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Three days of Islamic intermittent fasting: impact on repeated-sprints performance and related metabolic responses

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Background: This study examined the effects of 3-days of Islamic intermittent fasting (IF) on physical performance and metabolic responses to treadmill repeated-sprints (RS). **Methods:** Twenty-one active healthy male Muslim adults (29.8±5.9 years, endurance and team-sports based training 4±1.5 times/week) performed 2-RS sessions [2sets: (5×5-s maximal-sprints (25-s recovery/sprints), 3-minutes recovery/sets]. Fed/Control session (CS) and fasting session (FS assessed at the 3rd day of the 3-d IF) were counter-balanced. **Main Outcome Measures:** Maximum sprinting power (MaxSP) was assessed using an instrumented treadmill. Serum lipids profile (TC, TG, HDL, and LDL), glucose, free fatty acids (FFA), insulin, cortisol, and blood lactate BLC were assessed pre- and post-exercise sessions. **Results:** MaxSP decreased significantly in FS (1349±59 W) compared to CS (1408±57; p=0.011 W). Specifically the FS showed a significant reduction in MaxSP at runs 1 and 2 of 2nd set ($p \leq 0.030$). Insulin decreased in CS post-exercise (p=0.030) but not in FS. FFA were always higher in FS than control at pre- and at post-exercise ($p<0.001$ and p=0.003, respectively). HDL was higher in FS (1.32±0.05 (mmol/l)) compared to CS (1.25±0.05 (mmol/l)) at post-exercise (0.039). IF did not affect BLC, whereas, TG decreased both at pre- and post-exercise (p=0.008, and p=0.012, respectively). **Conclusion:** During RS, performance is impacted in the initial runs of the second set when fasting. In intermittent fasting conditions, repeated sprints sessions are accompanied by an increased reliance on lipids metabolism through oxidation of FFA.

Keywords: Lipids profile; Repeated-sprints ability (RSA); Physical performance; Qatar; Anaerobic exercise; Active adults.

In silico evaluation of Molecular Structure geometry, Vibrational Spectra and Substitution Effect of Thiohydantoin

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ABSTRACT:

In this study the Molecular geometry optimization, vibrational frequencies, energy gaps, net charges, dipole moments and heats of formation Thiohydantoin at the ground state, in present work, we have been calculated and performed by using the Molecular Mechanics, PM3, ab initio/HF and DFT/B3LYP methods basis set in order to obtain optimized geometrical parameters are in good agreement with experimental values. Comparison of the obtained fundamental vibrational frequencies of Thiohydantoin result by DFT/B3LYP (6-311G++ (d, p)) method, are in a close agreement with the experimental data. Ab initio/HF with 6-31G basis set was used to investigate the effects of a variety of substituants (methyl ,dimethyl, trimethyl ,and chloride ,dichloride ,trichloride) on the electronic properties of thiohydantoin derivatives. Detailed vibrational wave number shifts and vibrational mode analyses were reported.Thiohydantoins are sulfur analogs of hydantoins with one or both carbonyl groups replaced by thiocarbonyl groups . Among the known Thiohydantoins, 2-Thiohydantoins are most notably known due of their wide applications as hypolipidemic, anticarcinogenic , antimutagenic , antithyroidal antiviral (e.g., against herpes simplex virus, HSV) , human immunodeficiency virus (HIV) and tuberculosis), antimicrobial(antifungal and antibacterial), anti-ulcer and anti-inflammatory agents, as well as pesticides. Additionally, 2-Thiohydantoins have been used as reference standards for the development of C-terminal protein sequencing, as reagents for the development of dyes preparative methods for 2-Thiohydantoins include the reactions between thiourea and benzil, amino amide and diimidazole thiocarbonate, and others.However, the above methods often suffer from one or more synthetic limitations for large-scale preparation of 2-Thiohydantoin derivatives due to their use of expensive, moisture sensitive and/or highly toxic starting materials and reagents. Moreover, the methods developed for combinatorial synthesis and used to prepare 2-Thiohydantoin derivatives in small quantities for purposes like biological testing may not be feasible when operated on a large scale.

Gamma-aminobutyric acid production through GABA shunt by the introduction of synthetic scaffolds in recombinant Escherichia coli

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Abstract:
Nylon 4 is a biodegradable polymer which can be produced from the monomer of pyrrolidone. Gamma-aminobutyric acid (GABA) is a precursor of pyrrolidone used for the production of bioplastics. In this study, Escherichia coli were engineered to produce gamma-aminobutyric acid from glucose via an alternative novel pathway by the introduction of synthetic scaffolds. The GABA pathway constructed contained succinate dehydrogenase, succinate-semialdehyde dehydrogenase and GABA aminotransferase to redirect the Krebs cycle flux to GABA production. By introduction of a synthetic scaffold, production of 0.64 g/L GABA was achieved at 30oC and pH 6.5. Final GABA concentration was increased by 11.3% via the inactivation of competing pathways, and higher initial glucose concentration led to the enhanced final GABA concentration of 1.01 g/L. This work was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, grant number: PJ01111601) by RDA, and Basic Science Research Program by the Ministry of Education (NRF-2014R1A1A2054726).

Genomic Characterization and Antimicrobial Resistance Profiles of *Escherichia coli* O157 and O157:H7 Isolated from Modified Atmosphere Packaged (MAP) Beef Meat

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Abstract:

The aim of this study is to detect *Escherichia coli* O157 and O157:H7 strains using conventional culture methods, confirmation via mPCR, determining the genomic characterization and phenotypic antimicrobial resistance profiles in samples of isolates obtained from Modified Atmosphere Packaged (MAP) minced and cubed beef meat products sold in the Samsun province. A total of 100 MAP meat (50 minced-50 cubed beef meat) samples were used as material in this study. It was detected that from the analysis that out of the 50 MAP minced beef meat samples 1 (1/50-2%) was contaminated with *E. coli* O157, 1(1/50-%2) with *E. coli* O157:H7 and from the 50 MAP cubed beef meat samples 1 (1/50-2%) was contaminated with *E. coli* O157. After conducting genotyping it was determined that *E. coli* O157 isolate obtained from the MAP minced beef meat samples carried stx_1, stx_2 genes; *E. coli* O157:H7 isolate carried stx_1, stx_2, *eaeA* and *hylA* genes while *E. coli* O157 isolate obtained from the MAP cubed beef meat samples only carried the stx_2 gene. From the phenotypic antimicrobial resistance profile that both *E. coli* O157 isolates were found only resistant against streptomycin whereas *E. coli* O157:H7 isolates were resistant to streptomycin, cephalothin, and tetracycline. As a result: in order to protect public health it is suggested; to keep in mind that products must meet proper hygienic and technical conditions during sale and storage; also in consideration of some isolates being resistant to antibiotics, use of uncontrolled antibiotics should be prevented.

Keywords: MAP minced and cubed beef meat, *Escherichia coli* O157:H7, mPCR, Antimicrobial resistance

Anti-inflammatory Effect of the *Strobilanthes crispus* methanolic extract on Lipopolysaccharide-stimulated RAW 264.7 Macrophages

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Abstract

Background, Inflammation is rapid response by body to deal with injuries, foreign particles and damaged cells. An unattended inflammation could lead to complication in cerebrovascular, cardiovascular system, joint and intestines. However, currently available conventional drugs exhibited adverse effects on many organ systems besides treating inflammation. *Strobilanthes crispus*, a native plant is believed to have anti-inflammatory property as it has been used in folk medicine to treat various diseases. Nevertheless, no scientific studies have been conducted to prove this traditional claim. Hence, this study focused on investigating the anti-inflammatory property of *S. crispus* on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages experimental model. **Methods,** The maximum non-toxic dose (MNTD) of *S. crispus* methanol extracts and optimum LPS concentration were determined prior to determination of anti-inflammatory effect of *S. crispus*. MNTD of *S. crispus* was determined using MTT assay and the optimum LPS was determined based on the production of nitric oxide (NO) using Griess reaction. Finally, the anti-inflammatory effect of *S. crispus* was determined by examining the NO and cytokines levels, namely interleukin-6 (IL-6) and interleukin-10 (IL-10) using Procarta immunoassay kit. **Results,** The MNTD for *S. crispus* leaves and stem extracts was 160 µg/mL and 1.5 µg/mL, respectively. The optimum LPS needed to induce maximum inflammation was 1 µg/mL. Upon pre-treatment with half MNTD (1/2MNTD) of leaf extract, the production of NO was significantly reduced while MNTD of stem extract resulted in an increase in IL-10 level. On the other hand, no significant reduction of IL-6 production was seen upon treatment except for indomethacin, which acted as the positive control drug. **Conclusions,** The present results showed that *S. crispus* could possess anti-inflammatory properties on lipopolysaccharide-stimulated RAW 264.7 macrophages through suppression of NO production and increase in IL-10 level.

Influence of ligands and protein binding to structure and flexibility of human dipeptidyl peptidase III

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Dipeptidyl peptidase III (DPP III), the sole member of the M49 family of metallopeptidases, is a two-domain zinc-dependent exopeptidase which takes part in the intracellular protein metabolism.[1] Human DPP III (h.DPP III) is also related to some pathophysiological processes like cataractogenesis[2], tumor growth[3] and influenza virus infection[4] but mechanisms are still unknown. Hast et al.[5] revealed that DPP III contributes to the activation of Nrf2 by binding to its negative regulator, repressor protein, Keap1. Nrf2-Keap1 signalling pathway is major regulator of cytoprotective responses to oxidative and electrophilic stress.

In order to rationalize experimental data and to understand behaviour of h.DPP III in the physiological conditions we performed an exhaustive *in silico* study of h.DPP III and its complexes with small ligands and Keap1.

A range of molecular dynamics simulation techniques, conventional, accelerated and steered were used to investigate the h.DPP III conformational landscape and the influence of ligand binding on the protein structure and dynamics.[6-10] According to the results, the compact forms of h.DPP III are more stable, but the open and partially closed states, spanning a wide range of conformations, can more effectively recognize the substrate which preferentially binds to the five-stranded ß-core of the lower DPP III domain. The simulations indicated existence of a dynamic equilibrium between open and semi-closed states and revealed two ways of the protein closure, leading to two distinct compact structures. The simulations clearly indicate that the mode of closure is determined by presence of a ligand.

Recently we have built the complex between h.DPP III and Keltch domain of Keap1. According to the preliminary simulations it seems that h.DPP III binds to Keap1 *via* the flexible loop of the upper, catalytic domain, containg an "ETGE" motif. The presence of the partner protein hinders the transformation of h.DPP III from the open to a semi-closed form observed during the protein simulation in solvent.

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DNA Binding Specificity of N-Terminal-Tetramethylguanidine-N-Methylpyrrole-N-Methylimidazole Asymmetric Hairpin Polyamides (TMG-AHP)

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Abstract

Long-term, persistent infection with high-risk strains of human papillomavirus (HPV) is the precursor of most cervical carcinomas and an increasing number of oropharyngeal squamous cell carcinomas. While vaccines are available for patients under twenty-six years old and protect from up to seven of 15-20 high-risk viral forms, there are no agents for the definitive antiviral treatment of pre-existing or future carcinogenic HPV infections. To treat current or future HPV infections, drugs that selectively target HPV but do not damage the host are necessary.

The antiviral candidates employed in the present study, NV1078 and NV1087, represent a subclass of long antiviral N-methylpyrrole N-methylimidazole (Py-Im) polyamides that have a modified N-terminal capping group, 1,1,3,3 tetramethylguanidine (TMG), and an asymmetric hairpin polyamide (AHP) structure. It has been demonstrated (Edwards, TG and Fisher, C, unpublished) that the two compounds possess exceptional antiviral activity against three high-risk HPV strains (HPV16, HPV18, and HPV31). NV1078 and 1087 eliminate or dramatically decrease the viral load of infected keratinocytes that maintain viral genomic DNA, or episomes. The TMG-AHPs, higher homologs of natural products distamycin A and netropsin, function by interfering with natural virus-host interactions and by stimulating the host cell's DNA Damage Response to destroy episomal viral DNA selectively.

The interactions between the above mentioned polyamides and the long control region of viral HPV16 and HPV18 DNA fragments were studied to determine the DNA sequence specificity and binding sites of the polyamides, to help establish the TMG-AHP's mechanism of action. Quantitative deoxyribonuclease I (DNase I) footprinting was used, along with affinity cleavage, to map and measure the interactions between fluorescently labeled DNA and the active TMG-AHPs. Binding locations on DNA were detected and quantified by capillary electrophoresis performed as a function of TMG-AHP concentration.

Results demonstrate that TMG-AHPs bind 13 nucleotides and do so with nanomolar affinity to the expected binding sites, as well as to one-, two-, and even three-mismatch binding sites. Moreover, it was observed that these AHPs have a conclusive preference within the LCR for the region adjacent to and overlapping the E1 and E2 viral protein binding sites, where both polyamides bind in a cluster. Data indicate that viral replication for HPV could be disturbed by the TMG-AHPs binding at numerous sites with high affinity over the sequence 7880-30 of the HPV16 viral LCR and at the preferred region of HPV18 LCR (7841-43). Coverage of viral DNA by AHPs is very high: for HPV16, the TMG-AHP binds 58% of the 365 bp from nt 7662-122, with similar results for HPV18. We expect this extensive binding by rigid TMG-AHPs to affect the structure of negatively-supercoiled viral episomes in dramatic fashion.

Keywords: HPV, hairpin polyamides, DNase I footprinting, affinity cleavage, capillary electrophoresis

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Cyclic lipodepsipeptide syringomycin E channels in heterogenic lipid membranes

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Syringomycin E (SRE) is a cyclic lipodepsipeptide that is produced by various strains of the plant bacterium *Pseudomonas syringae pv. syringae* (Sinden et al., Physiol. Plant Pathol., 1971; Gross and DeVay, J. Appl. Bacteriol., 1977). SRE forms anion-selective voltage-gated ion channels in planar lipid bilayers (Feigin et al., J. Membr. Biol., 1996; Schagina et al., Membr. Cell Biol., 1998; Ostroumova et al., FEBS Lett., 2005). We tested the effects of the dipole modifier agents, phloretin and RH 421, on conductance and lifetime of the single SRE channels in model heterogeneous sphingolipid-containing membranes. Virtually solvent-free bilayer lipid membranes were prepared using monolayer-opposition technique from equimolar mixture of phosphoethanolamine, phosphoserine and sterols (cholesterol or ergosterol) and 20 mol % sphingolipids (sphingomyelin, N-stearoyl-phytosphingosine or N-stearoyl-sphinganine) in 0.1 M KCl (pH 7.4). We demonstrated that the effects of dipole modifiers on SRE channel conductance and lifetime were dependent upon sphingolipid type and were not affected by membrane sterol content. A homogenous population of syringomycin pores was observed in lipid bilayers comprising phytosphingosine or sphinganine, while two types of channels with different sensitivity to dipole modifiers were found in sphingomyelin-containing bilayers. This finding might be due to differences in the lateral heterogeneity of the sphingomyelin and phytosphingosine- or sphinganine-containing membranes, suggesting that the distribution of SRE and dipole modifiers between ordered and disordered lipid domains differs between bilayers comprising these sphingolipids. The work was supported by the Russian Foundation of Science (# 14-14-00565), the Russian Foundation for Basic Research (# 16-04-00806) and SP-69.2015.4.

Indole-3-Acetic Acid Induced Alterations in Antioxidant Enzyme Activity of *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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Indole-3-acetic acid (IAA) is of great importance among natural auxins which promote plant growth and development. On the other hand, IAA has negative impacts on nontarget organism such as insects by altering development, survival, longevity, reproductive potential, hemolymph metabolites, and apoptotic indices. Microbial infections or pollutants/toxins such as UV radiations and pesticides trigger production of reactive oxygen species (ROS). When ROS is not sufficiently reduced by enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferases (GSTs), they cause damage to the organism by reacting with macromolecules of biological importance, such as lipids, proteins, and DNA, eventually leading to cell death. In the current study, we aimed to investigate the effects of various doses (0, 50, 500, 1,000, 5,000, and 10,000 ppm) of IAA on the antioxidant enzyme activity in *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae, which is an ubiquitous pest of honey bee colonies. Laboratory colonies of the greater wax moth, *G. mellonella* were established from adults reared at 25 ± 5°C, 60 ± 5% RH, and with a photoperiod of 12: 12 (L:D) h at Kocaeli University, Turkey. SOD activity was determined using commercial available assay kits. Absorbance was read in a microtiter plate and determined at 450 nm using xanthine and xanthine oxidase systems. GST activity was determined with 1-chloro-2,4-dinitrobenzene and reduced glutathione as substrates. The assay was carried out in a 96-well microtiter plate and absorbance was measured continuously at 340 nm for 5 min. CAT activity was determined by measuring the decrease in absorbance over a 3-min period at 240 nm due to hydrogen peroxide decomposition. Treatment with IAA in diet resulted in a decrease in the activities of CAT, SOD, and GST at higher doses of 500, 1,000, 5,000, and 10,000 ppm with respect to control. The most effective decrease was observed in CAT and SOD activity at 500 ppm by >40% and in GST activity at 1,000 ppm by >60%. Increased oxidative stress kills cells either by necrosis or by apoptosis. Several reports have demonstrated that plant growth regulators, including IAA, cause an increase in apoptotic indices in insects. These findings indicated that IAA treatment in *G. mellonella* larvae may lead to excessive apoptosis by increasing ROS production and decreasing activity in antioxidant defense systems.

Keywords: Catalase, *Galleria mellonella*, Glutathione S-transferase, Indole-3-acetic acid, Superoxide dismutase.

Gibberellic Acid Induced Genomic Variations in *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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Gibberellic acid (GA_3) is a planth growth regulator (PGR) which stimulates plant growth and development in a compliance with other plant hormones. Previously, we investigated genotoxic effects of GA_3 on hemocytes of *Galleria mellonella* L. (Lepidoptera: Pyralidae) by using comet assay. Our results revealed that genetic damage index of hemocytes of GA_3 treated *G. mellonella* larvae was increased significantly compared to control at all tested doses (50-5,000 ppm). In the current study, genotoxic effects of GA_3 on the genomic DNA of *G. mellonella* were investigated by using random amplification of polymorphic DNA by the polymerase chain reaction (RAPD-PCR). *G. mellonella* is ubiquitous pest of honey bee colonies. Among all the developmental stages, only larval forms of *G. mellonella* feed on honey combs and cause damage in apiculture. Laboratory colonies of the greater wax moth, *G. mellonella* were established from adults reared at 25 ± 5°C, 60 ± 5% RH, and with a photoperiod of 12: 12 (L:D) h at Kocaeli University, Turkey. Newly hatched larvae of *G. mellonella* were reared on various doses (0, 50, 500, 1,000 and 5,000ppm) of GA_3 treated synthetic diet. Genomic DNA of *G. mellonella* larvae was extracted by phenol-chloroform-isoamyl alcohol method. The RAPD-PCR technique is sensitive to shape of the temperature profile, type of polymerase and concentration of Mg^{2+}, Taq, and DNA. Reactions were standardized and all PCR reactions were run on the same thermal cycler. This is the first study to use RAPD-PCR technique to identify genomic variations in *G. mellonella*. We used OPA-1 to 10 primers. In the control group of OPA-1 to 10 have 15, 10, 12, 9, 11, 9, 9, 9, 15, 12 bands with different molecular weights, respectively. All primers showed DNA length polymorphism at all tested doses. The observations presented here lead us to conclude that GA_3 has genotoxic effects on *G. mellonella.* Our findings were compatible with our previous study that showed genotoxicity of GA_3 by comet assay. Thus, we suggested that both RAPD-PCR and comet assay can be used to investigate genotoxic effects of chemicals such as PGRs.

Keywords: *Galleria mellonella*, Genotoxicity, Gibberellic acid, Polymorphism, RAPD-PCR.

Haptoglobin as an Antioxidant

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Among many environmental factors acting on biological objects, influence of non-ionizing electromagnetic radiation (EMR) is the most interesting in terms of oxidative metabolism. This interest is futher enhanced by the increasing penetration of technologies related to the EMR, in particular, microwave, into people's lives; today, radiation sources for medical (diagnostics, physiotherapy), appliances (TV, microwave devices) and military (radar systems) purposes fill the habitat of all living things. The participation of free radical oxidation processes is suggested to occur in the mechanism of microwaves' effects which finds more evidence in experimental facts. This mechanism may be mediated by free radical chain process, including lipid peroxidation. For inhibition of lipid peroxidation process, there is an antioxidant system in tissues and organs. Haptoglobin as an antioxidant involved in the regulation of hormonal processes and lipid peroxidation. One of the functions of haptoglobin is to bind the hemoglobin released from disrupted erythrocytes and thereby prevent iron loss by organism. The complex with hemoglobin works as peroxidase. It also concurrently breaks cathepsin C and cathepsin BnL and limits utilization of oxygen by pathogenic bacteria.

The aim of our experimental investigation is to study the effect of total body irradiation with non-ionizing EMR on blood haptoglobin level.

Investigations were carried out on Wistar rats weighting 250-300 g. Animals were divided into experimental and control groups. The experimental group of animals irradiated with 460 MHz EMR from apparatus "Volna-2" (Russia). Animals were exposed to EMR for 20 minutes daily to 4 weeks at a flux density of 30 mW/sm^2. The content of haptoglobin was determined by the method Prohurovskaya & Movshovich (1972).

Results show that prolonged exposure of animals up to 3 weeks at the above flux density leads to increased serum haptoglobin to 34.72±1.87mg%, whereas control animals' level of haptoglobin was 24,05±0.73 mg % ($p<0,05$). After 4 weeks irradiation, serum haptoglobin level decreased to 23.78±3.08 mg%. Earlier in our experiments, we have shown that EMR 460 MHz for a prolonged irradiation under the same conditions leads to increased concentrations of lipid peroxidation products in blood of rats. It is known that increased lipid peroxidation leads to the release of heme iron, which catalyzes hydroxyl radicals' formation. It is also known that in various oxidizing effects on red blood cells, there have been observed oxidation and denaturation of hemoglobin (the formation of the so-called Heinz bodies), accompanied by the release of heme/hemin (Ferriprotoporphyrin IX). It is well known that the exogenous hemin easily integrated into the membrane, destabilizing it and causing hemolysis. On the other hand, peroxidase activity of oxy- and methemoglobin with haptoglobin binding increases by 20-30 times. So, haptoglobin level determining in plasma can have great diagnostic value. Furthermore, binding of haptoglobin to hemoglobin may probably prevent hemoglobin participation in free radical reactions.

Transcriptional regulation of Oct4/Sox2 in *Hoxb1* and *Fgf4* DNA complexes - an *in silico* approach

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Abstract:

Stem cells are characterized by their infinite division, and the capability to differentiate into any other cell types. Both of these properties are highly dependent on Oct4 (Octamer-binding transcription factor 4) and Sox2 (sex determining region Y (SRY)-box 2) proteins, whose interaction on various transcriptional regulators perpetuates the pluripotency of the cells. The Oct4 and Sox2 can induce the expression of *Hoxb1* and *Fgf4* genes as their promoters have Oct4/Sox2 binding sites. In this study, the structural mechanism of transcriptional regulation of Oct4/Sox2 proteins in *Hoxb1* (Oct4/Sox2^{0bp}) and *Fgf4* (Oct4/Sox2^{3bp}) complexes has been elucidated by molecular dynamics simulations along with binding free energy studies. Our results indicate that both proteins exhibit a variable conformational sampling space with distinct hot spot residues on these promoters. In addition to this, dynamic cross-correlation matrices are significantly different for both complexes with exposed and closed structural conformation of these proteins on *Fgf4* and *Hoxb1* promoter complexes respectively. Binding free energies are higher for *Fgf4* complex, as well as linker region in Oct4 display higher fluctuation in *Fgf4* promoter complex. These conformational variations plausibly explain the qualitative binding and transcriptional potential of Oct4/Sox2 heterodimer on these target genes.

Comparative phytochemical analyses and *in-vitro* antioxidant activity of aqueous and ethanol extracts of *Simarouba glauca*

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ABSTRACT

Aims: The study was conducted to evaluate the phytochemical and antioxidant potentials of ethanol and aqueous leaf extracts of *Simarouba glauca* vis-à-vis standard antioxidants.

Study design: True experimental study.

Place and Duration of Study: Department of Biochemistry, University of Benin, Benin City. Nigeria, between August and October 2015.

Methodology: Samples were harvested, air dried, pulverized and extracted with aqueous and absolute ethanol; freeze dried at the National energy commission centre, University of Benin. Total phenol content was determined by Folin-ciocalteau method, tannin determined according to Folin and Denis methods while flavonoids content was determined according to the methods described by Ebrahimzadeh *et a*l. DPPH radical scavenging activity was conducted based on the ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. Reducing power activity of extracts was conducted based on test samples extract' ability to reduce ferricyanide to ferrocyanide indicated in the colour change. Total antioxidant activity of ethanol and aqueous leaf extracts was determined based on the ability of the sample to reduce the ferric-tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)-TPTZ) at low pH. Hydroxyl radical activity of extracts

was conducted on the principle based on the ability of test samples to reduce H_2O_2 in the presence of 1,10-phenanthroline. Trolox equivalent antioxidant activity of extracts was conducted based on the ability of test sample to scavenge 2,2'azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^+$) radical generated based on the principle of decolourization. Nitric oxide (NO.) radical scavenging activity of S, glauca leaf extracts was estimated based on the ability of test samples to scavenge radicals generated by the reaction of naphthylethylenediamine dihydrochloride. Butylated hydroxytuolene (BHT), Ascorbate, Quercetin and Trolox were standard antioxidant.

Results: DPPH radical scavenging activity yielded aqueous and ethanol extracts IC_{50} values of 3.2144 and 4.9100 µg/ml respectively. Reducing power activity yielded (aqueous and ethanol extracts) EC_{50} of values 60.3233 and 60.1000 µg/ml respectively. Total antioxidant activity yielded (ethanol and aqueous extracts) IC_{50} values of 52.4320 and 68.8201 µg/ml respectively. Hydroxyl radical activity yielded (ethanol and aqueous extracts) IC_{50} values of 49.3130 and 50.2341 µg/ml respectively. Trolox equivalent antioxidant activity yielded (ethanol and aqueous extracts) IC_{50} values of 45.2015 and 52.0721 µg/ml respectively. Nitric oxide scavenging activity yielded aqueous IC_{50} value of 14.2102 µg/ml but ethanol extract yielded no inhibition concentration at 50 percent.

Conclusion: The study showed that aqueous and ethanol leaf extracts of *S. glauca* demonstrated substantial amount of biochemically valuable phytochemicals and antioxidant potential capable of scavenging reactive oxygen species.

Keywords*: Simarouba glauca, Phytochemicals, Oxidants, Radical Scavenging properties.*

Antidiabetic, Anti-α-Amylase and Anti-α-Glucosidase Effects of Olive Leaf Extract

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Abstract:

Diabetes is a global health problem that all nations suffer from all over the world. Diabetes Mellitus is a heterogenic syndrome group accompanied by partly or totally lack of insulin hormone or it is characterized with hyperglycemic concomitant with resistance against insulin. Keeping blood glucose level in a certain level is important for diabetic individuals. Because of this some medicine were developed. Although many medicines treat disease they have inevitable side effects. Recently scientifically proven that some nutrition is effective to prevent and treat diseases in natural way which increases the importance of nutrition support to protect people's health. Therefore, using functional nutrition's as a nutraceutical and therapeutic way is becoming popular. Olive products have a special importance in Mediterranean diet and olive leaf is used to treat diabetes by local people in Turkey. Oleuropein is a main compound consisting of olive leaf's polyphenolic profile. It is a natural bioactive compound coming from secoiridoid groups.

The purpose of this study is to investigate the antidiabetic effects of olive leaf extract (OLE) and α-Amylase and α-Glucosidase enzyme inhibition of OLE on *in vivo* experimental diabetes. In order to achieve this one control group, one diabetic group, three diabetic OLE group (*25-50* and *100* mg/kg bw), one diabetic infusion group, and one Acarbose group were formed by using *Wistar albino* rats. All diabetic groups were created with STZ-induced.

Blood glucose levels, percentage of $HbA_{1c,}$ pancreas α-Amylase and intestinal α-Glucosidase enzymes activities of rats were evaluated. Blood glucose level of all groups that administered OLE was decreased compared to diabetic group, but statistically significant difference was only found in OLE+25 group. Although percentages of HbA_{1c} increased in diabetic group, it decreased in OLE, infusion and Acarbose groups. α-Amylase activity decreased statistically in all diabetic treated groups compared to diabetic group. Similarly, although decrease in α-Glucosidase activity was observed, statistically significant difference is only determined in OLE-25 group.

Findings of this study revealed that OLE is effective in controlling blood glucose level and this effect is more efficient on 25 mg/kg dose. Controlling hyperglycemia helped to decrease percentages of HbA_{1c}. It is assumed that OLE could have an insulin-like effect. It might also be effective on decreasing blood glucose level by inhibiting carbohydrate digestion enzyme, α-Amylase and α-Glucosidase, or down regulating gene expressions of those enzymes. Considering the fact that OLE does not have any side effects, it could be a better alternative for fitoformulation than the antidiabetic drugs.

Protective Effect of Astaxanthin against Oxidative Stress in SH-SY5Y Cells Stimulated with Lipopolysaccharide

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Astaxanthin (ASTA), is a red-orange carotenoid with powerful antioxidant that occurs naturally in a wide variety of living organisms. Recently, ASTA studies have focused on several biological functions such as radical scavenging, singlet oxygen quenching, anti-carcinogenesis, anti-diabetic, anti-obesity, anti-inflammatory and immune enhancement activities. More importantly, there is evidence demonstrating that ASTA confers neuroprotective effects in experimental models of chronic neurodegenerative disorders, and neurological diseases.

In this study, SH-SY5Y cells were pre-treated with ASTA for 1 h, washed, and then treated with LPS for an additional 24 h. Control cells did not contain ASTA and/or LPS. We investigated the potential role protective of ASTA in restoring physiological conditions in SH-SY5Y cells stimulated with LPS (10 mg/ml). We evaluated the effect of ASTA and LPS on cell viability in the SH-SY5Y cell line. An MTT reduction experiment showed that ASTA did not exhibit any cytotoxic effects on SH-SY5Y cells at concentrations of 10 mM. Hence, for all experiments this concentration of ASTA was employed.

Our results show that pre-treatment with ASTA (10 mM) for 1 h attenuates the LPS-induced toxicity and ROS production. The beneficial effect of ASTA is associated with a reduction intracellular O_2^- production by restoring the antioxidant network activity of superoxide dismutase (SOD) and catalase (CAT), which influence HO-1 expression.

Consequently ASTA exerted a protective effect against LPS induced cytotoxicity. We accordingly hypothesize that ASTA has therapeutic properties protecting SH-SY5Y cells from LPS-induced inflammatory and oxidative stress.

Evaluation of The Rapid Serum Tube for Troponin I on the Cobas E601 Analyzer

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Background: Recently developed BD Vacutainer(®) Rapid Serum Tube (RST) (Becton–Dickinson and Company, Franklin Lakes, NJ) provides rapid clotting time allowing fast serum separation.

Aim: The aim of this study is comparison of Troponin I results in RST with BD Vacutainer(®) Serum Separating Tubes II Advance Tube (SST).

Methods: Serum samples were collected with intravenous cannula simultaneously into BD Vacutainer(®) RST and BD Vacutainer(®) SST Tubes from patients admitted to emergency department over a 1 month period. Troponin I (Roche Diagnostics, Mannheim, Germany) were analysed on the Roche Cobas e601 (Roche Diagnostics, Mannheim, Germany) analyzer. The obtained datas were evaluated by the way of regression analysis and Bland Altman graphics.

Results: The comparison with the RST samples and the SST samples, equations were found as y=1.007x-0.02 in lineer regression analysis.

Conclusion: The RST samples provide acceptable performance for Troponin I analysis. In order to reduce the turnaround time for Troponin I on emergency department, using the RST will be convenient .

Keywords: Blood specimen collection, serum, Troponin I

An insight to dioxygenases from 3-nitrotoluene degrading Strain *Diaphorobacter* sp. DS2

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A large number of bacterial strains have been isolated for the degradation of nitroaromatic compounds which are major pollutants of our industrialized world. *Diaphorobacter sp.* Strain DS2 is one such example which mineralizes 3-nitrotoluene (3-NT) and uses it as a sole source of carbon and energy. The degradation pathway of 3-NT involves two major dioxygenase systems. The first step involves, 3-nitrotoluene dioxygenase (3-NTDO) which is a multi-component enzyme system that converts 3-NT to methyl catechol. In the second step, it is further converted to 2-hydroxymuconate semialdehyde by catechol-2, 3-dioxygenase (C23O). C23O is an extradiol type dioxygenase leading to aromatic ring fission. Previously, our group solved the crystal structures of the ferredoxin and oxygenase components of 3-NTDO with 2.4Å and 2.9Å resolution respectively from *diaphorobacter* strain DS2 to gain a further insight about the enzyme. We then carried out site-directed mutagenic studies of 3-NTDO based on the active site information from nitrobenzene dioxygenase (NBDO) and naphthalene dioxygenase (NDO). These mutants have the ability to catalyse wide range of nitroaromatic and aromatic substrates. This study can further be applied to enzyme mediated synthesis of compounds that are otherwise not so feasible via conventional methods. Most recently, we amplified, sequenced and analysed C23O for its evolutionary patterns. Whole genome sequencing of *diaphorobacter* sp. DS2 revealed its genome size to be 4.5 Mb. A detailed study is presently being carried out to mine for potential biosynthetic pathways that may be involved in degradation of such classes of compounds.

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Biochemical identification of an anaerobic oral microflora in healthy and autistic children

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Abstract

Considering a proportion of 10% oral bacterial assessment with a precision of 9% and a confidence coefficient of 95%, forty-three subjects were tested. Preliminary examination included morphological identification followed by staining of pure colony by Gram stain. Confirmatory bacterial identification was done using API Rapid ID 32 A Kit (bioMérieux® SA, Marcy-l'Etoile, France). Biochemical identification of bacteria in collected plaque samples from healthy and autistic samples was done using kit for anaerobes using 29 miniaturized enzymatic tests and a database. Eleven different bacterial species were identified from collected samples in both groups, however *Prevotella intermedia* and *Porphyromonas gingivalis* were not detected in any of the samples.

Conformational study of *Bacteroides thetaiotaomicron* dipeptidyl peptidase III

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Bacteroides thetaiotaomicron, a Gram-negative anaerobe, is a dominant member of human intenstinal microbiota[1]. As such, it is of great importance in understanding the symbiotic host-bacterial relationship within the human intestine. Dipeptidyl peptidase III isolated from *Bacteroides thetaiotaomicron* (Bt.DPP III) is a two-domain zinc exopeptidase from the M49 family. Members of this family, characterized by their HEXXGH motive, cleave dipeptidyl residues from the N-terminus of their substrates. This conserved region contains two His residues that coordinate the Zn ion along with Glu449 and Glu476. The crystal structure[2] of Bt.DPP III reveals a two-domain molecule, with a cleft inbetween, strongly resembling the human DPP III, despite their low sequence identity (~23%).

In this work we used classical and accelerated molecular dynamics simulations (MD) to examine the long-range conformational changes of the enzyme over the course of 200 ns and compared them to its human counterpart. In order to determine the best method for the enzyme and complex description severa force fields, ff03, ff12SB and ff14SB were utilized. We identified two distinct Bt.DPP III conformers, open and closed. Special emphasis has been placed on the zinc ion coordination flexibility, since the existing data for human DPP III suggests the high plasticity of the Zn^{2+} coordination[3].

The syntetic substrates Arg-Arg-2-naphtylamide and Lys-Ala-2-naphtylamide were docked into the active site of the wild type protein and of the C450S mutant and the solvated complexes were simulated for at least 200 ns at room temperature. In order to understand the enzyme-ligand interactions the results of simulation were compared with the experimental data[4].

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Antibacterial Activity and Development of LbL films Containing Peptide of the Cry1Ab16 Toxin from *Bacillus thuringiensis* for Food Control Applications

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Introduction. Peptides are potential candidates to meet the needs of the modern world in relation to diagnosis, disease monitoring, quality control in industry, and more recently, detection of genetically modified organisms (GMOs) and food security through the development of biosensors. An important group of proteins are insecticidal crystal proteins (ICPs) such as Cry1Ab toxin. The peptide PcL342-354C was obtained from the Cry1Ab16 toxin present in *Bacillus thuringiensis* (Plácido et al., 2016).

Objectives. Synthesis, characterization and antibacterial activity of LbL films containing peptide of the Cry1Ab16 toxin from *Bacillus thuringiensis* for food control and biotechnological Applications. Materials and Methods. Cry1Ab16 protein sequence was retrieved from the NCBI database (access number AAK55546) and peptide was selected by *in silico* evaluation based on their sequence and immunogenicity. Peptides was manually synthesized using a solid phase approach with Fmoc/t-butyl chemistry. To estimate the secondary structure of the peptide by Far-UV CD spectroscopy. To MIC of peptide, realized the broth microdilution test. Therefore were used three strains of *Escherichia coli*: *E. coli* ATCC 259922, *E. coli* ATCC 35218 and *E. coli* ML1 (clinical specimen) with concentrations ranging from 3.90-500 μg/mL. After determining the MIC proceeded to the determination of MBC. LbL films

(peptide and cashew gum) were tested against *Escherichia coli* (NCTC 9001) in a ring diffusion method is a kind of semi-quantitative test to the antimicrobial activities of the films deposited onto glass plates. Representative films and morphological effect of peptide on *E. coli* ATCC 25922 were examined using AFM. The analysis was carried on the samples in vibrating (tapping) mode. Imaging was performed using a TT-AFM instrument.

Results and Discussion. The PcL342-354C peptide showed a MIC equal to 31.25 μg/mL for *E. coli* ATCC 25922 and *E. coli* ML1 and MIC equal to 15.62 μg/mL for *E. coli* ATCC 35218. The MBC for all strains used was equal to 250 μg/mL, proving a potential antibacterial activity against Gram-negative bacteria. The morphological effect of peptide at MIC concentration on *E.coli* was significant. The morphology of the Cashew gum/PcL342-354C LbL film was analysed using atomic force microscopy (AFM) presented a nanometer regular surface in addition to maintain antimicrobial activity against *E. coli*. In general, the thorough characterization of the peptide and peptide/ cashew gum film showed that this system is not only homogeneous and low cost, but is also reproducible, which opens prospects for application of this film in sensors among other biotechnological applications.

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NISCH AND *CDH1* PROMOTER HYPERMETHYLATION, SMOKING AND LUNG CANCER

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Background: The high mortality of lung cancer is attributable to presence of metastatic disease in nearly two-thirds of patients at diagnosis. Detection of early stage lung cancer amenable to curative resection could boost survival.

Cancer specific methylation patterns of tumor suppressor genes, which precede precursor lesions, could possibly herald earlier diagnosis.

Objective:

- Evaluate frequency of promoter hypermethylation of *NISCH* and *CDH1* in cfDNA of lung cancer patients and correlation with clinicopathological variables.
- Evaluate possible correlation between *NISCH* methylation and smoking
- Compare serum nischarin levels among lung cancer cases, smoker and nonsmoker controls.

Materials and Methods: Forty histopathologically confirmed lung cancer cases, thirty smoker and thirty non-smoker controls were enrolled. Plasma cfDNA was extracted and subjected to bisulfite treatment followed by MS-PCR. Serum nischarin levels were estimated using double antibody sandwich ELISA (Qayee bio). Statistical analysis was performed using SPSS22.0.

Results: The promoter hypermethylation of both *NISCH* and *CDH1* was significantly higher in lung cancer patients ($P<0.05$). *NISCH* was methylated more frequently in non-cancerous smokers as compared to lifelong nonsmoker controls ($P<0.05$) but was independent of the smoking status of cancer cases. Pack years and packs per day were significantly higher in the methylated group. No significant association was found type or duration of smoking or with staging or histological grading. No signification correlation of serum nischarin levels with cancer status, smoking or *NISCH* methylation was found.

Conclusions:

- *NISCH* and *CDH1* are highly methylated in plasma cfDNA of lung cancer patients and hence could be used as a part of blood-based biomarker panel for early diagnosis.
- Since *NISCH* is highly methylated in both high risk smoker controls as well as cancerous-nonsmokers, *NISCH* methylation may mark the convergence of varied etiologies of lung cancer. It may be investigated as a universal therapeutic target for lung cancers regardless of clinicopathological heterogeneity.

Serum Neutrophil Gelatinase Associated Lipocalin levels in Algerian type 2 diabetes patients

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Abstract:

Lipocalin2 known as neutrophil gelatinase associated lipocalin (NGAL),is belonging to lipocalin family. Previous studies of NGAL have been focused on its role as a biomarker in diverse diseases,

including metabolic disease and diabetic nephropathy. However, the association of NGAL with diabetic vascular complications is still unclear.

The aim of our study is to compare and correlate serum NGAL with serum and urine creatinine,creatinine clearance and microalbuminuria(alb/crea),as biomarkers of glomerular injury in type2 diabetic patients.

This study included 17males and 13females with an age range between 20-78years old. These type2 diabetic patients were treated in diabetology unit of military hospital of Algiers.

Creatinine, creatinine clearance, glycated hemoglobin,fast serum glucose levels and microalbuminuria (Alb/Creat) were evaluated and correlated to serum NGAL.

The results showed that NGALis positively correlated with serum creatinine , microalbuminuria ratio (alb/crea),glycated hemoglobin, creatinine clearance and inversely correlated with glucose level and urinary creatinine.

In conclusion, serum levels of NGAL might become a new tool for the evaluation of renal involvement in the progression of diabetes and to complete this work urine NGAL must be evaluated..

The LINK to a Ying and Yang Tale of Enzyme Allostery

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Diaminopimelate (DAP) pathway is responsible for *de novo* synthesis of an essential amino acid *L*-lysine and its immediate metabolic precursor *meso*-DAP among plants and bacteria. The first committed step of the pathway is catalysed by dihydrodipicolinate synthase (DHDPS). DHDPS is an allosteric enzyme that binds *L*-lysine in a classical feedback process, but the underlying molecular mechanism is poorly understood.

Analytical ultracentrifugation studies on DHDPS from the wheat plant *Triticum aestivum* and the bacterial pathogen *Vibrio cholerae* (*Vc*) intriguingly show *L*-lysine induced quaternary structural transition. *L*-lysine dissociates wheat DHDPS tetramers to dimers in a cooperative manner, but induces association of dimers to tetramers in the bacterial ortholog (*Vc*-DHDPS) non-cooperatively. SAXS analyses subsequently demonstrate that the lysine-bound *Vc*-DHDPS tetramer surprisingly adopts a unique quaternary architecture. A low resolution X-ray structure (3.3 Å) reveals that the lysine-bound *Vc*-DHDPS forms a perpendicular back-to-back dimer-of-dimers that is yet to be seen for a DHDPS structure to date. We, therefore propose a new allosteric phenomenon coined the ***L**igand-**I**nduced dis/associatio**N** by lysine (K)* **(LINK)** model that displays molecular disparity across the plant and bacterial kingdoms.

Enzyme Encounter Complexes Functioning in Lysine Biosynthesis

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Enzyme encounter complexes have recently been of interest as a possible drug target, and are defined as a substrate transfer in a catalytic pathway.

The diaminopimelate (DAP) biosynthesis pathway is essential to plants and bacteria, including cyanobacteria, but is absent in animals. The pathway thus represents a promising target for the discovery of new herbicides and antimicrobial agents, including environmentally important anti-cyanobacterial agents. The DAP pathway commences with a condensation reaction catalysed by dihydropicolinate synthase (DHDPS) followed then by a reduction reaction catalysed by dihydrodipicolinate reductase (DHDPR). These enzymes are well characterised in Gram negative bacteria, Gram positive bacteria and plants, but little is known about the structure and function of cyanobacterial orthologues. We therefore set out to characterise DHDPS and DHDPR both in solution and the crystal state from the environmentally and industrially important cyanobacterial species, *Anabaena variabillis* (Av).

AvDHDPS and AvDHDPR were successfully cloned, expressed, purified to homogeneity, and crystallised. The crystal structures of the recombinant enzymes were subsequently determined to a resolution of 1.9 and 2.8 Å, respectively, showing that both enzymes adopt canonical bacterial homo-tetrameric structures.

Subsequent kinetic analyses of DHDPS and DHDPR from *A. variabilis* compared to the Gram negative *Escherichia coli*, and plant *Vitis vinifera*, reveal that the optimal reaction rate occurs when DHDPS is coupled with DHDPR from the same species. This suggests that DHDPS-DHDPR form species-specific encounter complexes during catalysis.

In silico modelling shows electrostatic matching when DHDPS and DHDPR from the same species are modelled together as a proposed encounter complex model. Likewise, electrostatic mismatching is observed when modelling enzymes from two different species.

Biophysical evidence of complex formation was obtained using analytical ultracentrifugation. Further validation of encounter complex formation is currently being pursued *ex vivo* using polyclonal antibodies raised against recombinant enzymes with promising results.

The results of this study have afforded new knowledge of an exciting class of transient protein-protein interactions (i.e. encounter complexes), that offer potential to be targeted for species-specific inhibition.

Association of Oxidized Low Density Lipoprotein Receptor 1 (OLR1) Gene Polymorphism and Serum Levels of Oxidized LDL and Paraoxonase in Patients with Metabolic Syndrome

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Background: Metabolic syndrome (MetS) is a cluster of the most dangerous cardiovascular disease risk factors: abdominal obesity, raised fasting plasma glucose, high serum Triglyceride, low HDL-C and high blood pressure. The factors that characterize MetS are also associated with the atherosclerotic process, in which an important role is played by oxidized LDL. OLR1 is a cell surface endocytosis receptor that recognizes, internalizes and degrades oxidized LDL in vascular endothelium and plays a role in the pathogenesis of atherosclerosis. Paraoxonase is a pleiotropic enzyme with the capacity to neutralize highly toxic xenobiotic compounds, such as paraoxon; and also attributes to the antioxidant properties of HDL.

Objective: The aim was to explore the association of *OLR1* gene polymorphism in patients with MetS and to measure the serum levels of oxidized LDL and Paraoxonase in patients with MetS in Indian population - a population with an increased prevalence of coronary artery disease and Type 2 Diabetes Mellitus.

Materials and methods: Forty cases fulfilling the IDF diagnostic criteria and 40 age and sex matched healthy controls were genotyped for *OLR1* gene (SNP: IVS4–73C>T, rs3736234) by RFLP-PCR. Serum oxidized LDL and Paraoxonase were estimated by ELISA. Association between the gene polymorphism and occurrence of MetS was estimated by Odds ratio, which was calculated by Unconditional Logistic Regression models.

Results: The T allele of *OLR-1*: IVS4-73 C>T SNP is associated with significantly increased risk of developing MetS (OR: 14.79, 95% CI: 1.80-121.2, $p < 0.05$). Of all the components and features of Metabolic Syndrome, there is a positive association of the TT genotype with waist circumstance ($p = 0.0017$ in comparison to CT genotype). Serum oxidized LDL and Paraoxonase levels were significantly increased in the cases as compared to controls ($p < 0.0001$, 0.0003 respectively), but no association of these two parameters were found with the SNP.

Conclusion: The intronic SNP: IVS4-73 C>T of *OLR1* gene has significantly increased risk of developing MetS. Also the risk genotype TT was significantly associated with increased waist circumstance, indicating the association of *OLR1* gene with obesity. Oxidized LDL and the antioxidant Paraoxonase might contribute in the pathogenesis of MetS.

The influence of novel Bisnaphthalimidopropyl derivatives (BNIPs) on proliferation, DNA damage, cell cycle progression and apoptosis in triple negative breast cancer cells *in vitro*.

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Bisnaphthalimidopropyl (BNIP) derivatives are a family of DNA intercalating compounds that exert anti-cancer activity against a variety of cancer cell lines *in vitro* [1,2]. Variations in the linker sequence have been found to improve the aqueous solubility and cytotoxic activity of BNIP derivatives, thus enhancing their potential application as anticancer drugs [3,4]. The aim of this study was to investigate the effect of three novel BNIP derivatives against triple negative human breast carcinoma cells and their mode of action, with regards to cell death. Bisnaphthalimidopropyl-piperidylpropane (BNIPPiProp), bisnaphthalimidopropyl- ethylenedipiperidine dihydrobromide (BNIPPiEth) and (*trans(trans)*-4,4'-methylenebis-cyclohexylamine *(trans,trans*-bisnaphthalimidopropyl diaminodicyclohexylmethane or *trans,trans*-BNIPDaCHM) were synthesised and characterised for the first time. The chemical structure of the BNIP derivatives was confirmed by Thin Layer Chromatography (TLC), Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS) while their DNA binding properties were determined by UV and fluorescence spectroscopy. Cytotoxicity of BNIP derivatives was assessed against human breast cancer MDA-MB-231 cells by MTT assay [5], resulting in IC_{50} values between 1.4-3.3 μM after 24 hours treatment. In addition, using the

single cell gel electrophoresis (COMET) assay [6], it was found that BNIPPiProp, BNIPPiEth and *trans,trans*-BNIPDaCHM, showed significant DNA damage to treated MDA-MB-231 cells after 24 hours treatment. Intracellular reactive oxygen species (ROS) accumulation was studied by flow cytometry, showing that the generation of ROS levels was induced after 4, 8 and 12 hours treatment with BNIP derivatives. After cell synchronisation, cell cycle distribution was conducted using propidium iodide (PI) staining of MDA-MB-231 cells and indicated that *trans,trans*-BNIPDaCHM induces sub-G1 cell cycle arrest, which has been previously associated with apoptotic cell death [7]. In particular, an increase of 139% and 142% in sub-G1 cell population after 24 hours treatment was observed with 1 μM *trans,trans*-BNIPDaCHM and 6 μM Camptothecin (positive control for sub-G1 arrest), respectively, compared to untreated cells. The above findings indicate that BNIPPiProp, BNIPPiEth and *trans,trans*-BNIPDaCHM exhibit dose-dependent cytotoxic effects *in vitro* (*trans,trans*-BNIPDaCHM>BNIPPiEth>BNIPPiProp), induce DNA damage and enhance ROS accumulation. Further investigation to confirm apoptotic cell death is on going and this could define the potential use of BNIP derivatives as anti-cancer agents in the future.

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Synthesis of new Co(II) complexes with 3-methoxysalicylaldehyde based hydrazones as a possible approach in the treatment of cobalt poisoning

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Hydrazones play an important role in bioinorganic chemistry as they easy form stable complexes with most of the transition metals. Many hydrazones are used as chelating agents in medical treatment for reducing the toxic effects of metals. By coordinating with metal ions these compounds can promote the excretion of the metals out the body.

Cobalt ions are essential to human body as the part of Vitamin B_{12}. Although cobalt is important for human health excess of cobalt can be harmful.

Two new Co (II) complexes with 3-methoxysalicylaldehyde-4-hydroxybenzoylhydrazone and 3-methoxysalicylaldehyde isonicotinoylhydrazone have been synthesized as a possible approach in the treatment of harmful health effects of cobalt poisoning. The hydrazones reacted with cobalt ions as monobasic tridentate ligands to yield mononuclear complexes with 1:2 metal:ligand molar ratio. The cobalt complexes were characterized by elemental analyses and IR spectroscopy. The spectral data of the complexes were interpreted on the basis of comparison with the spectra of the free ligands. This analysis revealed coordination to the metal ion through phenolic-oxygen, azomethine-nitrogen and amide-oxygen atoms. The complexes are quite stable and therefore these aroylhydrazones can be used as chelators in the cases of poisonings with cobalt.

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Design, molecular properties and *in vitro* cytotoxic activity of 3,5-dichlorosubstituted salicylaldehyde benzoylhydrazones

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Salicylaldehyde benzoyl hydrazone (SBH) belongs to a class of hydrazones of the type R'-CH=N-NH-CO-R which possess a high antiproliferative activity. The common method for the synthesis of SBH is the Schiff base condensation between salicylaldehyde and benzhydrazide. Various derivatives of SBH have been designed in order to discover new more effective antiproliferative compounds. The inserting of the halogen atoms in the molecules of different hydrazones strongly influences the biological activity of the compounds.

Novel 3,5-dichlorosubstituted salicylaldehyde benzoylhydrazone derivatives were designed by varying the type of substituents at 4th position of hydrazide moiety. The molecular properties of the compounds, important for drug pharmacokinetics in the human body, were assessed with the Lipinski's rule of five. *In silico* evaluation of the value of logP (partition coefficient) and the remaining parameters of drug similarity, as well as the topological polar surface area and absorption percentage, were used to find the lead candidates with encouraging properties for further elaboration. Some of the investigated 3,5-dichloro substituted hydrazones were further tested for *in vitro* cytotoxicity on a K-562 chronic myeloid leukemia cell line by MTT-test. The bioassay results demonstrated that the compounds exhibit concentration-dependent cytotoxic effects at low micro molar concentrations. The values of IC_{50} are less, but comparable to these of Cisplatin and much lower to these of Melphalan. The results confirm that the compounds are potential candidates for future drug discovery study.

Acknowledgment: Thanks are due to Medical Science Fund at the Medical University – Sofia, Bulgaria (Grant 35/2015) for the financial support.

Identification of Novel Inhibitors of Cyclooxygenase 2 by Molecular Docking Studies

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Abstract:

Computational methods that predict the structure and specificity of ligand-protein interactions can yield deep insight into the structural biology of many biological pathways. Molecular docking is computational tool commonly applied in drug discovery projects and fundamental biological studies of protein-ligand interactions. Traditionally, molecular docking is used to screen a collection of small molecules against a receptor and predict the binding affinity of the ligand-receptor complex and thus identify potential active ligands.

Here, chemical constituents from selected Indonesian medicinal plants have been virtually screened for their efficacy in inhibiting COX-2 - an enzyme play important roles in pain, inflammation and cancer. Molecular docking studies were performed using Molegro Virtual Docker (MVD) software on 202 compounds found in ten selected Indonesian traditional medicinal plants *(Ceibapentandra (L.)Gaertn, Acorus Calamus L, Ipomoea Batatas (L.), Ananas Comosus (L.) Merr, Citrus Aurantifolia, Lantana Camara, Morinda Citrifolia L, Moringao Leifera Lamk, Plantago Major L, Rosa Damascena Miller)* against COX-2 enzyme.

These docking studies revealed high binding affinity of compounds - acubin, apigenin, syringin and salicylic acid against cyclooxygenase-2, (PDB ID 4COX), thus, could be potent inhibitors of COX-2 and can be further investigated for *in-vitro* and *in-vivo* activity.

These molecular docking calculations not only explore the probable binding conformations of compounds within the active site of protein but it provide further useful information in understanding the structural & chemical features of COX-2 inhibitors in designing and finding new potential inhibitors.

High prevalence of extremely-drug resistant clinical Acinetobacter isolates in Karachi-Pakistan expressing Metallo-β-Lactamases

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ABSTRACT

Acinetobacter are Gram negative bacteria which are increasingly recognized as opportunistic nosocomial pathogens with a remarkable ability to acquire resistance to different classes of antibiotics. Resistance to β-lactam antibiotics among the clinical *Acinetobacter* has increased markedly in recent years, making the treatment problematic particularly among the critically ill patients. Acinetobacter species have been implicated in a wide range of nosocomial infections including bacteremia, pneumonia, meningitis and urinary tract infections.

The aim of this study was to determine antibiotic resistance profile and molecular characterization of clinical isolates *Acinetobacter* species. For this purpose, antibiotic resistance profile of117 *Acinetobacter* isolates from clinical samples was evaluated against a panel of antibiotics and the strains were subjected to molecular characterization for different resistance genes.

Of the 117 isolates, 109 were identified as *A. baumannii* whilst 8 as other species of *Acinetobacter*. Identification of the isolates was confirmed by the amplification of blaOXA-51 gene. All the *A. baumannii* isolates were extremely-drug resistant, showing increased resistance to carbapenems. A PAN-resistant strain was also identified, resistant to colistin as well. Among the metallo-β-lactamases (MBLs), VIM gene was amplified in 29 (22%) isolates and NDM-1 gene was detected in 5 (4.5%) strains.

Increasing reports of XDR and PAN-resistant *A. baumannii* strains in Pakistan is an alarming situation and stresses the need for the implementation of appropriate infection control strategies. The health care system in Pakistan is not well-established and over the counter availability of antibiotics and indiscriminate use further compounds the problem. A national surveillance system should be established in order to monitor the emergence of drug resistance and study the epidemiology of *A. baumannii* and other species of *Acinetobacter* in Pakistan.

Keywords: Acinetobacter, Extremely drug resistant, *bla*NDM-1, *bla*VIM, colistin, carbapenem

Transformation of selenite by the rhizobacterium *Azospirillum brasilense* with the formation of selenium nanoparticles

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In the recent years, the use of different biological systems (including bacteria) has been widely developing for the synthesis of various compounds, particularly for the synthesis of nanoparticles. The basis of this approach is to utilise various biochemical processes instead of classical chemical and physical processes. This is part of "green chemistry", a special field that aims at minimising harm to the environment in the synthesis of chemical compounds.

Bacteria of the genus *Azospirillum* occur in close association with many higher plants and are among the most intensively studied plant-growth-promoting rhizobacteria (PGPR). They have high resistance and adaptability to different unfavourable environmental conditions [1, 2].

For the first time we demonstrated the ability of two strains of *Azospirillum brasilense* (Sp7 and Sp245) to reduce selenite (SeO_3^{2-}) to the red amorphous modification of elementary selenium [3]. A significant accumulation of selenium by bacterial cells was shown using X-ray fluorescence (XRF). The presence of intracellular electron-dense spherical nanoparticles with sizes 50–400 nm was observed using transmission electron microscopy (TEM). Electron energy loss spectroscopy (EELS) showed those electron-dense nanospheres to consist of elementary selenium. The observed selenium nanoparticles (SeNPs) were heterogeneous in size and mainly located intracellularly [3].

The conditions of SeNPs synthesis for these bacteria were further optimized for strain *A. brasilense* Sp245 to produce extracellular SeNPs homogeneous in size. The SeNPs obtained were isolated and characterised by dynamic light scattering (DLS), UV-Vis spectrophotometry and TEM. The zeta potential of these SeNPs was found to be –(23.7…21.5) mV. The size of SeNPs was shown to depend on the initial concentration of Na_2SeO_3. SeNPs most homogenous in size, with an average diameter of 80 nm, were formed at 10 мМ Na_2SeO_3. Similar results were obtained for the other strain, *A. brasilense* Sp7.

Different mechanisms of SeO_3^{2-} reduction and the formation of SeNPs are discussed. They can include either enzymatic or chemical reduction, i.e. involving cellular biomolecules as reducing agents. Some of the possible mechanisms are Painter-type reactions, thioredoxin reductase system, dissimilatory reduction and others.

Research is ongoing to explore the possibility of using microbiologically synthesised SeNPs in biology and medicine, in particular, for the treatment of various diseases, including cancer. Microbial reduction of Se oxyanions can be helpful in water and soil bioremediation, production of biologically active food additives and in green synthesis of various SeNPs.

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Diabetes and mi-RNA

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Abstract:

Diabetes is the most common metabolic disorder and is recognized as one of the most important health threats of our time. MicroRNAs (miRNAs), a family of endogenous small noncoding RNA molecules that regulate gene expression. They play a key role in post-transcriptional regulation by selectively binding complementary messenger RNAs, thus affecting translation. MicroRNAs regulate many biological cellular functions and are often deregulated during diseases. Recent research has suggested that miRNAs play a critical role in the pathogenesis of diabetes. The aim of this review is to describe the role of miRNAs in diabetes. The pancreatic beta-cell and its endocrine product insulin play a central role in glucose homeostasis and the pathogenesis of diabetes. A large number of miRNAs have been implicated in normal pancreatic development and function. The most important roles of miRNAs in associated by diabetes are regulation of beta-cell function, pancreas development, beta-cell regeneration and regulation of autoimmunity. MiR-375, one of the most abundant miRNAs present in islet cells. It has been demonstrated that miR-375 negatively regulates glucose-stimulate insulin secretion. Several other miRNAs are able to influence the function of adult beta-cells. For instance, miR-9 has been demonstrated to be necessary for optimal insulin release in response to glucose. Another microRNA able to modulate of the insulin secretory machinery is miR-124a. MiRNAs also control insulin signalling in target

tissues, including the liver, skeletal muscle and adipose tissues. MiR-145 and miR-126 have been shown to reduced expression of IRS-1 in cells. Some other miRNAs lowered Glut4 levels in tissue that induced insulin resistance and reduced glucose uptake that leads to hyperglacemia. MiRNA regulation is critical for the prevention of autoimmunity that have been shown role of microRNA in events associated to the control of (auto) immune response in type 1 diabetes. In addition, because of changes in serum microRNA profiles have been shown to occur in association with diabetes, some reports also discuss the potential use of microRNAs as blood biomarkers. The discovery of potential specific biomarkers may help predict or detect the development and progression of diabetes at an early stage, and therefore allow timely intervention to delay subsequent complications. In conclusion, the deregulation of miRNA function has been linked to diabetes, although it is not yet fully certain whether this is a cause or effect of the pathology. Therefore to gain insights of their function of miRNA need to be more researched that helps define appropriate therapeutic intervention in the management of diabetes.

Keywords: Diabetes, mi-RNA

Features of miRNA binding sites in mRNAs of *Bos taurus* ZNF family transcription factors

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Abstract:
miRNA binding sites in the mRNA nucleotide sequences of 247 transcription factor (TF) genes of *Bos taurus* ZNF family was established. miRNAs binding sites are located in the 5'UTRs, CDSs and 3'UTRs of mRNAs. miRNA binding sites in mRNAs were selected with the value ΔG/ΔGm equal or greater than 85%. miR-574 was identified from 749 *Bos taurus* miRNAs, it has binding sites with mRNAs of *KLF7, SNAI2, WIZ, ZFP1, ZFP91-1, ZNF175, ZNF6772, ZSCAN29-1* genes. The value ΔG/ΔGm varied from 85 to 95%. The starts of miR-574 binding sites with mRNAs of *KLF7, ZFP91-1, ZNF6772* genes are located through two nucleotides. miR-574 binding sites are located in the 3'UTR. miR-1260b has binding sites with mRNAs of 29 genes (*BCL11B-1, CTCF-1, DPF2-1, EGR3, FEZF2, PRDM13, PRDM14, PRDM6, SALL1, SP6-2, SP8, WIZ, ZFX-1, ZFX-2, ZKSCAN1, ZNF219, ZNF335, ZNF341, ZNF384, ZNF48, ZNF513, ZNF618-1, ZNF653, ZNF668, ZNF746, ZNF75, ZNF805, ZNF774, ZSCAN20-1*) which are located in the 3'UTRs, CDSs and 5'UTRs. The value ΔG/ΔGm varied from 85 to 91%. The starts of miR-1260b binding sites in mRNAs of *ZNF618-1* target gene are located through one nucleotide in the 5'UTR and with mRNAs of *ZNF384, BCL11B-1* genes through three nucleotides in the CDSs. We found miR-2881, miR-2882, miR-2885, miR-3141 and miR-6528 binding sites in the nucleotide sequences of mRNAs of 27, 17, 23, 40 and 21 target genes, respectively. The value ΔG/ΔGm for these miRNAs varied from 85 to 96%. We also established binding sites for families of miRNAs: miR-1777 (miR-1777a, miR-1777b), miR-2284 (miR-2284aa, miR-2284b, miR-2284c, miR-2284f, miR-2284h-5p, miR-2284i, miR-2284l, miR-2284m, miR-2284o, miR-2284p, miR-2284s, miR-2284v, miR-2284z), miR-2285 (miR-2285ae, miR-2285af, miR-2285g, miR-2285o, miR-2285p, miR-2285q, miR-2285, miR-2285w, miR-2285y) and miR-2325 (miR-2325a, miR-2325c) with mRNAs of *Bos taurus* ZNF family TF genes.

Keywords: miRNA, mRNA, transcription factor, ZNF

The importance of the biomarkers, ADA, CRP and INF-γ, in diagnosing pleural effusion etiologies.

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Abstract: The aim of the present study is to investigate the clinical utility of biomarkers Adenosine deaminasa (ADA), C-reactive protein (CRP) and Interferon gamma (INF-γ) in the differentiation of exudative and transudative pleural effusion, and in the differentiation of the three types of exudative pleural effusion. The study enrolled 250 patients with pleural effusion that were admitted in hospital, from 2012-2015. The patients with pleural effusion were classified based on Light's criteria, on biochemical and on cytological analyses, as exudative (130), and transudative (120). The patients with exudative pleural effusion were categorized as: malignant, tuberculosis and parapneumonic. The patients went thoracentesis and venous blood samples, under aseptic conditions, and from each subject were collected in syringe at least 30 ml of pleural fluid. The measurement of pleural fluid and venous blood were done within 24 hours. To measure the levels of CRP in blood and liquid were used the test of CRP with COBAS 6000 Roche company. To measure the levels of ADA was used the colorimetric method of Giusti Gallant and for INF-γ was used the commercial enzyme-linked immunosorbent assay (ELISA) test. The Mann-Witney U statistical test for non-parametric data was used for the role that ADA and CRP plays in the differentiation of exudative and transudative pleural effusion. The values of ADA and CRP differ significantly between the two types of effusion ($p<0.05$). For the accuracy of the test was used the ROC curve analyses, and based on the area under the curve, ADA biomarker in pleural fluid is a better test for the differentiation of exudative from transudative pleural effusion. The Kruskal-Wallis H statistical test for non parametric data demonstrated that the values of ADA and CRP in serum and pleural fluid differ significantly between the three groups of exudative pleural effusion, with $p<0.05$. The values of ADA differ significantly when comparing malign with tuberculosis and malign with parapneumonic pleural effusion. The major differences for CRP biomarker were seen in the comparison of malign and parapneumonic pleural effusion. The Chi-square statistical test for the nominal data of INF-γ test demonstrated that, INF-γ in pleural fluid is a significant test for the differentiation of the three types of exudative pleural effusion and INF-γ in serum does not play a role for this differentiation. As a conclusion, for the differentiation of exudative and transudative pleural effusion ADA biomarker is a better test for this differentiation. The biomarkers ADA, CRP in serum and pleural fluid, and INF-γ in pleural fluid, plays a significant role for the differentiation of the three types of exudative pleural effusion.

Is 7qh+ a New Human Chromosomal Variation?

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The heterochromatic segments on the long arms of chromosomes 1, 9, 16 and Y have been defined as secondary constriction (qh) regions. In the literature heterochromatic segments on the chromosome 7 has not been reported yet. We report a 9 year old boy admitted to Department of Pediatric Neurology, Ondokuz Mayis University Faculty of Medicine with the complaints of epilepsy and the suspicious for Fragile-X. According to the WISC-R IQ test he had borderline mental retardation. Patient was referred to Department of Medical Biology, Section of Medical Genetics for genetic analysis. Cytogenetic analysis was performed using Giemsa banding and karyotyping was based on the International System for Human Cytogenetic Nomenclature. We used C-band technique for detecting possible inversion and staining heterochromatic and centromeric region. Molecular cytogenetic analysis was done with fluorescence in situ hybridization (FISH) method. Molecular genetic analysis performed for examining expansion of the fragile-X CGG repeat, TP-PCR assay was performed and all amplicons were evaluated on an ABI3130XL Genetic Analyzer System by Fragment analysis. Genetic examinations of parents were also done. As a result of this study, cytogenetic analysis of patient's of peripheral blood cultures showed 46,XY, 7qh+. FISH analysis revealed 46,XY. ish 7q11.23 (ELNx2), centromeric 7 and 7q11 sinyalization were normal. However DAPI staining showed increased heterochromatic region. FMR1 gene CGG repeats have been observed in normal range. The results of parental cytogenetic analysis showed that father have normal 46,XY. The mother has the

same chromosomal variation 46,XX, 7qh+ however she was phenotypically normal and healthy.

FISH analysis of mother showed 46,XX. ish 7q11.23 (ELNx2), centromeric 7 and 7q11 sinyalization were normal and DAPI staining showed increased in heterochromatic region. In addition, FMR1 gene CGG repeats were in normal range for mother.

This increased centromeric heterochromatin region of chromosome 7 may be a new variation. This variation may be occurred due to a translocation between heterochromatin regions of other chromosomes in previous generation individuals. To our knowledge, this is the first study revealed 7 qh+ chromosomal variation in the literature.

Biological Characterization of Anticoagulant Molecule Isolated from *Vipera lebetina* Venom

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Abstract

Snake venom is a mixture of various pharmacologically active proteins and peptides that affect different physiological systems, mostly hemostatic and cardiovascular. Many of these components are highly specific for diverse molecular targets and thus constitute potential therapeutic and diagnostic agents. Viperid snake venoms are particularly rich in proteins that disturb the platelet aggregation, blood coagulation cascade and fibrinolysis, acting as pro-coagulants that activate coagulation factor X (FX), prothrombin and fibrinogen, or as anticoagulants, degrading fibrin or fibrinogen molecules, inhibiting coagulation factors and interfering with platelet aggregation. Among these components, serine proteases, metalloproteases, phospholipases A2, L-amino acid oxidases, 5'-nucleotidases, disintegrins and C-type lectin-like affect several steps of hemostasis at many different points. Some of them are of special research interest due to their potential application as biochemical tools in coagulation research. Fibrin(ogen)olytic enzymes as serine or metalloproteases, can break down fibrin clots and help to prevent further clot formation by their action on fibrinogen. These enzymes offer promising tools in the treatment of thrombosis. In the present study we describe the purification and characterization of VLACII, an anticoagulant protease from *Vipera lebetina* venom. Purification was achieved by gel-filtration chromatography on Sephadex G-75 followed by ion-exchange chromatography on DEAE Sephadex A-50 and reversed-phase high performance liquid chromatography (RP-HPLC) on C8 column. The purified enzyme named VLACII, with molecular mass estimated to 21 kDa when tested on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). VLACII preferentially degraded the Bβ-chain of fibrinogen, but not Aα and γ chains. This fibrinogenolytic enzyme showed esterasic activity with specific activity estimated at 27.40 (UDO/min/mg) on BAEE (N-α-Benzoyl-arginine-ethyl-ester-hydrochloride). The isolated VLACII acting on plasma coagulation showed an anticoagulant potency that was corroborating with fibrinogenolytic activity. The purified molecule did not present hemorrhagic activity. These results suggested that VLACII has potential as an anticoagulant that could be used in a clinical setting and it may be useful for treating thrombotic disorders.

Keywords: Venom, *Vipera lebetina*, Anticoagulant, Fibrinogenolytic.

Effects of deltametrin on antioxidant enzymes of *Arthrospira platensis* Gomont

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Pesticides are toxic not only for harmful organisms but also for other terrestrial and aquatic organisms. Deltamethrin are widely used as insecticides, and have become well-known in the world. The aim of this study is to determine the effects of deltametrin pesticide on growth and antioxidant enzymes activities [Superoxide dismutase (SOD), Glutatione reductase (GRX) and Ascorbate peroxidase (APX)] of *Arthrospira platensis* Gomont. Therefore 0 µg/mL, 0.5 µg/mL, 1 µg/mL, 1.5 µg/mL, 2 µg/mL concentrations of deltametrin were inoculated in *A. platensis* cultures which contain 1 µg/mL chlorophyll-*a*. OD 560 and chlorophyll-*a* contents were measured in every 24 hours spectrophotometrically and observed during seven days. At the end of the seventh day, 2 mL of cultures were centrifuged, and then pelets were extracted by K_2HPO_4 buffer for superoxide dismutase (SOD) and glutatione reductase (GRX) and by TrisHCl – EDTA - ascorbic acid buffer for ascorbate peroxidase (APX) enzyme activity assays. Supernatants were treated for superoxide dismutase (SOD), glutathione reductase (GRX) and ascorbate peroxidase (APX) enzyme activities and were measured spectrophotometrically. Triplicate measurements were made. LSD analysis was done for statistical analysis using the SPSS 20.0 software. OD 560 and chlorophyll-*a* values were significantly different ($p < 0.05$) between control group and other concentrations of deltametrin at the seventh day. Changes of OD 560 and chlorophyll-*a* for each concentration was significantly different ($p < 0.05$) among seven days. Although ascorbate peroxidase (APX) and glutatione reductase (GRX) enzyme activities were not changed, superoxide dismutase (SOD) enzyme activities were decreased significantly ($p < 0.05$) with increasing concentrations; except for concentration of 0.5 µg/mL. Therefore, deltametrin pesticide has negative effects on growth and superoxide dismutase (SOD) enzyme activities of *A. platensis.*

Keywords: *Arthrospira platensis,* Superoxide dismutase (SOD), Glutatione reductase (GRX), Ascorbate peroxidase (APX), chlorophyll-*a*, OD-560

ANALYSIS OF GLUTATHIONE S-TRANSFERASE 1 (GSTO1) ALA140ASP GENETIC POLYMORPHISM IN TURKISH POPULATION

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Introduction:

Glutathione S-Transferases (GSTs) are important multifunctional antioxidant enzymes playing role in phase II reactions. The Glutathione S-Transferase omega 1 is expressed by *GSTO1* gene located in chromosome 14q11.2 (Whitbread et al., 2003). Ala140Asp single nucleotide polymorphism, a missense mutation, occurs in *GSTO1* gene exon 4. The 419th nucleotide cytosine is converted to adenine, so the amino acid alanine is substituted to aspartic acid. The aim of this study is to determine the genotype and allele frequencies of GSO1 Ala140Asp genetic polymorphism in Turkish Population and compared them with other populations.

Materials and Methods:

Blood samples were collected from 130 healthy individuals with collaboration of Gülhane Military Medical Academy, Department of Neurology, Ankara. The main method for this study is PCR-RFLP technique. For this technique, the whole DNA isolated from human blood samples by the method of Lahiri and Shnabel was amplified by PCR methods and the products were digested by restriction enzyme, Cac8I. PCR products and restriction endonuclease digestion products were analyzed with agarose gel electrophoresis technique.

Results and Discussion:

The distribution of GSTO1 Ala140Asp genotype in Turkish population was 47.7% wild type (CC), 36.2% heterozygous mutant (CA) and 16.1% (AA). The wild type allele frequency result of present study (C:0.658) is closer with another study for Turkish population(C:0.689) (Ada et al., 2012). However, as regard to another study for Turkish population, the frequencies of these alleles are considerably different compared to our results (Takeshita et al., 2009). The wild type allele frequency is 0.915 and the minor one is 0.085 (Takeshita et al., 2009). This frequency closer to Taiwan, Chinese and Italian population, is higher than our results. The reason of this may result from the nature of study population. Also, our population results for GSTO1 Ala140Asp polymorphism are similar to German, American and Serbian populations (C allele: 0.680, 0.655 and 0.637, respectively). These

results have shown that Turkish population's allele frequency of GSTO1 Ala140Asp single nucleotide polymorphism is similar with other white race population.
Key words: Glutathione S Transferase, Ala140Asp, Genetic Polymorphism, Turkish population

The Evaluation Nano Calcium Silicate Cements Performance for Palpation

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Abstract:

Root canal therapy includes cases of dental treatment that causes complications such as infectious of bone. Therefore, a substance that can also combined excellent sealing with appropriate tissue response is necessary.

In recent years, studding on the addition of various calcium silicate cement base for making these cements bioactive as a root canal filling materials is done ; however, influence of addition tri-calcium phosphate on the reform Bioactivity and other properties of these cements in this article was not research yet, which revealed that adding calcium phosphate significantly affects the properties of silicate cement in a positive way.

After the cement powder components were mixed with double distilled water, complex physical and chemical reactions happen that in this reactions, the silicates of the cement products hydrations and with solving phosphate compounds and precipitate calcium phosphate such as hydroxyapatite, a hard solid mass produce.

SEM images of composite cement shows the spherical particles of hydroxyapatite after one day immersed in SBF. The results showed that calcium phosphate phase is a resource for encourage and accelerate the apatite layer on the surface of the composite cement. The researchers reported that the hydrolysis of α-TCP after 15 days was completed.

As a result, cement made in this study with more tests can used for dental procedures. This cement has ability to setting itself, high strength, biocompatibility and the ability of inject.

Keywords: nano calcium silicate, cement, composite, biocompatibility

In Vitro Evaluation of Effects of Fullerene Nanoparticles On the Cytotoxicity and DNA-Damaging Effects of Pesticides In IMR-90Human Lung Fibroblast Cell Line

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Abstract—Pristine fullerene nanoparticles (nC60) have received considerable attention in the last years due to their potential for use in biomedical and electronic industries. Presence of nC60 in environmental water samples has been shown and it is estimated that higher amounts of nC60 will be produced and released to the environment in the near future. There are some evidence that nC60 can enhance the adverse effects of organic and inorganic substances via facilitating their cellular uptake. In the present study, we aimed to evaluate the effects of aqueous suspensions of fullerene nanoparticles on the cytotoxic and genotoxic effects of pesticides deltamethrin and fenitrothion, in human lung fibroblast cell line IMR-90. Characterizations of nC60 were performed prior to experiments using TEM, DLS and zeta potential measurements. Intracellular uptake of nC60 was analyzed using an immunofluorescence staining technique. The clonogenic assay was used to measure cytotoxicity whereas, micronucleus, comet and gH2AX tests were used to assess chromosome, single and double stranded DNA damages, respectively. As a result we observed that nC60 itself has low cytotoxic and genotoxic effects on IMR-90 cells with IC50 value >1000 mg/L. Our further revealed that co-exposure with non-toxic concentrations of nC60 significantly increased the cytotoxicity and genotoxicity of both pesticides. These effects could be due to increased cellular exposure to deltamethrin and fenitrothion via internalization of higher amount of pesticides adsorbed on nC60 as demonstrated by immunofluorescence staining. Our findings indicate that, in addition to the analysis of potential effects of nanoparticles, the evaluation of the possible effects of their interaction with other environmental contaminants is crucial.

Antiepileptic Agent Levetricetam Interacts with Dipalmitoylphosphatidylcholine Lipids

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Levetricetam [(*S*)-α-ethyl-2-oxo-1-pyrrolidine acetamide] (LEV), marketed under the trade name Keppra, is a medication that was approved by the US Food and Drug Administration in 1999. It is widely prescribed in the treatment of epilepsy and used for partial onset, myoclonic, or tonic-clonic seizures (1-2). Even though LEV is suggested to suppress epileptic seizures in effective fashion, its mechanism of action is still unknown. In the literature there are some reports stating that it exerts its action, which proceeds in different ways such as binding to a synaptic vesicle glycoprotein, SV2A, (3), inhibiting presynaptic calcium channels (4) and impeding impulse conduction across synapses (5). In the view of its high bio-availability (around 100%) and low protein binding capacity (10%), it has also potential to exert its activity by binding membrane lipids. In order to gain new insight for this we tested the possibility of interaction of LEV with a simplified model system called dipalmitoylphosphatidylcholine (DPPC) multilamellar vesicles (MLVs) at agent concentrations (0-1-5-10-20 mol%) using differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy. The results showed that LEV at concentrations used (10-20%) causes significant change in lipid phase behavior, lipid dynamics (fluidity), lipid acyl chain flexibility (order), hydration state of the head group and/or the region near the head group of DPPC MLVs. These results clearly revealed that LEV changes the structural and dynamical parameters of neutral DPPC lipid vesicles and locates within the bilayer. This may also imply LEV itself has capacity to show its action on PC lipids.

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Unraveling Autoimmune Diabetes by Using Genetically Modified Mouse Models: From mechanism dissection to clinical application

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Insulin-dependent diabetes mellitus (IDDM) is a T cell-mediated autoimmune disease. To delineate the protective roles of some immune modulatory molecules, such as soluble decoy receptor 3 (DcR3), cytotoxic T lymphocyte antigen 4 (CTLA4), program death ligand 1 and 2 (PD-L1 and 2), heme oxygenase 1 (HO-1), and chemokine receptor D6 in the autoimmune process and to search for potential preventive and/or therapeutic targets in this disease, we have generated (a) insulin promoter (pIns)-sDcR3 transgenic non-obese diabetic (NOD) mice, (b) pIns-single chain anti-CTLA4 transgenic NOD mice, (c) pIns-single chain anti-4-1BB transgenic NOD mice, (d) pIns-PD-L1 transgenic NOD mice, (e) pIns-HO-1 transgenic NOD mice, and (f) pIns-D6 transgenic NOD mice and demonstrated their immunomodulatory potential and underlying mechanisms. Meanwhile, to explore the modulatory potential of interleukin-12, 23 and 27 on autoimmune diabetes, we have generated following transgenic, knockout and knockdown NOD mice: (1) Th1 and Th2 doubly transgenic (2) IL-12 knockout (3) IL-23 knockdown (4) IL-27 knockdown NOD mice. Our results revealed that 20% IL-12-deficient NOD mice still developed autoimmune diabetes, the diabetic incidence of IL-23 knockdown NOD mice is lower than that of control littermates, and the number and percentage of Th1 cells are dramatically decreased and Th17 cells are increased in IL-27 knockdown mice, indicating a differential role of IL-12 cytokine family in modulating Th1 and Th17 cell development during autoimmune diabetogenic process. Making full use of these unique mouse strains, we are quantitatively and qualitatively investigating the immunopathogenic mechanisms of autoimmune diabetes and providing valuable information for the development of novel immunotherapies.

Comparative histological, genetic and cytological studies of carbon tetrachloride induced model of liver cirrhosis, its prevention and treatment

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Cirrhosis is an advanced stage of liver fibrosis that is accompanied by distortion of the hepatic vasculature. Hepatic parenchymal and nonparenchymal cells are involved in the initiation and progression of liver fibrosis and cirrhosis. Abberrant DNA methylation and stem cells differentiation changes also are the main hallmarks of liver cirrhosis.

The goals of this work were:

1. Studying of the possible anticirrhotic effect of grape seed extract and its synergy with a well-known anticirrhotic drug.
2. Comparative study of the structural and functional characteristics of rat liver tissue and hematopoetic stem cells under normal, cirrhotic and its prevention/treatment conditions.

Studies were made on Wister rats, weighing 130~150 g. They were housed in a room at 22±2°C and 12 hours light/dark cycle and given food and water ad libitum. All animal experimental procedures performed according to Directive 2001/20/EC.

Liver cirrhosis was induced by carbon tetrachloride.

As natural antioxidant extract from "Vitis Vinifera Satira Hayreniq" grape seeds was used.

Urdoxa was used as a comparative drug, well known in medical practice: used to treat predominantly cholestatic liver disorders.

For morpho-functional characterization of liver tissue DNA metylation and hystological (light microscopic) studies have been done.

Hematopoietic colony-forming cell assay was used to study bone morrow stem cells differentiation potency.

In the experiment grape seeds extract, used separately and in combination with Urdoxa, has demonstrated high effectiveness in treatment and prevention of liver cirrhosis.

IL-10 deficiency leads to increased diet-induced body mass gain due to mitochondrial abnormalities and decreased oxygen consumption

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The increasing prevalence of obesity around the world is an important public health issue. Several studies have shown that obesity, which results from an imbalance between caloric intake and energy expenditure, is linked to a chronic, low-grade systemic inflammation that involved the actions of a number of cytokines and inflammatory factors. The anti-inflammatory cytokine, interleukin-10 (IL10), has a key role as a regulator of obesity-induced inflammation. Since non-shivering thermogenesis in brown adipose tissue (BAT) is important to whole body energy balance, changes in BAT activity may lead to obesity. Studies have identified a link between inflammation and BAT disorders; however, no previous study has addressed the putative connection between BAT thermogenesis, IL-10 and obesity. In the present study, we used IL10-deficient (IL10d) mice fed on a high-fat diet to evaluate a number of parameters related to body adiposity and energy expenditure. Despite the fact that body mass gain was significantly higher in IL10d mice, there were no differences in caloric intake and spontaneous activity, as compared to wild-type. Most of the energy balance difference between wild-type and IL10d was due to reduced O_2 consumption/CO_2 production. This was accompanied by reduced BAT size and reduced isolated-BAT mitochondria respiration, which was mostly due to reduced uncoupled respiration. Therefore, we determined UCP1 expression, which was significantly reduced in the BAT of IL10d mice. Because of the defective function of IL10d mice BAT mitochondria, next, we performed transmission electron microscopy which revealed major morphological abnormalities as mitochondrial enlargement with irregular cristae and swelling. Because IL10d mice are chronically inflamed, we hypothesized that mitochondria abnormalities could be a result of inflammation. In fact, TNFa levels are increased in BAT of IL10d mice and the immunoneutralization of TNFa resulted in an almost complete correction of the morphological abnormalities of IL10d BAT mitochondria. Thus, in the absence of IL10 there is an impairment of whole-body thermogenesis leading to increased body mass gain. At least part of the defective thermogenesis is due to severe inflammatory damage of BAT mitochondria.

Molecular and cellular effects of silica nanoparticles in human glioblastoma cell line LN-229

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OBJECTIVES:

Application of silica nanoparticles (SiO_2 NPs) in biomedical areas has recently become an intensively investigated field of research. Contemporary medicine offers new perspectives in SiO_2 NPs utilization including imaging, drug delivery and cancer therapy. However, despite the extensive exploration of SiO_2 NPs, their potential toxicological implications and molecular modes of action are still incompletely understood. In this respect, in order to elucidate if SiO_2 NPs might be considered as a potential cytostatic agent in brain tumor therapy, we examined the molecular and cellular effects of SiO_2 NPs exposition in human glioblastoma cancer cell line LN-229.

METHODS:

The effect of 24 and 48 h exposure of LN-229 cells to spherical porous SiO_2 nanoparticles at different concentrations was investigated. The MTT assay was performed to evaluate the viability of SiO_2 NPs-treated cells. Flow cytometry analysis was used to determine apoptosis ratio. Relative expression levels of key pro-apoptotic, pro-inflammatory, and oxidative stress-related factors, such as *PUMA*, *NOXA*, *CHOP*, *BIM*, *BAX*, *COX2*, *IL-1β*, *SOD1*, *SOD2* and *CAT* were evaluated using q-RT-PCR method.

RESULTS:

The exposure of LN-229 cells to 5-15 nm SiO_2 nanoparticles resulted in evident dose- and time-dependent reduction of cell viability. Also, a strong pro-apoptotic effect of SiO_2 NPs treatment at the determined dosages (50 and 100 μg/ml) was observable. A significantly increased ratio of early apoptotic cells was noticed after 24 h of SiO_2 NPs exposition, while the proportion of apoptotic cells shifted towards late apoptosis after 48 h of treatment. The expression levels of pro-apoptotic genes varied significantly. *BAX* and *PUMA* expressions stayed unaffected by SiO_2 nanoparticle treatment, while a remarkable up-regulation of *CHOP* and *NOXA*, and down-regulation of *BIM* mRNA were observed. Moreover, *COX2*, *IL-1β* and *SOD2* showed marked overexpression, while *SOD 1* and *CAT* transcripts were significantly down-regulated.

CONCLUSIONS:

Our findings demonstrate that SiO_2 NPs can negatively affect glioblastoma cells via the induction of various cellular and molecular effects. The exposure to SiO_2 NPs may alter inflammatory and oxidative stress status, and eventually enhance apoptotic cell death of glioblastoma LN-229 cells. Our preliminary data suggest that silica nanoparticles may plausibly be used as a cytostatic agents in brain cancer therapy. However, more detailed analysis of SiO_2 NPs effects is still required to comprehensively solve the nature of these compounds.

Effects of zinc on antioxidant enzymes of *Scenedesmus ellipsoideus* Chodat

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Although Zinc is an essential mineral, higher concentrations are toxic for ecosystem and living organisms. The aim of this study is to determine the effects of zinc on growth and antioxidant enzymes activities [Superoxide dismutase (SOD), Glutatione reductase (GRX) and Ascorbate peroxidase (APX)] of *Scenedesmus ellipsoideus* Chodat. *S. ellipsoideus* was isolated from Lake Ketence (İzmit) and cultured in BG11 medium. 0 mg/mL, 1 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL concentrations of zinc were inoculated in *S. ellipsoideus* Chodat cultures which contain 1 µg/mL chlorophyll-*a*. OD 560 and chlorophyll-*a* contents were measured in every 24 hours spectrophotometrically and observed during seven days. At the end of the seventh day, 2 mL of cultures were centrifuged, and then pelets were extracted by K_2HPO_4 buffer for superoxide dismutase (SOD) and glutatione reductase (GRX) and by TrisHCl – EDTA - ascorbic acid buffer for ascorbate peroxidase (APX) enzyme activity assays. Supernatants were treated for superoxide dismutase (SOD), glutathione reductase (GRX) and ascorbate peroxidase (APX) enzyme activities and were measured spectrophotometrically. Triplicate measurements were made. LSD analysis was done for statistical analysis using the SPSS 20.0 software. OD 560 values were significantly different ($p < 0.05$) between control group and each concentration of zinc at the seventh day. Chlorophyll-*a* values were significantly different ($p < 0.05$) between control group and 6 mg/mL and 8 mg/mL concentrations of zinc at the seventh day. Changes of OD 560 and chlorophyll-*a* for 6 mg/mL and 8 mg/mL were significantly different ($p < 0.05$) from other concentrations among seven days. Although ascorbate peroxidase (APX) and glutatione reductase (GRX) enzyme activities were not changed, superoxide dismutase (SOD) enzyme activities were altered significantly ($p < 0.05$). SOD activity was significantly increased at 4 mg/mL and, then was significantly decreased 8 mg/mL accordingly to control. Therefore, zinc has effects on growth and superoxide dismutase (SOD) enzyme activity of *S. ellipsoideus*.

Keywords: Zinc, Superoxide dismutase (SOD), Glutatione reductase (GRX), Ascorbate peroxidase (APX), *Scenedesmus ellipsoideus* Chodat

The differential effects of Smad3 phosphorylation events on tumorigenesis and metastasis in breast cancer cells

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Numerous studies have shown that the transforming growth factor-β (TGF-β) plays a dual role in cancer. TGF-β often displays tumor suppressive functions in normal cells and early carcinoma by inhibiting cell cycle, inducing apoptosis and preventing cell immortalization. However, as tumors develop and progress, TGF-β signaling switches to promote cancer progression and metastasis through its effects on the tumor microenvironment, enhanced invasive properties, and inhibition of immune cell function. Smad3, a key intracellular mediator of TGF-β signaling, also functions as both a positive and negative regulator in carcinogenesis. In the canonical TGF-β signaling, Smad3 is phosphorylated by activated type I TGF-β receptor at the C-tail SSXS motif. In addition to C-tail, serine/threonine phosphorylation sites in the Smad3 linker region are phosphorylated by proline-directed kinases, such as CDK, c-Jun, p38 MAPK, Erk and GSK-3β, which are often highly expressed in cancer cells. Here we show that mutation of the Smad3 linker phosphorylation sites by adenoviral infection significantly suppresses tumorigenesis by inhibiting primary tumor growth, but increases lung metastasis of breast cancer cell lines. In contrast, the blockade of Smad3 C-tail phosphorylation promotes tumorigenesis, while markedly inhibiting metastasis to the lung of tail-vein-injected breast cancer cell lines. *In vitro* studies demonstrate that the mutation of Smad3 linker phosphorylation sites greatly intensifies the TGF-β-induced responses, including growth arrest, apoptosis, epithelial-mesenchymal transition, and invasive activity. These results suggest that the canonical TGF-β signal, which is mediated through C-tail phosphorylation, is modulated by negative inputs from the linker phosphorylation. Interestingly, the blockade of Smad3 linker phosphorylation reduces the size of putative cancer stem cell population by exhibiting suppression of mammosphere formation, downregulation of embryonic transcription factors, such as Oct4, Nanog and Sox-2, and induction of apoptosis by repressing an anti-apoptotic protein Bcl-2. Taken together, our results demonstrate a critical role of the counterbalance between Smad3 C-tail and linker phosphorylation in tumorigenesis and metastasis, implicating a new aspect to therapeutic intervention of breast cancer targeting the TGF-β signal pathway.

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Cytoplasmic DRAK1 overexpressed in head neck cancer cells renders resistance to TGF-β1 tumor-suppressive effects by interrupting Smad3/4 complex.

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide and a highly malignant cancer associated with poor prognosis and low overall survival. Early stage HNSCC is easily curable through surgery, radiation therapy and chemoradiation, whereas patients with advanced HNSCCs at late stages frequently present high mortality rates with little opportunity for receiving effective treatment. Because chemotherapy for advanced HNSCC is often ineffective, finding molecular mechanism and discovering new therapeutic targets in HNSCC are required. It has been known that resistance to TGF-β1-mediated growth inhibitory effects in advanced HNSCCs is caused by the deletion or downregulation of key components of TGF-β signaling. However, the resistance of advanced HNSCCs, which harbor intact components of the TGF-β signaling pathway, to TGF-β tumor suppressor activity via negative regulators of TGF-β is poorly understood. Here, we describe a role of DRAK1 (DAP kinase-related apoptosis-inducing kinase 1) as a negative regulator of TGF-beta signaling pathway in head and neck cancer cells. DRAK1 is significantly overexpressed in primary head and neck tumors and cell lines. Overexpression of DRAK1 decreased the TGF-β1-induced transcriptional activity and expression of tumor suppressor target genes, including p15, p21, whereas DRAK1 silencing enhanced. Furthermore, endogenous depletion of DRAK1 enhanced the TGF-β1-induced cell growth inhibition and suppressed the tumorigenic ability of head and neck cancer cells in vivo. Interestingly, the gain- or loss-of-function of DRAK1 did not change the TGF-β1-induced phosphorylation of Smad2/3. DRAK1 specifically binds to Smad3 through its kinase domain, thereby blocking recruitment of Smad4 and translocation of Smad3 into nucleus. Thus, our findings suggest that aberrant expression of DRAK1 increases tumorigenic potential through the inhibition of TGF-β1 tumor suppressor activity in head and neck cancer cells.

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Inhibition of dipeptide uptake by reducing p38MAPK-Smad3 signaling is a potential therapeutic target for CML treatment

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Although tyrosine kinase inhibitors (TKIs), such as imatinib mesylate, dasatinib, and nilotinib, have significantly increased the survival rate of chronic myelogenous leukemia (CML) patients, therapeutic targeting of CML stem cells which is responsible for recurrence of CML disease remains elusive. A nutrient supply specifically required for CML stem cell maintenance could provide a candidate target for a novel therapy for eradicating CML stem cells. However, to reduce the harmful side effects of such molecular targeting on normal haematopoiesis, it is essential to understand the mechanism that distinguish CML stem cells from normal haematopoietic stem cells (HSCs). Thus, we carry out a global metabolic comparison of normal HSCs with the corresponding stages of CML stem cells in tetracycline-inducible CML-affected mice using sophisticated metabolomics techniques. Interestingly, increased dipeptide transporter activity sustains CML stem cell maintenance by providing an alternative nutrient supply, while this mechanism is not normally observed in normal HSCs. CML stem cell maintenance is also influenced by the TGF-β pathway, and Smad3, a downstream effector of TGF-β signaling is known to have a significant role in regulating cell fate. Here, we demonstrate that post-translational modification of Smad3 by non-canonical phosphorylation at Ser208 is crucial for CML stem cell activity *in vivo*. Importantly, we also provide the evidence that this non-canonical Smad3 phosphorylation is mediated by dipeptide-triggered activation of p38MAPK. Our results demonstrate that dipeptide species support CML stem cell maintenance by activating p38MAPK–Smad3 signaling *in vivo*, and thus point towards a potential therapeutic target for CML treatment.

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Identification of KIAA1324 as a novel tumor suppressor of gastric cancer progression

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Gastric cancer is the fourth most common type of cancer and the second leading cause of death from cancer worldwide. Recent advances in genome and transcriptome analysis have contributed to the identification of many potential cancer-related genes. Furthermore, biological and clinical investigations of the candidate genes provide us with a better understanding of carcinogenesis and development of cancer treatment.
Here, we report the suppressive role of *KIAA1324* in the gastric cancer. We performed transcriptome sequencing to identify novel gastric cancer-related genes, and found that *KIAA1324* was significantly downregulated in most gastric cancer tissues and cell lines. Histone deacetylase was involved in the downregulation of *KIAA1324*. Furthermore, gastric tumor tissue microarray from 428 patients suggested that suppressed *KIAA1324* levels were associated with poor prognosis in gastric cancer patients. To assess the *in vivo* role of the KIAA1324, we performed xenograft assay and demonstrated that KIAA1324 dramatically reduced gastric tumor formation and also inhibited development of pre-existing tumors. KIAA1324 also decreased cancer activities such as proliferation, invasion, drug resistance and induced apoptosis in gastric cancer cells. To find regulatory mechanism of KIAA1324-induced apoptosis, we identified GRP78 (glucose-regulated protein 78 kDa) as a KIAA1324-binding partner through protein interaction analysis. KIAA1324 decreased oncogenic activities of GRP78 by inhibiting GRP78–caspase-7 interaction and suppressing GRP78-mediated AKT activation, thereby inducing apoptosis. In conclusion, our study suggests a tumor suppressive role of KIAA1324 via inhibition of GRP78 oncoprotein activities and provides new insight into the diagnosis and treatment of gastric cancer.

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FAM3B suppresses the tumorigenesis of gastric cancer via preventing the AKT signaling pathway

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Gastric cancer is recorded as the fourth most frequent cancer and the second leading cause of cancer death worldwide. Combined use of molecularly targeted therapy with chemotherapy may offer improved outcomes for gastric cancer patients. PANcreatic-DERived factor (PANDER or FAM3B), a member of the FAM3 family, is a newly discovered cytokine molecules. FAM3B expression is first identified in the islets of Langerhans of the endocrine pancreas and also is relatively high in colon and stomach. FAM3B induces apoptosis of pancreatic islets via caspase 3-dependent mediated mechanism. However, the biological function of FAM3B in gastric cancer has still remained elusive. Here we show that the week expression level of FAM3B in 11 out of 14 human cancer tissues samples, which is 78.5%, including the gastric cancer cell lines. Ectopic expression of FAM3B leads to inhibition of cell growth and reduction of single cell colony forming activity. In the xenograft model, overexpression of FAM3B significantly reduced the tumor formation of gastric cancer cells. FAM3B not only induces the decrease of AKT phosphorylation level, which is involved in cell survival, but also upregulates p21, an inhibitor of cell cycle progression. We also demonstrated that the gene expression of FAM3B is epigenetically regulated in gastric cancer cell lines. The treatment of MS-275, a synthetic inhibitor of histone deacetylase, rescued FAM3B gene expression. In conclusion, our data suggest the role of FAM3B as a putative tumor suppressor during gastric carcinogenesis through inducing the cell growth arrest and downregulting the oncogenic AKT signaling pathway in gastric cancer.

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