (LH), follitropin (FSH), and chorionic gonadotropin (human placental gonadotropin, hCG) that has essential role in the development and function of tyroid and gonads. CGA gene is located on 6q14-q21.Rs6631 in CGA has strong association with men infertility. Studies have shown that miR-1302 can negatively regulate CGA and substitution of T with A may interfere this process. This miRNA can band with rs6631-A more strongly than rs6631-T. Study of this polymorphism, may help to find a cure for idiopathic men infertility. Tetraprimer technique is an appropriate way to study this polymorphism; because it is faster and cheaper than ordinary PCR. Also, laboratories with low equipment can use this method. Primers designed by the use of Primer1 and then checked by Oligo7 software. By the use of these primers and tetraprimer technique, this polymorphism can be studied for the first time in Iran.

Key words: Men infertility, miRNA, SNP, Primers

P-39 Study the Association of G1793A Polymorphism in *MTHFR* Gene with Male Infertility

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Methylene tetra hydropholate reductase gene has an essential role in folate metabolism. This gene is located on chromosome 1 and contained 12 exons. The gene contains many single nucleotide polymorphisms. The aim of this study was to investigate the association of G1793A polymer-phism with male infertility. In this study, 132 fertile and 118 infertile men referring to IVF centers in North of Iran were selected. 2cc bloods were collected from all subjects. Genomic DNA was extracted by DNGplus (Cinnagen, Iran). G1793A genotyping was performed by PCR-RFLP. Our data revealed that there is no significant association between A allele and male infertility (OR: 1.0070, 95% CI; 0.4021 to 2.5224, P= 0.9880). So G1793A could not be a risk factor for male infertility.

Key words: Male infertility, *MTHFR* gene, single nucleotide polymorphism, G1793A

P-40 The Evaluation Needed for Resuscitation of Newborns During the Years 2013 and 2014 in Rouhani Hospital of Babol

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The tranfer of inhalant to independent breathing outside the uterus is required. Nearly 10% need to stimulate breathing for the start of breathing, but less than 1% need advanced resuscitation. Whereas, Rouhani Hospital of Babol is a level 3 center. Therefore, we attempted to determine the need for resuscitation of newborns in the hospital too. This retrospective study on infants older than 24 weeks of gestation who were alive at the end of 2 years were at the center. Information on maternal and neonatal data were extracted from report files and analyzed using SPSS software. In this study, 5674 of 986 newborn with Apgar lower of 9, average gestational age was 35.55 weeks and an average weight of 2611.23 gr were born alive that 80% were delivered by caesarean method, 42% were

outcomes the first infants and the neonatal anomalies 3.8% and 53.50% have high-risk pregnancy, especially of twin 12.1%, diabetes 9%, hypertension and preeclampsia 8.8%, PROM 2.5%, IUGR 1.9%, preterm delivery 1% and 18.2% had other causes. 11.35% the first steps of resuscitation, 4.91% positive pressure ventilation, 1.98 chest compressions and 0.1% injected drugs were needed. 37.3% were hospitalized in the NICU. It seen high rates of caesarean sections and referral of this center can be result of more than of need to resuscitation of infants about 7%. Thus reducing unnecessary caesarean sections and trained team

Key words: Newborn, resuscitation, caesarean, Apgar

P-41 Application of Real-Time PCR by FRET Probes for ΔF508 Mutation Detection in Cystic Fibrosis Disease

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Cystic fibrosis is a non-contagious genetical disease among children. ΔF508 mutation of CFTR gene, is one of the prevalent mutations of this disease. Clinical attributes of this disease include deficiency in the digestive and pulmonary system of child reducing its complications whichcan be assisted by quick diagnosis. Therefore, a reliable method to diagnose mutation can be very important. In this study, we surveyed Real-Time PCR method in the presence of FRET probes in order to identify this mutation. 20 samples of peripheral blood of the patients referred to 17 Shahrivar Center of Rasht City were collected following the designation of sequences of FRET probes for ΔF508 mutation and, then DNA extraction, Real-Time PCR reaction on the DNA samples was executed, and the results were interpreted through analysis of melting curve. Through the study and analysis of the melting curve, it was specified that each of the normal and mutant allele creates its own specific melting curve and, thus, normal and mutant alleles were diagnosable since cystic fibrosis disease is a fatal disease among the children, our study showed a reliable, simple and sensitive method to diagnose ΔF508 mutation.

Key words: Real-Time PCR method, FRET probes, ΔF508 mutation, cystic fibrosis

P-42 The Effect of Causes of Infertility on ART Outcomes in Patients that Referred in Infertility Center of Fatima Zahra

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Infertility is one of the important health problems in a social community. Therefore, the study of factors affecting the success rate of pregnancy in couples is very important. The aim of this study was to evaluate the cause of infertility on ART outcomes in patients referred to Fatemeh Zahra Infertility Center of Babol. This cross-sectional study was done on 50 patients with various causes of infertility, between 2009 and 2010 in Fatemeh Zahra infertility Center. Information of demographic and outcome of treatment in patients were extracted from the patients, medical records and their data were analyzed using SPSS software. 62 patients were enrolled and 12 patients were excluded, finally 50 patients with diagnosis of infertility to treatment through cycles of ART, was evaluated. Out of 50 patients, 13 (26%) patients had successful pregnancies, and 37 (74%) patients were treatment failures. Factors of infertility were 4% due to the unknown factors, 44% male factor, 36% women factor and 16% male and female factors that the highest rates of pregnancy on ART were male factor 6 (46.5%), female factor 5 (38.5%), male and female factors 2 (15%) and unknown factors were related to no pregnancies. This study suggests that from the causes of infertility in couples, the male factor has the best response to ART. To increase the success rate of ART studies should be performed on the other causes of infertility and its treatment.

Key words: ART, pregnancy, infertility, male factor, female factor

P-43 The Assessment of Association of Prolonged Jaundice with Polymorphism of UGT1A1 Gene (G71R) in Gilbert's Syndrome

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Jaundice is a common condition during the neonatal period. Prolonged jaundice occurs in a large number of breastfed infants. Considering the impact of genetic factors on the incidence of jaundice, the aim of this study was to determine the association between prolonged jaundice and G71R polymerphism in Gilbert's syndrome. This case-control study was conducted in Taleghani children`s Hospital of Gorgan. The case group consisted of 87 patients with jaundice, aged more than 2 weeks with indirect bilirubin level higher than 10 mg/dL. Acute diseases (hypoxia, hemolysis or sepsis) and the use of phenobarbital and other medications of motherswere the exclusion criteria. The control group consisted of 81 newborns without jaundice, referring to Taleghani Children Hospital. The two groups were matched in terms of age and sex. DNA extraction was performed through the "salting out" method. CTPP-PCR was applied to amplify the polymorphism of G71R region. Overall, 84% and 64%of subjects in the case and control groups were males, respectively. The distribution of Gilbert genotype was not significantly different between the two groups. (P=0.772). Hence, there was a correlation between prolonged icterus in males with UGT1A1 G71R polymorphism (P=0.03). In the case group, 5.7% of the subjects were homozygous, 83.9% were heterozygous, and 10.3%were normal. In the control group, 3/7% of the participants were homozygous, 84% was heterozygous, and 12.3% was normal. Our findings show that there is no association between prolonged jaundice and G71R polymorphism even though a relationship was revealed between males and mentioned polymorphism.

Keywords: UGT1A1 gene polymorphism, prolonged neonatal, icter, Gilbert syndrome.

P-44 Aniline Pentamer-Modified Polyurethane for Cardiac Tissue Engineering

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Myocardial infarction (MI) is a major cause of morbidity and mortality worldwide. MI runs a series of complex processes including abnormalities in the electrical function of the cardiovascular system. One of the challenges that remained in cardiac tissue engineering is that poor conductivity of the patch can limit its ability to couple transplanted cells electrically to the local host myocardium. Electroactive scaffolds have the ability to integrate transplanted cells with the host tissue in a synchronized behavior. A novel biodegradable electroactive polyurethane containning aniline pentamer was synthesized and fully characterized by spectroscopic methods. To tune the physico-chemical properties and biocompatibility, the prepared sample was blended with polycaprolactone. The behavior of the prepared samples against L929 mouse fibroblast and human umbilical vein endothelial cells were evaluated. The effective contribution of conducting segments in final materials regarding cell functions was compared with corresponding non-conductive material. *In vitro* degradation tests conducted in phosphate-buffered saline and DPPH free radical assay were used for the evaluation of antioxidant property of the electroactive oligoaniline-embedded polyurethane. Presence of electroactive moieties and the electroactivity behavior of the prepared films were confirmed by UV–visible spectroscopy and cyclic voltammetry. A conventional four probe analysis demonstrated the electrical conductivity of the films in the semiconductor range (~10^{-5} S/cm). MTT assay showed that the electroactive polyurethanes are not toxic. The percentage of DPPH scavenging was recorded almost 58.9% after 15 min. *In vitro* degradation tests proved that the films were also biodegradable. Our study highlighted the potential application of electroactive polyurethanes as a substrate to direct electrical signals on cell activities for tissue engineering applications. Our data demonstrated that the inherently electrical conductive substrates are non-toxic and support cell proliferation and attachment combined with antioxidant property.

Key words: Myocardial infarction, cardiac patch, electroactivity, polyurethane

P-45 Fabrication of Engineered Cardiac Tissue as a New Tool for Drug Screening and Diseased Heart Studies

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Harmful heart drug effects cause important problem in clinical therapies. Engineered tissues are a good platform for *in vitro* drug screening. For this purpose, a method named engineered heart tissue (EHT) has been developed. Culturing cardiomyocytes in a collagen matrix creates a coherently contractile EHT model for using in pharmacological studies. The cardiac cells were isolated from 11-day old embryonic chicken by enzymatic digestion and then engrafted in collagen matrix. These cells then were casted in 8-well polycarbonate mold with a $5*10^6$ cardiomyocyte density in each well (mold, N=4). Tissue formation process was observed using inverted microscope.

To evaluate tissue characteristics, monophasic action potential, pulse rate (PR) and force measurement were done in each tissue before and after injection of β-adrenergic (epinephrine 0.1 μM). Electrophysiological tests were recorded using silver electrodes. Contractile forces of EHT were also measured by an isometric transducer. EHT showed spontaneous contractile characteristics from 0.2±0.001 μN to 0.4±0.0014 μN with a significant response to β-adrenergic stimulation (epinephrine, 0.1 μM). Tissue recordings revealed that PR increased from 437.7±3.45 ms to 839.8±3.07 ms ($p<0.0001$) before and after epinephrine and action potential duration (APD) decreased from 206.6±6.73 ms to 187.9±2.05 ms ($p<0.02$). It seems that EHT contains many physiological characteristics of a cardiac tissue and acts as a functional model, suitable for use in regenerative medicine and drug tests.

Key words: Engineered heart tissue, drug screening, electrophysiological tests.

P-46 The *In vitro* Effects of Coculturing ofRat Fetal Lung Tissue on Maturation of Rat Immature Oocytes

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In this study, the *in vitro* effects of coculturing of rat fetal lung tissue on maturation of rat immature oocytes have been considered. Immature oocytes were prepared from ovaries of 6-8 weeks old female mice (race NMRI) following the intraperitoneal injection of 5 IU PMSG. Obtainedoocytes were dividedintotwo groups including control andexperimental groups. The control group was cultured in α-MEM medium containing 10% FBS. The number one and two experimental groups along with obtained fetal lung tissues from 10-13 days old rats were cultured. For the maturationof immatureoocytes, theywere incubatedfor 24 h with 5% CO_2 at 37°C. Maturation processwas monitored by inverted microscope and then the data were analyzed using SPSS software and Tukey-Dancan test. Maturation rate of oocytes in the control and the experimental groups numbers one and two were 46%, 56% and 65%, respectively, which in turn clearly indicated asignificant increase ($P<0.05$) in obtained values in experimental groups compared to the control group. Coculturing of immature oocytes with fetal rat lung tissue due to the ownership of fibroblast and retinoic acid as growth factors, leading to the development of germinal vesicle stage oocytes into the metaphase II and possibly improves maturation rate of oocytes in the laboratory conditions.

Key Words: Maturation, lung tissue, co-culture, egg mouse

P-47 Combined Analysis of Two Common Polymorphisms at 9p21 Locusin Coronary Artery Disease Patients in North of Iran

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Coronary artery disease (CAD) including myocardial infarction (MI) as its complication, is one of the most common heart disease worldwide and also in Iran, with extreme mortality. CAD is multifactorial and twin and family studies at different loci have demonstrated that role of genetic factors in the progression of CAD. Many genome wide association studies (GWAS) have reported the significant association of locus 9p21 polymorphism with coronary artery disease. This study investigated two polymorphisms of this locus for determining their role as genetic risk factor in (CAD) in population of North of Iran. rs10757274 and rs1333042 on chromosome 9p21 were genotyped in 103 CAD subject (angiography positive) and 102 control subjects (angiography negative) originating from North of Iran with using 5`-exonuclease TaqMan genotyping assays. rs10757274 showed statistically significant association with coronary artery risk (P=0.009) but the risk allele G was found to be more abundant in control (angiography negative) group. By contrast, rs1333042 did not show association with CAD between groups. Also, the GG haplotype decreased the risk of CAD, and the presence of G allele appeared to be advantageous in CAD patients. Our findings confirmed the association in only one of 2 variants at chromosome 9p21 with CAD in Iranian patients, but due to the abundance of the risk allele G at rs10757274 in (angiography negative) group, this conflicts with the existing data. Also, the highest frequency of GG haplotype with stenosis less than 50%, suggests that genetic polymorphisms in 9p21 locus, may be helpful for determining susceptibility to CAD in Iranian patients.

Keywords: 9p21 locus, atherosclerosis, variants, angiography, risk factor, Iranian

P-48 Comparison of the Observed Heterozygosity and Expected Heterozygosity Repeats Four STR-loci in DNA Microsatellites in Mazandaran Ethnicity

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DNA fingerprinting is currently the most sensitive and accurate known method of determining identity as one of the basic tests in laboratory identification of forensic medicine and police in the world. Short Tandem Repeats or STRs in micro-satellite DNA is suitable markers for forensic genetic purposes. In this study, we assessed the status of locus repeat of 4 STR-loci, namely, D8S1179, D7S820, CSF1PO, TH01was studied in a Mazandarani. A total of 102 unrelated subjects with ethnic group among Iranian population where in three generations had lived before in Mazandaran. The whole blood samples were taken from the subjects. The samples were transferred onto FTA paper and drying them will be completed DNA extraction. Polymerase chain reaction (PCR) amplification multiplex of DNA was then denatured using ABI 3500 genetic analyzer analyzed. Peaks plotted by the ABI, which represent the number of STR repeats in individual samples, were analyzed using the proprietary software. The analysis performed with the highest observed heterozygosity at locus D8S1179 of 0.833 and a minimum at locus CSF1PO value is 0.657. The expected heterozygosity was also the locus D8S11790.825 and the minimum value is related to the CSF 0.69, respectively to evaluate the frequency data that can be useful for forensic objectives.
Key words: DNA finger printing, observed heterozygosity, STR, micro-satellite DNA

P-49 Histopathological Study of Angiogenesis in Rat Model of Alzheimer's Disease

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Alzheimer's disease is a progressive neurodegenerative disorder which causes an irreversible and progressive memory loss, behavioral changes and loss of intellectual abilities of human. and may lead to cerebrovascular damage, injury and extensive neuronal degeneration of synapses and reducing neurotransmitters in the brain of patients. By injecting beta-amyloid in rat hippocampus in scores of spatial memory test, Y-MAZE was used. After stabilization of disease in terms of behavior and histopathology, survey of angiogenesis status, especially in hypocampus was performed. According to our observations, angiogenesis in the group that was treated by beta-amyloid, considerably increased than control group. Since the angiogenesis is essential for the process of organ regeneration and healing and also can reduce tissue damages caused by hypoxia and ischemia and other pathological disorders, so increasing angiogenesis may be helpful to reduce defects caused by the disease.
Key words: Alzheimer, rat, angiogenesis, histopathology

P-50 Microscopic Surgery and Biotherapy Science: Cellular and Molecular Biodebridment Using MDT Method

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Nowadays, the medical society due to many political and economic issues as well as problems such as high cost of modern medicine, resistance to antibiotics, insufficiency of the patient's organ functional problems (insufficiency of renal, liver, immune system, self-medication, many superstitions associated with organ transplantation and so on), needs an important review. The philosophy of medicine reviewed the middle ages (Medieval) principles-based treatment according to local amenities and inspiration from nature as biotherapy or the use of natural living, adivine blessing treatment is very important. Maggot from Lucilia sericataflies or larvae in the treatment of diabetes and incurable wounds such as pressure or bedsore wound, the necrotic cells and molecules from degradation of necrotic cells were convert to a soup appearance. This application for destroying damaged necrotic material caused by secreting various enzymes from alimentary secretion of maggot that break down and eliminate compounds has been obtained as a result of wound healing. The larvae secretory substances are allantoin, urea, phenylacetic acid, phenylacetaldehyde, NH4OH, calcium carbonate, proteolytic enzymes, and seraticin antimicrobial

enzymes, providing larvae for maggot therapy in a closed cycle in accordance with the terms of sterile condition and final sterilizing put on the wounds and prepared for the specific procedure provided closed aerobic chamber in the wound dressing and consumption of aerated complete wound and larvae each day 3 times dressing up to 3 days must change all maggot with a new larvae to almost to full recovery changes. Larvae necrotic cell destruction mechanism and its related liberated molecules as well as moving and stimulating wound healing promotes recovery vessels (hyperemia and new vascular jobs). This method has been commonly used in Iran since 2000, but the methods of doing business in Babylon from 2010 production of larvae, larval therapy for patients with diabetes, pressure ulcers bed sore or so on began.

Keywords: larval therapy, maggot therapy, biotherapy, fly larvae, Lucilia sericata

P-51 Study of the Hypoglycemic and Hypolipidemic Activities Of The Alcoholic Extract Of Swertia longifolia boissin Streptoz-otocin induced Diabetic Adult Male Wistar rats

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The history of obesity and diabetes has been reported since the ancient times and many physicians use herbs to treat diabetes. So, the aim of this study was to investigate the effect of alcohol extract of the aerial parts of Boiss Swertia Longifolia on fat and blood glucose levels in streptozotocin-induced diabetes. In this study, 35 male Westar rats were divided into five groups: one control, one diabetic control, and three experimental diabetic groups (n=7). The control group received normal daily water and food, the diabetic control group received drug solvent and three experimental groups received the following in order: 100mg/kg, 200ml/kg of alcohol extract of Swertia Longifolia Boiss and Glibenclamide (10mg/kg) through tube feeding, respectively. To induce diabetes, Streptozotocin (60 mg/kg) was injected intra peritoneally. After 21 days, blood samples were collected from all groups and then blood factors were measured and analyzed. In general, the results suggest that glucose and lipid profiles, including cholesterol and LDL levels in STZ-treated control group than in the control group, has been increased. While all of these factors, in the recipient groups of plant extracts of sage Mountain, have decreased. In addition, insulin levels, showed significant increase in all experimental groups receiving plant extracts of sage Mountain ($P<0.05$). Consumption of alcoholic extract of aerial parts of Swertia Longifolia Boiss by reducing blood fat, and increasing insulin levels, may have beneficial effects on diabetes and hyperlipidemia.

Key words: Diabetic, Insulin, Cholesterol, Swertia Longifolia

P-52 Degradation Effect of Morphine on Microtubules Eye Lens Synthesis in Drosophila Melanogaster

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Morphine is an alkaloid extracted from opium and is a powerful opioid that serves as an analgesic and adjuvant anesthesia, antitussive, anti diarrheal drugs. Morphine use in women during pregnancy or before that, sometimes leads to congenital anomalies among newborn babies. Alkaloids affect on polymerization and depolimerization of microtubule and inhibit their assembly by binding soluble tubulins. Colchicine and morphine are both considered as a microtubule destabilizer factors. Microtubules play a major role in the differentiation of the eye lens. Colchicine, is the cause of the congenital eye disorders too, by inhibition of microtubule polymerization, therefore, morphine can have the same effect in utero, due to its alkaloid nature. In this study, we examined the effect of morphine on the differentiation of the eye lens in Drosophila melanogaster compared with colchicine. In this study, tw o groups of Drosophila melanogaster flies as a sample and one group as a control group were studied. The first and second groups, respectively, were cultured in a medium containing 100 mg of morphine and 2 mg of colchicine and until achieving two consecutive generations of their offspring. Offsprings were compared with the control group and the positive control group after transformation. According to the results, the morphogenic eyes of the flies cultured in medium containing colchicine and morphine were similar and the significant difference was observed between morphine group and control group. Presence of morphine increased abnorma-lities in melanogaster offspring's eye lens. Morphine can cause inhibition of microtubule polymerization and leading to eye disorders in the Drosophila melanogaster.

Keywords: Morphine, Congenital eye Disorders, Differentiation of the eye lens.

P-53 Determination of Plasma Malondialdehyde and Total Antioxidant Capacity (TAC) Level in Alzheimer's Patients and Healthy Persons

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Various studies have shown oxidative stress is one of the most important indicators in the pathogenesis of Alzheimer's disease (AD). Therefore, we studied the plasma levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) of Alzheimer's patients in comparison with control group. Case-control study was carried out on 30 patients with Alzheimer's disease and control groups, respectively, with an average age of 81 and 77.8. The samples were collected from the Sari Senior Center who have distinct groups using Mini-Mental State Examination (MMSE). The plasma levels of malondialdehyde and total antioxidant capacity was measured by spectrophotometric method. Plasma levels of malondialdehyde significantly increased in AD patients in comparison to control group, while the total antioxidant capacity was significantly reduced in patients. There was not any correlation between MMSE and parameters. There was a negative correlation between MDA and TAC (r=-0.26*, P value =0.04). Based on receiver operative characteristic (ROC) curve, TAC is a good marker for distinguishing patients. The area under ROC curve (AUC) in plasma of patient for MDA and TAC were 0.63 and 0.67, respectively. Our results corroborate the link between damage caused by oxidative stress and Alzheimer's disease and these markers may contribute in the etiology of AD. Thus, reconnaissance of these markers provides the condition for more effective treatment of patients.
Keywords: Alzheimer's disease, Oxidative stress, Malondialdehyde, TAC.

P-54 The First Investigation of *COX-2-1195 A>G* Polymorphism in Iranian Migraine with Aura and Migraine without Aura Patients

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Migraine is a common debilitating headache with current head pain attacks which is associated to temporal changes of the head blood vessels diameter and contribute to physical activity dysfunctions in chronic pain phase. According to criteria of International Headache Society (IHS) migraine has been classified into two main categories, migraine with aura (MA) and migraine without aura (MO). This study was performed with the aim of investigating the association of COX-2-*1195A →G* genetic polymorphism and the risk of migraine susceptibility in the control and case groups. Genomic DNA of blood samples were purified from 100 migraine patients and 100 controls in this study. By using the appropriative COX-2-1195A→G (rs89466) primer in PCR process, the expected region of subject's *COX-2* gene was amplified, then enzyme digestion was performed using RFLP manner and *Pvu II* restriction enzyme. After analyzing the data with SPSS software, it was shown that the frequency of the *COX-2-1195AG* and *COX-2-1195GG* genotypes in migraine cases were significantly higher than in controls and this demonstrates that there is a direct relation between this polymorphism and migraine susceptibility. Regarding to the acquired results in this research as the first study in Iran, there is expectancy to achieve better results of relevancy of *COX-2* gene and migraine by repeating this experiments on more and extensive samples in the different parts of world.
Keywords: Migraine, Polymorphism, Iran, *Cox-2* gene, Cyclooxygenase.

P-55 Effect of Nerve Growth Factor Concentration on Primary Cultured Schwann Cell Morphology and Proliferation

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Nerve growth factor (NGF) is known to improve differentiation, cell survival, and neurite out growth after nerve injuries. In this study, possible involvement of NGF with different concentrations in morphology and proliferation of Schwann cells in the first week were investigated. Here, primary cultured Schwann cells from neonatal rat sciatic nerves were exposed to the NGF at concentrations ranging from 0.001 to 100 ng/ml for 18 hours. Cell morphology was observedby a scanning light microscope. Furthermore, Bromo deoxy uridine (BrdU) assay and ELISA were applied to quantify the proliferating cells. The results revealed an increase of Schwann cells proliferation in response to NGF, where as the morphology of Schwann cells was not affected. This increase was significantly

promoted in 0.25 ng/ml of NGF, but not at lower concentrations. Further analysis of NGF presence in cultured cells showed that the proliferation of Schwann cells peaked at 5 ng/ml NGF up to 30% in comparison to the control group. It is declared that a neurotrophic factor like NGF can increase proliferation, and also accelerate cell development in-vitro. Based on our study, the incorporation of Schwann cells and NGF can be extended in situations that we need more Schwann cell population especially in nerve transplantation.

Key words: Schwann cells; Nerve Growth Factor.

P-56 Homocysteine Intracerebroventricular Injection Induces Apoptosis in the Substantia Nigra Cells and Parkinson Like Behavior in Rat

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Parkinson Disease is a degenerative disorder of the central nervous system. The motor symptoms of Parkinson's disease result from the death of dopamine-generating cells in the substantia nigra, a region of the midbrain; the cause of this cell death is unknown. Homocysteine (Hcy) is a non-protein amino acid. It is homologs of the amino acid Cysteine. Elevated levels of homocysteine in plasma have been associated with a number of disease states. Hyper homocysteinemia may cause some neurovascular disorders as stroke. In our experiment, Hcy (2 µmol/µl) was injected intracerebroventricularly (i. c. v) in rat, five days later, locomotor activity was measured with digital open field apparatus, After decapitation of rats and brain removal, slices of parts of brain were prepared and apoptosis was investigated in substantia Nigracells by immunohistochemical analysis and apoptotic markers (Bax) and caspase measured. Hcy could decrease locomotor activities significantly in rats as well as it could induce apoptosis in substantia nigra cells. These results suggest that Hcy is a neurotoxic metabolite and may induce cell death in some nuclei in the brain and Parkinsonism.

Key Word: Homocysteine, Rat, Parkinsonism, Immunohistochemistry, Apoptosis

P-57 Association of 565C/T polymorphism in ABCA1 Gene with Serum C-reactive Protein Levels and Incidence of Atherosclerosis

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ATP-binding cassette transporter A1 (ABCA1) is a membrane integral protein and a member of the ABC transporter superfamily that by facilitating the active transport of lipids to apoA-I plays an important protective role against atherosclerosis. The interaction of apoA1 with ABCA1, in addition to promoting the uptake of lipids, causes the activation of Janus Kinase 2 (JAK2) / signal transducer and activator of transcription 3 (STAT3) signaling pathway. Migration of STAT3 to the nucleus inhibits the expression of interleukin (IL) -1β, IL6 and tumor necrosis factor (TNF) -α inflammatory cytokines. Genetic disorders in the ABCA1 gene lead to reduced lipid transport out of the cell and increased production of inflammatory cytokines which ultimately results to the production of C-reactive protein (CRP). Therefore, the increased levels of CRP indicate the development of atherosclerotic lesions. The role of mutations in ABCA1 gene in the development and expansion of atherosclerosis has not yet been clearly identified. The purpose of this study was to investigate the association between 565C/T polymorphism in ABCA1 gene with changes in CRP levels in an Iranian population for the first time. A population consisting of 100 patients with hypercholesterole-mia, hypertriglyceridemia and 99 normal subjects from northern part of Iran enrolled to the study. ABCA1 gene was amplified by polymerase chain reaction (PCR) and 565C/T single nucleotide polymorphism was determined and evaluated with RFLP. The CRP levels were measured with high sensitive method by hs-CRP kit. Genotype distribution between patient and control groups have significant differences (P= 0.008). TT genotype compared with CC independently showed increased levels of CRP (P= 0.002; (OR) = 2.682; 95% (CI) = 1.387-5.264). The TT genotype of 565C/T ABCA1 gene polymorphism is indepen-dently associated with increased levels of CRP and incidence of atherosclerosis in Iranian patients.

Key words: ABCA1, 565C/T polymorphism, CRP, PCR-RFLP, atherosclerosis.

P-58 Bioinformatic Analysis of a Derived Protein From β-2 glycoprotein I as a Vaccine Candidate for Antiphospholipid Syndrome

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Antiphospholipid syndrome (APS) is known as an important autoimmune disease defined by a combination of thrombo-embolic complications and the presence of antiphospholipid antibodies (aPL) in the blood. Human β–2 glycoprotein I is an important plasma protein secreted from liver which has been implicated in the binding of antiphospholipid antibodies to negatively charged phospholipids, a process considered as an important risk factor for the development of thrombosis. It seems that subunit vaccine targeting multiple domain of this significant antigen may act as ideal approach for prevention and treatment of APS. The most effective domains of β-2 GPI for induction of immunologic responses in APS include domain I, II and V. Silico design is an essential tool for vaccine evaluation prior to experimental studies. Therefore, immunogenic epitopes of β-2 GPI domains were determined. Then, B cell and T cell selected epitopes were applied for constructing a chimeric protein. The chimeric gene structure, its mRNA, and deduced protein were analyzed by bioinformatic software. Finally, modeling was done to predict the 3D structure and validation of the predicted protein was evaluated by ramachandran plot. The B and T cell epitopes of mentioned antigens were predicted by ABCpred and CTLPred. Then, MHC binding properties of selected epitopes were determined. The chimeric DNA was constructed according to the highest score of the MHC binding epitopes of β-2 GPI by the glycine rich linker. The predicted 3D structure of chimeric protein showed that most of the dominant epitopes were folded individually. Subsequently, validation experiment showed that most residues of chimeric protein locate in favorable regions of ramachandran plot. Finally, this predicted protein was able to induce T CD4+ and CD8+ cells immune responses. In silico analysis, indicate that this chimeric protein can be effectively expressed and utilized as a vaccine against APS.

Key words: Antiphospholipid syndrome, Recombinant vaccine, β-2 glycoprotein I

P-59 *MYH7* and *MYBPC3* Genes Polymorphisms in Iranian Patients with Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is the most common form of Mendelian-inherited heart disease which affects 0.2% of the global population. HCM is characterized by left or right ventricular hypertrophy, which is usually asymmetric and involves the interventricular septum. HCM is also the most-common cause of sudden cardiac death in individuals younger than 35 years of age. The clinical phenotype is highly variable and ranges from lifelong absence of symptoms to rapidly progressive heart failure or early sudden cardiac death, sometimes with little or even no hypertrophy. This cardiomyopathy is familial in the majority of cases and is transmitted as an autosomal-dominant trait. Much progress has been made in the elucidation of the genetic basis of HCM, resulting in the identification of more than 900 individual mutations in over 20 genes. Interestingly, most of these genes encode sarcomeric proteins, such as myosin-7 (also known as cardiac muscle β-myosin heavy chain; MYH7), cardiac myosin-binding protein C (MYBPC3), and cardiac muscle troponin T (TNNT2). In this study, 110 blood specimens from 5 family including patients and their relatives were collected and *MYH7* exon 22 and *MYBPC3* exon 3 were analyzed by DNA extraction and PCR-SSCP. Direct sequencing of PCR products from samples with altered pattern in SSCP was done to identify probable mutations. There was not any mutation in sequenced exons. Our results showed that mutations in these exons of *MYH7* and *MYBPC3* genes may not have any key role in studied patients and this is necessary to study other exons for better assessment. Generally, the analysis should start systematically by testing *MYH7* and *MYBPC3* and then focused on candidate genes such as*TNNT2* and *TNNI3*. In severe phenotypes, several mutations should be searched.

Key Words: Hypertrophic cardiomyopathy, MYH7, MYBPC3, polymorphism, PCR-SSCP

P-60 The Effect of Urine on Lymphocyte's Viability and Function

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Inflammatory cells could infiltrate to any tissue such as urinary tracts, washed away by urine. However, due to the presence of harsh condition in urine, leukocytes cannot be alive in urine for a long time. In this study, we determined the viability rate

of human PBMC (Peripheral Blood Mononuclear Cell) in fresh urine and their responses to PHA. PBMCs were prepared by ficoll-hypaque gradient centrifugation method from one individual (female, age: 24). The 1×10^6 of isolated cells was dispensed in 1 ml urine. 6 Molar urea and RPMI-1640+FBS10% were used as negative and positive control, respectively. After 20, 60 and 120 minutes the viability of these cells was measured by trypan blue dye exclusion assay. 1×10^5 of PBMC were isolated from urine and cultured as triplicate in RPMI-1640`supplemented with FBS 10% and 1. 5% PHA under standard cell culture condition for 48 hr. MTT assay was performed to determine the PBMC response to PHA. These experiments were repeated three times independently. We observed no significant difference between the viability rates of the PBMC incubated in urine with positive control after 20, 60 and 120 minutes. Overall, there was a significant difference in trends of viability rate between three groups ($p<0.05$). Our results show that not only PBMC remained remarkably alive in urine after 120 minutes, but also these cells can respond to PHA by agglutination and proliferation until 60 minutes after incubation in urine, which was confirmed by MTT assay. In addition urine can be considered as a poisonous fluid for PBMC, these cells survive for about 2h and even maintain their function for 60 minutes in this fluid.

Key words: Urine, Lymphocyte, Viability

P-61 Chronic Diseases Associated with Blood Factors in 25-60 Years Old Couples in Babol

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In recent years, care to prevent chronic diseases was emphasized. This study aimed to determine the association between chronic diseases and blood factors. This cross-sectional stndy was conducted in Spring 2014, with a research population of 25-60 year old couples who were selected by random cluster. Blood pressure two times within 15 minutes of sitting at home and their blood samples amount 5cc was prepared and measured in the Health Center laboratory of babol. The data collected through a researcher-made questionnaire, its validity and reliability were confirmed. The collected data were analyzed by SPSS17 software. Of the 470 women with a mean age of 36.5±4.3 years, 38 (8%) patients had heart disease, 23 (4.8%) patients diabetes, 170 (35.8%) patients relative risk diabetes, 28 (5.9%) patients thyroid and 62 (13.1%) patients from nerve disease. Of the 439 men with a mean age of 42.6±6.3 years, 23 (5.2%) patients had heart disease, 22 (5%) patients diabetes, 132 (30%) patients relative risk diabetes, 13 (2.9%) patients thyroid and 33 (7.4%) patients nerve disease. The mean of sistolic and diastolic blood pressure, fasting blood sugar, cholesterol and triglyceride levels of women consecutive follows 110.5±14.9, 71.3±11.1, 92.8±25.2, 185.1±40.7 and 147.9±74.5 and for men were 111.9 ± 14.6, 70.5±10.3, 92.7±21.1, 186.5±42.6 and 168.8 ± 100.6. There was a significant relationship between the risk of cardiovascular disease in men and the sistolic and diastolic blood pressure and cholesterol, between diabetic men and diastolic blood pressure and fasting blood sugar, between relative risk diabetic men and fasting blood sugar ($p<0.05$). There was a significant relationship between the diabetes in women with fasting blood sugar and triglyceride, between relative diabetic women and fasting blood sugar ($p<0.05$). The results showed that measurement of blood factors in some diseases was higher and periodical care should be considered, especially in relative risk diabetic patients.

Keywords: Blood factors, chronic diseases, Sistolic blood pressure, Diastolic blood pressure, Fasting blood sugar

P-62 Evaluation of Human Papillomavirus Genotype 16 Pseudovirion Production in Mamalian Cell Line

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Cervical cancer, the most common cancer affecting women in developing countries, is caused by human papillomaviruses (HPV) infection. At present, the most promising vaccine against HPV-16 infection is based on the L1 protein. The L1 VLP vaccines have fundamental drawbacks such as type restriction and relatively high cost of production, therefore, efforts to development of second-generation HPV vaccines are ongoing. One of the second-generation HPV vaccine candidates is based on the L2 protein. The purpose of this project is the synthesis of HPV16 pseudovirions through the expression of genes encoding L1 and L2 proteins of HPV16 in mammalian cells and evaluation of the pseudovirions performance by packing a reporter gene (EGFP) and infect new cells with this structure. E. coli DH5α was

transformed by P16 and pEGFP-N1 plasmids. Plasmids were extracted and purified using silica dioxide. HEK 293FT cells were co-transfected by plasmids above and expression of HPV16 pseudo virions in these cells was determined by fluorescent microscopy and flowcytometry. Pseudovirions produced by gel chromatography were extracted and purified and 293FT cells were transduced with these pseudovirions in order to evaluate the infecting potency. The result was detected by fluorescence microscopy and image of the pseudovirions surface was prepared by the atomic force microscopy (AFM). Co-transfection of P16 plasmid containing L1 and L2 genes of HPV16 and pEGFP-N1 reporter plasmid into HEK 293FT cells was successful. Expression of HPV16 pseudo-virions was confirmed by fluorescence microscopy and flowcytometry and transduction of 293FT cells was successful. The results showed that simul-taneous transfection of P16 plasmid containing L1 and L2 genes of HPV16 and pEGFP-N1 reporter plasmid in mammalian cells leads to spontaneous assembly of pseudovirions containing the reporter gene.

Keywords: Cervical cancer, virus-like particles, HPV16 pseudovirions

P-63 Effect of Arbutin on the Lipid Peroxidation and Antioxidant Capacity in the Serum of Cyclosporine Treated Rats

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Cyclosporin A (CsA) is a potent immunesuppre-ssant drug with therapeutic and toxic actions. The use of CsA is limited by its toxicity. Several researchers proposed that oxidative stress could play an important role in CsA-induced toxicity. Arbutin has recently been shown to possess antioxidative and free radical scavenging abilities. The present study was designed to investigate *in vivo* effects of arbutin on the lipid peroxidation and antioxidant capacity in the serum of cyclosporine treated rats. In this study, adult male Wistar rats were divided into six groups (n=8/group): (I) control (no CsA and arbutin administration), (II and III) were treated *subcutaneously (*Sc) with arbutin (50, 100 mg/kg/bw) respectively, (IV) adminis-trated CsA (25 mg/kg/bw) intraperitoneally (IP), (V and VI) received the combination of CsA (25 mg/kg/bw) IP and arbutin (50, 100 mg/kg/bw) Sc daily, respectively. At the end of the treatment (after 3 weeks) serum lipid peroxidation was measured by thiobarbituric acid-reacting substances (TBARS) and serum total antioxidant capacity (Ferric Reducing Ability of Plasma [FRAP]) was assayed based on spectrophotometric method. Result showed TBARS had been significantly increased by CsA administration compared with control rats. Arbutin (50mg/kg/bw) completely prevented this effect, but arbutin (100 mg/kg/bw) alone or in combination with CsA significantly increased lipid peroxidation compared with controls. Our data indicate that arbutin (50mg/kg/bw) had protective effect in the CsA-induced toxicity but high concentration of arbutin (100mg/kg/bw) showed meaningful oxidative and lipoperoxidative effects.

Key words: Cyclosporin A, Oxidative stress, Arbutin, Anti-oxidant.

P-64 Determination of the Optimum X-Ray Irradiation Condition of 6MV Linear Accelerator for the Lymphocyte Inactivation in Bone Marrow Transplantation

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Nowadays CO-60 devices are utilized for the inactivation of lymphocytes in the prevention of graft-versus-host disease (GVHD). Due to problems such as radioactive source age, problems in source replacement and protection risks, we are looking to replace linear accelerator (Linac) for this objective. However, since the x-ray spectrum of 6 MV Linac is different compared to CO-60 device, careful determination of the x-ray irradiation condition is required. Firstly, venous blood of the right-handed people with blood group O^+ was diluted with hanks buffer then it was passed on ficole tubes for the isolation of the mononuclear cells. After irradiation with Cobalt and accelerator, the samples were exposed to different radiation doses of Linac in the sterile condition. The proliferative responses of exposed cells were examined with MTT assay in comparison with the control. The average

percentage of cell survival in each delivered dose and also the required dose of radiation for the inhibition of lymphocyte proliferation and the optimal dose of radiation were obtained. Two important points in the exposure of blood and blood products by linear accelerator is to determine the appropriate dose of lymphocyte inactivation without damage to other blood cells and the other is designing an appropriate exposure manner. The linear accelerator for these two purposes and finally the use in bone marrow transplant is a very good replacement.

Keywords: GVHD, Linac, Bone marrow transplantation

P-65 Investigation of Radio Protective Effect of the Combined Famotidine and Cimetidine Regime on Survival of Gamma-Irradiated Mice through Oral Method

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Ionizing radiation causes harmful effects on cells through penetration into biological tissues by transferring their energy in critical macromolecules such as DNA, cell membrane proteins and lipids. Drugs known as radiation protection are investigated to reduce the harmful effects of radiation. In this study, Famotidine and Cimetidine, are investigated through the ideal method of prescribed radio protective drug (i. e. orally administered). This study is done in three steps on NMRI mice. Firstly, in order to determine the LD50 in mice, groups are irradiated with gamma rays. In the next step, to determine the optimal dose of protective agents, appropriate doses of the drugs administered to mice and then placed under the LD50 and mortality is recorded daily for 30 days and viability determined. In this research, drug doses by gavage for 3 days before irradiation every 12 hours as well as 2 to 3 hours before irradiation. In the third step, the optimal dose of drugs was given by gavage, separated and combined, and mice were exposed to total body irradiation under 6-10 Gy gamma rays and LD50/30 radiation was calculated by Probit. Then, DRF was obtained by ratio of LD50/30 of the drug-received group to the LD50/30 of the group which only received radiation. Due to the presence of sulfur in the structure of these drugs, it is expected that both drugs in each three doses tested to determine the optimal dose, caused to increase the survival rate of mice compared to the control groups without drugs and this increase amount is less than the state of using combination regime of drugs. Combination regimen format can lead to create additional effect of radio protection of mentioned drugs and also potential toxicity in the body is decreased to allow a reduction in the dosage of these drugs (at therapeutic doses).

Key words: Famotidine, Cimetidine, Combination regime, DRF, LD50/30

P-66 Determination of Radio Protective Effect of Royal Jelly against Ionizing Radiation-Induced DNA damage in Human Lymphocytes

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Royal jelly, as a dietary supplement, produced by honey bee has several features such as antioxidant and free radical scavenging roles. In this study, we investigated radio protective effect of royal jelly against ionizing radiation-induced DNA damage in human peripheral blood lymphocytes. 11 participants were recruited in this quasi-experimental study. Peripheral blood samples were taken from each participant at two time points. First sampling was done on admission time of each participant at Hazrat-e-Maryam Fertility Center and the second sampling was performed after daily consumption of 1000 mg royal jelly capsules for two weeks. The obtained blood samples from both the first and second samples were divided into two parts so, one part did not receive ionizing radiation and the other part was exposed to 2 Gy. DNA damage was measured by comet assay technique and was scored based on arbitrary unit. The results of our study revealed more DNA damage inirradiated blood samples compared to non-irradiated ones ($p<0.05$). In addition, taking royal jelly capsules for two weeks decreased both background and radiation-induced DNA damage in the second sample compared to the first one ($p<0.05$). Our results showed that royal jelly could be considered as a natural radio protector against ionizing radiation-induced DNA damage in human lymphocytes. This feature of royal jelly could be related to its antioxidant and free radical scavenging roles.

Key words: Royal jelly, DNA damage, Ionizing radiation, Radio protector

P-67 Aptasensor for Monitoring of ATP: Application of Aptamer Based on Novel Technologies

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Inside the cells, ATP is the most abundant high energy phosphate compound. ATP is needed for the energy requiring reactions in the cell for active cation transport across the membrane. ATP is an essential biochemical molecule in biological systems and plays an important role in metabolism. Also, aptamer is a specific small oligo nucleotide with binding properties. In addition, aptamer is used for biochemical molecular detection. In the first step, the sensor solution was prepareds in buffer. Solution of sensor was mixed in the presence of buffers. The samples were centrifuged and precipates were collected. To detect ATP, 200µl of sensor solution was added to ATP solution. After heating and cooling the solution color, change was measured and recorded with spectrophotometer. The introduced ATP detection method based on aptamer technologies was applicable in biological fluids. This method is useful in therapeutic application, molecular recognition, such as ATP release during several diseases.

Keywords: Aptamer, aptasensor, ATP

P-68 Fungal Aerosols Agents as New Occupational Hazards

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Fungi are occupational hazards in two main groups, allergenic or toxic agents forming bio aerosols, and agents causing zoonoses and other infectious diseases. Fungal aerosols are a main health problem in agriculture and agricultural industry, and may also be an occupational risk factor in many other work environments, such as: medical and veterinary facilities, diagnostic laboratories, libraries and many others. In this retrospective study, the authors did an extensive literature review of published studies about fungal aerosols and their role in diseases in Medline journals and virtual media during recent 50 years. Fungal aerosols are particles of organic dust and/or droplets suspended in the air. In the lungs of exposed workers, fungal aerosols evoke an inflammatory process mediated through the CD14 receptor and Toll Like Receptor 4 (TLR-4), leading to impairment of lung function, bronchitis, and asthma. The studies showed that occupational exposure to fungal aerosols is associated with an increased lung cancer risk. Spores, little particles (β-glucans), low molecular secondary metabolites (mycotoxins) of fungi pose an occupational hazard as a source of allergens and mycotoxicosis. Mycotoxins are regarded as potential factors of respiratory occupational risk in agriculture, especially at occasional high exposures. Also, they may exert an adverse effect on liver and other organs. β-glucan is an important agent causing the development of pulmonary diseases, both of an inflammatory and an allergic nature. β-glucan can induce Th1 as well as Th2 driven immune responses. Fungal aerosols can cause rhino conjunctivitis and dermatitis in workers that work in polluted environments. Potential health effects of fungal aerosol exposures are diverse including infectious diseases, acute toxic effects, allergies and cancer. Methods to assess bioaerosol exposures are available (culture and non-culture methods) ; however, these methods are still limited and they are generally not widely available. Therefore, more research is needed to establish better exposure assessment tools and reduce pollution or prevention of exposure.

Keywords: Occupational hazards, Fungal aerosols, Allergy, Mycotoxicosis, Fungal diseases

P-69 Fine Structure of Alpha Gliadin Genes and Designing a siRNA Cassette to Overcome Gluten Antropathy in Celiac Patient`s Diet

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Celiac disease is a genetic disorder in which the patients suffer from long-term lasting gastrointestinal problem in their digestive system. In this disease, the small intestine suffers from the reaction of immune system to food allergy and cause malabsorption of food ingredients. The studies showed that the reason for sensitivity in patients is related to a grain protein. These patients cannot tolerate gluten, which is a combination of two proteins of gliadins and glutenins. The main

allergic portion is Alpha gliadin as a subunit of Gliadin fraction. Alpha gliadin is the most abundant subunit of wheat protein. Today, modern biotechnologies can be used to silencing genes and inhibit the protein production. RNAi technology is one of the most important methods for the control of gene expression. In the present research, silencing of Alpha gliadin gene has been targeted. Therefore, α-gliadin gene coding sequence was assessed by bioinformatics methods, and then specific primers were designed by Primer-Blast software according to the gene fine structure and pre-designed cassette. After PCR amplification, the amplificant was extracted from agarose gel (0.8%) and ligated to pTG19 cloning vector and cloned in E. coli successfully. After sequencing and confirming the nucleotide sequence, the second part of RNAi cassette was designed and constructed. After access to the designed cassette, all targeted genes were evaluated by in silico studies. The results showed that, the Aria alpha gliadin based construct could have great application in the control of alpha gliadin gene expression in the majority of cereal crops species.

Keywords: Alpha gliadin, celiac, RNAi technology, Wheat

P-70 The Eeffect of Green Tea Consumption on Oxidative Stress in Alzheimer Patients

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Alzheimer's disease (AD) is the most prevalent degenerative disorder of the brain, among elderly individuals. Many studies indicate that oxidative stress is an important pathogenic factor in AD, by oxidizing macro molecules such as DNA, lipids and proteins. It also states that green tea as the most popular beverage in the world, is a rich source of antioxidant compounds that can remove oxygen species. In this study, the effects of green tea consumption on oxidative stress in AD, was investigated. In this clinical trial study, we enrolled thirty patients with severe probable AD. The diagnostic criteria was NINCDS/ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) criteria, with the MMSE ≤24 (Mini-Mental State Examination). Patients swallow four green tea pills per day for two months (2g per day in 2 divided doses). Venous blood sample was collected before and after administration in fasting status. All of serum samples were frozen at -80°C until assayed. Lipid peroxidation marker was measured using thio barbituric acid-reactive substances (TBARS) method. Also, total antioxidant capacity was determined using ferric reducing antioxidant power (FRAP) assay. Data collected were compared using paired t- test. The mean age of patients was 81 ±8.2 (±SD) years. The mean of MMSE Score (±SD) was 3.66±4.26. TBARS level was significantly reduced after two months of green tea consumption (P <0.002) and FRAP level significantly increased after two months (P<0.000). Conclusion: Frequent consumption of green tea in the diet can reduce oxidative stress in Alzheimer's patients.

Keywords: Alzheimer's disease, Antioxidant, Green tea, Oxidative stress

P-71 Prevalence of The Metabolic Syndrome among Baluch Ethnic Women

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Metabolic syndrome is a cluster of metabolic disorders that increases the risk of cardiovascular disease and diabetes. The main characteristics of the metabolic syndrome include high triglycerides, low HDL-C, hyperglycemia, high blood pressure and abdominal obesity. Due to the development of the heart disease among Iranian women in the recent years, the present study aimed to determine the prevalence of the metabolic syndrome in Baluch ethnic women of Chabahar. This cross-sectional study was conducted on 120 women with a mean age of 15-45, and the prevalence of metabolic syndrome was assessed according to the ATP- III in the city of Chabahar, Sistan and Baluchistan. Based on this study, the prevalence of metabolic syndrome in Baluch ethnic women of Chabahar was estimated 17.5%. The prevalence of each component of metabolic syndrome was as follows: glucose higher than or equal to 110 in 15 cases (12.5%), hypertension in 3 cases (2.5%), waist circumference more than or equal to 88 cm in 14 cases (11.7%), HDL less than 50 in 40 cases (33.3%), triglycerides more than or equal to 150 in 40 cases (20.8%). The findings of the present study show the high prevalence of the metabolic syndrome in Baluch ethnic women of 15-45 years old. Hence, essential steps should be taken for the prevention of suffering and identification of risk factors.

Key words: Baluch ethnic, Metabolic syndrome

P-72 SIRT1 Gene Expression in Plasma Levels of Granulysin and Biochemical Factors During and after Holey Ramadan Fasting

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Currently, the calorie restriction has been accepted as a method to increase longevity and quality of life. Initial studies have shown that calorie restriction can increase the expression of Sirtuin including selective internal radiation therapy (SIRT). SIRT1 with diverse mechanisms regulates metabolism. Additionally, SIRT1 reduces inflammation and consequently increases longevity. In the present study, we have evaluated the effect of Ramadan fasting as a model of calorie restriction on the mRNA expression of SIRT1, biochemical factors of blood and Granulysin plasma level. Forty-three fasting male volunteers with a mean age (41/15±13/6) were included in this study. Blood samples were taken on the final week of Ramadan (August, 2013), and four months later. mRNA was extracted from peripheral blood mononuclear cells (PBMCs) and used for cDNA synthesis. SIRT1 Gene expression was evaluated by Real-Time PCR method. Additionally, the serum levels of glucose, urea, cholesterol, triglyceride, LDL, HDL, C-reactive protein, and granulysin were measured. SIRT1 gene expression increased 4.63 folds in fasting state when compared to non-fasting state ($P<0.002$). Moreover, CRP levels in the final week of Ramadan compared to the non-fasting state significantly reduced ($P= 0.0111$). Although, granulysin plasma level in fasting state compared to non-fasting state increased, but this increase was not statistically significant ($P= 0.2905$). The results showed that CHL and LDL increased ($P=0.0027$ and $P=0.0001$ respectively), but urea was significantly during fasting ($P= 0.045$). Nevertheless, other factors (FBS, TG and HDL) did not show any significant changes. Previous studies showed that Ramadan fasting reduces inflammation. Our results indicate that Ramadan fasting has important effects on inflammation through up-regulation of SIRT1 mRNA and reduction in CRP and urea levels.

Keyword: Calorie restriction, Ramadan fasting, Sirtuin, Granulysin, gene expression

P-73 Effect of Captopril on Learning and Memory in Diabetic Rats with STZ

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Diabetes is a metabolic disorder because of decrease in insulin secretion or insulin resistance. One of the side effects of this disease is dysfunction of nervous system including memory and learning. The aim of this research was to determine the effect of captopril on memory and learning in streptozotocin-induced diabetic rats. In this study, 48 male Wistar rats with weight (200-250 g) were randomly divided into 6 groups (n=8 each group), the control group, diabetic group, captopril dose 50 mg/kg, captopril dose 100 mg/kg, diabetic rats treated with captopril dose 50 mg/kg and diabetic rats treated with captopril dose 100mg/kg. For the induction of diabetes, streptozotocin dose 50 mg/kg was injected intraperitoneally. After 30 days, memory of passive avoidance learning of each experimental group was controlled with the shuttle box. SPSS software was used for analyzing. The results show that in diabetic rats compared with the control group significantly reduced memory passive avoidance and diabetic rats receiving captopril compared with diabetic rats significantly increased. The results of this study suggest that captopril can be a factor for improving memory and learning, that is impaired by diabetes or it is appropriate for preventing memory disorder.

Keywords: Memory, Learning, diabetes, captopril

P-74 The Study of the Effect of Alcoholic Extract of Salvia Hydrangea on Pituitary-thyroid Gland Axis in Male Rats Hypercholesterolaemia

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Homeostasis Maintenance is Controlled by several factors, especially the pituitary thyroidaxis. This study aimed to evaluate the effect of alcoholic plant extracts of on cholesterol and activity of pituitary-thyroidaxis in male rats suffering from hypercholesterolaemia. In this experimental study, 35 Wistar rats in the weight range of 170±5 g in 5 groups (n=7) were selected as follows: Control group received normal diet, sham group received high fat diet and normal salin (2 ml/day), experimental groups received *salvia hydrangea* extract with doses (100, 200 and 400 mg/kg) and experimental groups were treated with high fat diet for 21 days. After the end of this period, blood sampling and measuring of the level of T3 was done, data were analyzed using SPSS-17 software. In control group, the level of cholesterol increased.

Cholesterol levels in all groups treated with extracts and T4 levels in the experimental group receiving the lowest dose of the extract showed a significant decrease. The results suggest that ethanol extract of salvia hydrangea reduce cholesterol thyroid hormones and increase TSH probably due to the presence of flavonoid compounds and the effects on negative feedback mechanisms of thyroid hormone. Thus, it is possible to treat hyperthyroidism by salvia hydrangea and reduce blood fat, although further studies are needed for definitive conclusions.

Key words: Salvia Hydrangea, Cholesterol, Rat, Thyroid

P-75 A Pilot Molecular Imprint Nanosensor for Detection of Hemoglobin A1C

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Diabetes mellitus is a chronic disease characterized by abnormally high level of serum glucose. A useful technique for assessing the control of diabetes is using the measurement of glycosylated hemoglobin. Hemoglobin is a protein composed of four chains. Hemoglobins are tetramers made up of two α-like subunits and two β subunits. HbA1c accumulate within the erythrocyte, it is used as an indicator of the success of long-term blood glucose control in diabetes. Molecular imprintings act as a recognition site for target biomolcoule. Solution of sodium hydroxide and sodium borohydride were dissolved in a test tube, and then the solution was heated to 50ºC. After that, allyl-bromide was added. For the preparation of HbA1c imprinted polymer, the solution, and HbA1c were added. Electrophoresis for HbA1c was performed. The structural changes in each chain of Hb provide evidence for diabetes. In this regard, molecular imprint nanosensor showed a binding capacity and selectivity for each change in chain of Hb or HbA1c. Molecular imprinting is a technique for preparing polymer and for entrapping a specific molecule in glycosylated hemoglobin biological samples. Molecular imprint nanosensor is useful in the diagnosis of diabetes.

Keywords: Hemoglobin, Imprint, Nanosensor

P-76 The Effects of *Berberis Vulgaris* Root Extract on Lipids Concentrations in Rats

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Atherosclerosis is the major cause of coronary artery disease and high level of blood lipids, especially cholesterol, which is its major cause. The aim of this study was to investigate the effects of alcohol extract of *Berberis Vulgaris* root on the lipids concentrations in rats. In this experimental study, 40 Wistar rats in the weight range of 170±5 g in 5 groups (n=8) were selected as follows: Control group receiving normal diet, sham group receiving high fat diet and normal salin (0.2 ml/day) intraperitoneal for 21 days. The experimental groups receiving alcohol extract of *Berberis Vulgaris* root with minimum, moderate and maximum dose (75, 150 and 300 mg/kg). The experimental groups also treated with high fat diet for 21 days. After the end of this period, blood sampling was done. Data were analyzed by SPSS. Comparison of the results of statistical tests about the effect of alcohol extract on the blood fat shows that the level of Cholesterol, Triglyceride, and LDL in experimental groups was higher than the control group. While their level in alcohol extract receiving groups of *Berberis Vulgaris* root indicated a significant decrease as compared to experimental groups ($P<0.05$). The results show that hypolipidemic effect of alcohol extract of *Berberis Vulgaris* root is due to the presence of alkaloid compounds found in its root, especially Berberin, which makes it possible to inhibit cholesterol synthesis and excretion.

Key words: Hypolipidemic, Berberis Vulgaris, Rat, Cholestrol

P-77 The Effects of Extracts of Portulaca Oleracea and Eryngium Billardieri on Liver and Kidney Function of Rats with Hyperlipidemia

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Since liver has a key role in the metabolism and kidney in the homeostasis, assessment of these two critical organs is very important. Therefore, the aim of this study was to assess the effects of aerial parts extraction of Portulaca Oleracea and Eryngium Billardieri on the liver and kidney function of rats with hyperlipidemia. In this experimental study, 56

Wistar rats were divided into 8 groups (n=7). Control group had normal diet, sham group had high fat diet and the experimental group were hypercholestrolemic rats which received Portulaca oleracea extract with maximum dose (800), the mean dose (400), and the minimum dose (200 mg/kg) and Eryngium Billardieri extract with maximum dose (300), the mean dose (200), and the minimum dose of (100 mg/kg). After 21 days, blood sampling was performed and liver enzymes (Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total protein) renal function (creatinine, BUN) was assessed. Data were analyzed by SPSS. Results showed that cholesterol, alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) had increased in the sham group that received only fatty foods, while the experimental groups which received Portulaca Oleracea extracts, liver enzymes had decreased. Levels of ALT and ALP in experimental group which received the minimal dose of Eryngium Billardieri and also the levels of cholesrtol, TG and LDL in all experimental groups, which received the extract of Eryngium Billardieri, than sham groups, had a meaningful reduction. The level of BUN and creatinine in the groups receiving extract than sham group had no meaningful changes ($p \leq 0.05$). Regarding to antioxidant, hypoglycemic and hypolipidemic effects of the extract and its effect on reducing liver enzymes, the extracts of this plant can be recommended to improve liver function.

Key words: Portulaca Oleracea, Rat, Eryngium Billardieri

P-78 Antioxidant Activities of Aqueous Extracts of Chick Pea Seeds

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The present study investigated the antioxidant activity of seed protein extract from chickpeas (*Cicer arietinum* L. *)*. Chickpea seeds were soaked in extraction buffer for 3 h and ground using a blender. The aqueous extract was obtained by filtering the mixture through gaze filter and centrifuged. Then, the extract was precipitated with ammonium sulfate and centrifuged. Precipitates were solubilized in water and flown through a sephadex G-10. The aqueous solution was used for next analysis. Diphenylpicrylhydrazyl (DPPH) assay was used to measure free radical scavenging of chickpea seed extract. Briefly, 0.1 mM solution of DPPH in methanol was made and its absorbance was measured at 515 nm. 40 µl, the chickpea seed extract was added to 3 ml of methanolic DPPH solution. After 30 min, the absorbance was measured at 515 nm. After 30 min, the absorbance was measured at 515 nm using a spectrophotometer. The concentration of antioxidant that involved 50% DPPH free radical scavenging (IC_{50}) was analyzed. Then, the diagram of radical scavenging activity was drawn against the extract concentration. The IC50 value of extract was calculated 25.9 mg/ml, which was comparative with ascorbic acid 11.6 µg/ ml. From the obtained results, seed chickpea extract may be a worthwhile natural antioxidant source.

Keyword: chickpea extract, plant protein, antioxidant

P-79 Investigation of Association Between miR-152 Polymorphism with Breast Cancer in Isfahan Population

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Breast cancer is the most common cancer among women in the world. This cancer is one of the most common malignancies among women in Iran. Unlike many other diseases which have a single cause, breast cancer is the consequence of the interaction between numerous factors comprising both genetic characteristics and lifestyle factors. Lately, researchers have begun to notice the role of miRNA and their polymorphisms in breast cancer. miRNAs are small, single stranded and non-coding RNAs. The miRNAs deregulation in breast cancer was first reported in 2005. One of the causes of this dysregulation of miRNA expression is single nucleotide polymorphisms in those genes. This study aimed to investigate the possible relationship of the rs12940701 polymorphism in miR-152 gene and breast cancer, using the method based on RFLP-PCR for genotyping 200 cases and 100 controls. By using the SGD data base, DdeI enzyme was recognized suitable for the identification of this polymorphism. Through the NCBI website, the desired gene sequence was gotten and Oligo7 software was used for designing primers. The length and T_m of primers was studied by Oligo7 software. To evaluate the specificity of primers and its lack of binding too their parts of the genome Blast program was used and RFLP-PCR can be optimized for the investigation of this polymorphism.

Key words: Breast cancer, miR-152, RFLP-PCR

P-80 Frequency of the Meth- ylenetetrahydr ofolate reductase 677 CT Mutation in an Iranian Population in Mazand-aran

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Metylenetetrahydrofolate reductase (MTHFR) gene is one of the main regulatory enzymes involved in folate metabolism, genome stability, DNA synthesis and repair, cellular cycles and remethylation reactions. In the present study, we determined the frequency of MTHFR C677T polymorphism of this gene in a healthy population in Mazandaran. We studied 100 unrelated healthy subjects of Mazandaran province. A total of 5 ml of peripheral blood was taken from individuals. Genomic DNA was extracted using DNA TM Kit. The MTHFR C677T mutation was determined by PCR-RFLP method. Frequency of CC, CT and TT genotypes of MTHFR C677T gene polymorphism were respectively 59%, 37% a 4% (0.05>P). The frequency of C allele in Mazandaran population was 0.77 and the frequency of T allele was 0.23. Results of the present study might be important in understanding the distribution of C677T MTHFR polymorphism in Mazandaran population. This is the first report of its own kind in Mazandaran population. Moreover, these results can be helpful in predicting the risk of diabetes, cancer, recurrent abortion, male infertility and cardiovascular diseases.

Key words: Methylenetetrahydrofolate reductase, C677T, Polymorphism, Mazandaran population

P-81 Efficiency of Rivaroxaban (xarelto) in Chronic Non Valvular Atrial Fibrillation (Clinical Supervision)

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The atrial fibrillation (AF) is an important independent risk factor of thromboembolic complications. Therefore, anticoagulant therapy is necessary for prevention of ischemic events in all forms of AF. During EINSTEIN-PE research for prevention of venous tromboembolism, the use of an oral anticoagulant rivaroxaban (Xarelto), which is a high-selective direct inhibitor of X-factor plays the central role in coagulation cascade. So, the key goal of this program is to determine to the established profile and safety of Xarelto in routine clinical practic for patients with non valvular AF during the observed 6 months period within the research named XANTUS-XL. During the observed period (6 months), we involved 56 patients with non-valvular AF. They were evaluated according to the approved protocol of AF. There were 33 men and 23 women. The ages were 54 to 69 years. The mean age was 61 years, average height and body weight were 174.2±3.5 cm and 78.1±2.1 kg. According to CHA2DS2Vasc scale, the average mark coefficient was 2.5, that is the indication for carrying out oral anticoagulant therapy. On HAS-BLED scale, the average mark coefficient was 1, that represents low risk of bleeding. Xarelto's preparation applied in a dose 20 mg per day from the moment of arrival of the patient to hospital. In all patients, laboratory analyses were carried out at the first visit of physician and continued for 3 months after discharge. All routine blood tests and coagulogram (including PT, INR and PTT) monitored. 25% of patients were on acetilsalicylic acid, and 75% of patients were on amiodaron therapy. Patients on Xarelto did not have bleeding during treatment in hospital and in control out of hospital. Good tolerance was shown in all patients. In patients with mild renal failure, partial protrombin time (PTT) increased by 1.3 times. Mean INR levels reached from 2, 0 to 2, 65; 3 patients had symptoms of anemia – Hb's level to 107 g/l, erythrocytes – to 3, 5x1012/l, and in biochemical blood tests, activity of serum transaminases were normal. Xarelto (rivaroxaban) 20 mg per day has established safety profile in routine clinical practice during the observed six-month period.

P-82 Does Myocardial Scan with Technetium Lead to Radioadaptive Response among Patients Who are Undergoing Thallium Scan? (Acytogenetic study)

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Myocardial perfusion scan is a technique which provides detailed images of revascularization and the heart activity by using low, level ionizing radiation. There is no doubt that lower doses of ionizing radiation can lead to cell resistance again the high doses and this effect is considered as radio adaptive response. Radio pharmaceutical 99mTc MIBI and 201TL are used in diagnosing heart disease. Technetium energy, with 6/02 hours half-life, is 140 Kevand Thallium energy, with 73 hours half-life, is about 68-82 Kev. According to the MIRD, the efficient absorption dose of Thallium is $2/3\times10^{-1}$ msv/mBq and this dose in the technetium is around $8/5\times10^{-3}$ msv/mBq. So, because of higher efficient absorption dose in Thallium than Technetium, it is supposed that Thallium induces more DNA damage than Technetium. Blood samples of the 90 participants of nuclear medicine department were taken and classified into four groups. First group called control group includes patients who do not receive any dose. The second group involves the patients who receive Technetium and do not have any radio pharmaceutical. The third group consists of the patients who receive Thallium and do not have any radiopharmaceutical. And the fourth group includes the patients who receive Technetium before receiving Thallium injection. The amount of DNA damage in peripheral blood lymphocytes would be analyzed by using comet assay. It is supposed that blood samples from the fourth group would have less DNA damage level than the second and third groups and the difference would be significant. This information would lead to prove radiation versatility in patients who do the cardiac imaging with Technetium and Thallium subsequently.

Key words: Myocardial perfusion Scan, Technetium, Thallium, Radioadaptive response

P-83 Effects of aged garlic extract on circulating and Heart Tissue irisin in an in aging rat model of chronic kidney disease

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This study aimed to investigate the effects of aged garlic extract on circulating and heart tissue irisin in an aging rat model of chronic kidney disease. For this purpose, 42 male aged rats (40-50 weeks old) with an average initial weight of 250-300 were randomly divided into 6 groups: 1- control, 2- saline, 3- sham, 4- garlic, 5- doxorubicin and 6- doxorubicin- garlic. Doxorubicin was used to induce chronic kidney disease. The supplement groups orally (gavage) received one ml per kg of body weight of garlic extract daily for 8 weeks. The rats were anesthetized 48 hours after the last exercise session and after 10-12 hours of fasting, plasma and heart tissue were immediately separated, and stored in a freezer at -70 °C to measure heart and plasma irisin levels. The data were analyzed using one-way ANOVA and Tukey's post-hoc test. The research results showed that garlic extract administration caused no significant changes in plasma irisin levels and heart tissue in healthy and doxorubicin-induced aged rats ($P>0.05$).

Keywords: Garlic extract, Irisin, Aged rats, Chronic kidney disease

P-84 Growth Inhibition and Induction of Apoptosis in HeLa cancer and Fibroblasts Normal Cells Lines Influence Silver Nanoparticles

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Silver nanoparticles have earned great applications in nanotechnology. In addition to antibacterial properties, they have strong antifungal and antiviral effects and they are popular in medicine currently. Despite extensive applications, there is not enough information about their potenital harmful effects on human health. The purpose of this study was to evaluate the growth inhibition and induction of apoptosis in HeLa cancer and fibroblast normal cell lines exposed to silver nanoparticles. HeLa cells were acquired from Pasteur Institute and cultured in RPMI1640 medium and fibroblast cells in DMEM containing 10% fetal bovine serum (FBS) and penicillin/streptomycin. Then, the efect of increasing concentrations of silver nanoparticles (20, 40, 60, 80 and 100 µg/ml) on the cells at 24 and 48h were evaluated using MTT assay, staining with acridine orange, and ethidiumb bromide. The results showed significant decline changes in the vital activity of HeLa and normal fibroblast cells with all studied concentrations after 24 and 48 h. This indicate that silver nanoparticles have high cytotoxic effects on the tested cell lines. Furthermore, the results of staining wth acridine orange and ethidium bromide confirmed the occurrence apoptotic cell death. Since the nanoparticles caused the decrease of the vital

activity of fibroblast cells, they could not be consider safe at the studied concentrations for treatment puroposes. Finally, it is suggested to perform further studies to identify the mechanism of action of silver nanoparticles in HeLa cancer and normal fibroblast cells, at cellular-molecular level, to obtain more detailed and accurate results.
Key words: Silver nanoparticles, HeLa cells, fibroblast, cytotoxic, MTT assay.

P-85 The Effect of Arbutin Supplementation on Kidney Total Oxidant and Antioxidant Status in Alloxan-induced Diabetic Rat

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Objective**:** Diabetes mellitus is a well known metabolic disorder engaged in the etiology of injuries mediated by oxidative stress. The aim of the present study was the effect of arbutin on total oxidant status (TOS) and total antioxidant (TAS) status the kidney tissue of diabetic rat. Methods: For this purpose, 28 male Wistar rats with an average weight of 195 to 220 g were randomly divided into 4 groups (7 rats per group) of control, diabetic, arbutin, diabetic + arbutin. Diabetes was induced with alloxan (90 mg/kg, intraperitoneally, ip) in rats and arbutin (50 mg/kg, subcutaneously) was administered for 5 days/week. Induced-diabetes significantly increased TOS and decreased TAS ($P \leq 0.05$) in rat kidney tissue ($P \leq 0.05$). Results**:** Diabetes increased TOS and TAS decreased kidney tissue of diabetic group compared to the control group ($P \leq 0.05$). Six weeks of supplementation with arbutin associated with an significant decrease in TOS ($P \leq 0.05$) and elevated TAS ($P \leq 0.05$) levels was compared with diabetes group. Conclusion: A combined arbutin supplementation can play a major role against kidney oxidative stress by modulating total oxidant and antioxidant status in diabetic rats.
Key words: Swimming training, Arbutin, TOS, TAS, Diabetes.

P-86 Differential Pattern of Cytokine Production in Uveitis Disease, Evidence Linked to Involvement of Cytokine Network in the Pathology of Uveitis

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Studies revealed that the immune responses of uveitis patients can be affected by the alteration of immune system factors; however, the main influenced immune genes are yet to be fully understood. Therefore, the main aim of this study was to identify the serum levels of drastic inflammatory cytokines including IL-17A and IFN-γ amongst patients suffering from uveitis in comparison to healthy controls. This was a cross-sectional study design. Peripheral blood specimens were collected from 38 Iranian uveitis patients along with 43 healthy students as control subjects. The serum levels of IL-17A and IFN-γ were assessed using enzyme-linked immunosorbent assay (ELISA) technique. Results were analyzed using SPSS software package version 18. The results showed that the serum levels of IL-17A and IFN-γ significantly increased in uveitis patients in comparison to healthy controls. According to the results of the present study, the increased IL-17 levels in the uveitis patients may be responsible for the increase of inflammation in patients and disease progression and increase INF-γ can have a critical regulatory role in the suppression of IL-17A and immunity response adjustment.
Key words: Uveitis, IL-17A, IFN-γ, ELISA, Cross-sectional.

P-87 Garlic Extract Normalized Lipid Profile and Changed Expression of LXR Alpha in Intestine and Liver

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In this study, we investigated the effect of garlic on lipid profile, glucose as well as liver X receptor α (LXRα), is an important regulator of cholesterol, triglyceride and glucose homeostasis, in intestine and liver of mice. N-Mary male mice were divided into 3 groups randomly (n=8): group1 received chow + 2% cholesterol + 0.5% cholic acid, group 2: chow + 4% (w/w) garlic extract + 2% cholesterol + 0.5% cholic acid, and group 3: chow only. After one month of treatment, mice were anesthetized, blood was collected from their heart, and the first 10 cm of the small intestine and liver were

removed. Glucose was measured by a glucometer; other biochemical factors were measured by enzymatic methods. LXR expression was checked by RT-PCR and western blotting. Compared with hypercholesterolemic mice, garlic extract markedly, low- reduced cholesterol density lipoprotein cholesterol (LDL-C), triglycerides, very low density lipoprotein-cholesterol (VLDL-C), atherogenic index, alanine aminotranferease (ALT) and aspartate aminotransferase (AST) (all of them P<0.05). LXR protein and mRNA in the intestine increased in garlic-extract treated group compared with chow group (P<0.05), while in the liver, only mRNA of LXR increased in hypercholesterolemic control mice (P<0.05). This experiment showed that garlic increased LXRα expression in the intestine. These effects probably have main role in declining serum triglyceride and cholesterol.

Key words: Cholesterol, Garlic, LDL-C, LXR

P-88 Walnut Reduced Lipid Profile and Increased PPARα Expression in Diabetic Rat

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Diabetes Mellitus has appeared as a universal burden. Studies have reported that mortality from Coronary Heart Disease (CHD) in diabetic patients is 2-4 times higher than nondiabetics. In this respect, walnut is a treatment which has beneficial effects on CHD risk factors. PPARα plays an important role in the regulation of lipid metabolism. This study was aimed to evaluate the effects of walnut on lipid profile as well as PPARα protein levels in rats. Animals were divided into 3 groups randomly (n=6) ; Group 1: Received chow only (control), Group 2: Diabetic rats + chow, Group 3: Diabetic rats + chow supplemented with 4% of whole walnuts. After four weeks, rats were sacrificed, blood was collected; lipid profiles as well as SREBP-1c protein levels were determined by western blotting. Compared with diabetic rats, walnut significantly decreased serum cholesterol (P<0.01), LDL-c (P<0.01), triglyceride (P<0.001) and VLDL-c (P<0.001) and also increased HDL-c (P<0.05) compared with diabetic. Moreover, PPARα protein level significantly increased (P<0.05) in walnut group compared with diabetic group (P<0.05). The findings showed that walnut administration in diet clinically decreases atherosclerosis risk factors. Lipid profile reduction might be due to the reduction of PPARα by this medical treatment in liver.

Key words: Cholesterol, Walnut, LDL-C, PPARα

P-90 Flaxseed Normalized Lipid Profiles and Expression of LXRa

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The aim of this study was to examine the effect of flaxseed on lipid profiles in diabetic rats, focusing on intestinal LXR alpha. Animals were randomly divided into 3 groups of 8 rats each. group1: rats + chow diet (control), group 2: diabetic rats + chow diet (diabetic control), and group3: diabetic rats + chow diet + 4% flaxseed (w/w) (flaxseed group). After one month the rats were sacrificed, blood was collected; lipid profiles were determined enzymatically as well as mRNA and protein levels of SR-BI were determined by RT-PCR and western blot respectively. Compared with diabetic control (group 2), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglyceride, and very low density lipoprotein cholesterol (VLDL-C) (all of them P<0.01) significantly decreased in flaxseed group (group 3). Intestinal LXRa mRNA significantly increased (P<0.001) in flaxseed group treatment compared with diabetic animals (group 2). Levels of LXR a significantly increased in flaxseed group (P < 0.05). In conclusion, flaxseed significantly reduced TC, LDL-C, TG, VLDL-C and atherogenic index, as compared with the diabetic rats (group 2). On the other hand, flaxseed led to up-regulation of LXRa in the intestine of rats.

Key words: Flax seed, Lipid profiles, Diabetic rats, LXR

P-91 Flaxseed Reduced NPC1L1 and Increased ABCG5 and ABCG8 Genes in the Intestine of Diabetic Rats

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The aim of this experiment was to examine the effect of flaxseed on gene expression of intestinal transporters: Niemann-Pick C1 like 1 (NPC1L1), ATP-cassette binding proteins G5 (ABCG5) and G8 (ABCG8). Animals were randomly divided into 3 groups 8 rats in each group: group1; normal diet, group2; diabetic rats, and group3; diabetic rats + 4% (w/w) flaxseed. After one-month, the rats were sacrificed, blood was collected; lipid profiles were determined enzymatically, and mRNA levels were determined by RT-PCR. Compared to diabetic rats, flaxseed significantly decreased total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides, very low density lipoprotein cholesterol (VLDL-C) and atherogenic index (all $P<0.05$). Intestinal NPC1L1 mRNA significantly decreased ($P<0.01$) in flaxseed group treatment compared with diabetic animals. Intestinal ABCG5 and ABCG8 mRNAs significantly increased ($P<0.001$) in flaxseed group treatment compared with diabetic animals. In conclusion, flaxseed significantly reduced lipid profile and atherogenic index, as compared with the diabetic group. Flaxseed treatment also led to downregulation of NPC1L1 mRNA and up-regulation of ABCG5 and ABCG8 mRNAs in the intestine of rats.

Key words: ABCG, ABCG8, Cholesterol, Flax, NPC1L1

P-92 Combination of Ezetimibe and Garlic Reduces Serum Lipids and Intestinal NPC1L1 Expression More Effectively

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The aim of this experiment was to study the influence of garlic combined with ezetimibe on lipid profile as well as intestinal NPC1L1 expression in normal and hyper cholesterolemic mice. A total of 40 mice randomly were divided into 5 groups: Group 1: hyper cholesterolemic group (received 2% w/w cholesterol + 0.5% w/w cholic acid in their diet), Group 2: garlic group (hyper cholesterolemic diet + 4% w/w garlic extract), Group 3: ezetimibe group (hyper cholesterolemic diet + 0.005% w/w ezetimibe), Group 4: combination group (hyper cholesterolemic diet + 0.005% w/w ezetimibe + 4% w/w garlic), Group 5: control (chow only). Serum LDL-C and total cholesterol (TC) levels significantly decreased in ezetimibe, garlic (both $p<0.05$), and combination groups ($p<0.001$). Also, triglycerides and VLDL-C were significantly lower in garlic and combination groups ($p<0.05$). Liver enzymes (ALT and AST), also significantly decreased in garlic, ezetimibe (both $p<0.05$) and combination groups ($p<0.001$) in comparison with the hypercholesterolemic animals. Analysis of semiquantitative RT-PCR results showed that the levels of NPC1L1 were also significantly less ($p<0.01$) in the garlic, ezetimibe, and combination groups ($p<0.001$) compared with the controls. Based on the results, the combination of garlic and ezetimibe can lower serum lipids and liver enzymes more effectively in hyper cholesterolemic mice. This experiment disclosed that a possible mechanism for the beneficial effects of garlic and ezetimibe combination in lowering plasma LDL-C and TC is inhibition of intestinal cholesterol absorption. More research might be necessary to show the efficacy and the exact mechanism of this coadministration.

Key words: Cholesterol, Garlic, Ezetimibe, Herbal Medicine, Hyper cholesterolemia, NPC1L1

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