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THE EFFECTS OF HIGH LEVEL NOISE AND α–ADRENOBLOCKER ON THE OXIDATION INTENSITY OF WHITE RAT BLOOD

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Noise action can exacerbate a number of disorders, many of which are associated with the increase of stress hormones release and oxidative stress development. It is well known that the intensification of free radical oxidation (FRO) in cells is a universal mechanism of cell response on stress factors of different origin. It was shown, that proteins as well as lipids undergo modification as the result of FRO processes intensification and products of proteins oxidation serve as a more informative markers of oxidative damage of cells compare with lipids.

The aim of this investigation is assessment of the new α_2-adrenoblocker Mesedin (2-(2-methylamino-4-thiazolyl)-1,4-benzodioxan hydrochloride, synthesized in the Institute of Fine Organic Chemistry, NAS RA as a regulator of the oxidation intensity of white rats plasma and erythrocyte membranes (EM) proteins, lipid peroxidation intensity and α–tocopherol (α–T) content in the studied samples under condition of noise action. Investigations were carried out on white male rats weighing 150-200 g. The animals underwent noise influence (91 dBA) with maximal energy in the region of average and high frequency during 8 hours (acute acoustic stress). To estimate protein oxidation intensity the spectrophotometric method based on the reaction of protein oxidation carbonyl derivatives (modified proteins) and 2,4-dinitrophenylhydrazine (2,4-DNPH) with 2,4-dinitrophenylhydrazones formation was used. Fluorimetric method has been used to determine α–T content in the studied samples.

The data obtained revealed an increase of carbonyl derivatives of protein oxidation both in plasma and EM proteins under the noise action correspondingly by 18% and 102%. Mesedin administration to the intact animals only slightly decreased the content of modified proteins in plasma. The administration of Mesedin to the animals 10 hrs prior the noise action leads to the sharp decrease of protein carbonyl derivatives content (76.5 and 47%) correspondingly in the plasma and EM. The observed changes were correlated with α-T content in the studied samples.

The results obtained revealed the carbonyl protein increased formation both in plasma and EM under the noise action. Administration of α_2-adrenoblocker Mesedin to the animals prior the noise action significantly prevents oxidation of blood proteins components, more expressed in EM and reveals noticeable regulatory effect under the acute acoustic stress conditions.

Phytochemical study on Ziziphora clinopodioides Lam essential oils wild-growing in the Armenian flora and grown up in the conditions of a hydroponics.

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Abstract:

The objects of this study are the wildly-growing grass of the species Ziziphora clinopodioides Lam. collected in the flowering phase in April-July 2013 from the mountains of the villages Voghjaberd, Hankavan, Arzakan and the grass Ziziphora cultured in the hydroponic conditions .

For the first time, the numerical characteristics of the merchandising quality control for raw material of the Ziziphora clinopodioides Lam. were defined from wild-growing plants (Voghjaberd, Hankavan, Arzakan) and grown up in a hydroponics.

The highest value of the essential oil yield differs raw material collected in the condition of hydroponics 1.25 ± 0.01%, for the relative density differs raw material collected in the vicinity of the village Hankavan-0.977 ± 0.001, for the refractive index differs raw materials collected in the vicinity of the village Arzakan 1.490 ± 0.003.

The highest values for the total extractive composition /50 ° spirits / and humidity differs raw material collected in Arzakan 29.0 ± 0.01% , 8.8 ± 0.15%, respectively, for the ash - hydroponics 9.1 ± 0.02%.

By the method of gas chromatography-mass spectrometry, the above studied samples revealed for the first time more than 80 components, among which there were the following main components:(±) pulegone (16,62-25,71%),verbenone (7,78-14,33%), eucalyptol (8,94-12,98%), DL (±) menthol (1,48-10,02%), isomenthone (3,42-8,05%), I-menthone (3.53 - 7,02%), D-menthone (5,13-6,85%), DL-carvone (3,18-6,57%), D (±) limonene (1,3-6,47%), thymol (0,73-5,41%).

QSAR modeling of plasmin inhibitors controlled by the spacer Hydantoin

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Abstract:
A unique feature of the new line of the plasmin inhibitors is that the interaction between the plasmin inhibitors and key sub sites in plasmin can be controlled by a spacer like Hydantoin (2 ,4 imidazolidinedione). The application of the novel chemotype provides further evidence on the importance of hydantoin as the spacer.
In this study 18 Hydantoins derivatives were investigated for the QSAR study in order to understand which properties are closely correlated with the activity of plasmin inhibitory. A multiple linear regression procedure was used to correlate between molecular descriptors pol, log P, MV, SAG , HE, MW and FR. The best QSAR model was further validated by a leave one-out technique as well as by calculation of statistical parameters for established theoretical models. High agreement between experimental and predicted data obtained in the validation procedure, indicates good quality of our QSAR model.

Keywords:

Hydantoin_ MLR_ plasmin inhibitors _QSAR.

IS CANCER risk higher for garage workers?

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Cancer risk is higher in India especially people who are occupationally exposed to hazardous materials. Environmental gene interaction in carcinogenesis is well demonstrated by phase I and II enzymes that are involved in the metabolism of carcinogens. This study looked at the significance of genetic polymorphism in *CYPIAI*, *GSTTI* and *GSTMI* genes in automobile workers were non addicted and had no family background with cancer development, along with elemental imbalances and intensity of DNA damage. *CYPIAI* was determined by PCR RFLP method and *GST* polymorphism by PCR method. In case of garage workers 90% workers are polymorphic for the *CYPIAI* m2 allele. About 60% of workers are *GSTTI* null and only 2% are *GSTMI* null. But in case of control population about 95% people bear the *CYPIAI* normal allele and similarly 96% control had normal *GSTTI* and *GSTMI* allele. Garage workers have to work in an environment where they are continuously exposed to the risk of inhaling different Poly Aromatic Hydrocarbons (PAH) due to occupational exposure. Waste water from garage was found to contain a significant amount of PAH, by high resolution mass spectroscopy and this water was randomly handled by garage workers without proper protective clothing resulting in occupational exposure. Due to this they are in higher risk of cancer development. This cancer development risk is associated with intensity of DNA damage which was determined by single cell gel electrophoresis; conventionally known as comet assay. Study revealed that garage workers had significantly higher DNA damage intensity ($p<0.001$) than control population as workers are exposed to different types of xenobiotics especially mutagenic PAHs . Though there is no association between tumor development time between occupational exposure to PAH and genotype, of *GSTMI*, *GSTT1*, and *CYP1A1* since null genotype individuals may possibly be poor detoxifiers with reduced ability to reactive carcinogenic intermediates. Levels of trace elements like Cu, Zn and Se of both garage workers and controls were obtained by ED-XRF and the results showed that garage workers were suffering from elemental imbalances. They were suffering in Selenium and Zinc deficiency and concentration was significantly less than control group ($p<0.001$). Besides Cu and Zn ratio was higher in garage workers. This higher Cu:Zn was a sign towards risk of carcinoma development. In this present study it was observed that in Eastern India somehow a major portion of garage workers are polymorphic in *CYPIAI* and *GSTTI , GSTMI* and due to occupational hazards they are in higher risk of cancer development which also enhanced by their elemental imbalances.

Synthesis and comparative evaluation of cytotoxicity *in vitro* of new platinum complexes with 3-amino-α-tetralonespiro-5'-hydantoin

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Cisplatin is one of the most successful compounds in the fight against cancer. Recently, interest was directed towards the development of cisplatin analogues which possess N-heterocyclic carrier ligands, coordinated to the cytotoxic platinum(II) moiety, instead of one or both of the am(m)ines. Hydantoins form a large group of derivatives widely applied in medicine and pharmacy, especially as anticonvulsants, antiarrhythmics, antibacterial drugs, cytotoxic agents etc.

A new *cis*-[$Pt(NH_3)LCl_2$], where L is 3-amino-α-tetralonespiro-5'-hydantoin was synthesized and studied. The molecular formula of the complex was confirmed by the elemental analysis, melting point and IR spectra. The results show that the coordination of the ligand with metal ion was realized by nitrogen atom of the amine group. On the basis of the results from the physicochemical investigation, the most probable molecular structure of the platinum complex was proposed.

This compound as well as previously prepared and studied Pt(II) and Pt(IV) complexes with general formulae *cis*-[PtL_2Cl_2] and *cis*-[PtL_2Cl_4], where L is the same ligand 3-amino-α-tetralonespiro-5'-hydantoin were investigated for cytotoxicity *in vitro* on HL-60 and SKW-3 human tumour cell lines. The results showed that all complexes exerted concentration dependent antiproliferative activity.

Design, drug-likeness and cytotoxicity of some bromo-salicylaldehyde aroylhydrazones

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Aroylhydrazones derived by the Schiff base condensation between salicylaldehyde and different hydrazides possess diverse pharmacological activities such as antimicrobial, antibacterial, anti-inflammatory, analgesic, antifungal, anti-tubercular, antiviral, anticancer, antioxidant etc. Various substitutions in the molecules have been made in order to improve their biological activity. Inclusion of a bromine atom in some hydrazones greatly increases the activity of the compounds.

In this work we present the comparative evaluation of *in silico* biological activity of a series of nine various bromo-derivative hydrazones. The compounds were designed by varying the position of bromo-substituent in salicylaldehyde moiety and the type of substituents at 4 position of hydrazide moiety. The drug relevant properties of the studied compounds, important for drug pharmacokinetics in the human body, were evaluated with the Lipinski's rule of five. The value of logP and the remaining parameters of drug similarity were calculated by the method based on group contributions. The approach is used only as a first step in drug discovery, to find the lead candidates with encouraging properties for further elaboration.

Some of the investigated bromo-derivative hydrazones were tested for *in vitro* cytotoxicity on a HL-60 acute myeloid leukaemia and SKW-3 T-cell leukaemia cell lines by MTT-test. The bioassay results demonstrated that the compounds exhibit concentration-dependent cytotoxic effects at low micromolar concentrations. The values of IC_{50} for 5-bromosalicylaldehyde-4-hydroxybenzoylhydrazone and 5-bromosalicylaldehyde isonicotinoyl-hydrazone are comparable to these of Cisplatin, but lower to these of Melphalan. Results confirm that the compounds are potential candidates for future drug discovery study.

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Association between TP53 gene ARG72PRO polymorphism and gastric cancer in Fars province, Iran.

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Keywords: TP53, polymorphism, gastric cancer

Introduction:
Gastric cancer as the 3rd most common malignancy in Iran, accounts for ~50% of all GI cancers who cause 55% of all cancer-related deaths in Iran. The rates of GC reported from Fars province, Iran, are among the highest in the world. Upper gastrointestinal cancer accounts for more than 50% of all cancer deaths in this area. Codon 72 polymorphism of the tumor suppressor gene TP53 has been associated with a higher risk in the development of several types of cancer. The polymorphism results in a variant protein with either an arginine (CGC) or a proline residue (CCC). We aimed analyze the association of the TP53 codon 72 polymorphism with the risk of developing gastric cancer in a high-risk population around the world.
Materials and Methods: We enrolled 87 patients with mean age 65.9 (range: 37-87; std.=11.1) affected with primary gastric cancer (GC) and same age- and sex-matched healthy control participants. The analysis has been done by PCR-RFLP on DNA extractions from peripheral blood.
Results: In case group the genotype was 16.1%, 42.5%, and 41.4% for Arg/Arg, Arg/Pro, and Pro/Pro, respectively. And for controls those were 18.5%, 40.2%, and 41.4%. In comparing case and control group, no significant correlation was found (p=0.9). Also, there was any significant correlation between codon 72 status and pathologic data.
Conclusion: Because of the high frequency of GC in our province, the investigations about the role of genetic susceptibilities for GC are very important. In spite of finding no relationship between P53 polymorphisms, studying other genetic variations is recommended.

Prevention of age associated excitotoxicity of glutamate in brain by multivitamin containing vitamin B6 and vitamin B12 and folic acid

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Glutamate (Glu) is known as an excitatory amino acid neurotransmitter which interacts with N-methyl-D-aspartate (NMDA) receptors for basal excitatory synaptic transmission. Neurophysiological studies indicated that Glu causes many forms of synaptic plasticity such as long-term ostentation and depression, which are thought to influence learning and memory. However, excessive levels of extracellular Glu in the nervous system are excitotoxic and lead to neuronal death and several neurodegenerative processes. Several lines of evidence suggested that increased extracellular Glu, can give rise to many potentially damaging mechanisms which may be pathologically important. While there is little doubt that the high level of Glu is neurotoxic, diminutive evidence points towards the enzymatic control of Glu metabolisms of the brain. Since multivitamin B supplementation has been recommended as adjunctive treatment in Alzheimer's disease, this study was undertaken to investigate the efficacy of vitamins B6, vitamin B12 and folic acid on the activities of GS, GAD, GOT and GPT in aging rat brain. Male Wistar rats (3 and 30 months old) were used. The animals were injected with vitamins B6, vitamin B12 and folic acid (10mg/Kg/day) for 30 days and the day after last injection the animals were killed by decapitation after a mild anesthesia. Forebrains were removed and homogenized in phosphate buffer. The activities of the enzymes were measured in the supernatant. The first part of this in vivo study sought to measure the specific activities of GS, GAD, GPT and GOT in the brain as a function of the age of the rats. The enzyme activities in aged rat brain were considerably lower compared to young animals. Vitamin B6 induced activation of GAD, GOT and GPT in both ages, but, the differences were more pronounced in aged animals. Vitamin B12 and folic acid stimulate the activity of GS in both young and old animals, but had lintel effects on GAD, GOT and GPT of both ages. These results are consisted with the effect of the biologically active form of vitamin B_6, pyridoxal 5- phosphate, which acts as the cofactor for the activity of GAD, GOT and GPT. Folic acid and vitamin B12 have fundamental roles in brain function at all ages, especially the conversion of homocysteine to methionine, which is essential for neuronal nucleotide synthesis. However, the higher rate of activation of the enzymes in the brain of aged animals might be resulted from either; lower availability of the vitamins in aged animals, or; the lower affinity of the enzymes for the vitamin metabolites, due to the posttranslational modifications of the enzyme proteins as consequences of aging. It is concluded that Glu metabolism might be considered as a therapeutic target for prevention of neurodegenerative disorders and age related symptoms.

Key words; Aging, Excitatory amino acids, Glutamate, Multivitamin B, Neurotransmitter

Plasma levels of C-reactive protein a cardiovascular risk factor indicator in Sudanese overweight and obese adults.

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Abstract:

Background: C-reactive protein is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes. This study aims to test whether overweight and obesity are associated with low-grade systemic inflammation as measured by serum C-reactive protein level.

Methods: The study involved 20-60 years old Sudanese adults divided in 3 groups according to their body mass index (BMI). Blood samples were drawn, Serum specimens for the measurement of CRP were analyzed using a high-sensitivity CPR test.

Results : The sample of the present study has included 41 males and 20 females with an age range between 18 and 52 years , the sample was divided into 3 groups according to their body mass index into 21 normal weight (BMI=22.17±1.45) , 20 overweight (BMI= 27.68± 1.15) and 20 obese (BMI= 34.15± 3.54) . The normal weight group had the lowest levels of C-reactive protein(2.15 ±2.52 mg/l) and obese the highest(2.87 ± 2.22 mg/l) . Plasma levels of C-reactive protein showed a positive and significant correlation with body mass index ($p< 0.05$) .

Conclusion: In conclusion, this study has shown, in Sudanese adults a positive and significant relationships between levels of CRP and measures of obesity(BMI) , These findings suggest a state of low-grade systemic inflammation in overweight and obese persons , the result of this study extend recent observations made by other investigators.

Keywords: Obesity; C-reactive protein; Inflammation; Body Mass Index.

THE STUDY OF CHEMICAL STRUCTURE OF THE ARMENIAN FLORA'S APRICOT GUM

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Abstract:

Recently in industrial purposes there is a wide use of gelatin, modified cellulose, dextrins and their synthetic analogues, which are anyway imperfect and have side effects. In this point of view the plantar polysaccharides become a plot of increasing interest of researchers. These substances make up the main moiety of a man nutritional range and because of that are widely applicable in the food and confectionary production. It comprises multiple common groups of organic compounds, which are together with proteins and fats are of vital significance for activity of all the living organisms.

Nowadays, there is a necessity to develop specific methods of standardization of the apricot gum, Gummi Armeniacae (GA). Usage of GA of native origin is as an alternative for Gummi Arabicae, known for its significance as an effective emulsifier, stabilizer and the dietary fiber in medicine and food industry. Because of that we set forth an aim to investigate the GA chemical structure and, so, to develop its new standardization method for GA Specification creation.

By means of the column chromatography the gum polar fraction's (placed onto Al2O3 of IV activity) purification rational method was developed, what could be also used for the gums other polar (arabinic and basorinic) fractions purification and identification purposes.

By means of the HPLC method the GA was standardized. The following monosaccharides: arabinose, galactose, glucose, xylose and ramnose neutral sugars and the glucuronic sugar were identified in the GA hydrolyzate.

In the native GA the low-molecular substances (catechol, hydroquinone and pyrogallol) were detected, what testify about the cambium layer participation in the tree's gum formation process.

Renoprotective effects of angiotensin receptor blocker and stem cells in acute kidney injury: Involvement of inflammatory and apoptotic markers

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Cisplatin, Cis-diamminedichloroplatinum (CDDP), is a platinum-based chemotherapy drug, and its chemotherapeutic use is restricted by nephrotoxicity. Inflammatory and apoptotic mechanisms play a central role in the pathogenesis of CDDP-induced acute kidney injury (AKI). The aim of this study was to compare the therapeutic potential of candesartan, angiotensin II receptor blocker, versus bone marrow-derived mesenchymal stem cells (BM-MSCs) in a rat model of CDDP-induced nephrotoxicity. Adult male Wistar rats (n=40) were divided into four groups; Normal control: received saline injection, CDPP group: received CDDP injection (6 mg/kg single dose), Candesartan group: received candesartan (10 mg/kg/day) for 10 days + CDDP at day 3, and Stem cells group: received BM-MSCs intravenously one day after CDDP injection. The rats were sacrificed seven days after CDDP injection. Significant elevation in serum creatinine and urea, renal levels of tumor necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1, renal expressions of nuclear factor kappa B (NF-kB), p38-mitogen-activated protein kinase (MAPK), caspase-3 and Bcl-2-associated x protein (Bax) were found in CDDP-injected rats when compared to normal rats. Both candesartan and BM-MSCs ameliorated renal function and reduced significantly the inflammatory markers (TNF-α , NF-kB, p38-MAPK and MCP-1) and apoptotic markers (caspase-3 and Bax) in renal tissue after CDDP injection. Candesartan as well as BM-MSCs have anti-inflammatory and anti-apoptotic actions and they can be used as nephroprotective agents against CDDP-induced nephrotoxicity. BM-MSCs is more effective than candesartan in amelioration of AKI induced by CDDP.

Olive leaf extract in cyclophosphamide induced hemorrhagic cystitis in rats.

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The possible uroprotective effect of oleuropein against cyclophosphamide (CYP) induced hemorrhagic cystitis (HC) was investigated in a rat model. HC was induced in rats by an intraperitoneal (i.p) injection of a single dose of 150 mg/kg CYP. Forty male Sprague Dawley rats were treated for 10 days and divided into 4 groups: Normal control group: received saline; Oleu group: received 30 mg/kg/day oleuropein orally; CYP group: treated with saline and injected with CYP at day 7; Oleu +CYP group: rats received 30 mg/kg/day oleuropein orally plus CYP at day 7. At the end of the experiment, nitric oxide (NO), reduced glutathione (GSH), Catalase (CAT), tumor necrosis factor (TNF)-α and vascular endothelial growth factor (VEGF) levels in bladder homogenates in addition to the bladder gene expression of intercellular adhesion molecule-1 (ICAM-1) were measured. Histopathological examination of bladder tissues was also performed. CYP injection produced a significant decrease in bladder GSH, CAT and a significant increase of NO, TNF-α , VEGF and ICAM-1 bladder contents when compared to control rats. Significant elevation in bladder GSH, CAT with a significant reduction of bladder NO, TNF-α and VEGF levels along with downregulation of ICAM-1 expression were determined with administration of oleuropein when compared to CYP treated rats. A significant improvement in bladder mucosal changes was observed in Oleu group when compared to CYP group. Oleuropein has a considerable uroprotective effect against CYP induced HC in rats by increasing antioxidant defense mechanism and decreasing inflammation evident by attenuating NO, TNF-α, VEGF bladder levels and ICAM-1 bladder gene expression.

Cytotoxicity of doxorubicin: inhibition of critical proteins

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Doxorubicin (DOXO), an anthracycline antibiotic which isolated from *streptomyces peucetius*, more than forty years ago, displays antitumor activity against wide range of solid tumors and leukemia. DOXO is an intercalator drug and as we know, the major biological effect of these agents is provocation of DNA damage which could induce permanent alterations in genomic information. The anticancer activity of DOXO is accompanied by severe toxic side effects which attributed to various factors but the general consensus is that DOXO generates free radicals. However the effect of DOXO on ROS-scavenging enzymes activity remains largely unknown. The goal of the present study was to evaluate the effect of DOXO on the activity of three ROS-scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), peroxidase and all components of respiratory chain (cyt b_{560}, b_{595} and d) in *Salmonella typhymurium* as a model. *Salmonella typhymurium* strain 3507 was harvested after 24h culture at 37ºC in rotary shaker, in liquid enriched medium in the presence of increasing DOXO concentrations from 1 to 150µg/ml. increasing DOXO concentrations led to an inhibition of CAT, SOD and peroxidase activity. CAT activity dropped to 70% of the control with 1µg/ml DOXO and to 30% in cell grown in 150µg/ml DOXO. SOD activity decreased from 95% in 1µg/ml to 30% in 150µg/ml and peroxidase activity decreased progressively from 85% in 1µg/ml DOXO to 21% in 150µg/ml of the drug. Besides, all components of respiratory chain were also inhibited by DOXO. It has been reported that anti-ROS such as CAT and SOD decreased apoptosis induced by DOXO. In the other hand we should keep in mind that DOXO therapeutic role is attributed to its intercalating ability, thus while DOXO could have a direct effect on critical proteins of the cell it could interfere in programmed cell death even before the DNA damage.

Doxorubicin makes a complex with peroxidase: spectroscopic studies

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There is an increasing interest in interfacing the studies on drug-protein interaction based on biochemical techniques to find the mechanisms of drug action and protein sensitivity. The goal of these studies is to develop novel pathways that optimally treat clinically significant diseases. Doxorubicin that also known as Adriamycin, is an intercalator drug commonly used in cancer therapy. There are a number of studies that focus on intercalating features of Doxorubicin and its derivatives, but little is known on its interaction with proteins. The purpose of this work was to assess the ability of DOXO to induce alterations in structure and function of horseradish peroxidase C as a model of ROS-scavenging enzymes which has a critical role in cell survival. Peroxidase activity was assayed by following H_2O_2-dependent oxidation of o-dianisidine at 460 nm, the electronic absorption spectra were recorded for 300-700 nm and Intrinsic fluorescence was detected for excitation wavelength of 297 nm and was recorded for 300-700 nm. All measurements were performed in citrate buffer 0.1M pH 4 at 37ºC. Assays for peroxidase showed that the enzymatic activity decreased as DOXO concentrations increased, (1-150µg/ml) going from 96% activity of control in 1µg/ml to 15% in 150µg/ml of DOXO. The Lineweaver-Burk plots showed the noncompetitive and mixed manner of inhibition for HRPC. Electronic absorption spectrum results for 403nm indicated that three molecules of DOXO bind to HRPC in two different binding sites. The first molecule binds independently in the presence of 1-100µg/ml and two other molecules bind in cooperative manner in the presence of 100-150 µg/ml of DOXO. Indeed fluorescence studies showed that the only tryptophan of HRPC quenched by drug-protein interaction and the complex of DOXO-HRPC is static. Thus Doxorubicin, a drug with antitumor ability, can display the key enzymes of the cell and make a direct effect on vital functions.

In-Vitro Production of Limonoids through Callus Cell Suspension cultures of *Melia Azedarach* L

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Abstract:

In present study, callusing response from different explants of chinaberry (*Melia Azedarach* L) and production of limonoids (secondary metabolites) through *in-vivo* and *in-vitro* callus cell suspension cultures has been investigated. For callus induction different explants including immature flowers, nodular stem sections, leaves and fruits were sterilized and then inoculated on MS media, supplemented 3.0% sucrose, and various concentrations of plant growth regulators including 2, 4-Dichlorophenoxyacetic acid, 6-Benzylaminopurine, 1-Naphthaleneacetic acid, Gibberellic acid separately and all in combination, solidified with 1.2% agar 26±2°C and 14/10 L/D photoperiod. The best callusing response (73.3%) was observed in stem sections on MS medium supplemented with 3.0% sucrose, 1.0mg/l 2,4-D, 3.0mg/l NAA and 5.0mg/l GA_3 and callus was transferred to cell suspension culture media having same plant growth regulators (PGRs) used in solid media. Highest amount of limonoids were determined through cell suspension culture at 28^{o}C (141.7µg/ml) from both biomass and broth media (63.19± 2.81) as compared to various other parameters like different pH, temperature and chemicals. From the direct source the total limonoids (80.17±8.98µg/ml) were determined in leave methanol extracts. Total limonoids from water and methanol extract of different ex-plants, callus and cell suspension cultures were quantified through UV-visible spectrophotometer at wavelength 577nm. Production of total limonoids both extra and intra cellular through cell suspension cultures of newly proliferating fresh callus was highest as compared to direct source of different ex-plants and dry callus extracts.

Keywords: *Melia Azedarach* L, Limonoids, Secondary metabolites, Callus, Cell suspension culture.

Characterization of *Listeria monocytogenes* isolated from water buffalo milk and dairy products

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Abstract:

Listeria monocytogenes is an important pathogen that causes listeriosis, leading to septicaemia, encephalitis, meningitis and gastroenteritis, particularly in the elderly and immunosuppressed individuals. Potential pathogens can be transmitted to human through the consumption of contaminated dairy products. Especially raw milk and raw milk cheese has been associated with outbreaks of human listeriosis.

The objective of the study was to assess the presence of *Listeria monocytogenes,* serotyping and investigate the antibiotic resistance in water buffalo milk and dairy products.

A total of 188 samples, including 97 water buffalo milk, 54 water buffalo cheese, 37 water buffalo clotting cream were obtained from Samsun city, Turkey. Samples were analysed using the standard procedure EN ISO 11290-1 and *L.monocytogenes* isolates were confirmed for the presence of genes encoding for hemolysin gene (*hylA*) by polymerase chain reaction (PCR).

Out of 188 samples, *Listeria* spp. was detected in 47(25%) samples in which 7 (3.7%) were positive for *L. monocytogenes.* A total of 13 *L. monocytogenes* isolates obtained from water buffalo milk and water buffalo cheese. However *L. monocytogenes* was not identified in any of the water buffalo clotting cream. Among 13 *L. monocytogenes* isolates, five isolates were identified as serotype 1/2a (or 3a), four isolates were 1/2b (or 3b), one isolate was 1/2c (or 3c), and the other tree were 4b (or 4d, 4e). The isolates showed high resistance to tetracycline and oxytetracycline (69% and 53%). However, there was no resistance to amoxicillin/clavulanic acid and chloramphenicol.

The results presented in this study indicate the potential risk of contamination of raw water buffalo milk and traditional buffalo cheese in terms of showing the presence of potential serotype 4b and multi-drug resistant *L. monocytogenes* serotypes. Thus, it is imperative that preventative measures including the implementation of good hygiene practice and good manufacturing practice should be applied during the preparation of foods in addition to ensuring the cold chain during storage.

Hepatocyte-derived microRNAs as predictors of rejection after liver transplantation

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ABSTRACT

Background and Aim: HCC is a major health problem in Egypt. Liver transplantation is a lifesaving and effective treatment of end-stage liver failure. However transplant recipients can suffer from serious side effects of long-term immunosuppression and they can lose their allografts because of rejection.

Subject and Methods: Blood samples were taken from 30 HCC patients on top of HCV who prepared to liver transplantation process. Also 50 blood samples from controls were taken.

Serum was separated for detection of: miR-122, miR-194 and miR-148a by real time RT-PCR.

Results: The mean values of miR-122 and miR-148a show significant difference between rejected and non-rejected patients [($p=0.001$) & ($p=0.027$) respectively].The mean values of miR-122, miR-194 and miR-184a were significantly overexpressed in HCC patients compared with healthy control subjects [($p<0.001$) & ($p=0.002$) & ($p=0.0019$) respectively].

Conclusion: MiR-122, miR-194 and miR-148a are overexpressed in HCC patients on top of HCV also miR-122 and miR-148a can be used as predictors of rejection after liver transplantation.

Keywords: HCC, miR-122, miR-194, miR-148a

GENETIC DIVERSITY AND CONSERVATION STRATEGIES OF *LILIUM CANDIDUM* L. POPULATIONS IN MARMARA REGION OF TURKEY

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Abstract:

Lilium candidum is an economically important plant. Due to overcollection from nature and destruction of its habitats, it is included in the "vulnerable" category of danger. In the present study, genetic diversity of the five different populations of *L. candidum*, naturally growing in Marmara region of Turkey was investigated. These populations were compared with Kuşadası population that was selected for comparasion group in Aegean region of Turkey and conservation strategies were developed in order to protect the species. RAPD analysis was performed on DNA extracted from the leaves of each replicate of samples.

Twenty-one primers were screened, but only six primers gave clear, reproducible banding patterns and selected were further analysis. Jaccard's genetic distances were calculated and dendogram was generated using the UPGMA algorithm. The dendogram obtained were classified into two main groups and three subgroups. Genetic distance and the polymorphic band ratio was determined as 0,0464–0,3619 and 74,47 % respectively between the populations.

The populations that had the lowest polymorphism ratio were, "Nusret" (19,15%) and "Şabla" (19,15%). The populations that had the highest polymorphism ratio was found as "Keçidere" (25,53%). According to our findings priorly the population of "Keçidere" and then all of the other populations in Marmara region of Turkey should be conserved *in situ*. In addition conservation of this species in botanical gardens, gene banks and agrosystems will support *in situ* management.

Keywords: *Lilium candidum*, genetic diversity, conservation, RAPD-PCR, endangered.

Pesticide-induced Genotoxicity Detected by RAPD and SDS-PAGE on *Glycine max* L.

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With fast economic development and industrialization, genotoxic chemicals were produced and distributed in the environment. These chemicals adversely affect living organisms, often lead to serious diseases to human being. In this study, genotoxic effects of the fungicide Pomarsol Forte WP 80, the insecticide Arrivo 25 EC and the herbicide The End EC were examined for root growth, total soluble protein content, RAPD (randomly amplified polymorphic DNA) profiles and SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) of whole cell proteins were used as endpoints of genotoxicity in soybean (*Glycine max* L.). Median EC (effective concentration) values were calculated according to relative reduction in root length (T/C%) after treatment for 72 h. Total soluble protein content was significantly decreased at 9.6 M Arrivo 25 EC while increased less significantly ($P<0.05$) at 0.4 M The End EC treatment. 20 RAPD primers were used, 18 of them produce band patterns, among them 11 RAPD primers found to produce unique polymorphic band patterns and subsequently were used to produce a total of 308 bands. Percentage of polymorphism was found as 20%. The changes in RAPD profiles of root after treatment was included variations as gain and/or loss of bands compared with the control group. Genomic template stability changed in RAPD profiles at various pesticide concentrations. SDS-PAGE analysis for total protein profile showed that there were differences between the treatment groups. Statistical differences between the groups were compared with one-way analysis of variance (ANOVA) and Tukey's HSD test ($P<0.05$).

Keywords: DNA polymorphism, genotoxicity, soluble protein, SDS-PAGE, *Glycine max*.

CONSTRUCTION AND CHARACTERIZATION OF A NEW CHIMERIC XYLANASE ACTIVE AT HIGH TEMPERATURE AND pH

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Xylan is the second most abundant polisaccaride in nature. Xylanases are hemicellulolytic enzymes,which are responsible for the degradation of the heteroxylans constituting the lignocellulosic plant cell wall. Endoxylanases, a group of xylolytic enzymes, are used commonly in industry especially at paper and pulp industries. In specially, xylanases from thermophiles are expected to play a significant role in industrial processes, since they are thermostabe and resistant to pH changes.

In this study, the xylanase genes of *Bacillus halodurans* C-125 and *Geobacillus* sp. TF16 were amplified with PCR. These genes were cloned into the pGEMT Easy cloning vector and the sequences of the genes were revealed. *Geobacillus* sp. TF16 xylanase gene was cloned into pET28a(+) expression vector and expressed in *E. coli* BL21 (DE3). The purification of TF16 xylanase has been performed by ammonium sulphate precipitation and ion-exchange column chromatography. Molecular weight, optimum pH, optimum temperature and kinetic parameters of the enzyme were determined.

Chimeric xylanase genes were constructed from *B. halodurans* C-125 and *Geobacillus* sp. TF16 genes with a method based on DNA shuffling. These chimeric genes were named as GeoInH2CTer, GeoInH2CTer2, GeoIntraH2Int3 and GeoIntraH2Int4. Chimeric genes were cloned into pET28a(+) expression vector. Although no expression had been seen for GeoInH2CTer and GeoInH2CTer2, expression in *E. coli* BL21 (DE3) has been succeed for GeoIntraH2Int3 and GeoIntraH2Int4. Optimum temperature, optimum pH and kinetic parameters of GeoIntraH2Int3 and GeoIntraH2Int4 were determined and compared with wild type xylanase and other xylanases in literature. The analyses of the pH stability were revealed that both GeoIntraH2Int3 and GeoIntraH2Int4 were retain most of their activity at high pH values even pH: 12.

Keywords: *Bacillus halodurans* C-125, *Geobacillus* sp. TF16, DNA shuffling, Chimeric gene

Vitamin D receptor gene (VDR) polymorphism in type 2 diabetes among Meccan population of Saudi Arabia.

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Abstract

Diabetes mellitus (DM) is a major public health problem worldwide including Saudi Arabia, impacting the morbidity and quality of life. Both environmental and genetic factors play important roles in the mechanisms mediating the development of Type 2 Diabetes Mellitus (T2DM). Vitamin D has important immunemodulatory properties, and depletion, or relative vitamin D resistance, could play a part in the etiology of both the type 1 and 2 diabetes through effects on insulin secretion. Ours and several other recent studies in humans and mouse model indicate the important role of Vitamin D pathway in pancreatic function and diabetes (Elfasakhany et al. unpublished data; Tizaouy et al., 2014, Zhao et al., 2014, Zhu et al, 2014; Liao et al., 2014). We report here single nucleotide polymorphism studies in the Vitamin D receptor (VDR) gene and an association between VDR polymorphisms and the risk of T2DM in the Mecca/Jeddah region of Saudi Arabia. Since Vitamin D and its receptor complex play the role of a transcription factor in regulating the β-cell insulin secretion, it is important to investigate the association between VDR polymorphisms and genetic susceptibility to T2DM in the defined Saudi population. Hundred eight clinically diagnosed T2DM patients from the King Abdullah Medical City, Mecca and hundred ten healthy control volunteers from the Saudi population in Mecca region that has never been previously studied were recruited for genetic association study. Genomic DNA was extracted from peripheral blood and genotyped for the single nucleotide polymorphism (SNPs) of FokI (T/C), BsmI (A/G) and TaqI (C/T) by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis, and new generation sequencing. Results will be presented at the conference regarding the genotype distribution and allelic frequencies between the diabetic subjects and the healthy controls. These genetic polymorphisms results may be useful for a PCR based genetic screening for susceptibility to T2DM in Saudi population.

Keywords: Vitamin D, Type-2 Diabetes Mellitus, VDR receptor, polymorphism, genetic screening.

A multi-functional basic factor isolated from *Cerastes cerastes* venom CC2-PLA2: its activities as pro-inflammatory, platelet aggregation-inhibiting and anti-blood clotting related to its factor X binding

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Viperidae venoms are a potent bio-source of various bioactive components. These venoms contain abundant hydrolytic enzymes that could affect directly or indirectly the haemostasis process through diverse mechanisms leading to pro- or anti-coagulant disorders. Several of these molecules including snake venom phospholipases (sPLA2) are of biomedical interest as anti-thrombolytic tools in coagulation research, diagnosis and/or therapeutics. Phospholipases A2 hydrolyze the sn-2 fatty acids of membrane phospholipids leading to a variety of lipid mediators. They play also multiple roles for maintenance of membrane phospholipids' homeostasis. In this work, three-step of chromatographies and proteomic approach have been used to isolate and characterize a novel phospholipase A_2 called CC2-PLA_2 from the venom of *Cerastes cerastes*. This phospholipase A_2 has been purified to an extent of about fifty folds and its molecular mass was estimated to 13 534 Da. CC2-PLA_2 is a basic protein with pI of 9. Its catalytic activity depends on the presence of Ca^{2+} ions which are directly involved in the binding of molecule to its receptor. CC2-PLA_2 identification and LC-MALDI-MS/MS analysis were undertaken after the protein reduction, alkylation and double hydrolyze with lysine-C endopeptidase and trypsin. Tryptic fragments of LC-MS/MS analyzed CC2-PLA_2 showed sequence similarities with other snake venom PLA_2. CC2PLA2 presents only 51% (61/120 amino acid residues) sequence homology with the first PLA_2 (gi |129 182CBE2 506|) previously purified from the same venom. The isolated CC2-PLA_2 exhibited platelet-inhibition activity and induced an inflammatory response characterized by leukocytosis in the peripheral blood. Many other activities are also exhibited by CC2-PLA_2. This molecule could display multiple functions; some of them are beneficial such as antiplatelet and anticoagulant activities but others are pathophysiological as haemolytic, edematous and pro-inflammatory activities. Our results showed that mechanistically, CC2-PLA_2 act by inhibiting platelet aggregation and/or by blocking plasmatic factor Xa (FXa) which leads to prevent the release of thrombin from prothrombinase complex. It is also able to hydrolyze the negatively charged phospholipids, which are cofactors for prothrombinase system. Inflammation induced by CC2-PLA2 was accompanied by a release of inflammatory mediators such as IL-6, eosinophil peroxydase and proteins of complement. Obtained results indicated also that CC2-PLA_2 induced a release of high level of pro-inflammatory (IL-6) cytokine with no effect on anti-inflammatory cytokine (IL-10). Furthermore, eosinophil peroxydase activity and hemolytic complement effect increased in peripheral blood. Mononuclear and neutrophil cells were found predominant in the induced leucocytosis following CC2-PLA_2 administration into animals. CC2-PLA2 as a multifunctional enzyme may have a potent clinical application as an anti-thrombolytic agent.

Keywords: *Cerastes cerastes*, basic CC2-PLA2, LC-MALDI-MS/MS analyses, Anti-platelet aggregation, Inflammatory mediators.

LAPTM4B gene expression and polymorphism as diagnostic markers of breast cancer In Egyptian patients.

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[(4)] Mohamed El-Marzouky: Lecturer of General Surgery, Faculty of Medicine, Cairo University.

Abstract:

Objectives: Investigate the association between LAPTM4B gene polymorphism and the risk of breast cancer among Egyptian female patients. Also, measurement of its serum level to evaluate its significance as a diagnostic marker for breast cancer.

Design and Methods: A case control study done on 88 breast cancer, 40 fibroadenoma and 80 healthy subjects. Genotyping of the LAPTM4B polymorphism was determined by PCR. Serum LAPTM4B level was measured using ELISA.

Results: There was a significant difference in the (*1/2 + *2/2) genotypes in breast cancer patients (59.1) compared to the control subjects (43.8%) (P = 0.047; OR=1.86; 95% CI = 1.01-3.43). The frequency of the allele 2* of the LAPTM4B gene was significantly higher in breast cancer patients (36.4%) than in the control (25.6%) (p =0.034; OR=1.66; 95%CI=1.04-2.65). Genotypes (*1/2+ *2/2) were significantly associated with differential classification of TNM. Serum level of LAPTM4B was significantly higher in breast cancer than in control and fibroadenoma and in fibroadenoma patients than control. In breast cancer patients, serum LAPTM4B was significantly higher in stage III and in large tumor size. Serum LAPTM4B was significantly higher in cancer patients genotypes (*1/2+ *2/2).

Conclusions: Genetic polymorphism of LAPTM4B is a potential risk factor for the development of breast cancer; Serum LAPTM4B may be used as a diagnostic and prognostic marker for breast cancer.

Sequencing, cloning and expression of an oxidative enzyme from the lichen, *Cladonia uncialis*

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Lichens, symbiotic associations between fungal and algal partners, produce a wide variety of natural products with diverse structures. Many lichen natural products possess bioactivities and have found applications as medicines, poisons, dyes, and aphrodisiacs by various indigenous peoples around the world. Modern methods for extracting useful molecules from biomatter are unworkable for lichen because these organisms grow extremely slowly. The use of heterologous gene expression has been frustrated by the absence of lichen whole-genome sequencing and the lack of mapping of gene clusters with specific metabolic products. We report the *de novo* whole-genome sequencing of the lichen, *Cladonia uncialis*, the first for any species of lichen. Over 60 biosynthetic gene clusters have been identified, putatively associated with production of polyketides, lantipeptides, terpenes, non-ribosomal peptides, and chimeric natural products. We also report the cloning and expression in *E. coli* of a p450-type oxidative enzyme from *C. uncialis*. Based on known metabolites produced by *C. uncialis* and the domain architecture of an adjacent polyketide synthase gene, we speculate that this enzyme is responsible for the dimerization of two molecules of methylphloroacetophenone to usnic acid. We will also report on the results of the oxidation of methylphloracetophenone with this enzyme.

Relationship between polyphenol structure , antibacterial activity and their interaction with antibiotics

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Abstract

Phenolics compounds are defined, chemically, as compounds that have one or more hydroxyl groups attacheddirectly to a benzene. Most of polyphenolsknown for theirbroad-spectrumantimicrobialactivity. The aim of thisstudyis to evaluatethe antibacterial effect of 16 phenolic compounds (phenolic acids and flavonoids) and threederivatives of polyphenols, using the diffusion method on agar against ESBL resistant strains (*Escherichia coli, Klebsiellapneumoniae* and *Proteusvulgaris*). The antibiogram against 14antibiotecs (ATB) showed that only 5ATB present a zone of inhibition, and from polyphenols there is 12 are active against*E. coli*, 5against*K. pneumonia* and 5against*P. vulgaris*. The resultsshwedthatpropyl gallate (ester of gallicacid) is the mostactive anti-bacterial agent (zone of inhibition 18.75 ± 0.25 mm against*E. coli*). The relationshipbetween the structure and activitywasestablished, werethe position of the hydroxyl (-OH) isprobablyresponsible for suchactivity or inhibition of activity. In synergy test, the active molecules were added to the active ATBs, the results show that additive, potential, antagonist effects were found.

Key words: Polyphenols, flavonoids, phenolic acids, antibacterial, synergy.

Responses of Biochemical Parameters to Drought Stress of Medicinal Important Plant: *Amsonia orientalis*

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Recent global climate, drought is one of the major environmental factors that can limit the growth and physiological characteristic of plants (Wu et al. 2012). Drought stress will result in oxidative damage due to over production of reactive oxygen species (ROS). ROS, damage RNA and DNA, inhibit enzyme, and cause membrane damage with basically four forms: singlet oxygen (O_2), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^\cdot$) (Helena and Carvalho 2008). Plants have defense systems such as antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD). (Foyer and Noctor 2000). In this study drought effect on biological system of *Amsonia orientalis* was studied. *A. orientalis* has a great medicinal importance because of its strong antimicrobial, anticancer and antitumor effect (Acemi et al. 2012). Antioxidant enzyme activities of drought stress treated *Amsonia orientalis* were analyzed by spectrofotometrically and electrofotometrically. Prolonged drought stress was gradually decreased H_2O_2 content of plant but, at the increased drought conditions H_2O_2 was increased nearly control level. Besides, MDA content was increased at increased drought level. Drought stress was also caused increased total SOD activity (168%) and POD activity (139%) but, CAT activity was nearly unchanged when the compared control. Activity staining of SOD, POD and CAT of drought stressed *A. orientalis* were analyzed by Native-PAGE. SOD electrophoregams showed three bands drought treated total plant extract. The first band could be namely as Mn-SOD because it wasn't inhibited either KCN or H_2O_2 whereas later two bands were also unaffected by KCN but strongly inhibited by H_2O_2. Hence, they were identified as Fe-SOD. POD electrophotograms of control, and drought stressed plant showed that *A. orientalis* has a single isoform of POD activity. Intensity of POD activity on gels was increased at increased stress treatment. For CAT, electrophoretic picture indicated that there was only one isoform of CAT in total plant extract. Amount of total proteins drought stressed extracts of *A.orientalis* were analyzed by SDS-PAGE. Intensity of bands was significantly decreased at increased UV-B stress. Although intensity was changed new protein bands (29, 40 50 and 55 kDa) were visually at under drought stressed plant. Expression or inhibitions of might be related the stress conditions.

Amber influence on plant cells fluorescence

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In spite of well-known unique biological properties of amber not so many scientific studies related to amber influence on plant cells were conducted.The aim of the research was to determine changes of relative fluorescence in both somatic and immature gametic plant cells in presence of either amber micro-particles or different amber components, like an amber acid (aspirit of amber) and sodium succinate dibasic hexahydrate. Cell relative fluorescence was measured with flow cytometer using 488 nm exciting laser light. Measurements of three parameters were used for analysis: percentage of fluorescent cells within all analysed cells, the mean cell fluorescence in 530 nm range and the mean cell fluorescence in 585nm range. On the first stage of the research callus culture initiated from leaves of *Linum usitatissimum* was used. It was discovered that the relative fluorescence of somatic cells was very close to amber self-fluorescence range; therefore for the second stage of research gametic cells were chosen. Microspore (one nucleus stage) cell cultures of following plant species were investigated: *Cyclamen persicum, Hordeum vulgare* and *Argyranthemum frutescens*. Experimental variants included the following: 1) cell culture control group without any additions of amber suspension, 2) amber suspensions control groups without cell culture (one group with amber microparticles, one group with amber acid and one group with sodium succinate dibasic hexahydrate), 3) cell cultures incubated in suspensions with amber micro-particles, with amber acid and with sodium succinate dibasic hexahydrate. Cell fluorescence was dependent on cultivation duration. Significant increasing in the relative cell fluorescence was observed in gametic cell cultures of all mentioned plant species after incubation in suspension of amber particles as well as in the amber components.

Keywords: plant cell fluorescence; flow cytometry; amber micro-particles

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The Impact Of Morus Alba Leaves Extract On Diabetes-Induced Complications In Rats

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ABSTRACT

Diabetes mellitus is one of the most common endocrine diseases. Researchers all over the world are exploring herbal supplements to control diabetes and its complications. This study evaluated the antidiabetic action of *Morus alba* leaves extract through its effect on hyperglycaemia, DNA damage and apoptosis of brain cells due to oxidative stress in diabetes. Moreover, evaluate the effect of diabetes on neurotransmitters levels of streptozotocin-induced diabetic rats.

Application of crude water extract of *Morus alba* resulted in amelioration of the alterations of serum glucose as well as neurotransmitters including acetylcholine (ACE), nor-adrenaline (NAD), serotonin (S-HT), histamine (HS), dopamine (DA) and gamma amino butyric acid (GABA). Furthermore, *Morus alba* leaves leaf extract display hypoglycemic effect, diminish DNA damage and apoptosis of brain cells of diabetic rats.

In conclusion ,our results suggest that The protective effect of *Morus alba* leaves extract could be attributed to the hypoglycaemic, and antioxidative potential of flavonoids, the major components of the plant extract.

Keywords: Morus alba,ACE,NAD,DA,GABA

Characterization of haem-binding secreted protein GroEL (Chaperonin) in *Helicobacter pylori*

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Abstract

Helicobacter pylori is a gram-negative spiral bacterial, it has been associated with peptic ulcers, gastritis, duodenitis and it is believed to be the causative agent of gastric cancer. The sources such as human lactoferrin, haem and haemoglobin (Hb) can support the *H. pylori* growth. However, the process of indirect iron acquisition by *H. pylori* is still not fully understood. We have evidences of a cytoplasmic protein that is secreted and has a high affinity for iron, because this protein was purified by haem-affinity chromatography and its capacity of binding iron was demonstrated. This protein was identified by mass spectrometry as *H. pylori* GroEL chaperonin (HpGroEL). Additionally, we found 60% similarity when HpGroEL was compared with *E. coli* GroEL chaperonin (EcGroEL). We think that the capacity of iron-binding is a specific characteristic of HpGroEL, because EcGroEL did not bind iron. The 3-D models of both proteins, submitted to PyMol program, showed that they are structurally conserved and that histidine and tyrosine amino acid residues are distributed through their structures. However, the data cannot explain how the haem is binding HpGroEL protein. To answer this question and determine which amino acids of the protein HpGroEL are interacting with the haem, we performed a sequence analysis and molecular modeling simulation with the program I-tasser to indicate the subcellular localization *HpGroEL*. Also molecular docking of *HpGroEL* with haem was performed using the software Moe to indicate the amino acid residues of *HpGroEL* that are involved in the binding of haem. We propose that *H. pylori* secretes proteins in order to withstand the extreme environment presents in the stomach. Our overall results represent the effort to explain the importance of indirect iron acquisition by *H. pylori*. Perhaps iron helps the bacterium to resist the acidic environment presents in the human stomach, and this mechanism is vital for *H. pylori* during the infection process.

Characterization of haem and haemoglobin binding membrane protein (Spbhp-37*)* in *Streptococcus pneumoniae*

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Abstract

Streptococcus pneumoniae is the main causative agent of bacterial pneumoniae, sinusitis and otitis media. It also can cause invasive diseases such as meningitis, bacteremia and septicemia. This pathogen causes considerable morbidity and mortality throughout the world, especially among young infants, elderly and immunocompromised individuals. Haemoglobin (Hb) and haem can support the *S. pneumoniae* growth and viability as sole iron sources. Unfortunately, the mechanism of Hb and haem-uptaking in this bacterium has been poorly studied. We have purified seven membrane proteins by haem-affinity chromatography and two of them: Spbhp-22 and Spbhp-37 (*S. pneumoniae* binding haem-protein) were identified as potential *S. pneumoniae* haem- and Hb-binding proteins. Additionally, Spbhp-37 was identified as lipoprotein by Mass spectrometry. Interestingly, several proteins required for virulence in gram-positive bacteria are lipoproteins, for instance FhuD, which acts as iron-siderophore transporter. Spbhp-37 was immunolocalized by transmission electron microscopy using specific antibodies raised against the recombinant protein. The expression of *spbhp-37* gene was increased when bacteria were grown in media culture supplied with haem or Hb as only iron source. Besides, we performed the molecular docking of Spbhp-37 with both, Hb and haem using the software Moe in order to identify the amino acid residues involved in the binding of these iron sources. In summary, in this work we are presenting the first findings that attempt to explain the mechanism involved in iron acquisition of this pathogen.

Establishment and Characterization of a Dentigerous Cyst Cell Line

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Abstract

The ectomesenchymal tissues involved in tooth development and their remnants are the origin of different odontogenic lesions including tumors and cysts of the jaws, with a wide range of clinical behaviors. Dentigerous cyst (DC) represents approximately 20% of all cases of odontogenic cysts and it has been demonstrated that it can develop benign and malignant odontogenic tumors. DC is characterized by bone destruction of the area surrounding the crown of a tooth which has not erupted and it contain is liquid. The treatment of odontogenic tumors and cysts usually are partial or total removal of the jaw, causing important secondary co-morbidities. However, molecules implicated in DC pathogenesis as well in its development to odontogenic tumors remains unknown. A cellular model may be useful to study these molecules, but that model has not been established yet. Here, we reported the establishment of a cell culture derived from a dentigerous cyst. This cell line was named DeCy-1. In spite of its ectomesenchymal morphology, DeCy-1 cells express epithelial markers such as cytokeratins 5, 6, and 8. Furthermore, these cells express the ODAM protein, which is present in odontogenesis and in dental follicle, indicating that DeCy-1 cells derived from odontogenic epithelium. Analysis by electron microscopy of this cell line showed that it has a high vesicular activity, suggesting that DeCy-1 could secrete molecules that may be involved in DC pathogenesis. Thus, secreted proteins were analyzed by PAGE-SDS where we observed approximately 11 bands. In addition, the capacity of these secretions to degrade proteins was analyzed by gelatin substrate zymography. A degradation band of about 62 kDa was found in these assays. Western blot assays suggested that the matrix metalloproteinase 2 (MMP-2) is responsible of this protease activity. Thus, our results indicate that the establishment of a cell line derived from DC is a useful in vitro model to study the biology of this odontogenic lesion and its participation in the development of odontogenic tumors.

Bone gamma-carboxy glutamic acid-containing protein and Retinol Binding Protein-4, are they different in medullary thyroid carcinoma patients?

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Abstract:

Background: Medullary thyroid carcinoma (MTC) is the third most common of all thyroid cancers (5-8%). Medullary thyroid carcinoma (MTC) derives from Para follicular C cells and may developed in either sporadic (75%) or autosomal dominant hereditary form (25%). Osteocalcin (OC), also known as a bone gamma-carboxy glutamic acid-containing protein (BGLAP), is a bone protein which is synthesized by osteoblasts. Retinol Binding Protein-4 (RBP-4) is an adipokine in the circulation. Adipokines could regulate inflammation, immunity and carcinogenesis. The aims of this study were analysis the correlation between MTC and plasma levels of OC and RBP-4.

Material & methods: Forty six MTC patients and 44 individuals as control group were studied. The mean age of cases was 34±11.3 years old (Mean ±SD) and in control group was 38±9.3. After informed consent, 10 ml of blood from the antecubital vein obtained and plasma was isolated. The plasma OC and RBP-4 concentration were measured by sandwich ELISA method. Obtained results were analyzed by SPSS version 16 with independent t-test method.

Results: The plasma OC concentration were 33.1±3.5 and 12.5±1.2 ng/ml (Mean ±SD) and Odds Ratio (OR) value was 1.0 among patients and control group respectively 1.04. In patients, mean plasma level of RBP-4 was 82.5±2.7 and in control group was 22.8±1.6 μg/ml and OR value was 2.1. The confidence interval was 95%. These differences of plasma levels were statistically significant (P= 0.001).

Conclusion: This study has shown differences between plasma level of OC and RBP-4 in two mentioned groups. These increased levels were also seen in males and females affected with medullary thyroid carcinoma. As these increased levels were observed in both gender and different ages, so they could be related to medullary thyroid carcinoma and they are independent of sex and age, it may be consider that plasma concentration of OC and RBP-4 had potency for helping in diagnosis or confirmation of medullary thyroid carcinoma across the other markers.

Key words: Medullary thyroid carcinoma, OC, RBP-4.

Alert for an HPV epidemic of oral cancer due to widespread chewable tobacco usage

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Abstract

Background:
Oropharyngeal cancer is the second leading malignancy after breast cancer in Pakistan. This was attributed to extensive use of several precarious chewable tobacco formulations. A study conducted here in 2008 on oral cancer showed HPV the causative agent. Since human papillomavirus (HPV), as proven, plays a pivotal role in oropharyngeal cancer the link between the HPV and chewable tobacco is not known. Therefore, this study was designed to investigate the relationship of chewable tobacco usage with HPV and its oncogenic strains in the lower socioeconomic class where the popularity of chewable tobacco is like an epidemic.

Methods:
Oral samples were collected from camps during awareness campaigns on oral hygiene/hazards of chewable tobacco. Subjects addicted to chewable tobacco formulations such as naswar, gutka, pan, areca nut with or without oral lesions, having no febrile conditions were included. DNA was extracted and PCR were performed. SPSS version 20.0 was used for analysis. Frequencies and percentages were calculated for qualitative and mean & standard deviation for numerical variables. Pearson chi-square with 95% confidence level was used, p-value less than 0.05 was taken as significant.

Results:
A total of 1000 subjects and 450 controls (age 28.90±12.163) were included in this study. Out of 1000 (831 males and 169 females), 204(20%) were HPV positive whereas, 13, 6 and 6 subjects were positive for HPV16, HPV18 and both HPV16&18 respectively, compared to only 14 (6.42%) in the controls. Overall 631 were chewing a single and 369 multiple forms. Significant association was found between different forms of chewable tobacco with Leukoplakia, Erythroplakia, Rough mucosa and Trismus.

Conclusions:

Usage of chewable tobacco formulations is associated with high frequency of HPV infection OR= 7.981 (CI 4.587-13.89), which is a threat for an epidemic of oropharyngeal cancer. In this era of globalization, these habits are not restricted to geographical vicinity and appear around various areas throughout the world. All these products contain Nicotine and people with or without addictive personalities fall victim to its dependence. Nicotine preys on genetically, mentally and socially predisposed individuals. Once addiction is established the other carcinogens in Gutka, Naswar, Paan and Arica nut erode the oral mucosa making the environment conducive for HPV to anchor in the basal epithelium, where the virus replicates in synchrony with the S-phase of the host. HPV-associated oral cancer is affecting a population younger than that typically is affected by HPV-independent oral cancer. Recommended tools are: Large-scale educational interventions; Marketing candies with similar flavor or taste; Awareness campaigns through media and educational institutes regarding information on oral hygiene, awareness of risk factors and symptoms; Population screening; Participation of family physicians and medical students for early detection of oral cancer; Enforced bans on manufacturing, advertising, promotion and sponsorship of tobacco products.

Keywords: Chewable Tobacco; HPV; Oral Disorders ; Ethnicity.

Phikud Navakot Increased the Expression of Antioxidant Enzymes and TNF Alpha in Isoproterenol-Induced AMI Rats

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Phikud Navakot (NVK), the main constituent of Yahom Navakot widely using for treatment of cardiovascular disorders such as dizziness and fainting, is a set of nine herbs namely *Angelica dahurica*, *Atractylodes lancea*, *Ligusticum chuanxiong*, *Angelica sinensis*, *Artemisia vulgaris*, *Saussrea costus*, *Picrorhiza kurrooa*, *Terminalia chebula*, and *Nardostachys jatamansi*. Some herbs in NVK have been demonstrated to possess an antioxidant activity as well as anti-inflammation, however the effect of NVK on acute myocardial infarction (AMI) has not been clarified. The aim of the present study is to investigate the effects of NVK on isoproterenol (ISO)-induced AMI in male Sprague Dawley rats. NVK (254.5 mg/kg BW/day) was given intragastrically through a fine feeding tube for 28 days. AMI rats were induced by subcutaneous injection of ISO (5 mg/kg BW) on the 26th and 27th day. The increment of serum cardiac marker troponin I, ST-segment elevation on the electrocardiogram, and pathological changes of the heart were observed starting 24 h after the last ISO injection in confirming myocardial injury in rats. The expression of antioxidative enzymes: superoxide dismutase (SOD)1 and SOD2 as well as heme oxygenase (HO-1) was determined by Western blot analysis. The release of proinflammatory cytokine tumor necrosis factor alpha (TNF-α) in serum was measured by ELISA. The results showed that NVK at the dose of 254.5 mg/kg BW caused an increase in the expression of SOD1, SOD2 and HO-1 in cardiac tissues of AMI rats when compared to the control AMI rats. NVK at the same dose showed a significant decrease in serum TNF-α . The results suggest antioxidant and anti-inflammatory activities of NVK in ISO-induced AMI rats.

Association of cholinergic muscarinic 2 receptor gene polymorphisms with learning skills among medical and fine arts students

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The cholinergic muscarinic 2 receptor (*CHRM2*) belongs to the superfamily of G-protein-coupled receptors. It is thought to be involved in neuronal excitability, synaptic plasticity, and feedback regulation of acetylcholine (ACh) release. It has been implicated in higher cognitive processing including learning and memory. The gene is located on chromosome 7q and contains a number of SNPs. Polymorphism at the *CHRM2* gene had shown a significant association with intelligence. The present study investigates single nucleotide polymorphisms in the *CHRM2* gene among students who have shown intelligence in sciences and arts. Medical students were chosen to represent students having skills in sciences whereas fine arts students represented skill in arts. A total of two hundred blood samples were withdrawn from medical students (100 samples) and fine arts students (100 samples) and extracted for DNAs by using Flexigene DNA kit. Primers for detecting SNPs in the *CHRM2* gene were designed using the program from http://www.ncbi.nlm.nih.gov/projects/SNP/. Genotyping was performed by using real-time PCR followed by high-resolution melting (HRM) analysis and the genotype was confirmed by sequencing. The difference in genotype distribution was analyzed by using Pearson's Chi-square test implemented in SPSS program version 11.5. Significant level was set at $p<0.05$. The results revealed that two SNPs in a intronic region of *CHRM2* gene, rs2061174 and rs6948054, showed significant differences ($p<0.05$) in genotype distribution among medical and fine arts students. The rs2061174 showed significant at *p*-value =0.001, OR and 95%CI were 3.78 (2.00-7.14), whereas the rs6948054 was significant at *p*-value =0.012, OR and 95%CI were 2.50 (1.32-4.77). The two SNPs in *CHRM2* gene, rs2061174 and rs6948054, may be used as a biomarker to differentiate learning skills among sciences and arts in ethnic Thai.

Comparison between a Droplet Digital PCR and Real Time PCR Method in Quantification of HBV DNA

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Abstract

HBV infection causes a potential serious public health problem. The ability to detect the HBV DNA concentration is of the importance and improved continuously. By using quantitative polymerase chain reaction (qPCR), several factors in standardized; source of material, calibration standard curve and PCR efficiency are inconsistent. Digital PCR (dPCR) is an alternative PCR-based technique for absolute quantification using Poisson's statistics without requiring a standard curve. Therefore, the aim of this study is to compare the data set of HBV DNA generated between dPCR and qPCR methods. All samples were quantified by Abbott's real time PCR and 54 samples with 2 -6 log10 HBV DNA were selected for comparison with dPCR. Of these 54 samples, there were two outlier samples defined as negative by dPCR. Of these two, samples were defined as negative by dPCR, whereas 52 samples were positive by both the tests. The difference between the two assays was less than 0.25 log IU/mL in 24/52 samples (46%) of paired samples; less than 0.5 log IU/mL in 46/52 samples (88%) and less than 1 log in 50/52 samples (96%). The correlation coefficient was r=0.788 and *P*-value <0.0001. Comparison to qPCR, data generated by dPCR tend to be the overestimation in the sample with low HBV DNA concentration and underestimated in the sample with high viral load. The variation in DNA by dPCR measurement might be due to the pre-amplification bias, template. Moreover, a minor drawback of dPCR is the large quantity of DNA had to be used when compare to the qPCR. Since the technology is relatively new, the limitations of this assay will be improved.

Keywords; Hepatitis B virus, real time PCR, digital PCR, DNA quantification.

The associations between serum resistin level and concentration of 25 – hydroxyvitamin D in Saudi diabetic patient

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Objectives: The aim of this study was to investigate associations between serum levels of an adipokine namely; resistin, and concentration of 25 – hydroxyvitamin D in Saudi diabetic patient.

Material and Methodology: 108 diabetic patients were recruited for cross-sectional study at King Fahad Medical City, Riyadh, Kingdom of Saudi Arabia. Fifty of the participants were males and 58 were females. Forty nine subject was selected as control. Blood samples were analyzed for biochemical parameters. Serum resistin level and 25 – hydroxyvitamin D were measured for the participants including those who are diabetics and at high risk to develop atherosclerotic cardiovascular diseases.

Result: Serum resistin levels in control group was 14.41 ± 11.5µg/ml compared to 20.21 ± 16.94 µg/ml in diabetic group $P \leq 0.001$. For type -2 diabetes mellitus it was19.53± 17.35 µg/ml whereas in type – 1 diabetes mellitus it was 25.22 ± 14.82 µg/ml P = 0.393. Vitamin 25 (OH) D level in diabetic subjects was 41.95 ± 44.8 whereas type -2 was 47.26 ± 41.98 compared to 46.88 ±33.76 for type -1. The level of resistin in those who are taking vitamin D supplementation was 19.25 ± 16.37µg/ml; and the resistin level in the group abandoned from taking vitamin D supplementation was 17.79 ± 14.4 µg/ml; P value =0.545. No association was found between resistin level and Vitamin 25 (OH) D supplementation in Saudi diabetic patients.

Conclusion: Several in vitro and in vivo studies have confirmed that adipoikneresistin have numerous important functions in the body. In this study showed no significant correlation was found between resistin level in diabetic patients and Vitamin 25 (OH) D in type 1 and type -2 diabetes mellitus. The level of serum resistin showed slight increase in diabetic patients compared to control group and it was higher in type -1 diabetes mellitus. Tremendous efforts are needed to explore the physiological mechanism of resistin action in metabolic disorders.

Keywords: Resistin, 25 –hydroxyvitamin D, Diabetes mellitus, Kingdom of Saudi Arabia

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Isolation,identification and characterization of a new chitinolytic strain: source of bimolecular

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Abstract

Marine and sedimentary microorganisms have interesting molecular adaptability and thus constitute an important source of unconventional bioactive molecules from biochemical and molecular mechanisms. Hydrolytic enzymes of these organizations offer major benefits and provide new opportunities for the improvement or creation of new biotechnological processes. Consider the case of chitinolytic or chitinase enzymes that hydrolyze, depolymerize, chitin. They have been the subject of biological study and are grouped into families based on their similarity of amino acid sequences. Chitinases have generated interest in various biotechnology applications due to their ability to degrade chitin in the cell wall of fungi and insects, which has led to the use as antifungal agents and insecticides. The research conducted in this study are part of this axis and aim to screen the chitinolytic strains and select a candidate bacteria for the production of chitinases low cost using a marine biomass (colloidal chitin) as the only carbon source and nitrogen. Among the seven strains grown on rich medium colloidal chitin, a labeled "SM 50" showed a degree of hydrolysis of chitin 94%. The morphological study of the strain has described as a bacillus, mobile, Gram negative. The biochemical and phylogenetic identification revealed a percentage of 98% of similarity with *Shewanella gaetbuli*. To enhance bacterial growth and the production of chitinases, the physico-chemical parameters were optimized. The results have mounted a very significant production of chitinases to pH 8, at a temperature of 30 ° C and a salinity of 30 mg / l. The insecticidal activity tests chitinases against weevil wheat and fungicide against *Fusarium oxysporium* causes Fusarium wilt wheat has been made. The results revealed a broad spectrum antifungal (inhibition zones) and insecticidal activity (death rate) in function of the doses tested.

Keywords: *Shewanella gaetbuli*, characterization, Chitinases, Extraction, Fusarium

Effect of Sodium Butyrate on the Surrounding Chromatin Environment in amplified CHO cells

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The requirement for complex, therapeutic proteins that possess the proper folding and correct post-translational modifications has resulted in mammalian cells, in particular Chinese hamster ovary (CHO) cells, being widely used in the biopharmaceutical industry. In the creation of mammalian cell lines plasmid DNA carrying the gene of interest integrates randomly into the host cell genome. Integration into different chromatin domains results in variable levels of gene expression between cell lines due to gene silencing mechanisms. Variable and unstable recombinant protein expression makes mammalian cell line development a long and laborious process. Therefore there is a necessity to overcome these gene silencing mechanisms, with the intention to accelerate process development of recombinant protein production in mammalian systems. Ubiquitous Chromatin Opening Elements (UCOEs) are DNA elements naturally found upstream of specific housekeeping genes, which maintain an open chromatin structure, therefore diminishing instability of production by preventing transgene silencing over long term culture.

In this study CHO-DG44 cell lines were transfected with GFP with the inclusion of a 8 kb UCOE in expression constructs. Cells were amplified to 250 nM MTX and grown continuously for 80 days in the absence of MTX selection. The cells were exposed to 2.5nM NaBu, growth characteristics, GFP fluorescence and mRNA expression were analysed by flow cytometry and qRT PCR respectively.

Results showed that NaBu treatment enhanced the level of GFP fluorescence and inhibited cell growth within 24 hours. The increase in GFP fluorescence was partly due to an increase in GFP mRNA. However, the observed increase in mRNA levels cannot fully account for the increase in GFP fluorescence seen as these were not proportional. Moreover, the effect caused by NaBu treatment varied between the cell lines tested. These findings indicate that the ability of NaBu to influence different cellular processes suggests that the observed effects of NaBu may not necessarily be due to deacetylation of histones associated with the integrated transgene.

Expression and cellular localization of P2X4 and P2X7 purinergic receptors in the superficial dorsal horn of rats suffering from chronic inflammatory pain

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There is a general agreement that interleukins play a crucial role in spinal pain processing mechanisms. By a purinergic receptor-dependent manner activation of nociceptive primary afferents results in interleukin-1βeta (IL-1β) release that contributes significantly to neuronal mechanisms of chronic pain. It was previously reported that P2X4 and P2X7 receptors may be the key mediators regulating the cytokine secretion, especially the IL-1β processing. Although the contribution of interleukins to the development of chronic inflammatory pain is widely accepted, our present knowledge concerning the role of P2X4 and P2X7 receptors in the pain processing mechanisms of spinal dorsal horn are insufficient. Thus, in the present experiment we investigated the expression and cellular distribution of these receptors in the superficial spinal dorsal horn in adult male rats suffering in chronic inflammatory pain evoked by unilateral plantar injection of complete Freund adjuvant (CFA). For this purpose in the first part of our experiments single immunoperoxidase experiments were performed to identify and characterize the purinergic protein expression in the superficial spinal dorsal horn in chronic pain conditions. Our results demonstrated an enhancement of purinergic protein expression in the substantia gelatinosa of gray matter following CFA injection. In the last part of our investigations to allow us a better understanding double fluorescent labelings followed by quantitative analysis were implemented for evaluating the distribution of purinergic receptors in chronic pain. According to our findings purinergic receptors were abundantly expressed by glial cells in chronic inflammatory pain compared to control. After CFA administration considerable increase of purinergic protein expression was confirmed by Western blot analysis. P2X4 and P2X7 receptors may have a critical role in the enhanced chronic inflammatory nociceptive transmission associated with the dorsal spinal horn. A deeper understanding of the molecular mechanism by which the purinergic receptors triggers IL-1β release, may open new perspectives for the treatment of inflammatory pain.

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Expression, purification and functional study of the retrotransposon-derived human protein PEG10

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The *Paternally Expressed Gene 10* (PEG10) is a retrotransposon-derived imprinted gene in the human genome. This gene is located close to the *Sarcoglycan Epsilon Gene* (SGCE) in a head-to-head orientation. PEG10 is derived from the Ty3/Gypsy family of retrotransposons but it lost its LTR sequences and ability to retrotransposition. Strong expression of human PEG10 was shown in numerous organs such as brain, kidney, lung, and testis. PEG10 was found to be essential for embryonic development in mice. Previous works have demonstrated that mutation in the coding sequence of the gene is lethal in embriological age due to the defects of placenta development. Overexpression or prolonged expression of PEG10 was observed in malignancies, such as pancreatic cancer and embryonic kidney Wilms tumor. It was also reported that PEG10 plays a role in the formation of hepatocellular carcinoma and both in acute and chronic lymphocytic leukemia preventing the apoptosis. The PEG10 mRNA encodes for at least two protein isoforms, the reading frame 1 (PEG10- RF1) and the reading frame 1 and 2 (PEG10- RF1/2), which are translated by a typical retroviral -1 ribosomal frameshift mechanism. The RF1 encodes for the *gag*-like protein and the RF1/2 encodes for the *gag-pol*-like protein that are common in retroviruses and retroelements. The RF1 protein contains a highly conserved $CX_2CX_4HX_4C$ zinc finger consensus sequence. The RF2 protein contains an Asp-Ser-Gly sequence which corresponds to the consensus active-site motif of retroviral aspartic proteases. The activity of the protease is probably needed for its strong oncogenic effect to induce cell proliferation and prevent apoptosis. Based on these facts the proteolytic enzyme of PEG10 may be regarded as a crucial chemotherapeutic target.

Our aim was to characterize and investigate the less studied PEG10 protease starting with its production. The amplified RF1, RF1/2 and PEG10 protease sequences were cloned into pGEX-4T-3 bacterial expression vectors, and the expressed proteins underwent a purification step. We performed a protease activity assay for PEG10 using its own Gag substrate (RF1 recombinant protein), and synthetic oligopeptide substrates resembling the natural cleavage sites of proteases belonging to Ty3/Gypsy retrotransposon family. The RF1/2 coding sequence was cloned into a pQE-TriSystem expression vector, as well. 293T mammalian cells were transfected to study the PEG10 protease expressed in eukaryotic expression system and examine its supposed posttranslational modifications. Molecular weight of the identified protein obtained by Western blot analysis was higher than the weight expected by software calculation. We suppose that this difference is caused by a possible posttranslational modification of which nature need to be explored.

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Effects of Some Pyrimidine Derivatives and Pomegranate Juice on Male Rat kidney Injuries Induced by Diethylnitrosamine and Carbon tetrachloride

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ABSTRACT:

The kidney possesses most of the common xenobiotic metabolizing enzymes, and is thus able to make an important contribution to the body's metabolism of drugs and foreign compounds. The effect of pyrimidine derivatives 6-amino-2-thiouracil (ATU), 2-thiouracil (TU) and 5-flurouracil (5FU), and pomegranate juice (PJ) on kidney nitric oxide (NO), malondialdehyde (MDA), DNA fragmentation (DNAF), caspase-3 levels and kidney function tests in rats treated with diethyl nitrosamine (DEN) and carbon tetra chloride CCl_4 was studied. The effect of PJ on rat not treated with DEN and CCl_4 was studied also. Administration of rats with DEN and CCl_4 caused an elevation in the levels of NO, MDA, DNAF, caspase-3 and kidney function tests, compared to the control. Treatment of rats with PJ pre, during, and post DEN and CCl_4 administration improved kidney function and decreased the levels of NO, MDA, DNAF, and caspase-3 activities better than that in DEN-5FU, DEN- ATU, DEN-TU groups compared to the DEN group, indicates that PJ reduced the oxidative stress and apoptosis induced by DEN and CCl_4 better than that in 5FU, ATU, TU. Administration of healthy rats with PJ only for 5 weeks not induced oxidative stress and apoptosis for kidney tissues. Treatment with 5FU after DEN and CCl_4 administration showed severe toxicity which was higher than that induced by DEN and CCl_4.

KEYWORDS:

apoptosis, diethylnitrosamine, DNA fragmentation, thiouracil, fluorouracil, pomegranate juice.

The Function of Axon Guidance Molecule Sema3A in Blood Vessel Navigation into the Molar Tooth Germ

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ABSTRACT-

Objective
To examine the role of Semaphorin3A in the navigation of blood vessels into the mandibular first molar tooth germ.

Materials and methods
A breeding colony of Sema3A knock-out CD-1 mice was established. Samples were collected at different embryonic and postnatal stages, PCR was performed to determine the genotype. Wild type (+/+), heterozygote (+/-) and knock-out (-/-) mouse heads were fresh frozen and embedded in OCT, and identical or serial sections were cut at 30 micron thickness. The topography, number and density of developing blood vessels inside and around the developing first mandibular molar tooth germ, were and will be investigated by immunohistochemistry using monoclonal mouse anti-Vegfr2 antibody.

Preliminary results
Developing blood vessels were found to be located in the peridental mesenchyme in the initiation and bud stage of the tooth germ. Subsequently, the blood vessels navigate into the papilla of the cap stage tooth germ, and intense ramification in the dental papilla was evident at the early bell stage. The blood vessels reach the cusp tips in the dental papilla and the enamel organ becomes vascularized in the newborn tooth germ.

Conclusion
My preliminary findings suggest that Sema3A has a minor role in angiogenesis during embryonic molar tooth development. Since nerve fibers and blood vessels course generally alongside one another in the body, and in Sema3a knock-out mice nerves are defasciculated at least until postnatal day 7 my next goal is to investigate angiogenesis in Sema3A deficient mice in the early postnatal stages PN3 and PN7, when the sensory and sympathetic nerve fibers start their ingrowth into the dental pulp, respectively.

DETECTION of Clostridium difficile and TOXIN GENES in SAMPLES of MODIFIED ATMOSPHERE PACKAGED MINCER and CUBES BEEF by PCR

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Aim: In this project conventional methods and multiplex PCR (mPCR) technique is used to know the prevalence of *Clostridium difficile* in samples of modified atmosphere packaged (MAP) minced and cubes beef, determining the genotype of toxins in the isolates and aimed determination of sensitivity to metronidazole, vancomycin and clindamycin antibiotics.

Matherial and Method: In the study 50 modified atmospheric packaged meat and 50 cubed meat samples of Bovine origin were collected randomly from the butcher and market in Samsun.

Results: Such that in 2 of 50 (4 %) minced beef samples and in 1 of 50 (2 %) cubed beef samples C. *difficile* factors has been identified. With the Multiplex PCR molecular evaluations, a total of five isolates known by conventional method were confirmed by PCR was verified *C.difficile* type gene. When *C. difficile* isolated properties evaluated 3 out of 5 shows toxigenic character, 2 in the *C. difficile* type B (*tdcB*), 1 in *C. difficile* type A (*tdcA*) toxin genes have been identified. When antibiotic resistance profile phenotypically analysed, only *C. difficile* type A (*tdcA*) toxin gene was found resistant against clindamycin all isolates were sensitive to vancomycin and metronidazole.

Conclusion: As results of research in modified atmosphere packaged (MAP) meats and cubes samples factor, toxin type and antibiotic resistance profile is determined for the first time in Turkey. The result of this study demonstrated that *C. difficile* factor detected in food of animal origin could be a potential danger to public health.

Keywords: Antibiotic resistance, *Clostridium difficile*, MAP cubes beef, MAP minced beef Multiplex PCR

MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN THE HIPPOCAMPUS AFTER THE DESTRUCTION OF DORSAL AMIGDALOFUGAL WAY

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With the object of studying genesis and functional significance of hippocampal theta-rhythm in this study under chronic experiments on rabbits in experimental conditioned drinking rabbit an attempt was made to elucidate the participation of brain limbic structure in the mechanisms of formation of hippocampal theta-rhythm. Destruction of dorsal amygdalofugal tract in contrast to ventral one has been found lead to full and irreversible blockade of hippocampal theta-rhythm. During, elektro- and chemostimulation (carbocholine, serotonine and noradrenaline) of different hypothalamic nuclei, amygdala, reticular formation and medial septum nucleus in intact of dorsal amygdalofugal tract is registered theta-rhythm of different frequency. Serotonin increased the power of oscillations within a 5 to 6 Hz range, where as noradrenaline shifted the maximum of distribution to a 4 to 5 Hz range. In contrast to the effects of biogenic amines, the effects of carbachol and strychnine took the form of intensified generation of high-amplitude theta waves with a frequency of 6.0-7.5 Hz. But after the destruction of hippocampal theta-rhythm activity only took-place durinq intrahippocampal (CA_3 field) application of strychnine and carbocholine rather than serotonine and noradrenaline.

Examination of the slices of the hippocampus and septum of experimental animals after coagulation of the dorsal amygdalofugal tract demonstrated that profound morphological changes were detected in both neurons and glial cells of these regions. Morphological studies developed deep degenerative changes just lyzis of Nissel matter, swelling of apical dendrites, hyperchromatism of nuclei, absence of tigroid matter in neurons and glial cells in different nuclei of hypothalamus, amygdala, reticulyar formation, medial septum nucleus and hippocampus under destruction of dorsal amygdalofugal tract. Neurons and glial cells are swelled. Biochemical assay by disk elektrophoreze showed disturbance of protein spectrum in all regions of hippocampus and medial septum nucleus under destruction of dorsal amygdalofugal tract. In none of these studied regions was not observed any protein fraction.

One of the factors which modulates the excitability of neurons in septo-hippocampal system is supposed may be disturbance of hypothalamo-hypophysial neurosecretory system under the influence of destruction of amygdala-hypothalamic relations.

Whole cell immunoassay based on Z-domains autodisplaying *E. coli* for multiple analyte detection

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Flow cytometry (FACS)-based immunoassays using *E. coli* cells with autodisplayed Z-domains were performed to (1) improve the sensitivity of the immunoassay through orientation control of antibodies with IgG binding autodisplayed Z-domains and (2) detect analytes with one measurement simultaneously. The Z-domain of protein A has been known to bind specifically to the Fc region of antibodies (IgGs). The Z-domain of protein A was expressed on the outer membrane of Escherichia coli by using "Autodisplay" technology as a fusion protein of autotransport domain. The E. coli with autodisplayed Z-domain was applied to the sandwich-type immunoassay as a solid-support of detection-antibodies against a target analyte.

The expression (autodisplay) of Z-domains on the outer membrane of *E. coli* was confirmed by fluorescence microscopy analysis. The SEM results showed that distribution of autodisplayed Z-domains was homogeneous. The *E. coli* cells were doubly transfected to express a fluorescent protein in the cytosol and the autodisplayed Z-domains on the outer membrane. The medical diagnosis of heart infarction biomarker, troponin-I, was demonstrated using the Z-domains autodisplaying *E. coli*. The sensitivity of the *E. coli* cell-based immunoassay (9 ng/ml) was estimated to be higher than that of the magnetic bead-based assay (60 ng/ml). Then, *E. coli* cells with autodisplayed Z-domains were applied to the FACS-based simultaneous detection of two analytes. To demonstrate the immunoassay, human hepatitis B virus surface antigen and C-reactive protein, inflammation markers for real clinical diagnosis, were used as model analytes. In the simultaneous analysis of mixed samples, the deviation from the standard curve was calculated to be less than 10%.

FACS can be used to identify the immunoassay type by simultaneously detecting the fluorescence signal from the cytosol and the Z-domains on outer membrane, enabling the quantification of bound analytes after treatment with additional fluorescently labeled antibodies. These results show that this FACS-based immunoassay is feasible for the simultaneous detection of analytes.

Prediction of Gene Co-expression by Quantifying Heterogeneous Features

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Abstract

Prediction of gene co-expression has a great importance because of its role in explaining the molecular and functional mechanisms of the cells. For this reason, high performance methods should be developed to reduce errors. We have developed a novel method using heterogeneous features including gene expression values and various sequence-based features (SBF) via the random forest (RF) classifier to predict co-expressed genes. The proposed method, *SeqNet*, outperforms current state-of-the-art methods. Furthermore, the results indicated that the SBF are effective in the detection of co-expressed genes. However, the highest performance in predicting co-expressed genes was achieved by sequence-based features, along with gene expression data. This may be due to the ability of heterogeneous features prompt functional relationships between genes. Finally, we have concluded that SBF improve the performance of co-expressed genes prediction methods. The *SeqNet* can predict gene co-expression relationships when there is not enough gene expression data.

Keywords: Codon usage, Prediction of gene co-expression, Gene co-expression network, Machine learning methods, Random forest, Sequence-based features.

EFFECT OF ETHANOLIC EXTRACT OF KELADI TIKUS (*Typhonium flagelliforme)* ON THE LEVEL OF IFN γ (*Interferon gamma*), VEGF (*Vascular Endothelial Growth Factor*) AND CASPASE 3 EXPRESSION

A STUDY IN C3H MICE WITH ADENOCARCINOMA MAMMA

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Breast cancer treatment options including surgery, radiation therapy, chemotherapy and immunotherapy have not been effective. Besides, they have side effects. K*eladi Tikus* (*Typhonium Flagelliforme*) has been shown to improve immune system, suppress tumor growth and induce apoptosis. One of the parameters for immune system, tumor growth and apoptosis is IFNγ (Interferon γ), VEGF (Vascular Endothelial Growth Factor) and Caspase 3 respectively. The aim of this study was to examine the effect of the administration of *Keladi Tikus* tuber extract at the dose of 200 mg/kgBW, 400 mg/KgBW, and 800 mg/kgBW on the level of IFNγ, VEGF and caspase 3 expression.

In this experimental study using post test randomized control group design, 24 CH3 mice with tumor were randomly divided into 4 groups including control group and treated groups: treated with 0.2 cc extract of *Keladi Tikus* at the dose of 200 mg/kgBW, 400 mg/kgBW, 800 mg/kgBW respectively for 30 days. On day 31 the lymphatic tissue was taken and evaluated for its level of IFNγ, using ELISA. The tumor tissue was taken and subjected to immunohistochemistry staining for VEGF and caspase 3 expression evaluation. The data on IFNγ, VEGF and Caspase 3 expression were analyzed using One Way Anova with significant level of 0.05

One Way Anova resulted in $p<0.05$. LSD test showed that the level of IFNγ and Caspase 3 for control group was different from that of treated groups. There was no significant different between the treated group of 400 mg/KgBW and 800mg/KgBW. VEGF expressions for all the treated groups were significant .

In conclusion, the oral administration of ethanolic extract of *Keladi Tikus* (*Typhonium flagelliforme)* at the dose of 200mg/kgBW, 400 mg/kgBW,800 mg/kgBW increases IFNγ, Caspase 3 and decreases VEGF expression in C3H mice with adenocarsinoma mamma.

Keywords: Typhonium flagelliforme, IFNγ, caspase 3, VEGF, adenokarsinoma mamma

THE SELECTIVITY INDEX AND APOPTOSIS INDUCTION OF COMBINATION DOXORUBICIN AND ETHANOL EXTRACT OF *Dioscorea esculantae* L Burk. ON T47D BREAST CANCER CELL LINE

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Cochemotherapy studies on doxorubicin have been conducted however no satisfying result due to ineffectiveness to induce apoptosis and toxic to normal cells. Thus, another alternative cochemotherapy to doxorubicin is still needed. This present study was aimed to evaluate the selectivity of the combination of doxorubicin and ethanol extract of gembili (*D. esculanta* L Burk.) (EEG) on T47D breast cancer cell lines. The apoptosis induction indicated by the percentage of apoptotic cells, expression of caspase3 and cPARP-1.

This was an experimental study with a posttest only control group design. The ratio of doxorubicin and EEG in combination were 1:64; 1:32; 1:8; 1:4; 1:2; 1:1. The IC_{50} of each combination on Fibroblast cells was compared to the IC_{50} of each combination on T47D cells to determine the selectitivy index. The difference in selectivity among the combination groups statistically was analyzed using Anova with confidence interval 95%. Meanwhile, The percentage of apoptotic cells was analyzed using Annexin V/PI staining by flowcytometry. The expression of caspase 3 and cPARP-1 on T47D and Fibroblast cells were analyzed using immunocytochemistry staining.

The IC_{50} value of doxorubicin alone on T47D and Fibroblast cells were 2.54 µg/mL and 5.74 µg/mL respectively. The IC_{50} value of EEG alone on T47D and Fibroblast cells were 39.61 µg/mL. and 152.85 µg/mL respectively. The highest selectivity index was showed in the combination ratio of 1:4 (23.85) ($p<0.05$). The combination could increase the apoptotic cells by 77.78% compared to control on T47D cells ($p<0.05$). Meanwhile, the apoptotic mechanism was indicated by the increased caspase 3 (84.53%) and cPARP-1 (83.36%) expression on T47D cells but not in Fibroblast cells.

The combination of doxorubicin and EEG (1:4) increased the selectivity index (23.85) through apoptotic induction based on the increased cPARP-1 and caspase 3 expression on T47D cells.

Key words : Doxorubicin, ethanol Extract of Dioscorea esculanta L Burk., Apoptosis, cPARP-1, caspase 3

DETERMINATION OF ANTIOXIDANT ACTIVITY OF BLOOD SERUM UNDER CHRONIC EXPOSURE TO 460 MHs ELECTROMAGNETIC RADIATION

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Activation of lipid peroxidation as universal consequence of influense of various extreme agents, including electromagnetic radiation, on live system result in increased oxidative metabolism of complex organic structures. For inhibition of universal mechanisms of formation in lipid peroxidation of free radicals in the body, there antioxidant system, which includes in its membership the complex intracellular enzymes that counteract oxidative stress and neutralize free radicals. Imbalance between the amount of generated active oxygen and antioxidant defense mechanisms of cells leads to excessive formation conditions and generates radicals, which are often called “oxidative stress”. With a significant increase in the content of lipid peroxidation products endogenous antioxidant system becomes unable to maintain the balance of proantioksidants that leads to the development of oxidative stress – one of the universal mechanisms of tissue damage. Simultaneous study of both systems’ activity is a good tool for identifying the impact of the specificity of a particular environmental factor. In today's world, the electromagnetic radiation (EMR) in the radio and microwave bands have become an integral part of human activity, therefore, to study their effects on redox homeostasis has great relevance. This work was carried out in order to identify changes in the total oxidant and antioxidant activity in plasma and red blood cells under the influence of chronic exposure to 460 MHz EMR. White rats were irradiated for 1 month 20 minutes a day for of power density - 30 mVt/cm^2. Total oxidant and antioxidant activities of plasma and red blood cells were determined by the A.M.Goryachkovsky method (1996). Relatively high intensity exposure of rats results in a decrease in total plasma antioxidant activity (compared to control), whereas oxidant activity undergoes slight oscillations. A significant increase in antioxidant activity was observed in erythrocytes when rats were irradiated 460 MHz EMR. Decrease in the total antioxidant activity of plasma and persistent increase in the level of free radical oxidation products gives reason to talk the imbalance in the antioxidant defense system of the blood and the body as a whole, which is an unfavorable factor in the pathological process and requires effective measures of metabolic correction.

Thus, in the plasma due to decrease of total antioxidant activity is strengthening of formation free radical process in animals. These data support the idea that the biological effects of non-ionizing EMR can realize by free radical processes.

Cytotoxicity of doxorubicin: inhibition of critical proteins

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Doxorubicin (DOXO), an anthracycline antibiotic which isolated from *streptomyces peucetius*, more than forty years ago, displays antitumor activity against wide range of solid tumors and leukemia. DOXO is an intercalator drug and as we know, the major biological effect of these agents is provocation of DNA damage which could induce permanent alterations in genomic information. The anticancer activity of DOXO is accompanied by severe toxic side effects which attributed to various factors but the general consensus is that DOXO generates free radicals. However the effect of DOXO on ROS-scavenging enzymes activity remains largely unknown. The goal of the present study was to evaluate the effect of DOXO on the activity of three ROS-scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), peroxidase and all components of respiratory chain (cyt b_{560}, b_{595} and d) in *Salmonella typhymurium* as a model. *Salmonella typhymurium* strain 3507 was harvested after 24h culture at 37ºC in rotary shaker, in liquid enriched medium in the presence of increasing DOXO concentrations from 1 to 150µg/ml. increasing DOXO concentrations led to an inhibition of CAT, SOD and peroxidase activity. CAT activity dropped to 70% of the control with 1µg/ml DOXO and to 30% in cell grown in 150µg/ml DOXO. SOD activity decreased from 95% in 1µg/ml to 30% in 150µg/ml and peroxidase activity decreased progressively from 85% in 1µg/ml DOXO to 21% in 150µg/ml of the drug. Besides, all components of respiratory chain were also inhibited by DOXO. It has been reported that anti-ROS such as CAT and SOD decreased apoptosis induced by DOXO. In the other hand we should keep in mind that DOXO therapeutic role is attributed to its intercalating ability, thus while DOXO could have a direct effect on critical proteins of the cell it could interfere in programmed cell death even before the DNA damage.

Doxorubicin makes a complex with peroxidase: spectroscopic studies

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There is an increasing interest in interfacing the studies on drug-protein interaction based on biochemical techniques to find the mechanisms of drug action and protein sensitivity. The goal of these studies is to develop novel pathways that optimally treat clinically significant diseases. Doxorubicin that also known as Adriamycin, is an intercalator drug commonly used in cancer therapy. There are a number of studies that focus on intercalating features of Doxorubicin and its derivatives, but little is known on its interaction with proteins. The purpose of this work was to assess the ability of DOXO to induce alterations in structure and function of horseradish peroxidase C as a model of ROS-scavenging enzymes which has a critical role in cell survival. Peroxidase activity was assayed by following H_2O_2-dependent oxidation of o-dianisidine at 460 nm, the electronic absorption spectra were recorded for 300-700 nm and Intrinsic fluorescence was detected for excitation wavelength of 297 nm and was recorded for 300-700 nm. All measurements were performed in citrate buffer 0.1M pH 4 at 37ºC. Assays for peroxidase showed that the enzymatic activity decreased as DOXO concentrations increased, (1-150µg/ml) going from 96% activity of control in 1µg/ml to 15% in 150µg/ml of DOXO. The Lineweaver-Burk plots showed the noncompetitive and mixed manner of inhibition for HRPC. Electronic absorption spectrum results for 403nm indicated that three molecules of DOXO bind to HRPC in two different binding sites. The first molecule binds independently in the presence of 1-100µg/ml and two other molecules bind in cooperative manner in the presence of 100-150 µg/ml of DOXO. Indeed fluorescence studies showed that the only tryptophan of HRPC quenched by drug-protein interaction and the complex of DOXO-HRPC is static. Thus Doxorubicin, a drug with antitumor ability, can display the key enzymes of the cell and make a direct effect on vital functions.

Biochemical changes in the bee hemolymph following treatment with amitraz and tau-fluvalinate

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Since its discovery on *Apis mellifera* during the 60s, varroasis caused by the mite *Varroa destructor* was and remains to this day a great challenge that faces beekeeping. Indeed, this is the first officer of the weakening or loss of the colonies in the world. *Varroa* is treated by chemicals, natural and biotechnical methods. In Algeria, the beekeepers use of unapproved chemical treatments such as amitraz in gout and wooden strips impregnated with the tau-fluvalinate molecule (Klartan). The aim of this work was to determine the influence of these molecules on different metabolites (carbohydrates, proteins, and fats) hemolymph and whole body of honey bees.

The experiment is performed on ground during the month of September 2013, three experimental groups were installed: two groups of 20 bee colonies were treated with amitraz in gout and Klartan and an untreated control group. Samples of bees treated samples were carried out after 24 and 72 hours treatment along with a batch of untreated controls. The collection of hemolymph was performed by puncture (3µl per bee) in the membranes of inter segmental tergites 2 and 3 through microcapillary. The extraction of different metabolites (proteins, carbohydrates and fats) was performed according to the method of Shibko et al. (1966) on the whole body and hemolymph of worker bees *Apis mellifera intermissa*. The proteins were quantified according to the Bradford method (1976). The determination of total carbohydrates was performed according to the method Duchateau and Florkin (1959) while the lipids were quantified using the method of Goldsworthy et al., (1972).

Treatment with amitraz and tau-fluvalinate disrupts the biochemical metabolism of the bee, the results obtained show that it causes a drop in the carbohydrate content and an increase in the protein levels in the body and the hemolymph. By against lipids were not affected by the two molecules.
Our results confirm once the negative effects of the application of chemicals, mostly unregistered treatments can therefore lead to a weakening of bee colonies following side effects caused on the metabolism of the workers.

Keywords: *Apis mellifera*, hemolymph, side effects, Biochemistry

Expression and cellular localization of P2X4 and P2X7 purinergic receptors in the superficial dorsal horn of rats suffering from chronic inflammatory pain

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There is a general agreement that interleukins play a crucial role in spinal pain processing mechanisms. By a purinergic receptor-dependent manner activation of nociceptive primary afferents results in interleukin-1βeta (IL-1β) release that contributes significantly to neuronal mechanisms of chronic pain. It was previously reported that P2X4 and P2X7 receptors may be the key mediators regulating the cytokine secretion, especially the IL-1β processing. Although the contribution of interleukins to the development of chronic inflammatory pain is widely accepted, our present knowledge concerning the role of P2X4 and P2X7 receptors in the pain processing mechanisms of spinal dorsal horn are insufficient. Thus, in the present experiment we investigated the expression and cellular distribution of these receptors in the superficial spinal dorsal horn in adult male rats suffering in chronic inflammatory pain evoked by unilateral plantar injection of complete Freund adjuvant (CFA). For this purpose in the first part of our experiments single immunoperoxidase experiments were performed to identify and characterize the purinergic protein expression in the superficial spinal dorsal horn in chronic pain conditions. Our results demonstrated an enhancement of purinergic protein expression in the substantia gelatinosa of gray matter following CFA injection. In the last part of our investigations to allow us a better understanding double fluorescent labelings followed by quantitative analysis were implemented for evaluating the distribution of purinergic receptors in chronic pain. According to our findings purinergic receptors were abundantly expressed by glial cells in chronic inflammatory pain compared to control. After CFA administration considerable increase of purinergic protein expression was confirmed by Western blot analysis. P2X4 and P2X7 receptors may have a critical role in the enhanced chronic inflammatory nociceptive transmission associated with the dorsal spinal horn. A deeper understanding of the molecular mechanism by which the purinergic receptors triggers IL-1β release, may open new perspectives for the treatment of inflammatory pain.

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The Effect of Silver Sulfide Nanoparticles (Ag_2S NPs) on Methane Production during Anaerobic Digestion of Waste Activated Sludge

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Many studies have been reported that the Ag_2S NPs were often found in waste activated sludge (WAS). However, little is known about the impact of Ag_2S NPs on WAS stabilization. The aim of this study was to investigate the effects of Ag_2S NPs and their released Ag^+ on WAS anaerobic digestion for methane production and the examination of the change of metanogenic population under different environments using qPCR. Four dosages (2, 50, 100, and 200 mg/g-TSS) of Ag_2S NPs were added to four different bottles containing WAS and were used to investigate the impact of Ag_2S NPs on methane production during WAS anaerobic digestion. At Ag_2S NPs dosages of 2, 50, 100 and 200 mg/g-TSS, the corresponding released Ag^+ concentrations were 1.6, 8.3, 18.9, and 28.8 mg/L, respectively. It was found that the relative amounts, compared to the control systems, of methane production in each WAS anaerobic digestion bottle adding 2, 50, 100, 200 mg/gTSS Ag_2S NPs were 99, 72, 48, and 24 %, respectively while the relative amount of methane production found in another four WAS anaerobic digestion bottles adding 1.6, 8.3, 18.9, 28.8 mg/L of Ag^+ (AgCl) were 99, 87, 59, 36 % of the control, respectively. Approximately 40% inhibition of CH_4 production was observed during anaerobic digestion of WAS due to the presence of 18.9 mg/L of Ag^+. When the Ag^+ was 28.8 mg/L, a much lower methane production (36% of the control) was obtained. It can be seen that the impact of Ag_2S NPs on methane production mainly resulted from the dissolved Ag^+. This indicates that the released Ag^+ from Ag_2S NPs played an important role on the adverse effect of Ag_2S NPs on the performance of WAS anaerobic digestion. Moreover, the volatile fatty acid (VFA) concentration was 398, 488, 862, 2,962, and 6,128 mg/L for control and four WAS anaerobic digestion bottles adding 1.6, 8.3, 18.9, 28.8 mg/L of Ag^+. Higher VFA concentration could decrease a significant growth on methanogen, resulting in low methane production. The average numbers of total gene copy of methanogens analyzed from samples taken from WAS anaerobic digestion bottles adding Ag_2S NPs of 2, 50, 100, and 200 mg/gTSS were approximately 2.98×10^8, 3.28×10^7, 8.64×10^6, and 9.89×10^4 copies/mL, respectively. Obviously, the more Ag_2S NPs appeared in sludge anaerobic digestion system, the less methanogens remained. In addition, the reactive oxygen species (ROS) concentration was 102, 146, 178, and 208 % of the ROS concentration found in the control systems. The ROS induced by Ag_2S NPs was considered as a toxicity, which caused the loss of cell viability and an adverse effect on methane production.

It can be seen that significant inhibition of WAS anaerobic digestion was obtained when the bioreactor was subjected to Ag_2S NPs at the final concentration is approximately or higher than 100 mg/gTSS. Supporting evidence includes reduced biogas production, accumulation of VFAs and ROS, and decrease in methanogenic population.

Biotransformation of progesterone and pregnenolone by *Aspergillus candidus*

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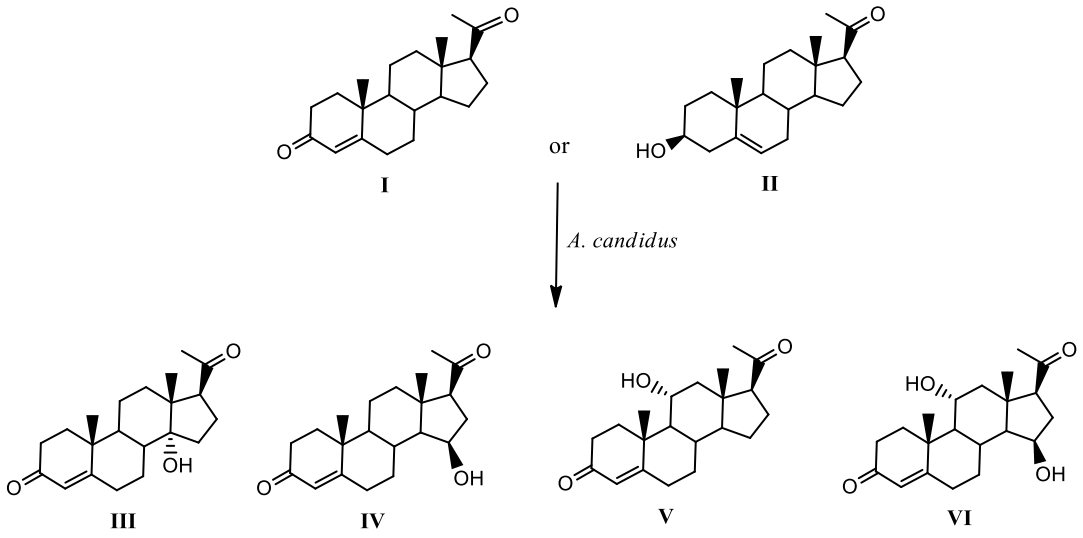
Fungal steroid biotransformation has been an important process of preparing new steroid derivatives with potential pharmacological activities due to their high regio- and stereoselectivity. A number of efforts are still made to improve the efficiency of fungal steroid biotransformations and to obtain new useful reactions and species [1].

Aspergillus is an extremely important fungal genus concerning mycotoxins, pathogenicity, fundamental eukaryotic genetics and biotechnological exploration [2]. *Aspergillus* species are ubiquitous fungi found in soil, water, and decaying materials. A few *Aspergillus* species are considered pathogenic to humans and animals [3].

The fungus *Aspergillus candidus* is a moderately xerophilic white mold, a food contaminant in cereals and an opportunistic pathogen for humans [4]. As far as steroid biotransformations by *Aspergillus candidus* are concerned, it has not been found any literature work on steroids.

In this work, progesterone **I** and pregnenolone **II** were incubated with *Aspergillus candidus* MRC 200634 for 5 days. Incubations of both substrates with *A. candidus* afforded 14α-hydroxyprogesterone **III**, 15β-hydroxyprogesterone **IV**, 11α-hydroxyprogesterone **V** and 11α,15β-dihydroxyprogesterone **VI**. Higher yields were obtained from the incubation of progesterone **I.**



The metabolites were separated by column chromatography. Structure determinations of the metabolites were performed by comparing melting points, NMR and IR spectra of starting materials with those of metabolites.

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THE EFFECT OF GARLIC (*ALLIUM SATIVUM*) ON LIPID PROFILE IN RABBITS

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ABSTRACT–

Objective: This study was conducted to investigate the cholesterol-lowering property of garlic (*Allium Sativum*) in whole blood of egg yolk induced hypercholesterolemia in rabbits. **Methods**: Forty rabbits of both sexes of 13.1 ± 28.4 weeks of age with average body weights of 1251.9 ± 512.2g were used for the experiment. The animals were divided into eight groups comprising control and seven experimental groups with 5 rabbits per group. The animals were acclimatized with grower's mash for one week after which the control group was fed with grower's mash and the seven experimental groups were fed with grower's mash supplemented with 10% egg yolk, 20% egg yolk, 2% garlic, 4% garlic, 10% egg yolk + 2% garlic, 20% egg yolk + 2% garlic, and 20% egg yolk + 4% garlic respectively for five weeks. Animals were phlebotomized through prominent ear veins and blood samples (2 ml) were collected from rabbits in each group before and after the treatment (diet administration) to assay for total cholesterol (TC), HDL-cholesterol and triglycerides (TG) using the CardioChek® analyzer; the LDL-cholesterol was determined using Friedewald formula. **Results**: The TC analysis shows that there was no significant difference between the control and the treatment groups ($P>0.05$). The HDL-Cholesterol analysis indicates no significant difference between the control and the treatment groups ($P>0.05$) except the group that received 10% egg yolk + 2% garlic supplementation ($P<0.05$). The LDL-Cholesterol analysis show significant difference exist between the control and all other treatment groups ($P<0.05$) except the group that received 2% garlic supplementation where a decrease ($P>0.05$) was observed. The results of TG analysis show no significant difference between the

control and the treatment groups that received 10% egg yolk, 2% garlic or 10% egg yolk + 2% garlic supplementations ($P>0.05$). However, there was significant increase ($P<0.05$) in the TGs of the treatment groups that received 20% egg yolk, 4% garlic, 20% egg yolk + 2% garlic or 20% egg yolk + 4% garlic compared to the TG of the control group. **Conclusion:** While egg yolk supplementation did not induce hypercholesterolemia; it was observed that garlic powder supplementation did not demonstrate significant hypocholesterolemic effect on the lipid profile of rabbits.

Keywords: Garlic (*Allium sativum*), Cholesterol, Grower's mash.

Regulation of function of thylakoid complexes by redox and energy state of chloroplasts

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Abstract–

Photosynthetic electron transport is performed by a chain of redox components electrochemically connected. It is efficiency depends on the activity of photosystem with interaction with dark reaction of photosynthesis. Plants developed different acclimation mechanisms that maintain photosynthesis under stress conditions. Recent studies indicate that redox signal from photosynthetic electron transport and reactive oxygen species play a central role in the regulation of acclimation and responses to stress. The imbalance between reduced and oxidized forms can change the photosystem stoichiometry. Cytochrome b6f complex, kinases and phosphatases activities and expression of photosynthesis gens are regulated by the redox state of PQ pool and energetic status of chloroplasts. Changes in illumination, dark-light shift and inhibitors was used to induce different responses in organization of chloroplasts of plants represented different metabolic types (C3 and C4-mesophyll and bundle sheath also red alga chloroplasts) to identify mechanisms responsible for chloroplast acclimation.

Protective Effects of Highly Expressed Recombinant Human EC-SOD against ROS, UVB-Induced Apoptosis and DNA–Damage in Human Keratinocytes.

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ABSTRACT–

Human extracellular superoxide dismutase (hEC-SOD) is a tetramer protein protects the extracellular space from oxidative stress by catalyzing the dismutation of biologically toxic superoxide anion into hydrogen peroxide and oxygen. Difficulty in obtaining large quantity of active recombinant (rhEC-SOD) has slowed its clinical applications although different systems have been used for its expression. In this work, transformed cells with His6-tagged rhEC-SOD were grown by fed-batch fermentation yielding a final dry weight of 16.47.6 g/L as an inclusion body which comprised 43.17% of total protein. Inclusion bodies of the rhEC-SOD were solubilized, refolded and purified by Immobilized Metal Affinity (IMAC) and gel filtration Chromatography (GFC). With 2 L of fed-batch fermentation, 56mg rhEC-SOD could be produced (purity 98%) with a total activity of 317.33 U. Appearance of the purified rhEC-SOD as a monomer form (26 kDa) directed the work to examine the role of the signal peptide at the N-terminal region in inducing the tetramer formation of this protein. In the predicted motif of the α-helix of the N-terminal region, amino acid substitutions of (M20D, V24D, W28A and V31D) lead to the complete disruption of the tetramer form of rhEC-SOD protein. However, no contribution could be detected with the mutations of (D12A, W16A and A22D). Partial disturbance of the tetramerization appeared with the mutations of (R34A and I17D). Protective effects of rhEC-SOD against reactive oxygen species (ROS), UVB-Induced apoptosis and DNA fragmentation were analyzed in human HaCaT keratinocytes. rhEC-SOD had a scavenging activity of 30% and 14% against H_2O_2-induced ROS and UVB-induced ROS, respectively. Also, rhEC-SOD could reduce the UVB-induced nuclear fragmentation index from 21 to 9 (43%). In addition, the DNA damage and fragmentation index decreased from 1.54 to 1.15 in UVB irradiated upon the treatment of keratinocyte HaCaT cells with rhEC-SOD protein before UVB irradiation.

EFFECT OF INDOL-3 ACETIC ACID ON THE BIOCHEMICAL PARAMETERS OF *Achoria grisella* HEMOLYMPH AND *Apanteles galleriae* LARVA

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ABSTRACT

Biochemical structures such as lipid, protein, sugar, and glycogen are known to play a pivotal role on the relationship between host and its parasitoid. Any changes in these parameters may have potential to alter the balance of the host-parasitoid relation. Taking this into account, the effects of an agriculturally used plant growth regulator, *indol-3 acetic acid* (IAA) on the biochemical parameters of the host and parasitoid were investigated. *Achoria grisella* Fabricus (Lepidoptera: Pyralidae) is a serious pest and causes harmful impacts on honeycomb. Endoparasitoid *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) feeds on the hemolymph of the *A. grisella* larva and finally causes mortality of the host. Different concentrations (2, 5, 10, 50, 100, 200, 500, and 1,000 ppm) of IAA were added to the synthetic diet for feeding host larvae. Protein, lipid, sugar, and glycogen contents in hemolymph of host and totally in parasitoid larvae were determined by Bradford, vanillin-phosphoric acid, and hot anthrone tests using UV visible spectrophotometer, respectively. Protein level in host hemolymph increased upon supplement of each doses of IAA except for 10 ppm. IAA application enhanced the level of sugar at 100 and 200 ppm whereas a decrease was observed in lipid at 5, 10, 200, and 1,000 ppm doses in host. All doses were effective on the parasitoid larvae. Nevertheless, the most effective dose was 50 ppm, which showed an increasing effect on glycogen but decrasing effect on lipid. Similarly, 1000 ppm increased the protein level and 100 ppm reduced the level of sugar. Our study indicates that application of IAA resulted in different effects on the amount of biochemical structures associated with the hemolymph of pest species and its natural enemy. Therefore, results showed that not only the target but also the non-target organisms exposed to widely-used plant growth regulators may be affected which, in turn may also change the host-parasitoid interaction.

Keywords: *Achoria grisella*, *Apanteles galleriae*, Indol-3-Acetic Acid, biological control.

Association of 105A/C IL-18 Gene Single Nucleotide Polymorphism with House Dust Mite Allergy In An Atopic Filipino Population

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ABSTRACT–

Allergy is a multifactorial disease affecting a significant proportion of the population. It is developed through the interaction of allergens and the presence of certain polymorphisms in various susceptibility genes. In this study, the correlation of the 105A/C single nucleotide polymorphism (SNP) of the IL-18 gene and house dust mite-specific IgE among Filipino allergic and non-allergic population was investigated. Atopic status was defined by serum total IgE concentration of ≥100 IU/mL, while house dust mite allergy was defined by specific IgE value ≥ $\overline{X}$ +1SD of IgE of nonatopic participants. Two hundred twenty match-paired Filipino cases and controls aged 6-60 were the subjects of this investigation. The level of total IgE and Specific IgE were measured using Enzyme-Linked Immunosorbent Assay (ELISA) while Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) analysis was used in the SNP detection. Sensitization profiles of the allergic patients revealed that 97.3% were sensitized to *Blomia tropicalis, 40.0% to Dermatophagoides farinae*, and 29.1% to *Dermatophagoides pteronyssinus*. Multiple sensitization to HDMs was also observed among the 47.27% of the atopic participants. Any of the allergy classes of the atopic triad were exhibited by the cases (allergic asthma: 48.18%; allergic rhinitis: 62.73%; atopic dermatitis: 19.09%), and two or all of these atopic states are concurrently occurring in 26.36% of the cases. A greater proportion of the atopic participants with allergic asthma and allergic rhinitis were sensitized to *D. farinae*, and *D. pteronyssinus,* while more of those with atopic dermatitis were sensitized to *D. pteronyssinus than D. farinae.* Results show that there is overrepresentation of the allele "A" of the 105A/C IL-18 gene SNP in both cases and control groups of the population. The genotype that predominate the population is the heterozygous "AC", followed by the homozygous wild "AA", and the homozygous variant "CC" being the least. The study confirmed a positive association between serum specific IgE against *B. tropicalis* and *D. pteronyssinus* and the allele "C" (*Bt P=0.021, Dp P=0.027*) and "AC" (*Bt P=0.003, Dp P=0.026*) genotype. Findings also revealed that the genotypes "AA" (*OR:1.217; 95% CI: 0.701-2.113*) and "CC" *(OR, 3.5; 95% CI: 0.727-16.849)* increase the risk of developing allergy. This indicates that the 105A/C IL-18 gene SNP is a candidate genetic marker for HDM allergy among Filipino patients.

Phenolic Compounds Characterization and Antioxidant Capacities of Different Cultivars of Peach (*Prunus persica* L.) leaves: A Comparative Study

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Several studies suggested that diet rich in fruits and vegetables may reduce the risk of developing some chronic diseases such as cardiovascular diseases, diabetes, neurodegenerative disorders and cancers. Peaches (*Prunus persica* L.) are one of the most consumed fruits on the Mediterranean diet. Peach leaves are also used in traditional medicine. The leaves are anthelmintic, insecticidal, sedative, diuretic, demulcent, expectorant, vermicidal and are used in leucoderma and in piles. Leaf paste is used to kill worms in wounds and fungal infections. The treatment of gastritis, whooping cough and chronic bronchitis is carried out internally with leaves. Information available about the phytochemical content of leaves is not always complete.

The aim of this study is the characterization of phenolic compounds present in foliar extracts of seven peach varieties cultivated in Algeria and the study of their antioxidant activity. Polyphenols were extracted with acetone, quantified and identified by HPLC-MS/MS analysis. Several phenolic compounds were identified in peach leaf extracts including cinnamic acids and flavonols. The different varieties present highly variable concentration in phenolic compounds quantified by HPLC analysis. Cultivars can be classified into two groups based on phenolic contents. Dixered, Flavorcrest, Tebana, Romea and Red Top present the higher concentration in phenolic compounds with values ranging from 320.6 to 392.2 mg/g DWE (dry weight extract). Cardinal and Spring Belle constitute the second group with concentration range between 140 and 146 mg/g DWE. In order to assess the antioxidant capacity of the foliar extracts, we carried out several tests acting by different mechanisms: Folin-Ciocalteu (F-C), Oxygen Radical Absorbance Capacity (ORAC) and 2,2-DiPhenyl-PicrylHydrazyl radical (DPPH). Results show that there are significant differences between peach foliar extracts of different varieties. Positive correlations were found between the different antioxidant capacities obtained with the different methods. These results allow us to conclude that peach leaves are a good source of antioxidants.

Keywords: Rosaceae, polyphenol, leaf, HPLC-MS/MS, flavonols, antioxidant capacity.

Comparative evaluation of antioxidant activities, total phenolic and flavonoids contents in two Algerian plants (*Borago officinalis* and *Urtica dioica*)

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Abstract–

Oxidative stress is induced by a large variety of oxygen free radicals, including reactive oxygen species (ROS). Accumulating clinical and experimental evidence indicates that ROS plays an essential role in the pathogenesis of airway inflammation.

The aim of our study is to demonstrate some antioxidant and antiradical properties of two local medicinal plants "Borago officinalis" and "Urtica dioica", renowned for their use in the pathogenesis of airway inflammation.

Aqueous and hydroalcoholic extracts of borage and nettle from Annaba region (Algeria) were evaluated for their antioxidant and antiradical properties by three different methods: DPPH radical scavenging, test NBT and total antioxidant activity, total phenolic and flavonoid content.

The extracts of borage and nettle showed an important antiradical or antioxidant properties, the most active extract with lower IC50 being ethanolic extract of borage which contained the highest amount of phenolics (94,09 ± 1,72mg gallic acid/g dry extract) followed respectively by ethanolic extract of nettle which contained the highest amount of flavonoids (39,96 ± 2,56mg quercetin/g dry extract), aqueous extract of borage and nettle.

A high and significant correlation existing between each method of antioxidant activity and total phenolic content of plants, indicating that total phenolic content is the major contributor to the antioxidant activity of plants. Also there is a high correlation between the three methods, proving coherent results.

Platelet-rich plasma protects tenocytes from adverse effects of Hydrogen Peroxide: a preliminary study.

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Platelet-Rich Plasma (PRP) and Platelet-rich plasma releasate (PDGFs) have shown activity in tenocytes and healing tendons[1]. However few studies have been performed to establish their efficacy against oxidative damage[2]. Oxidative stress is proposed to contribute to the pathogenesis of tendon degeneration and in the impair of tendon healing. There are a lot of potential source of Reactive oxygen species (ROS) either in the tendon or in its proximity especially during physical exercise[3]. In this study the effects of different concentrations of PRP and PDGFs in tenocytes were evaluated to establish their real efficacy and the most effective dose for tissue healing. A key parameter of PRP is its platelet concentration[1]. In addition, tenocytes were exposed to hydrogen peroxide (H_2O_2) in presence or absenceof PRP or PDGFs to test their possible protective effects on oxidative stress. PRP (2.0 10^6 platelets/μL) was obtained from ACD anticoagulated blood samples, while the PDGFs were prepared by incubating for 1 hour at 37°C the platelet pellet in 22 mM $CaCl_2$ (f.c. 2.0 10^6 PLT/μL). Tenocytes were obtained from equine superficial digital flexor tendon and cultured in Dulbecco's modified Eagle's medium (DMEM) + 10% Fetal bovine serum (FBS),+ 100 U/mL of penicillin and 100 μg/mL of streptomycin at 5% CO_2 and 37°C in humidified atmosphere. To test the effect of PRP, cells were seeded at a density of $15x10^3$ cells/well in 96-well plates and allowed to adhere for 24 hours at 37º C. Different concentrations of PDGFs or PRP (5%, 10%, 15 % in DMEM) were used alone or in combination with different concentration of H_2O_2 for 24 hours. The effects of PRP and PDGFs on the viability/proliferation of equine tenocytes were evaluated by the WST-8 assay (which measures the activity of dehydrogenase enzymes in the mitochondria of living cells). Both autologous preparation induced significant increase in cell viability, at every doses tested ($P<0.05$). Furthermore, no statistically significant differences between the doses of PRP used were observed on cell viability. However, the effect of PRP is always greater than PDGFs. This may be due to plasma proteins, such as fibronectin, which are known to increase cell proliferation. Hydrogen peroxide induced a significant ($P<0.05$) reduction in tenocyte viability in a dose dependent-manner starting from 500 μM. On the contrary, PRP and PDGFs were able to prevent this detrimental effects ($P<0.01$). The obtained results, although preliminary, suggest that PRP could protect tenocytes from the injury induced by oxidative stress. Additional analysis such as expression of collagen I and II by qPCR as well as MMP2 and MMP9 by zymography are still in progress

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Description of the new family of *Caldicoprobacteraceae:* Isolation of new thermophilic anaerobic strains from an Algerian thermal aquifer

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Abstract :

The family *Caldicoprobacteraceae* belongs to the order *Clostridiales*, phylum *Firmicutes*. It embraces the genus *Caldicoprobacter* which contains three recognized species: C. oshimai, *C. algeriensis*, and *C. guelmensis*, together with *Acetomicrobium faecale* which should be reclassified within the genus *Caldicoprobacter* as *C. faecale*, comb. nov. Members of the family are defined by a wide range of morphological and chemotaxonomic properties including the cellular fatty acids content. They are all strictly anaerobic thermophilic heterotrophic rod-shaped bacteria using sugars, but not proteinaceous compounds. *Caldicoprobacter algeriensis* and *C.guelmensis* were isolated from Algerian hot springs, where as *C. oshimai* was isolated from sheep feces. Interestingly, this latter bacterium had clone OTU4 (99.5 % similarity)retrieved from cow feces enrichment cultures as its closest phylogenetic relative, thus demonstrating that similar microorganisms, despite being most probably dormant, might prevail in herbivore feces.

With the exception of *A. faecale*, all other *Caldicoprobacter* species displayed a xylanolytic activity at 70 C that was found extracellular for *C. algeriensis* and *C. guelmensis*. In this respect, these species may be used to degrade hemicellulosic material, known as the second most abundant component of plant fiber. *Acetomicrobium faecale*, which should be reassigned to a *Caldicoprobacter* species (see above), was shown to ferment arabinose, xylose, and ribose while producing equimolar amounts of acetate and ethanol. It was therefore suggested to be a potential candidate for the biotechnological production of ethanol since neither yeasts nor *Zymomonas mobilis* can use these sugars.

Keywords: Thermophilic anaerobic strains, hot spring, Algeria

Algoriphagus trabzonensis sp. nov., isolated from fresh water in Turkey

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Two Gram-negative, lack of motility, catalase- and oxidase- positive, MS7^T isolated from fresh water in Trabzon, Turkey. Bacteria were isolated from fresh water in the provinces of Izmir, Turkey and were characterized in order to determine their phylogenetic positions. The strain was designated as MS7^T. The strain grew optimally at 30°C and pH 7.4 and in the presence of 2.0 % NaCl. 16S rRNA gene sequence analysis revealed that the strain belonged to the genus *Algoriphagus*; strain MS7^T showed highest sequence similarity to strain *Algoriphagus alkaliphilus* 97.09 %, *Algoriphagus terrigene* 96.4 %, *Algoriphagus jejuensis* 96 %, *Algoriphagus boritolerans* 96.08 % and *Algoriphagus aquatilis* 95.1 %. The major fatty acids of strain MS7^T were iso-C15 : 0 (30.14%) and and summed future 9 (16:0 10-methyl and/or iso C17:1 w9c; 18.75 %). Polar lipid analysis revealed a diphospatidyglycerol, a phosphatidylglycerol, a phosphatidylmonomethylethanolamine, a phosphatidylethanolamine, a variety of unidentified aminophospolipids, unknown phospholipids and unidentified aminolipids. The major isoprenoid quinone was MK-7. The DNA G+C contents for *Algoriphagus alkaliphilus* AC74^T and strain MS7^T were calculated using linear regression analyses of Tm against the G+C content of the standard DNA (i.e. E. coli K-12 DNA). The DNA G+C content of MS7^T was 41.6 mol%, a value consistent with that of members of the genus *Algoriphagus*. To determine the degree of genomic DNA relatedness between *Algoriphagus alkaliphilus* AC74^T and strain MS7^T, DNA–DNA hybridization was performed spectrophotometrically by DNA reassociation kinetics. The level of DNA–DNA relatedness between strain MS7^T and *Algoriphagus alkaliphilus* AC74^T was 41 %, which is clearly below the 70 % threshold accepted for species delineation. Thus, our results support the placement of strain MS7^T within a separate and previously unrecognized species. Based on these data, the strain is considered to represent a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus trabzonensis* sp. nov. is proposed. The type strain is MS7^T (=NCCB 100372^T=LMG 26290^T).

Characterization of Philippine Drug-susceptible and Multi-drug Resistant Mycobacterium tuberculosis Isolates through Combined 15-loci MIRU-VNTR Genotyping and Mutation Analysis of Drug Resistance Genes

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Molecular genotyping, an important strategy to characterize bacterial strain infecting a patient, allows identification of *M. tuberculosis* complex members with varied responses to anti-mycobacterial therapy. M. tuberculosis Interspersed Repeating Units – Variable Number of Tandem Repeats (MIRUVNTR) is a fast, reproducible and cost-effective PCR-based method capable of differentiating MTb strains. This study focused in evaluating the utility of MIRU-VNTRs to discriminate fifty-four MTB isolates from the Lung Center of the Philippines through amplification of twelve MIRU-VNTRs and three Exact Tandem Repeats (ETRs). Digital codes were determined per isolate through calculation of VNTR repeats and analyzed using online MIRU-VNTRplus program. Hunter Gaston Discriminatory Indices suggest that five out of fifteen (33.33%) MIRU-VNTRs are highly discriminatory (>0.75). All MIRU-VNTRs and ETRs except ETRC had HGDI indices ≥0.5 suggesting good resolving power. MIRU-VNTR profile of LCP isolates supplemented with mutation data of *rpoB*, *katG* and *gyrA* genes obtained through gene sequencing lead to identification of four clusters closely related to East African-Indian strain which confirmed previous reports regarding the existence of a distinct Manila family of MTb strains. These four clusters namely EAI-M1 to EAI-M4 are characterized by increasing propensity to develop drug resistance. *rpoB*, *katG* and *gyrA* mutations observed in the Philippine isolates were highly similar to reported literature. Our results show that combined 15-loci MIRU-VNTR genotyping strategy and mutation profiling of drug resistance-related genes could could serve as a molecular epidemiology tool to characterize and monitor the drug susceptible and multi-drug resistant MTb strains in the country.