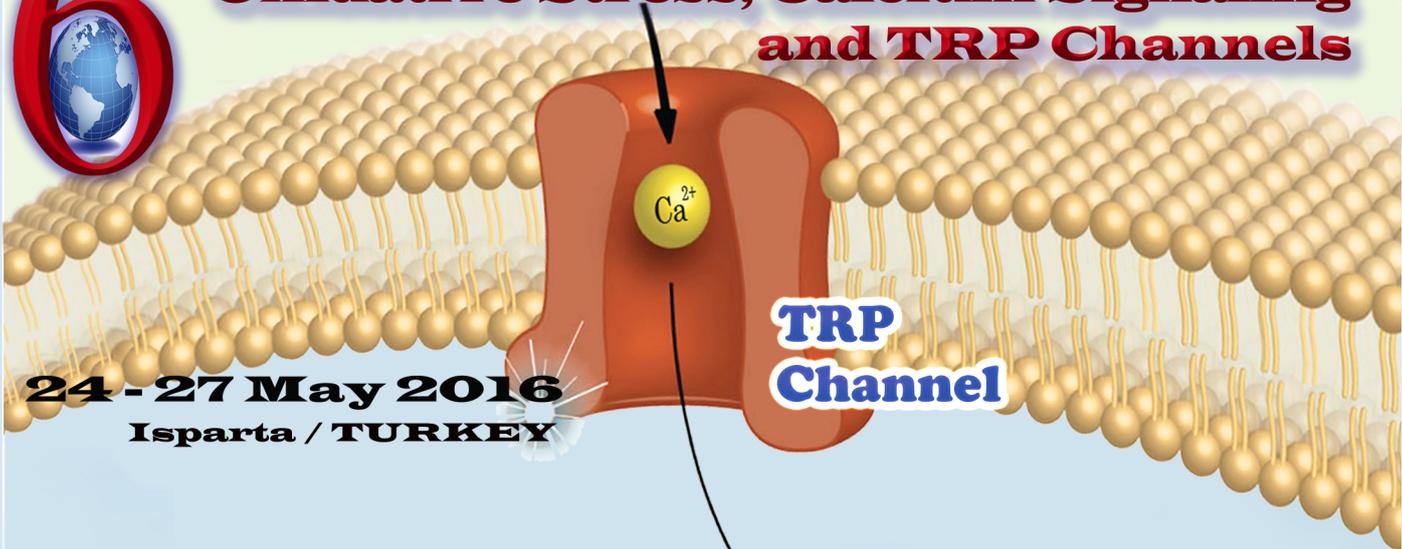


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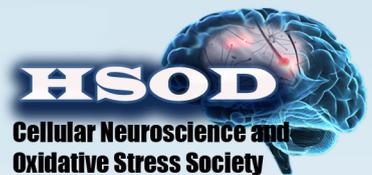


**TRP
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**24 - 27 May 2016
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EDITOR IN CHIEF

Mustafa Nazıroğlu,
Department of Biophysics and Neurosciences,
Medical Faculty, Suleyman Demirel University,
Isparta, Turkey.
Phone: +90 246 211 37 08. Fax: +90 246 237 11 65
E-mail: mustafanaziroglu@sdu.edu.tr

Associate Editors

A. Cihangir Uğuz and Ömer Çelik,
Department of Biophysics, Medical Faculty,
Suleyman Demirel University, Isparta, Turkey.
E-mail: biophysics@sdu.edu.tr

Managing Editors

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Areas of particular interest are four topics. They are;

A- Ion Channels (Na⁺- K⁺ Channels, Cl⁻ channels, Ca²⁺ channels, ADP-Ribose and metabolism of NAD⁺, Patch- Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience

(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD⁺ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels, role of TRPM2 channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress

(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

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Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide synthase, ageing, antioxidants, neuropathy, traumatic brain injury, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.



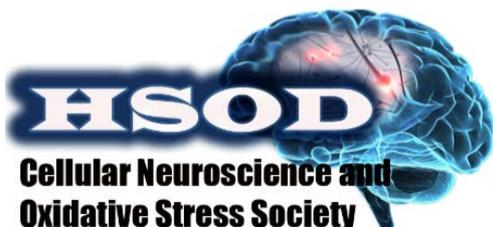
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CONFERENCE

▶ Conference No. 1

Multiple roles of Orai1 calcium channels

James W. Putney, Gary S. Bird, Felicity M. Davis, Pooja Desai, Diane D'Agostin, Natacha Steinckwich-Besançon

Calcium Regulation Group, Signal Transduction Laboratory, National Institute of Environmental Health Sciences-NIH, Research Triangle Park, NC USA.

Numerous physiological functions are initiated or regulated by a rise in cytoplasmic calcium, a process known as calcium signaling. In general, Ca^{2+} signals arise from the release of Ca^{2+} from intracellular stores, from entry of Ca^{2+} across the plasma membrane, or more commonly a combination of the two. One of the most widely encountered mechanisms of Ca^{2+} signaling involves the intracellular release of Ca^{2+} from endoplasmic reticulum by inositol 1,4,5-trisphosphate coupled to the entry of Ca^{2+} via store-operated Ca^{2+} channels [1]. Store-operated Ca^{2+} entry is activated when the release of Ca^{2+} stores lowers endoplasmic reticulum luminal Ca^{2+} concentration which in turn activates a Ca^{2+} sensor molecule STIM1 (or STIM2). STIM1 aggregates and collects at endoplasmic reticulum-plasma membrane junctions where it interacts with store-operated channels composed of Orai1 (or Orai2 or 3) subunits [2]. Orai1 is expressed in a long (α) and short (β) form due to alternative translation initiation. While both forms can form functional store-operated channels, only the long form can form non-store-operated arachidonic acid/leukotriene C4 regulated channels [3].

To better understand the role of store-operated channels in various physiological functions, mouse models have been generated with deletions of the key proteins, STIM1 or 2, or Orai1, 2 or 3. Our laboratory has focused primarily on mice lacking the predominant store-operated channel subunit, Orai1 [4]. These mice

are deficient in both innate and acquired immunity, in bone formation and resorption, in keratinocyte differentiation and wound healing, and in lacrimal secretion. Surprisingly, female Orai1 knockout female mice are fertile. However, pups born to Orai1 knockout females do not survive due to a failure of lactation in the dams. This appears due to a loss of Ca^{2+} oscillations in mammary gland myoepithelial cells [5]. On the other hand, male Orai1 knockout mice are sterile. This results from a loss of sperm late in spermatogenesis. The use of mouse models with tissue-specific deletions or modifications of STIM and Orai genes should yield important new information on physiological and pathological roles of store-operated channels.

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▶ Conference No. 2

Signalling to and from IP3 receptors

Colin W Taylor

Department of Pharmacology, University of Cambridge, Cambridge, UK.

Inositol 1,4,5-trisphosphate receptors (IP_3R) are intracellular cation channels that mediate release of Ca^{2+} from intracellular stores. Most mammalian cells express IP_3R . Binding of IP_3 to an IP_3R primes it to respond to Ca^{2+} , which then triggers opening of the channel. This allows IP_3R s to regeneratively propagate intracellular Ca^{2+} signals via Ca^{2+} -induced Ca^{2+} release. In this talk, I will briefly discuss the structural basis of how IP_3

binding initiates IP₃R activation, and consider how dynamic regulation of IP₃R distribution contributes to intracellular Ca²⁺ signalling.

▶ Conference No. 3

Optogenetic manipulation of neuronal circuits regulating feeding behavior

Deniz Atasoy

Janelia Research Campus HHMI -Virginia USA
İstanbul Medipol University, Medical School,
Department of Physiology, İstanbul

New tools for mapping and manipulating molecularly defined neural circuits have improved the understanding of how the central nervous system regulates appetite. Activation of starvation-sensitive AGRP neurons can rapidly elicit behavioral state similar to food deprivation, which present an entry point for reverse-engineering neural circuits for hunger. We mapped functional synaptic interactions of AGRP neurons with multiple cell populations in mice and probed the contribution of these distinct circuits to feeding behaviour using optogenetic and pharmacogenetic techniques. We have also developed tools for detailed structural analysis of AGRP neuronal connections using serial-section electron microscopy. Our results characterized some basic features of functional and anatomical circuit organization for AGRP axon projections.

Keywords: AGRP neurons; optogenetic.

▶ Conference No. 4

Altered calcium handling in duchenne muscular dystrophy and the role of TRP channels

Heinrich Brinkmeier

Institute of Pathophysiology, University Medicine

Greifswald, Karlsburg, Germany

Lack of the cytoskeletal protein dystrophin and some of its binding partners are the underlying effects of Duchenne muscular dystrophy (DMD) and its mouse model *mdx*. Dystrophin deficient muscle fibres show unregulated Ca²⁺ influx and increased Na⁺ influx. Subsequently fibres degenerate due to intracellular Ca²⁺-dependent pathological processes. There is good evidence that dysregulation of ion channels and ion transport processes substantially contribute to the altered ion homeostasis in DMD muscle. Cell physiological techniques allowed the characterization of mechanosensitive cation channels, store operated channels and Ca²⁺-leak channels as Ca²⁺ entry pathways in DMD/*mdx* muscle fibres. However, the molecular identification of the involved channels and transporters turned out to be difficult and was only partially successful. In skeletal muscle STIM1 and Orai1 are amongst other proteins important for store-operated Ca²⁺ entry (SOCE). Both, loss of function of Orai1 and gain of function of STIM1 can cause genetic muscle diseases. TRPC1, C3, C6, TRPV1, V2, V4 and TRPM7 are expressed in mouse skeletal muscle, but their functional roles are incompletely understood. Deficiency of TRPC1 leads to fast fatigability of isolated mouse muscles indicating a role of TRPC1 in SOCE. Overexpression of TRPC3 in the mouse causes a myopathy resembling *mdx* muscular dystrophy. TRPV1 has been localized in the SR membrane and its overactivity causes muscle hypertrophy. Finally, TRPV4 is presumably the major mechanosensitive cation channel of the sarcolemma. Genetic deletion of single TRP channels in mice does not severely affect muscle structure and function. In contrast, overexpression or overactivity of some of the above mentioned channels can be very harmful and may be a key factor in the pathomechanism of Duchenne muscular dystrophy.

▶ Conference No. 5

A new intracellular signaling molecule free Zn²⁺ mediates endoplasmic reticulum stress in hyperglycemic cardiomyocytes

Belma Turan

Studies have shown important besides dissident roles for cytosolic free Zn^{2+} level ($[Zn^{2+}]_i$) in different cell types. The Zn^{2+} releases during cardiac cycle into cytosol of cardiomyocytes and results mostly cytosolic free $[Zn^{2+}]_i$ increases via triggering production of reactive oxygen/nitrogen species (ROS/RNS). However, it has been not clearly shown how there are associations between increased ROS/RNS production and $[Zn^{2+}]_i$ under hyperglycemic condition in cardiomyocytes. However, it is known that many signaling pathways are mediated with either hyperglycemia, increased oxidative stress or intracellular ionic levels such as $[Zn^{2+}]_i$ and $[Ca^{2+}]_i$. Moreover, we, previously, have demonstrated that increased level of ROS/RNS contributes to direct and/or indirect damaging effects on cardiomyocytes in diabetes, providing a close relationship between both increased and deleterious effects of intracellular basal $[Zn^{2+}]_i$ besides $[Ca^{2+}]_i$ in the heart. Additionally, we also presented that increased $[Zn^{2+}]_i$ in cardiomyocytes, either under hyperglycemia or directly Zn^{2+} -exposure, hyperphosphorylated RyR2-macromolecular complex together with a marked activation in a transcription factor nuclear factor- κ B. It is also known that ER stress is one of the underlying mechanisms of not only diabetic cardiac dysfunction but also dilated cardiomyopathy and failing heart in humans. Furthermore, experimental evidence demonstrates the requirement for $[Zn^{2+}]_i$ for proper ER function, because Zn^{2+} deficiency upregulates the ER stress response in mammalian cells, indicating a conserved requirement for Zn^{2+} in proper ER function. In this regard, we have shown that the levels of ER-targeted cytoprotective chaperones such as GRP78 and calnexin, unfolded protein response signaling protein CHO/Gadd153 besides the levels of calpain, Bcl-2, phospho-Akt, PUMA, and PML in hearts from the diabetic rats are markedly changed while any treatment of diabetic animals with either a β -blocker, timolol or an antioxidant, NAC have significant preventive role against hyperglycemia induced oxidative stress associated the changes via regulation of cytosolic $[Zn^{2+}]_i$ in cardiomyocytes. Therefore, our data have pointed out the importance of well-controlled $[Zn^{2+}]_i$ in a proper S(E)R function in diabetic subjects

► Conference No. 6

Phospholipids reduce the oxidative stress and improve the heart function after myocardial infarction

Adelina Curaj, Sakine Simsekyilmaz, Mareike Staudt, Andreas Götzenich, Andeea Urs, Mircea Leabu, [Elisa A. Liehn](#)

Institute for Molecular Cardiovascular Research (IMCAR), Aachen University Hospital, Germany

Despite considerable progress over the last period, acute myocardial infarction continues to remain the major cause of morbidity and mortality worldwide. Phosphatidylserine (PS) is a phospholipid component of the inner leaflet of cell membranes and seems to be a messenger for the cellular death and an important destruction signal for the macrophages¹. On the other hand, PS has demonstrated some usefulness in treating cognitive impairment² but also speeded up recovery, prevented muscle soreness, improved well-being, and might possess ergogenic properties³. In our studies, we also found an up-regulation of the PS in *cxcr4*^{+/-} deficient hearts, as an adaptation to the poor blood supply present in these mice⁴. The best known mechanism which is described to protect the heart in the absence of oxygen is the ischemic preconditioning. Ischemic preconditioning is an experimental technique gaining resistance against the loss of blood supply in many tissues⁵. By a short (under 5 minutes) and repeated (two or three times) impairment of the blood supply, the tissue or the organ becomes robustly protected from a predicted severe ischemic insult. In this regard, we believe that PS can play an important role in myocardial preconditioning and protection of the heart from ischemia.

The effect of PS was tested *in vitro* on isolated cardiomyocytes undergoing hypoxia. After 3 hours of preincubation with PS, the cardiomyocytes increased significantly their protection, indicating no stress signals as analysed by Alamar blue staining after 5

hours of ischemia. Using a mouse model of myocardial infarction, we tested the effect of oral administration of PS on myocardial function and biology. We did not detect any significant changes in fatty acids or lipid composition after one week PS administration in any analysed organs. Moreover, the blood analysis showed normal and unchanged parameters after in all groups. The pre-treated with PS one week before myocardial infarction induction showed a significantly preserved heart function and reduced infarction size. Starting the administration after myocardial infarction induction will also reduce infarction size, however the hearts remain with a significantly diastolic dysfunction. No differences in apoptosis were detected, as measured by TUNEL staining in infarcted myocardium. Inflammation was also reduced, do probably reduced tissue damages after PS administration. mRNA extraction from isolated cardiomyocytes after hypoxia and myocardium after myocardial infarction showed a significant up-regulation of main player of preconditioning program, as protein kinase C type β , hemo-oxygenase, as well as Hypoxia-inducible factor (HIF)-1.

We demonstrated that PS can be used to activate preconditioning program of the heart, to reduce to assure a significant protection from ischemia *in vitro* and *in vivo*. We strongly believe that PS can be used in the treatment of heart failure to protect the cardiomyocytes and to adapt them to chronic hypoxic conditions, preserving their function. Comparing with other methods used in present, as remote ischemia or opioide administration, PS administration seems to be easy to performe with minimal side effects, no professional supervision being necessary. Since PS are already in clinical use for some cognitive diseases, we believe that our findings would be for a high relevance for the cardiovascular community, with immediately clinical implication.

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► Conference No. 7

Consequences of ATAD3 hemi-deletion in mice: Effects on training and obesity

Denis Rousseau

Department of Biology, Science Faculty, Joseph Fourier University, Grenoble, France

ATAD3 is a wondering mitochondrial ATPase as ubiquitous and essential for mitochondrial biogenesis, therefore vital at first zygote expression. However, ATAD3 precise function is still unknown even if it may serve the transport of lipid-protein rafts from the reticulum to mitochondria (ref).

To evaluate the role of ATAD3 expression level at animal scale, we have used ATAD3^{+/-} mice with a C57bl6j genetic background, which live normally, to test their physiological capacities when involving mitochondrial biogenesis, (i) the running and training performances, and (ii) the reaction to high-fat/high-sucrose diets.

In males, we found that ATAD3 depletion decreases running and training performances, heart weight and body weight, especially at adipose tissue level, but surprisingly increases the gripping strength and notably modifies the circadian activity. This phenotype is associated with a lower mitochondrial mass in muscle and a decreased oxidative stress in the brain. In females, we observed that ATAD3 depletion favours body weight increase upon high-fat/high-sucrose diet.

Our data lead today to the conclusion that ATAD3 miss of function is potentially involved in pathologies such as obesity and myopathies but also certainly in other disorders such as neuropathies since ATAD3 is expressed the more in the brain. Then, we would like to

search today for neuronal disorders in these mice.

▶ Conference No. 8

Non-selective cationic channels and calcium entry mechanisms in the cardiovascular system

David J. Beech

School of Medicine, University of Leeds, Leeds LS2 9JT, UK

The sensing of physical forces arising due to blood flow is key in the maturation and remodeling of blood vessels which are required in embryonic development and adult life but the mechanisms by which the physical forces are sensed have been elusive; indeed, is there a specific force sensing protein and, if so, how does it sense force and generate appropriate downstream signals? We have revealed how calcium-permeable non-selective cationic channels formed by assembly of Piezo1 proteins act as sensors of physiological force and determinants of vascular structure in both development and adult physiology (Li et al 2014). Global or endothelial-specific disruption of mouse Piezo1 profoundly disturbed the developing vasculature and was embryonic lethal within days of the heart beating. The importance of Piezo1 channels as sensors of blood flow was shown by Piezo1 dependence of shear stress-evoked ionic current and calcium influx in endothelial cells and the ability of exogenous Piezo1 to confer sensitivity to shear stress on otherwise resistant cells. Downstream of this calcium influx there was protease activation and spatial reorganization of endothelial cells to the polarity of the applied force. It will be discussed how Piezo1 functions as a physiological force sensor which is essential for normal vascular development.

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▶ Conference No. 9

Diversity and specificity of Ca²⁺ entry pathways in the vascular system

Mohamed Trebak

Department of Cellular and Molecular Physiology, College of Medicine, PennState University, Hershey, PA, USA

Orai proteins (Orai1-3) form a family of highly Ca²⁺ selective plasma membrane channels that are regulated by stromal interacting molecules (STIM1 and STIM2); STIM proteins are Ca²⁺ sensors located in the membrane of the endoplasmic reticulum (ER). STIM and Orai proteins are expressed in vascular smooth muscle and constitute the molecular components of the ubiquitous store-operated calcium entry (SOCE) pathway that mediate the Ca²⁺ release-activated Ca²⁺ (CRAC) current. STIM/Orai proteins also encode store-independent Ca²⁺ entry pathways in smooth muscle. Altered expression and function of STIM/Orai proteins have been linked to vascular and airway pathologies including restenosis, hypertension and atopic asthma. We used smooth muscle and endothelial-specific knockout mice to determine the contribution of STIM and Orai to vascular disease. Here, I will present recent findings from our laboratory on the role of STIM and Orai proteins in vascular remodeling and disease.

Keywords: Calcium ion; STIM/Orai proteins; vascular system

▶ Conference No. 10

Screening of TRPC channel activators identifies novel neurotrophic piperazine compounds

Seishiro Sawamura, Masahiko Hatano, Yoshinori

Takada, Kyosuke Hino, Tetsuya Kawamura, Jun Tanikawa, Hiroshi Nakagawa, Hideharu Hase, Akito Nakao, Mitsuru Hirano, Rachapun Rotrattanadumrong, Shigeki Kiyonaka, Masayuki X. Mori, Motohiro Nishida, Yaopeng Hu, Ryuji Inoue, Ryu Nagata and Yasuo Mori

Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, Japan (S.S., M.H., Y.T., H.H., M.H., R.R., S.K., M.M., Y.M.); Department of Technology and Ecology, Hall of Global Environmental Studies, Kyoto University, Kyoto, Japan (S.K., Y.M.); Sumitomo Dainippon Pharma Co., Ltd. (Y.T., K.H., T.K., J.T., H.N., R.N.); Division of Systems Medical Science, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Japan (A.N.); Division of Cardiocirculatory Signaling, Okazaki Institute for Integrative Bioscience (National Institute for Physiological Sciences), National Institutes of Natural Sciences, Japan (M.N.); Department of Physiology, School of Medicine, Fukuoka University Fukuoka, Japan (Y.H., R.I.)

TRPC3 and TRPC6 channels activated by diacylglycerol (DAG) play important roles in neurotrophic effects of brain-derived neurotrophic factor (BDNF), the most abundant and widely distributed neurotrophin in the central nervous system, which restores neurological deficits in various disease models. However, therapeutic potential of BDNF is limited by its poor pharmacokinetic profile. Elucidation of a framework for designing small molecules, that elicit BDNF-like activity via TRPC3 and TRPC6, establishes a solid basis to overcome this limitation. We discovered a group of piperazine-derived compounds that activate DAG-activated TRPC3/TRPC6/TRPC7 channels through library screening. The compounds [4-(5-chloro-2-methylphenyl)piperazin-1-yl](3-fluorophenyl)methanone (PPZ1) and 2-[4-(2,3-dimethylphenyl)piperazin-1-yl]-*N*-(2-ethoxyphenyl)acetamide (PPZ2) activated recombinant TRPC3/TRPC6/TRPC7 channels in a dose-dependent manner, but not other TRPCs in human embryonic kidney cells. PPZ2 activated native TRPC6-like channels in smooth muscle cells isolated from the rabbit portal vein. Also, PPZ2 evoked cation currents and Ca²⁺ influx in rat cultured cerebellar granule neurons.

Strikingly, both compounds induced BDNF-like neurite growth and neuroprotection, which were abolished by a knockdown or inhibition of TRPC3/TRPC6/TRPC7 in cultured neurons. Inhibitors of Ca²⁺ signaling pathways impaired neurite outgrowth promotion induced by PPZ compounds. PPZ2 increased activation of the Ca²⁺-dependent transcription factor, cAMP response element-binding protein (CREB). These findings suggest that Ca²⁺ signaling mediated by activation of DAG-activated TRPC channels underlies neurotrophic effects of PPZ compounds. Thus, piperazine-derived activators of DAG-activated TRPC channels provide important insights for development of a new class of synthetic neurotrophic drugs.

▶ Conference No. 11

Functional Interaction between TRP channels and anoctamin1

Makoto Tominaga

Division of Cell Signaling, Okazaki Institute for Integrative Bioscience

TRP channels are nonselective cation channels with high Ca²⁺ permeability. We found physical and functional interaction between TRPV4 and anoctamin1 (ANO1), a Ca²⁺-activated chloride channel, in HEK293T cells and choroid plexus epithelial cells (CPECs) in mice. Chloride currents induced by a TRPV4 activator were markedly increased in an extracellular calcium-dependent manner in HEK293T cells expressing TRPV4 with ANO1 and in CPECs. We also found physical interaction between TRPV4 and ANO1 in both cell types. Cell volume changes were induced by ANO1-mediated chloride currents in parallel with membrane potential changes, and the cell volume was significantly decreased at negative membrane potentials by TRPV4-induced ANO1 activation. These physical and functional interactions between TRPV4 and ANO1 can modulate water transport in the choroid plexus. Similar physical and functional interaction between TRPV1 and ANO1 was found in HEK293T cells and mouse sensory neurons. Capsaicin-evoked inward currents were significantly inhibited by a

specific ANO1 antagonist T16Ainh-A01 (A01) in mouse DRG neurons, indicating that capsaicin-induced inward currents are composed of two components; a TRPV1-mediated cation influx and an ANO1-mediated chloride efflux. In addition, capsaicin-evoked action potential was drastically inhibited by A01. Furthermore, pain-related behaviors in mice treated with capsaicin were significantly reduced by the concomitant administration of A01. These results indicate that the TRPV1-ANO1 interaction is a significant pain-enhancing mechanism in the peripheral nervous system. Thus, TRP channel/anocamin complex could play many important roles in various tissues.

► Conference No. 12

Role of TRPC5 in multidrug resistance of breast cancer

Xin Ma^{1,2}, Jian Jin², Xiaoqiang Yao¹.

¹School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong, China.

²School of Medicine and Pharmaceutics, Jiangnan University, Wuxi, Jiangsu, China

Tumor cells develop multidrug resistance during chemotherapy. Furthermore, drug resistance can be transferred from drug-resistance cancer cells to non-resistant cells via extracellular vesicles. However, the underlying mechanisms of chemoresistance and its intercellular transfer are not fully understood. In the present study, we found that the Ca²⁺-permeable channel TRPC5 is overproduced in the adriamycin-resistant breast cancer cell line MCF-7/ADM, and that knockdown of TRPC5 markedly reduced the growth of human tumor xenografts in an athymic nude mouse model. We explored the underlying reasons for the role of TRPC5 in promoting breast cancer cell chemoresistance and uncovered the following novel mechanisms: 1) TRPC5 stimulates the production of a drug pump P-glycoprotein via NFATc3; 2) TRPC5 promotes angiogenesis by enhancing VEGF release; and 3) TRPC5 promotes the formation of extracellular vesicles, which is important for transfer of chemoresistant properties from chemoresistant cells to

non-resistant cells. Furthermore, under the chemotherapy treatment, MCF-7/ADM cells acquired endothelial cell phenotype through a Notch4-dependent epithelial-mesenchymal transition (EMT), rendering these cells with the properties of endothelial cells for new vessel formation. Taken together, these results suggest a critical functional role of TRPC5 in cancer cell chemoresistance. It could potentially lead to TRPC5-based treatment strategies for cancer chemoresistance and tumor growth.

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► Conference No. 13

Melatonin and apoptosis induced by intracellular calcium overload

Javier Espino, Ana B. Rodríguez, José A. Pariente

Department of Physiology, Neuroimmunophysiology and Chrononutrition Research Group, Faculty of Science, University of Extremadura, 06006 Badajoz, Spain

Apoptosis is a gene-regulated form of cell death that is critical for normal development and tissue homeostasis. A major component of the apoptotic machinery involves a family of aspartic acid-directed cysteine proteases, called caspases, which cleave multiple protein substrates *en masse*, leading the loss of cellular structure and function, and ultimately resulting in cell death. In addition, numerous reports suggest that aging is accompanied by alterations in the apoptotic behaviour of a variety of cell types and tissues. On the other hand, the calcium ion is one of the cellular signalling mechanisms most widely used by different cell types, and with the greatest number of physiological and pathological implications. Alterations of calcium homeostasis, particularly excessive and prolonged increases in cytosolic free calcium concentration ($[Ca^{2+}]_c$), are early signs that precede other morphological and functional alterations responsible for

the development of irreversible damage in various tissues. In fact, it has been reported that sustained elevation of intracellular calcium plays a role in cell death. The proapoptotic effects of calcium are mediated by a diverse range of calcium-sensitive factors that are compartmentalised in various intracellular organelles, including endoplasmic reticulum and mitochondria.

The pineal gland hormone melatonin regulates seasonal and circadian rhythms of mammals and functions as a powerful free radical scavenger, but emerging evidence suggests that it may be involved in other important processes such as the protection of human leukocytes and other cell types against damage-induced apoptosis. Recent convincing evidence suggests that the so-called intrinsic pathway might represent the main target of melatonin to antagonize apoptosis in human leukocytes and in other tumor cell lines and *in vivo* models.

Here, we have evaluated the effect of melatonin on apoptosis evoked by increases in $[Ca^{2+}]_c$ in human leukocytes. Our results show that treatment of neutrophils with the calcium mobilising agonist FMLP or the specific inhibitor of calcium reuptake thapsigargin induced a transient increase in $[Ca^{2+}]_c$. FMLP and thapsigargin increased the caspase-9 and -3 activities and the active forms of both caspases. The effect of FMLP and thapsigargin on caspase activation was time-dependent. Similar results were obtained when lymphocytes were stimulated with thapsigargin. This stimulatory effect was accompanied by activation of the proapoptotic protein Bax and release of cytochrome *c*. However, when leukocytes were pre-treated with melatonin, all apoptotic features indicated above were significantly reversed, suggesting that melatonin reduces caspase-9 and -3 activities induced by increases in $[Ca^{2+}]_c$ in human leukocytes, which is produced through modulation of Bax activation. The protective effects on leukocyte apoptosis resulting from melatonin administration depends on melatonin's antioxidant action, as melatonin treatment substantially prevented intracellular reactive oxygen species (ROS) production induced by thapsigargin and FMLP. Finally, melatonin was able to delay endoplasmic reticulum stress-induced apoptosis in aged leukocytes and may counteract, at the cellular level, age-related degenerative phenomena linked to oxidative stress.

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▶ Conference No. 14

The road to understanding titin phosphorylation and oxidation in heart failure with preserved ejection fraction

Nazha Hamdani
Bochum, Germany

Understanding the mechanisms underpinning diastolic stiffness has been a major research focus over the last decade. Abnormal diastolic left ventricular (LV) function with impaired relaxation and increased diastolic stiffness is characteristic of heart failure with preserved ejection fraction (HFpEF). HFpEF accounts for more than 50% of all cases of heart failure (HF) in ageing Western societies and is closely associated with co-morbidities such as obesity, diabetes and arterial hypertension. To date, all large multicentre trials of HFpEF treatments have produced disappointing results. This outcome suggests that a "one size fits all" approach to HFpEF may be inappropriate and supports the use of tailored, personalized therapeutic strategies with specific treatments for distinct HFpEF phenotypes.

One of the most important mediators of diastolic stiffness is the giant myofilament protein titin. Titin isoform transitions and post-translational modifications are major modulators of titin-based stiffness and contribute to a large degree to diastolic stiffness. Post-

translational modifications of titin, including phosphorylation and S-glutathionylation, with a particular emphasis on titin phosphorylation by cyclic guanosine monophosphate-dependent protein kinase G (cGMP-PKG). Recent evidence suggests that comorbidities common to HFpEF promote a systemic inflammatory state that contributes to endothelial dysfunction, reactive oxygen species production (ROS), nitrosative stress, and depressed NO bioavailability. All these events contribute to the modulation of specific redox-sensitive signal transduction pathways and lead to modifications such as phosphorylation and oxidation (including the S-glutathionylation of titin). These changes may be related to a decrease in NO bioavailability, which results in an imbalance in several important signalling pathways including the cGMP-dependent PKG pathway.

► Conference No. 15

TRPM2 and TRPV1 channels: Potential drug targets for treating epilepsy*

Mustafa Naziroğlu^{1,2}

¹Neuroscience Research Center (NÖROBAM), Suleyman Demirel University, Isparta, Turkey

²Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Epilepsy has 2-3% incidence worldwide. However, epileptic seizures are partially controlled by present antiepileptic drugs. Calcium ion accumulation in hippocampal neurons has long been known as a major contributor to the etiology of epilepsy. The Ca²⁺ permeable transient receptor (TRP) melastatin 2 (TRPM2) and vanilloid 1 (TRPV1) are part of the TRP family, members of the melastatin and vanilloid subfamilies, respectively. TRPV1 is activated by the pungent (capsaicin) nature of hot chili peppers. On the other hand, poly (ADP-ribose) polymerase (PARP) pathways through the production of ADP-ribose and NAD⁺ serve an important role in activating the TRPM2 channel. Nonetheless, both TRPV1 and TRPM2 are activated and potentiated by oxidative stress. TRPM2 and TRPV1 channels are primarily expressed in the

DRG, dentate gyrus, and hippocampal CA1 and CA3 regions. Growing interest in the therapeutic potential of TRPM2 and TRPV1 continually provides support for the hypothesis that TRPM2 and TRPV1 channel inhibition likely underlies many of the benefits associated with epilepsy (1-3), including improved antioxidant and modulated calcium ion entry. A limited number of recent reports have implicated TRPV1 in the induction or treatment of epilepsy suggesting that this may be new area for potential drugs targeting this debilitating disease. Thus activation of TRPV1 by oxidative stress, resiniferatoxin, cannabinoid receptor (CB1) activators (i.e. anandamide) or capsaicin induced epileptic effects, and these effects could be reduced by appropriate inhibitors, including capsazepine (CPZ), 5'-iodoresiniferatoxin (IRTX), and CB1 antagonists. It has been also reported that CPZ and IRTX reduced spontaneous excitatory synaptic transmission through modulation of glutaminergic systems and desensitization of TRPV1 channels in the hippocampus of rats. Taken together, findings in the current literature support a role for calcium ion accumulation through TRPM2 and TRPV1 channels in the etiology of epileptic seizures, indicating that inhibition of TRPV1 in the hippocampus may possibly be a novel target for prevention of epileptic seizures.

Keywords: Anadamide, Calcium ion, Epilepsy, Seizures, TRPM2 channel, TRPV1 channels.

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Comparison of the oxidative stress-induced ionic currents with the other identified ionic currents in neurones of the ventral cochlear nucleus

Ramazan Bal

Department of Physiology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

All auditory nerve fibers terminate in the cochlear nucleus (CN). Three major cell types, octopus, bushy and stellate cells are recognized in the ventral cochlear nucleus (VCN). Synaptic responses and the firing pattern of octopus cells are mainly governed by their unusual biophysical characteristics. In vitro recording condition, octopus cells respond to square current pulses at the onset of the stimulus with a single action potential followed by a steady state depolarization. Onset responses of octopus cells mainly result from the large resting conductances of octopus cells, mixed-cation conductance (g_h) and low-voltage activated potassium conductance (g_{KL}). Octopus cells are also equipped with prominent calcium channels. At rest octopus cells have unusually low input resistances, as a consequence of the activation of g_h and g_{KL} . Whereas, stellate cells have high input resistances (~ 180 MOhm) as a consequence of low amplitudes of g_h and g_{KL} .

An average maximum mixed-cation conductance (g_h) was 150 nS and 41% of the maximum conductance (61.5 nS) was activated at resting potential (-61.8 ± 1.7 mV). In order to counterbalance, comparable amplitude of the low-voltage activated potassium conductance (~ 70 nS) was activated at rest, the maximum low-threshold K conductance (g_{KL}) was large, 514 ± 135 nS though. Whereas, at rest ADPR induced an inward currents of 136.8 ± 22.4 pA from octopus cells, which corresponds to 5.1 nS TRPM2 conductances. However in stellate cells, the resting mixed-cation conductance (g_h) was 5.2 nS at resting potential (-64.2 ± 2.1 mV). The low-voltage activated potassium conductance activated at rest was ~ 6.6 nS. ADPR induced an inward current of 25.8 ± 7.5 pA, which corresponds to 1.18 nS TRPM2 conductance at resting membrane potential.

The proportions of the TRPM2 conductance to the voltage gated conductances in octopus is $\sim 4/100$ ($5.1/(61.8 + 70)$). Whereas the proportions of the TRPM2 conductance to the voltage gated conductances in stellate cells is $\sim 1/10$ ($1.18/(5.2 + 6.6)$).

In conclusion, oxidative stress-induced TRPM2 channels in stellate cells have higher contribution to the resting conductance, when compared to that in octopus cells. So TRPM2 channels appear to be more likely to have a modulatory role in setting membrane potential and controlling resting excitability.

Keywords: TRPM2 channels; Oxidative stress; Ventral cochlear nucleus.

Proton sensing, TRPC channels and the cerebellum

Maike Glitsch
Oxford

Canonical Transient Receptor Potential (TRPC) channels fulfil a range of different functions in the brain, ranging from neuronal survival, development and mediating slow synaptic events. In the cerebellum, TRPC3 has been shown to underlie the slow excitatory postsynaptic current in Purkinje cells following parallel fibre stimulation, and this current is required for proper motor coordination. TRPC3 and 6 have furthermore been implicated in granule cell growth cone turning and survival in culture, whilst TRPC4 and 5 are thought to regulate granule cell dendritic tree development.

We have compared TRPC subunit expression levels throughout postnatal cerebellar development in whole cerebellum and in primary cerebellar granule cells and find striking differences in expression patterns. Furthermore, knock-out of a proton-sensing G protein coupled receptor significantly altered expression level of two TRPC subunits, in agreement with previous findings obtained in transformed cerebellar neurons.

Finally, we investigated physiological roles for TRPC channels in cerebellum and find that, consistent with its

changes in expression level during development, one TRPC subunit appears to be involved in migration of cerebellar neurons.

► Conference No. 18

Exercise improves vascular function in type 2 diabetes: But how?

Ismail Laher

Faculty of Medicine, Dept. Pharmacology and Therapeutics, University of British Columbia, Vancouver, Canada V6T1Z3

Improving coronary function in diabetic hearts is a key to preventing ischemic cardiac events. Exercise is a key component in the management of patients with type 2 diabetes, but the effects of exercise on diabetic coronary function are relatively unknown. We investigated myogenic tone and endothelial function in isolated mouse coronary arteries, since these are important components of the forces that determine vascular fluid dynamics in the myocardium. We hypothesized that exercise in db/db mice reduces the extent of pressure-induced myogenic constriction in coronary arteries while also improving endothelial function. The db/db mouse is a frequently used rodent model of type 2 diabetes.

We used pressurized mouse coronary arteries isolated from hearts of control and db/db mice that were sedentary (no exercise) or exercised for 1 h/day on a motorized exercise-wheel system (set at 5.2 m/day, 5 days/wk). The exercise protocol lasted 8 weeks and blood samples were collected at sacrifice for later analysis of biochemical markers.

Exercise reduced the body weights of db/db mice by approximately 10% without affecting hyperglycemia or plasma insulin levels. These moderate levels of exercise decreased the levels of whole body oxidative stress, as measured by plasma 8-isoprostane levels. Myogenic regulation of arterial diameter stimulated by increased transmural pressure, and smooth muscle responses to the vasoconstrictor U-46619 (a thromboxane agonist) or the vasodilator sodium nitroprusside (an endothelium-

independent dilator) were unaffected by exercise. Moderate levels of exercise restored ACh-simulated, endothelium-dependent coronary artery vasodilation in db/db mice, an effect that was related to increased expression of Mn SOD and decreased nitrotyrosine levels in hearts of db/db mice.

Exercise improved coronary artery function in db/db mice, an animal model of type 2 diabetes. The beneficial effects of moderate levels of exercise was independent of changes in myogenic tone or hyperglycemic status and primarily involved increased nitric oxide bioavailability due to decreased free radical production and improved free radical metabolism.

► Conference No. 19

Oxidative stress in insulin-secreting cells, a putative role in diabetes

Pierre Maechler

Department of Cell Physiology and Metabolism, University of Geneva Medical Center, Geneva, Switzerland

In pancreatic beta-cells, mitochondrial metabolism translates glucose sensing into signals regulating insulin secretion. Chronic exposure of beta-cells to excessive nutrients impairs its function and is postulated to contribute to the development of type 2 diabetes. Such gluco-lipotoxicity is associated with elevated production reactive oxygen species (ROS) from over-stimulated mitochondria. Mitochondria are both a source of ROS and a sensitive target of oxidative attacks. We recently established stress-specific molecular targets in INS-1E beta-cells and human islets exposed to gluco-lipotoxic conditions and oxidative stress [1, 2]. Acute oxidative stress impairs β -cell function, resulting in mitochondrial inactivation normally coupling glucose metabolism to insulin secretion [3]. Even one single acute oxidative stress can induce beta-cell dysfunction lasting over days, explained by persistent damages in mitochondrial components accompanied by subsequent generation of endogenous ROS of mitochondrial origin [4]. Interestingly, stressed cells can recover over weeks, becoming more resistant to a new oxidative attack [4],

pointing to a mitohormetic response possibly implicating UCP2 [5].

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Young Speakers

▶ Young Speaker No. 1

Increased oxidative stress parameters in children with moderate iodine deficiency

Ozgur Pirgon¹, Ibrahim Halil Topkaya²

¹Department of Pediatric Endocrinology and Diabetes, Suleyman Demirel University, Faculty of Medicine, Isparta, Turkey

²Suleyman Demirel University, Faculty of Medicine, 4th Grade Medical Student, Isparta, Turkey.

Iodine is a part of thyroid hormones and has been reported to act directly as an antioxidant or induce indirectly antioxidant enzymes. This study aimed to assess the urinary iodine concentration and its relationship with the antioxidant and oxidative stress capacity in healthy school-aged children.

196 students from 5 primary schools randomly selected between 9-12 years (mean age 10.2 ±1.2 years) were enrolled into the study. Urinary iodine levels were measured by spectrophotometry with Sandell-Kolthoff reaction. Total antioxidant status (TAS) and total oxidant status (TOS) were analysed from urine samples. The ratio of TOS to TAS was regarded as an oxidative stress index (OSI), an indicator of the degree of oxidative status.

Children in 54% (107) had iodine deficiency (ID) and the majority of them (30%) had mild ID. There was not any severe iodine deficient child in the population (<20 µg/L). Urine TAS levels were significantly lower in the moderate ID group than in the mild ID group (6.5±4.1 vs. 11.3±4.1 µmol, p<0.001) and than iodine sufficient group (11.0±5.3 µmol, p<0.001). TOS levels and OSI were found higher in the moderate ID group than the mild ID group (4.8±2.1 vs. 3.7±2.1 µmol, p<0.001) and the iodine sufficient group (4.8±2.1 vs. 3.4±2.5 µmol, p<0.001). In moderate ID group, low urine iodine levels

exhibited significant negative correlations with OSI (r=-0.660) and TOS (r=-0.248) and a positive correlation with TAS (r=0.475).

We found that children with moderate ID were exposed to more oxidative burden than children with mild ID or iodine sufficiency. Increased systemic oxidative stress induced by moderate ID could cause development of iodine deficient related complications and diseases. Iodine supplementation could have a beneficial role in the prevention of oxidative stress.

Key words: iodine, iodine deficiency, oxidative stress, total antioxidant status, children.

▶ Young Speaker No. 2

Investigation of toxic effect of acrylamide on fetal brain development and protective potentials of N-Acetylcysteine

Mehmet Erman Erdemli¹, Yusuf Turkoz¹, Eyup Altinoz²

¹Department of Medical Biochemistry, Medical Faculty, Inonu University, Malatya, Turkey

²Department of Medical Biochemistry, Medical Faculty, Karabuk University, Karabuk, Turkey

Acrylamide (AA) is a commercial chemical especially neurotoxic for humans and animals. When food is cooked in high temperatures plenty of AA is formed. Individuals, who are fed on these nutrients or consume industrial products that contain AA, are exposed to AA and toxic effects are observed in these individuals. The objective of the present study is to investigate possible toxic effects of AA applied during pregnancy on brain development of the fetus of the rats by analyzing fetal brain tissue oxidative stress parameters-

Four groups were formed with 9 pregnant rats each as control, AA, N-acetylcysteine, AA + N-acetylcysteine groups. Caesarian section was implemented on the 20th day of pregnancy, Malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and BDNF levels were analyzed and histopathology

examinations were performed in brain tissues of the fetuses obtained by caesarian section from all groups.

If AA group's data compared with the control group, it was determined that AA caused degeneration in neuron structures in fetal brain tissue and caused hemorrhagic damages; dramatically decreased BDNF levels; increased MDA, SOD, and decreased GSH, GSH-Px, CAT levels ($p<0.05$). On the other hand, it was determined that the N-acetylcysteine, suppressed the effects of AA on fetal development and fetal brain tissue damage in above mentioned parameters ($p<0.05$).

AA passed through the placenta of pregnant rats and caused oxidative stress in fetal brain tissue. However, N-acetylcysteine applied with AA removed the toxic effects of AA with its free radical sweeping antioxidant effect, and suppressed oxidative stress as a result and brought BDNF levels back to normal.

▶ Young Speaker No. 3

Alterations in thymic morphology and antioxidant biomarkers in 60-day-old male rats following exposure to a continuous 900-MHz electromagnetic field during adolescence

Ali Kulaber¹, Gökçen Kerimoğlu¹, Ersan Odacı¹, Şafak Ersöz²

¹Department of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

²Department of Pathology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

Numerous devices that emit electromagnetic fields (EMF) are now part of daily life. The effects of exposure to such EMF at varying frequencies on living cells, tissues and systems are now the subject of significant scientific research (1, 2, 3). The present study was therefore intended to investigate changes occurring in thymic tissue of male rats exposed to the effect of 900 Megahertz (MHz) EMF in postnatal days 22-59, inclusive. Control (CG), sham (SG) and EMF

(EMFG) groups consisting of eight male Sprague Dawley rats each, aged 21 days, were established for this purpose. No procedure was performed on CG rats. SG rats were kept in a Plexiglas cage every day for 1 hour between postnatal days 22 and 59, inclusive, but without being exposed to EMF. EMFG rats were kept in the same cage for the same periods as the SG rats, but were exposed to 900 MHz EMF. Rats were sacrificed on postnatal day 60. The thymuses were removed and divided into two parts. The right half of each thymus was stained with H&E for histological investigation. The other half was used for biochemical analyses. Oxidant/antioxidant parameters were studied at biochemical analysis. According to our results MDA levels in EMFG increased significantly compared to CG and SG ($p=0.004$ and $p=0.004$, respectively). CAT levels in CG increased significantly compared to SG and EMFG ($p=0.004$ and $p=0.004$, respectively). Normal morphological structure was impaired in EMFG sections at histological examination. On the basis of the study findings it may be concluded that 900 MHz EMF applied for 1 hour a day every day on postnatal days 22-59, inclusive, can lead to an increase in tissue MDA and pathological changes in male rat thymic tissue.

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▶ Young Speaker No. 4

Is the hot chili pepper consumption through activation of TRPV1 channel risk for epilepsy?

İrem Karaer¹, Nur Fidan Çam¹, Mustafa Nazıroğlu²

¹Student, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

²Neuroscience Research Center, Süleyman Demirel

University, Isparta, Turkey

Approximately 50 million people in the World suffer from epilepsy and 20-30% of these patients spend seizure which cannot be controlled with available drugs. Because of this scientists are heading to searching the main reasons of epilepsy. The TRPV1 cation channels has been identified as a polymodal transducer molecule on a sub-set of primary sensory neurons which responds to various stimuli including noxious heat ($> 42\text{ }^{\circ}\text{C}$), protons, oxidative stress, osmotic pressure and vanilloids such as capsaicin, the hot ingredient of chili peppers. When TRPV1 channels activated Na^+ and Ca^{2+} entry occur resulting increased neuronal excitability (1). The studies between the years of 2010-2014 behavioral observations are done inducing epilepsy models in rats and EEG recording is observed (2,3).

In a recent study, we observed involvement of apoptosis and Ca^{2+} accumulation through TRPV1 channels in the PTZ-induced rat model by measuring intracellular free calcium concentration, current densities of TRPV1, apoptosis, latency time of epilepsy, caspase 3 and caspase 9 values (4).

In conclusion, current literature results indicate that capsaicin consumption and calcium accumulation through TRPV1 channel play physiologically relevant roles in the regulation of epileptic seizures. This interaction of capsaicin may play an important role in epilepsy associated with activation of TRPV1 channels.

Keywords: Epilepsy; TRPV1 channels; Oxidative stress; Apoptosis; Capsaicin.

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Oral Presentations

▶ Oral Presentation No. 1

Effects of long term pre and postnatal exposure to 2.45 GHz wireless devices on developing male rat kidney

Ayça Esra Kuybulu¹, Faruk Öktem², İbrahim Metin Çiriş³, Recep Sütçü⁴, Ahmet Rifat Örmeci⁵, Selçuk Cömlekçi⁶, Efan Uz⁷

¹Department of Pediatric Nephrology, Suleyman Demirel University Faculty of Medicine, Isparta, Turkey

²Department of Pediatric Nephrology, Bezmi Alem University Faculty of Medicine, Istanbul, Turkey

³Department of Pathology, Suleyman Demirel University Faculty of Medicine, Isparta, Turkey

⁴Department of Biochemistry, Katip Celebi Faculty of Medicine, Izmir, Turkey

⁵Department of Pediatrics, Suleyman Demirel University Faculty of Medicine, Isparta, Turkey

⁶Department of Electronics and Communication, Suleyman Demirel University Faculty of Engineering, Isparta, Turkey

⁷Department of Biochemistry, Suleyman Demirel University Faculty of Medicine, Isparta, Turkey

The aim of the present study was to investigate oxidative stress and apoptosis in kidney tissues of male wistar rats that pre- and postnatally exposed to wireless electromagnetic field (EMF) with an internet frequency of 2.45 GHz for 3 months.

The study was conducted in three groups of rats which were prenatal, postnatal and sham exposed groups. Oxidative stress markers and histological evaluation of kidney tissues were studied.

Renal tissue malondialdehyde (MDA) and total oxidant (TOS) levels of prenatal group were high, and total antioxidant (TAS) and superoxide dismutase (SOD)

levels were low. Spot urine NAG/creatinine ratio was significantly higher in pre- and postnatal groups ($p < 0.001$). Tubular injury was detected in most of specimens in postnatal groups. Immuno-histochemical analysis showed low intensity staining with Bax in cortex, high intensity staining with Bcl-2 in cortical and medullar areas of prenatal group (p values, < 0.001 , 0.01 and 0.001 , respectively) when compared with sham group. Bcl2/Bax staining intensity ratios of medullar and cortical area was higher in prenatal group than sham group ($p = 0.018$, $p = 0.011$).

Based on this study, it is thought that chronic pre- and postnatal period exposure to wireless internet frequency of EMF may cause chronic kidney damages; staying away from EMF source in especially pregnancy and early childhood period may reduce negative effects of exposure on kidney.

Key Words: Apoptosis, oxidative damage, wireless, neonates, prenatal exposure

▶ Oral Presentation No. 2

The effect of insulin-like growth factor-1 over expression on muscle and oxidative damage to proteins

Hatice Tohma^{1,2,3}, Peter Arthur², Miranda Grounds³

¹Department of Chemistry, Faculty of Art and Sciences, Erzincan University, Erzincan, Turkey

²School of Biochemical, Biomolecular and Chemical Sciences, Australia

³School of Anatomy and Human Biology; University of Western Australia, Australia

Sarcopenia is a condition where muscle mass and strength declines with progressing age. Although extensively studied, how oxidative insult results in decreased in muscle mass and function is yet to be determined. Oxidative stress might have a role in sarcopenia associated muscle loss which may be a consequence of the accumulation of molecular oxidative damage to proteins.

If oxidative stress is connected to muscle wasting, then maintaining muscle mass could be reflected in oxidative stress during aging. In the present study, we used muscle specific insulin-like growth factor-1 (IGF-1) over expressing mice to investigate reversible protein thiol oxidation and irreversible protein carbonyl formation in age related muscle wasting. To determine the protein thiol oxidation and protein carbonylation we used 2 tag technique and protein carbonylation assay, respectively.

In this study, IGF -1overexpression in mice partially prevented age related muscle mass loss. Changes in protein thiol levels and or changes in protein carbonyl levels were not consistently associated with changes in muscle mass. These results suggest that changes in protein thiol oxidation are unlikely to be linked to loss of muscle mass with aging.

Keywords: Sarcopenia; Oxidative stress; insulin-like growth factor-1.

▶ Oral Presentation No. 3

Regulation of intracellular free Zn^{2+} in ventricular cardiomyocytes with subcellular organelles

Erkan Tuncay¹, Aysegul Toy¹, Guy A. Rutter², Belma Turan¹

¹Department of Biophysics, Faculty of Medicine, Ankara University, Ankara, Turkey;

²Division of Diabetes Endocrinology and Metabolism, Department of Medicine, Imperial College London, UK

The total intracellular free Zn^{2+} level in cells is comparable to that of Ca^{2+} while its about 70% is compartmentalized in subcellular organelles, with most of its remainder bound to cytosolic proteins. It has been also shown that the resting level of intracellular free Zn^{2+} ($[Zn^{2+}]_i$) can be increased with different external stimuli such as thiol-reactive oxidants or high glucose, that of them, in turn, contribute to oxidant-induced alterations in EC-coupling of mammalian cardiomyocytes. Since Zn^{2+} is important in cellular physiology, very little is known how $[Zn^{2+}]_i$ is controlled

and stored intracellularly in cardiomyocytes. Therefore, in the present study we aimed to investigate possible role of two Zn^{2+} -transporters on regulation of $[Zn^{2+}]_i$ in cardiomyocytes. First, we monitored cytosolic (CY), mitochondrial (MitC) and endoplasmic reticulum (ER) levels of free $[Zn^{2+}]$ in cardiomyocytes by using genetically-encoded FRET-based sensors. Our data showed that ER revealed higher $[Zn^{2+}]$ level compared to the other compartments in freshly isolated left ventricular cardiomyocytes. Second, we tested the localisation and expression levels of two Zn^{2+} -transports ZnT7 and ZIP7 in the freshly isolated rat ventricular cardiomyocytes. Third, we examined the role of ZnT7 and ZIP7 on regulation of cytosolic free $[Zn^{2+}]$ in the cardiomyocytes under hyperglycemic condition. Our Western-blot data have shown that the protein level of ZIP7 increased in either cardiomyocytes isolated from streptozotocin-induced diabetic rat heart or high glucose (25 mM; 24-hours) incubated cells. However, the protein level of ZnT7 is decreased in these cells, significantly. Our immunofluorescence analysis also demonstrated that ZIP7 and ZnT7 are localised in ER. Consequently, our present data showed that ER is a Zn^{2+} pool and ZIP7 and ZnT7 have a crucial role in regulation of cytosolic Zn^{2+} in mammalian cardiomyocytes.

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▶ Oral Presentation No. 4

An investigation on the effects of intermittent hypoxia in streptozotocin-induced diabetic cardiac function

Ali Doğan Dursun¹, Erkan Tuncay², Yusuf Olgar², Fırat Akat¹, Ayhan Tanyeli¹, Derya Güzel¹, Ferda Topal Çelikkan³, Metin Baştuğ¹, Hakan Fıçıcılar¹, Belma Turan²

Departments of ¹Physiology, ²Biophysics, ³Histology and Embryology, Faculty of Medicine, Ankara University, Ankara, Turkey.

Diabetes Mellitus (DM) is an important disorder with an increase of mortality and morbidity due to its impact on the cardiovascular system. Either acute or chronic

hyperglycemia first initiates repeated acute changes in cellular metabolism, and then followed by cumulative long-term changes in macromolecules. Intracellular Ca^{2+} -homeostasis is regulated via Ca^{2+} -release from intracellular stores, mainly from SR through ryanodine receptors, RyR2 and SERCA2-mediated Ca^{2+} -reuptake in cardiomyocytes (1). Any change in this regulation underlies diabetic cardiomyopathy under hyperglycemia (2) with important contribution of changes in phospholamban (PLB) and/or CaMKII levels/activities, SERCA2 and RyR2 in this homeostasis (3). In our previous study, we have shown that "chronic intermittent" hypoxia could cause an improvement in cardiac function in experimental animal studies via reducing diabetes-induced heart-tissue injury and increasing the capillarity as well as significant protection against diabetes-induced depressed left ventricular developed pressure (4,5). Therefore, in here, we first aimed to examine whether intermittent hypoxia has beneficial effect on altered parameters of intracellular Ca^{2+} -homeostasis at cellular level in freshly isolated left ventricular cardiomyocytes from diabetic rats. We used 11 week-old, Wistar male rats that are grouped as control, intermittent hypoxia (IH), diabetes (DM) and DM+IH (IH; ~3,000 m height 6-h daily, for 6-wk). We measured intracellular basal level of Ca^{2+} and transient Ca^{2+} changes in Fura-2 loaded resting cells and electrically stimulated cells, respectively. We also monitored L-type Ca^{2+} -channel currents by using patch-clamp technique. Compared to the controls, the basal Ca^{2+} level was significantly higher in DM group while the amplitude of Ca^{2+} -transients of this group was significantly smaller. Interestingly, IH exposure to DM group induced a marked decrease in L-type Ca^{2+} -channel currents although it is not different in DM group comparison to the control with no change in IH group. Additionally, we observed significant further decrease in the amplitude of Ca^{2+} -transients (even less than the DM group) together with decreased basal Ca^{2+} level (similar to the control level) in IH+DM group. Our data on induction of less Ca^{2+} release from SR via decreased L-type Ca^{2+} -channel currents under IH exposure are supporting the change in Ca^{2+} -homeostasis at cellular level in cardiomyocytes. These phenomena may further underlie the decreased basal Ca^{2+} level in IH+DM group. Therefore, one can suggest that IH exposure in DM cardiomyocytes may play beneficial role via compensating the increased basal Ca^{2+} level in

hyperglycemic cells. Our biochemical data associated with decreased mRNA level of CaMKII with no change in either mRNA or protein expression level of SERCA and PLB in IH+DM group, compared to high-phosphorylated level of CaMKII in DM group, are also supporting our electrophysiological findings. As conclusion, IH exposure in DM rats playing a compensatory role on altered Ca^{2+} -homeostasis at cellular level, beneficial action of IH, at most, seems to be associated its effect on hyperglycemia-associated decreased elasticity properties of the heart tissue at organ level.

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▶ Oral Presentation No. 5

Capsaicin- and menthol-induced Ca^{2+} oscillations and Ca^{2+} waves in breast and prostate cancer cells

¹Laszlo Pecze, ²Michael Dougoud, ²Christian Mazza, ¹Beat Schwaller

¹Department of Medicine, University of Fribourg, Fribourg, Switzerland. laszlo.pecze@unifr.ch.

²Department of Mathematics, University of Fribourg, Fribourg, Switzerland

The TRP agonists capsaicin (50 μM) for TRPV1 and menthol (50 μM) for TRPM8 applied to prostate and

breast cancer cells in vitro elicited intercellular Ca^{2+} waves and/or intracellular Ca^{2+} oscillations. The oscillations indicate that thermo-sensitive TRP channels are involved in the transmission of extracellular mitogenic signals derived from the tumor milieu to the intracellular signaling machinery. In order to decipher the presumed function of Ca^{2+} waves spreading among cultured cells, a mathematical model was developed. This model allows to numerically modifying the connectivity probability between neighboring cells, the extracellular diffusion rate of Ca^{2+} ions and the individual sensitivity of cells to an agonist. The simulations demonstrate that the most sensitive cells are the wave initiator cells and moreover that Ca^{2+} waves play an important role in the harmonization of evoked responses.

▶ Oral Presentation No. 6

Sono-Photodynamic Therapy: An alternative cancer treatment

Mehmet Dinçer Bilgin

Adnan Menderes University, Medical Faculty,
Department of Biophysics, Aydın, 09010, Turkey

Photodynamic therapy (PDT) is noninvasive and could be an alternative treatment for cancers. Photodynamic therapy is a type of photochemotherapy, which mainly bases on the principle of singlet oxygen generation. This therapy leads to the necrosis or apoptosis of cancer cells radiated by an appropriate wavelength of visible light, which activates a photosensitive agent localized in the target tissue in the presence of molecular oxygen. PDT is specific to the target tissue, well-tolerated, allows treatment of multiple lesions in the same session, and is not associated with cumulative toxicity. PDT is applied for the treatment of various cancers and pre-cancerous disorders. However, PDT has two recognized disadvantages; (1) PDT can apply only to the superficial lesions of tissues, (2) PDT-treated patients tend to suffer long-lasting skin sensitivity. Similar to PDT, sonodynamic therapy (SDT) is based on preferential uptake and/or retention of a sonosensitizing drug in tumor tissues and subsequent activation of the drug by ultrasound irradiation. SDT is a developing treatment

and promising for the treatment of cancer. Ultrasound irradiation can penetrate deeply into tissues and has focused energy on the site of malignancy with minimal damage to peripheral healthy tissue. Sono-photodynamic therapy (SPDT), which combines light and ultrasound to activate the sono-photosensitizer to generate both photochemical and sonochemical effects, that enhances the antitumor efficacy on various cancers compared with PDT and SDT alone. But the potential applications and mechanisms of this therapy have not been fully explained. In this work, the possible applications and mechanisms of sono-photodynamic therapy as well as of molecular, cellular and tumor responses of sono-photodynamic therapy are discussed.

▶ Oral Presentation No. 7

Curcumin inhibits apoptosis by regulating intracellular calcium release, reactive oxygen species and mitochondrial depolarization levels in ARPE-19 cells

Handan Bardak¹, Abdülhadi Cihangir Uğuz^{2,3}, Yavuz Bardak¹, Mustafa Nazıroğlu^{2,3}, İshak Suat Övey³

¹Department of Ophthalmology, Haydarpaşa Numune Research and Training Hospital, Istanbul, Turkey

²Department of Biophysics, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

³Neuroscience Research Center, Süleyman Demirel University, Isparta, Turkey

Our eyes are increasingly exposed to light from many sources, most of which contain high energy level that can easily penetrate to the organelles. High energy levels can trigger oxidative stress conditions. Under stress conditions, apoptotic cascade can be triggered and cellular viability can easily be lost. Curcumin, a polyphenolic compound derived from the dietary spice turmeric, a yellow substance obtained from the root of the plant *Curcuma longa* Linn, possesses diverse pharmacological effects, including anti-inflammatory, antioxidant, and antiproliferative activities. The aim of the current study is to determine whether or not curcumin has any protective effect against blue light irradiation.

For this purpose, we evaluated the intracellular calcium release mechanism, PARP, procaspase -3 /-9 protein expression levels by western blotting, reactive oxygen species levels, apoptosis levels, caspase-3 / -9 activation levels by spectrofluorometry, mitochondrial membrane depolarization levels, and oxidative stress parameters. Retinal pigment epithelial (ARPE-19) cells were divided into four main groups. The cells in curcumin and curcumin + blue light groups were incubated with 20 μ M curcumin. Blue light exposure was performed for 24 hours in an incubator.

Lipid peroxidation (LP) and cytosolic free Ca^{2+} concentrations were higher in the blue light exposure group than in the control group; however, their levels were determined as significantly lower in the curcumin and curcumin + blue light exposure groups than in the blue light group alone. PARP and procaspase-3 levels were significantly higher in blue light group. Curcumin administration significantly decreased PARP and procaspase-3 expression levels. Reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) values were lower in the blue light exposure group, although they were higher in the curcumin and curcumin + blue light exposure groups. Caspase-3 and -9 activities were lower in the curcumin group than in the blue light group. Moreover, vascular endothelial growth factor (VEGF) levels were significantly higher in the blue light exposure group.

In conclusion, curcumin strongly induced regulatory effects on oxidative stress, intracellular Ca^{2+} levels, VEGF levels, PARP expression levels and caspase-3 and -9 values in an experimental oxidative stress model in ARPE-19 cells.

Keywords: Apoptosis, ARPE-19 cells, blue light damage, Ca^{2+} signaling, Curcumin, oxidative stress

Oral Presentation No. 8

Disrupted calcium signaling and periodic leg movements during sleep in hypertensive-obstructive sleep apnea patients; a correlation study

Mohammad Torabi-Nami^{1,2*}, Samrad Mehrabi^{2,3}, Bijan

Zare⁴, Farshad Nazaraghiaie⁵, Sabri Derman⁶

¹Department of Neuroscience, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

²Sleep Disorders Laboratory, Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

³Division of Pulmonology, Department of Internal Medicine, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

⁴Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

⁵Department of Human Consciousness and Yogic Sciences, Mangalore University, India

⁶Sleep Disorders Unit, American Hospital, Koç Foundation, Istanbul, Turkey

Disrupted calcium signaling has been suggested as a possible early event in neuropathies and specific neurological symptoms such as what seen in restless leg syndrome (RLS). A considerable proportion of RLS patients tend to suffer from Periodic Leg Movement in Sleep (PLMS). On the other hand, the sleep-disordered breathing in patients with obstructive sleep apnea syndrome (OSAS), not only often coexists with hypertension but the condition also contributes to isolated leg movements during sleep. We hypothesized that PLMS symptoms in hypertensive OSAS patients may at least partly be related to the use of calcium channel blockers (CCBs) by these patients. To test such a possible correlation, we retrospectively evaluated 79 consecutive hypertensive patients (81% male) with a recent diagnosis of OSAS who underwent full polysomnography (apnea-hypopnea index >5 events/h) at our hospital-based sleep disorders laboratory. Smokers, diabetics, those who were taking hypnotics/benzodiazepines and patients with congestive heart failure were excluded. All subjects were on at least one antihypertensive drug including angiotensin-converting enzyme inhibitors (23%), beta-blockers (31%), angiotensin receptor blockers (17%), diuretics (7%) and CCBs (22%). Multiple regression analysis revealed that the use of CCBs ($p=0.028$) was the only treatment-related variable inversely correlated with total sleep time (TST) whereas positively correlated with isolated leg movements during sleep characterized as PLMS ($p=0.041$). Since the particular calcium channel and

exact calcium signaling pathway involved in PLMS has not been yet identified, further investigations on the nature of this correlation appear warranted. Moreover, the hypothesis that TRPC5 and TRPC6 channels are amongst the main players involved in the effect of CCBs upon induction or aggravation of PLMS symptoms in hypertensive, OSAS patients needs to be tested in well-designed future studies.

Keywords: Calcium Channel blockers, PLMS, Hypertension, OSAS, Calcium Signaling

▶ Oral Presentation No. 9

The effects of stem cells applications on the oxidative stress and apoptosis during implantation

Işıl Aydemir, Pınar Kılıçaslan Sönmez, Mahmud Özkut, Dila Hatun Sal, Fulya Gülbağça, Suna Saygılı, Mehmet İbrahim Tuğlu

Department of Histology and Embryology, Faculty of Medicine, Manisa Celal Bayar University, Manisa, Turkey.

Blastocyst implantation is an important mechanism for the beginning of pregnancy. The complex cellular and molecular factors released from endometrium and blastocyst plays a crucial role during implantation (1). Oxidative stress is one of these factors. Nitric oxide synthase (NOS) is marker of oxidative stress and NOS activity involved in the decidua, capillaries and nerves of uterus during pregnancy (2). Damaged molecular events cause infertility problems. In our study, we aimed to determine effects of bone marrow derived mesenchymal stem cell (BMSC) application into the rat endometrium via oxidative stress and apoptosis. Female rats were divided into three groups which were saline (n:7), media(n:7), BMSC in media (n:7). After vaginal smear technique, 200 µL saline, 200 µL culture media and 1×10^6 BMSC/200 µL culture media were injected into the uterine and periton of female rats when they were in estrous cycle. The pregnant female rats on the 7th day were sacrificed and uterine samples removed and were stained with heamatoxylin-eosin histochemically, anti-eNOS and anti-iNOS immunohistochemically, with TUNEL for apoptosis. H-score results were determined

using One-Way ANOVA test statistically. The intrauterine BMSC application to the uterine was decreased the both eNOS and iNOS immunoreactivities whereas intraperitoneal BMSC administration was diminished the number of apoptotic cells. Recently, stem cell treatments became more common in clinical area for several diseases (3). According to our results, distribution of oxidative stress markers and cell death were changed by application of stem cells. It is suggested that stem cell treatments can be used in case of infertility due to oxidative stress.

Keywords: Mesenchymal stem cell, Implantation, Endometrium, Oxidative stress, Apoptosis.

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▶ Oral Presentation No. 10

Effect of TRPM2 antagonist ACA on the okadaic acid induced neurodegeneration

Murat Çakır¹, Halil Düzova¹, Elif Taşlıdere², Suat Tekin¹

¹Department of Physiology, Faculty of Medicine, Inonu University, Malatya, Turkey.

²Department of Histology and Embryology, Faculty of Medicine, Bezmialem University, İstanbul, Turkey.

Okadaic acid (OKA) is a specific protein phosphatases inhibitor and it can cause the accumulation of phosphorylated forms of tau protein and induce impairments in cognitive function, neuronal morphology, glial function and free radical homeostasis (1). Transient receptor potential melastatin 2 (TRPM2) is an oxidative stress sensing calcium-permeable channel that is thought to contribute to calcium dysregulation associated with neurodegenerative diseases, including Alzheimer's disease (2-3).

This study was aimed to investigate the neuroprotective effect of TRPM2 inhibitor N-(p-Amylcinnamoyl) anthranilic Acid (ACA) in a neurodegenerative model induced by OKA. OKA was administrated adult male Sprague Dawley rats (n=50) via intra-cerebro-ventricular injection (icv) to induce neurotoxicity in these experimental models. Rats (320-380 g) were randomly divided into five groups : i) control , ii) sham-opera (injected icv with artificial cerebrospinal fluid (aCSF), iii) ACA (ACA intraperitoneally (ip) 25 µg/kg/day for 13 days), iv) OKA (OKA was dissolved in aCSF and injected via icv (200 ng) in a volume of 10 µl bilaterally) and v) OKA+ACA (treated with icv with OKA (200 ng) and ACA ip 25 µg/kg/day for 13 days).

We observed irregular borders, acidophilic cytoplasm, and shrunken and degenerated heterochromatic nucleus at neurons in cortex and hippocampus of OKA treated rats. In addition, caspase-3 positive cells were observed in the cortex and hippocampus. When OKA+ACA rats compared to OKA rats , it was found that numbers of degenerated neurons and caspase 3 positive cells of cortex and hippocampus regions were significantly reduced (p<0.0001).

In conclusion, we observed that ACA might provide some protections against OKA induced neurodegeneration.

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► Oral Presentation No. 11

Effect of TGF-beta type 1 receptor kinase inhibitor, LY- 364947 on angiogenesis in liver cirrhosis model in rats

Özge Çağlar¹, Necmiye Canacankatan¹, Figen Doran²

¹Department of Biochemistry, Faculty of Pharmacy, Mersin University, Mersin, Turkey

²Department of Pathology, Medicine Faculty, Çukurova University, Adana, Turkey.

Cirrhosis is common and progressive condition characterized by severe scarring of the liver. Transforming growth factor (TGF) beta type 1 is one of the major profibrogenic mediators which take part in the development of liver cirrhosis. *Angiogenesis*, the formation of new blood vessels from the existing vasculature has been linked to progression to cirrhosis. In this study, we aimed to determine the effect of the TGF-β type I receptor kinase inhibitor, LY-364947 on angiogenesis in liver cirrhosis.

Six groups were included as Control, Cirrhosis, Cirrhosis + DMSO, Cirrhosis + LY-364947 (100µg/kg/week), Cirrhosis + LY- 364947 (300µg/kg/week), after Cirrhosis + LY- 364947 (100µg/kg/week). Experimental liver cirrhosis was developed by N-Nitrosodiethylamine. Control, Cirrhosis, Cirrhosis+DMSO, Cirrhosis+LY- 364947 (100µg/kg/week), and Cirrhosis+LY- 364947 (300µg/kg/week) groups were killed when cirrhosis was established by evaluation of histopathological investigations in rats which were chosen randomly from the Cirrhosis group. After cirrhosis was established LY- 364947 was administered for 4 weeks to rats after Cirrhosis+LY- 364947 (100µg/kg/week) group.

Angiogenesis was evaluated by measurement of vascular endothelial growth factor (VEGF), TGF-β1 and angiostatin levels. The evaluations were carried out by colorimetric methods according to the assay instructions. Histopathological investigations were also carried out.

TGF-β1 levels were decreased and angiostatin levels were increased significantly by LY- 364947 (300µg/kg/week). TGF-β1 levels were also decreased by LY- 364947 (100µg/kg/week). Although LY- 364947 (300µg/kg/week) suppressed VEGF levels, it was not significant. Histopathological findings indicated that cirrhosis development was reduced by LY- 364947 (300µg/kg/week).

In conclusion, LY- 364947 may be suggested as a promising antiangiogenic agent in liver cirrhosis by suppressing TGF- β 1, altering angiostatin and reducing liver cirrhosis.

Keywords: liver cirrhosis, LY- 364947, TGF- β type I, VEGF, Angiostatin

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Oral Presentation No. 12

The effect of swimming exercise on age-related electrical and mechanical changes in female rat myocytes.

Nihal Ozturk¹, Yusuf Olgar², Semir Ozdemir²

¹Technical Sciences Vocational School, Akdeniz University, Antalya Turkey.

²Department of Biophysics, Medical Faculty, Akdeniz University, Antalya Turkey.

The effects of exercise on electrical and mechanical remodeling of female heart during senescence remain unclear. Therefore, in our study, we examined the impact of swimming exercise on age-related electrical and contractile alterations that may occur in old female rat ventricular myocytes. The rats were grouped as Sedentary-young (4 month), sedentary old (4 month) and exercised old (24 month). Exercised rats were trained 2 hours/day for 8 weeks. Experimental recordings were measured from freshly isolated left ventricular myocytes at 36 \pm 1 °C. There was significant increase in myocyte size as well as prolongation of action potential duration and suppression of transient outward potassium current in aged rats. The swimming exercise had no remarkable effect on those age-related changes of ventricular myocytes. On examination of the heart's mechanical activity, significant decrease in the amount of fractional shortening was measured in aged myocytes. Although it was not effective on slowed

relaxation kinetics, swimming exercise achieved remarkable improvement in fractional shortening. In conclusion, despite swimming exercise had no effect on the electrical properties of old female rat myocytes, it elicited significant improvement in the contractility. Thus the effects of exercise on mechanical activity may be associated only with structural improvement of myocardium.

Keywords: Exercise; Ventricular myocytes; Age-related Electrical and Mechanical Changes

Protective effects of pterostilbene and magnetic field on renal ischemic injury

Ozlem Bozkurt^{1,2}, Hatice Keser¹, Mehmet Dincer Bilgin^{1,2}, Feride Severcan³

¹Department of Biophysics, Medical Faculty, Adnan Menderes University, Aydın, Turkey.

²Department of Biophysics, Institute of Health Sciences, Adnan Menderes University, Aydın, Turkey.

³Department of Biological Sciences, Middle East Technical University, Ankara, Turkey

Ischemia reperfusion injury is one of the most important pathological mechanisms observed after organ transplantation (1). Although the application of magnetic field to prevent ischemic injury has been proposed as a new treatment strategy, controversial results have been reported (2). This study aims to determine the therapeutic role of 50 Hz low frequency magnetic field and pterostilbene, an antioxidant which is a derivative of resveratrol, on renal ischemic injury.

The rats were divided into control, ischemia-reperfusion, 50 Hz low frequency magnetic field treatment and 10 mg/kg dose of pterostilbene injection together with magnetic field treatment groups. Treatment was applied for 5 consecutive days after the induction of renal ischemia and 24 hours of reperfusion. After 5 days, the kidneys were homogenized and the homogenate was analyzed by Fourier transform infrared (FTIR) spectroscopy.

Ischemia-reperfusion injury caused a decrease in lipid and protein amount, lipid/protein ratio; a decrease in the level of unsaturated lipids and unsaturated/saturated lipid ratio; an increase in membrane fluidity and lipid peroxidation in components of rat kidneys. Moreover, ischemia-reperfusion injury led to an alteration in the structure of lipids and proteins and a higher content of long chained lipids were observed in kidneys of ischemia-reperfusion group. 50 Hz frequency magnetic field application was not sufficient to repair all ischemia-induced alterations, however pterostilbene injection together with magnetic field treatment

successfully restored ischemia-induced changes in all of the investigated parameters.

Pterostilbene treatment together with magnetic field application can be a promising prevention for ischemic injury.

Keywords: Renal ischemia reperfusion injury, low frequency magnetic field, antioxidant, pterostilbene, FTIR spectroscopy

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Exposure to 900 MHz electromagnetic field during the adolescent period can cause oxidative stress in the male sprague dawley rat liver

Gökçen Kerimoğlu, Ersan Odacı

Department of Histology and Embryology, Medical Faculty, Karadeniz Technical University, Trabzon, Turkey.

Technological advances and advertising are causing increased use of devices such as wireless internet modems, television and mobile phones that produce electromagnetic fields (EMF). Mobile phones are today the main source of EMF in adolescents because of overuse among young people and children (1). Several studies have reported that EMF leads to oxidative damage in the heart, kidney, brain and other tissues (2). This study was therefore designed to research the effects of exposure to 900 MHz EMF on the adolescent rat liver. Twenty-four male Sprague Dawley rats, aged 21 days, were used. Rats were equally and randomly divided into three groups, a control group (CG), a sham group and an EMF-exposed group (EG). EG rats were exposed to 900 MHz EMF for 1 h/day throughout the

adolescent period (between postnatal days 21 and 59) in a special box. SG rats were also kept in the box for 1 h/day without EMF exposure. The whole-body specific absorbance rate was calculated as 0.0093 W/kg. All rats were sacrificed and their livers removed on the postnatal 60th day. Biochemical analyses showed increase malondialdehyde and glutathione levels and decreased catalase levels in EG compared to the other groups. Analyses of histological slides stained with hematoxylin and eosin revealed loss of hepatocytes, dilated sinusoids and paleness around pericentral regions, and increased apoptotic and mitotic figures in the parenchyma. The study results revealed that exposure to EMF throughout the adolescent period can result in oxidative damage in the male adolescent rat liver.

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▶ T. Oral Presentation No. 3

The effects of smoking on manganese, selenium and hemorheological parameters

Fatma Ates Alkan¹, Denizhan Karis¹, Gulfidan Cakmak², Alev Meltem Ercan¹

¹Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

²Department of Respiratory Medicine, Haseki Education and Research Hospital, Istanbul, Turkey

Smoking is a significant risk factor in fatal diseases including cardio-cerebrovascular diseases and sudden death (1). Manganese (Mn) and selenium (Se) are essential trace elements involved in the mechanisms of cellular antioxidant defense by means of oxygen transport via Fenton-type reaction (2,3,4). The structure and number of erythrocytes that play important role in blood flow that take effective place in tissue oxygenation. The increase of hematocrit as a result of probable hypoxia may effect oxygen delivery index

(ODI) and erythrocyte deformability index (Tk) (5). The aim of this study is to investigate the relationship between serum levels of Mn and Se with blood viscosity (BV), plasma viscosity (PV), fibrinogen, ODI, Tk as hemorheological parameters in current-smokers without diagnosis of chronic obstructive pulmonary disease (COPD). The individuals (n:128) were divided as ex-smokers, current-smokers and non-smokers. Mn and Se levels were analyzed using inductively coupled plasma-optical emission spectrophotometer. BV and PV were measured via viscometer at 37°C. Plasma fibrinogen level was measured by Clauss method. Mn levels, BV, PV, and fibrinogen were significantly higher in current smokers than control groups; whereas Se levels were lower in current-smokers than control groups. In conclusion, increase in Mn, BV, PV, fibrinogen and decrease in Se both may result from the inflammatory response in oxidative defense mechanism. The decrease of ODI and Tk without statistical significance might be effective in the alteration of BV and PV in current-smokers without diagnosis of COPD. Our results point out that hemorheological parameters and levels of serum manganese and selenium may be used as markers of tissue oxygenation and oxidative stress before the clinic onset of COPD.

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Changes in testicular morphology and oxidative stress biomarkers in 60-day-old Sprague Dawley rats following exposure to continuous 900 MHz electromagnetic field for 1 hour a day throughout adolescence

Hatice Hancı¹, Gökçen Kerimoğlu, Ersan Odacı

Department of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

Following reports of several reversible/irreversible pathologies in living tissues caused by the effects of electromagnetic field (EMF) at different frequencies, the impact on human health of EMF emitted by mobile phones in particular is now the subject of intense investigation. Since cell phones are used by adolescents more than adults, they are also more exposed to EMF than adults. However, data concerning the effects of exposure to EMF during the adolescent period are still insufficient (1, 2). EMF can damage both testicular morphology and biomarkers. The purpose of this study was performed to investigate the 60-day-old male rat testis following exposure to 900-megahertz (MHz) EMF throughout the adolescent period using histopathological and biochemical analysis methods. Twenty-four Sprague Dawley rats aged 21 days were randomly and equally (n=8) divided into three groups. No procedure was performed on the control group (Gr-CNT) rats. The sham group (Gr-SHM) rats were held in the EMF-cage without EMF exposure. The EMF group (Gr-EMF) rats were exposed to 900-MHz EMF for 1 hour each day inside the EMF-cage during the adolescent period. Testis were extracted and divided into right and left hemispheres at postnatal day 60. The left halves were used for biochemical analyses and the right hemispheres for histopathological evaluation. Biochemically, malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were analyzed as oxidative stress markers. Histopathologically, apoptotic index was determined in TUNEL-stained sections. Histopathological damage scores and observations were analyzed in hematoxylin

and eosin stained sections. Biochemical analyses revealed increased MDA, SOD, CAT levels in Gr-EMF compared to Gr-CNT and Gr-SHM, while Gr-CNT GSH levels decreased significantly compared to Gr-EMF and Gr-SHM. Gr-EMF rats exhibited histopathological changes, decreased seminiferous tubule diameters, germinal epithelium and a higher apoptotic index. Our results show that changes may occur in morphology and oxidative stress biomarkers in the rat testis following exposure to 900 MHz EMF throughout the adolescent period.

Key words: testicles, rat, electromagnetic field, cell phone

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Biochemical evaluation of the effects of aspirin and indomethacin on renal and hepatic tissue in ovariectomized rats

Saliha Aksun¹, Murat Aksun², Ömer Demir³, Hasan Orhan Çetin¹, Recep Sütcü¹, Buket Demirci⁴

¹İzmir Katip Çelebi University, Medical Faculty, Department of Medical Biochemistry

²İzmir Katip Çelebi University, Medical Faculty, Department of Anesthesiology and Reanimation

³İzmir University Medical Faculty, Department of Medical Pharmacology

⁴Adnan Menderes University, Medical Faculty, Department of Medical Pharmacology, Aydın

It is clear that age related antiinflammatory drug taking is common and uncontrolled especially during menopause. The purpose of the study is to evaluate to effect of aspirin and indomethacin on renal and hepatic tissue. Superoksid dismutaz (SOD), catalase, glutathion peroksidase (GpX) and malondialdehyd(MDA) had been

evaluated of the balance between the oxidant and antioxidant system at kidney and liver tissues taken from female rats gone to menopause during the 18 month period after ooferectomia. Blood urea, creatinine, cystatin C, aspartate transaminase(AST), Alanine transaminase(ALT) had been studied for the evaluate renal and hepatic function.

Indo and aspirin groups liver MDA levels were significantly higher than aged and young groups (respectively; $3,12 \pm 0,49$; $2,90 \pm 0,83$; $2,79 \pm 0,46$; $2,47 \pm 0,92$ nmol/mg protein). GpX and catalase levels were higher (respectively; GpX, $6,57 \pm 1,45$; $6,09 \pm 2,20$; $5,54 \pm 1,20$; $5,26 \pm 1,98$ U/mg protein; catalase; $200,7 \pm 105,1$; $208,5 \pm 128,9$; $172,8 \pm 108,0$; $193,3 \pm 124,0$ katal/mg protein) and SOD levels were higher in indomethacin and aspirin groups. While compared with the young rats, ovariectomized rats renal tissue MDA levels tended to increase. But firstly aspirin and then indomethacin kidney MDA levels were higher than in all (asp: $6,48 \pm 1,35$; indo: $5,91 \pm 1,10$; aged rats: $5,51 \pm 1,52$; young rats: $5,08 \pm 0,72$ nmol/mg protein).

Aspirin group average SOD level was $6,92 \pm 2,18$ (U/mg protein) and as higer than others. Indo GpX is greater than others ($4,43 \pm 1,09$ U/mg protein).

Highest urea, creatinine and cystatine C levels were in ovariectomized rats. AST was higher in indomethacin, ALT was higher in aspirin group accordingly to old group

In our study we saw mostly, both of the oxidant and antioxidant levels were higher in drug use. This situation can be explained by more working antioxidant system for compansation. On the other hand we have to discuss two issues, because of the higher antioxidant levels in aspirin group according to elderly, we could consider aspirin may be play a protective role for kidney. Another aspect is, while there is no deterioration in tissue function is it a warning of increased oxidative stres?

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T. Oral Presentation No. 6

Effects of Wi-Fi (2.45 GHz) exposure on oxidative stress in brain and liver of rats during pregnancy and the development of newborns*

Ömer Çelik^{1,2}, Mehmet Cemal Kahya³, Mustafa Nazıroğlu^{1,2},

¹Neuroscience Research Center, Süleyman Demirel University, Isparta, Turkey

²Department of Biophysics, Medicine Faculty, Süleyman Demirel University, Isparta, Turkey

³Department of Biophysics, Medicine Faculty, İzmir Katip Çelebi University, İzmir, Turkey

Excessive production of reactive oxygen substances (ROS) and reduced antioxidant defense system through electromagnetic radiation (EMR) exposure may lead to oxidative brain and liver damage further degradation of membranes during pregnancy and development of rat pups. In a recent study (1), we were able to observe changes on oxidative stress values in brain and liver of 2.45 GHz-EMR exposed newborn rats between 1st and 3rd weeks after birth because brain of the rats are developing during synaptogenesis period (first 3 weeks after birth). In addition, reports of EMR exposure on oxidative stress in brain and liver of rats are conflicting (1,2). The present study in rats during pregnancy and the development of newborns between 1st and 3rd weeks was designed to determine the effects of 2.45 GHz exposure on the brain and liver oxidative injury induced by EMR.

Sixteen pregnant rats and their 48 newborns were equally divided into control and EMR groups. EMR groups were exposed to 2.45 GHz EMR (one hour/day in five days of each week) from pregnancy to 3 weeks old. Brain cortex and liver samples were taken from the newborns and mothers at the end of 3rd weeks. In the EMR groups, lipid peroxidation level in the brain and liver were increased by the EMR exposure although the

glutathione peroxidase (GSH-Px) activity, vitamin A, vitamin E and β -carotene concentrations were decreased in the brain and liver by the exposure. Reduced glutathione (GSH) and vitamin C concentrations in brain were also lower in the EMR group than in control although their concentrations did not change in liver.

In conclusion, these results demonstrated that Wi-Fi (2.45 GHz) device induces oxidative toxicity through GSH, GSH-Px and antioxidant vitamin concentration decrease in brain and liver of rat pups during development. The results of lipid peroxidation and antioxidant indicated that brain was more sensitive to the oxidative injury as compared to liver. However, further investigations in human and babies are required to clarify the mechanism of action of the applied EMR exposure and oxidative stress on the rat brain and liver as well as to establish the biological significance of the observed phenomena.

Keywords: Brain; Electromagnetic radiation; Glutathione; Liver; Oxidative stress; Antioxidant vitamins.

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T. Oral Presentation No. 7

Altered maternal serum dynamic thiol-disulfide interchange reactions in gestational diabetes mellitus

Melahat Yildirim^{1*}, Esengül Turkyılmaz², Gulcan Daultekazın², Busra Demir Cendek³, Murat Alisik⁴, Pervin Baran⁴, Aysegül Cinkaya², Ayşe Filiz Avsar Yavuz¹

¹Yildirim Beyazıt University, Department of Obstetrics and Gynecology, Bilkent, Ankara, Turkey

²Ataturk Training and Research Hospital, Department of Obstetrics and Gynecology, Bilkent, Ankara, Turkey

³Etilik Zübeyde Hanim Women's Health and Education Hospital, Department of Obstetrics and Gynecology, Ankara, Turkey

⁴Ataturk Training and Research Hospital, Department of Biochemistry, Bilkent, Ankara, Turkey

*Correspondence: melahatyildirim@yahoo.com

Gestational diabetes mellitus (GDM) is carbohydrate intolerance that occurs or is first recognized during pregnancy. Oxidative stress is defined as a perturbation in the balance between the production of reactive oxygen species and antioxidant defenses leading to tissue damages in organisms. Limited number of studies have been conducted to date the association between oxidative stress and the pathogenesis of GDM. In this study, we aimed to evaluate the relationship between GDM and dynamic thiol/disulfide homeostasis which basically shows the oxidative stress in body by using a newly developed and automated analysis method.

Study population consisted of body mass index and gestational age-matched pregnant women. Patients were subdivided into 3 groups based on their response to glucose challenge test (GCT) and oral glucose tolerance test (OGTT) results: (1) control group (normal GCT test results n= 87); (2) Impaired glucose tolerance (IGT) group (n= 37); (3) GDM group (n=22). Maternal serum native thiol, total thiol, and disulphide levels were compared among three groups.

Maternal serum thiol values were found decreased and disulphide levels were increased in GDM groups were compared to control and IGT groups (Thiols: 398.19±30.49 μ mol/L for controls, 395.92±35.52 μ mol/L for IGT, and 371.89±41.14 μ mol/L for GDM groups, p= 0.002). Disulphides for control group; 17.47±4.38 μ mol/L, for IGT group; 19.27±3.34 μ mol/L, for GDM group; 25.46±4.21 μ mol/L, (p=0.001). Disulphide/thiol ratio was found to be increased in GDM group comparing to other groups (Disulphide/thiol ratio: 0.044±0.012 for controls, 0.049±0.009 for IGT group, 0.068±0.0103 for GDM group p=0.001)

Thiol- disulphide balance has shifted to the oxidative side in pregnant women with GDM. This condition may affect the fetus adversely due to increased oxidative stress. Therefore blood glucose regulation becomes crucial in these patients.

▶ T. Oral Presentation No. 8

Effects of long-term exposure mobile phones and Wi-Fi-induced electromagnetic radiation on plasma sex hormone levels and uterine oxidative stress in pregnant rats and their offspring

Murat Yüksel¹, Mustafa Nazıroğlu², Mehmet Okan Özkaya¹

¹The Department of Obstetrics and Gynecology, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

²The Department of Biophysics, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

There is growing interest in the increase in EMR-induced environmental pollution. Because modern Wi-Fi devices work at a frequency of 2450 MHz and mobile phone devices in many countries work at frequencies of 900 and 1800 MHz. Several recent reports involving different tissues and blood have indicated that EMR exposure modifies cellular antioxidant levels and hormone homeostasis in humans and animals. Although the pathophysiologic mechanisms that are responsible for such effects remain unknown, common theories include changes in temperature, membrane permeability, and ROS production. In addition, current reports on the relationship between serum reproductive hormone levels and EMR exposure in humans and animals are conflicting. Hence, we aimed to investigate the effects of 900, 1800, and 2450 MHz EMR on oxidative stress and hormone homeostasis in the blood and uterus of rats.

Thirty-two rats and their forty newborn offspring were divided into the following 4 groups according to the type of EMR exposure they were subjected to: The control, 900 MHz, 1800 MHz, 2450 MHz groups. Each group was exposed to EMR for 60 min/day during the

pregnancy and growth periods. The pregnant rats were allowed to stand for 4 generations (total 52 weeks) before, plasma and uterine samples were obtained. During the 4th, 5th, and 6th weeks of the experiment, plasma and uterine samples were also taken from the developing rats.

Although uterine lipid peroxidation increased in the EMR groups, uterine glutathione peroxidase activity (4th and 5th weeks) and plasma prolactin levels (6th week) in developing rats decreased in these groups. In the maternal rats, plasma prolactin, estrogen and progesterone levels decreased in the EMR groups, while plasma total oxidant status, and body temperatures increased. There were no changes in the levels of reduced glutathione, total antioxidants, vitamin A, vitamin C, and vitamin E in the uterine and plasma of maternal rats.

In conclusion, although EMR exposure decreased the prolactin, estrogen, and progesterone levels in the plasma of the maternal rats and their offspring. EMR-induced oxidative stress in the uterine of maternal rats increased during the development of offspring. Mobile phone- and Wi-Fi-induced EMR can be one of the causes of increased oxidative uterus injury in growing rats and decreased hormone levels in maternal rats.

Keywords: Antioxidants; Uterine; Hormone; Growing rat; Electromagnetic Radiation.

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▶ T. Oral Presentation No. 9

Effects of iron deficiency on oxidative stress and apoptosis in placenta of patients with anemia

Raziye Desdicioglu¹, Fevziye Burcu Sirin², Fatma Simsek³, Gonca Gul Gulbas Tanrısever⁴, Recep Sutcu⁵, Sefa Kelekci⁶

¹Yıldırım Beyazıt University, Medical School, Department of Obstetrics and Gynecology, Ankara, Turkey

²Katip Celebi University, Atatürk Training and Research Hospital, Department of Biochemistry, Izmir, Turkey

³Katip Celebi University Medical School Department of Histology and Embriology, Izmir, Turkey

⁴İpekyolu, Obstetrics Gynecology and Pediatrics Hospital Van, Turkey

⁵Katip Celebi University, Medical School Department of Biochemistry, Izmir, Turkey

⁶Katip Celebi University, Medical School, Department of Obstetrics and Gynecology, Izmir, Turkey

Iron deficiency anemia (IDA) is the most common nutritional disorder in the world. Anemia is associated with some pregnancy complications. The exact pathogenetic mechanism is unknown caused by the complications of iron deficiency anemia. In our study, markers of oxidative stress, antioxidant enzymes levels and the rate of apoptosis were evaluated in the placental tissue in women with IDA and control group. We aimed to investigate the possible pathogenetic mechanisms of iron deficiency anemia cause of complications.

In our study, placental tissues of 12 anemic (hemoglobin value 11gr/dl.less than) and 22 normal pregnant women are histologically assessed. Malondialdehyde (MDA) and activity of antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were measured in placental tissues. Apoptotic changes were also examined by Tunel method.

In our study, SOD and GSH-Px activities were increased in the placental tissue in anemic patients ($P < 0,05$). The MDA level and CAT activity did not differ in both groups. The rate of apoptotic cells were significantly higher in anemic placentas than in controls. According to our study, apoptotic cell rate and antioxidant enzyme activities were increased in placenta of anemic patients. Hence, oxidative stress and placental apoptosis may be another mechanism of iron deficiency related adverse pregnancy outcomes.

Keywords: Pregnancy, Iron deficiency anemia, Placental apoptosis, Placental oxidative stress.

Changes of oxidative stress and apoptosis in preeclamptic pregnant women: Manual microarray study

Arzu Şahin¹, Havva Erdem², Ahmet Karataş³, Nilüfer Kadioğlu², Handan Ankaralı⁴

¹Department of Physiology Medical Faculty, Ordu University, Ordu, Turkey

²Department of Pathology, Medical Faculty, Ordu University, Ordu, Turkey

³Department of Obstetrics and Gynecology, Medical Faculty, Abant İzzet Baysal University, Bolu, Turkey

⁴Department of Biostatistics, Medical Faculty, Duzce, Turkey.

Preeclampsia is one of the major causes of maternal-foetal mortality and morbidity. In this study the aim was to determine whether preeclampsia is associated with an increase in placental apoptosis, oxidative stress and angiogenesis with altered expression of mediators such as caspase-3, Bcl-2, FGF, MMP-9, and MPO. This retrospective study used formalin-fixed tumour samples taken from pregnant patients who were diagnosed with preeclampsia between 2010 and 2011 in the department of Pathology, Medical Faculty. Statistically significant positive relationships were found between MMP-9 and groups ($P = 0.024$), between MMP-9 and caesarean section (C) ($P = 0.006$), between BMI and groups ($P = 0.050$) and between baby weight and groups ($P = 0,006$).

We did not find a statistically significant relationship between other stains and other prognostic parameters such as weight (during pregnancy), haemoglobin, haematocrit, gestational week, and weight of placenta.

In this study, significant relationships were found between MMP-9 and preeclampsia (PE)s group, between MMP-9, C and BMI and PEs groups and between baby weight and groups. It was found that FGF-2 and bcl-2 levels tended to be elevated in N group when compared to PE term placental samples. No relationship was found between apoptosis and placenta in the late trimester.

Keywords: hypertension, placenta, FGF, MMP-9, MPO, bcl-2, caspase-3.

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► T. Oral Presentation No. 11

Effects of oxidative stress and angiogenesis of smoke in placentas of pregnant smokers: Manuel microarray study

Ali Aslan¹, Ahmet Karataş², Havva Erdem³, Nilüfer Kadioğlu⁴, Handan Ankaralı⁵, Özlem Admış⁶

¹Department of Physiology, Medical Faculty, Ordu University, Ordu, Turkey.

²Department of Obstetrics and Gynecology, Medical Faculty, Abant İzzet Baysal University, Bolu, Turkey.

³Department of Pathology, Medical Faculty, Ordu University, Ordu, Turkey.

⁴Department of Pathology, Bozüyük State Hospital, Bilecik, Turkey.

⁵Department of Biostatistic, Medical Faculty, Duzce University, Duzce, Turkey.

⁶Department of Biochemistry, Medical Faculty, Duzce University, Duzce, Turkey.

Prenatal smoking remains a common habit. Pregnant smokers damage both themselves and fetus. This damage is evaluated with FGF, MMP-9, MPO study. 68 pregnant woman's placenta which sent to pathology laboratory between years 2010 to 2011 and their clinical histories were included in the study. 28 woman were smoker (S), 40 woman were non-smoker (NS). FGF, MMP-9, MPO sections of the cases were examined retrospectively. Number of cigarettes, age, systemic disease rate, mean abortus number, blood pressure, hemoglobin and hematocrit, apgar, pathological parameters were evaluated. Statistically significant positive relationships were found between MMP-9 (grade 1) and NS groups ($p=0,039$). There were not stain of S groups with MMP-9. There were found range of 15% stain of MMP-9 (grade 1). Systemic diseases

were S more than NS ($p=0,049$). There were observed NS groups more than S groups grade 2 FGF stain, but statistically, there were not significant ($P=0,791$). There were observed NS groups more than S groups grade 0 MPO stain and more S groups than NS groups grade 1 MPO stain, but statistically, there were not significant ($P=0,640$). As a result, effects of smoking on fetus has been demonstrated with the MPO, MMP-9, and FGF. More comprehensive studies are needed to reveal the relationship between fetus and smoking.

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► T. Oral Presentation No. 12

Raloxifene and tamoxifen attenuates PARP activity, cytokine and oxidative stress levels in the brain and blood of ovariectomized rats

Betül Yazğan¹, Yener Yazğan², İshak Suat Övey², Mustafa Nazıroğlu^{2,3}

¹Department of Physiology, Medical Faculty, Adıyaman University, Adıyaman, Turkey.

²Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, Turkey.

³Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey.

It has been well known that 17 β -estradiol (E_2) has antioxidant role on neurological and behavioral systems in brain. Raloxifene (RLX) and tamoxifen (TMX) are selective estrogen receptor modulators. E_2 deficiency stimulates mitochondrial function for producing apoptosis and reactive oxygen species (ROS) production although RLX and TMX may reduce the mitochondrial ROS production via their antioxidant properties in the brain and erythrocyte of ovariectomized (OVX) rats. We aimed to investigate the effects of estradiol, raloxifene and tamoxifen on the oxidative stress,

apoptosis and cytokine production in the brain and erythrocyte of OVX rats.

Forty female rats were divided into five groups: First group was used as control. Second group used as OVX. Third, fourth and five groups used OVX+estrogen, OVX+TMX and OVX+RLX, respectively. Estrogen, tamoxifen and raloxifene were subcutaneous given to these three groups for 14 days after OVX-induction.

Brain and erythrocyte lipid peroxidation levels were high in the OVX group although they were low in OVX+E₂, OVX+RLX and OVX+TMX treatments. OVX+E₂, OVX+RLX and OVX+TMX treatments increased the lowered glutathione peroxidase activity in erythrocyte and brain, and reduced glutathione and vitamin E concentrations in brain. β -carotene, vitamin A, procaspase 3 and 9 in brain, and TNF- α and interleukin (IL)-1 β levels in the plasma of five groups were not statistically changed by the treatments. However, plasma IL-4 levels and brain PARP activity were high in the OVX group and their levels were decreased by the OVX+E₂, OVX+RLX and OVX+TMX treatments.

In conclusion, we observed those E₂, RLX and TMX administrations are beneficial on oxidative stress, inflammation and PARP level in the serum and brain of OVX rats by modulating antioxidant system, DNA damage and cytokine production.

Keywords: Brain; Cytokine; Apoptosis; Oxidative stress; Selective estrogen receptor modulators.

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Effects of *Hypericum perforatum* on TRPM2 and TRPV1 channels in sciatic nerves and dorsal root ganglia of sciatic nerve injury-induced rats

Fuat Uslusoy¹, Mustafa Nazıroğlu^{2,3,4}, Bilal Çiğ³, İshak Suat Övey⁴

¹Department of *Plastic* and Reconstructive Surgery, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

²Neuroscience Research Center, Suleyman Demirel University, Isparta Turkey

³Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

⁴Department of Neuroscience, Institute of Health Sciences, Suleyman Demirel University, Isparta, Turkey

Peripheral nerve injury may result in neuropathic pain, which is characterized by spontaneous pain through increase of cytosolic Ca²⁺ entry. Oxidative stress and overload Ca²⁺ entry have important roles on apoptosis in dorsal root ganglion (DRG) neurons after sciatic nerve injury (SNI). Recently we observed regulator roles of *Hypericum Perforatum* (HP) on apoptosis and Ca²⁺ entry through TRPM2 and TRPV1 channels antioxidant property in the DRGs of spinal cord injury-induced rats due to its antioxidant property (1,2). We investigated the protective property of HP on TRPM2 and TRPV1 channels in SNI-induced DRG and sciatic nerve neurons of rats.

Rats were divided into five groups as control, sham, sham+HP, SNI and SNI+HP. The HP groups received 30 mg/kg HP for 4 weeks after SNI induction. The SNI-induced TRPM2 and TRPV1 currents and cytosolic free Ca²⁺ concentration were increased by SNI induction although they were reduced by HP treatment.

In conclusion, increase of TRPM2 and TRPV1 channel activation in the SNI-induced DRG and sciatic nerve neurons were decreased by HP treatment. Our findings may be relevant to the etiology and treatment of SNI by HP.

Keywords: *Hypericum perforatum*; Sciatic nerve injury; Calcium ion; TRPM2 channel; TRPV1 channel.

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▶ T. Oral Presentation No. 14

Melatonin administration can inhibit TRPM2 currents in Transfected CHO Cells

Omer Celik

Department of Biophysics Medical School of Suleyman Demirel, Isparta, Turkey.

The calcium ion (Ca²⁺) concentration in the extracellular fluid is 10 to 20 thousands fold higher than intracellular fluid. Even a small increase of Ca²⁺ concentration inside the cell can affect cellular functions. Transient receptor potential (TRP) channels are a group of non-selective cation channels that have crucial functions in cellular systems. These channels have been divided into six subfamilies in mammalian and one of them, TRPM channels are well known as activated by cellular oxidative stress. TRPM2, one of the member of TRPM ion channel family, is activated by hydrogen peroxide, β-NAD and TNFα. The activation mechanism of TRPM2 channels involves production of ADP-ribose, which binds to an ADP-ribose binding region in the TRPM2 C-terminus (1). Changing oxidative stress status in the cell activates the TRPM2 channels and causes the Ca²⁺ ions to flux from extracellular matrix into the cell through these channels (2). TRPM2 channels are widely expressed in many cell types including brain, dorsal root ganglion neurons and hematopoietic cells. Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone that produced and released by the pineal gland. It has been considered a potent antioxidant that detoxifies a variety of ROS in many pathophysiological states. In this study it was aimed to investigate the effect of melatonin on hydrogen

peroxide activated TRPM2 channel currents using by Patch-clamp technique.

Patch-clamp is an electrophysiological method allowing the researchers to measure and record the ionic currents from the different types of ion channels which are embedded into cell membrane. Cells were exposed melatonin by two ways extracellular incubation (300 μM and 1 mM) and inside the patch pipet (200 μM). Activation of TRPM2 channels were propagated by extracellular hydrogen-peroxide (10 mM) administration. In this study TRPM2 transfected Chinese Hamster Ovary (CHO) cells were divided into 3 group as control, extracellular melatonin and intracellular melatonin. Three recordings (melatonin as control, hydrogen peroxide and melatonin + hydrogen peroxide) were obtained from all groups. Whole cell patch-clamp recording, calcium signaling and apoptosis experiments were performed for all groups.

Patch-clamp and recordings and calcium signaling experiments showed that extracellular and intracellular melatonin applications may have a modulator role on TRPM2 channel currents in transfected CHO cells. By the patch-clamp experiments it was determined that in the melatonin administrated groups the level of TRPM2 currents arising from Ca²⁺ flow were significantly (p<0.001) lower than hydrogen peroxide administrated group. Moreover, calcium signaling experiments verified our patch clamp findings. Apoptosis levels were significantly (p<0.001) decreased in melatonin groups comparing with control and hydrogen peroxide groups. As a result intracellular and extracellular melatonin applications may inhibit and have a protective and regulator role against cellular oxidative stress induced TRPM2 channel currents in a dose-dependent manner. Although our patch-clamp and calcium findings demonstrate that extracellular and intracellular melatonin administrations inhibited TRPM2 currents in transfected cells they should be proved by also knock-out animal experiments.

Keywords: TRP cation channels, Patch-clamp technique; Calcium ion;

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Assessment of pollution with oxidative stress biomarkers in fish culture

Ayşegül Bildik, Mehmet Ersin Kartal

Department of Nutrition and Dietetics, Faculty of Health Sciences, Zirve University, Gaziantep, Turkey.

In this study oxidative stress biomarkers were measured in liver and muscle tissues of rainbow trout (*Oncorhynchus mykiss*) to assess exposure level of trout farms to pollutants in Kemer Dam and Fethiye. Samples which produced by fish farming were collected from cages in Kemer Dam located in Bozdoğan limits and concrete cages in Fethiye. Liver and muscle tissues of samples were homogenized in 1:5 w/v of cold homogenization solution for 10 min at pH 7.4. Homogenates were centrifuged at 500 g for 15 min, the fatty layer removed and the obtained supernatant centrifuged at 12,000 g for 20 minutes then microsomal and cytosolic fractions were prepared by centrifuging the supernatant at 100000 g for 60 min (1). The supernatant containing cytosolic fraction used to determine total protein level, glutathione-S-transferase (GST) activity (2) and reduced glutathione (GSH) as phase II enzymes (3), malondialdehyde (MDA) product of lipid peroxidation (4) as an oxidative stress parameter and acetylcholinesterase (ACHE) inhibition as a neuromuscular parameter (5) of oxidative stress. And pellets containing microsomal fraction were resuspended in a ratio of 0.5 ml of buffer per g of liver tissue in 100 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer pH 7.4, containing 150 mM KCl, 20% w/v glycerol and used to determine ethoxyresorufin-O-deethylase (EROD) induction as a phase-I enzyme (1). To assess seasonal variations samples were collected in January and July. Physical and chemical properties of water samples collected from trout farms were measured and results assessed within these five biomarkers. As a result of assessment of all datas, significant increases were detected in EROD induction, GST activity, MDA level and ACHE inhibition in concrete cages in Fethiye when compared with Kemer Dam ($p < 0,05$). When all datas

were analyzed according to seasonal alterations of two sites respectively, in case results adduced that Kemer Dam exposed to pollution on July more than January; concrete cages in Fethiye exposed to pollution on January more than July. A significant increase of ACHE inhibition on July according to increase of ammonium nitrogen level were detected in both two sites ($p < 0,05$). Otherwise according to significant increase of turbidity, a significant increase detected in oxidative stress parameters GSH, MDA level and GST activity in Kemer Dam ($p < 0,05$).

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The investigate of the effects of copper, zinc and bicarbonate on antioxidant statue in rats at intermittent hypobaric hypoxia.

Duygu Tarhan, Şefik Dursun

Department of Biophysics, Cerrahpaşa Medical Faculty, İstanbul University, İstanbul, Turkey.

Intermittent hypobaric hypoxia is defined as to be below the normal values of partial oxygen pressure at tissue due to hypoxia periods (1). It can lead to increased reactive oxygen species (ROS) which causes oxidative damage to DNA, lipids and proteins. The effect of antioxidant defense mechanism varies from tissue to tissue under hypoxic conditions (2-3). The aim of our study was to investigate the effects of bicarbonate (HCO_3), copper (Cu) and zinc (Zn) on antioxidant statue

in blood, liver and kidney tissues under hypoxia. Forty adult male Sprague Dawley rats randomly divided into five groups (Control, Hypoxia, Hypoxia+Zn, Hypoxia+Cu and Hypoxia+HCO₃). Rats were exposed hypoxia a daily 8 hours for 5 days/week until completing 3 weeks in hypoxia cabine at a simulated pressure of 400-500 mmHg. The animals of Hypoxia+Zn, Hypoxia+Cu and Hypoxia+HCO₃ experimental groups received through drinking water 30 mg/kg Zn, 7 mg/kg Cu and 3 mmol/kg NaHCO₃, respectively. At the samples of blood, liver and kidney taken from rats at the end of experiment were measured the values of GSH (Glutathione) and MDA (Malondialdehyde) by spectrophotometer. According to statistical evaluated, the kidney GSH values showed a significant increase in all experimental groups (p<0.01). Increase in the plasma GSH values is also due to kidney cells. As a result of our investigation it is seen that the antioxidant response of kidney cells to hypoxia are more effective than applied dose levels of zinc, copper and bicarbonate ions at the study.

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► T. Oral Presentation No. 17

Effects of curcumin and melatonin on bone formation in orthopedically expanded suture in rats:

A biochemical, histomorphometric, and immunohistochemical study

Mine Gecgelen Cesur¹, Kanat Güllü², Fevziye Burcu Şirin³, Meryem Akpolat⁴, Gözde Öğrenim¹, Afra Alkan⁴, Gökhan Cesur⁵

¹Department of Orthodontics, Faculty of Dentistry, Adnan Menderes University, Aydın, Turkey.

²Department of Histology and Embryology, Faculty of Medicine, Bulent Ecevit University, Zonguldak,

Turkey.

³Department of Biochemistry, Faculty of Medicine, Katip Çelebi University, İzmir, Turkey.

⁴Department of Biostatistics, Faculty of Medicine, Yıldırım Beyazıt University, Ankara, Turkey.

⁵Department of Physiology, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey.

Rapid maxillary expansion (RME) is a common orthodontic treatment used to correct transversal arch deficiency (1,2). Antioxidant administration with RME procedure accelerate the bone formation processes in an expanding suture so that the retention appliances could be removed earlier (3,4). Consequently, the aim of this study was to investigate the effects of curcumin (CUR) and melatonin (MEL) on new bone formation following RME in a rat study model using biochemical, histomorphometric, and immunohistochemical techniques. The rats (32, 12-week-old adult male, Wistar albino) were randomly divided into the following 4 groups (n=8 each): no expansion (NE), expansion only (EO), expansion plus MEL (MEL). CUR and MEL were given to the rats during the 5 day expansion and 12 day retention period. After the sacrifice of animals, biochemical, histomorphometric and immunohistochemical examinations were performed. Serum bone alkaline phosphatase levels in the MEL group were statistically (p<0.01) higher than the EO group. Serum glutathione peroxidase and catalase activities in CUR and MEL group were significantly (p<0.05) higher than the OE group. Inflammatory cell infiltration, new bone formation, and capillary intensity parameters demonstrated statistically significant differences between the groups (p<0.05). The immunohistochemical findings revealed that IL-1, IL-6, and TNF- α H scores showed considerable differences between the groups (p<0.001). The highest IL-1, IL-6, and TNF- α H scores were found in the EO groups than in the other groups (p<0.05). In conclusion, CUR and MEL treatments may be effective in accelerating new bone formation in the RME procedure and that may be beneficial in preventing relapse following the RME procedure.

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▶ T. Oral Presentation No. 18

The effects of aluminum administration in different doses and durations to rats on oxidative damage and tissue histology

Bahar Öztürk Kurt¹, Semra Özdemir¹, Gamze Tanriverdi², İsmail Seçkin²

¹Istanbul University Cerrahpasa Medical Faculty Department of Biophysics, Istanbul

²Istanbul University Cerrahpasa Medical Faculty Department of Histology and Embryology, Istanbul

Aluminum (Al) is a toxic element and humans are constantly exposed to it as a result of an increase in industrialization^{1,2}. The aim of the study was to investigate effects of Al exposure in different durations and doses on oxidant-antioxidant system and histological parameters. Al chloride (AlCl₃) was injected intraperitoneally five times a week to 40 male Wistar Albino. Rats were divided into five groups: 1.group (3 weeks, 8 mg/kg AlCl₃), 2.group (6 weeks, 8mg/kg AlCl₃), 3.group (3 weeks, 16mg/kg AlCl₃), 4.group (6 weeks, 16mg/kg AlCl₃) and control. Malondialdehyde (MDA) and glutathione (GSH) levels were measured in liver and kidney tissues. For histological evaluation, Caspase-3 positive cells were counted and apoptotic index was calculated. Liver MDA and GSH levels were higher (p<0,05) in first group than third and fourth groups. Liver GSH levels in the second and fourth groups were lower than the control (p<0,05). While the kidney MDA levels were increased; kidney GSH levels were decreased in first and third groups according to the control (p<0,05). Increased connective tissue in central vein and portal areas were determined

in experimental liver tissues. Tubule damage and glomerular degeneration were observed in experimental kidney tissues. The apoptotic index were increased in experimental groups (p<0,001). Although Al causes oxidative damage in liver and kidney tissues, lipid peroxidation preventive mechanisms become activate by increase of exposure time and dose. The results of the present study show that the effects of Al on oxidative stress and apoptosis are dependent on dose and duration.

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▶ T. Oral Presentation No. 19

In vitro effects of duloxetine on TRPM2 and TRPV1 channels in the hippocampus and dorsal root ganglion of rats

Arif Demirdağ¹, Mustafa Nazıroğlu^{2,3}, İshak Suat Övey²

¹Department of Psychiatry, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

²Department of Neuroscience, Health Science Institute, Suleyman Demirel University, Isparta, Turkey

³Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey

Overload of Ca²⁺ entry and excessive oxidative stress in neurons are the two main causes of depression. Activation of transient receptor potential (TRP) vanilloid type 1 (TRPV1) and TRP melastatin 2 (TRPM2) during oxidative stress has been linked to neuronal survival (1). Duloxetine (DULOX) in neurons reduced the effects of Ca²⁺ entry and reactive oxygen species (ROS) through glutamate receptors, and this reduction of effects may also occur through TRPM2 and TRPV1 channels. Although the effects of DULOX on Ca²⁺ entry through glutamate receptors support its antidepressant properties (2), its mechanisms of action

(besides the monoaminergic system) are not fully understood. Hence, we investigated whether TRPM2 and TRPV1 channel dependent pathways— such as oxidative stress, apoptosis, and mitochondrial depolarization—may be involved in the effect of DULOX treatments in the hippocampal and DRG neurons of rat.

Freshly isolated hippocampal and DRG neurons were incubated for 24 hours with DULOX. In whole-cell patch clamp and intracellular free calcium ($[Ca^{2+}]$) concentration (Fura-2) experiments, cumene hydroperoxide and ADP-ribose-induced TRPM2 currents in the neurons were inhibited by N-(p-amylocinnamoyl) anthranilic acid (ACA), and capsaicin-induced TRPV1 currents were inhibited by capsazepine (CPZ) incubations. TRPM2 and TRPV1 channel current densities, $[Ca^{2+}]$ concentration, apoptosis, caspase 3, caspase 9, mitochondrial depolarization, and intracellular ROS production values in the neurons were lower in the DULOX group than in controls. In addition, the above values were further decreased by DULOX+CPZ and DULOX+ACA treatments.

In conclusion, in the in vitro experimental model, TRPM2 and TRPV1 channels are involved in the Ca^{2+} entry-induced oxidative neuronal death in the hippocampus and DRG, and reduced channel activity by DULOX treatment may account for the neuroprotective activity against apoptosis and Ca^{2+} entry. Our results indicate that DULOX plays a neuronal modulation role against oxidative stress-induced apoptosis Ca^{2+} mobilization through TRPM2 and TRPV1 channels in hippocampal and DRG neurons. DULOX may become an important pharmacological target in the treatment of hippocampal oxidative injury and pain induction.

Keywords: Apoptosis, Duloxetine, Oxidative stress, Pain, TRPM2, TRPV1

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The neuroprotective role of dexmedetomidine on calcium signaling, oxidative stress and apoptosis in cerebral ischemia-induced rats: The involvement of TRPM2 and TRPV1 channels

Hatice Akpınar¹, Mustafa Nazıroğlu^{2, 3}, İshak Suat Övey², Bilal Çiğ², Orhan Akpınar⁴

¹Department of Anesthesiology and Reanimation, Faculty of Dentistry, Suleyman Demirel University, Isparta, Turkey

²Department of Neuroscience, Institute of Health Science, Suleyman Demirel University, Isparta, Turkey

³Neuroscience Research Center, University of Suleyman Demirel, Isparta, Turkey

⁴Department of Microbiology, Faculty of Dentistry, Suleyman Demirel University, Isparta, Turkey

Dexmedetomidine (DEX) has sedative, analgesic and anesthetic properties and it has also neuroprotective, antioxidant and voltage gated calcium (VGCC) channels agonist effects in brain and spinal cord injuries. Calcium ion permeable TRPM2 and TRPV1 channels are activated in the hippocampus and DRG neurons by oxidative stress. The role of oxidative stress and Ca^{2+} influx through VGCC and NMDA receptors on pain and cerebral ischemia has been known for a long time (1-3). However, there is no report on Ca^{2+} influx through TRPM2 and TRPV1 channels in rats with cerebral ischemia. The molecular mechanism(s) underlying neuronal injury through spinal cord and cerebral ischemia injuries remains poorly understood. To address this gap, we tested the effects of DEX treatment on apoptosis, oxidative stress, and Ca^{2+} influx through TRPM2 and TRPV1 channels in the DRG and hippocampus of rats with cerebral ischemia.

Fifty-six rats were divided into five groups. Placebo was given to control, sham control and ISC groups, respectively. ISC was induced in the third group. DEX and ISC+DEX groups received intraperitoneal DEX (40 μ g/kg) at 3rd, 24th and 48th hours after ISC induction. The hippocampal and DRG neurons also were stimulated in vitro with a TRPM2 channel agonists

(ADP-ribose and cumene hydroperoxide) and a TRPV1 channel agonist (capsaicin). We found that DEX was fully effective in reversing ISC-induced TRPM2 and TRPV1 current densities as well as, cytosolic calcium ion accumulation the neurons. In addition, DEX completely reduced ISC-induced oxidative toxicity by intracellular ROS production and mitochondrial membrane depolarization. The DEX and ISC+DEX treatments also decreased poly (ADP-ribose) polymerase, caspase 3, caspase 9 activities, and apoptosis, levels in the hippocampus.

In conclusion, results suggest that apoptosis, oxidative stress and intracellular Ca²⁺ signaling through activation of TRPM2 and TRPV1 channels in hippocampal and DRG neurons of cerebral ischemia-induced rats are decreased in hippocampal and DRG neurons by cerebral ischemia induction. Its inhibitory effect on cerebral ischemia-induced TRPM2 and TRPV1 channel activation should be considered a potential pharmacological target for itching caused by cerebral ischemia-mediated activation of pain and brain injuries.

Keywords: Apoptosis; Dexmedetomidine; Cerebral ischemia; Oxidative stress; TRPM2; TRPV1.

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▶ T. Oral Presentation No. 21

Selenium potentiates the anticancer effect of cisplatin against oxidative stress and calcium ion signaling-induced intracellular toxicity in MCF-7 breast cancer cell: Involvement of TRPV1 channel

Esin Sakallı Çetin¹, Mustafa Nazıroğlu^{2,3,4}, Bilal Çiğ^{2,4}, İshak Suat Övey^{2,4}, Pınar Aslan Koşar⁵

¹Department of Medical Biology, Faculty of Medicine, Mugla Sitki Kocman University, Mugla, Turkey,

²Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey,

³Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey,

⁴Department of Neuroscience, Health Science Institute, Suleyman Demirel University, Isparta, Turkey,

⁵Department of Medical Biology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Transient receptor potential vanilloid type 1 (TRPV1) is a calcium-selective ion channel (1). Calcium signaling is a main cause of proliferation and apoptosis of breast cancer cells (2). Selenium, an essential dietary trace element, is implicated as an anticancer agent in several cancers, including breast cancer (3). Although previous studies have implicated the TRPV1 channel, the synergistic inhibition effects of selenium (Se) and cisplatin in cancer and the suppression of ongoing apoptosis have not yet been investigated in MCF-7 breast cancer cells. This study investigates the anticancer properties of Se through TRPV1 channel activity in MCF-7 breast cancer cell line cultures when given alone or in combination with cisplatin. The MCF-7 cells were divided into four groups: the control group, the Se-treated group (200 nM), the cisplatin-treated group (40 µM) and the Se+cisplatin-treated group. The intracellular free calcium ion concentration and current densities increased with TRPV1 channel activator capsaicin (0.01mM), but they decreased with the TRPV1 blocker capsazepine (0.1 mM), Se, cisplatin, and Se+cisplatin incubations. However, mitochondrial membrane depolarization, apoptosis, and the caspase 3, and caspase 9 values increased in the Se-treated group and the cisplatin-treated group, although Western blot (procaspase 3 and 9) results and the cell viability levels decreased with the Se and Se+cisplatin treatments. Apoptosis and caspase-3 were further increased with the Se+cisplatin treatment. Intracellular reactive oxygen species production increased with the cisplatin treatment, but not with the Se treatment. This study's results report, for the first time, that at a cellular level, Se and cisplatin interact on the same intracellular toxic cascade, and the combination of these two drugs can result in a remarkable anticancer effect through modulation of the TRPV1.

Keywords: Apoptosis; Breast cancer; Calcium signaling; Cisplatin; Oxidative stress; TRPV1 channel.

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▶ T. Oral Presentation No. 22

Protective effect of caffeic acid phenethyl ester against chlorpyrifos-induced hepatotoxicity in mice

Hacı Ahmet Deveci¹, Gökhan Nur¹, Mahmut Karapehlivan²

¹Gaziantep University, Vocational School of Higher Education in İslahiye, Gaziantep

²Kafkas University, Faculty of Veterinary Medicine, Biochemistry Department, Kars

In this study, we aimed to investigate the protective effect of Caffeic acid phenethyl ester (CAPE) on paraoxonase (PON1) activity, levels of nitric oxide (NO), total sialic acid (TSA) and total antioxidant/oxidant capacity (TAC and TOC) in liver tissue mice treated with chlorpyrifos-ethyl (CPF).

The study 35 male Swiss albino mice divided into 5 equal groups as following, intraperitoneal injection of saline for control (C) group mice, subcutaneous 80 mg/kg chlorpyrifosethyl for chlorpyrifos-ethyl (CPF) group, intraperitoneal injection of 10 µmol/kg CAPE for caffeic acid phenethyl ester (CAPE) group, subcutaneous 80 mg/kg chlorpyrifos-ethyl and intraperitoneal 10 µmol/kg CAPE for CPF+CAPE group, 10% ethanol diluted in physiological saline solution for ethanol (E) group was applied intraperitoneally for 21 days. All the mice were fed with the normal chow and tap water *ad libitum*. At the end of

the study, PON1 activity, levels of TSA, NO, TAC and TOC in the liver tissue were analyzed.

Levels of liver TAC and PON1 activity were statistically lower ($P<0.001$) in CPF group than in other groups. Also, levels of liver TOC, TSA and NO in CPF group were statistically higher ($P<0.001$) than in other groups. In conclusion, CAPE showed protective effect on PON1 activity, levels of NO, TSA, TAC and TOC in liver tissue and prevented the degenerations in liver tissue of chlorpyrifos-ethyl.

Keywords: Caffeic acid phenethyl ester, Nitric oxide, Paraoxonase activity, Total oxidant/antioxidant capacity, Total sialic acid.

▶ T. Oral Presentation No. 23

17β-Estradiol inhibites nitric oxide-cGMP-dependent pathway but may activate independent pathway in small intestine of ovariectomized rat*

Sevcan Sevimli¹, Aziz Bulbul²

This study is a partial summary of the PhD dissertation of the first author

¹Usak School of Health, Department of Nursing, Usak University, TR-64200 Usak - TURKEY

²Department of Physiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR-03200 Afyonkarahisar - TURKEY

This study was designed to investigate the effect of 17β-estradiol on small intestinal motility of ovariectomized rats and the possible role of nitric oxide (NO) in this activity. A total of 24, 3 to 6 month-old female Sprague Dawley rats were ovariectomized and divided into four groups as one control and three experimental groups. The control group received 0.2 mL, sesame oil once daily for three days, whereas the experimental groups were treated with 25, 50 and 100 µg/rat intramuscular 17β-estradiol, respectively. The rats were sacrificed by cervical dislocation under anesthesia 18 h after the termination of last treatment. Immediately, duodenum, jejunum and ileum were isolated for organ bath

contractility experiments to evaluate isometric smooth muscle motility *in vitro*. It was observed that application of 100 µg/rat 17β-estradiol showed a decreasing tension in duodenum, whereas none of the different doses of 17β-estradiol showed any significant difference in jejunum. The application of 50 and 100 µg/rat 17β-estradiol decreased the spontaneous contractile tension of ileum. However, L-arginine (10⁻⁵M), 8-Br-cGMP (10⁻⁶) and SNP (10⁻³M) decreased the spontaneous contractions of smooth muscle of duodenum, jejunum and ileum. Moreover, it was demonstrated that 17β-estradiol decreased the relaxing activity of L-arginine and 8-Br-cGMP but increased the activity of SNP in dose dependent manner. In conclusion, it is suggested that 17β-estradiol has a relaxative effect in duodenum and ileum and particularly inhibits the activity of cGMP-PK. However, endogenous NO-NOS pathway mediated by 17β-estradiol may play a key role in secretory and/or ciliary activity of intestine.

▶ T. Oral Presentation No. 24

Determining the effect of electromagnetic field generated by the high voltage power line on cells of rat spermatogonia and rat antioxidant parameters

M.Cihan Yavas¹, Engin Deveci², H.Murat Bilgin³, İbrahim Kaplan⁴, Uğur Şeker², M.Salih Çelik⁵, Veysi Akpolat¹, Yaşar Yıldırım⁶, Zülfikar Yılmaz⁶, İsmail Yıldız⁷, M.Zülküf Akdağ¹

Dicle University, Medical Faculty, Dept of Biophysics¹, Dept of Histology and Embryology², Dept of Physiology³, Dept of Biochemistry⁴, Dept of Nephrology⁶, Dept of Biostatistics⁷. Diyarbakir, Turkey
Selahaddin Eyyübi University, Vocational School of Health Services⁵ Diyarbakir, Turkey

The main aim of our study is to investigate the effect of the electromagnetic field generated by the high voltage (HV) line on the testicles tissue and the serum biochemistry of wistar albino male rats. It was also studied the protective activity of melatonin (MEL) and Ganoderma Lucidum (GI) against oxidative damage induced by electromagnetic field. Melatonin has a particular ability to neutralize free radicals and prevent

tissue damage associated with oxidative stress (1). Ganoderma extract has a wide range of biological activities, including anticancer activity, antioxidation and immune-stimulation (2).

In the present study, 64 wistar albino rats were divided into 8 equal groups (n:8). During the 26-day-experiment; Group 1: HV, Group 2: HV+ GI, Group 3: HV+MEL, Group 4: Sham-control. During the 52-day-experiment; Group 5: HV, Group 6: HV+ GI, Group 7: HV+MEL, Group 8: Sham-control groups were formed. Melatonin 10 mg/kg was administered on daily basis intraperitoneally and GI 20 mg/kg by oral lavage. To those rats in the 26-day- and 52-day-experiment groups (Groups 1, 2, 3, 5, 6 and 7) were administered ELF-EMF (Extremely low frequency-electric and magnetic fields) 8 hours a day. The average magnetic field intensity (2,48 µT) and electric field (80,3 V/m) in the Plexiglas cages was measured. In the final stage of the research, the tissue sections taken from the rats' testicles were evaluated and counted under the light microscope by administering routine histology, immunohistochemistry-PCNA and TUNEL methods. The blood samples from the rats were analyzed for changes in the serum NO (nitric oxide), TAS (total antioxidative status), TOS (total oxidative status) and OSI (oxidative stress index) parameters.

In conclusion; our study indicates that the oxidative effect generated by high voltage causes changes in the rats' serum biochemistry; in a number of degenerative deteriorations in the histological structure of the testicles. Furthermore, it's detected that the oxidative effect generated by high voltage produced negative effects in the formation of spermatogonia, and that GI partly preserves these effects and Melatonin is more active in their preservation.

Keywords: High Voltage, ELF-EMF, PCNA, TUNEL Assay, Spermatogonia, Antioxidant, Melatonin, Ganoderma lucidum

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▶ T. Oral Presentation No. 25

The effects of meal frequency and calorie restriction on oxidant-antioxidant systems in rats.

Hasan Basri Savas, Fatih Gultekin.

Department of Medical Biochemistry, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey. E-mail: drhbs63@gmail.com

In living organisms, oxidant and antioxidant systems are in a balance. Reactive products formed continuously by exogenous and endogenous sources are rendered harmless by the antioxidant system. Oxidative stress is an etiological factor in aging and development of various neurodegenerative diseases. In the present study, we aimed to investigate the effects of meal frequency and calorie restriction on oxidant-antioxidant systems in rat's serum and adipose, striated muscle tissues and liver. 24 adult male, Wistar albino rats weighing 200-250 gr obtained from Suleyman Demirel University, Experimental Animals Laboratory were included in the study. Rats were divided into three groups based on nutrition; Ad libitum (AL) (n=8), two meal group (TM) (n=8) and two meal and calorie restriction group (TM-CR) (n=8). The nutrition regulation was performed to all groups After 4 week pilot study, for 20 weeks, 7 days a week, a total of 60 minutes a meal. Serum and tissues of rats was isolated at the end of experiment. These adipose, striated muscle tissues and liver were homogenized. Total antioxidant status (TAS) and total oxidant status (TOS) and were determined through Erel method (1,2) by a biochemical auto-analyser equipment (Beckman Coulter AU 5800. USA), using a commercial kit (Rel Assay Diagnostic Turkey). Oxidative stress index was calculated using the formula (OSI) = TOS/TAS. Statistical analyses were carried out using SPSS package program. <0.05 was regarded as statistically significant. When TAS, TOS and OSI values were compared by nonparametric

Kruskall Wallis test and Bonferroni corrected Mann-Whitney U test. There are significant differences between AL and TM, AL and TM-CR in adipose tissue, between AL and TM in liver of rats. Calorie restriction and the sparse meal frequency increase the activity of antioxidants and can reduce oxidative stress. Thus, many diseases caused by oxidative stress can be prevented by correctly regulating of the feeding.

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▶ T. Oral Presentation No. 26

Oxidative status and plasma amino acids and fatty acids in patients with beta thalassemia major

Tuğba Koca¹, Duran Canatan¹, Ahmet Rifat Örmeci¹, Yavuz Savaş Koca², Handan Duman¹, Aslı Baykal³, Mustafa Akçam¹

¹Süleyman Demirel University, School of Medicine, Department of Pediatrics, Isparta, Turkey.

²Süleyman Demirel University, School of Medicine, Department of General Surgery, Isparta, Turkey.

³Akdeniz University, School of Medicine, Department of Biochemistry, Antalya, Turkey.

Oxidative damage and increasing of lipid peroxidation are caused by chronic iron overload in patients with beta thalassemia major. Fatty acids are important structural elements for palmitoylation of membrane proteins which constitute a great part of natural membranes. Oxidative damages caused by reactive oxygen derives in thalassemic erythrocytes can be determined with lipid

peroxidation, protein oxidation, and antioxidant system elements. The aim of study was to evaluate the relationship between amino acid and fatty acid levels with iron overload, lipid peroxidation and antioxidant enzymes in beta thalassemia major.

A total 40 patients with beta thalassemia major with regular blood transfusion and chelating agents were included to the study. The levels of serum amino acid, fatty acid, and ferritin were measured. Oxidative status was evaluated by measuring catalase, glutathione peroxidase, superoxide dismutase, and lipid peroxidation as malondialdehyde.

C16-palmitoyl levels were low in 14 (32.5%) patients. The levels of other fatty acids and amino acids were within the normal range. The mean levels of malondialdehyde and ferritin were higher in patients with low C-16 palmitoyl levels than the patients with normal levels. This elevation was statistically significant ($p=0.02$ and $p=0.01$, respectively).

The high levels of ferritin and malondialdehyde in the patients with low C16-palmitoyl levels might be caused by this fatty acid's preventative effect on oxidative stress.

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▶ T. Oral Presentation No. 27

The effects of ferulic acid against oxidative stress and inflammation in formaldehyde-induced hepatotoxicity

Fethullah Gerin¹, Hayriye Erman¹, Mustafa Erboga², Umit Sener³, Ahsen Yilmaz¹, Hatice Seyhan¹, Ahmet Gurel¹

¹Department of Biochemistry, Medical Faculty, Namik Kemal University, Tekirdag, Turkey

²Department of Embriology-Histology, Medical

Faculty, Namik Kemal University, Tekirdag, Turkey

³Department of Physiology, Medical Faculty, Namik Kemal University, Tekirdag, Turkey

Ferulic acid (FA) is a natural compound that has a phenolic group. Health benefits of phenolic compounds like FA attract the interest of researchers because of their antioxidant potentials. This study was designed to elucidate the protective effects of FA on formaldehyde-induced hepatotoxicity by measuring some routine biochemical parameters and oxidative stress-related parameters in addition to YKL-40 in male Wistar rats.

Twenty-one 16-week-old Wistar albino rats were handled under standard laboratory conditions. The groups were set as follows; Group 1(control): saline was administered intraperitoneally at doses of 50 mg/kg, during 10 days; Group 2 (formaldehyde): formaldehyde was administered intraperitoneally at doses of 10 mg/kg during 10 days; Group 3 (formaldehyde + FA): formaldehyde and FA were administered intraperitoneally during 10 days at doses of 10 mg/kg and 50 mg/kg, respectively. Tissue SOD, CAT, GSH-Px activities and tissue MDA levels were measured. Also, serum YKL-40, TNF- α , IL-6, IL-1 β , IL-8, total protein, albumin, total bilirubin concentrations and AST, ALT, ALP, and LDH activities were measured. Histological specimens were examined in light microscopy.

We suggested that FA can be used as a promising hepatoprotective agent against formaldehyde toxicity because of the obvious beneficial effects on oxidative stress parameters.

FA, as an anti-inflammatory and anti-oxidative compound, shows its hepatoprotective effects by decreasing the production of inflammatory cytokines, and YKL-40, and increasing the activities of antioxidant enzymes. FA also alleviated degeneration due to formaldehyde toxicity. Consequently, it can be implemented that FA is a promising pharmacological agent for tackling risks associated with hepatic damage.

Cytotoxic and apoptotic effects of two newly synthesized Pd(II) complexes on human leukemia cell lines in vitro

¹Neslihan Tekin, ²Kamile Öztürk, ³Talat Baran, ²Barış Kerimoğlu, ²Mehtap Tarhan, ³Ayfer Menteş

¹Department of Biotechnology and Molecular Biology, Faculty of Science and Letters, Aksaray University, Aksaray, Turkey

²Department of Biology, Faculty of Science and Letters, Aksaray University, Aksaray, Turkey

³Department of Chemistry, Faculty of Science and Letters, Aksaray University, Aksaray, Turkey

Palladium (Pd)-containing complexes are being explored as promising antitumor agents, yet their biological activity has hardly been examined. Here newly synthesized two Pd(II) complexes were investigated for their cytotoxic and apoptotic properties against two human leukemia cell lines (*HL-60* and *K562*).

Pd(II) complexes (Pd-5a and Pd-6a) with 5a and 6a as ligands were synthesized and characterized by ¹H-NMR and FT-IR. The cytotoxicity of the compounds were quantified using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. Bax, Bcl-2, caspase 3, and p53 expression levels were estimated using Real-time quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR).

Here we show that Pd-5a and Pd-6a complexes have important cytotoxic activity on human leukemia cell lines. RT-PCR indicated that Bax and caspase 3 expression levels were increased after 24 h treatment with Pd-5a and Pd-6a complexes in both *HL-60* and *K562* cells at some selected dose. p53 levels were increased in *K562* cells. Furthermore, Bcl-2 expression level decreased after 24 h treatment with Pd-5a and Pd-6a complexes in *K562* cells at all selected dose. In *HL-60* cells, only one selected dose (25 µM) decreased the expression level of Bcl-2.

The results obtained in the present investigation indicate

that these two newly synthesized Pd(II) complexes have apoptotic effects at appropriate doses through p53 and Bax genes and might represent a novel potentially active agents for the management of some selected human leukemia cell lines.

Keywords: Apoptosis; Pd(II) complexes; Cancer; Cell culture.

Acknowledgments

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Lignosus rhinocerotis (Cooke) Prevents Neurotoxic Effects of Cisplatin on Cultured Sensory Neurons

Ramazan Üstün¹, Elif Kaval Oğuz², Ayşe Şeker¹, Sıddık Keskin³

¹Yuzuncu Yıl University, Faculty of Medicine, Department of Physiology, Van, Turkey

²Yuzuncu Yıl University, Faculty of Education, Division of Science Teaching, Van, Turkey

³Yuzuncu Yıl University, Faculty of Medicine, Department of Biostatistics, Van, Turkey

Cisplatin is effective chemotherapy drug in cancer treatment, induces peripheral neuropathy in 30% of patients. Peripheral neuropathy is the dose limiting side effect, which has no preventative therapy. *Lignosus rhinocerotis* (tiger's milk mushroom) is used as traditional medicine to relieve cough, asthma and chronic hepatitis in Malaysia. The purpose of this study is to examine the regenerative effect of *Lignosus rhinocerotis* on cultured sensory neurons exposed to the toxic effect of cisplatin.

Sensory neurons were grown in primary culture following enzymatic and mechanical dissociation of dorsal root ganglia from 6-8-week-old mice. Neuron

culture was incubated in the moist condition with 5% CO₂ and at 37°C for 24 hours.

Groups:

1) *Cisplatin*: Neurons were exposed to cisplatin (10µg/ml) for 24 hours.

2) *Lignosus r. extract + cisplatin*: Neurons were treated with lignosus r. extract (30 µg/ml) one hour prior and then exposed to cisplatin (10µg/ml) for 24 hours.

3) *K252a + Lignosus r. extract + cisplatin*: Firstly neurons were treated with k252a (inhibitor of trkA) (100nM) one hour prior and then treated with lignosus r. extract (30 µg/ml) for 1 h and finally exposed for 24 hours to cisplatin (10µg/ml)

4) *Control*: No application were made

Microscopic visualization: To visualize the death and survival of the neurons, propidium iodide (7.5µM) and calcein AM (1µM) were added to the culture medium. The preparations were transferred to Zeiss Cell Observer microscopy system. Mozaix mode is chosen for visualizing the survival and the dead neuron cells and changes in the neuritis. Two proportion Z test is used for comparison of groups' proportions for survival rates of neurons, survival rates of neuritis and rates of fragmentation. In addition, Wilcoxon test is also used to compare before and after values of axon length.

At the end of the study, the survival rates of neurons in the groups (cisplatin, lignosus, k252a, control) were observed as 275/313, 204/206, 274/277 and 265/269, respectively. The survival rates of neuritis were also recorded as 90/261, 193/206, 272/277, 265/269, respectively. The rates of fragmentation in neuritis were found 67/261, 8/206, 5/277, 1/269 in the groups.

There was no statistically significant differences among the lignosus, k252a and control groups in terms of rates of survival neuron, neurite and fragmentation as well as axon length. However, cisplatin group was found different from the other three groups ($p < 0,01$).

As a conclusion, it can be stated that Lignosus rhinocerotis (cooke) prevents neurotoxic effects of cisplatin on cultured sensory neurons and it has therapeutic potential.

Key words: Lignosus, cisplatin, dorsal root ganglion neuron

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▶ T. Oral Presentation No. 30

Investigation of effect of sphingosine-1-phosphate receptors on MCF-7 and MDA-MB-231 breast cancer cells behavior

İbrahim Uğur Çalıř, Didem Turgut Cosan

Department of Medical Biology, Faculty of Medicine, Eskiřehir Osmangazi University, Eskiřehir, Turkey

Sphingolipids such as sphingomyelin, ceramide, sphingosine and sphingosine-1-phosphate (S1P), which belong to the sphingoid based lipid family, have important structural functions in regulation of cell membrane fluidity. It is also referred to as a bioactive lipid mediator and they play role in various stages of cancer.

Breast cancer is the most common cancer among women. One in eight women will be faced with breast cancer in their lifespan. The main cause of death in breast cancer patients is metastasis.

In this study five groups were created from each of MCF-7 and MDA-MB-231 cells. Non-silencing group, non-target silencing group, S1P1 siRNA treated group, S1P3 siRNA treated group, S1P1 and S1P3 siRNA treated groups were examined for cellular proliferation, adhesion, viability and lateral motility at 24th, 48th and 72nd hours of application.

In MCF-7 and MDA-MB-231 cells, viability of the cells in groups was found to be reduced at all hours, by silencing of S1P1 and S1P3 either alone or together. While there was reduction in adhesion of MCF-7 cells in S1P1-and-S1P3- silencing group, adhesion of MDA-MB-231 cells was increased in S1P1-and-S1P3-

silencing group. Lateral motility of both MCF-7 and MDA-MB-231 cells was statistically reduced in S1P1-silencing, S1P3-silencing and S1P1-and-S1P3-silencing groups at every hours.

S1P and its receptors play critical roles in cancer progression and metastasis. Interaction between these molecules is held responsible for reduced survival rates and development of drug resistance in breast cancer. Through understanding this interaction, discovery of new therapeutic agents to inhibit S1P and its receptors would provide opportunities for new therapeutic strategies against cancer and its metastasis.

Poster Presentations

▶ Poster No. 1

Efficacy of vitamin c in prevention of chemoradiation-induced mucositis

Farshad Seyed Nejad^{2,1}, Amir Reza Rezaie², Parya Solati²

¹Radiation Oncology Department, Shahid Madani Hospital, Tabriz University of Medical Sciences, Iran

²Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Iran

Mucositis is one of the most common side effects of cancer-treatment which manifests as erythematic and painful ulcers in mucus of the patients receiving chemotherapy and radiotherapy. The aim of this study is to find out the efficacy of Vitamin C in prevention of mucositis in patients with head and neck cancers who receiving chemoradiation.

This randomized double blind clinical trial was done in 60 patients with Head and Neck cancer receiving chemoradiation in dosage of 60-72 Gy at Shahid Madani Hospital of Tabriz since December 2012 till June 2013. Patients of intervention group received 250mg Vitamin C per oral daily. Evaluation of severity of mucositis was done via WHO common Toxicity criteria weekly for four weeks. Statistic data was analyzed by SPSS software.

Significant decrease in frequency (21.4% vs. 50%) and severity of mucositis (0.4 vs. 1.25) was seen in Vitamin C group (($P < 0.05$).

Vitamin C consumption during chemoradiationtherapy could reduce the frequency and severity of mucositis related to chemoradiation in patients with head and neck cancer.

Key words: Chemoradiation, Mucositis, Vitamin C

▶ Poster No. 2

Investigation of serum NO and ADMA levels in thyroid dysfunction

Arzu Şahin¹, Mustafa Gül², Ömer Akyol³, Abdulkadir Yıldırım⁴, Fikret Çelebi⁵, Serap Yıldırım²

¹Department of Physiology Medical Faculty, Ordu University, Ordu, Turkey

²Department of Physiology Medical Faculty, Atatürk University, Erzurum, Turkey

³Department of Internal Medicine, Şile Public Hospital, Istanbul, Turkey

⁴Department of Biochemistry Medical Faculty, Atatürk University, Erzurum, Turkey

⁵Department of Physiology Veterinary Faculty, Atatürk University, Erzurum, Turkey

The deterioration of endothelial function, is the first sign of coronary artery disease (1). NO, major vasodilator released from endothelium, previously known as endothelium-derived relaxing factor, is a small, reactive and free radical molecule having regulatory function both intracellularly and extracellularly. The activity of the enzyme NOS, which is responsible for NO synthesis, is regulated by an endogenous inhibitor, namely ADMA molecule. In recent studies; increased plasma levels of ADMA were shown to be associated with the endothelial dysfunction and increased atherogenesis (1,2). The aim of this study was to investigate serum nitric oxide (NO) and asymmetric dimethyl arginine (ADMA) levels in patients with thyroid disorders. A total of 150 thyroid patients and 50 healthy individuals were included in the study, who were divided into three groups as the control (n=50), hyperthyroid (n =75) and hypothyroid (n=75) groups. Serum levels of TSH, FT3 and FT4 levels were measured by using the chemiluminescence method, nitrite/nitrate by was measured by the spectrophotometric method using commercial kit, and ADMA was measured by ELISA method. Serum levels of glucose, creatinine, triglycerides, cholesterol, BUN, HDL cholesterol and LDL cholesterol were measured using the spectrophotometric methods. As for the average NO values of the groups, we determined significantly higher NO levels in the hypothyroid group

than the hyperthyroid group ($p = 0.049$). ADMA levels were statistically significantly higher in the hyperthyroid group than the other groups ($p = 0.000$). Permanent damages may occur as a result of late detection of cardiovascular disorders, which is one of the major complications of thyroid diseases. Endothelium, which is the main regulator of vascular homeostasis, serves to maintain a balance between vasoconstriction and vasodilatation, between inhibition and stimulation of migration of smooth muscle cell proliferation, and between thrombogenesis and fibrinolysis (1). In this study the most important cause of increased ADMA levels in hyperthyroidism patients may be reduced NO and free oxygen radicals which increase in hyperthyroid situations. Additionally thyroid diseases may affect the synthesis of ADMA, a species of methyl arginine related to protein metabolism."

Keywords: Thyroid disorder, NO, ADMA, Endothelial dysfunction

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► Poster No. 3

Relationship between plasma viscosity and oxidative stress parameters in patients with ascending aortic aneurysms

Bahar Öztürk Kurt¹, Sinan Göçer², Cengiz Köksal³, Meltem Ercan¹

¹Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul, Turkey

²Ministry of Health Batman Regional Government Hospital, Batman, Turkey

³Department of Cardiovascular Surgery, Kartal Kosuyolu Reseach and Training Hospital, Istanbul, Turkey

An ascending aortic aneurysm (AAA) is basically the

*"ballooning" of the aorta in the region where the aorta first exits the heart*¹. We aimed to investigate plasma viscosity (PV), fibrinogen, nitric oxide (NO) and asymmetric dimethylarginine (ADMA) parameters and their relationship between each other in AAA patients. The study group was composed of 23 patients who underwent surgical correction for AAA (63±8 years). The control group consisted of 30 patients without cardiological problem (72±10 years). Blood samples were taken preoperatively and postoperatively on the seventh day to obtain plasma for measurements. Pre-op PV level was statistically higher than post-op and the control ($p<0.05$). The statistically significant increase was determined in post-op fibrinogen level according to the pre-op ($p<0.05$). Fibrinogen and ADMA levels in the study groups were lower than the control ($p<0.001$). Post-op NO level was found to be lower than the pre-op ($p<0.05$). Vasodilation causes changing in blood flow and intravascular coagulation and also the formation of fibrin. Due to fibrin deposition, some coagulation factors especially fibrinogen is consumed. Because of these reasons, while pre-op PV was increased; pre-op fibrinogen level was decreased. The decrease in the level of ADMA led to the increase in the level of nitric oxide. Nitric oxide is potent inhibitor of platelet aggregation and adhesion to the vessel wall². Because of the distribution of blood flow, the pre-op nitric oxide level was increased. Our research showed that PV and osmotic stress parameters might be crucial for diagnosis, treatment and follow-up of patients with aneurysm.

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► Poster No. 4

Determination of antioxidant properties of oenin and callistephin

Zübevir Huyut¹, Şükrü Beydemir², İlhami Gülçin², Mehmet Ramazan Şekeroğlu¹

¹Department of Biochemistry, Medical Faculty, Yüzüncü Yıl University, Van, Turkey

²Department of Chemistry, Faculty of Science, Atatürk

University, Erzurum, Turkey

Flavonoids are potent and important antioxidants in our diet (1,2). In this study, we aimed to determine the antioxidant capacities of Oenin and Callistephin which are natural flavonoids (3).

In different concentrations (10-30 µg/mL), some antioxidant methods including ferric ions (Fe^{3+}) reducing antioxidant power (FRAP assay), cupric ions (Cu^{2+}) reducing antioxidant power (CUPRAC assay), 1,1-diphenyl-2-picryl-hydrazyl radicals (DPPH) scavenging, 2,2-azino-bis-(3-methyl benzo-thiazoline-6-sulfonic acid) radicals ($\text{ABTS}^{\cdot+}$) scavenging, N-N'-dimethyl-p-phenylenediamine-dihydrochloride radicals ($\text{DMPD}^{\cdot+}$) scavenging, superoxide anion radical ($\text{O}_2^{\cdot-}$) scavenging assays, and total antioxidant capacity of Oenin and Callistephin were determined according to the previous studies (4,5) and compared to α -Tocopherol, which is used as standard antioxidant compound in food and pharmaceutical applications.

Oenin and Callistephin demonstrated effective Fe^{3+} and Cu^{2+} reducing activities. Also, both natural flavonoids scavenged $\text{ABTS}^{\cdot+}$, $\text{DMPD}^{\cdot+}$ and DPPH radicals in a range of 15-35% higher than α -Tocopherol. IC_{50} values belonging to $\text{ABTS}^{\cdot+}$ and $\text{O}_2^{\cdot-}$ radicals scavenging activity of Oenin and Callistephin were lower than that of α -Tocopherol. At the same, inhibition capacity of Oenin and Callistephin in linoleic acid emulsion oxidation were found higher than that of α -tocopherol.

The results obtained from our study clearly showed that both biologically active flavonoids were more effective in terms of the free radical scavenging activities and Fe^{3+} and Cu^{2+} reducing abilities and total antioxidant activity. These results revealed that they were potent antioxidant compounds compared to α -tocopherol. They may be used for preservative in food and pharmaceutical applications.

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Poster No. 5

Investigating of the relationship between the second trimester screening biochemical markers and complications and anomalies in pregnant women.

Hasan Basri Savas¹, Seyit Ali Kose², Mesut Guler³, Fatih Gultekin¹.

¹Suleyman Demirel University, Medical Faculty, Medical Biochemistry Department. Isparta, TURKEY.

²Suleyman Demirel University, Medical Faculty, Department of Obstetrics and Gynecology. Isparta, TURKEY.

³Suleyman Demirel University, Medical Faculty. Isparta, TURKEY.

We aimed to investigate the relationship between the alpha-fetoprotein (AFP), unconjugated estriol (uE3), the β -subunit of Human chorionic gonadotropin (beta hCG), maternal age and Down syndrome, neural tube defects and trisomy 18 risk levels and prenatal maternal or fetal complications, postnatal chromosomal anomalies, emergence of high or low birthweight births, in pregnant women with triple test. The pregnancy complications were investigated retrospectively and post pregnant chromosomal abnormalities, neural tube defects (NTDs), high and low birth weight have been found in patient following, in 82 patients of Suleyman Demirel University Research and Education Hospital, Department of Obstetrics and Gynecology, Isparta, at the 2010-2012 year. AFP, uE3 and beta hCG levels were measured by chemiluminescent method and multiple of median (MoM) values were calculated. The obtained data were evaluated by nonparametric correlation, Mann-Whitney U test and multiple logistic regression analysis, using SPSS 17.0 software. The average age of the women in the study group was 27.7. The risk has been identified, for Down syndrome in 8 patients, for NTD in 10 patients, for trisomy 18 in 3 patients, over 1/250. The number of patients with preeclampsia was 2; gestational diabetes mellitus (DM)

was 8 during pregnancy. While the number of babies born with low birthweight was 13, high birthweight infants' number was 11. The relationship between each Mom value of beta hCG, uE3, AFP and low birth-weighted was statistically significant ($p < 0.05$). In our study, post-natal Down syndrome incidence was only 1, in 10 patients which Down syndrome risk was over 1/250 in the triple screening test. The Down syndrome risk of the patient was calculated 38/10000 which has postnatal Down syndrome, in triple screening test. Besides, none of the patients who were calculated NTD risk over 1/250, has post-natal NTD, the risk of NTD for 1 patient who has postnatal NTD was calculated 1/10000. Biochemical parameters measured for the triple test and can give significant insight in terms of complications and chromosomal abnormalities that may occur in pregnancy and postnatal term. But a false positive rate should be taken into consideration. Similar studies confirm our data (1, 2). Advanced clinical trials are recommended for increasing the safety of pregnancy screening tests.

Keywords: Triple screening test, chromosomal abnormalities.

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► Poster No. 6

Anti-tumor necrosis factor alpha (infliximab) attenuates apoptosis, oxidative stress and calcium ion entry through modulation of cation channels in neutrophils of patients with ankylosing spondylitis

Yunus Ugan¹, Mustafa Nazıroğlu^{2,3}, Mehmet Şahin¹, Mehmet Aykur³

¹Department of Internal Medicine, Division of Rheumatology, Faculty of Medicine, Suleyman Demirel

University, Isparta, Turkey

²Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey

³Department of Microbiology, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey

Ankylosing spondylitis (AS) is an inflammatory rheumatological disease that has a high incidence of 1% worldwide. AS is known to be associated with increased neutrophil activation and oxidative stress, however, the mechanism of neutrophil activation is still unclear. We have hypothesized that the antioxidant and anti-tumor necrosis factor properties of infliximab may affect intracellular Ca^{2+} concentration in neutrophils of AS patients. The objective of this study was to investigate the effects of infliximab on calcium signaling, oxidative stress and apoptosis in neutrophils of AS patients.

Neutrophils collected from 10 patients with AS and 10 age-matched control subjects. In a cell viability test, the ideal non-toxic dose and incubation time of infliximab were found as 100 μ M and 1 hour, respectively. In some experiments, the neutrophils were incubated with the voltage gated calcium channel (VGCC) blockers verapamil+diltiazem (V+D) and the TRPM2 channel blocker 2-aminoethyl diphenylborinate (2-APB).

Intracellular Ca^{2+} concentration, lipid peroxidation, apoptosis, caspase 3 and caspase 9 values were high in neutrophils of AS patients and were reduced with infliximab treatment. Reduced glutathione level and glutathione peroxidase activity were low in the patients and increased with infliximab treatment. The intracellular Ca^{2+} concentrations were low in 2-APB and V+D groups.

In conclusion, In conclusion, we have shown here the effects of the anti-TNF- α drug infliximab on calcium signaling, oxidative stress and apoptosis in neutrophils from AS patients. Infliximab was seen to inhibit intracellular Ca^{2+} flux through modulation of TRPM2 and VGCC channels in neutrophils. We have suggested here a new concept in the mechanism of action of infliximab where by the anti TNF- α drug may improve neutrophil activity in AS patients by regulating intracellular signaling pathways and protecting them against apoptosis and oxidative stress.

Keywords: Ankylosing spondylitis; infliximab;

apoptosis; calcium signaling; oxidative stress; neutrophil.

► Poster No. 7

Investigation on the cross-talk between β_3 -Adrenergic receptor and Zn^{2+} signalling in diabetic cardiomyocytes

Belma Turan, Erkan Tuncay, Esmâ Nur Okatan,

Ankara University Faculty of Medicine Department of Biophysics, Ankara Turkey

Zinc, is an essential catalytic-structural component in thousands of enzymes involved in several cellular signalling pathways. Although the intracellular free Zn^{2+} level ($[Zn^{2+}]_i$) is very low (about less than 1-nM) in mammalian cardiomyocytes, studies have shown that its level is increased by 70% under hyperglycemia. Previous studies have also demonstrated that β -adrenoceptors (AR) play important role in development of diabetes-induced cardiac dysfunction. In this regard, we previously have shown that the subtypes of this system, β_1 – and β_2 -AR were downregulated while β_3 -AR subtype was upregulated in diabetic cardiomyocytes. Therefore, in this study, we aimed to investigate whether upregulation of β_3 -AR and increased $[Zn^{2+}]_i$ have functional relationship in diabetic cardiomyocytes. In Langendorff-perfused heart studies, the contractile force is decreased about 25% with Zn^{2+} exposure under in vitro condition, which is in similar range obtained in hyperglycemic condition in the similar heart preparations. Furthermore, under in vitro hyperglycemic condition, we measured marked increased $[Zn^{2+}]_i$ (about 50%) level in FluoZin-3 loaded isolated cardiomyocytes. Furthermore, we showed that not β_1 - or β_2 -AR but β_3 -AR agonist leads to increase $[Zn^{2+}]_i$ through nitric oxide (NO) pathway. Moreover, we observed two-fold increased negative inotropic response to β_3 -AR agonist exposure in diabetic rat heart preparation. Our Western-blot data showed that the protein levels of some signalling proteins in β_3 -AR pathway such as NO-products, GSK-3 β enzyme or Akt are supporting the electrophysiological data. Since GSK-3 β , which is activated by the changes in $[Zn^{2+}]_i$ level via PI3K/Akt pathway, is able to cause

cardiomyocyte dysfunction. To this end, we also measured protein levels of the PI3K/Akt pathway components, in here, as conclusion our data can propose that β_3 -AR/NO pathway activation and concomitant increased $[Zn^{2+}]_i$ -induced PI3K/Akt pathway activation may be one of the molecular mechanism underlying the diabetic cardiomyopathy.

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► Poster No. 8

Investigation of ghrelin oxidative stress and obesity relationship in serum

Sevilay Tarakçı Zora¹, Nesrin Emekli², Türkan Yiğitbaşı², Gözde Ülfer²

¹Istanbul Aydin University, Vocational School of Health Services, İstanbul, Turkey

²Istanbul Medipol University, Institute of Health Sciences, Medical Biochemistry Department, Istanbul, Turkey

Ghrelin has been reported to increase appetite and cause obesity. Also, the literature shows that ghrelin increases in hunger and decreases especially after meals that are high in glucose and fat ratio (1,2). The degradation of structure and function of macromolecules and enzymes with the release of reactive oxygen species as a result of oxidative stress lead to cellular damage in the organism (3). Insulin resistance and the metabolic syndrome criteria is a condition observed in obesity (4). The study was designed to analyze ghrelin impact in the obese and non-obese individuals in terms of oxidative stress and insulin resistance. In this study the serums of 61 obese and 24 control groups with ages younger than 18 and older than 75 people were used. Ghrelin by Elisa method; the total oxidant and antioxidant by Erel Method and insulin resistance was performed by HOMA-IR method. Ghrelin values $110,434 \pm 25,22$ pg/mL in obese; $110,780 \pm 25,46$ pg/mL in non-obese ($p>0,05$). Oxidative stress level and insulin resistance were found to be higher in obese ($p<0,05$). There was no significant difference of total oxidant levels in obese and control group. The analysis of total antioxidant and

oxidative stress levels showed a significant relationship, ($p < 0,05$). While, this study has shown that there is no association between ghrelin and oxidative stress level, there was found a positive correlation between insulin resistance and oxidative stress index. In conclusion, these results have suggested that ghrelin has no any effect in the increased oxidative stress in obesity.

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► Poster No. 9

3,4-Dihydroxy-5-methoxy benzoic acid demonstrate powerful antioxidant capacities than α -tocopherol and trolox as standard compounds

Zübeyir Huyut¹, Şükrü Beydemir², İlhami Gülçin²

¹Department of Biochemistry, Medical Faculty, Yüzüncü Yıl University, Van, Turkey

²Department of Chemistry, Faculty of Science, Atatürk University, Erzurum, Turkey

Phenolic compounds are among the most important natural antioxidant class in daily human diet (1). In this study, the antioxidant property of 3,4-dihydroxy-5-methoxy-benzoic acid (DMBA) derived from phenolic acids was investigated for the determination of its antioxidant characteristics. Also, these activities were compared to α -Tocopherol and Trolox as standard and common antioxidant molecules.

In different antioxidant concentrations (10-30 $\mu\text{g/mL}$), ferric ions (Fe^{3+}) reducing antioxidant power (Fe^{3+} - Fe^{2+}

transformation assay), cupric ions (Cu^{2+}) reducing antioxidant power (CUPRAC assay), 1,1-diphenyl-2-picryl-hydrazyl radicals (DPPH) scavenging assay, 2,2-azino-bis-(3-methyl-benzo-thiazoline-6-sulfonic acid) radicals (ABTS⁺) scavenging assay, superoxide anion radical ($\text{O}_2^{\cdot-}$) scavenging assay, ferrous ions (Fe^{2+}) chelating activity of DMBA were determined according to the methodologies described previously (2-5).

Fe^{3+} and Cu^{2+} reducing power of DMBA were high in range of 13-57% respectively. Also, ABTS⁺, DPPH and $\text{O}_2^{\cdot-}$ anions radicals scavenging activities of DMBA were found 32, 26 and 49% higher than α -Tocopherol and Trolox, respectively. So IC_{50} values of ABTS⁺, DPPH and $\text{O}_2^{\cdot-}$ radical scavenging activity of DMBA was lower according to α -Tocopherol and Trolox. In addition, DMBA showed more potent activity in terms of ferrous ions (Fe^{2+}) chelating activity compared to α -Tocopherol and Trolox.

The results obtained from this study indicated that DMBA had higher antioxidant activity when compared to α -Tocopherol and Trolox. We suggest that it may be used as a preservative additive for food and other applications.

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The effects of 40 Hz pulsed magnetic fields on H₂O₂-induced Ca²⁺ responses in the vascular smooth muscle cells

Figen Çiçek, İlknur Baldan, Bora Taştekin, İsmail Günay

Department of Biophysics, Faculty of Medicine, Çukurova University, Adana, Turkey

Today, biological systems are exposed directly or indirectly to the electromagnetic field forces with various intensity and frequency. Despite the studies which show the effects of these forces on some cellular functions, action mechanisms still have not well understood.

In electrically excitable cells the changes on magnetic and thereby electrical fields may have unpredicted effects on signal transduction mechanisms. As it has been shown earlier that low frequency pulsed magnetic field (PMF) have altered voltage gated Ca²⁺ channels activation. Because Ca²⁺ is one of the key regulators of the cell homeostasis, we examined the effect of PMF on the cytosolic Ca²⁺ signals in apoptotic processes.

In this study, vascular smooth muscle cells (VSMCs) were isolated from the thoracic aorta of rats which were exposed to PMF (40 Hz-1.5 mT/day) in vivo for 30 days or control animals without any treatment. Secondary cell cultures were used between the passages of 2-3.

The effects of PMF were tested with an apoptosis inducer H₂O₂. The 1 mM H₂O₂ induced Ca²⁺ responses were measured in Fura 2-AM loaded cells with a CCDI camera system. Measurements were analysed according to their baseline fluorescence intensity and slopes fitted to response amplitudes of H₂O₂. We observed that in PMF treated group baseline of Ca²⁺ tended to be higher according to the control groups. Also 1 mM H₂O₂ application caused a statistically significant increase in the slopes of cytoplasmic Ca²⁺ responses. These differences in Ca²⁺ responses may reflect a change in physiological regulation for the cells with the PMF exposures.

Keywords: Pulsed magnetic fields; Oxidative stress; Smooth muscle cells; Calcium ion

Biochemical alterations with bee products in kidney tissues of nitric oxide synthase inhibited rats

Zeliha Selamoglu¹, Mehmet Fuat Gulhan², Engin Sahna³

¹Department of Biotechnology, Faculty of Arts and Science, Nigde University, Nigde, 51240 Turkey.

²Department of Medicinal and Aromatic Plants, Technical Sciences Vocational School, Aksaray University, Aksaray, 68100 Turkey.

³Department of Pharmacology, Faculty of Medicine, Firat University, Elazig, Turkey.

In this study, the effects of propolis, caffeic acid phenetil ester (CAPE) and pollen on biochemical parameters such as paraoxanase (PON1) activity, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), asymmetric dimethylarginine (ADMA) and nuclear factor kappa B (NF-κB) in kidney tissues of rats induced by *N*ω-Nitro-L-arginine methyl ester (L-NAME) have been investigated.

L-NAME for 28 days and the propolis, CAPE and pollen for the last 14 days with L-NAME together were given to rats. L-NAME (40 mg/kg, intraperitoneally), NOS inhibitor for 28 days to occur hypertension, propolis extract (200 mg/kg/days, by gavage), CAPE (50 μM/kg/days, intraperitoneally) and pollen extract (100 mg/kg/days, by gavage) the last 14 of 28 days were given to rats. In the 0th, 14th and the 28th days of study, rats' blood pressure (BP) has been measured. At the end of the 28th day, decapitated rats' kidney tissues have been taken and biochemical parameters analysed.

BP of L-NAME with pollen group was lower ($P < 0.05$) than the other additional groups (L-NAME with CAPE and propolis groups). PON1 activity and TAS level significantly decreased in L-NAME group compared to control ($P < 0.05$), but these parameters increased ($P < 0.05$) in L-NAME+(propolis, CAPE, pollen) groups

compared to L-NAME group. TOS, ADMA and NF- κ B levels increased ($P<0.05$) in L-NAME group compared to control, but decreased ($P<0.05$) in L-NAME+(propolis, CAPE, pollen) groups compared to L-NAME group.

In conclusion, oxidative alterations occurring in kidney tissues of hypertensive rats may be prevented by natural bee products (propolis and pollen) and CAPE that one active compound of propolis.

Keywords: Hypertension, L-NAME, Oxidant /Antioxidant Status, Oxidative stress, Propolis, CAPE, Pollen, Rat, Kidney.

► Poster No. 12

Transcriptional and translational approaches of propolis, caffeic acid phenethyl ester and pollen treatment to N^ω-nitro-L-arginine methyl ester induced rats

Zeliha Selamoglu¹, Mehmet Fuat Gulhan², Merve Duruyurek¹

¹Department of Biotechnology, Faculty of Arts and Science, Nigde University, Nigde, 51240 Turkey

²Department of Medicinal and Aromatic Plants, Technical Sciences Vocational School, Aksaray University, Aksaray, 68100 Turkey

We studied the effects of propolis, caffeic acid phenethyl ester (CAPE) and pollen on total RNA and total protein levels in adrenal medulla, heart, hypothalamus of N^ω-Nitro-L-arginine methyl ester (L-NAME) induced rats.

L-NAME for 28 days and the propolis, CAPE and pollen for the last 14 days with L-NAME together were given to rats. L-NAME (40 mg/kg, intraperitoneally), NOS inhibitor for 28 days to occur hypertension, propolis extract (200 mg/kg/days, by gavage), CAPE (50 μ M/kg/days, intraperitoneally) and pollen extract (100 mg/kg/days, by gavage) the last 14 of 28 days were given to rats. After the treatments, decapitated rats' adrenal medulla, heart and hypothalamus were removed and total RNA and total protein levels analysed.

Total RNA and total protein levels were found significantly decreased ($P<0.05$) in adrenal medulla, heart and hypothalamus of nitric oxide synthase blocked rats caused by L-NAME compared to control. These parameters in these tissues of L-NAME+ (plus; propolis, pollen and CAPE) groups compared to the L-NAME group have increased ($P<0.05$).

These results suggest that in L-NAME can block in transcriptional and translational levels in rats' adrenal medulla, heart and hypothalamus. Determination of total RNA and total protein levels was undertaken in order to obtain preliminary information about the transcriptional and translational approaches. In this study, it has been observed that bee products have the ability can be modulate in transcriptional and translational levels in rats.

Keywords: L-NAME, Total RNA, Total Protein, Propolis, CAPE, Pollen, Rat.

► Poster No. 13

The effect of bovine serum albumin and fetal calf serum on sperm quality, DNA fragmentation and lipid peroxidation of the liquid stored rabbit semen

Serpil Sarıözkan¹, Gaffari Türk², Fazile Cantürk³, Arzu Yay⁴, Ayşe Eken⁵, Aytaç Akçay⁶

¹Department of Reproduction and Artificial Insemination, ⁶Department of Biostatistics, Faculty of Veterinary Medicine, ³Department of Biophysics, ⁴Department of Histology and Embryology, Faculty of Medicine, ⁵Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey

²Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Fırat University, Elazığ, Turkey

The aim of the present study was to determine the effects of the bovine serum albumin (BSA) and fetal calf serum (FCS) on sperm quality, DNA fragmentation and lipid peroxidation of liquid stored rabbit semen stored up to 72 h at 5 °C. Each pooled and diluted

ejaculate was split into three groups containing BSA (5 mg/ml), FCS (10%) or no additive (control). The extender supplemented with BSA and FCS did not improve the percentages of motility and acrosomal abnormality during 48 h. The additives BSA and FCS had a significant effect in the maintaining of plasma membrane integrity between 48 and 72 h storage period, compared to the control ($P < 0.01$). The supplementation of BSA and FCS had a protective effect on motility ($P < 0.05$), plasma membrane integrity ($P < 0.01$) and acrosomal integrity ($P < 0.01$) at 72 h compared to the control. The supplementations with BSA and FCS led to a reduction in DNA damage of rabbit sperm at 48 and 72 h during storage period, compared to the control ($P < 0.001$). Although supplementation of BSA and FCS caused significant ($P < 0.01$) decreases in malondialdehyde (MDA) level at 48 h and 72 h, they significantly ($P < 0.01$) increased the glutathione peroxidase (GPx) activity up to 72 h when compared to the control group. In conclusion, BSA and FCS supplementation to liquid stored rabbit semen provide a protection for spermatozoa against cool storage-induced DNA damage and plasma membrane integrity by their antioxidative properties.

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► Poster No. 14

Role of extracellular and intracellular Ca^{2+} on relaxations induced by ethanol in the smooth muscle

Derya Kaya, Naciye Yaktubay Döndaş

University of Çukurova, Faculty of Medicine, Department of Pharmacology, Adana, Turkey.

Since worldwide alcohol consumption, the effect of ethanol is important in gastrointestinal tract (GIT). The

effect of ethanol may change from one tissue to another in the GIT (1). So, in this study we aimed to clarify the role of extracellular and intracellular calcium (Ca^{2+}) on relaxations induced by ethanol in isolated mouse gastric fundus. Mice (Swiss *albino*) of either sex were used in this study. After killing the mice by cervical dislocation, the gastric fundal strips were prepared by longitudinal incision and mounted under 0.5 g tension in an organ bath filled with *Tyrode's* solution. The bath medium was maintained at 37 °C and gassed with %95 O_2 and 5% CO_2 . Experimental data were recorded by an isometric transducer. Ethanol (164 mM) caused reproducible relaxations in isolated mouse gastric fundal strips. These relaxations were significantly inhibited by verapamil (10-500 μ M), a blocker of L-type Ca^{2+} channels, and ruthenium red (10-100 μ M), a blocker of ryanodine receptors (intracellular Ca^{2+} channels) in a concentration dependent manner. The results of experimental data suggest that extracellular Ca^{2+} influx and intracellular Ca^{2+} release may play a role on relaxations induced by ethanol in the isolated mouse gastric fundal smooth muscle.

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► Poster No. 15

Molecular mechanism of frog esophageal relaxations induced by electrical field stimulation and sodium nitroprusside

Naciye Yaktubay Döndaş, Nuran Ögülener

University of Çukurova, Faculty of Medicine, Department of Pharmacology, Adana, Turkey.

In our previous study, we showed the role of nitric oxide (NO) on relaxations induced by ultraviolet light (366 nm) in mouse gastric fundus (1). In the present study, we aimed to investigate further insights into the mechanism of NO-mediated relaxations in different tissue and relaxant agents. So in this study we investigated the role of Na^+ - K^+ -ATPase on relaxations

induced by electrical field stimulation (EFS; endogenous NO, released by EFS) and sodium nitroprusside (SNP; an effective donor of nitric oxide, exogenous NO) in frog esophageal tissue.

Both male and female frogs (*Rana pipiens*) weighing 15-25 g were used in this study. Upper esophageal segments were removed after the animals were decapitated and pithed. The esophageal strips were prepared by circular incision and mounted under 0.5 g tension in an organ bath filled with Tyrode solution. The bath medium was maintained at 25 °C and gassed with %95O₂ and 5%CO₂. The muscular movements in these experiments were recorded isotonicly on a smoked kymograph drum with a linear frontal writing lever. The strips were precontracted with methacholine (1μM; a cholinergic agent) and relaxed with EFS (5Hz, 25V, 1ms) or SNP (1μM). N^o-nitro L-arginine (L-NA; 1-1000 μM), an inhibitor of NO synthase, inhibited EFS-induced relaxations in a concentration dependent manner. However it failed to affect SNP-induced relaxations in the related tissue. On the other hand ouabain, a selective inhibitor of Na⁺-K⁺-ATPase, significantly decreased EFS- and SNP-induced relaxations in a concentration dependent manner. Similarly, 1H-[1,2,4] oxadiazolo-[4,3-a] quinoxalin-1-one (ODQ), a selective inhibitor of guanylyl cyclase, dramatically decreased EFS- and SNP-induced relaxations. The results suggest that Na⁺-K⁺-ATPase may play a critical role on relaxations induced by EFS (endogenous NO) and SNP (exogenous NO) in the isolated frog esophageal strips.

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► Poster No. 16

Effects of clothianidin exposure on sperm quality, testicular apoptosis and fatty acid composition in developing male rats

Ramazan Bal¹, Gaffari Türk², Ökkeş Yılmaz³, Ebru Etem⁴, Tuncay Kuloğlu⁵, Gıyasettin Baydaş⁶, Mustafa Naziroğlu⁷

¹Department of Physiology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey, ²Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, ³Department of Biology, Faculty of Science, ⁴Department of Medical Biology, Faculty of Medicine, ⁵Department of Histology and Embryology, Faculty of Medicine, Firat University, Elazığ, Turkey, ⁶Council of Higher Education, Ankara, Turkey, ⁷Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Clothianidin (CTD) is one of the latest members of the synthetic organic insecticides, the neonicotinoids. In the present study, it was aimed to investigate if daily oral administration of CTD at low doses for 90 days has any deleterious effects on reproductive functions of developing male rats. Animals were randomly divided into four groups of six rats each, assigned as control rats, or rats treated with 2 (CTD-2), 8 (CTD-8) or 32 (CTD-32) mg CTD/kg body weight by oral gavage. The significant decreases of the absolute weights of right cauda epididymis and seminal vesicles, and body weight were detected in the animals exposed to CTD administration at 32 mg/kgBW/day. Epididymal sperm concentration decreased significantly in CTD-32 group and the abnormal sperm rates increased in CTD-8 and CTD-32 groups when compared to control group. The testosterone level was significantly decreased in CTD-32 group when compared to control group. The administration of all CTD doses resulted in a significant decrease in the level of glutathione. Significant increments were observed in sperm DNA fragmentation and the TUNEL positive apoptotic cell numbers in CTD-32 group. In groups CTD-8 and CTD-32, only docosapentaenoic, arachidonic, palmitic and palmitoleic acids were significantly elevated when compared to control. The ratios of 20:4/18:2 and 18:1n-9/18:0 were decreased when rats exposed to CTD. It is concluded that low doses of CTD exposure during critical stages of sexual maturation had moderate detrimental effects on reproductive organ system and more severe effects are likely to be observed at higher dose levels.

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▶ **Poster No. 17**

Melatonin potentiates chemotherapy-induced apoptosis in HeLa cells.

Roberto Pariente, Ignacio Bejarano, José Antonio Pariente, Ana Beatriz Rodríguez, Javier Espino.

Department of Physiology, Neuroimmunophysiology and Chrononutrition Research Group, Faculty of Science, University of Extremadura, 06006 Badajoz, Spain.

This study was intended to evaluate the effect of melatonin on the cytotoxic and pro-apoptotic actions of various chemotherapeutic agents in cervical cancer HeLa cells.

MTT assay was used to evaluate cell cytotoxicity and caspase-3 activity was determined from the cleavage of its specific fluorogenic substrate. Apoptotic cell death (redistribution of phosphatidylserine in the presence of propidium iodide), activation of caspase-9, and production of reactive oxygen species (ROS) were determined by flow cytometry. Finally, DNA fragmentation was qualitatively analyzed by agarose gel electrophoresis.

We found that both treatment for 24 h with melatonin (1 mM) or three of the chemotherapeutic drugs tested, namely cisplatin (CIS, 20 μ M), 5-fluorouracil (5-FU, 1 mM), and doxorubicin (20 μ M), induced a decrease in HeLa cell viability. Furthermore, melatonin significantly increased the cytotoxic effect of such chemotherapeutic agents. Consistently, co-stimulation of HeLa cells with any chemotherapeutic agent in the presence of melatonin further increased caspase-3 activation, particularly in CIS- and 5-FU-challenged cells. Likewise, concomitant treatments with melatonin and CIS significantly enhanced the ratio of cells entering mitochondrial apoptosis due to reactive oxygen species (ROS) overproduction, substantially augmented the population of apoptotic cells, and markedly enlarged DNA fragmentation compared to the treatments with CIS alone. Nonetheless, melatonin only displayed

moderate chemosensitizing effects in 5-FU-stimulated HeLa cells, as suggested by slight increments in the percentage of cells stimulated for ROS production and in the proportion of early apoptotic cells compared to the treatments with 5-FU alone.

Our findings provided evidence that in vitro melatonin strongly enhances CIS-induced cytotoxicity and apoptosis in HeLa cells and, hence, the indoleamine could be potentially applied to cervical cancer treatment as a powerful synergistic agent.

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▶ **Poster No. 18**

Effect of Heat Shock Proteins (HSP) on cell viability and caspases activation in human epitheloid cervix carcinoma HeLa cells

Ishak Suat Övey¹, Roberto Pariente², Ignacio Bejarano², Ana Beatriz Rodríguez², Mustafa Naziroglu¹, José Antonio Pariente²

¹Department of Neuroscience Research Centre (NOROBAM), Medical Faculty, Suleyman Demirel University, TR-32260, Isparta (Turkey) and ²Department of Physiology, Neuroimmunophysiology and Chrononutrition Research Group, University of Extremadura, 06006-Badajoz (Spain).

In the present study, we investigated the effect of heat shock proteins (HSP) on apoptotic cell death on human epitheloid cervix carcinoma HeLa cell line.

Cells were heat shocked (HS) for 1 hour at 42 °C, followed by incubation at 37 °C for 1, 2, 4, 6 and 24 hours. Cell viability was determined using the MTT assay and caspase -3, -8 and -9 enzymatic activities were determined by fluorimetric methods using the specific fluorogenic substrates.

Our results show that the heat shock at 42 °C of HeLa cells produces a decrease in cell viability which was maintained for 24 h. However, when HeLa cells were

pre-incubated with 100 nM geldanamycin (GA) (HSP90 inhibitor), 100 nM 17-(allylamino)-17-demethoxygeldanamycin (17AAG) (HSP90 inhibitor) or 1 μ M pifithrin- μ (PFT- μ) (HSP70 inhibitor) for 24 hours, cell viability was significantly ($p < 0.05$) lower than that induced by heat shock at all times tested. Similar results were obtained when caspase-3, -8 and -9 enzymatic activities were determined, showing that pre-treatments with the three HSP inhibitors increase cell cytotoxicity induced by heat shock.

Our results suggest that HSP evoked by heat shock have protective activities on HeLa cells, and are involved in cell survival. The inhibition of HSP with specific inhibitors induce cell cytotoxicity and activation of caspases.

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▶ Poster No. 19

The investigation of levels serum total sialic acid, high density lipoprotein and paraoxonase activity in sheep naturally infected with foot and mouth disease

Haci Ahmet Deveci¹, Gökhan Nur¹, Abdulsamed Kükürt², Merve Alpay³, Mahmut Karapehlivan²

¹Gaziantep University, Vocational School of Higher Education in Islahiye, Gaziantep

²Kafkas University, Faculty of Veterinary Medicine, Biochemistry Department, Kars

³Düzce University, Faculty of Medicine, Biochemistry Department, Düzce

We aimed to investigate of levels total sialic acid (TSA), high density lipoprotein (HDL) and Paraoxonase (PON1) activity in sheep naturally infected with infected foot and mouth disease.

Zoonotic diseases are major public health problem which transmitted from animals to humans. FMD (foot and mouth disease) is a viral, zoonotic and acute

infection that influence all ruminants such as cattle, sheep, and goats. Under normal conditions, there is a balance between antioxidant system and reactive oxygen species. Oxidative stress is known as imbalance when the increment of oxidants. This stress is a part of molecular mechanisms on cellular and tissue damage at diseases. Oxidative stress may be an important initiator of the infection in animals by reducing antioxidants that are required for an active immune response.

Our research material were provided from Gaziantep-Islahiye city, were designed with 30 sheep (15 healthy and 15 infected) which were between 2-4 age. Clinical symptoms such as high fever, high amount of saliva, vesicles in tongue in mouth and nail were detected in FMD infected animals. The blood samples were taken from V.jugularis in animals, then centrifuged for serum separation. At the end of the study, PON1 activity, levels of TSA and HDL in serum were analyzed.

Serum PON1 activity and HDL levels of FMD infected group were significantly lower than control group although total sialic acid levels show opposite higher effect compare to healthy group. This difference observed between healthy and infected sheep were found to be statistically significant ($P < 0.001$).

Keywords: Foot and mouth disease, High density lipoprotein, Oxidative stress, Paraoxonase activity, Sialic acid.

▶ Poster No. 20

Histopathology and biochemical analysis of in the testes puberty period of rats applied with capsaicin

Gökhan Nur¹, H. Ahmet Deveci¹, Mümtaz Nazlı², Hadi Attaran³, Özlem Nur¹, Oğuz Merhan⁴

¹Gaziantep University, Vocational School of Higher Education in Islahiye, Department of Veterinary, Gaziantep.

²Mugla Sıtkı Koçman University, Faculty of Medicine, Department of Histology and Embryology, Mugla

³Gazi University, Faculty of Pharmacy, Department of Toxicology, Ankara.

⁴Kafkas University, Faculty of Veterinary Medicine, Department of Biochemistry, Kars.

In this study, biochemical and histopathological changes in the capsaicin treated rat testes which were in their puberty phase were examined. The study consisted of 3 groups, Capsaicin (CAP), Sham and Control, where there were 10 rats per group; in total 30 Sprague-Dawley rats were used.

The CAP group rats were injected subcutaneously with 1 mg / kg dose of capsaicin in 10% ethanol which was dissolved in distilled water after the addition of 1% Tween 20. The Sham group was subcutaneously injected with 0.3cc solution consisting of 10% ethanol, 1% Tween 20 and distilled water which acted as a carrier, whereas the Control group did not receive any injections.

The rats in CAP and sham group were injected for one week and received no injections for the following week. Thereafter, all the rats in the group were sacrificed by cervical dislocation under diethyl ether anesthesia, and testes were taken. Triple staining method for histological staining on sections obtained from testis was used. Testicular histologic sections are evaluated in terms of appearance. No changes had been observed in connective tissues and seminiferous tubules in sham and control groups. In the CAP group gaps between the tubular cell layers, invagination in the tubular cell wall tubular degradation to the integrity and fragmentation in the connective tissues was observed. The level of malondialdehyde in the testicular tissues of capsaicin group was increasing, on the other hand, the levels of glutathione and nitric oxide decreased. In conclusion, this study has led to histopathological changes in the applied dose of capsaicin on the testicular tissue and biochemical parameters were observed caused toxic effects occur when considering.

Keywords: Capsaicin, Testes, Histopatology, Malondialdehyde, Glutathione, Nitric oxide

▶ Poster No. 21

Tetramethrin induce total oxidant /total antioxidant activity and sialic acid levels on

hepatic histopathology formation aganist to protective effect of caffeic acid phenethyl ester in mice

Gökhan Nur¹, H. Ahmet Deveci¹, Abdülsamed Kükürt², Özlem Nur¹

¹Gaziantep University, Vocational School of Higher Education in Islahiye, Department of Veterinary, Gaziantep.

²Kafkas University, Faculty of Veterinary Medicine, Department of Biochemistry, Kars.

Tetramethrin is a synthetic pyrethroid, known as an insecticide used to target insects. In this study, effects of tetramethrin combinations with sialic acid levels and antioxidant activities on liver histopathological changes against mice groups in different conditions were planned to evaluate.

Thirty Swiss albino mice (*Mus musculus*) were used for the study. Tetramethrin 10 mM in DMSO dissolved in 10 mm/kg was injected intraperitoneally tetramethrin group I. The group II was designed by administration 10 µM/kg caffeic acid phenethyl ester (CAPE) dissolved in 1% ethanol then 10 mm/kg tetramethrin dissolved in DMSO were intraperitoneally given to the animals. No injection applied to the control group. Blood samples from all experimental groups were taken at the end of the experiments. Total oxidant, total antioxidant and sialic acid levels were measured for obtained blood serum samples. Also, liver tissue samples were taken for histological examination under light microscope.

As a result, we found extensive necrosis areas based on liver degeneration in tetramethrin group but the degenerations were reduced in CAPE experimental group. However, total oxidant and sialic acid levels were significantly increased while total antioxidant levels were decreased in tetramethrin group.

In conclusion, tetramethrin has dose-dependent genotoxic effects in liver tissue although CAPE has limited protective effect. CAPE could be used for treatment against to tetramethrine-induced oxidative toxicity.

Key words: CAPE, Sialic acid, Total oxidant level, Total antioxidant level, Tetramethrin

The effect of electromagnetic field generated by the high voltage power line on serum paraoxanase (PON1) levels in wistar albino male rats

Mehmet Cihan Yavaş¹, İbrahim Kaplan²

Dicle University, Medical Faculty, Dept of Biophysics¹,
Dept of Biochemistry², 21280, Diyarbakır, TURKEY

In this study male wistar albino rats were given high voltage. In doing so, we aimed to research the effect of the extremely low frequency-electromagnetic field (ELF-EMF) generated by the high voltage power (HV) line on the serum paraoxanase (PON1) levels. Recent studies on the subject report that reactive oxygen species might be active in the mechanisms of ELF-EMF effect (1). Paraoxanase, high-density lipoprotein and serum total antioxidant capacity parallel alterations reflect their similar functions as measurement of antioxidant system (2).

The study included 24 adult male wistar albino rats, weighing 320 grams on average. They were randomly grouped into 3 as n:8. Group 1: Sham-control, Group 2: 26 days HV and Group 3: 52 days HV. A high voltage (10kV) experiment mechanism was set up (electrical field 80,3 V/m, magnetic field intensity 2,48 µT). The electromagnetic field was measured with the aid of a Spectran device NF5035, with reference to the method of 6-minute measurement. PON1 blood serum levels of the rats (U/L) were measured spectrophotometrically (3).

In the final phase of the study the average PON1 serum levels of the experiment and the control groups were compared. A primary finding of our study, which involved exposure to high voltage, shows that the PON1 levels of the 26-day and 52-day application groups decreased compared to the control groups, which resulted in a significant change between the groups (p<0,01). As a corollary, our study suggests that the exposure to ELF-EMF generated by the high voltage line may cause a reduction in the exposure of PON1 activity. Such kind of a change may be related with the

increase that the electromagnetic field caused in oxidative stress. We've noticed that short-term exposures, compared to long-term ones, may lead to lower PON1 activity. Therefore, our study also points to the importance of further research in this regard.

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The effects of melatonin application on oxidative stress and cognitive functions in experimental epilepsy model

Ferhat Şirinyıldız¹, Gökhan Cesur¹, Yüksel Yıldız¹,
Özlem Bozkurt², Cenk Orak¹, Gül Taşlı Yeşilçayır¹,
Rauf Onur Ek¹

¹Adnan Menderes University, Faculty of Medicine,
Department of Physiology, Aydın, Turkey

²Adnan Menderes University, Faculty of Medicine,
Department of Biophysics, Aydın, Turkey

Epilepsy is defined as temporary symptoms caused by the abnormal, excessive and synchronous activity of the brain (1). *Pentylentetrazole* (PTZ) is an agent that affects on reticular formation neurons and cortex neurons to initiate tonic-clonic epileptic seizures (2). The purpose of our study is to define dose dependent effects of *Melatonin* (MEL) against cerebral oxidative stress and cognitive functions in epileptic rats.

12-14 week-aged 40 male Wistar Albino rats were divided into 4 groups (n=10); Control Group (C), PTZ Group (PG, Epilepsy, 35mg/kg(i.p.)), M25 (Treatment, 35mg/kg(i.p.) PTZ+25mg/kg(i.p.) MEL), M100 (Treatment, 35mg/kg(i.p.)PTZ+100mg/kg(i.p.)MEL). Epileptic score determination (ESD) (3), cerebral total oxidant capacity, t maze open field escape and t maze food finding tests were performed and data was evaluated statistically.

Totally 12 injections were administered in 23 days. On injection days, ESD was performed. T maze open field escape and t maze food finding durations were tested on the 21th day. There were significant differences in intragroup and intergroup measurements of ESD ($p<0,05$). Significant scores were found for t maze open field escape test between the groups ($p<0,05$). But there was no significant difference in between groups for t maze food finding test ($p>0,05$). Total oxidant status in cerebrum samples were significantly different upon administration of different doses of MEL ($p<0,05$).

Melatonin has a dose depended therapeutic effect on epileptic seizure scores and total oxidant status induced by PTZ. Furthermore, melatonin has positive effects on cognitive functions revealed by t maze open field escape test.

Keywords: Pentylentetrazole, Epilepsy, Melatonin, Rat

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▶ Poster No. 24

Protective effects of 20(S)-Ginsenoside Rg3 in diabetic neuropathy in rat sciatic nerve

Serap Oktay¹, Ozlem Bozkurt^{1,2}, Mehmet Dincer Bilgin^{1,2}

¹Department of Biophysics, Institute of Health Sciences, Adnan Menderes University, Aydın, Turkey;

²Department of Biophysics, Medical Faculty, Adnan Menderes University, Aydın, Turkey.

Diabetic neuropathy, the most important chronic complication of diabetes, affects the patient's life quality and mortality and does not have a known effective treatment (1). 20 (S) ginsenoside Rg3, an active ingredient of ginseng saponins, has been reported to enhance insulin secretion and to have protective effects on diabetic renal damage (2). This study aims to evaluate the neuroprotective effects of 20(S)-Rg3 on experimental diabetic neuropathy.

Adult male Wistar rats were randomly assigned for control, untreated diabetic and 20(S)-Rg3 treated diabetic groups. Diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg), and controlled by the presence of hyperglycemia (>300 mg/dl). Rats in 20(S)-Rg3 treated group received a 5mg/kg/day dose of 20(S)-Rg3 by oral gavage for 5 weeks. Tail flick and hot plate tests were conducted to record nociceptive changes at 3rd and 4th week. At the end of the treatment, nerve conduction velocities (NCV) and distal latencies were determined.

20(S)-Rg3 treatment restored the decrement in body weight in diabetic rats. Moreover, ginsenoside treatment significantly reduced plasma glucose levels. Also 20(S)-Rg3 treatment decreased both tail flick and hot plate latencies significantly in diabetic rats, revealing its protection against nociceptive behavior. Diabetic rats showed a significant decrease ($p<0.001$) in nerve conduction velocities and distal latencies as compared to the control rats. However, nerve conduction velocities and distal latencies were improved by the treatment of 20(S)-Rg3.

The findings highlighted the possible neuroprotective effects of 20(S)-Rg3 treatment on diabetic neuropathy.

Keywords: 20(S)-Ginsenoside Rg3, diabetic neuropathy, nerve conduction velocity, hot plate test, tail flick test.

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► Poster No. 25

Comparison of protective effects of melatonin and amifostine in brain damage due to gamma radiation

Suat Çakına¹, Tefik Gülyaşar², Alaattin Özen³, Şule Parlar⁴, Ziya Çukur⁵, Nükhet Kürkcü⁴, Cem Uzal⁴, Seralp Şener³

¹Health Services Vocational School, Canakkale Onsekiz Mart University, Canakkale, Turkey

²Department of Biophysics, Faculty of Medicine, Trakya University, Edirne, Turkey

³Department of Radiation Oncology, Faculty of Medicine, Eskişehir Osmangazi University, Eskişehir, Turkey

⁴Department of Department of Radiation Oncology, Faculty of Medicine, Trakya University, Edirne, Turkey

⁵Experimental Animal Unit, Trakya University, Edirne, Turkey

Melatonin, reaching to its highest concentrations in the nucleus of the cell, protects DNA in the nucleus from free radical damage induced by ionizing radiation (1). Amifostine (WR-2721, Ethyol) is rapidly converted *in vivo* to the active free thiol and inorganic phosphate by vascular endothelial cell alkaline phosphatase. The free thiol, which is preferentially taken up by normal tissues relative to tumor tissues, is a potent scavenger of oxygen-free radicals resulting from irradiation, thereby preventing the formation of harmful hydroperoxide radicals that damage DNA and increase risk of cell death (2).

The purpose of this study was to investigate the protective effects of melatonin and amifostine against organ damage induced by whole-body irradiation in rats.

Fifty rats were divided into five groups: control,

radiotherapy alone, radiotherapy+amifostine, radiotherapy+melatonin, radiotherapy+ amifostine+ melatonin. Intraperitoneal amifostine (200 mg/kg) and intraperitoneal melatonin (10 mg/kg) was administered to 30 minutes before irradiation. Rats were irradiated with a single dose of 8 Gy on whole body. 72 hour after exposure to irradiation the animals were decapitated. Total antioxidant capacity (TAC) and total oxidant status (TOS) were determined in the serum by spectrophotometric method using a commercial kit. Oxidative stress index (OSI) was found with the ratio of TOS to TAC.

Addition of amifostine and/or melatonin to radiotherapy increased TAC and TOS significantly ($p<0.05$). However, the TOS level is very small increase and OSI was no statistically in Group 5 (radiotherapy+amifostine+melatonin).

We suggest that amifostine+melatonin have some protective effects in brain tissues against whole body irradiation.

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► Poster No. 26

miRNA-521 expression levels and manganese superoxide dismutase activities to response ionizing radiation in patients with prostate cancer

Selim Öğüt^{1,2}, Birsen Aydemir¹, F. Behice Cinemre³, Hakan Cinemre⁴, Didem Karaçetin^{2,5}, Haldun Şükrü Erkal⁶, Yağmur Karakoç²

¹Department of Biophysics, Faculty of Medicine, Sakarya University, Sakarya, Turkey

²Department of Radiotherapy, School of Health, Istanbul Gelişim University, Istanbul, Turkey

³Department of Biochemistry, Faculty of Medicine, Sakarya University, Sakarya, Turkey

⁴Department of Internal Medicine, Faculty of Medicine, Sakarya University, Sakarya, Turkey

⁵Istanbul Education and Research Hospital, Radiation Oncology Clinic, Istanbul, Turkey

⁶Departments of Radiation Oncology, Faculty of Medicine, Sakarya University, Sakarya, Turkey

MicroRNAs (miRNA) are short noncoding RNA that regulate many cellular pathways. miRNAs primarily bind to the 3'UTR of an mRNA and either represses its translation or may result in the degradation of the mRNA. A single miRNA may have several mRNA targets (1-3). In recent studies it has been investigated the role of miRNAs in radiation response in prostate cancer cell lines. However, the role of miRNA, and how miRNAs integrate into the radiation signaling pathways is largely unknown. Manganese superoxide dismutase (MnSOD) is a mitochondrial anti-apoptotic and antioxidant enzyme that dismutates superoxide radicals. Increased activities of MnSOD are known to protect against radiation induced oxidative damage. It is known that MnSOD may be an indirect target of miR-521 (4). miR-521 plays an important role in radiation response by altering the levels of anti-oxidant protein, MnSOD in prostate cancer cells. The aim of this study was to determine the MnSOD activities and miRNA-521 expression levels in the patients with prostate cancer before and after radiation therapy. Thirty-two patients who underwent external beam radiotherapy for prostate cancer were conducted in this study. Blood samples were taken on admission before initiation of radiation therapy and after completion of radiation therapy. Circulating levels of miRNA-521 expression were analyzed by quantitative reverse transcription-PCR. MnSOD activities were measured by enzyme-linked immunoassay. In 10 of 32 patients, miRNA-521 expression levels were decreased (31%) while MnSOD activities were increased after radiation therapy of prostate cancer patients. This study showed that miRNA-521 may have an important role in efficiency of prostate cancer radiotherapy.

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► Poster No. 27

Manganese superoxide dismutase activities and circulating levels of zinc, magnesium, calcium, iron in patients with prostate cancer before and after radiation therapy

Selim Ögüt^{1,2}, Birsen Aydemir¹, F. Behice Cinemre³, Hakan Cinemre⁴, Nazlı Eyvan Topçu¹, Didem Karaçetin^{2,5}, Haldun Şükrü Erkal⁶

¹Department of Biophysics, Faculty of Medicine, Sakarya University, Sakarya, Turkey

²Department of Radiotherapy, School of Health, Istanbul Gelişim University, Istanbul, Turkey

³Department of Biochemistry, Faculty of Medicine, Sakarya University, Sakarya, Turkey

⁴Department of Internal Medicine, Faculty of Medicine, Sakarya University, Sakarya, Turkey

⁵Istanbul Education and Research Hospital, Radiation Oncology Clinic, Istanbul, Turkey

⁶Departments of Radiation Oncology, Faculty of Medicine, Sakarya University, Sakarya, Turkey

Trace elements play an important role in the maintenance of genome stability in human body. Many endogenous antioxidant enzymes contain trace elements including superoxide dismutase, catalase, glutathione peroxidase. These enzymes have roles in the detoxification of reactive oxidative species induced by

ionizing radiation in the cells (1-3). Zinc, copper, manganese, and iron are main trace elements that have protective roles against radiation-induced DNA damage (4). The purpose of this study was to examine the manganese superoxide dismutase (MnSOD) activities and zinc, calcium, magnesium and iron levels in patients with prostate cancer before and after radiation therapy. Thirty patients who underwent external beam radiotherapy for prostate cancer was conducted in this study. Blood samples were taken on admission before initiation of radiation therapy and after completion of radiation therapy. Circulating activities of MnSOD and element levels were measured by enzyme-linked immunoassay and colorimetric methods, respectively. MnSOD activities were found to be higher in the after radiotherapy patients group when compared to before radiotherapy patient group. Additionally, no significant differences were found between the groups as zinc, calcium, magnesium and iron levels were taken into consideration. In this study we showed that the levels of MnSOD were increased after radiotherapy of cancer patients and it may have protective role against side effects induced by ionizing radiation.

This work was supported by the Research Fund of the University of Istanbul Gelişim.

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► Poster No. 28

The role of circulating selenium and selenoprotein-P (SeP) levels in gestational diabetes mellitus

Ayşe Yücel¹, Elif Erdoğ an¹, Birsen Aydemir¹, F. Behice Cinemre², Hakan Cinemre³, Tevfik Gülyaş ar⁴, Mustafa

Yıldız⁴, Abdullah Tüten⁵, Ali Rıza Kızıler⁶, Nevin Yılmaz⁵

¹Department of Biophysics, Medical Faculty, Sakarya University, Sakarya, Turkey

²Department of Biochemistry, Medical Faculty, Sakarya University, Sakarya, Turkey

³Department of Internal Medicine, Medical Faculty, Sakarya University, Sakarya, Turkey

⁴Department of Biophysics, Medical Faculty, Trakya University, Edirne, Turkey

⁵Department of Obstetrics and Gynecology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

⁶Department of Biophysics, Medical Faculty, Namık Kemal University, Tekirdağ, Turkey

It is known that there is a relationship between dysregulation of Selenoprotein P(SeP) synthesis and type 2 diabetes due to formation of insulin resistance. SeP has antioxidant and metabolic functions in the body(1-2). Recently, it has been demonstrated that SeP played an important role in glucose metabolism and the regulation of insulin sensitivity as a new hepatokine (1). Selenoprotein P is a major selenoprotein in plasma, containing at least 40% of the total plasma Se. Unlike any other selenoprotein, human selenoprotein P contains ten selenocysteine residues and it is believed to be responsible for the distribution of Se to body tissues. It is also thought to be a scavenger of peroxynitrite (3). Se serves many important functions in the human body. The key one is its antioxidative function. The protective role of selenium results from its presence in glutathione peroxidase (GPx) and thioredoxin reductase (TrxRs), namely in the active center of antioxidative enzymes (4). In this study we aim to investigate a relationship between SeP and selenium(Se) in gestational diabetes. 66 gestational diabetic pregnant and 50 healthy pregnant women were studied. Serum SeP levels were measured by ELISA assay. Selenium levels were measured by graphite furnace atomic absorption spectrometry. SeP, HbA1c and fasting blood glucose levels were higher in gestational diabetic pregnant compared to healthy pregnant women. Additionally, no significant differences were found between two groups as selenium levels were taken into consideration. As a conclusion, the increasing serum SeP levels might be an independent factor for the deterioration of glucose metabolism in gestational diabetes.

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▶ Poster No. 29

The importance of circulating selenium and selenoprotein-W (SeW) levels in the pathogenesis of preeclampsia

Elif Erdoğan¹, Birsen Aydemir¹, F. Behice Cinemre², Hakan Cinemre³, Tevfik Gülyaşar⁴, Mustafa Yıldız⁴, Abdullah Tüten⁵, Ali Rıza Kızıler⁶, Nevin Yılmaz⁵

¹Department of Biophysics, Medical Faculty, Sakarya University, Sakarya, Turkey

²Department of Biochemistry, Medical Faculty, Sakarya University, Sakarya, Turkey

³Department of Internal Medicine, Medical Faculty, Sakarya University, Sakarya, Turkey

⁴Department of Biophysics, Medical Faculty, Trakya University, Edirne, Turkey

⁵Department of Obstetrics and Gynecology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

⁶Department of Biophysics, Medical Faculty, Namık Kemal University, Tekirdağ, Turkey

Preeclampsia is a disease characterized by hypertension, proteinuria and edema during pregnancy. It causes intrauterine growth retardation, premature birth, fetal and maternal mortality. The selenium takes place in the structure of selenoproteins which are mostly showing oxidoreductase activities in human. This element, as a constituent of selenoproteins, activates anti-carcinogenic factors, prevents diseases of the cardiovascular systems as well as exhibits anti-proliferative and anti-inflammatory activities (1). Some studies were reported that selenoproteins W (SeW) was

associated with fetal development (2-4). SeW expression in the fetal muscle and heart tissue depends on fetal selenium levels. Although, SeW function is not completely elucidated, it may be related with oxidoreduction reactions. Our aim was to compare levels of serum SeW and selenium (Se) between preeclamptic and healthy pregnant women. 60 patients presented to Obstetrics and Gynecology outpatient clinic for following of preeclampsia has been studied. Serum SeW levels were measured by using ELISA assay. Selenium levels were measured in all cases by graphite furnace atomic absorption spectrometry. We found that SeW, fetal weight, total protein and albumin levels significantly reduced in the preeclamptic pregnant compared to the healthy pregnant ($p < 0.01$, for each). Systolic and diastolic blood pressure, and selenium levels were significantly increased in preeclamptic patients when compared to healthy control group ($p < 0.001$, for each). We concluded that the altered levels of selenium and SeW in preeclampsia may play a role in the occurrence of fetal and maternal complications.

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▶ Poster No. 30

Effects of sonodynamic therapy on prostate cancer cell line

Mehran Aksel^{1,2}, Özlem Bozkurt², Esin H. Değirmenci³, Ali Özmen⁴, Mehmet Dinçer Bilgin²

¹Institute of Health Sciences, Adnan Menderes University, Aydın, Turkey.

²Department of Biophysics, Medical Faculty, Adnan Menderes University, Aydın, Turkey.

³Department of Biology, Institute of Natural and Applied Sciences, Adnan Menderes University, Aydin, Turkey.

⁴Department of Biology, Faculty of Arts and Sciences, Adnan Menderes University, Aydin, Turkey.

Sonodynamic therapy is a new modality on cancer treatment that uses synergetic effect of low intensity of ultrasound and a chemical agent called sonosensitizer for the induction of cell death in malign tumors (1). The mechanism of sonodynamic therapy is based on the activation of reactive oxygen species and thus eliciting oxidative stress which preferentially induces apoptosis in malignant cells. The aim of this study is to assess the effectiveness of sonodynamic therapy on prostate cancer cell lines.

We performed an *in vitro* study using PC3 and LNCaP cells, which are most commonly used prostate cancer cell lines. The cells were divided into control group, only ultrasound subjected group, only sonosensitizer subjected group and sonodynamic therapy group (subjected to both sonosensitizer and ultrasound). Methylene blue and aluminium (III) phthalocyanine tetrasulfonate were used as sonosensitizers (2, 3).

The cytotoxic effect of sonodynamic therapy was determined by MTT assay which revealed that the sonodynamic therapy enhanced the loss of viable cells in prostate cancer cell lines compared with ultrasound and drug alone groups. Propidium iodide/hoechst33342 staining showed that prostate cancer cells with apoptotic morphological characteristics were significantly increased in sonodynamic therapy groups. The generation of intracellular reactive oxygen species was also confirmed by using muse analyzer. The results indicated that sonodynamic therapy significantly inhibited tumor growth and induce an increase in intracellular reactive oxygen species, resulting in cellular apoptosis on prostate cancer cell lines.

These findings support that sonodynamic therapy offers a new approach to the treatment of prostate cancer.

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▶ Poster No. 31

Effect of *Leontice leontopetalum* extract on cell proliferation in diabetes-induced pancreatic beta cell line

Celal Guven¹, Eylem Taskin Guven², Mufide Ahbab³, Leyla Sener⁴, Önder Yumrutaş⁵, Neslihan Abacı⁶, Mustafa Pehlivan⁷, Handan Akcakaya⁴

¹The Department of Biophysics, Faculty of Medicine, University of Adiyaman, Adiyaman-TURKEY

²The Department of Physiotherapy and Rehabilitation, School of Health Sciences, Istanbul Bilim University, Istanbul-TURKEY

³The Department of Biology, Faculty of Science and Literature, Hacettepe University

⁴The Department of Biophysics, Faculty of Medicine, University of Istanbul, Istanbul-TURKEY

⁵Department of Medical Biology, Faculty of Medicine, Adiyaman University, Adiyaman- TURKEY

⁶Department of Genetics Institute for Experimental Medicine (DETAE), İstanbul University; İstanbul-Turkey- TURKEY

⁷Department of Hematology, Gaziantep University, Gaziantep- TURKEY

Diabetes is a defect in glucose metabolism due to insulin deficiency. Insulin-dependent diabetes in the other word, type-1 is tried recovery the pathologic effect and complication related with diabetes by drugs. But, the synthetic drugs have been reported to have side effects on the body (1). One of the alternative therapeutic methods is herbal medicine. *Leontice leontopetalum* (LL) belongs to Berberidaceae, and its extract has been shown to have antiepileptic and antispasm (2). But, there are no studies on the effect of LL on diabetes. The aim of the study was to investigate of the proliferative effect of LL's extract on β -cell-treated with STZ.

Human pancreatic beta cell (1.1B4) line was used the current study. LL's extracts (1, 10, 100, and 1000 µg/ml) were supplemented in media for 24 hours and/or after STZ treatment (10 mM). So, totally four groups were created as Control, STZ, *L. leontopetalum*'s extract and STZ+LL. Cells proliferation were shown by using xCelligence. Insulin content was measured at 1.1, 8.4 and 16.7 mM glucose concentrations.

All LL's concentration caused to decrease cell proliferation. But, 10 mM STZ was not significantly affected by cell proliferation. STZ+LL group also gave rise to decline cell proliferation. STZ and STZ+LL extract led to decrease insulin content at all glucose concentrations.

STZ caused diabetes by reducing cell proliferation and insulin content, unfortunately *L. leontopetalum* has no protective effect on diabetes. The project was financially supported by the Research Foundation of Adiyaman University (TIPFBAP/2014-0006).

Keywords: Diabetes, Leontice leontopetalum, Insulin, Cell Proliferation

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► Poster No. 32

Effects of N-acetylcysteine treatment on CoCl₂-induced apoptosis and oxidative stress levels in SH-SY5Y neuroblastoma cells

Ahmi Öz¹, Ramazan Çınar¹, Ömer Çelik¹, A. Cihangir Uğuz¹, Mustafa Nazıroğlu¹

Department of Biophysics, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

Hypoxia and ischemia directly affect the cells and make

more tendence to reactive oxygen species (ROS) production triggered cell death. Cobalt chloride (CoCl₂) is a mimic of hypoxia model in cell culture experiments. SH-SY5Y neuroblastoma cells, a secondary cell line which widely using as *in vitro* model of Alzheimer's and Parkinson's diseases. N-acetylcysteine (NAC) is a powerful antioxidant because of functional cysteine groups in its chemical structure, soluble in liquid phase and revealed that has promoter effect on intracellular reduced glutathione (GSH) levels. Hence, we aimed to investigate possible regulator effects of NAC administration on CoCl₂ induced and ROS triggered apoptosis processes in neuronal like SH-SY5Y cells. For the aim, we performed apoptosis, intracellular ROS production and mitochondrial depolarization assays, caspase-3 and -9 activity analysis, GSH, glutathione peroxidase (GSH-Px) and lipid peroxidation (LP) analyses in control, CoCl₂, pre- and post-NAC treated groups.

We observed that CoCl₂ incubation increased apoptosis, MDA and intracellular ROS production levels, but decreased cell viability (MTT), GSH and GSH-Px values. Although both NAC treated groups show positive effect on intracellular antioxidant redox system it has not been found any correlation between NAC treatment and either increasing of cell viability or decreasing of apoptosis. In conclusion, CoCl₂ causes elevation in ROS production, pre-NAC treatment seems to have more beneficial effects on intracellular antioxidant system in SH-SY5Y cells in compared with post-NAC treatment.

Keywords: N-acetylcysteine; Apoptosis; Oxidative stress; SH-SY5Y neuroblastoma cells

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Effects of argan oil on mitochondrial oxidative stress and functional parameters in acrylamide treated rats liver

Rahime Er and Birsen Aydın

Department of Biology, Science and Art Faculty, Amasya University, Amasya, Turkey.

Argan oil (AO), which is extracted from argan almonds, is well known for its cosmetic, pharmaceutical and nutritional virtues. Cardioprotective effects and managing dyslipidemia due to its high unsaturated fat composition and antioxidant compounds were demonstrated in different studies, however mitochondria mediated hepatoprotective effect is still unclear.

Acrylamide (ACR) is a chemical frequently used in both industrial and synthetic processes and may be produced during food processing. Female Sprague Dawley rats were exposed to ACR (50 mg/kg i.p), argan oil (6 ml/kg o.p) and ACR along with AO for three times a week during 30 days. Mitochondrial superoxide dismutase (Mn-SOD) and glutathione peroxidase activities along with reduced glutathione level were decreased, while the lipid peroxidation and protein carbonyl levels were increased in the ACR treated rats. Mitochondrial TCA cycle enzymes (isocitrate dehydrogenase, α -ketoglutarate dehydrogenase), electron transport chain complex activities (NADH dehydrogenase, succinate dehydrogenase, cytochrome oxidase), MTT reduction and ATP level were also decreased after ACR treatment. The administration of ACR along with AO normalised almost all these mitochondrial parameters. The ameliorating effect of argan oil in the present study can be partially correlated to its antioxidant activity. The results of the study concluded that argan oil may be effective to improve the function of mitochondria in acrolein treated rat mitochondria, suggest its possible therapeutic application against mitochondria mediated liver failure.

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The possible role of pentraxin superfamily in patients with major depression

Abdullah Akpınar¹, Bilal Tanrıtanır¹, Kadir Demirci¹, Hikmet Orhan², Suleyman Akif Carsancaklı³

¹Department of Psychiatry, ²Biostatistics, ³Microbiology, Medical Faculty, Suleyman Demirel University, Isparta, Turkey.

Pentraxin-3 (PTX3), serum amyloid p component (SAP), C-reactive protein (CRP), neuropentraxin 1 (NP1) and neuropentraxin 2 (NP2) belong to the pentraxin superfamily (1). Inflammatory and oxidative abnormalities have been found in major depression but pentraxin superfamily has not been studied in this disorder (1,2). The present study aimed to investigate the levels of pentraxin superfamily and the possible association of depressive symptoms in patients with major depression (MD).

The study group included 60 patients with MD and 30 healthy controls. The severity of depressive symptoms was evaluated using the Hamilton's 17-item Depression Rating Scale (HDRS).

The mean levels of NP1 and NP2 in patients with MD were significantly lower than that in controls. There was no significant difference in the levels of PTX3 and SAP. PTX3 level significantly correlated with the HDRS-2

(feeling of guilt) subscale score. *SAP* level did significantly correlated with the HDRS-8 (retardation). *NP2* level did significantly correlate with the HDRS-14 (genital symptoms).

The main results of this study *NP1* and *NP2* levels were lower in MD. These molecules were found to be involved for neurogenesis (neuromodulation, synaptogenesis and synaptic plasticity) and neurotoxicity (Alzheimer's disease, in hypoxic-ischemic brain damage) in preclinical and clinic studies (3,4). Low levels of *NP1* and *NP2* levels may indicate insufficient neurogenesis in MD. The establishment of inflammatory abnormalities and oxidative stress in the etiopathogenesis of MD might also provide the rationale for studies of therapies directed at the modulation of this process.

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► Poster No. 35

Oxidative effect of thiacloprid and d-tubocurarine on *Rana ridibunda* gastrocnemius muscle

Yusuf Çamlıca¹, Esra Pekoğlu¹, Serap Yalın²

¹Department of Biology, Faculty of Science and Letters, Mersin University, Mersin, Turkey

²Department of Biochemistry, Faculty of Pharmacy, Mersin University, Mersin, Turkey

Reactive oxygen species (ROS) and reactive nitrogen

species (RNS) increase in vertebrates as a result of in vivo exposure to different pesticides (1). Such mediator reactives are: hydrogen peroxide (H_2O_2) formed by partial reduction of the oxygen; superoxide anion (O_2^-); hydroxyl (OH) radicals and nitric oxide (NO). RNS and ROS can cause damage to biological systems by reacting with, for example, DNA, proteins and cell membranes (2). In this study, the effect of neonicotinoid insecticide thiacloprid and its antagonist d-tubocurarine on the amount of thiobarbituric acid reactive substances and their effects on catalase enzyme activity was investigated in frog gastrocnemius muscle. The isolated gastrocnemius muscle was subjected to four different doses of thiacloprid (1×10^{-3} , 1×10^{-4} , 1×10^{-5} and 1×10^{-6} M) for 120 minutes. The muscles were also treated with a 1×10^{-5} M thiacloprid and 1×10^{-4} M d-tubocurarine mixture, with 1×10^{-6} M thiacloprid and 1×10^{-5} M d-tubocurarine mixture. The muscle tissues in the control group were maintained in Ringer's solution for 120 minutes. Each dose group was studied with an equal number of preparations ($n = 5$). Based on the research results, it was determined that 1×10^{-3} , 1×10^{-4} , 1×10^{-5} , 1×10^{-6} M thiacloprid and the mixture of 1×10^{-6} M thiacloprid and 1×10^{-5} M d-tubocurarine decreased the amount of thiobarbituric acid reactive substances of the striated muscle tissue compared to the control group ($P < 0.05$). 1×10^{-3} ($P < 0.001$) and 1×10^{-4} M thiacloprid ($P < 0.05$) decreased the catalase enzyme activity in muscle tissue. This study provides important data for the potential of thiacloprid and d-tubocurarine creating oxidative stress in skeletal muscle, for assessing the possible effects on non-target organisms and for assessing their environmental risks.

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► Poster No. 36

Oxidative effect of acetamiprid and d-tubocurarine on frog nerve tissue

Yusuf Çamlıca¹, Salih Cüfer Bediz¹, Serap Yalın²

¹Department of Biology, Faculty of Science and Letters, Mersin University, Mersin, Turkey

²Department of Biochemistry, Faculty of Pharmacy, Mersin University, Mersin, Turkey

Target region of neurotoxic insecticides are the enzymes in the insect nervous system, ion channels or ion receptors (1). Insecticides have different effects on the target areas and they exhibit considerable diversity in terms of enzyme inhibition, agonist or antagonist effect on receptor and ion channel modulation (2). The aim of this study is to investigate the toxic effects of acetamiprid and d-tubocurarine, on the sciatic nerve of *Rana ridibunda* by using biochemical methods. Frog sciatic nerves were isolated after making them spinal. Four different concentrations of acetamiprid solution (1×10^{-3} , 1×10^{-4} , 1×10^{-5} and 1×10^{-6} M) were applied on sciatic nerves for 120 minutes. In addition, the sciatic nerve tissues were maintained for 120 minutes in the mixture of 1×10^{-3} M acetamiprid and 1×10^{-2} M d-tubocurarine, the mixture of 1×10^{-5} M acetamiprid and 1×10^{-4} M d-tubocurarine, the mixture of 1×10^{-6} M acetamiprid and 1×10^{-5} M d-tubocurarine. Nerve tissues in the control group were maintained in Ringer's solution for 120 minutes. The agonist and antagonist effects were studied in the experimental group an equal number of subjects ($n = 5$). The results of the colorimetric analysis revealed that application of 1×10^{-3} M acetamiprid on sciatic nerve significantly reduced the catalase activity and the level of acetylcholinesterase compared to that of control group. In contrast, it was determined that, the same dose of the insecticide significantly increased the level of malondialdehyde on nerve tissue compared to that of control group. The significant reduction of catalase and acetylcholinesterase in the sciatic nerve tissue due to acetamiprid while causing significant increase of malondialdehyde indicates that this insecticide, depending upon the oxidative stress, causes damage on peripheral nerves in high doses.

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Poster No. 37

Selenium attenuates apoptosis, inflammation, and oxidative stress in the blood, and brains of aged rats with scopolamine-induced dementia

Kadir Demirci¹, Mustafa Nazıroğlu^{2, 3}, İshak Suat Övey², Hasan Balaban¹

¹Department of Psychiatry, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

²Department of Neuroscience, Institute of Health Science, Suleyman Demirel University, Isparta, Turkey.

³Neuroscience Research Center, University of Suleyman Demirel, Isparta, Turkey.

Decreased acetyl choline concentrations, increased inflammation, apoptosis, and oxidative stress are implicated in the etiology of Alzheimer's disease (AD). A potent antioxidant, selenium might modulate dementia-induced progression of brain and blood oxidative and apoptotic injuries. The present study explores whether selenium protects against experimental dementia-induced brain, and blood oxidative stress, apoptosis levels, and cytokine production in rats with scopolamine (SCOP)-induced dementia.

Thirty-two rats were equally divided into four groups. The first group was used as an untreated control. The second group was treated with SCOP to induce dementia. The third and fourth groups received 1.5 mg/kg selenium (sodium selenite) and SCOP+selenium, respectively. Dementia was induced in the second and fourth groups by intraperitoneal SCOP (1 mg/kg) administration.

Brain, plasma, and erythrocyte lipid peroxidation levels as well as plasma TNF- α , interleukin (IL)-1 β , and IL-4 levels were high in the SCOP group though they were low in selenium treatments. Selenium and selenium+SCOP treatments increased the lowered glutathione peroxidase activity and reduced glutathione, β -carotene, vitamins A and E concentrations in the brains, erythrocytes and plasmas of the SCOP group. SCOP increased the apoptotic values as active caspase-

3, procaspase-9, and PARP, while selenium and selenium+SCOP treatments decreased them.

In conclusion, selenium induced protective effects against experimental dementia-induced brain, and blood oxidative injuries and apoptosis through regulation of cytokine production, the antioxidant vitamin, glutathione concentrations, and glutathione peroxidase activity.

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► Poster No. 38

Effect of chronic organophosphate toxicity on Na⁺ K⁺ ATPase activity in rat hippocampus and cell numbers in the cornu ammonis

Orhan Başı¹, Ali Aslan², Umut Serkan Söztanacı³, Osman Fikret Sönmez⁴, Hüseyin Avni Uydu⁵, Ersan Odacı⁶

¹Department of Anatomy, Faculty of Medicine, Ordu University, Ordu, Turkey

²Department of Physiology, Faculty of Medicine, Ordu University, Ordu, Turkey

³Department of Anatomy, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey

⁴Department of Neurosurgery, Samsun Training and Research Hospital, Samsun Turkey

⁵Department of Medical Biochemistry, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey

⁶Department of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

Organophosphate compounds are used widely in

farmland. In developing countries, occupational or accidental exposure to organophosphate compounds cause diseases or death. In our study we aimed to determine whether there were changes in the number of pyramidal cells in the hippocampus and to investigate the Na⁺ K⁺ ATPase activity of adult rats with long term exposure to fenthion which is one of the organophosphate compounds.

We used 18 adult wistar albino rats. Rats were randomly divided into 3 groups as experiment, sham and control. The experimental group (n:6) was fed with a normal diet and 30 mg/kg fenthion in 1 ml saline were given by gavage. The sham group (n:6) was fed with a normal diet and 1 ml saline were given by gavage. The control group (n:6) was subjected to normal nutrition. At the end of 30 days the rats were sacrificed. The left hemisphere was cut and stained with cresyl violet. The pyramidal cell numbers in the cornu ammonis region of the hippocampus were calculated. The hippocampus in the right hemisphere was homogenized and centrifuged. The obtained supernatant was investigated for Na⁺ K⁺ ATPase activity.

The pyramidal cell counts in the experimental group were observed to be statistically lower at the compared to the number of granular cells in the sham and control groups. The Na⁺ K⁺ ATPase activities were observed to be high level of significance in the control and sham groups compared to the experimental group.

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► Poster No. 39

Effect of chronic organophosphate poisoning on antioxidant enzyme activity in the rat hippocampus

Orhan Başı¹, Ali Aslan², Umut Serkan Söztanacı³, Osman Fikret Sönmez⁴, Hüseyin Avni Uydu⁵, Ersan Odacı⁶

¹Department of Anatomy, Faculty of Medicine, Ordu University, Ordu, Turkey

²Department of Physiology, Faculty of Medicine, Ordu University, Ordu, Turkey

³Department of Anatomy, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey

⁴Department of Neurosurgery, Samsun Training and Research Hospital, Samsun Turkey

⁵Department of Medical Biochemistry, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey

⁶Department of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

Misuse of organophosphate compounds (OC) is a serious issue for community. High dose exposure of organophosphate is a well-known clinical manifestation. However, chronic exposure of OC causing neurotoxicity but its mechanisms is not understood. OC are specifically used as an insecticide and pesticide. The usage and availability of organophosphates are very common and easy, therefore its poisoning is getting an important health issue in developing countries.

Therefore, we aimed to investigate SOD, CAT, MDA and GR enzyme activities in the adult rats hippocampus, which was exposed to an OC, fenthion.

We used 18 adult wistar albino rats, whose body weights ranging from 270-300 g. Rats were randomly divided into 3 groups as experiment, sham and control. The experimental group (n:6) was fed with a normal diet and 30 mg/kg fenthion in 1 ml saline were given by gavage. The sham group (n:6) was fed with a normal diet and 1 ml saline were given by gavage. The control group (n:6) was subjected to normal nutrition. At the end of 30 days the rats were sacrificed. The right hemisphere of the brains were removed. Dissected hippocampus was homogenized and SOD, CAT, MDA, GR activity were examined.

SOD levels are statistically lower at the experimental group. CAT levels are statistically higher at the control group. MDA levels are statistically higher at the experimental group. GR levels of the control groups are higher than the experimental group.

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Poster No. 40

Investigation of the effect of *Rheum Ribes L.* on oxidative stress in diabetic rats

Metin Yıldırım¹, Mehmet Berköz³, Ülkü Çömelekoğlu², Merih Akkapulu¹, Ali Erdiñç Yalın¹, Serap Yalın¹

¹Mersin University, Faculty of Pharmacy, Department of Biochemistry, Mersin, Turkey

²Mersin University, Faculty of Medicine, Department of Biophysics, Mersin, Turkey

³Yuzuncu Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Van, Turkey

Rheum ribes L., a perennial plant in the family Polygonaceae, has many bioactivities and is often used in traditional therapy. One of its bioactivities is known to be its anti-diabetic effect. The phenolic compound profile of *Rheum ribes L.* and the flavonoid structures that it contains make this plant a potential source of antioxidants. This study aims to examine the effect of *Rheum ribes L.* on the oxidative stress in experimental diabetic rats. In this study, 36 rats were distributed in to 6 different groups. Group I was the control group; Group II is the diabetes group to which was intraperitoneally administered single dose of streptozotocin (40 mg/kg); in Group III rats were made diabetic and given the infusion of *Rheum ribes L.* by gavage for 15 days; in Group IV rats were made diabetic and given the ethanol extract of *Rheum ribes L.* by gavage for 15 days; in Group V rats were not made diabetic but given the infusion of *Rheum ribes L.* by gavage for 15 days; in Group VI rats were not made diabetic and given the ethanol extract of *Rheum ribes L.* by gavage for 15 days. After these practices, experimental animals were sacrificed, and the liver of each animal were taken. These tissues were homogenized and superoxide dismutase, catalase, malondialdehyde and glutathione peroxidase levels were examined on the obtained tissue homogenizers. According to our results, *Rheum ribes L.* was found to

reduce the oxidative stress and increase the levels of the antioxidant enzyme As a result, we suggest that extract of *Rheum Ribes L.*, may be used to treat diabetes.

Keywords: Rheum Ribes L., Antioxidant, Diabetes Mellitus

▶ Poster No. 41

Determination of AChE enzyme activity on brain tissue of Goldfish (*Carassius auratus*) exposed to ultrasonic irradiation

Harika Eylül Esmer¹, Güllü Kaymak², Şeyma Tartar¹, N.Cenk Sesal¹, Figen Esin Kayhan¹

¹Department of Biology, Faculty of Sciences and Arts, University of Marmara, Istanbul 34722, Turkey.

²Department of Biology, Faculty of Science and Arts, University of Sakarya, Sakarya 54187, Turkey.

Water treatment has been recommended as an intervention strategy to reduce microbial risks associated to water. Commercial water treatments mostly depend on chemical agents; although scientists search for environmental friendly alternatives to chemical biocides. Ultrasound usage is classified as a non-chemical strategy to water cleaning. Ultrasound works by the phenomenon of acoustic cavitation which occurs after sound waves above the frequency of 20 kHz. In the present study, ultrasound applied with a horn-type sonicator (UP100H; Hielscher GmbH, Teltow, Germany) operating at a fixed frequency of 30 kHz and a nominal power output up to 100 W was used in 20L aquarium. In our study, ultrasound usage was investigated in cleaning frequency of ultrasoud, effects on brain tissue of Goldfish (*Carassius auratus*). Goldfish were acclimatized for two weeks in stock tanks under laboratory conditions. Tap water free from chlorine was used. During the acclimatization period, fish were fed with pellet twice a day. The 4 experiment study groups were composed as; ultrasound exposed to each group and 5 zebrafish were studied at 1day, 4 days and 7 days of exposed and the last group was composed as the control group. The experiment was repeated 3 times. Oxidative stress determined by AChE activity and spesific activity by Ellman's method. The results

showed us, AChE enzyme activity according to control group; in the first day insignificant increase, in the fourth day significant increase and in the seventh day significant decrease. AChE spesific enzyme activity gave similar results. As a conclusion this study identified that; ultrasonic treatment is efficient. Even though in the first day induced oxidative stress wasn't observed, in the following days enzyme was effected by ultrasonic irradiation.

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Keywords: Ultrasound, Goldfish, Oxidative stress, Brain, AChE

▶ Poster No. 42

Prevent of mitochondrial damage-induced via adriamycin in fibroblast by melatonin

Celal Guven¹, Eylem Taskin Guven², Handan Akcakaya³

¹The Department of Biophysics, Faculty of Medicine, University of Adiyaman, Adiyaman, Turkey

²The Department of Physiotherapy and Rehabilitation, School of Health Sciences, Istanbul Bilim University, Istanbul, Turkey

³The Department of Biophysics, Faculty of Medicine, University of Istanbul, Istanbul, Turkey

Adriamycin (ADR) is a drug for solid tissue cancer patient's treatment. However, it has some side effects on noncancerous tissues. The therapy of its undesirable actions on cytotoxicity is still found. However, its cytotoxicity might relate to increasing reactive oxidant status (ROS), mitochondrial dysfunction. Melatonin (MEL) is reported to have antiapoptotic and antioxidant effects. The aim of this study was to investigate that Melatonin reverses the cytotoxicity of adriamycin through a decline apoptosis. Total four groups were created as a control, MEL, ADR, co-treated MEL with ADR. The level of some proteins including cytochrome-c, mitochondrial membrane potential (MMP) was

measured. Oxidative stress index (OSI) was calculated. Apoptotic cell number was counted by TUNEL. ADR gave rise to elevated OSI. Although ADR initiated to apoptosis by increasing the cytochrome-c level, MEL+ADR was modulated the cytochrome-c level. Although ADR caused to elevate MMP, MEL reversed to ADR's effect on MMP. Cell loss by apoptosis-mediated oxidative stress is the important mechanism of cytotoxicity induced by ADR, and the alternations were attenuated to concomitant MEL with ADR. Consequently, MEL has protective effects on ADR induced cytotoxicity may be a candidate agent to protect the cell in ADR chemotherapy.

Keywords: Adriamycin, Apoptosis, Melatonin, Mitochondrial Membrane Potential, Oxidative stress

► Poster No. 43

The Effect of *Salvia officinalis* on experimental inflammation and antioxidant system

Umut Kerem Kolaç¹, Emre Entok², Derya Üstüner³, Neslihan Tekin⁴, Hilmi Özden⁵, Murat Dinçer⁶, Emine Çolak¹, Mehmet Cengiz Üstüner¹

¹Department of Medical Biology, Medical Faculty, Osmangazi University, Eskişehir, Turkey

²Department of Nuclear Medicine Medical Faculty, Osmangazi University, Eskişehir, Turkey

³Vocational School of Health Services, Osmangazi University, Eskişehir, Turkey

⁴Department of Biotechnology and Molecular Biology, Aksaray University, Aksaray, Turkey

⁵Department of Anatomy, Medical Faculty, Osmangazi University, Eskişehir, Turkey

⁶Department of Medical Oncology, Medical Faculty, Osmangazi University, Eskişehir, Turkey

Inflammation is a complex biological answer playing a critical role in pathogenesis of many diseases. Free radicals and lipid peroxidation products produced during inflammation cause harmful mutations and post translational changes of key proteins. Lipopolysaccharide (LPS) is an endotoxin and located

in outer membrane of gram-negative bacteria. *Salvia officinalis* (Lamiaceae) with its high flavonoid and phenolic acid content is an anti-inflammatory herb having powerful antioxidant activity. The aim of this study was to investigate anti-inflammatory and antioxidant activity of *Salvia officinalis* in acute inflammation. Thus we used experimental lipopolysaccharides (LPS)-induced model. Forty-two female *Wistar albino* rats were divided into 6 groups. *Salvia officinalis* (sage) extract (10mg/kg and 30mg/kg) was orally administrated to rats in order to treat inflammation formed after intraperitoneal LPS (1mg/kg) application. ¹⁸F-fluoro-deoxy-D glucose (0.8 ml/kg) was administrated and lung and liver ¹⁸F-FDG-uptake was calculated. Lung, kidney and erythrocyte superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) enzyme activities and malondialdehyde (MDA) levels were determined. Lung and liver ¹⁸F-FDG uptake were found to be higher in inflammation group than control group. MDA levels in erythrocyte hemolysate, lung and kidney tissues were found to be significantly higher in inflammation group compared to treatment groups, whereas lung and hemolysate SOD activities were determined significantly lower in inflammation group compared to groups treated with *Salvia officinalis*. Likewise CAT and GPx activities in inflammation control group were significantly lower. In conclusion, *Salvia officinalis* was found to be efficient in reducing free oxygen radical production and ameliorating inflammation induced by LPS in rats.

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► Poster No. 44

Effect of the extenders and supplements (Equex-Stm, EDTA and BSA) on oxidative stress parameters of ram semen

Ömer Varışlı¹, Abdullah Taşkın²

¹Department of Reproduction and Artificial Insemination, Kirikkale University, Faculty of Veterinary Medicine, Kirikkale, Turkey.

²Department of Genetics, Harran University, Faculty of Veterinary Medicine, Sanliurfa, Turkey.

The main objective of the current study was to evaluate the effects of extender type and supplements on the oxidative stress parameters of Ram semen.

In this study, ejaculates of 4 Awassi rams were used. Ejaculates were taken by artificial vagina twice a week during breeding season. The ejaculates that have been taken were used. The semen specimens were diluted by Tris, TES, TL-HEPES and DPBS extenders supplemented with different chemicals such as Equex-STM, EDTA and BSA. Oxidative status of sperm samples were assessed by measuring serum lipid hydroperoxide (LOOH) and total oxidant status (TOS). Antioxidative status was assessed by measuring serum free sulfhydryl groups (SH total thiol), ceruloplasmin (CP) and total antioxidant capacity (TAC). Oxidative stress index (OSI) was also calculated. Analyses were performed after dilution.

The TOS level was low in TL-HEPES, TEST and DPBS supplemented with BSA and EDTA. TAS was the same in all groups ($p>0.05$). Ceroplasmin was higher in the groups which supplemented with BSA and EDTA. Lipid peroxidation was lower in the BSA and EDTA supplemented groups. In conclusion, Equex-STM doesn't cause any significant differences in oxidative status. BSA and EDTA showed significant help to stabilize of semen oxidative parameters.

► Poster No. 45

The Effect of pretreatment procedures on the oxidative and antioxidative status of ram semen

Ömer Varışlı¹, Serkan Erat², Abdullah Taşkın³

¹Department of Reproduction and Artificial Insemination, Kirikkale University, Faculty of Veterinary Medicine, Kirikkale, Turkey.

²Department of Animal Breeding and Husbandry, Kirikkale University, Faculty of Veterinary Medicine, Kirikkale, Turkey.

³Department of Genetics, Harran University, Faculty of Veterinary Medicine, Sanliurfa, Turkey.

Small amounts of reactive oxygen species (ROS) in sperm are important for capacitation, hyperactivation and acrosome reaction. But excessive ROS is responsible for mammalian sperm damage. Antioxidants supplementation in sperm extender is widely used and very popular in semen chilling and cryopreserving. But in most studies, oxidative and antioxidative analysis shows quite differences.

The aim of this study was to compare pretreatment protocol of the oxidative stress and antioxidative status of ram semen. For this study, four healthy and fertile rams were used. Sperm samples of the rams were obtained by using an artificial vagina and pooled. The specimens were diluted by semen extender and divided into two parts. The first part (control-A) is directly analyzed. The other semen samples (group B) were transferred into a 2-ml beaker in ice water and sonicated for 10 s and repeated 6 times at intervals of 30 s to separate sperm head and tail. Oxidative status of sperm samples were assessed by measuring serum lipid hydroperoxide (LOOH) and total oxidant status (TOS). Antioxidative status was assessed by measuring serum free sulfhydryl groups (SH total thiol), ceruloplasmin (CP) and total antioxidant capacity (TAC). Oxidative stress index (OSI) was also calculated.

SH, TAS and OSI were significantly different ($p<0.01$) in both groups. SH and TAS levels were significantly higher in group B compared to control group, whereas OSI level was lower in group B. As a result, the pretreatment of semen before analyses can significantly effect results.

► Poster No. 46

Effect of silibinin on the histology of spleen in streptozotocin-induced diabetic rat

Tuğba Semerci Sevimli¹, Murat Sevimli², Nurten Özçelik¹

¹Department of Medical Biology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

²Department of Histology and Embryology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

The spleen is one of the most important lymphoid organs, which has significant roles in cellular and humoral immunity. Diabetes causes some morphological changes and extensive paranchymal fibrosis in the spleen. The mechanism of these changes are related to the increased apoptosis of immune cells due to increased oxidative stress (1). Diabetes also causes significant depletion in the white pulp, dilatation in the blood vessels and collagen depositions in the spleen. (2). Silibinin is a potent flavonoid antioxidant derived from *Silybum Marianum*. Previous studies have demonstrated the antioxidant effects of silibinin on different pathologies and organs (3). The aim of this experimental study was to investigate the effects of silibinin on Streptozotocin (STZ)-induced diabetic rat spleen through histochemical methods. There were five groups in our study as follows; The Control group, Diabetic Group, Treatment Group 1 (diabetic group treated with 100 mg/kg silibinin), Treatment Group 2 (diabetic group treated with 200 mg/kg silibinin), and the Silibinin Group (no diabetes but 5 rats treated with 100mg/kg and 5 rats treated with 200 mg/kg silibinin). STZ was administered at a dose of 65mg/kg through intraperitoneal injection and Silibinin was administered through gastric gavage for 4 weeks. Our study is the first study investigating the effects of silibinin in the diabetic rat spleen. We found that silibinin slightly restores the deterioration in the general architecture of the spleen and reduces paranchymal fibrosis. This effect can be attributed to the antioxidant effects of silibinin, but the molecular mechanism of this effect remains unknown.

Key Words: Diabetes, Silibinin, Spleen

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Effect of silibinin on the histology of liver in streptozotocin-induced diabetic rat

Tuğba Semerci Sevimli¹, Murat Sevimli², Nurten Özçelik¹

¹Department of Medical Biology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

²Department of Histology and Embryology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

Diabetes is an important cause of oxidative stress. Furthermore, this oxidative damage is responsible for many complications of diabetes. Cellular damage and cell death due to oxidative damage lead to hepatic steatosis and hepatocyte degeneration (1). Silibinin is a potent flavonoid antioxidant derived from a plant called *Silybum Marianum* (2). The aim of this experimental study was to investigate the effects of silibinin on liver damage of Streptozotocin (STZ)-induced diabetic rats through histochemical methods. There were five groups in our study as follows: The Control group, Diabetic Group, Treatment Group 1 (diabetic group treated with 100 mg/kg silibinin), Treatment Group 2 (diabetic group treated with 200 mg/kg silibinin), and Silibinin Group (no diabetes, but with 5 rats treated with 100mg/kg and 5 rats treated with 200 mg/kg silibinin). STZ was administered at a dose of 65mg/kg through intraperitoneal injection and Silibinin was administered through gastric gavage for 4 weeks. We found that histologically, there was a decrease in hepatic steatosis and liver damage in silibinin-treated diabetic groups. Diabetes causes microvascular and macrovascular pathologies that affect many organs and systems. One mechanism for these effects is oxidative stress. Silibinin is a potent flavonoid antioxidant, which is also known as a hepatoprotective agent (3). As in the other previous studies, we found that silibinin improved hepatic injury in diabetic rats, but the molecular mechanism of this effect is still unknown.

Key Words: Diabetes, Silibinin, Liver

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► Poster No. 48

Oxidative stress parameters in zebrafish heart tissue after exposed to fluoxetine

Güllü Kaymak¹, Meliha Koldemir Gündüz², Seyma Tartar³, Harika Eylül Esmel³, Mehtap Çevik², Belgin Süsleyici Duman², Figen Esin Kayhan³

¹Department of Biology, Faculty of Science and Arts, Sakarya University, Sakarya, Turkey

²Division of Biology, Department of Molecular Biology, Faculty of Science and Arts, Marmara University, Istanbul, Turkey

³Division of Biology, Department of Zoology, Faculty of Science and Arts, Marmara University, Istanbul, Turkey

Fluoxetine (FLX) is a selective serotonin (5-HT) reuptake inhibitor (SSRI) and the active ingredient of the largely prescribed antidepressive drug Prozac. Due to its excretion by patients and the direct disposal into wastewaters, FLX has been found at detectable levels in surface waters surrounding wastewater and has one of the highest acute toxicity levels of any human pharmaceuticals for non-target aquatic organisms. FLX concentrates in fish tissues and notably in the heart. Zebrafish (*Danio rerio* Hamilton 1822) are small cyprinid fishes which have long been used as models and as a robust platform for toxicology research. This is attributed to its fast reproductive capacity.

Zebrafish were acclimatized for two weeks in stock tanks under laboratory conditions. Tap water free from chlorine was used, during the acclimatization period; fish were fed ad libitum with pellet twice a day. The six experiment study groups were composed as; 145 ng (daily dose) fluoxetine exposed to each aquarium tank and five zebrafish were studied at 15 min., 30 min., 60 min., 4 days and 8 days of exposure and the last group

was composed as the control group. The experiment was repeated three times. After exposure to fluoxetine, heart tissue of zebrafish was homogenized and used for malondialdehyde (MDA), catalase (CAT) and total protein measurements done with spectrophotometric methods.

The results show that protein levels were increased in all experiment groups, except 4. day that we didn't observe any significant difference, compared to control group. In all experiment groups, the MDA levels were found to be decreased. When compared to control group, CAT enzyme activity significantly decreased only in the 60 min. group, in the other groups we didn't observe a significant difference. In conclusion, our results indicate a rapid and robust effect of fluoxetine in reversing biochemical alterations and the long term fluoxetine treatment may inhibit the antioxidative defense mechanism.

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Keywords: zebrafish, fluoxetine, heart, oxidative stress.

► Poster No. 49

Lipid peroxidation in the liver tissue of common carp (*Cyprinus carpio*, Linnaeus 1758) in sapanca lake naturel condition.

Güllü Kaymak¹, Figen Esin Kayhan², Nazan Deniz Yön Ertuğ¹

¹Department of Biology, Science and Art Faculty, Sakarya University, Sakarya, Turkey.

²Department of Biology, Science and Art Faculty, Marmara University, İstanbul, Turkey.

Sapanca Lake has an economical importance in terms of its aquatic products also water is taken from the Lake for domestic and industrial needs. Sapanca is one of the few lakes in Turkey, which provides drinking water, but it is exposed to heavy urbanization because of its natural beauty and its proximity to the metropolitan Istanbul. Sapanca Lake is polluted from highways near the coast

and also from waste water from settlement areas around the lake. Common carp, a model aquatic organism, was used as an experimental animal. In this study, lipid peroxidation indicating oxidative stress in carp (*Cyprinus carpio*, Linnaeus 1758) liver tissue was used to investigate the effects of domestic and industrial pollution in Sapanca Lake. For this purpose, fish samples were taken from the Lake with professional fisherman in every month of 2015 and the samples were brought to the laboratory in cold chain. After dissection, liver tissues of carp were homogenized and used to determine malondialdehyde (MDA) level with spectrophotometric methods. MDA is one of the most frequently used indicators of lipid peroxidation. The results show that MDA level increased in summer especially on July. This could be due to reduction of rainfall and rise of water temperature. Lots of creeks, which are water supply of Sapanca Lake, dry in summer and thus Lake's volume contracts. So that pollution is concentrated more. Thus, aquatic ecosystems can be considered as an indicator of health in both animals and humans.

Acknowledgement: This project has been supported by Sakarya University Scientific Research Commission (BAPKO) with 2015-50-02-007 project number.

Key words: Lipid peroxidation, common carp, Sapanca Lake, oxidative stress.

▶ Poster No. 50

Effect of silibinin on the histology of kidney in streptozotocin-induced diabetic rat

Tuğba Semerci Sevimli¹, Murat Sevimli², Nurten Özçelik¹

¹Department of Medical Biology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

²Department of Histology and Embryology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

Diabetes is one of the most important causes of end-stage renal failure. The main reason for renal damage in diabetes is metabolic and hemodynamic changes induced by hyperglycemia(1). However, increasing

evidence shows that more complex mechanisms play a role in diabetic nephropathy. Oxidative damage is one of these mechanisms. Hyperglycemia increases the production of reactive oxygen species in the kidney (2). Silibinin is a potent flavanoid antioxidant derived from a plant called *Silybum Marianum*. Previous studies have indicated the antioxidant effects of silibinin on different pathologies and organs(3). The aim of this experimental study was to investigate the effects of silibinin on the kidney tissue of Streptozotocin (STZ)-induced diabetic rats through histochemical methods. There were five groups in our study as follows: Control group, Diabetic Group, Treatment Group 1 (diabetic group treated with 100 mg/kg silibinin), Treatment Group 2 (diabetic group treated with 200 mg/kg silibinin), and Silibinin Group (no diabetes but 5 rats treated with 100mg/kg and 5 rats treated with 200 mg/kg silibinin). STZ was administered at a dose of 65mg/kg by intraperitoneal injection and Silibinin was administered by gastric gavage for 4 weeks. In contrast to some previous studies, we could not find any significant restorative effect of silibinin on renal damage. The diabetic group and the treatment groups demonstrated similar histological findings. These conflicting results can be related to the differences between the experimental designs. The role of oxidative stress in diabetic rat kidney damage is clear, but silibinin has no effect on these molecular mechanisms.

Key Words: Diabetes, Silibinin, Kidney

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▶ Poster No. 51

Effect of a potent antioxidant phytochemical, silibinin, on the histology of the heart in streptozotocin-induced diabetic rat

Tuğba Semerci Sevimli¹, Murat Sevimli², Nurten Özçelik¹

¹Department of Medical Biology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

²Department of Histology and Embryology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey.

Diabetic cardiomyopathy is one of the most important complications of diabetes. The acute stage effects of diabetes on the heart and the aorta are not sufficiently known. Diabetes causes microvascular and macrovascular pathologies that affect many organs and systems. The heart is one of these organs. The most important mechanism for these effects is oxidative stress that results from decreased blood flow (1). Silibinin is a potent flavanoid antioxidant derived from a plant called *Silybum Marianum* (2). The aim of this experimental study was to investigate the effects of silibinin on the heart tissue of Streptozotocin (STZ)-induced diabetic rats through histochemical methods. There were five groups in our study as follows: The Control group, Diabetic Group, Treatment Group 1 (diabetic group treated with 100 mg/kg silibinin), Treatment Group 2 (diabetic group treated with 200 mg/kg silibinin), and the Silibinin Group (no diabetes, but 5 rats treated with 100mg/kg and 5 rats treated with 200 mg/kg silibinin). STZ was administered at a dose of 65mg/kg by intraperitoneal injection and Silibinin was administered through gastric gavage for 4 weeks. We found that histologically, there was a slight decrease in damage on the heart tissues of silibinin-treated diabetic groups. Silibinin seems to cure some deleterious effects of diabetes on heart tissue. Silibinin is a potent flavonoid antioxidant. Previous studies have demonstrated the antioxidant effects of silibinin on different pathologies and organs (3). In our study, the restorative effect of silibinin on diabetic heart damage can be attributed to the antioxidant properties of silibinin.

Key Words: Diabetes, Silibinin, Heart

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Poster No. 52

Alpha-Lipoic acid prevents brain injury in rats administered with valproic acid

İsmet Burcu Turkyilmaz¹, Bahar Bilgin Sokmen², Refiye Yanardag¹

¹Department of Chemistry, Faculty of Engineering, Istanbul University, Istanbul, Turkey.

²Department of Chemistry, Faculty of Arts and Sciences, Giresun University, Giresun, Turkey.

Valproic acid (VPA, 2-propyl pentanoic acid) is a short chain fatty acid. It has been widely used as an antiepileptic drug for many years. Although it is widely used for the treatments of neurologic disorders, exposure to VPA was reported as causing brain injury in some cases (1,2). Besides VPA also increases free radical generation that causes many side effects in various organs (3). Alpha-lipoic acid is a sulfur containing coenzyme and can act as a powerful antioxidant in both fatty and aqueous phases in both its reduced or oxidized forms. It can reduce oxidative stress by redox generation of other antioxidants (4). In our study, the effect of alpha-lipoic acid on VPA induced brain injury was studied. Rats were divided into four groups. Group 1 is control group, Group 2 is rats given alpha-lipoic acid (50 mg/kg), Group 3 is rats receiving VPA (0,5 g/kg), Group 4 is rats given VPA and alpha-lipoic acid. While VPA was applied by intraperitoneally, alpha-lipoic acid was administered by gavage technique for fifteen days. On the 16th day, all the animals were sacrificed under anesthesia. Brain tissues were taken, homogenized in saline to make 10% (w/v) homogenate and centrifuged. In supernatants, brain glutathione (GSH), advanced oxidized protein product (AOPP), nitric oxide (NO) and protein levels, catalase (CAT) and superoxide dismutase (SOD) activities were determined. GSH levels, CAT and SOD activities were found to be reduced while increased levels of AOPP and NO were observed in VPA group. However, administration of alpha-lipoic acid reversed

these effects in VPA group. In conclusion, we can say alpha-lipoic acid protects VPA induced brain injury in rats.

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► Poster No. 53

Serum osteopontin and bone sialoprotein levels in patients with tendinopathy

Cüneyt Tamam, Serdar Hira, Uğur Demirpek, Mehmet Gem

Department of Biochemistry, Tatvan Military Hospital, Bitlis, Turkey

The pathogenesis of tendinopathy remains unclear. Small integrin-binding ligand N-linked glycoproteins, a family of non-collagenous proteins including osteopontin (OPN) and bone sialoprotein (BSP), were initially thought to be limited to mineralized tissue but recent studies showed that they are more widely distributed and are expressed in nonmineralized tissues (1,2). Musculoskeletal tissue cells are categorized as the same functional unit developed from the mesenchymal stem cells (3). This theoretical background led us to hypothesize that OPN and BSP could be involved tendinopathy pathogenesis. The aim of this study was to investigate relationship between serum OPN and BSP levels and tendinopathy disease. 39 female tendinopathy patients and 39 female healthy volunteers were recruited for this prospective observational study. Serum OPN and BSP levels were measured using enzyme-linked immunosorbent assay. We also measured body mass

index and erythrocyte sedimentation rate (ESR), white blood cells (WBC) and neutrophil lymphocyte ratio (NLR). There was no significant differences in serum BSP levels between two groups ($41,83 \pm 52,03$ vs. $53,64 \pm 53,06$ ng/mL, $p=0,276$). There was also no significant differences in serum OPN levels between two groups ($57,37 \pm 21,61$ vs. $77,72 \pm 72,14$ ng/mL, $p=0,363$ respectively). There were no significant differences in WBC, NLR and ESR values between two groups ($p=0,897$, $p=0,795$, $p=0,405$ respectively). There was no correlation between serum BSP levels and OPN, WBC, NLR and ESR levels in patients group. Patients with tendinopathy had a negative correlation between serum OPN levels and NLR levels. The results of this study have indicated that BSP and OPN levels are not involved in pathogenesis of tendinopathy.

Keywords: Tendinopathy; SIBLING; Osteopontin; BSP.

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► Poster No. 54

Effect of insulin like growth factor-1 (IGF-1) on oxidative stress during wound healing

Tuğçe Özmen, Şule Coşkun Cevher

Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey

Wound healing consist of three phase including hemostasis, inflammation, proliferation and remodeling (1). The process of wound healing is regulated by varieties of different growth factors, cytokines and hormone (2). In the line with this information, we planned to evaluate the relationship between IGF-1 therapy and oxidative stress in dermal tissue followed by wounding.

Wistar-albino rats (250-300 g) were used in the experiments. Excisional wounds were made under anesthesia in rats. The animals were divided into 4 groups: Control (n=6), untreated wounds (n=6), control BSA (n=6) and IGF-1 treatment (1.5 ng/ml) (n=6). All animals were sacrificed under anesthesia on the 3rd day. Tissue malondialdehyde (MDA) and nitric oxide (NOx) levels were determined.

The MDA and NOx levels were increased in the tissue in the inflammation phase of wound healing by IGF-1 treatment when compared to un-treated group.

In conclusion, the oxidative stress values were increased in the wound healing process by IGF-1 treatment.

Keywords: Oxidative stress; Wound; Inflammation; IGF-1.

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► Poster No. 55

Protective effects of hydrated C₆₀ fullerene nanostructures against rotenone-induced oxidative cytotoxicity in rat astrocytes

Can Ali Ağca¹, Artem A Tykhomyrov², Svetlana V. Kirichenko³, Victor S Nedzvetsky³.

¹Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Bingöl University, Bingöl, Turkey.

²Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (NASU), Kyiv, Ukraine.

³Department of Biophysics and Biochemistry, Dnipropetrovsk National University, Dnipropetrovsk, Ukraine.

C60 fullerene and their hydrated nanostructures are proposed for prevention as well as treatment of various pathological conditions caused by oxidative stress. It has been earlier demonstrated that hydrated C₆₀

fullerene (C₆₀HyFn) nanostructures exert neuroprotective effects through reduction of reactive oxygen species (ROS) and decrease of excessive astrocytosis in a brain (1). In the present study, we aimed to clarify whether C₆₀HyFn could diminish ROS generation stimulated by respiratory chain inhibitor rotenone as an inductor of oxidative cytotoxicity in the primary astrocytes cell culture. Incubation of astrocytes with rotenone in the range of concentration of 0.5-5 μM for 24 h caused dose-dependent cell death. Pre-treatment of cell culture with C₆₀HyFn in the final concentration of 0.5 μM for 24 h significantly enhanced viability of astrocytes incubated with rotenone. Pre-treatment of cells with 1 μM of C₆₀HyFn considerably diminished rate of ROS formation induced by toxicant as it was shown with using of dihydroethidium dye for fluorescence analysis. Western-blot analysis showed decrease in glial fibrillary acidic protein (GFAP) level indicating suppression of cell activity of astrocytes exposed to the high dose of rotenone (5 μM). In contrast, treatment with lower dose of rotenone (0.5 μM) lead to increasing level of GFAP, as toxic effect. C₆₀HyFn treatment provided normalization of astrocytic marker level regardless concentration of rotenone indicating partial recovery of astrocyte functioning disturbed by oxidative stress. These data highlight C₆₀HyFn as novel promising candidate for preventing ROS-mediated injury of CNS cells in which mitochondrial dysfunction of glial cells plays crucial role.

Keywords: Oxidative stress; Retina; Rotenone; Mitochondria.

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► Poster No. 56

Hydrated C₆₀ fullerene attenuates oxidative stress and acts as glia-protector in retina of diabetic rats

Victor S Nedzvetsky¹, Artem A Tykhomyrov², Irina V. Prischepa¹, Can Ali Ağca³

¹Department of Biophysics and Biochemistry, Dniepropetrovsk National University, Dnipropetrovsk, Ukraine.

²Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (NASU), Kyiv, Ukraine.

³Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Bingol University, Bingol, Turkey.

Oxidative stress plays important role in the development of diabetic retinopathy. Retinal glial cells provide neurons with main metabolites and protect them from injures. Hydrated C₆₀ fullerene (C₆₀HyFn) has been demonstrated to be powerful antioxidant in CNS, however its possible cytoprotection directed to retinal cells remains unknown. This study was organized to elucidate potential of nanostructures of hydrated C₆₀ fullerene (C₆₀HyFn) to reduce lipid peroxidation and reactive gliosis induced by hyperlicemia in retinal tissues. Diabetes was induced by single injection of streptozotocin (STZ) (50 mg/kg) to adult Wistar rats. STZ-injected rats were divided on two groups: diabetes and diabetes+C₆₀HyFn. Part of diabetic and healthy control rats consumed 60 nM solution of C₆₀HyFn as drinking water for 90 days. Malondialdehyde (MDA) content was measured as oxidative stress marker, glial fibrillary acidic protein (GFAP) levels were detected by western blot (WB) and immunocytochemistry (IHC) as a marker of reactive astrocytosis. Significant elevation of MDA concentration was observed in retinas of diabetic rats compared with control ($P < 0.01$) at the end of the experiment. Increased retinal GFAP expression (2.1 folds) was observed by WB in diabetic group versus control ($P < 0.05$), and reactive gliosis development in the inner retinal layer was confirmed by IHC. In retinas of diabetes+C₆₀ group animals, C₆₀HyFn supplementation significantly decreased MDA concentration and normalized GFAP expression compared with non-treated diabetic rats. C₆₀HyFn exerts protective effects against oxidative damages in diabetic retina through inhibition of lipid peroxidation and provides recovery of glial functions.

Keywords: Oxidative stress; Diabetes; Retina.

Effect of endurance training on oxidative stress levels in basketball players

Recep Soslu, Fazile Nur Ekinçi Akdemir, Ali Ahmet Doğan

Department of Physical Education and Sports Bartın University Bartın, Turkey

Regular physical activity on health is known to have made many beneficial effects (1). But to increase the production of different types of exercise SA, an antioxidant that affect the immune system in different ways and it creates oxidative stress, and therefore was determined to bring cellular damage (1,2). Oxygen consumed mitochondria cytochrome oxidase and 95-99% water and the remainder is converted into the (% 1-5) are expressed in part of superoxide radicals generated from mitochondria during infiltrating oxygen consumption (3,4). The increase in free radical production that occurs during prolonged submaximal aerobic exercise, it was found that basically stems from the huge increase in oxygen consumption (5). This study was designed to determine whether endurance training basketball in how it affects the free radical formation.

In this study, Atatürk University School of Physical Education and Sports in studying, playing basketball actively participated voluntarily in 14 male basketball players. Target heart rate of athletes that make up the research groups were determined by Karvonen method. Athletes, for 8 weeks, the period of 3 days per week for 45-60 minutes and heart rate of 50-70% has been applied intensity running exercises. First, blood samples were taken at the end of the third and sixth week 3 times in total. In serum taken from blood samples of SOD, GSH, CAT and MDA levels were examined.

Blood samples taken during the first week as a result of the findings obtained in this study SOD, GSH, CAT and MDA levels were no significant results. However, only the second and third measurements of SOD and MDA levels were found to be significant in terms of the difference between them and the first statistical measure

($p < 0.001$), whereas there was no association with GSH and CAT.

Overall results that suppress lipid peroxidation occurs due to aerobic training is examined and increase antioxidant enzyme levels that could be said to be more effective in improving performance in terms of the positive impact it.

Key words: Endurance Training, Oxidative Stress, Free Radicals, Basketball

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► Poster No. 58

Oxidative Stress and *CYP1A1*, *GSTP1* gene polymorphism in smoking pregnant and their cord blood

Fuat Dilmeç¹, A. Ziya Karakılıç², Sevgi Acun², Hakim Çelik², Ahmet Özer³, Mustafa Zerin²

Departments of Medical Biology¹, Physiology², Clinical Genetics³ Faculty of Medicine, Harran University, Sanliurfa

Polycyclic aromatic hydrocarbons in tobacco-smoke are catalyzed by the cytochrom-P450, and trigger the production of harmful metabolites which are subsequently detoxified by glutathione-S-transferase-P1. This process may be affected by certain polymorphisms of *CYP1A1* and *GSTP1*, the genes encoding crucial cytochrom-P450 enzymes, causing alterations in their activities. We aimed to investigate possible interactions between tobacco-smoking and *CYP1A1*, *GSTP1* gene polymorphisms on redox balance

in blood samples of pregnant and newborn cords.

Eighty blood samples were collected from 20 smoking and 20 non-smoking pregnant women and the cords of newborns. The frequencies of *CYP1A1* (NM_000499.2, GI:1543), and *GSTP1* (NM-000852.2, GI:2950) polymorphisms, arylesterase (ARES), paraoxonase (PON1), total thiol (T-SH), ceruloplasmin (CP), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD) along with lipid peroxide (LOOH), total oxidant (TOS) and antioxidant status (TAS) and oxidative stress index (OSI) were determined. Data were analysed using SPSS v11.5.

Plasma TAS ($p=0.031$) and CP ($p=0.009$) in smoking mothers, and LOOH ($p=0.043$), TOS ($p=0.046$) and OSI ($p=0.035$) levels in non-smokers were elevated. While TAS ($p=0.022$) levels were higher in cordons not exposed to tobacco-smoke, T-SH ($p=0.035$), LOOH ($p=0.030$), TOS ($p=0.040$) and OSI ($p=0.004$) levels were higher and CAT activity ($p=0.045$) was lower in newborn cordons of smoking pregnant. There was no significant difference between the polymorphism frequencies and oxidative parameters in pregnant and their fetuses (*CYP1A1*: c.*1189 CT genotype: 45.0% and 50.0%, T allele: 22.5% and 30.0%, respectively; *GSTP1*:c.313 A>G genotype: 50.0% and 20.0%, G allele: 35.0% and 25.0%, respectively).

Tobacco smoking may play an important role on redox balance by decreasing antioxidant defense and increasing oxidative stress. No statistical difference was determined between smoking and oxidative stress by means of *CYP1A1*: c.*1189 CT and *GSTP1*:c.313AG gene polimorpisms in pregnant and the cord, but there is a need for further studies in larger cohorts.

Keywords: *CYP1A1*, *GSTP1*, Smoking, Polymorphism, Oxidative Stress

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► Poster No. 59

Increasing of oxidative stress and decreasing of antioxidant enzyme activities in patients with acute ischemic stroke

A. Ziya Karakılıç¹, G. Şahika Gökdemir², M. Tahir Gökdemir³, Abdullah Taşkın⁴

Departments of Physiology¹, Emergency Medicine³ and Biochemistry⁴, Faculty of Medicine, Harran University; Department of Physical Therapy², Mehmet Akif İnan Training and Research Hospital, Şanlıurfa, Turkey

Free oxygen radicals may damage to balance of oxidants and antioxidants, and may increase the oxidative stress in cerebrovascular components. Increasing oxidative damage lead to insufficiency in physiological functions of antioxidants in cerebrovascular components, and so may play an important role in ethiopathogenesis of ischemic stroke. This study was carried out to investigate the oxidative stress index (OSI), total oxidant (TOS) and antioxidant (TAS), status, arylesterase (ARS), paraoxanase (PON), lipid peroxides (LOOH), thiol (SH) and seruloplasmin (CP) levels in patients with ischemic stroke.

This study was performed on Training and Research Hospital at Harran University. By courtesy of ethical committee, the blood samples were taken from 70 patients (69.57±12.38) and 50 healthy (65.72±8.43) controls. The values of ARS, PON, TAS, TOS, LOOH, SH and CP were measured and OSI was calculated. Data were analyzed using SPSS software.

The values of OSI, LOOH and TOS significantly increased ($p<0,001$, $p<0,030$, $p<0,005$, respectively), while the levels of TAS, ARS, PON and SH decreased ($p<0,019$, $P<0,006$, $P<0,001$ and $p<0,036$, respectively) in patients with ischemic stroke. But, the values of CP were statistically not affected ($p<0,356$) in patients compared with the controls.

In conclusion, the values of TOS, OSI and LOOH were increased while the levels of ARS, PON, TAS and SH significantly decreased in ischemic stroke. Based upon these results, we think that the decreasing of antioxidant defenses and the increasing of oxidative stress are important in ethiopathogenesis of ischemic stroke; however, there is a need for more detailed studies to assess all molecular mechanisms induced in patients with ischemic stroke.

Key words: Oxidative stress, seruloplasmin, thiol, ischemic stroke.

*This study was partially founded by the Commission of Scientific Research Projects at Harran University.

▶ Poster No. 60

Protective effects of dexpanthenol on trinitrobenzene sulfonic acid-induced colitis in rats

Suna Demirtaş¹, Ferhat Şirinyıldız¹, Cenk Orak¹, Gül Taşlı Yeşilçayır¹, Rauf Onur Ek¹, Gökhan Cesur¹, Özlem Bozkurt², Mehran Akse², Yüksel Yıldız¹

¹Adnan Menderes University, Medical School, Physiology Department, Aydın, Turkey

²Adnan Menderes University, Medical School, Biophysics Department, Aydın, Turkey

Oxidative stress and inflammation are both considerable risk factors in the pathogenesis of inflammatory bowel disease. This study aims to determine the effects of dexpanthenol (DXP) through its antioxidant and anti-inflammatory properties on intestinal injury in rat colitis model induced by trinitrobenzene sulfonic acid (TNBS) administration.

Thirty Wistar-Albino female rats were divided into 4 groups as DXP control, sham control, colitis and DXP treated colitis groups. Colitis was induced by the intrarectal administration of TNBS under anesthesia. After induction of colitis, the animals in DXP control and DXP treated colitis groups were subjected to intraperitoneal DXP (500 mg/kg/day) injection for 3 days. Rats were sacrificed on day 4 after induction of colitis, and their intestinal tissues were analyzed biochemically and histopathologically.

DXP caused a significant decrease in intestinal injury as determined by the histological scores. Tissue malondialdehyde levels were higher in the colitis group in comparison to the sham and DXP treated colitis groups. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) activities were lower in the colitis group compared to DXP control and

DXP treated colitis groups. The antioxidant enzyme activities were increased by DXP treatment compared to colitis group, where the increase was significant for GSH-Px activity but not for SOD and CAT activities. Furthermore, treatment with DXP also reduced elevations in myeloperoxidase activity observed in intestines of colitis group.

In conclusion, dexpanthenol seems to have anti-inflammatory and antioxidant properties. Therapy with DXP carries a potential to reduce the intestinal injury on TNBS-induced colitis in rats.

Keywords: Inflammatory bowel disease, TNBS, dexpanthenol, antioxidant, reactive oxygen species, antioxidant enzyme activity

▶ Poster No. 61

Comparative assessment of the cytotoxic effects of ferulic acid on leukemia, pancreas, prostate and thyroid cancer cells

Mücahit Seçme¹, Levent Elmas¹, Canan Eroğlu², Gülseren Bağcı¹, Yavuz Dodurga¹

¹Department of Medical Biology, School of Medicine, Pamukkale University, Denizli, Turkey.

²Department of Medical Biology, School of Medicine, Necmettin Erbakan University, Konya, Turkey.

Natural antioxidants present therapeutic potential for different disorders including cancer, diabetes, cardiovascular and neurodegenerative diseases. The researchers are always on the lookout for new agents to help them in their fight, especially against cancer. Ferulic acid (FA) is one of the most abundant phenolic acids in many plants and might be found in different concentrations in fruits and vegetables. FA presents many useful potential therapeutic effects such as hepatic, neuro and photoprotective effects, antimicrobial and anti-inflammatory activities, antioxidant content and free radical scavenging ability (1,2). The aim of this study is to evaluate the *in vitro* cytotoxic effect of FA in various human cancer cells such as the androgen-independent PC-3 and androgen-dependent LNCaP prostate cancer cells, MiaPaCa-2 pancreatic cancer

cells, TT medullary thyroid cancer cells and K562, Kasumi and HL-60 leukemia cells. Cytotoxic effects of FA in cells were determined by XTT and CellTiter Glo methods in time and dose dependent manner to find half maximal inhibitory concentration (IC₅₀) doses. IC₅₀ dose of FA were calculated as 300µM at the 48th PC-3 cells, 500µM at the 48th LNCaP cells, 500µM at the 72nd in MiaPaCa-2, 150µM at the 72nd in TT cells. A significant effect of FA on cell viability in leukemia cells could not be found in the range of 20-1000µM. In conclusion, these results demonstrated that FA decreases cell viability in various cancer cells and established that FA as a potential candidate chemical for further studies aimed at finding a better, more effective treatment approach for cancer.

Keywords: Ferulic acid; cancer; cell viability.

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▶ Poster No. 62

Acrylamide applied during pregnancy was caused by oxidative stress in the mother's blood

Mehmet Erman Erdemli¹, Zeynep Aksungur¹, Yusuf Türköz¹

¹Department of Medical Biochemistry, Faculty of Medicine, Inonu University, Malatya, Turkey.

A liberal amount of acrylamide is produced as a result of frying or baking foods in high temperatures (1) and individuals take certain amounts of acrylamide everyday by consuming these food items. Pregnant women are also exposed to acrylamide originating from food during pregnancy and their fetuses are probably affected (2). Vitamin E is a powerful antioxidant that detoxifies free radicals (3). Our hypothesis in the current study was that Vitamin E could protect the pregnant rats and their

fetuses from the toxic effects of acrylamide, applied during pregnancy.

The rats were divided into 5 different groups as control, corn oil, acrylamide, vitamin E, and acrylamide plus vitamin E, consisting of 8 pregnant rats in each group. Acrylamide was dissolved in drinking water and was administered to the acrylamide group in dose of 5 mg/kg body weight daily by oral gavage during pregnancy. Vitamin E was dissolved in corn oil and was administered to the E vitamin group in dose of 100 mg/kg body weight daily by oral gavage during pregnancy. Acrylamide plus vitamin E group was given the same dose of acrylamide and vitamin E. Rats were fed ad libitum during the experiment. On the 20th day of pregnancy, the blood samples were taken from pregnant rats and serum samples were obtained. The serum samples were used to determine the levels of malondialdehyde (end-product of lipid peroxidation), reduced glutathione, total antioxidant capacity, total oxidant capacity, and xanthine oxidase activity.

Acrylamide significantly increased the serum levels of MDA, TOS, XO and significantly decreased the serum levels of GSH, TAS compared to others groups ($p < 0.05$). Conversely, Vitamin E significantly increased the serum levels of GSH, TAS and significantly decreased the serum levels of MDA, TOS, XO compared to others groups ($p < 0.05$)

In conclusion, acrylamide, applied during pregnancy, caused oxidative stress in the blood of pregnant rats, but vitamin E were largely eliminate the toxic effects of acrylamide by increasing the serum levels of GSH, TAS and by decreasing the serum levels of MDA, TOS, XO. Therefore, we recommend to pregnant women to consume food enough, containing vitamin E, for protecting themselves from the toxic effects of food-borne acrylamide due to the widespread nature of fast-food culture in Turkey in today's life.

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► Poster No. 63

The effect of glutathione treatment on the biochemical and immunohistochemical profile in streptozotocin-induced diabetic rats

Fatmagül Yur, Semiha Dede¹, Turan Karaca, Sevim Çiftçi Yeğin, Yeter Değer, Hülya Özdemir

¹Department of Biochemistry, Veterinary Medical Faculty, Yuzuncu Yil University, Van, Turkey.

This study investigated the possible role of glutathione (GSH) in diabetic complications and its biochemical safety in experimental diabetic rats. Serum biochemical parameters and the histology of the pancreas were investigated. Seven rats were separated as controls. To create the diabetes in rats, 45 mg/kg single-dose streptozotocin (STZ) was administered i.p. The treatment was continued for 1 month. STZ was administered to the diabetes + GSH group, then reduced GSH, dissolved in isotonic salt solution (200 mg/kg), was applied i.p. two times a week. The GSH group received i.p. GSH. Serum biochemical parameters were determined by autoanalyzer. Immunohistochemical procedures were used to determine the percentage of the insulin-immunoreactive β -cell area in the islets of Langerhans. The biochemical parameters changed to different degrees or did not change. Pancreatic cells of the control and GSH groups were healthy, but in the diabetic and GSH-treated diabetic groups we found damage in different numbers. The results from these analyses show that GSH supplementation can exert beneficial effects on pancreatic cells in STZ-induced diabetic rats and can safely be used for therapy in and protection from diabetes and complications of diabetes.

Histological and biochemical alterations in the male rat bladder following exposure to 900 megahertz electromagnetic field for 1 hour a day on postnatal days 22-59 inclusive

Sibel Türedi, Gökçen Kerimoğlu, Ersan Odacı

Department of Histology and Embryology, Medical Faculty, Karadeniz Technical University, Trabzon, Turkey

The purpose of this study was to investigate the effects on rat bladder tissue of exposure to a 900 Megahertz (MHz) electromagnetic field (EMF) on postnatal days 22-59 inclusive using histological and biochemical methods. The study began with 24 male Sprague Dawley rats aged 24 days. These were then divided into three groups of eight rats each, control (CG), sham (SG) and EMF (EMFG). No procedure was performed on the control group. The EMFG rats were exposed to continuous 900 MHz EMF for 1 hour a day inside a Plexiglas cage, at the same times every day, on postnatal days 22-59, inclusive. The SG rats were placed inside the same cage for the same periods but were not exposed to EMF. At the end of the experiment (on postnatal day 60) the rats were sacrificed, and the bladder tissues were removed. These were stained with H&E and Masson trichrome for histomorphological evaluation. TUNEL was used to evaluate apoptosis. Tissues' oxidant/antioxidant parameters were investigated biochemically. Tissue malondialdehyde (MDA) levels in EMF increased significantly compared to CG and SG ($p=0.004$ and $p=0.006$, respectively), while catalase (CAT) activities decreased significantly compared to CG and SG ($p=0.004$ and $p=0.004$, respectively). Glutathione (GSH) levels also decreased significantly in EMFG compared to CG and SG ($p=0.004$ and $p=0.004$, respectively). No pathology was observed in CG tissues at histopathological evaluation, and the findings in SG were similar to those in CG. However, degeneration in the transitional epithelium, stromal irregularity and increased fibrotic tissue, edema and increased vascularization were observed in EMFG. In conclusion, the results show that exposure to continuous 900 MHz EMF for 1 hour a day on postnatal

days 22-59 inclusive can result in increased lipid peroxidation levels in rat bladder tissue, a decrease in antioxidant enzyme levels and pathological changes.

Keywords: Electromagnetic field; Bladder; Antioxidants; Oxidative stress.

Effects of erdosteine on transient receptor potential melastatin (TRPM) 2,8 ion channels on liver ischemia and reperfusion model in rats

Okan Tutuk¹, Recep Dokuyucu¹, Hatice Dogan¹, Sumeyye Tutuk³, Nilufer Bilgic², Gokhan Agturk¹, Bulent Gogebakan², Fatih Sefil¹, Cemil Tumer¹

¹Department of Medical Physiology, School of Medicine, Mustafa Kemal University, Hatay, Turkey.

²Department of Medical Biology, School of Medicine, Mustafa Kemal University, Hatay, Turkey.

³Department of Medical Biochemistry, School of Medicine, Mustafa Kemal University, Hatay, Turkey.

The aim of this study was investigated to the effects of erdosteine (Erd) on *TRPM 2,8* ion channels on liver ischemia/reperfusion (I/R) model in rats.

Fifty male Wistar albino rats were randomly divided into five groups (for each $n=10$); group I (control); group II (sham); group III (I/R; 45 min ischemia+45 min reperfusion); group IV (Erd; 100 mg/kg/day of Erd was given orally for 2 days) and group V (Erd+I/R; 45 min ischemia+45 min reperfusion; 100 mg/kg/day of Erd was given orally before application of I/R model for 2 days). At the end of reperfusion, rats were sacrificed and liver tissues were excised for molecular and biochemical analyses. Gene expression levels of *TRPM* ion channels and plasma levels of tumour necrosis factor alpha (TNF- α) and interleukin (IL)-6 were investigated on liver tissues. According to the statistical distribution analyses, Kruskal-Wallis test was used for statistical analyses. $p<0.05$ values was considered statistically significant.

In molecular examination, it was observed that *TRPM*

2,8 gene expression levels, TNF- α and IL-6 levels were significantly increased in I/R group as compared to the other groups. Also it was observed that plasma cytokine levels of the treatment group (Erd+I/R) were closer to the levels of control group by significantly decreasing in comparison with I/R group. In addition to this, it was found that *TRPM 2,8* gene expression levels were statistically decreased in treatment group (Erd+I/R) as compared to I/R group.

We think that Erd has an effect in a reducing direction on early reperfusion injury by influencing the proinflammatory cytokine levels and Erd supports antioxidant defense system in process of hepatic I/R injury. Thus, in the present study we showed that Erd inhibits the entry into cell of Ca^{2+} ve Mg^{2+} ions by reducing *TRPM 2, 8* gene expression levels. Based on these findings, it could be suggested that Erd may be used in treatment of liver disease associated with I/R injury by inhibiting especially oxidative stress and associating with entry of Ca^{2+} ion.

Key Words: Liver, ischemia reperfusion, erdosteine, TRPM 2, TRPM 8

Acknowledgement: Our study was supported by Mustafa Kemal University Scientific Research Projects Coordination Unit. (Number-12262)

► Poster No. 66

Deviations from oxidant/antioxidant levels and pathological changes in the male rat kidney following exposure to 900-MHz electromagnetic field (1 hour/per day) on postnatal days 22-59 inclusive

Sibel Türedi, Gökçen Kerimoğlu, Ersan Odacı

Department of Histology and Embryology, Medical Faculty, Karadeniz Technical University, Trabzon, Turkey

The objective of this study was to investigate the effect on male rat kidney tissue of exposure to 900 Megahertz (MHz) EMF applied on postnatal days 22-59 inclusive,

in terms of oxidant/antioxidant parameters and histopathological evaluation. Twenty-four male Sprague Dawley rats, aged 21 days, were used. These were divided equally into one of three groups; control (CG), sham (SG) or EMF (EMFG). CG was not exposed to any procedure. SG rats were kept inside a Plexiglas cage, without being exposed to the effect of EMF, for 1 h a day on postnatal days 22-59, inclusive. EMFG rats were exposed to continuous 900 MHz EMF for 1 h a day under the same conditions as the SG rats. All rats were sacrificed on postnatal day 60, and the kidneys were removed. Tissues were stained with H&E staining for histomorphological evaluation. The TUNEL method was used to assess apoptosis. In terms of biochemistry, oxidant/antioxidant parameters were studied. The study findings showed that tissue malondialdehyde (MDA) increased in EMFG compared to CG and SG ($p=0.004$ and $p=0.004$, respectively), while CAT levels decreased compared to CG and SG ($p=0.004$ respectively $p=0.004$). EMF group glutathione (GSH) levels decreased compared to CG ($p=0.004$), while an increase was observed in superoxide dismutase (SOD) values ($p=0.006$). At histopathological examination, CG kidney tissue exhibited normal morphology, and similar findings were observed in SG. In the EMF group, however, pathologies such as dilatation and vacuolization in the distal and proximal tubules, degeneration in glomerules and an increase in cells tending to apoptosis, edema, and periglomerular fibrosis were observed. In conclusion, on the basis of our findings we conclude that continuous exposure to the effect of 900 MHz EMF for 1 h a day on postnatal days 22-59 inclusive causes an increase in oxidative stress in rat kidney tissue, a decrease in antioxidant enzyme levels and various pathological changes.

► Poster No. 67

The use of controlled internal drug release for synchronization augmented oxidative and nitrosative stress and leptin levels in Georgian goats*

Mushap Kuru¹, Metin Ögün², Hasan Oral¹, Abdulsamed Kükürt², Mustafa Makav³, Recai Kulaksiz⁴

¹Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey

²Department of Biochemistry, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey

³Department of Physiology, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey

⁴Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey

The aim of this study was to investigate the effects of synchronized with the controlled internal drug release (CIDR) on oxidative and nitrosative stress and leptin levels in the breeding season of Georgian goats. For this purpose, 40 clinically healthy Georgian goats at ages 2-4 were intravaginally exposed to CIDR (Eazi-Breed CIDR[®]) on day 0, and they were injected with equine chorionic gonadotropin (Chronogest[®]) and prostaglandin F_{2α} (Dinolytic[®]) on day 9 of the experiment. CIDR was removed on day 11. Malondialdehyde (MDA) and nitric oxide (NO) levels were determined by colorimetric methods. Endothelial NO synthase activities (eNOS), total antioxidant capacity (TAC), total oxidant capacity (TOC), leptin and progesterone (P₄) levels were measured by commercial kits. NO, MDA, eNOS activities, TOS and P₄ levels were significantly high on day 11, and leptin concentrations were significantly high (P<0.001); additionally, TAS levels were statistically significantly lower (P<0.001) on breeding day of Georgian goats. NO, MDA, eNOS activities and TOS levels of pregnant and non-pregnant goats were different on days 11 and on the day of breeding (P<0.001). In conclusion, the administration of CIDR to Georgian goats was augmented oxidative and nitrosative stress and progesterone concentrations. Moreover, the serum leptin concentrations increased and TAS levels decreased in the goats on breeding day.

Keywords: Nitric oxide, eNOS, leptin, progesteron, CIDR, Georgian goat.

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The role of caffeic acid phenethyl ester (CAPE) on transient receptor potential melastatin (TRPM) 6,7 ion channels on hepatic ischemia reperfusion injury in rats

Recep Dokuyucu¹, Okan Tutuk¹, Hatice Dogan¹, Nilufer Bilgic², Bulent Gogebakan², Fatih Sefil¹, Cemil Tumer¹

¹Department of Medical Physiology, School of Medicine, Mustafa Kemal University, Hatay, Turkey.

²Department of Medical Biology, School of Medicine, Mustafa Kemal University, Hatay, Turkey.

Molecular steps of cell death that occurs as a result of ischemia reperfusion injury haven't been known exactly. Because of this reason we aimed to investigate the role of CAPE which has antimicrobial, antioxidant and anti-inflammatory effects on TRPM 6,7 ion channels on hepatic I/R injury in rats.

Fifty male Wistar albino rats were classified as group I: control (n=10); group II: sham (n=10); group III: CAPE (n=10); group IV: I/R (n=10); grup V: I/R+CAPE (n=10). Animals of groups IV, V were subjected to 45 minutes hepatic ischemia followed by 60 minutes reperfusion. CAPE (10 μmol/kg/day) was intraperitoneally given to the rats of group III and V for 7 days before ischemia. At the end of experiments, rats were sacrificed and liver tissues were excised for gene expression levels of TRPM 6,7 ion channels. The data were analyzed by ANOVA or Kruskal-Wallis tests. Statistical significance was considered to be p<0.05.

The statistical analysis showed that gene expression levels of TRPM 6 and 7 were significantly decreased in group CAPE when compared to group I/R. There was a statistical decrease for gene expression levels of TRPM 6 in treatment group CAPE+I/R when compared to group I/R, but the gene expression levels of TRPM 7 was not statistically significant between these groups.

The results of our study indicated that CAPE which has antioxidant and anti-inflammatory effects could change the gene expression levels of TRPM 6 and 7 by

preventing cell death against I/R injury and also we believe that it may be a therapeutic potential against I/R injury.

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▶ Poster No. 69

Investigation of the effects of lidocaine on transient receptor potential melastatin (TRPM) 2,8 cation channels on experimental hepatic ischemia-reperfusion injury model in rats

Recep Dokuyucu¹, Hatice Dogan¹, Okan Tutuk¹, Nilufer Bilgic², Gokhan Agturk¹, Bulent Gogebakan², Muzeyyen Izmirli², Fatih Sefil¹, Cemil Tumer¹

¹Department of Medical Physiology, School of Medicine, Mustafa Kemal University, Hatay, Turkey.

²Department of Medical Biology, School of Medicine, Mustafa Kemal University, Hatay, Turkey.

To investigate the role of lidocaine which is an important sodium channel blocker on TRPM 2,8 cation channels by performing experimental hepatic ischemia reperfusion (IR) model in rats.

Forty four male Wistar albino rats were divided into five groups; group I: Control (n=7); group II: Sham (n=10); group III: Lidocaine (Lid) (n=7); group IV: IR (n=10); group V: IR+Lid (n=10). Animals of groups IV and V were subjected to 45 minutes hepatic ischemia followed by 60 minutes reperfusion. Single dose lidocaine (2 mg/kg) was intraperitoneally given to the rats of the treatment group V before 10 min of reperfusion process. At the end of reperfusion process, rats were sacrificed and liver tissues were excised. Molecular analyses which are mRNA expression, RNA isolation, cDNA synthesis and qRT-PCR studies were performed with the purpose of determination of gene expression levels of *TRPM 2,8* ion channels in liver rats. The data was analyzed by Kruskal-Wallis test. Statistical significance was considered to be $p < 0.05$.

We found a statistical decrease for gene expression levels of both *TRPM 2* and *TRPM 8* in group V in comparison with group IV. Although there was not a statistical significance for gene expression levels of *TRPM 2* in treatment group V in comparison with group IV, there was a statistical decrease for gene expression levels of *TRPM 8*.

Previous experimental studies indicated that lidocaine is associated with apoptotic process and also results of our study revealed that lidocaine which is used a sodium channel blocker has an impact on gene expression levels of *TRPM 2* and 8. So we think that lidocaine may give beneficial results for treatment of hepatic ischemia reperfusion injury.

Keywords : Lidocaine, ischemia-reperfusion injury, TRPM 2, TRPM8

▶ Poster No. 70

Activation of TRPV4 cation channels in HEK 293 cells can be antagonized by the tarantula peptide toxin GsMTx-4

Christian SJ Kesselring, Jörg Eisfeld, Mirjam Krautwald, Yaxin Zhang, Martin Landsberger, Heinrich Brinkmeier

Institute of Pathophysiology, University Medicine Greifswald, Karlsburg, Germany

The transient receptor potential channel TRPV4 is a calcium conducting, osmosensitive cation channel functioning as a mechanosensitive channel in skeletal muscle. Since muscular mechanosensitive channels can be blocked by the peptide toxin GsMTx-4, we studied whether TRPV4 itself is sensitive to GsMTx-4. To this end HEK 293 cells were transiently transfected with a TRPV4-YFP construct. Intracellular Ca^{2+} concentrations were monitored with the fluorescent Ca^{2+} indicator Fura-2 and the data shown as Fura-2 fluorescence ratios after alternate excitation at 340 and 380 nm. Non-transfected cells (n=47) showed an average resting ratio of 0.39 (range 0.38 and 0.42), while TRPV4 transfected cells (n=524) showed on average an increased ratio of 1.11. Application of 4a-PDD (5 μ M) further increased

the fluorescence ratios to 1.74 with a peak or plateau at 1-3 min (n>40 cells, three independent experiments). The effect of 4a-PDD could be reversed within 10-30 min by application of the TRPV4 blocker HC-067047 (1 μ M) and by GsMTx-4 (5 μ M). Since TRPV4 overexpression caused disturbances of cellular Ca²⁺ homeostasis, even without stimulation by 4a-PDD, we decided to use better controlled inducible expression systems. Stable transfection of TRPV4-YFP in a Flp-In 293 T-REx cell line and a corresponding CHO cell line caused moderate TRPV4 expression in response to induction with doxycycline. Patch clamp investigations on these cell lines are in progress to study TRPV4 pharmacology. The results support the view that TRPV4 channels are sensitive to the mechanosensitive channel blocker GsMTx-4. The results may have impact for mechanosensation and neuromuscular function.

► Poster No. 71

Controlled internal drug release use for synchronization on paraoxonase activities and total sialic acid levels in Abasian goats*

Mushap Kuru¹, Abdulsamed Kükürt², Recai Kulaksız³, Hasan Oral¹, Nebi Çetin⁴, Mahmut Karapehlivan⁵

¹Department of Obstetrics and Gynecology, ²Department of Biochemistry, ³Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey

⁴Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Yüzüncü Yıl, Van, Turkey

⁵Department of Medical Biochemistry, Faculty of Medicine, University of Kafkas, Kars, Turkey

The aim of this study was to determine the effects of the use of Controlled Internal Drug Release (CIDR) on paraoxonase activities (PON) and total sialic acid (TSA) levels in Abasian goats. In this study, 30 Abasian goats, aged 2-4 years, were synchronized in breeding season. The CIDR device (Eazi-Breed CIDR[®]) was used for a period of 11 days. Two days before the removal of the CIDR from the vagina, it was injected with equine chorionic gonadotropin (Chronogest[®]) and

prostaglandin F2 alpha (Dinolytic[®]). Pregnancy diagnosed was performed 30 days after the breeding with transrectal ultrasonography. Blood samples were collected from the 30 goats (20 pregnant + 10 non-pregnant) 8 days before the synchronization on days 0 and 11, and on the day of breeding. The serum samples were used for the measurement of PON, cholesterol, triglyceride, high density lipoprotein (HDL) and TSA levels. PON, triglyceride, HDL and TSA concentrations were significantly differ for the day of measurement (P<0.001). PON, and HDL levels on day 11 were the lowest (P<0.001). TSA levels on day 11 were found to be higher than the other days (P<0.001). The triglyceride levels were decreased on the day of breeding (P<0.001). At the HDL levels on the day of 11, TSA levels on the day of breeding were differ between the pregnant and non-pregnant goats (P<0.05). In conclusion, CIDR used for estrus synchronization of the goats reduced the serum PON and HDL levels, and increased the TSA and triglyceride levels.

Keywords: Paraoxonase, Total sialic acid, Synchronization, CIDR, Abasian goats.

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► Poster No. 72

Application of initiated chemiluminescence in monitoring of oncological process

Iryna Oliynyk

Department of Biophysics, science and higher mathematics, Lviv Medical Institute, Lviv, Ukraine

The present work aims to analyze the possibilities of application of initiated chemiluminescence (ICL) method in monitoring of treatment of oncological processes of mucous membrane of mouth and larynx. It is established, that ICL can be used in this case as inexpensive and highly informative express-method. We use the induction of CL by hydrogen peroxide with iron sulfate (II). To check the possibility of application of this method, 248 experiments on the analysis of the CL of blood serum of persons with oncological diseases of

mucous membranes were performed as well as and 306 experiments for formation of control group of healthy people. Patients with oncological deceases were divided into 5 groups: «before medical treatment», «one month after the radical operation», «after irradiation», «patients without evidence of tumour progression (after treatment)», «patient with recurrent tumour». All results obtained are compared with data of ICL of healthy people that form the control group. We found an essential metabolic difference only in those kinds of OP which are characterized by two maxima on chemiluminograms. These OP are only 12.81% in group of patients with oncological diseases. It is established the possibility of application of ICL methods in monitoring the quality of operation and control over the medical treatment at different stages when two maxima of ICL are present. Despite the fact that study of chemiluminograms with two maxima are mostly informative at present, this doesn't exclude the existence of other methods of quantitative analysis of kinetics of ICL and encourage the investigation of such methods. Any OP introduces changes into the functionality of organism and they should be reflected in ICL.

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► Poster No. 73

Effect of doxycycline/collagen coaxial nanofibers on oxidative stress in the wound healing I. malondialdehyde and glutathione levels

Serdar Tort¹, Esra Oğuz², Tuğçe Özmen², Fatmanur Tuğcu Demiröz¹, Şule Coşkun-Cevher², Füsün Acartürk¹

¹Department of Pharmaceutical Technology, Faculty of Pharmacy, Gazi University, Ankara, Turkey

²Department of Biology, Science Faculty, Gazi

University, Ankara, Turkey

Wound healing includes three overlapping phases: Hemostasis/inflammation, proliferation/repair and remodeling (1). Nanofibrous wound dressings have useful properties for wound healing phases, such as less inflammation, cytocompatibility, high porosity and drug loading capacity (2). The aim of the present study was the assessment of electrospun nanofibers applied once or daily on oxide stress parameters of acute wound in rats.

Wound dressings were prepared by coaxial electrospinning method. Collagen was loaded to the core and doxycycline was loaded to the shell of coaxial nanofibers. Wistar rats (n=12) were used for wound healing studies. Rats were divided into two groups. After shaving of the dorsal hair, full thickness excisional wounds were created using 1 cm biopsy punches. Group I were treated with doxycycline/collagen coaxial nanofibers only once after initial wounding. Group II were treated with same nanofibers once daily. After three days, rats were sacrificed and tissues were excised. Tissues malondialdehyde (MDA) and glutathione (GSH) levels were determined by spectrophotometric measurements.

MDA levels were found 26.2 nmol/g and 24,35 nmol/g in Group I and II, respectively and there was no significant difference ($p>0,05$) between the two groups. GSH levels were found 1.58 $\mu\text{mol/g}$ and 0.846 $\mu\text{mol/g}$ in Group I and II, respectively. There was significant difference between the two groups ($p<0,05$).

In conclusion, doxycycline/collagen coaxial nanofibers enhanced antioxidant capacity of wound in rats especially in group I. Only once applied nanofibers may be effective on wound healing process rather than once daily application.

Keywords: Wound healing; Nanofibrous; Oxidative stress

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Evaluation of paraoxonase activity and total sialic acid levels in pregnant cow with subclinical paratuberculosis

Mustafa Makav¹, Abdulsamed Kükürt², Mushap Kuru³, Hüseyin Avni Eroğlu⁴, Hacı Ahmet Deveci⁵

¹Department of Physiology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

²Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

³Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

⁴Department of Physiology, Faculty of Medicine, Kafkas University, Kars, Turkey

⁵Department of Medical Services and Techniques, Vocational School of Higher Education in Islahiye, Gaziantep University, Gaziantep, Turkey

Known as Johne's Disease, bovine paratuberculosis caused by *Mycobacterium avium subsp. Paratuberculosis* is a kind of infection characterized with the bowel wall thickening, the chronic enteritis unresponsive to treatment, and cachexia. Having the effect role to the etiology of the Crohn's disease, this infection is known as an important zoonotic as well. Herewith, Diagnosis of the disease and the related studies are vital for human health. In this study, it was aimed to determine to Paraoxonase Activity (PON), Total Sialic Acid (TSA) and Malondialdehyde (MDA) in pregnant cows with subclinical paratuberculosis.

In the study, it was used two groups; one of them is the study group:12 pregnant cows among 2-6 years old and with subclinical paratuberculosis determined with ELISA Kit, and the second of them is the control group: similarly old 12 health pregnant cows. Based upon the blood samples, the level of PON, TSA and MDA was measured in the method of colorimetric.

According to the data, when being statistically compared with the control group, it was determined that there was a significant decrease ($P<0.001$) in PON in the study group. However, it was determined that there was a significant increase ($P<0.001$) in TSA. Although

there was a numerical difference in MDA, it was not found statistically significant ($P>0.05$).

In conclusion, there was a decrease in PON, and an increase in TSA in pregnant cows with subclinical paratuberculosis. In this line, it could be proposed to study the measurement of TSA and PON in subclinical infections.

Keywords: Paratuberculosis, Paraoxonase Activity, Total Sialic Acid, Malondialdehyde

Serum paraoxonase activity and lipid profile in bulls with foot and mouth disease

Hacı Ahmet Deveci¹, Mustafa Makav², Abdulsamed Kükürt³, Erdoğan Uzlu⁴, Gökhan Nur¹, Şemistan Kiziltepe⁵, Mahmut Karapehlivan⁶

¹Department of Medical Services and Techniques, Vocational School of Higher Education in Islahiye, Gaziantep University, Gaziantep, Turkey

²Department of Physiology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

³Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

⁴Department of Internal Medicine, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

⁵TC Ministry of Food, Agriculture and Livestock, Provincial Directorate, Kars, Turkey

⁶Department of Medical Biochemistry, Faculty of Medicine, Kafkas University, Kars, Turkey

The study was comprised of 12 bulls, aged between 18-36 months, was found the severe symptoms of Foot-and-mouth disease (FMD) and 10 clinically healthy bulls of similar age. Serum total paraoxonase activity (PON), triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels were colorimetrically measured. Moreover; it was were found the acute fever, anorexia, vesicular lesions in the mouth and feet of infected animals with consequent excessive salivation, lameness and reduced productivity as clinical signs. Serum PON activity ($p<0.001$), triglyceride

($p < 0.01$), total cholesterol ($p < 0.01$), HDL ($p < 0.01$), LDL ($p < 0.01$) and VLDL ($p < 0.01$) levels were found statistically significant in the FMD group when compared to the healthy ones. All in all, paraoxonase activity and lipid profile in serum were affected so significantly in bulls with foot and mouth disease.

Keywords: Paraoxonase activity, HDL, LDL, Foot-and-mouth disease, Bulls

► Poster No. 76

Effect of apelin on oxidative stress and obesity in serum of obese patients

Fatma Betül Daşgın Fakıoğlu¹, Nesrin Emekli¹, Sevilay Tarakcı Zora², Türkan Yiğitbaşı¹, Gözde Ülfer¹

¹Medical Biochemistry Department Department of Medical Biochemistry, Institute of Health Sciences, Istanbul Medipol University, Istanbul, Turkey.

²Istanbul Aydın University, Vocational School of Health Services, İstanbul, Turkey

Obesity is the medical condition in which body fat is accumulated excessively in the body. Body fat is the source of some adipokins and in turn plays role in energy balance of the body. In the study we investigated the effect of the apelin adipokinin on antioxidant system and some parameters related to energy metabolism. For this purpose, samples were acquired from clinically obese ($n=61$, BMI > 24.9) and non-obese ($n=24$, BMI between 18.5-24.9) cases, obtained from volunteers with the age range of 18 to 75.

Apelin amount was investigated using ELISA, total oxidant and antioxidant levels were measured via colorimetric testing, biochemical parameters such as glucose, insulin, LDL-cholesterol, HDL-cholesterol and triglycerides (TG) levels were measured via photometric testing. HbA_{1c} and CRP levels were quantified *in vivo* immunochemiluminescence. Insulin resistance was calculated via HOMA-IR method and oxidative stress index was assigned using Erel's method.

Results show no significant difference between the apelin levels of obese and non-obese samples ($p > 0.05$).

Moreover, apelin level shows no correlation with the various biochemical parameters mentioned ($p > 0.05$). Oxidative stress, TG, glucose levels, insulin resistance and the HbA_{1c} seems to have significantly higher value in samples acquired from obese cases ($p < 0.05$). As a conclusion to this study, we discuss that more scientific research needs to be conducted to understand the effect of apelin on obesity metabolism at a clinically significant level.

Keywords: Obesity, apelin, oxidative stress, adipose tissue.

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► Poster No. 77

The effects of melatonin and L-Carnitine on lipid profile and oxidative stress in type 2 diabetic rats

Derya S. Salmanoğlu¹, Tugba Gurpinar¹, Kamil Vural¹, Nuran Ekerbicer², Ertan Darıverenli¹, Ahmet Var³

¹Department of Pharmacology, ²Physiology and ³Biochemistry, Faculty of Medicine, Celal Bayar University, Manisa, Turkey.

Oxidative stress is thought to play a major role in the development of diabetic cardiovascular disease. It was suggested that antioxidant treatment might be an important therapeutic option for preventing vascular complications in diabetes mellitus. Melatonin and L-carnitine have strong antioxidant properties and ameliorating effects on lipid profile.

Diabetes induced with high fat diet (for 8 weeks) and multiple low doses intraperitoneal injection of STZ

(twice, 30 mg/kg/d i.p). The animals in the experimental group were randomly assigned as follows: Control group (C), high fat diet (HFD), STZ-induced diabetic group (HFD + STZ), HFD + STZ diabetic group received melatonin (10mg/kg/d i.p), HFD + STZ diabetic group received L-carnitine (0.6 g/kg/d i.p), and HFD +STZ diabetic group received glibenclamide as positive control (5mg/kg/d, oral). The serum fasting blood glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride and malondialdehyde (MDA) levels were tested. Also, glutathione peroxidase (GPx), superoxide dismutase (SOD) levels activities were determined in rat liver.

According to our study melatonin and L-carnitine treatment decreased fasting blood glucose, total cholesterol, and LDL levels. MDA levels significantly decreased with the melatonin treatment whereas SOD levels were not significantly changed between the groups. The results suggest that especially melatonin treatment has significant beneficial effects in the oxidative stress in diabetes.

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▶ Poster No. 78

Implementation of an automated patch clamp system for the study of Nav1.7 channels and TRPV4 channels stably expressed in CHO cells

Jörg Eisfeld¹, Mirjam Krautwald¹, Thomas Knott², Heinrich Brinkmeier¹

¹Institute of Pathophysiology, University Medicine Greifswald, Karlsburg, Germany

²Cytocentrics Bioscience GmbH, Rostock, Germany

The transient receptor potential channel TRPV4 is

regulated by various stimuli, such as mechanical stress, temperature and chemical substances. To study the regulation and pharmacology of TRPV4 channels in detail, an automated patch clamp system (Cytospatch⁴) was applied. Major advantages of the system are its ability of temperature control, temperature variation in a wide range and its small solution and drug consumption. To establish the automated patch clamp system we recorded whole cell Na⁺ currents from CHO cells stably expressing the human Nav1.7. The sodium currents showed the same electrophysiological characteristics as known from manual patch clamp experiments. Na⁺ inward currents were maximal upon square voltage pulses going from -90 mV to -10 mV. The currents were reversibly inhibited by ranolazine in a concentration dependent manner. Complete block of Nav1.7 currents was achieved by tetrodotoxin (TTX) application at 1 μM. To study TRPV4 currents, we expressed the TRPV4 in a CHO cell line that expresses the tetracycline regulated transactivator. In this cell line expression of the gene of interest, coding for the TRPV4-YFP fusion protein, can be induced by the addition of doxycycline to the culture medium. Small TRPV4 currents could be recorded in the whole cell configuration by ramp protocols going from -100 mV to +100 mV. Currents could be slightly stimulated by application of 4α-PDD and antagonized by the TRPV4 blocker GSK 2193874. We conclude that automated patch clamp is probably suitable for the study of TRPV4 channels, but CHO cells show only marginal expression.

▶ Poster No. 79

Chard (*Beta vulgaris* L. var. *cicla*) ameliorates the adverse effects of valproic acid toxicity in small intestine

Burcin Alev¹, Sevim Tunali², Unsal Veli Ustundag¹, Hazal Ipekci¹, Hazal Hazineci¹, Ozan Ozcan¹, Bahar Kuruca¹, Ebru Emekli-Alturfan¹, Tugba Tunali-Akbay¹, Refiye Yanardag², Aysen Yarat¹

¹Department of Basic Medical Sciences, Biochemistry, Faculty of Dentistry, Marmara University, Maltepe, Istanbul, Turkey

²Department of Chemistry, Faculty of Engineering,

Istanbul University, Avcilar, Istanbul, Turkey

Valproic acid (VPA) is an antiepileptic drug and has severe toxic effects in experimental animals and humans (1). Chard (*Beta vulgaris* L. var. cicla) has antioxidant, antidiabetic, antitumor and hepatoprotective effects (2). The aim of this study was to examine the effects of chard on VPA-induced small intestine damage. Female Sprague Dawley rats were grouped as; control, chard given control (100 mg/kg/day, by gavage); VPA (500 mg/kg/day, intraperitoneally) and chard given VPA (100 mg/kg/day chard by gavage, 500 mg/kg/day VPA, intraperitoneally). The aqueous extracts of chard leaves were given 1 h prior to administration of VPA for seven days. Biochemical parameters such as glutathione (GSH), malondialdehyde (MDA) as an index of lipid peroxidation, sialic acid (SA) levels and glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), tissue factor (TF) activities were determined in small intestine homogenates. Decreased small intestine GSH levels, SOD and GST activities, increased MDA, SA levels and TF activity were detected in the VPA group compared to the control group. Chard administration significantly reversed GSH, MDA levels and TF activity. However, it caused decreases in SOD and GST activities and increase CAT activity compared to the VPA group. This depletion may be associated with enhanced glutathione metabolism. Based on our results chard may be suggested to reverse the oxidative stress in VPA-induced small intestine toxicity.

Keywords: Valproic acid, small intestine, antioxidant-oxidant parameters

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► Poster No. 80

The effects of white cabbage on rat skin in amiodarone toxicity

Burcin Alev¹, Ismet Burcu Turkyilmaz², Unsal Veli Ustundag¹, Hazal Ipekci¹, Ebru Emekli-Alturfan¹, Tugba Tunalı-Akbay¹, Refiye Yanardag², Aysen Yarat¹

¹Department of Basic Medical Sciences, Biochemistry, Faculty of Dentistry, Marmara University, Maltepe, Istanbul/ Turkey

²Department of Chemistry, Faculty of Engineering, Istanbul University, Avcilar, Istanbul/ Turkey

Cabbage (*Brassica oleracea* L. var. capitata) is one of the most important vegetables with high nutritional value. It is a source of anticancer glucosinolates. Moreover, it also has antimicrobial, anti-inflammatory and antioxidant properties and has high levels of flavonoids and anthocyanins (1,2). Cabbage may protect from the side effects of amiodarone (AMD) which is used for the treatment of arrhythmias (3). In this study, we aimed to investigate the effects of cabbage aqueous extract on rat skin in AMD toxicity. Female Sprague-Dawley rats were randomly divided into four groups as follows; control group receiving corn oil; cabbage extract (500 mg/kg/day) given group; AMD (100 mg/kg/day) given group; AMD + cabbage extract (in same doses for both) given group. Cabbage extract and AMD were given by gavage to rats for 7 days. AMD was given to the animals one hour after the cabbage extract administration. All animals were fasted overnight and on the 8th day they were sacrificed under anesthesia. Skin samples were taken from animals and homogenized in saline. Oxidant-antioxidant biochemical parameters were determined in homogenized skin samples. Decreased skin glutathione (GSH) levels, superoxide dismutase (SOD), glutathione S-transferase (GST) and tissue factor (TF) activities, increased malondialdehyde (MDA) and sialic acid (SA) levels were found in the AMD group compared to control group. Cabbage extract administration significantly reversed MDA levels and TF activity. However, it caused decreases in GSH levels, SOD and GST activities compared to AMD and control groups. As cabbage administration decreased MDA levels, the depletions in antioxidants may be associated with enhanced glutathione metabolism to protect from increased oxidative stress due to AMD administration. Based on our results white cabbage may protect skin from oxidative stress in AMD induced skin toxicity.

Keywords: White cabbage, amiodarone, skin, antioxidant-oxidant parameters

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▶ Poster No. 81

Antioxidant effects of astaxanthin in neurodegenerative diseases

Nilüfer Genç Özdamar, Nurhan Gümral

Department of Physiology, Medical Faculty, Süleyman Demirel University, Isparta, Turkey

Brain aging constitutes an important aspect of the aging process. Considerable experimental evidence that brain aging takes part in the development of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease (1). Cognitive disorders (Alzheimer's, Parkinson's, etc.) are the unresolved problems of an aging society. For example Alzheimer's disease in the population has 17% between 65-69 years, the incidence increases with age and in people after the age of 95 about 50% (2). The underlying cause of the aging process is not illuminated enough by now. However, it can be said that it is complex and multifactorial. One of the theories that explain this process is the oxidative stress theory (3). This theory, as to date; progressive oxidative damage due to excessive accumulation of reactive oxygen species and free radicals comprise lead to physiological dysfunction. Astaxanthin (ASTX), a pink -orange carotenoid pigment, naturally exists in many aquatic animals, such as shrimp, crab and salmon (4). *Haematococcus pluvialis* is believed to accumulate the highest levels of astaxanthin in nature (it can accumulate >30 g of astaxanthin kg⁻¹ dry biomass). Studies have shown that ASTX has antioxidant, antitumor, anticancer,

antidiabetic, neuroprotective and immunomodulatory properties. It has been reported that ASTX decreased malondialdehyde and nitric oxide levels markedly restored the GSH-PX and SOD activities, markedly inhibited neuronal degeneration (4) in experimental brain pathologies.

Keywords: Oxidative stress; Astaxanthin; Neurodegenerative diseases; Antioxidants.

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▶ Poster No. 82

Protective effects of zinc, selenium and glutathione on the lipid peroxidation induced by hypoxia in HEK293 cells

Dilek Düzgün Ergün, Nural Pastacı Özsoğacı, Şefik Dursun, Derviş Özçelik

Department of Biophysics, Cerrahpaşa Medical Faculty, Istanbul University, Istanbul, Turkey.

Hypoxia is defined as the reduction or lack of oxygen in organs, tissues or cells. This can be one of the reasons of increased lipid peroxidation as documented by increased production of indicators of oxidative stress in cells (1). Zinc (Zn) and selenium (Se) are nutritionally essential in a trace amount for living organisms and they show antioxidant properties. Glutathione (GSH) is referred to as a antioxidant preventing damage to important cellular components caused by reactive oxygen species (2,3). However, it is unknown what changes are induced by hypoxia in human embryonic kidney 293 cells (HEK293). In this study, we aimed to investigate the protective effects of Zn, Se and GSH on the lipid peroxidation (LPO) induced by hypoxia in

HEK293 cells. For this purpose, HEK293 cells were divided into five study groups as: Normoxia, Hypoxia, Hypoxia+Zn, Hypoxia+Se and Hypoxia+GSH. Cells all of groups were incubated for 48 hours at 37°C in 5%CO₂. Cells in normoxia and hypoxia groups were placed in an normoxic conditions using normoxic gas mixture (20%O₂, 5%CO₂ and balance N₂) and in hypoxic conditions using hypoxic gas mixture (5%O₂, 5%CO₂ and balance N₂) for 30 min, respectively. After normoxic and hypoxic gas mixture exposure, lipid peroxidation in the cells lysate sample was analyzed. Lipid peroxidation levels were significantly higher in hypoxia group than normoxia group. Also lipid peroxidation levels in hypoxia+Zn, hypoxia+Se and hypoxia+GSH groups were statistically lower than hypoxia group. The results show that hypoxia induced oxidative stress via LPO and Zn, Se and GSH reduced effects on oxidative stress of hypoxia. Our findings provide evidence that Zn, Se and GSH significantly ameliorate oxidative stress induced by hypoxia in HEK293 cells.

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► Poster No. 83

Effects of zinc and selenium on electromagnetic field-induced oxidative stress in HEK293 cells

Nural Pastacı Özsoğacı, Dilek Düzgün Ergün, Derviş Özçelik

Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

There is a widespread use of 2.45 GHz frequency radiation in parallel to technological developments such

as industrial, medical, military and especially education and training. Therefore, societies are increasingly exposed to electromagnetic fields (EMF). Recent studies show the effects of EMF on biological systems. Some of the adverse effects of electromagnetic field on tissues are oxidative stress and cell damage (1,2). Zinc (Zn) and selenium (Se) are trace elements which found in all organisms and antioxidant enzymes, consequently these elements protect against oxidative stress by acting as cofactors for antioxidant enzymes (3). The present study was designed to investigate the effects of 2.45 GHz EMF on lipid peroxidation and the protective effects of different Zn and Se levels in human kidney embryonic cells (HEK293). For this purpose six main group are formed as; control, EMF, 50µM Zn+EMF, 100µM Zn+EMF, 100nM Se+EMF, 200nM Se+EMF. The cells of EMF groups were exposed 2.45 GHz EMF for 1 hour. Element groups were incubated at CO₂ incubator with different levels of Zn and Se for 48 hour and thereafter they were exposed 2.45 GHz EMF for 1 hour. After the experimental procedure lipid peroxidation levels were measured of each groups. Lipid peroxidation levels were significantly higher in all EMF groups than control group, nevertheless Zn and Se treatment significantly reduce the lipid peroxidation of EMF groups. In conclusion, our findings show that EMF cause the oxidative stress in HEK293 cells by increasing the levels of lipid peroxidation. The supplementation of Zn and Se may make protective effect against oxidative stress which induced by EMF in HEK293 cells.

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Impaired morphology and oxidative stress biomarkers in the male rat spleen following exposure to continuous 900 MHz electromagnetic field for 1 hour a day throughout adolescence

Gökçen Kerimoğlu¹, Ersan Odacı¹, Şafak Ersöz²

¹Department of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

²Department of Pathology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

The purpose of this study was to investigate changes occurring in the male rat spleen exposed to the effect of 900 Megahertz (MHz) electromagnetic field (EMF) during adolescence using histological and biochemical methods. Twenty-four male Sprague Dawley rats, aged 21 days, were used. These were divided equally into one of three groups; control (CG), sham (SG) or EMF (EMFG). CG was not exposed to any procedure. SG rats were kept inside a Plexiglas cage, without being exposed to the effect of EMF, for 1 h a day on postnatal days 22-59, inclusive. EMFG rats were exposed to continuous 900 MHz EMF for 1 h a day under the same conditions as the SG rats. All rats were sacrificed on postnatal day 60, and their spleens were removed. Tissues were stained with H&E. Oxidant/antioxidant parameters were studied in terms of biochemistry. CAT and GSH levels in EMFG decreased significantly compared to CG and SG ($p=0.004$ and $p=0.004$, respectively for CAT; $p=0.004$ and $p=0.004$, respectively for GSH). MDA and SOD levels in CG decreased significantly compared to SG and EMFG ($p=0.004$ and $p=0.004$ respectively for MDA; $p=0.006$ and $p=0.004$ respectively for SOD). At histological examination, splenic tissue exhibited normal capsules and trabeculae in CG and SG, and normal lymphocyte and reticular cells. However, histopathological changes were observed in white pulp of the rat spleen in EMFG. We conclude that exposure to 900 MHz EMF during adolescence can cause pathological and biochemical changes in the male rat spleen.

Keywords: Electromagnetic field; Spleen: Antioxidant; Oxidative stress.

Iron induced intracellular calcium changes in K562/Dox

Yunus Emre Halis, Leman Yalçıntepe Güneştutar

Department of Biophysics, Istanbul Faculty of Medicine, Istanbul University, Capa-Istanbul, Turkey

Cancer disease may re-occur during the use of chemotherapeutic agents (eg : doxorubicin) in cancer treatment process and sometimes drug resistance mechanisms may develop against drugs even which had not been maintained (1). P-gp (P-glycoprotein) plays an important role in these kinds of drug resistance mechanisms. Drug resistant cells are exposed to physiological and genetic modifications (2). The regulation of iron and calcium levels inside the cell is our focus and we aimed to observe whether these levels are modified. K562 cells were maintained with doxorubicin (<250nM). Drug resistance development process was analyzed by flow cytometry via P-gp expression and %52,62 P-gp expression was observed. Then intracellular Ca^{2+} regulation (accumulation, uptake, depletion) was analyzed via iron in both doxorubicin resistant K562D and K562S sensitive cells with/without iron chelator (DFO) (100 μ M) treatment. Iron had no significant effect on K562D cells viability while DFO had. Increased DFO doses inclined reduced cell viability. Calcein-AM fluorescent dye was used to analyze intracellular iron chelation and iron uptake. We demonstrated that increased doses of iron caused increased Ca^{2+} levels in K562S and K562S/DFO cells. Controversially, K562D and K562D/DFO cells showed decreased Ca^{2+} levels pattern. DFO caused more sensitivity to iron in both sensitive and resistant cells. The presence of calcium inhibitors, DFO treated or non-treated K562 and K562D cells were maintained with iron. DFO increased the sensitivity to iron in both groups of cells. In conclusion, these results indicate that DFO may be used combined with chemotherapeutic agents to reverse drug resistance potential of cancer cells.

Keywords: Iron; Doxorubicin; Drug resistance

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► Poster No. 86

Total antioxidant/oxidant status and oxidative stress index in serum and saliva of children with sickle cell disease

Mine Öztürk Tonguç¹, Selma Ünal², Onur Bobuşoğlu³, Gürbüz Polat³

¹Süleyman Demirel University Faculty of Dentistry, Department of Periodontology, Isparta, Turkey

²Mersin University Faculty of Medicine, Department of Pediatrics, Mersin, Turkey

³Mersin University Faculty of Medicine, Department of Biochemistry, Mersin, Turkey

Sickle cell disease (SCD) is a chronic inflammatory disease in which vaso-occlusive crisis and endothelial dysfunction are present (1). Sickling and hemolysis disturb microcirculation with resultant oxidative and inflammatory stress in SCD (2). The aims of the study were to investigate the serum and salivary total antioxidant and oxidant status and oxidative stress index levels in children with SCD and to compare them with their healthy counterparts.

Forty-three children with SCD and 43 healthy children were included in the study. The blood and saliva samples were collected. Total oxidant status (TOS) and total antioxidant status (TAOC) levels in serum and saliva were measured by an automatic colorimetric method. The oxidative stress index (OSI) a novel biomarker for oxidative stress was calculated as $[TOS / (TAOC \times 100)]$. The serum TOS and OSI level was increased in children with SCD whereas serum TAOC levels significantly decreased in comparison with the controls ($p < 0.05$). While the serum OSI values of the

SCD patients were significantly higher, there was no statistically significant difference between the groups regarding salivary OSI values. Besides, serum TAOC level positively correlated with salivary TAOC level and negatively correlated with serum and salivary OSI levels. In conclusion, sickle cell disease increases oxidative stress in blood but it has no effect on oxidative stress in saliva. The antioxidant systems in saliva may be effective on elimination of oxidative stress in the mouth.

Keywords: sickle cell disease, oxidative stress, saliva, oxidative stress index

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► Poster No. 87

The role of oxidative stress in association of metabolic syndrome and periodontitis

Mine Öztürk Tonguç

Department of Periodontology, Faculty of Dentistry, Süleyman Demirel University, Isparta, Turkey

Periodontitis, a chronic infection characterized by chronic inflammation in the tissues surrounding the teeth, is formed by a large number of pathogenic microflora. Inflammatory mediators such as C-reactive protein (CRP), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) levels and products derived from oxidative damage increase in blood in individuals with periodontitis (1). It is known that periodontal disease, associated with hyperlipidemia, type 2 diabetes mellitus and cardiovascular disease, results in systemic inflammation, oxidative stress and endothelial dysfunction (2). Metabolic syndrome is a combination of obesity, hypertension, impaired glucose tolerance or diabetes, hyperinsulinemia, and dyslipidemia. In these systemic conditions increased

serum levels of pro-inflammatory cytokine and products derived from oxidative damage are observed (3). Oxidative stress may act as a potential common link explaining relationships between each component of metabolic syndrome and periodontitis. The aim of the presentation was to evaluate the role of oxidative stress in bi-directional relationship between metabolic syndrome and periodontitis.

Keywords: metabolic syndrome, oxidative stress, periodontitis, obesity, hyperglycemia

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▶ Poster No. 88

The electrophoretical determination of serum protein fractions in lycopene treated experimental diabetic rats

Veysel Yuksek , Semiha Dede, Ebubekir Ceylan

Department of Medical Laboratory Techniques, Vocational School of Ozalp, Yuzuncu Yil University, Van, Turkey.

This study was planned to determine the effects of lycopene treatment on serum protein fractions in experimental diabetic rats. In order to induce diabetes in rats in the diabetes (D) and diabetes + lycopene (DL) groups, rats were given 45 mg/kg single-dose streptozotocin intraperitoneally. Lycopene (10 mg/kg/day dissolved in sunflower oil) was administered to the rats in the lycopene-only (L) and DL groups. Blood glucose levels and HbA1c% in DL group and diabetes group increased ($p < 0.05$) compared to control and L group. Total protein, albumin, a1, a2, and b globulin fractions of diabetic and DL groups were lower

than control and L groups ($p < 0.05$). D group had lowest gamma (c) globulin levels among other groups ($p < 0.05$). The c globulin levels was slightly increased than diabetic groups (D and DL), but it was still lower than control and L groups ($p < 0.05$). The highest value of A/G ratio was observed in diabetic group. Similarly, the % level of A/G ratio of D group was higher than other groups. It was noted that the A/G ratio decreased and reached to control group levels after lycopene treatment.

Keywords: Serum protein fractions; lycopene; Diabetes

▶ Poster No. 89

The relationship between antioxidant capacity and serum copper and zinc levels in smoking individuals

¹Denizhan Karis, ¹Fatma Ates Alkan, ²Gulfidan Cakmak, ¹Alev Meltem Ercan

¹Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

²Department of Respiratory Medicine, Haseki Education and Research Hospital, Istanbul, Turkey

Smoking induces the oxidative stress in chronic obstructive pulmonary disease (COPD) via radical production from oxidative stress by chemicals in cigarette (1). The accepted efficacy of copper (Cu) and zinc (Zn) in organism play important role in oxidative balance related with nitric oxide (NO_x) and asymmetric dimethylarginine (ADMA). Zn is crucial in nucleic acid metabolism and is a fundamental anti-oxidant cofactor in endothelial cells aligning the biological systems (2). Cu is known to include pro-oxidative properties (3). Oxidant capacity in circulatory and respiratory system diminishes bioavailability of NO_x . ADMA, which is an inflammatory competitive inhibitor of NO_x synthase, increases in oxidative stress (4). We postulated to determine to analyze the relationship between Cu-Zn and NO_x -ADMA, as endothelial damage markers in smokers without clinical diagnosis of COPD, whether the organism is affected indirectly from smoking by means of oxidative stress. 128 individuals were divided as ex-smokers, current-smokers and non-smokers.

Plasma NO_x level was determined by enzymatic methods using commercial kits. ADMA concentration was analyzed by ELISA plasma assay. Cu and Zn levels were measured using inductively coupled plasma-optical emission spectrophotometer. The levels of NO_x variables of current-smokers were significantly lower than ex-smokers and non-smokers. In conclusion, this study indicates that smoking effects to play a significant role in oxidative stress. The initial endothelial dysfunction effect of diminishing NO_x - even with no symptoms of COPD - is a significant data. The levels of copper and zinc as anti-oxidant trace elements with no alterations may be considered as proceeding of the defense mechanism.

Keywords: Smoking; Antioxidant; Element; Nitric oxide.

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▶ Poster No. 90

Serum adenosine deaminase and deoxyadenosine deaminase activities in cattle naturally infected with *Babesia bovis*

Mehmet Berköz¹, Tahir Kahraman², Taraneh Öncel³,
Metin Yıldırım⁴, Ali Erdinç Yalın⁴, Serap Yalın⁴

¹Yuzuncu Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Van, Turkey

²Yuzuncu Yıl University, Faculty of Pharmacy,

Department of Biochemistry, Van, Turkey

³Pendik Veterinary Control and Research Institute, Parasitology Laboratory, İstanbul, Turkey

⁴Mersin University, Faculty of Pharmacy, Department of Biochemistry, Mersin, Turkey

Adenosine deaminase is an enzyme present in a great number of plants and animals, found from simple invertebrates to human beings. Adenosine deaminase and deoxyadenosine deaminase activities increase substantially during mitogenic and antigenic responses of lymphocytes and conversely lymphocytes blastogenesis. Various diseases characterized by the alteration of cell-mediated immunity can be affected by the serum activity of adenosine deaminase. *Babesia bovis*, a tick-borne haemoprotozoan parasite, causes severe and fatal infections in cattle at tropical and sub-tropical areas generating serious economic losses. In this study, we investigated the activity of adenosine deaminase and deoxyadenosine deaminase activities in cattle naturally infected with *Babesia bovis*. This study was conducted on 26 bovine naturally infected with bovine babesiosis in Marmara Region, Turkey. As a control group, 22 clinically healthy bovine reared under the same management and environmental conditions were also sampled. Data were analyzed using the SPSS v16.0 program. Statistical comparisons of the groups were made using *Independent T* test. Obtained p value of less than 0.05 was considered statistically significant. The data showed that serum adenosine deaminase and deoxyadenosine deaminase activities were markedly higher in *Babesia bovis* cases. Thus, increased production of adenosine deaminase confirms the presence of an inter-relationship between T cells and neutrophils in babesiosis. Recirculation of activated T-cells and macrophages may cause higher serum adenosine deaminase activity in cattle infected with *Babesia bovis* to avoid accumulation of toxic metabolites.

Keywords: *Babesia bovis*, adenosine deaminase, deoxyadenosine deaminase

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▶ Poster No. 91

The effect of electromagnetic field on oxidative stress parameters in rat brain tissue

M. Berkoz¹, B.A. Mamur², N. Aras², M. Yildirim³, Ü. Comelekoglu⁴, S. Yalin³

¹Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Van, Turkey

²Mersin University, Faculty of Medicine, Department of Medical Biology, Mersin, Turkey

³Mersin University, Faculty of Pharmacy, Department of Biochemistry, Mersin, Turkey

⁴Mersin University, Faculty of Medicine, Department of Biophysics, Mersin, Turkey

Extremely low frequency electromagnetic fields represent one of the environment factor that influence animal organism that that conduct the organism to stress. In this study we determined the oxidative stress parameters from rat brain tissues that were exposed to the Global System for Mobile Communication (GSM) cell phone rated at a frequency of 1800MHz. We divided female mature albino rats of Wistar strain in three groups two of which were sham and control. Third group was exposed to the RF-EMF for 2 h/day for 8 weeks. At the end of the study, the rats in all groups were sacrificed by cardiac puncture under ketamine and xylazine anesthesia. Brain tissues were separated and kept at -80 °C until superoxide dismutase (SOD) and catalase (CAT) activities and glutathione (GSH) malondialdehyde (MDA) levels were measured. Tissue protein contents were measured according to the method developed by Lowry et al. using bovine serum albumin as standard. The electromagnetic field led to a significant increase in malondialdehyde (MDA) levels and significant decrease in SOD and CAT levels in the brain tissue of rats ($p < 0.05$). There was no significant

difference in GSH levels in the same tissues ($p > 0.05$). In conclusion, electromagnetic field emitting from mobile phone might produce impairments in some oxidative stress parameters in the brain tissue of albino rats.

Keywords: Electromagnetic field, oxidative stress, antioxidant, brain

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▶ Poster No. 92

Relationship between pregnancy rate and serum sialic acid levels and paraoxonase activity after synchronization with progesterone releasing intravaginal device protocol in heifers

Mushap Kuru¹, Abdulsamed Kükürt², Hasan Oral¹, Mahmut Karapehlivan³

¹Department of Obstetrics and Gynecology, Faculty of Veterinary, Kafkas University, Kars, Turkey

²Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

³Department of Medical Biochemistry, Faculty of Medicine, Kafkas University, Kars, Turkey

In this study, it was aimed to determine the relationship between pregnancy rate and serum sialic acid levels and paraoxonase activity after progesterone releasing intravaginal device application in heifers.

In the current study, 160 heifers were synchronized with 5-day Co-Synch + Progesterone Releasing Intravaginal Device (1.55 g progesterone, PRID, PRID Delta®, Ceva,

Turkey) protocol. Blood samples were taken from heifers 10 days before starting synchronization (-10th day), PRID insertion day (0th day), PRID removal day (5th day) and artificial insemination day (8th day). Pregnancy examination was conducted with transrectal ultrasonography after 30 days of artificial insemination. Serum paraoxonase (PON) activity and sialic acid (SA) levels were measured by colorimetric methods.

The pregnancy rates were determined on the day of 30, and were 50% with transrectal ultrasonography. At the 5th day of the study, serum PON activity were significantly lower (P= 0.000) and SA levels were increased (P=0.000) with PRID application. PON activity (P=0.017) and SA levels (P=0.000) were statistically different between pregnant and non-pregnant groups at the end of PRID application (on day 5)

In conclusion, intravaginal progesterone releasing device application has influenced serum paraoxonase activity and sialic acid levels. Especially, PON activity and SA levels were varied significantly with PRID application on the 5th day levels and it has been concluded that this may be a fertility indicator. Based on the findings, it was assumed that further extensive study could be elaborated.

Keywords: Heifer, Paraoxonase activity, Sialic acid, Pregnancy, PRID.

▶ Poster No. 93

Orthodontic tooth movement and oxidative stress

Neslihan Ebru Şenışık

Department of Orthodontics, Faculty of Dentistry, Süleyman Demirel University, Isparta, Turkey

Under the mechanical forces at periodontal ligament (PDL), the distribution of the stress-strain increases and pressure and tension sides occur. At pressure side, excessive pressure of PDL causes circulatory impairment as ischemia and hypoxia in the early stages of orthodontic treatment. Both hypoxia and ischemia

induced the release of reactive oxygen species (ROS). The aim of this study is to review the contemporary literature regarding oxidative stress during orthodontic tooth movement. ROS are produced in metabolic and physiological processes. Harmful oxidative reactions which may occur in the organism enzymatically and non-enzymatically removed with antioxidative mechanisms. Increase of oxidants and decrease of antioxidants can't be blocked under certain circumstances, oxidative/antioxidative balance shifts towards the oxidative status. Oxidants and antioxidants that participate in bone remodelling are effective on oxidative stress rate. Nitric oxide is a highly reactive free radical and active during orthodontic tooth movement. Malondialdehyde that is used for the measurement of lipids that is damaged by free radicals and biomarker of the DNA damage (8-hydroxydeoxyguanosine) were evaluated in the ROS determination. Myeloperoxidase was evaluated during the correction of teeth irregularities. The reviewed studies indicated that orthodontic tooth movement and the materials that are used in orthodontic treatment did not induce ROS release.

In conclusion, results of oxidants and antioxidants on orthodontic tooth movement in literature are conflicting and the subject should be clarified by future studies.

Keywords: Orthodontic tooth movement; Oxidative stress; Antioxidant

▶ Poster No. 94

Effects of timokinon application on the apoptosis and DNA damage in rats exposed to acute swimming exercise

Selçuk Emekçi, Fatmagül Yur

Department of Nutrition and Dietetics, Fethiye School of Health, Muğla Sıtkı Koçman University, Muğla, Turkey.

The aim of this present study was to investigate the effects of thymoquinone application on the muscle damage and the markers of apoptosis such as enzymes

caspase 3, caspase 8, caspase 9 and 8-OHdG levels in rats exposed to acute swimming exercise. Thirty-two wistar rats were used in this study. An approval of local ethical committee was received for his study. Rats have been allocated randomly into 4 groups: control group 1 (n=8), acute exercise group 2 (n=8), acute exercise and timokinon treated group 3 (n=8) and only thymoquinone treated group 4 (n=8), those groups were treated for 7 days. Blood was collected from the heart and samples from tissues by a proper necropsy on the study day 7 from each of rats exposed to acute swimming exercise and immediately euthanized after general anaesthesia. Caspase 3, 8 and 9 and 8-OHdG levels were analysed in the homogenate gained from the muscle tissues. There was no statistical significant difference between control groups and other groups regarding caspase 3 activity. Caspase 8 level was high in the group exposed to acute swimming and treated with thymoquinone and treated only with timokinon compared to control group ($p<0.05$). Caspase 9 level was significantly low in the acute swimming exercise group ($p<0.05$) compared to acute swimming exercise and thymoquinone treatment group and only thymoquinone treatment group. 8-OHdG levels were significantly high ($p<0.05$) in the groups other than control group. It has been reported that the formation of reactive oxygen radicals (ROS) and free radicals as well as oxidation related damages in the muscle, liver, blood and other tissues has been increasing especially during the heavy exercises. Results obtained from this study showed that the application of timokinon and acute swimming exercise in rats increased 8-OHdG level and caspase 8 and 9 level in the muscle, and respectively damages in the muscle; however, the application of timokinon did not have a preventive effect.

Keywords: thymoquinone; Oxidative stress; Apoptosis; Swimming exercise.

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▶ Poster No. 95

Therapeutic effects of melatonin on cerebral SOD, CAT, MDA and TAS levels in experimental epileptic rat model

Ferhat Şirinyıldız¹, Gökhan Cesur¹, Yüksel Yıldız¹, Mehmet Dinçer Bilgin², Cenk Orak¹, Gül Taşlı Yeşilçayır¹, Rauf Onur Ek¹

¹Department of Physiology, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey

²Department of Biophysics, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey

Epilepsy is a very common neurological disease which arises from the abnormal firing of frontal cortex neurons (1). Pentylentetrazole (PTZ) is an agent that affects on reticular formation and cortex neurons to induce epileptic seizures. The purpose of our study is to define dose dependent effects of melatonin on cerebral antioxidant and oxidant parameters in PTZ-induced epileptic rat model.

12-14 week-aged 40 male Wistar Albino rats were divided into 4 groups (n=10) as control, epileptic, 25 mg/kg melatonin treated epileptic, 100 mg/kg melatonin treated epileptic groups. Epilepsy was induced by 35 mg/kg PTZ injection, i.p. A total of 12 injections were administered in 23 days. On injection days, epileptic score determination (ESD) (2) was performed. Cerebral superoxide dismutase (SOD) activity, catalase (CAT) activity, malondialdehyde (MDA) levels and total antioxidant status (TAS) levels were determined and data was evaluated statistically.

ESD levels were higher in epileptic group and decreased with melatonin treatment. SOD, CAT and TAS levels were observed to be higher in melatonin treatment groups in comparison to epileptic group, more precisely in 100 mg/kg melatonin group. MDA levels were found to be higher in epileptic group in comparison to all other groups.

Melatonin has a dose depended therapeutic effect to induce a decrease in epileptic seizures. Melatonin increases the antioxidant capacities and decreases lipid peroxidation in a dose dependent manner. Therefore, this study reveals the antioxidant power of melatonin.

Keywords: Pentylentetrazole, epileptic rat model, melatonin, antioxidant level, lipid peroxidation, epileptic score determination.

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► Poster No. 96

Purification, characterization, and sensitivity to pesticides of peroxidase from wheat (*Triticum aestivum* ssp. *vulgare*)

Sevgi Altın¹, Hatice Tohma¹, İlhami Gülçin², Ekrem Köksal¹

¹Department of Chemistry, Faculty of Science and Arts, Erzincan University, Turkey

²Ataturk University, Faculty of Sciences, Department of Chemistry, Erzurum, Turkey

The purification and characterization of peroxidase is currently growing of interested since peroxidases have implications in various industrial and biochemical areas. In this study, wheat peroxidase was purified and characterized using different techniques. Peroxidase from wheat was purified using (NH₄)₂SO₄ precipitation, dialysis, CM-Sephadex anion exchange chromatography and SDS-gel electrophoresis. Enzyme kinetics were studied using two substrates: guaiacol and hydrogen peroxide. Km and Vmax values were calculated from Lineweaver-Burk graph for each substrate patterns. Km values for guaiacol and hydrogen peroxide were 2,467 and 7,307 respectively. Enzyme activity has been enhanced 284 folds. The pH and temperature optima were 5,5 and 40°C, respectively. Peroxidase had molecular mass of 38,8 kDa as determined by SDS-PAGE. The enzyme was highly inhibited by citric acid and CTAB.

Keywords: Oxidative stress; Antioxidants; Pesticide;

► Poster No. 97

Effects of pulsed electromagnetic fields on the L929 cell behavior during *in vitro* wound healing

Suna Saygılı¹, Mehmet Gümüşay², Fulya Gülbağça¹, Dila Hatun Sal¹, Işıl Aydemir¹, Adnan Kaya², M. Ibrahim Tuğlu¹

¹Department of Histology and Embriology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

²Biomedical Technologies, Institute of Science, Izmir Kâtip Celebi University, Izmir, Turkey

Pulsed Electromagnetic Field (PEMF) therapy is used as an adjuvant wound healing therapy by inducing tiny electrical currents on tissue (1). Pulsed radio frequency energy (PRFE) system delivers electromagnetic signals to a target tissue without the intended generation of deep heat that is based on delivery of pulsed, shortwave radio frequency energy in the 13–27.12 MHz carrier frequency range (2). Despite the multitude of studies, the biological mechanisms of these systems are unclear. One of the biological end points that PEMF therapy regulates the levels of reactive oxygen species (3). Our PEMF system delivers 75 Hz frequency, 1 mT magnetic field intensity and square waveform to the tissue with a Helmholtz coil and our PRFE system delivers 27.12 MHz PSK modulation 13 dBm amplitude with a spiral antenna. L929 cell line is used to determine PEMF and PRFE application effects on wound healing. In our study, we aimed that effect of PEMF and PRFE exposures on the wound healing in the L929 cell line with wound model. Wound model was performed with needle of ppd syringe in shape of plus. Cultured cells were exposed to the PEMF for 5 hours. The proliferations of the cells are measured by MTT assay with formazan reaction. The wound healing was investigated by closure of the wound by the cell proliferation with cell morphology using inverted microscope images. At the end of the 24 hours L929 cells had more proliferative activity by PEMF exposure compared to control cultures.

Keywords: Pulsed electromagnetic field; Oxidative stress; Wound healing; Cell viability.

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▶ Poster No. 98

Effects of pulsed electromagnetic fields on the behavior of neural wound healing in NA2B cells

Mehmet Gümüşay¹, Suna Saygılı², Fulya Gülbağça², Dila Hatun Sal², Işıl Aydemir², M. İbrahim Tuğlu², Adnan Kaya¹

¹Department of Biomedical Technologies, Institute of Science, Izmir Katip Celebi University, Izmir, Turkey.

²Department of Histology and Embryology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey.

During the past 20 years, there has been growing interest in investigating the possible effects of extremely low frequency electric and magnetic fields (1). The effects of low frequency pulsed electromagnetic fields (PEMF) on wound healing in vitro were examined by using NA2B cells. The exposed and control cells were kept under the same condition. The exposed cells were applied PEMF for 5h/day to a 1 mT at 75 Hz frequency and square waveform for 3 days. The effects PEMF on cell proliferation, oxidative stress and TGF-beta were investigated. In Vitro scratch assay was performed to study cell migration and cell proliferation. a 'wound gap' in a cell monolayer was manually generated by a pipette tip with shape of plus, followed by monitoring the 'healing' of wound. Cell proliferation rates were determined by the acquired images of the gaps was taken at 0, 24, 48 and 72 h after the gaps were created. PEMF significantly enhanced the proliferation of NA2B in the culture medium at 75 Hz (P < 0.05). In conclusion, PEMF have been shown to be beneficial in repairing wounds, but the mechanism of action is

unclear (2). PEMF have been known to modulate the production of growth factors and effects intracellular calcium ion (Ca²⁺) (3). In conclusion, our results provide evidence to support the concept that PEMF decreases oxidative stress (4). This suggests that PEMF may facilitate wound healing. This finding not only shed light on the PEMF mechanism, but suggests that PEMF can be used in the treatment of chronic wounds.

Keywords: Pulsed electromagnetic fields; Wound healing; Cell proliferation; Oxidative stress.

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▶ Poster No. 99

A comparison with the tail-flick method on the analgesic activity of induced by different extremely low frequency electromagnetic field intensities

Ayşe Demirkazık Çaңçalar¹, Ercan Özdemir², Gökhan Arslan², Olca Kılınç¹, A Şevki Taşkıran²

¹Department of Biophysics, Medical Faculty, Cumhuriyet University, Sivas, Turkey.

²Department of Physiology, Medical Faculty, Cumhuriyet University, Sivas, Turkey.

One of the most consistent findings of exposure to extremely low frequency magnetic fields (ELFMF) has been the effect on nociception. In general, pulsed ELFMF decrease nociception (induce analgesia) (1,2). We aimed to compare the effects of analgesia activity by tail-flick (TF) method of different intensities of ELFMF magnetic field. Twenty four adult Wistar albino

male rats weighing 250-300 g were used. Before study procedure, rats were randomly assigned in four groups: sham (= exposed to no MF), exposed to 1 mT intensity of MF, exposed to 5 mT intensity of MF and exposed to 10 mT intensity of MF. Groups (sham, 1 mT, 5 mT, 10 mT) were left in selenoid within a magnetic field of 50 Hz and 1, 5, 10 mT intensities for 165 min., during 15 days. Then, before the rats exposed to Magnetic field was performed the TF test, 0., 30th, 90th and 120th minute. All data showed as mean % maximal antinociceptive effects (% MPE). The analgesic effects of the ELFMF were measured as TF latencies in all groups for each rat and converted to %MPE. Data of % MPE in sham group; first day- 5,01±0,53, in 1mT group- 5,61±0,39, in 5mT group- 5,13±0,44, in 10 mT group- 4,05±0,34. Datas of %MPE in sham group-seventh day- 5,37±0,51, in 1 mT group- 13,66±1,27, in 5 mT group- 25,89±3,00 and in 10 mT group- 25,37±2,41. Obtained data suggested that % MPE TF, progressively increased in seventh day and exposed to 5 mT intensity of MF group. In conclusion, we observed that the analgesic activity of induced 5 mT intensity of ELFMF optimum dose on pain and these effects had the highest value in seventh day.

Key Words: Magnetic Field, Selenoid, Analgesia, Tail flick test, Rat

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▶ Poster No. 100

The beneficial effect of silymarin administration against nicotine-Induced oxidative stress in mice

Mahmut Karapehlivan¹, Abdulsamed Kükürt², Hasan Özen³, İnan Kaya⁴, Tolga Kasaci¹, Hacı Ahmet Deveci⁵, Elnare Günal⁶, Metin Ögün², Ayla Özcan¹

¹Department of Medical Biochemistry, Faculty of Medicine, Kafkas University, Kars, Turkey

²Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

³Department of Pathology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

⁴Department of Biology, Faculty of Arts and Sciences, Kafkas University, Kars, Turkey

⁵Department of Medical Services and Techniques, Vocational School Of Higher Education in Islahiye, Gaziantep University, Gaziantep, Turkey

⁶Department of Emergency Medicine, Faculty of Medicine, Kafkas University, Kars, Turkey

The aim of this study was to investigate the protective actions of silymarin, a standard plant extract with strong antioxidant activity obtained from *Silybum marianum* on the development of oxidative stress induced by treatment with nicotine in mice.

Male *Swiss Albino* mice were used to form the four experimental groups (Control: C, Silymarin: S, Nicotine: N, Silymarin+Nicotine: S+N), each groups consists of 7 mice. Animals were treated with intraperitoneal injection of nicotine (1.5 mg/kg), and silymarin (100 mg/kg) for 7 days. Blood reduced glutathione (GSH) was measured via the whole blood. Plasma malondialdehyde (MDA), nitric oxide (NO), paraoxonase activity (PON), and total sialic acid (TSA) levels were determined with spectrophotometric methods. Statistical analysis was performed using an appropriate statistical program.

Plasma TSA concentration were found significance difference in the nicotine treatment groups when compared to control (P<0.001). Then, it was found statistically significant at the TSA levels and PON activity at the group injected silymarin plus nicotine when compared to control (P<0.001). Blood GSH levels and PON activity of nicotine-treated mice were found to be significantly lower than that of the control (P<0.001). Moreover, blood GSH levels were found to be similar in the silymarin plus nicotine treatment group when compared to control (P=0.808). Plasma TSA, MDA, and NO levels in N group were found significantly higher when compared to S+N, S, and C groups (P<0.001). It was found that there was significantly higher plasma TSA and MDA levels in S+N groups when compared to C and S groups (P<0.001).

In conclusion, it was found that the active components of silymarin have had protective effects against oxidative action of nicotine. Based upon these data and findings, this plant extract could be used as a dietary supplement for humans exposed to nicotine.

Keywords: Oxidative stress; Nicotine; Nitric oxide, Paraoxonase activity; Total Sialic Acid.

▶ Poster No. 101

Protective role of selenium on pain results in experimental rat model of fibromyalgia

Esra Yüksel¹, Mustafa Nazıroğlu^{2,3}, Mehmet Şahin¹, Bilal Çiğ³

¹Department of Internal Medicine, Division of Rheumatology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

²Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey

³Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Fibromyalgia (FM) is a clinical disease with the symptoms of generalized body pains (hyperalgesia and / or allodynia), tenderness of specific anatomical regions (tender points), chronic fatigue and sleep disorders. FM is one of the frequently seen chronic pain syndromes in the clinical setting. We investigated the effects of selenium (Se) on pain (hot plate and Von Frey) results in the rat experimental fibromyalgia model.

40 adult female Wistar albino rats were used in our study and they were equally divided into 4 groups as control, Se, FM and FM+Se groups. For induction of FM, 0.2 ml of hydrochloric acid (HCL) (pH 4.0) was injected into the gluteus muscle of FM, FM+Se groups. Control group received intramuscular 0.2 ml sterile saline water for 2 weeks although selenium groups received intraperitoneal sodium selenite (1.5 mg/kg over day) for 14 days (total seven doses). The claw movement to the mechanical stimulus was measured by Von Frey filaments. With the hot plaque (55 °C) method the fibromyalgia (pain) was also measured.

As compared to control group the pain measurements were statistically ($p < 0.001$) increased by induction of FM although they were decreased by Se treatments.

In conclusion, we observed that selenium has pain decreasing of the experimental fibromyalgia model.

Keywords: Fibromyalgia; Selenium; Von Frey; Hot Plate.

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▶ Poster No. 102

Serum Ischemia modified albumin changes in preeclampsia and comparison with serum total oxidant and antioxidant status

Saliha Uysal¹, Aysun Toker¹, Rukiye Ozcelik², Ummugulsum Can³

¹Department of Biochemistry, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

²Department of Obstetrics and Gynecology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

³Department of Biochemistry, Konya Training and Research Hospital, Konya, Turkey

Ischemia modified albumin (IMA) is a modified form of albumin. Several studies report that IMA is elevated in various hypoxia and oxidative stress related pathologies (1). Preeclampsia is one of the disease where oxidative stress is implicated in the pathogenesis (2). The aim of the study to examine serum IMA, total antioxidant status (TAS) and total oxidant status (TOS) levels in normotensive and preeclamptic women.

Study group of current study includes 40 preeclamptic and 20 age matched normotensive pregnant women. The influence of gestational age on these markers was also investigated. Serum IMA, TAS and TOS levels were measured by spectrophotometric methods.

Serum IMA and TOS levels were significantly higher in patients with preeclampsia compared to normal pregnant patients ($p= 0.000$), whereas we found similar TAS levels between two groups ($p= 0.304$). Serum IMA, TOS and TAS levels were also unchanged between mild and severe preeclamptic women ($p>0.05$). In conclusion, serum TOS and IMA levels appear to be significantly increased during preeclampsia. However, these levels did not correlate with disease severity. These results need to be confirmed by determining IMA formation and clearance especially during preeclamptic pregnancy.

Keyword: preeclampsia, ischemia modified albumin, oxidative stress

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► Poster No. 103

Oxidative stress markers in serum of patients with acute pancreatitis

Merve Sibel Güngören¹, İbrahim Kılınç², Saliha Uysal², Aysun Toker², Kadir Küçükceran³, Mehmet Ergin³

¹Biochemistry Laboratory, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

²Department of Biochemistry, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

³Department of Emergency Medicine, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

Acute pancreatitis (AP) is an inflammatory disease of the pancreas presenting with abdominal pain and usually associated with raise pancreatic enzyme level in the blood or urine (1). AP should be recognized as early as possible to start treatment, as it may end up with metabolic derangement, sepsis, multiple organ failure

and death (2). Oxidative stress has been implicated in the pathogenesis of acute pancreatitis (3). The aim of the study was to investigate serum IMA, total antioxidant status (TAS), total oxidant status (TOS) levels and thiol levels in patients with acute pancreatitis and compare these results with control group.

Study group of the current study includes 95 patients with the diagnosis of acute pancreatitis and 56 age-matched, otherwise healthy individuals. Serum IMA, TAS, TOS and thiol levels were measured by spectrophotometric methods.

Serum thiol and TOS levels were significantly higher in patients with acute pancreatitis compared to otherwise healthy group ($p< 0.05$), whereas there was no statistically significant difference in TAS and IMA levels between two groups ($p>0.05$). Ranson scores of patients were found to be correlated with serum thiol and TOS levels.

In conclusion, serum TOS and thiol levels appear to be significantly increased during acute pancreatitis. Moreover, levels of these parameters correlate with disease severity.

Keywords: acute pancreatitis, ischemia modified albumin, oxidative stress, thiol

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► Poster No. 104

Investigation of ischemia modified albumin levels in patients with periodontitis

Fatmanur Kazancı¹, Yudum Yaral Yılmaz¹, Ayşe Tayman², Sümeyye Cihanbeyoğlu¹, Nuray Kılıç¹, Hüseyin Tuğrul Çelik¹, Mehmet Namuslu¹, Ramazan Yiğitoğlu¹

¹Department of Medical Biochemistry, Medical Faculty, Turgut Özal University, Ankara, Turkey

²Department of Periodontology, Faculty of Dentistry, Ankara University, Ankara, Turkey

Periodontitis is a disease characterized by the loss of osteoid and connective tissue due to chronic inflammation. Ischemia modified albumin (IMA) is another form of albumin in which the N-terminus is altered through a series of chemical reactions. In addition to being a potential new marker of tissue ischemia, serum IMA has become a recent interest as an inflammation and infection marker (1). On this basis we evaluated the levels of serum IMA in patients with periodontitis.

Our study groups consisted of 16 patients with periodontitis and 12 healthy volunteers. Serum IMA levels were determined with the colorimetric method described by Bar-Or et al (2). All data were analyzed with the Statistical Package for Social Science (SPSS) computer program, version 16 Microsoft Windows. $p < 0.05$ was considered to indicate significance. Serum levels of IMA were significantly higher among cases in the patient group than in the control group ($p < 0,026$).

In this study, we tried to assess the role of serum IMA in the diagnosis of periodontitis. According to our results, the serum IMA levels are higher in the group of patients with periodontitis, but further studies of a higher population of patients are required to confirm our results. In addition to this, our study indicates that elevation in serum IMA levels may also be due to the local inflammatory conditions. Hence, the use of IMA for diagnostic purposes in ischemic conditions will be of relevance.

Keywords: Ischemia modified albumin, Periodontitis, Inflammation

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Impact of alpha lipoic acid on histopathological changes in liver caused by cigarette smoking

Nurhan Gumral¹, Rahime Aslankoc², Nurgul Senol³, Fatma Nihan Cankara⁴

¹Department of Physiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

²Department of Midwifery, Faculty of Health Sciences, Suleyman Demirel University, Isparta, Turkey

³Department of Nutrition and Dietetics, Faculty of Health Sciences, Suleyman Demirel University, Isparta, Turkey

⁴Department of Pharmacology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Cigarette smoking (CS) causes a variety of adverse effects on organs that have no direct contact with the smoke itself, such as the liver (1,2). The purpose of this study is to define the protective role of alpha lipoic acid (ALA) on histopathological changes in liver tissues caused by cigarette intoxication.

Twenty eight female Sprague Dawley rats were randomly divided into three groups: Control group (n=8), CS group (n=10; 12 cigarettes/day, 8 weeks), and CS+ALA group (n=10; 12 cigarettes/day+100mg/kg, 8 weeks). At the end of the study period, liver tissues were taken from all animals for the histopathological and immunohistochemical examinations.

In the light microscopic evaluation of Masson's trichrome stain, the control group showed normal histological structure of liver tissues. Medium levels of dilatation and hemorrhagic patches were found in liver sinusoids and vessels in CS group. High levels of necrotic patches, dilatation in sinusoids and vessels were found in CS+ALA group. Moreover, an extensive fibroblast growth and increase of collagen fiber, namely fibrosis, were observed in the same group. Also, the macroscopic and microscopic examinations determined neoplastic growth and tumorigenesis in liver in CS+ALA group. Immunohistochemical analyses support our histopathological findings. TNF- α via

immunostaining had a negative result in the control group. A big number of necrotic cells were observed in CS+ALA group. TNF- α immunopositive cells in CS group were found at medium level (+2), in CS+ALA group-at extensive level (+3).

In conclusion, it was observed that cigarette smoking caused sporadic structural changes in liver tissues. The administered of ALA (100mg/kg) has no protective effect against cigarette toxication on liver tissue. Future studies which will applied different doses of ALA can bring clarity to this issue.

Key words: Cigarette smoking, liver, alpha lipoic acid

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► Poster No. 106

Effects of treadmill and swimming exercises on oxidative stress parameters in cerebellum of rats

Recep Soslu¹, Fazile Nur Ekinçi Akdemir², Ali Özkan¹, Mutlu Türkmən¹, Murat Kul¹, Taner Bozkuş¹

¹Physical Education and Sport School, Bartın University, Bartın, Turkey

²Department of Nutrition and Dietetics, Health School, Agri İbrahim Çeçen University, Agri, Turkey

There are many benefits of regular physical activity on health. However, the different types of exercise increases free radical production in a few ways. In addition to the control of muscle activating muscle contractions stimulate the cerebral cortex and cerebellum area as well as play an important role in the basal ganglia. The purpose of this study was to find out the effects of both treadmill and swimming exercises on oxidative parameters of cerebellum of rats.

Fifty six male rats were divided into two groups and one of the groups was applied 15, 30 and 60 minutes daily swimming exercises for 7 days during 12 weeks, and the

second group was applied treadmill exercises for 5 days during the same period of 12 weeks. At the end of the application, the cerebellar tissue of the rats was removed. Tissues were stored in at -70°C until analysis and homogenized on the day analysis. In the cerebellar tissue samples were measured malondialdehyde (MDA) level, superoxide dismutase (SOD), and reduced glutathione peroxidase (GSH-Px) activities.

According to the results obtained, there was a statistically significant decrease in MDA levels and significant increase in SOD levels of rats which were applied swimming exercise.

In conclusion, it was found out that the exercises applied in different periods and volumes have oxidative stress levels of cerebellar tissue. Through application of different exercise programs, different parts of brain would be affected and special training programs can be developed in order to increase physical performance related to brain's functions.

Keyword: Cerebellum, Swimming Exercise, Treadmill, Oxidative Stress.

► Poster No. 107

The radioprotective effects of propolis and caffeic acid phenethyl ester on radiation-induced oxidative/nitrosative stress in brain tissue.

Seyithan Taysı¹, Elif Demir², Kadir Çınar³

¹Department of Medical Biochemistry, Medical Faculty, Gaziantep University, Gaziantep, Turkey.

²Division of Biochemistry, College of Health, Harran University, Şanlıurfa, Turkey

³Department of Neurosurgery, Sehitkamil State Hospital, Gaziantep, Turkey.

Head and neck cancer patients treated with radiotherapy suffer severe side effects during and following their treatment. Efforts to decrease toxicity of irradiation to normal tissue, organs and cells have led to searching for cytoprotective agent. Investigations for effective and non-toxic compounds with radioprotective capability

led to increasing interest in antioxidant such as Propolis and Caffeic acid phenethyl ester (CAPE) (1). The aim of this study was to evaluate the antioxidant and radioprotective effects of Propolis and CAPE on radiation-induced oxidative/nitrosative stress in the brain tissue. Fourty Sprague-Dawley rats were randomly divided into five groups. Group 1 (Irradiation (IR) + Propolis) received total cranium irradiation and propolis was given orally through an orogastric tube daily. Group 2 (IR+CAPE) received total cranium irradiation plus CAPE, was dissolved in dimethyl sulfoxide (DMSO) just before giving to the rats, intraperitoneally (IP) every day. Group 3 (IR) received 5 Gy of gamma irradiation as a single dose to total cranium plus 1 ml saline daily. Group 4 received daily plain DMSO. Group 5 received daily plain saline. At the end of the 10 day time period, xanthine oxidase (XO), nitric oxide synthase (NOS) activities, nitric oxide (NO[•]) and peroxynitrite (ONOO⁻) levels were significantly higher in IR group compared to all other groups. In conclusion, the results suggest the radioprotective ability of Propolis and CAPE involving prevention of radiation-induced oxidative/nitrosative damage.

Keywords: Nitrosative stress; Oxidative stress; Free radicals; Irradiation.

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▶ Poster No. 108

In vitro evaluation of thiol-disulfid homeostasis in radiation-exposed sera

Ural Koç¹, Mehmet Gümüş², Almila Şenat³, Özcan Erel³

¹Department of Radiology, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkey

²Department of Radiology, Faculty of Medicine, Yıldırım Beyazıt University, Ankara, Turkey

³Department of Biochemistry, Faculty of Medicine, Yıldırım Beyazıt University Ankara, Turkey

Reactive oxygen species (ROS) are chemically active and has been associated with numerous diseases. Radiation is one of the invisible cause of ROS production. Radiation sources excite or ionize the matter, which contribute the formation of oxidative stress. Thiols are organic compounds that contain a sulfhydryl group, have a pivotal role at state of oxidative stress. One of the main targets of ROS is thiol groups on sulfur containing amino acids such as methionine or cysteine. Thiols can undergo oxidation reaction via oxidants and form disulfide bonds when oxidative stress occurs. Those disulfide bonds can be reduced back to thiol groups, so dynamic thiol-disulfide homeostasis is maintained. The aim of this study was to evaluate a novel, easily calculated, readily available, and relatively cheap oxidative stress marker, thiol-disulfid homeostasis, in vitro acute radiation-induced sera. Thiol-disulfid homeostasis was examined in 55 sera. Sera were divided into two groups. One group was put at room conditions, the other was also same but exposed to radiation in a very short interval at 0 hour (hr), 3 hr, 6 hr. Total exposure dose was 300 miligray (mGy). Basal level (0 hr), room condition level at 6 hr and radiation exposed level at 6 hr were compared. Nevertheless, there was no significant difference between serum native and total thiols on radiation exposed group and room group, the mean values at radiation exposed group lower than the room group. The other parameters were not statistically significant. It may be related to energy transfer processes between x-ray and sera. This study is the preliminary result of one condition, so that it important to evaluate higher and lower doses exposed sera to understand the X-ray effects on sera thiol-disulfide homeostasis. If the relations will be shown in vivo it is important to get dose-response curve to use it in vivo conditions.

Keywords: Thiol-disulfid; Oxidative stress; Antioxidant; Radiation.

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Modulator role of non-steroidal selective estrogen receptor modulators on TRPM2 and TRPV1 channels in the hippocampus and dorsal root ganglion neurons of ovariectomized rats: Patch-clamp results*

Yener Yazgan¹, Mustafa Nazıroğlu^{1,2}

¹Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, Turkey.

²Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey.

Transient Receptor Potential (TRP) channels are mostly calcium ion (Ca²⁺) permeable non-selective cation channels. It is well-known twenty-eight subtypes of six TRP channel subfamilies found in human cells including TRPM2 and TRPV1. Both channels have oxidative stress related activation mechanisms and expressed by neurological tissues including dorsal root ganglia (DRG) neurons. 17β-estradiol (E₂) has antioxidant role on neurological and behavioral systems in nervous system. Raloxifene (RLX) and tamoxifen (TMX) are selective estrogen receptor modulators also have antioxidant properties. Ovarian resection model use for achieving E₂ deficient ovariectomized (OVX) rats. E₂ deficiency stimulates apoptosis and reactive oxygen species (ROS) production, although RLX and TMX may reduce the mitochondrial ROS production via their antioxidant properties of ovariectomized (OVX) rats. There are limited studies about activation/inhibition manner of these channels induced by antioxidants administration. Thus, we aimed to investigate the effects of E₂, RLX and TMX on the TRPM2 and TRPV1 channel currents by Patch-Clamp technique in DRG neurons of OVX rats.

Forty female rats were divided into five groups: First group was used as control. Second group used as OVX. Third, fourth and five groups used OVX+E₂, OVX+TMX and OVX+RLX, respectively. Estrogen, tamoxifen and raloxifene were subcutaneous given to these three groups for 14 days after OVX-induction.

TRPM2 cation channel currents were significantly higher than in the OVX group compared to control group. We showed that E₂, TMX and RLX administrations inhibited TRPM2 currents in OVX+E₂, OVX+TMX and OVX+RLX groups by whole-cell configuration of Patch-Clamp. TRPV1 cation channel currents also markedly higher in OVX group in compared to control group. After E₂, TMX and RLX treatments, the currents in OVX+E₂ group were notably lower than OVX group and observed that very close to control group. In OVX+RLX and OVX+TMX groups, it has been showed that TMX and RLX administrations completely inhibited the TRPV1 currents, and have more potential effects on TRPV1 channels compared to E₂ group.

In conclusion, we observed those of E₂, TMX and RLX administrations are useful on TRPM2 cation channel inhibition. Although E₂ treatment also has effect on TRPV1 inhibition, TMX and RLX administrations are seem to be have more potent inhibitor activity on TRPV1 channels in hippocampal and DRG neurons of OVX induced rats.

Keywords: TRPM2; TRPV1; Patch-Clamp; OVX method.

*The study was partially supported by the Unit of Scientific Research Project (BAP), Süleyman Demirel University (Project Number: BAP: 4135-YL2-14).

Protective effects of melatonin on heart injury in streptozotocin-induced diabetic rats

Ismet Burcu Turkyilmaz¹, Goksel Sener², Levent Kabasakal², Refiye Yanardag¹

¹Department of Chemistry, Faculty of Engineering, Istanbul University, Istanbul, Turkey.

²Department of Pharmacology, Faculty of Pharmacy, Marmara University, Istanbul, Turkey

Diabetes mellitus (DM) is a common metabolic disorder

and is characterized with many long-term complications such as neuropathy, nephropathy and vasculopathy. It has been demonstrated that free radicals play an important role for inducing DM and the imbalance between the anti-oxidant mechanisms and oxidant agents is the key of pathogenesis (1,2). Melatonin is a potent free radical scavenger and antioxidant and is shown to have beneficial effects on various DM induced pathologies (3). This study was designed to determine the possible protective effects of melatonin and/or insulin treatment on the biochemical changes in the heart tissues of streptozotocin-induced diabetic rats. We divided male rats into 5 groups. The control group received saline for 12 weeks. In diabetes group, diabetes was induced via a single dose of streptozotocin (60mg/kg) administered intraperitoneally and rats were given vehicle as solvent for melatonin every day for 12 weeks. In the third group, diabetic rats were treated with melatonin (10 mg/kg/day) for 12 weeks. The fourth group was diabetic rats given insulin (6U/kg) subcutaneously for 12 weeks. The fifth group is diabetic rats which received insulin and melatonin at the same dose and time. At the end of the experiment, animals were decapitated and heart tissues were collected. Tissues were homogenized in saline to make 10% (w/v) homogenate and were centrifuged. In supernatants, aspartate aminotransferase (AST), superoxide dismutase (SOD), myeloperoxidase (MPO) activities, protein carbonyl (PC) and protein levels were determined. The results show that heart AST and MPO activities and PC levels were found to be increased while SOD activity was decreased in diabetes group. Administration of melatonin and insulin reversed these aforementioned changes in diabetes group. We can conclude that, insulin and melatonin+insulin treatments may protect from diabetes-induced heart injury.

Keywords: Melatonin; Diabetes; Antioxidants; Oxidative stress.

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▶ Poster No. 111

Protective effects of endogenous and exogenous melatonin on oxidative toxicity in pinealectomized rats induced with organophosphate insecticide

Fatma Karakuş¹, Hakan Mollaoğlu¹, Tennur Atabay¹, Hatice Yalçinkaya¹, Efkân Uz², Muhittin Akyıldız³, Tolgahan Acar⁴, Ozan Ganiüsmen⁵, Hakan Cengiz^{6,7}, Nuray Öztaşan⁸, Sümeyra Kayan⁹, Nilüfer Genç Özdamar⁹

¹Department of Physiology, Faculty of Medicine, Sifa University, Izmir, Turkey

²Department of Biochemistry, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

³Department of Biochemistry, Faculty of Medicine, Sifa University, Izmir, Turkey

⁴Department of Anatomy, Faculty of Medicine, Sifa University, Izmir, Turkey

⁵Department of Neurosurgery, Faculty of Medicine, Sifa University, Izmir, Turkey

⁶Department of Biostatistics and Medical Informatics, Faculty of Medicine, Sifa University, Izmir, Turkey

⁷Department of Molecular Medicine, Institute of Health Sciences, Faculty of Medicine, Dokuz Eylül University, Izmir, Turkey

⁸Department of Physiology, Faculty of Medicine, Afyon Kocatepe University, Afyon, Turkey

⁹Department of Physiology, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

Fenthion is an organophosphate insecticide which has been used for many years. Yet studies on the harmful effects of fenthion and their prevention are still being conducted. The aim of this study was to evaluate toxicity induced through acute fenthion exposure and the protective quality of exogenous and endogenous melatonin by using following parameters; superoxide dismutase (SOD), catalase (CAT) and malondialdehyde

(MDA).

63 male Wistar-albino rats were separated into 6 groups. Control group (G1) had 8 rats while other groups were given 11 rats each: fenthion (G2), fenthion+melatonin (G3), pinealectomy (G4), pinealectomy+fenthion (G5), pinealectomy+fenthion+melatonin (G6). In order to observe the effects of endogenous melatonin, fenthion was administered orally one day before with a dosage of 54 mg/kg. Melatonin was administered for two days intraperitoneally during daytime in a single dose of 10 mg/kg. 24 hours following the application of fenthion, brain, liver and kidney samples were obtained.

Upon administration of fenthion on liver tissue, a significant increase in G2 CAT and MDA values was detected while no significant difference was observed in SOD enzyme activities. G6 Liver SOD enzyme activity recorded a significant decrease compared to G4 and G5. G1-G2-G3 kidney groups showed no significant difference in their CAT and SOD activities. Pinealectomized groups' CAT and SOD activities had a significant increase compared to other groups. Fenthion application on brain tissue resulted in a significant increase in (G2)'s SOD activity while application of melatonin in G3 caused it to move to control levels.

Administration of fenthion to rats caused an apparent oxidative damage in liver and brain tissues while the oxidative damage was mild in the kidney and therefore we have observed changes in SOD and CAT enzyme activities and an increase in the MDA levels. Exogenous melatonin which was given at midnight when the endogenous melatonin secretion was high greatly prevented oxidative damage in tissues however it was observed that pinealectomized rats did not see enough anti oxidative effect of exogenous melatonin.

Keywords: Melatonin; Organophosphate insecticide; Oxidative stress; Antioxidant.

▶ Poster No. 112

The effect of pulsed electromagnetic field application on oxidative stress in osteoporosis model induced by heparin

Olgun Topal¹, Seden Sert Zayıf², İltter İlhan², Müge Çina Aksoy¹, Duygu Kumbul Doğuç²

¹Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Süleyman Demirel University, Isparta Turkey

²Department of Medical Biochemistry, Faculty of Medical, Süleyman Demirel University, Isparta, Turkey.

Osteoporosis is a skeletal system disease characterized by increase in bone fragility and fracture risk as a result of decreased bone mass (1). Oxidative stress plays an important role in the pathogenesis of osteoporosis (2). The beneficial therapeutic effects of selected low-energy, time-varying magnetic fields, called pulsed electromagnetic fields (PEMFs), have been documented with increasing frequency since 1973 (3). In recent years PEMF are currently used in the treatment of spinal fusions, non-unions (4). PEMF is also suggested to be effective in the treatment of osteoporosis as well. In this study we induced an experimental osteoporosis model by using heparin injection. Aim of the current study was to investigate effects of PEMF on oxidative stress status in pathophysiology of osteoporosis. In the current study, adult male rats were divided into 3 groups as control (CG, n=10), experiment I (EIG, n=12) and experiment II (EIIG, n=12). Heparin was applied to the experimental groups for 33 days in order to induce secondary osteoporosis. SF was injected simultaneously to CG. At the end of injection period, 3 mm defect in the right femur of each animal was made by an operation on the 35th day. PEMF treatment was applied to EIG for 4 weeks at 1mT density, 7.3 Hz, 1 hour/day after operation. Afterwards all rats were sacrificed, the total oxidant (TOS)/antioxidant (TAS) capacity were analyzed in rats' serum with Rel TOS-TAS Diagnostics Assay kit. Statistical assessment was made by One Way ANOVA. TAS, TOS and oxidative stress index (OSI) levels of EI and EII groups were not found significantly different when compared to CG (p>0.05). In addition EIG showed no significant difference about oxidative status when compared to EIIG. In previous studies, PEMF on oxidative stress was shown to have conflicting effects. In conclusion, we observed no effect of PEMF on oxidative stress parameters. The gender and the age of the rats may be the facts that lead to the results. Also PEMF treatment time and level may be the other facts that would cause different results.

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▶ Poster No. 113

The consumption of propolis and royal jelly in preventing upper respiratory tract infections and as dietary supplementation in children

Sevda Yuksel¹, Sumeyya Akyol²

¹Department of Child Development, School of Health, Turgut Ozal University, Ankara, Turkey

²Department of Medical Biology, Faculty of Medicine, Turgut Ozal University, Ankara, Turkey

Propolis and royal jelly (RJ), two important honeybee products, have been used commonly all over the World as traditional and ethnopharmacological nutrients since ancient times. Both of them have a lot of active ingredients, which are known to be effective for several medical conditions. In this article, medical databases were searched for the usage of RJ and propolis in upper respiratory tract infections (URTI) and as a dietary supplementation, together and separately. 10-hydroxy-2-decenoic acid (10H2DA) is the most prominent active compound showing antimicrobial effect within RJ. Caffeic acid phenethyl ester (CAPE) is the most famous one that shows antimicrobial and anti-inflammatory effect within propolis. When compared with propolis, RJ was found to have richer content for all three main nutrients; proteins, carbohydrates, and lipids. More clinical, experimental, and basic studies are needed to find out the best-standardized mixture to cope with URTI in which RJ and propolis will be main ingredients

in addition to the other secondary compounds that have health-beneficial effects.

Keywords: Propolis, royal jelly, caffeic acid phenethyl ester, 10-hydroxy-2-decenoic acid, upper respiratory tract infections, nutrition.

▶ Poster No. 114

The effect of different Turkish song compositions on the sympathetic and immunobiochemical parameters

Sevda Yuksel, Fatmanur Hacıevliyagil Kazancı, Sefa Çiftçi, Merve Altan, Mustafa Yuksel, Hüsamettin Erdamar, Ramazan Yiğitoğlu

Turgut Ozal University, Health School, Ankara, Turkey.

Music is food for the soul but also it is a powerful tool in evoking emotions. Music is an effective concept that exists in every period of human life (1,2). Turkey is a rich country in musical heritage. Studies on the use of music for therapeutic purposes are increasing day by day. The Greek philosophers were convinced that music benefited health and specific recommendations were formulated for using music therapeutically against mental and physical illness (3). In this study, we aimed to evaluate the effects of different classical Turkish music styles (Saba, Muhayyer Kurdi, Hijaz, Ussak) on these parameters. Therefore we planned this research to accomplish all of these aims. Venous blood samples were collected before and after music listening and serum levels of serotonin, adrenalin, immunoglobulin A and cortisol were measured. Sixtyone healthy volunteers listened five different kinds of music (popular, rock, arabesque, European classical, classical Turkish music). To compare the same parameters in each volunteer before and after music listening, the Wilcoxon signed-ranks were performed as appropriate. For tests of significance, a p-value of <0.05 was considered to be statistically significant. All parameters were normally distributed. Our Statistical analysis results showed that cortisol level of the people listening Saba style decreased, Immunoglobulin A level decreased for the people who listened popular music and increased for the

group listening Nihavent style and adrenaline level increased significantly in all groups except for the people listening Ussak style of music.

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► Poster No. 115

Effects of folic acid treatment on body weight gain in pregnant women

Sumeyra Kayan¹, Nurhan Gumral¹, Fatma Selcen Cebe²

¹Department of Physiology, Faculty of Medicine, SuleymanDemirel University, Isparta, Turkey

²Department of Obstetrics Center, Medical Faculty, SuleymanDemirel University, Isparta, Turkey

Elevated oxidative stress increases the risk of obesity, is associated with perturbed maternal metabolism, raised plasma hormones, including leptin, insulin, and IGF-1, and the accumulation of inflammatory markers (1,2). The objective of our study was to evaluate whether folic acid exerts antioxidant effect on body weight gain in pregnant women.

The study was performed as a retrospective study in Suleyman Demirel University, Obstetrics Center between 2015-2016. In the current study weight gain of sixty five pregnant women who used folic acid during first trimester were compared with un-treated (control) twenty eight pregnant women. The statistical analyses were performed with the SPSS 21.0 program by using Mann Whitney U test.

The differences between these values at the 95% confidence interval (CI) was statistically significant ($p < 0,05$). This results show that the percentage of body weight difference between used folic acid pregnant (median=41,73) or not used folic acid pregnant

(median=59,23) was : ($U=567,5-p < 0,05$).

In conclusion, the current results show that maternal weight gain could be affected by the association between folic acid supplementation and the oxidative stress metabolism (3,4). However, further studies need to explore the benefits folic acid usage in preventing obesity.

Key words: Folic acid, pregnant weight gain, oxidative stress

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► Poster No. 116

Possible protective effects of agmatine and famotidine on MK-801-induced cytotoxicity in SHSY 5Y cell line

M. Betül Yerer-Aycan¹, Alim Hüseyin Dokumacı¹, Gökhan Ünal², Ceren Şahin², Feyza Arıcıoğlu²

¹Department of Pharmacology, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey

²Department of Pharmacology and Department of Psychopharmacology, Research Faculty of Pharmacy, Marmara University, İstanbul, Turkey

APO-1 / (Fas/CD95) cell surface receptor is a member of the nerve growth factor (NGF)/tumour necrosis factor (TNF) receptor superfamily and mediates apoptosis. Upregulation of APO-1 causes the programmed cell death (1). Agmatine is an endogen molecule and plays role on neuromodulation. There are contradictive reports about agmatine's effect on brain (neuroprotective or neurodegenerative (2). Famotidine

is H2 receptor blocker which use in gastro intestinal diseases. In addition, famotidine has Glycogen Synthase kinase (GSK) inhibitory effect which is important on neuromodulation (3). MK 801 is NMDA receptor antagonist and abnormal hypofunction of NMDA causes schizophrenia (4). Here in, we aimed to investigate effects of agmatine on APO-1/(Fas/CD95) regulation and cell viability in SHSY 5Y neuroblastoma cell line. SHSY5Y cells were incubated in DMEM, % 10 fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin at the conditions are 37 °C and % 5 CO₂. The cells were dispensed 10⁶ cells/well to 6 well plate and 12500 cells/well to 96 well plate. After overnight incubation, agmatine (100 µM), MK 801 (100µM) and famotidine (250, 100, 50 µM) were incubated for 24 hours.

Apo-1/Fas kit was used to determine Apo-1/Fas(CD95) levels. ELISA Apo-1/Fas kit has specific antibody coated onto wells. Firstly samples prepared to the directions of kit procedure. Protein levels measured with BCA protein assay and protein levels detected 1-5 mg/ml than experiment was performed to the kit procedure. Cell viability was assessed by SRB (Sulforhodamine B) colorimetric method for cytotoxicity screening.

The negative control MK 801 increased Apo-1/Fas levels and this result supported with cell viability assay. Famotidine decreased Apo-1/Fas dose dependent manner. Agmatine protected cells from the MK 801 induced toxicity.

In conclusion, we observed that agmatine and famotidine was protected SHSY 5Y cells from MK 801 induced toxicity. Therefore with regard to this preliminary results, it is thought that mechanism of famotidine and agmatine can be investigated by advanced experiments and might be beneficial in neurodegenerative disorders.

Keywords; Agmatine, famotidine, apoptosis, neuroblastoma.

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Poster No. 117

Investigation of saxagliptine and vildagliptine protective effect on amyloid beta (1-42)-induced cytotoxicity in SHSY 5Y cell line

Alim Hüseyin Dokumacı, M. Betül Yerer-Aycan

Department of Pharmacology, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey

Alzheimer's disease (AD) is progressive illness, is the leading cause of dementia in late adult life. AD characterized by intracellular neurofibrillary tangles and extracellular amyloid protein deposits contributing to senile plaques (1). Antidiabetic dipeptidyl peptidase-4(DPP-4) inhibitor saxagliptine and vildagliptine reported to ameliorative effect on streptozotocine (STZ)- induced AD model on rats. Kosaraju et al. (2) at al showed that saxagliptine increased the GLP-1 levels which is endogenous molecules and decreases amyloid beta protein and phosphorylated tau level. Behavioral studies support that vildagliptine and saxagliptine have ameliorative effect on cognition and memory (2,3).

SHSY5Y cells were incubated in DMEM with 10% fetal bovine serum and 100 U/ml penicillin+100 µg/ml streptomycin at the conditions are 37 °C and 5% CO₂. Cells were dispensed 10⁶ cells/well to 6 well plate. After overnight incubation, the cells were further incubated with vildagliptine (250, 100, 50 µg/mL), saxagliptine (200, 100, 10 µg/mL), Aβ1-42 (5µM) and memantine as NMDA receptor antagonist drug (100µM) for 24 hours. Commercial Apo-1/Fas kit was used to determine Apo-1/Fas(CD95) levels. ELISA Apo-1/Fas kit has specific antibody coated onto wells. Firstly samples prepared to the directions of kit procedure. Protein levels measured

with BCA protein assay and protein levels were detected 1-5 mg/ml than experiment was performed to the kit procedure.

Our positive control memantine (100 μ M) and saxagliptine (200 and 100 μ M dose) vildagliptine (50 μ M) decreased the Apo-1/Fas(CD95) levels comparing to control. In addition A β 1-42 induced toxicity was ameliorated with memantine 100 μ M, vildagliptine 50 μ M and saxagliptine 200 and 100 μ M dose.

As our preliminary results, saxagliptine has protective effect on A β 1-42 induced toxicity on SHSY 5Y cells. When we think that amyloid beta cytotoxic effect is a big problem in AD, new approaches for treatment of this disease is getting importance day to day.

Keywords: Alzheimer, vildagliptine, saxagliptine, amyloid beta.

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► Poster No. 118

Gallocatechin gallate inhibits the adipocyte differentiation

Oruc Allahverdiyev¹, Mehmet Berköz², Metin Yildirim³, Gülhan Bora⁴, Ömer Türkmen⁵

¹Yüzüncü Yıl University, Faculty of Pharmacy, Department of Pharmacology, Van, Turkey

²Yüzüncü Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Van, Turkey

³Mersin University, Faculty of Pharmacy, Department of Biochemistry, Mersin, Turkey

⁴Yüzüncü Yıl University, Faculty of Pharmacy, Department of Microbiology, Van, Turkey

⁵Yüzüncü Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Van, Turkey

Despite the fact that the effect of many catechin derivatives on adipocyte differentiation has been known, the effects of gallocatechin gallate on differentiation of 3T3-L1 pre-adipocyte to mature adipocytes have not been extensively enlightened. In this study, we aimed to investigate the effect of gallocatechin gallate application on transcription factors in adipocyte differentiation. Gallocatechin gallate was applied to 3T3-L1 pre-adipocytes at several concentrations (0, 50, 100, 150, 200 ve 250 μ M) in the cell culture medium and then treated with dexamethasone, isobutyl methylxanthine, biotin and insulin to induce the differentiation to mature adipocytes. Then, cell numbers, triglyceride content, glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity, and mRNA levels of transcription factors (PPAR γ and C/ERP α and SREBP-1c) were examined in both differentiated adipocytes and non-differentiated adipocytes as well as in adipocytes which were treated with gallocatechin gallate at various concentrations. While the gallocatechin gallate application statistically significantly reduced the pre-adipocyte cell number and triglyceride content ($p < 0.05$), it did not cause a significant change in GPDH activity ($p > 0.05$). Furthermore, despite there was a statistically significant decrease in PPAR γ and SREBP-1c mRNA levels of pre-adipocytes ($p < 0.05$), a significant change in C/ERP α mRNA levels was not detected ($p > 0.05$). These results demonstrate that gallocatechin gallate inhibits the differentiation in lipid cells by reducing gene expressions of transcription factors in differentiation of 3T3-L1 pre-adipocytes. For this reason, it can be concluded that consumption of green tea can be beneficial in reducing body weight and in inhibiting obesity development because of its reducing effect on lipid cell differentiation induced by gallocatechin gallate content.

Keywords: Adipocyte differentiation; 3T3-L1; Transcription factors; Gallocatechin gallate

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▶ Poster No. 119

Effects of thymoquinone on oxidant-antioxidant systems in the experimental acute pancreatitis model

Hasan Basri Savas¹, Betul Mermi Ceyhan¹, Yusuf Ilgin², Altug Senol²

¹Department of Medical Biochemistry, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey.

²Department of Internal Medicine, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey.

1. It was aimed to show the oxidant-antioxidant efficiency of the thymoquinone in experimental acute pancreatitis model. For this aim, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) levels were compared in experimental and sham group in rats.

42 adult male rats were divided into five groups as follows: Group 1: Sham 1; (S1; n = 6) received same volume of intraperitoneal saline with the pretreatment group. Group 2: Sham 2; (S2; n = 6) received same number and dose of intraperitoneal saline with the post treatment group. Group 3: Acute pancreatitis control group; (AP; n = 10) acute pancreatitis was induced by caerulein 50 mcg / kg / ip four times with 1 hour intervals. Group 4: pretreatment group; (PRE; n = 10) 20 mg / kg / ip thymoquinone was given, before 4 hours and 1 hour prior the creating of acute pancreatitis. After the administration of thymoquinone, caerulein was given 50 mg / kg / ip 1 hour intervals four times. Group 5: Posttreatment (POST; n = 10), acute pancreatitis was created by the administration caerulein 50 mg / kg / ip 1 hour by 4 times. 1 hour and 4 hours after the caerulein-

induced pancreatitis, 20 mg / kg / ip thymoquinone was given. Rats were sacrificed and serum TAS, TOS and OSI levels were measured using a commercial kit with auto-analyzer (1,2).

The TAS value of PRE were significantly higher compared to the AP and S2 group (p <0.05). The TOS value of PRE, POST, AP were significantly higher compared to the S1, S2 group (p <0.05). The OSI levels of AP were significantly higher compared to the PRE group (p <0.05).

It was shown that thymoquinone has an antioxidant activity in experimental acute pancreatitis model. Further studies may be useful for the researching new areas of the oxidant-antioxidant efficiency of thymoquinone.

Keywords: Thymoquinone, total antioxidant status; total oxidant status; acute pancreatitis.

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▶ Poster No. 120

The effect of thymoquinone on antioxidant vitamin levels in diabetic rats

Ayşe Usta¹, Semiha Dede², İbrahim Hakkı Yörük¹

¹Department of Chemistry, Faculty of Science, Yuzuncu Yil University, Van, Turkey

²Department of Biochemistry, Faculty of Veterinary Medicine, Yuzuncu Yil University, Van, Turkey

In this study, it were investigated that effects of thymoquinone (TQ) on antioxidant vitamin levels on experimental diabetic rats. Twenty-eight male Wistar-Albino rats (200-250 g) were used as material. The rats were divided into four groups of control (C), thymoquinone (T), diabetes (D) and diabetes+thymoquinone (DT). D and DT groups were treated with 45 mg/kg streptozotocin (STZ) (i.p). TQ

was administered as 30 mg/kg/21 day by oral gavage in DT and T groups. It was observed that the retinol concentrations significantly decreased in the diabetes and diabetes + TQ groups ($p \leq 0.05$). There was no difference between the group delivered TQ and the control group. It was identified that the tocopherol concentrations were lower in all study groups than in the control group, and it was identified that the reduction in the diabetes and diabetes + TQ groups was significant when compared to the control group ($p \leq 0.05$). It was identified that the vitamin D3 levels were significantly lower in the study groups when compared to the control group ($p \leq 0.05$). It was identified that the serum antioxidant vitamin levels decreased in the group with experimental diabetes and that TQ administration did not significantly affect the antioxidant vitamin levels significantly in the group with experimental diabetes.

Key words: experimental diabetes, antioxidant vitamins, thymoquinone

► Poster No. 121

Dexmedetomidine reduces plasma cytokine levels in cerebral ischemia-induced rats

Orhan Akpınar¹, Mustafa Nazıroğlu^{2, 3}, İshak Suat Övey², Hatice Akpınar⁴

¹Department of Microbiology, Faculty of Dentistry, Suleyman Demirel University, Isparta, Turkey

²Department of Neuroscience, Institute of Health Science, Suleyman Demirel University, Isparta, Turkey

³Neuroscience Research Center, University of Suleyman Demirel, Isparta, Turkey

⁴Department of Anesthesiology and Reanimation, Faculty of Dentistry, Suleyman Demirel University, Isparta, Turkey

Dexmedetomidine (DEX) has anxiolytic, sedative, and analgesic effects. DEX has been reported to exert inhibitory effect on inflammation both *in vitro* and *in vivo* studies. However, it is unclear that DEX modulates cerebral ischemia (ISC)-induced pro-inflammatory cytokine levels in rats. ISC-induced progression of cytokine production might be modulated by DEX as

potent antioxidant. The present study was conducted to explore whether DEX protects against plasma cytokine production in ISC-induced rats.

Forty rats were equally divided into five groups. The first and second groups were used as untreated and sham controls, respectively. The third group was operated to induce ISC. The fourth and fifth groups used as DEX and ISC+DEX and they received 40 µg/kg DEX at 3rd, 24th and 48th after cerebral ischemia induction.

Plasma IL-1β, TNF-α and IL-4 levels were high in ISC group although they were low in DEX treatments.

In conclusion, DEX induced protective effects against cerebral ischemia-induced plasma IL-1β, TNF-α and IL-4 through regulation of the cytokine production.

Keywords: Cerebral ischemia; Oxidative stress; Cytokine; Antioxidants.

► Poster No. 122

The effects of menopause and metabolic risk factors on periodontal parameters and oxidative stress markers in saliva

Esra Sinem Kemer Doğan¹, Fatma Yeşim Kırzıoğlu², Burak Doğan¹, Özlem Fentoğlu², Banu Kale³, Süleyman Akif Çarsancaklı⁴, Hikmet Orhan⁵

¹Department of Periodontology, Faculty of Dentistry, Mustafa Kemal University, Hatay, Turkey

²Department of Periodontology, Faculty of Dentistry, Suleyman Demirel University, Isparta, Turkey

³Division of Endocrinology, Department of Internal Medicine, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

⁴Department of Microbiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

⁵Department of Biostatistics and Medical Informatics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

The aim of the study was to evaluate the effects of

menopause and metabolic risk factors on periodontal parameters and saliva total antioxidant status (TAS) and total oxidant status (TOS) levels.

One hundred seventy six women between 30-70 years old were categorized according to the menopausal status as premenopause (PreM, n=86) or postmenopause (PostM, n=90). The number of the metabolic risk factors that defined as The US National Cholesterol Education Programme Adult Treatment Panel III criteria was calculated. Sociodemographics, biochemical and periodontal parameters were assessed. TAS and TOS levels in saliva were determined.

In the study population, the more metabolic risk factors, the higher periodontal parameter values, TOS level and lower saliva volume were observed. When analysed under the equal risk factor, periodontal parameters were significantly higher in group PostM than group PreM but higher TOS levels in group PostM than group PreM did not exhibit statistically difference, except having 3 risk factors. Significant correlations determined with canonical correlation analysis were found between the periodontal and systemic parameters.

In conclusion, increased oxidative stress depending on menopause and multiple risk factors may play a role in worsening of the periodontal health.

Keywords: Menopause; Metabolic risk factors; Periodontal parameters; Saliva; Oxidative stress

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► **Poster No. 123**

Effects of etodolac derivative compound, SGK 216, on oxidative stress-induced cell apoptosis in human leukemia cell line

Pınar Mega Tiber¹, Sevgi Koçyiğit¹, Pelin Çıkla-Süzgün², Ş.Güniz Küçükgül², Oya Orun¹

¹Department of Biophysics, Faculty of Medicine, Marmara University, Istanbul, Turkey

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, Istanbul, Turkey

Cancer is the most common unsolved health problem in the world. Chemotherapy and radiation therapy are used in the treatment of cancer. These methods have some side effects, so alternative methods are still being searched. Etodolac ((R,S) 2-[1,8-diethyl-1,3,4-tetrahydropyrano[3,4-b]indole-1-yl acetic acid] is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. It has inhibitory effects on cyclooxygenase-2 (COX-2) activation and similar to other COX-2 inhibitors, it shows anti-tumorigenic effects (1). In this study, 3-(2-(1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-yl)acetylhydrazono)-2-(4-fluorophenyl)-4-thiazolidinone (SGK 216), which is a derivative of etodolac, were evaluated against leukemia cancer cell line (K562) and control lymphocyte cells.

Using the MTT colorimetric method, SGK 216 was evaluated *in vitro* against the K562 cell lines, for cell viability and growth inhibition at different doses (10, 25, 50, 75 and 100 µM) following 24 hours incubation. Apoptosis was evaluated by mitochondrial membrane potential changes with JC-1 probe and ROS activities with 2',7'-dichlorofluorescein diacetate (DCFDA) probe both for K562 cancer cells and lymphocyte cells isolated from healthy donor.

In conclusion, current results indicate that, SGK 216 has anti-proliferative effects in a dose-dependent manner, with an IC50 value of 28 µM against K562 cell line. Comparing mitochondrial membrane potential between the cells showed that SGK 216 has more effect on K562 cells than control lymphocyte cells. In addition to anti-proliferative and apoptotic effects, SGK 216 also slightly increased ROS activities, strengthening its anti-tumorigenic role. Therefore SGK 216 can be a promising candidate for leukemia treatment.

Keywords: Etodolac derivative compound; Oxidative stress; Cancer; Apoptosis.

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► Poster No. 124

Determination of free oxygen radical scavenger activities and phenolic contents of *Nepeta nuda* subsp. *Lydiae*

Abdülmelik Aras¹, Ercan Bursal², Mehmet Dođru¹

¹Department of Chemistry, Faculty of Science, Dicle University, Diyarbakır, Turkey

²Department of Nursing, School of Health, Muş Alparslan University, Muş, Turkey.

Reactive oxygen species play important roles in many biological processes including metabolic pathways, cell signaling, immune response, and a variety of pathophysiological conditions. Antioxidant substances protect living organisms against oxidative damages of free radicals by donating a hydrogen atom or by chelating metals. Numerous of plants such as herbs, fruits and vegetables have been reported as main sources of natural antioxidants (1, 2). Phenolic acids are secondary metabolites widely found in the plants, are known to act as antioxidants (3).

The antiradical activities of extracts of *Nepeta nuda* subsp. *Lydiae* were determined by DPPH free radical scavenging method. Antioxidant activities of plant extracts were also determined by ferric reducing antioxidant power (FRAP) method. Especially, water extract of plant presented high antiradical potential on DPPH free radical scavenging method. The DPPH free radical inhibition percentages of extracts and standard antioxidants at the same concentration (30 µg/mL) decreased in the order of ascorbic acid (90.9%) > BHA (89.5%) > BHT (52.6%) > water extract (47.3%) > ethanol extract (26.5%). In the present study, the ferric ions reducing antioxidant capacities of extracts were low when compared to standard antioxidants. Phenolic compounds of *Nepeta nuda* subsp. *Lydiae* were also identified by UHPLC-ESI-MS/MS. The major phenolic compounds were found to be chlorogenic acid (1325±65

ppb), rosmarinic acid (238±11 ppb), and quinic acid (224±10 ppb). In conclusion, the leaves of *Nepeta nuda* have antioxidant and antiradical potential as well as rich phenolic content. Thus, it can be useful for preventing radicals and oxidative stress.

Keywords: Antioxidant; Phenolic contents; *Nepeta nuda*; Oxidative stress.

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► Poster No. 125

Determination of antioxidant and free oxygen radical scavenger activities of *Satureja boissieri* Hausskn ex Boiss

Ercan Bursal¹, Ömer Kılıç², Abdülmelik Aras³

¹Department of Nursing, School of Healths, Muş Alparslan University, Muş, Turkey

²Department of Park and Garden Plants, Technical Vocational College, Bingöl University, Bingöl, Turkey

³Department of Chemistry, Faculty of Science, Dicle University, Diyarbakır, Turkey

Free radicals are naturally exist in living systems; however, high amounts of free radicals can oxidise biomolecules, leading to tissue damage, cell death or degenerative processes, including aspects of ageing, cancer, cardiovascular diseases, arteriosclerosis, neural disorders, skin irritations and inflammation. Also, free radicals and lipid peroxides play an important role in oxidative stress. Antioxidants protect living organisms against oxidative damages of free radicals (1). Phenolic compounds play important roles in adsorbing and neutralizing free radicals, or decomposing peroxides.

Satureja taxa are widely used as herbal teas and spices

in different countries for their pleasant fragrance. It has been reported that the essential oils of *Satureja taxa* have anti-inflammatory, antispasmodic, antidiarrhea, antioxidant, antiviral, antibacterial and antifungal effects, mainly in vitro (2).

The aim of this study was to determine the antioxidant potency of *Satureja boissieri* Hausskn ex Boiss in vitro by analyzing the DPPH free radical scavenging activity method. The high radical scavenging percentages of water extract (42.5%) and ethanol extract (55.3%) of *S. boissieri* indicated that it might be beneficial for improving health and preventing some diseases. Also, we used UHPLC-ESI-MS/MS technique to determine the phenolic contents of *S. boissieri*. Hesperidin (5051±247 ppb) and rosmarinic acid (4364±214 ppb) were identified as the major phenolic compounds.

In conclusion, leaves of *S. boissieri* have high potential of biological activities and phenolic content so it can be a source for using in food industry as a food ingredient to produce functional food products.

Keywords: Antioxidant; Phenolic contents; *Satureja boissieri* Hausskn ex Boiss; Oxidative stress.

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▶ Poster No. 126

Protective effect of Quercetin on cyclophosphamide-induced lung toxicity in rats

Emin Şengül¹, Volkan Gelen², Semin Gedikli³, Seçkin Özkanlar⁴, Cihan Gür⁴, Adem Kara³, Fikret Çelebi¹, Ali Çınar¹

¹Department of Physiology, Veterinary Faculty, Atatürk University, Erzurum, Turkey.

²Department of Physiology, Veterinary Faculty, Kafkas University, Kars, Turkey.

³Department of Biochemistry, Veterinary Faculty, Atatürk University, Erzurum, Turkey.

⁴Department of Histology and Embryology, Veterinary Faculty, Atatürk University, Erzurum, Turkey.

Cyclophosphamide (CYP) is an anticancer agent that widely used in chemotherapy (1). It was suggested that CYP causes lung and testicular toxicity (2). In many studies, some antioxidants have possible protective effects against CYP's side effects (3,4). This study aimed that to investigate the protective effect of quercetin on CYP induced lung toxicity in rats.

Thirty-five male Sprague-Dawley rats weight 250-300 g were used for this study. The groups consisted a control and 4 experimental groups, respectively: Group I is control was given only intragastric (ig) solvent (corn oil) for 7 days. Group II is CYP and given ig corn oil for 7 days for placebo and after 7 days, single dose intraperitoneal (ip) CYP (200 mg/kg) was given on seventh day. Groups III and IV respectively, Quercetin doses 50 and 100 mg / kg dissolved in corn oil at doses was administered ig for 7 days and single ip injection of CYP (200 mg / kg) was administered on seventh day. The Group V, Quercetin 100 mg / kg dose was given to rats ig for 7 days. On the 8th day of the experiment, all groups of rats were anesthetized and intracardiac blood samples were taken. The collected lung tissues were used for analyzing of oxidative stress parameters (malondialdehyde, reduced glutathione and superoxide dismutase) and histopathological examinations.

In conclusion, the results of present study revealed the possible protective efficacy of quercetin against CYP induced lung damages in rats.

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Association between hypoxia inducible factor-1a (HIF1A) gene polymorphism and epilepsy

Gönül Zişan Şahin, Hatice Köse Özlece, Yunus Yılmaz, Serpil Can, Nergiz Hüseyinoğlu

Department of Medical Biology, Medical Faculty, Kafkas University, Kars, Turkey

Department of Neurology, Medical Faculty, Trakya University, Edirne, Turkey

Department of Pediatrics, Medical Faculty, Kafkas University, Kars, Turkey

Department of Physiology, Medical Faculty, Kafkas University, Kars, Turkey

Department of Neurology, Medical Faculty, Kafkas University, Kars, Turkey

Epilepsy is a frequently observed neurological disease characterized by repeated seizures as a result of excess discharge due to neuronal hyperexcitability (1). The molecular mechanisms underlying the pathogenesis of epilepsy are not exactly known. During each epileptic seizure, temporary hypoxia/ischemia may develop in the brain due to reduction in cerebral blood flow in the hippocampus and as a result neuronal cell death may occur (2). Additionally at high elevations, hypobaric hypoxia may develop linked to partial oxygen pressure reduction. Hypoxia inducible factor-1a (HIF1A) is an important transcription factor regulating the expression of genes providing cellular responses to insufficient oxygen. In an experimental rat model, an increase in HIF1A gene expression has been determined under hypoxic conditions, supporting that it may play a role in the epileptogenesis process (3,4). This study was planned with the aim of determining whether HIF1A gene polymorphism affects the predisposition to epilepsy in the Kars Caucasian population. This case-control study included 80 epileptic patients assessed with ILAE and 120 healthy controls. For DNA extraction the membrane-column method and Lab Turbo mini DNA isolation kit with silica membrane technology (Ct.No. LGD 480-220) was used. According to Hap-map data, 4 single nucleotide polymorphisms (SNP) of the HIF1A gene (rs10146037 rs11549465 C/T,

rs1957757 C/T, and rs12434438 A/G) were genotyped using the Iplex method on a MALDI-TOF MassArray. The genotype frequency of at least one G allele for rs12434438 SNP (A/G and/or GG) was found to be significantly high in epilepsy patients compared to control group [$p = 0.00117$, odds ratio (OR) 2.599, 95% confidence interval (CI) 1.451– 4.655]. The genotype frequency for at least one T allele for rs1957757 SNP (C/T and/or TT) was found to be significantly high in epilepsy patients compared to control group [$p = 0.00025$, odds ratio (OR) 4.984, 95% confidence interval (CI) 1.984– 12.518]. There was no association identified for the remaining two SNPs of the HIF1A gene tested (rs10146037 and rs11549465) ($p \geq 0.005$). In the results of our study we observed that there may be a association between HIF1A gene polymorphism and epilepsy, supporting the possible role of HIF1A in epilepsy pathophysiology.

Keywords: Factor-1a (HIF1A) gene polymorphism; Hypoxia; Epilepsy.

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Effects of estradiol, raloxifene and tamoxifen on caspase, PARP, TRPM2 and TRPV1 channel expression in hippocampus of ovariectomized rats

Yener Yazgan¹, Mustafa Naziroğlu^{1,2}

¹Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, Turkey.

²Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey.

The Ca²⁺ permeable transient receptor (TRP) melastatin 2 (TRPM2) and vanilloid 1 (TRPV1) are part of the TRP family, members of the melastatin and vanilloid subfamilies, respectively. TRPV1 is activated by different stimuli including capsaicin which is the pungent nature of hot chili peppers (1). However, the TRPM2 channel was activated by poly (ADP-ribose) polymerase (PARP) pathways through the production of ADP-ribose (2). Caspase activities and apoptosis levels are increased in hippocampus by activation of TRPM2 and TRPV1 channels (3). TRPM2 and TRPV1 channels are primarily expressed in the hippocampus and their expression levels in hippocampus were also affected in rats by ovariectomy (4). Tamoxifen (TMX) and raloxifene (RLX) are non-steroidal selective estrogen receptor modulators (SERMs) and they have neuroprotective and TRP channel modulator actions in the central nervous system. Hence, they may modulate caspase, PARP, TRPM2 and TRPV1 channel expression in hippocampus of ovariectomized (OVX) rats

Forty female rats were divided into five groups: First group was used as control. Second group used as OVX. Third, fourth and five groups used OVX+E₂, OVX+TMX and OVX+RLX, respectively. Estrogen, tamoxifen and raloxifene were subcutaneous given to these three groups for 14 days after OVX-induction. The caspase, PARP, TRPM2 and TRPV1 channel expression levels were performed by western blot analyses.

The procaspase 3, procaspase 9, PARP, TRPM2 and TRPV1 channel expression levels were high in the OVX group although they were low in OVX+ E₂, OVX+RLX and OVX+TMX groups.

In conclusion, we observed those of E₂, RLX and TMX administrations are beneficial on regulation of apoptosis, DNA damage (via PARP activity), TRPM2 and TRPV1 channel expression levels in the hippocampus of OVX rats.

Keywords: Hippocampus; PARP; Caspase; Selective estrogen receptor modulators, TRPM2 and TRPV1.

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▶ Poster No. 129

Doxorubicin and melatonin reduce apoptosis and mitochondrial oxidative stress in MCF-7 breast cancer cells: Involvement of TRPV1 channel

Pınar Aslan Koşar¹, Mustafa Nazıroğlu^{2,3}, İshak Suat Övey⁴ Bilal Çiğ⁴

¹Department of Medical Biology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

²Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

³Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey

⁴Department of Neuroscience, Health Science Institute, Suleyman Demirel University, Isparta, Turkey

Apoptosis is mediated by specific proteinases, namely caspases (1). The activation of caspases by cytosolic cysteine proteases results in apoptosis. TRPV1 is a Ca²⁺ permeable channel gated by oxidative stress and capsaicin (CAP) and modulated by melatonin (MEL) and capsazepine (CPZ). A combination of doxorubicin (DOX) and MEL may offer a potential therapy for breast cancer by exerting antitumor, anti-apoptotic effects and modulating Ca²⁺ influx and TRPV1 activity. We aimed to investigate the effects of MEL and DOX on the oxidative toxicity of MCF-7 human breast cancer cells, in addition to the activity of the TRPV1 channel and apoptosis. The MCF-7 cells were divided into the following six treatment groups: control, incubated with MEL (0.3 mM), incubated with 0.5 µM DOX, incubated with 1 µM DOX, incubated with MEL+0.5 µM DOX, or incubated with MEL+1µM DOX. The intracellular

free Ca²⁺ concentration was higher in the DOX groups than in the control, the concentration was decreased by MEL. The intracellular free Ca²⁺ concentration was further increased by treatment with the TRPV1 channel activator CAP (0.01 mM), it was decreased by the CPZ (0.1 mM). The intracellular production of reactive oxygen species, mitochondrial membrane depolarization, apoptosis level, procaspase 9, PARP activities, and caspase 3,9 activities were higher in the DOX and MEL groups than in the control. Apoptosis and the activity of caspase 9 were further increased in the DOX+MEL groups. In conclusion, the findings indicate that MEL supported the effects of DOX by activation of TRPV1 and apoptosis, as well as by inducing MCF-7 cell death.

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▶ Poster No. 130

The effects of 25-hydroxy vitamin D3 deficiency on oxidant-antioxidant system in human

Hüseyin Vural¹, Hasan Basri Savas¹, Betül Mermi Ceyhan¹

¹Department of Medical Biochemistry, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.

Vitamin D increases calcium absorption and accelerates the active transport of calcium. Vitamin D is obtained from diet and exposure to sunlight. Vitamin D deficiency causes to fracture, rickets, osteomalacia and osteoporosis (1).

The patients who admitted to Suleyman Demirel University Faculty of Medicine Research and Application Hospital in last one month were examined about 25-hydroxy vitamin D3 (vitamin D3) levels. 70 male and female, adult patients were included in the study. Groups were as follows: control (vitamin D3 normal: 20-100 ng / ml): n = 30, low vitamin D3 group (<20 ng / ml) n = 40. Total antioxidant status (TAS) and total oxidant status (TOS) in serum of the patients were evaluated by Modified Erel Method (2,3). Oxidative stress index (OSI) was calculated by total oxidant status (TOS)/total antioxidant status (TAS) formula. Statistical analysis was performed with SPSS.

TOS and OSI levels were significantly higher in low vitamin D group compared with the control although TAS and 25 OH vitamin D3 levels were (p <0.05) significantly lower in low vitamin D group compared with the control.

In conclusion, low 25 OH vitamin D3 levels had effects as increasing on oxidative stress and suppressive on antioxidant systems.

Keywords: 25-hydroxy vitamin D3; Total oxidant status; Oxidative stress index.

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▶ Poster No. 131

Cytomorphometric evaluation of patients with human papillomavirus high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) infected cervical epithelial cells using liquid-based cytology

Zehra Safi Öz¹, Nilüfer Onak Kandemir², Fürüzan Köktürk³, Şükrü Oğuz Özdamar²

¹Department of Medical Biology, Faculty of Medicine, Bulent Ecevit University, Zonguldak, Turkey

²Department of Pathology, Faculty of Medicine, Bulent Ecevit University, Zonguldak, Turkey

³Department of Biostatistics, Faculty of Medicine, Bulent Ecevit University, Zonguldak, Turkey

Human papillomavirus virus (HPV) is the main causal factor of cervical carcinoma. More than 100 HPV genotypes have been described and nearly 20 of them have been associated with cervical carcinoma (1). The aim of this study was to evaluate the nucleus/ cytoplasm ratio of High Risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) exfoliated epithelial cells prepared via Liquid based cytology.

In present study, 40 HR-HPV types infected patients' cervical smears and 34 control smears' with no infection agent prepared via liquid based system were evaluated for cellular and nuclear size. All areas were evaluated using image analysis software at a magnification of $\times 400$. The mean nucleus/cytoplasm ratio in HR-HPV types infected patients was smaller than the value in the control group but the difference between the groups was not statistically significant ($p > 0.05$).

In conclusion, HR-HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) affect the nuclear and cytoplasmic sizes. Light microscopic evaluation could be used to screen the changing of exfoliated epithelial cell' size prepared via Liquid based cytology. The nuclear/cytoplasmic ratio may reflect genotoxic impairments in the cervical epithelium in HR-HPV. Further studies are needed to investigate the potential role of HR-HPV on DNA damage and oxidative stress.

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Poster No. 132

Cannabinoid drug abuse problem: Is it most popular subject and potential source of oxidative stress?

Saliha Aksun

Department of Medical Biochemistry, Faculty of Medicine, Izmir Katip Celebi University, Izmir, Turkey.

Commonly known drug abuse tests are opioids, amphetamins, metamphetamins, kannabinoids (THC), barbiturates, benzodiazepine, cocaine, methylenedioxyamphetamine (ecstasy) and synthetic cannabinoids, usually urine specimens are used to do drug abuse testing. Hair, sweat, saliva, blood are other rarely using specimens to analyze. Despite a lot of synthetic cannabinoids are used in the world only few of them are measurable. It's using and serious toxic effects are quite high. We aim to inform some about abuse tests.

In our laboratory we used in vitro diagnostic immunassay systems for initial screening of drugs in urine (Dimension RxLclinical chemistry system, Siemens). All results are reported as semi-quantitatively according to their cut off levels. High level above the threshold is reported as a positive test result. To confirm a positive screening test, verification analysis should be done by mass spectrometry. A reading below the threshold is accepted as negative test result. In both situations incorrect results named as false negatively or false positively are very important for patient or social environment.

In the last year we reported nearly 100 amphetamine, 170 ecstasy, 45 cocaine, 130 opioids, 450 tetrahydracannabiol positive urine results but also we sure there were also so many synthetic cannabinoids results in there but we cannot able to say it.

Today in our laboratory unfortunately we cannot measure synthetic cannabinoids because of their high costs and it's difficulty inducing problem in biochemistry labs. Although the clinician can suspect using of that drug based on his clinical symptoms even

so it is necessary to determine it in laboratory to avoid false positively or false negatively clinical decision.

In conclusion, it is important to know who used abuse drug, what are the effect of these drugs on human body, does it break the balance of the oxidative and antioxidant systems?

Keywords: Drug abuse; Oxidative stress: Cannabinoid.

▶ Poster No. 133

Statin has no ameloriating effect of the apoptosis and testicular damage in diabetic animals

Nuran Ekerbiçer¹, Tugba Gurpınar², Muge Kiray³, Ali Riza Sisman⁴, Mehmet Ates⁵, Güven Güvendi³, Nazan Uysal³

Celal Bayar University, Department of Physiology¹, Department of Pharmacology², Faculty of Medicine, Manisa, Turkey

Dokuz Eylül University, Division of Behavioral Physiology Department of Physiology³, Department of Biochemistry⁴, College of Vocational School of Health Services Department of Pharmacology⁵ Faculty of Medicine, İzmir, Turkey

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are potent inhibitors of cholesterol biosynthesis and they are widely used to lower serum cholesterol levels in the prevention of cardiovascular diseases. Diabetes causes many functional and structural complications in testis, and cause testicular damage. Since the lipid metabolism affected besides carbohydrate metabolism statins are widely used by diabetic patients. Vascular endothelial growth factor (VEGF) and nerve growth factor beta (NGF- β) are important neurotrophic factors for male reproductive system. We aimed to investigate the correlation between testicular damage and testicular VEGF and NGF- β levels in statin treated diabetic rats.

Rats were divided into four groups: control group, diabetic group, statin group, diabetic group treated with

statin. Diabetes was induced by streptozotocin (STZ, 45 mg/kg/i.p.) in adult Wistar rats. Statin was given after two weeks of STZ induction by orogastric gavage for 2 weeks (10 mg/kg/day). Then testicular tissue was removed; testicular VEGF and NGF- β levels were measured by ELISA. Testicular damage was detected by using hematoxylin and eosin staining and periodic acid-Schiff staining, and apoptosis was identified by terminal-deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL). Seminiferous tubular sperm formation was evaluated using Johnsen's score.

Basement membrane was found to be thickened in seminiferous tubules and degenerated germ cells, seminiferous tubule diameter was found to be decreased both in the diabetic and statin groups but significantly more so in the diabetic group (statin $p < 0.003$, diabet $p < 0.0001$). Johnsen 's score which is used in the evaluation of spermatogenesis was decreased in both groups (both of $p < 0.0001$). Additionally, levels of VEGF and NGF- β were decreased in diabetic tissue (VEGF, $p < 0.0001$ in both groups; NGF- β $p < 0.001$ in statin group; $p < 0.0001$ in diabetic group). There were no significant ameloriating effects seen in statin treated diabetic group comparing to diabetic and statin groups.

These results suggest that the decrease of VEGF and NGF- β levels used is associated with the increase of the apoptosis and testicular damage. Statin treatment have no improving effects on testis.

Keywords: Statin; Apoptosis; Diabetes; Testis.

▶ Poster No. 134

Synergic effects of cannabinoid receptor reuptake and TRPV1 channel inhibitors on hippocampal calcium entry, apoptosis and oxidative stress in the in vitro 4-aminopyridine epilepsy model*

Mustafa Nazıroğlu^{1,2,3}, Afife Nur Taner⁴, Esra Balbay⁴, Bilal Çiğ^{1,3}

¹Department of Neuroscience, Institute of Health

Science, Suleyman Demirel University, Isparta, Turkey.

²Neuroscience Research Center, University of Suleyman Demirel, Isparta, Turkey.

³Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.

⁴Student, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.

Transient receptor potential vanilloid 1 (TRPV1) cation channels were mainly expressed in neurons including hippocampus which is main epileptic are in brain. In addition to capsaicin (CAP) and reactive oxygen species (ROS), TRPV1 channel is activated in the neurons by endogenous cannabinoid, anandamide. Although, our previous reports (1,2) indicate role of TRPV1 channels in the generation of epilepsy, role of anandamide on ongoing seizures through activation of TRPV1 have not yet been investigated. In the current study, we investigate the role of cannabinoid receptor reuptake (CRR) and TRPV1 blockade on the modulation of Ca^{2+} entry, apoptosis and oxidative stress in epileptic hippocampus.

Epilepsy was induced in adult hippocampal neurons by using in vitro 4-aminopyridine (4-AP) to trigger seizure-like activity model. The hippocampal neurons were stimulated in vitro with CAP although they were inhibited by capsazepine (CPZ) and anandamide reuptake inhibitor (AM404). We found that AM404 and CPZ were fully effective in reversing 4-AP-induced intracellular free Ca^{2+} concentration. The Ca^{2+} concentration in the neurons were lower in CPZ groups than in AM404 groups. In addition, the AM404 and CPZ treatments decreased intracellular ROS production, apoptosis, caspase 3, caspase 9 and mitochondrial membrane depolarization values in the hippocampus.

In conclusion, the results of the current study indicate that the synergic protective effect of cannabinoid receptor and TRPV1 channel antagonists on Ca^{2+} entry, oxidative stress and apoptosis in acute 4-AP-induced seizure model. However, modulator role of TRPV1 channel inhibition was more important in cannabinoid receptor inhibition. Current results represent a novel pharmacological approach towards the development of new drugs for the treatment of seizures and related syndromes resulting from overload Ca^{2+} entry and oxidative stress in the hippocampus.

Keywords: Anandamide; Apoptosis; Epilepsy; Hippocampus; TRPV1; Mitochondria.

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► Poster No. 135

Bioinformatical investigations of androctonus crassicauda scorpion toxins: Evidence for the role of aromatic cage

Suleyman Aydın¹, Ayça Çakmak²

¹Department of Pharmacology, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

²Department of Pharmacology, Faculty of Medicine, Bozok University, Yozgat, Turkey

Scorpion envenomations are one of the major health problems at many countries including Mediterranean countries and Turkey. There are more than one scorpion species in Turkey, *Androctonus crassicauda* being one of the most poisonous one on the world and in the Middle Eastern countries. Acute actions of the scorpion venom toxins are on cardiac and nervous systems. Main action of scorpion toxins including *Androctonus crassicauda* toxins are on ion channels. Some but not all of the toxins are identified, sequenced and are available via protein databanks.

The aim of this study was to investigate the sequence and aromatic amino acid motifs of the SCX8 toxin of *Androctonus crassicauda* scorpion venom.

The protein sequences were downloaded from uniprot database (www.uniprot.org). *Androctonus crassicauda* toxin sequences were extracted using slackware linux

operating system. Protr and Bio3d packages of the R programming language (cran.r-project.org) and clustalo were used for alignment of the toxin sequences. Swiss-model, Swiss Pdb-viewer was also used for 3D modelling and viewing.

SCX8 toxin consists of 66 amino acids and was observed to be rich in tyrosine and cysteine. 3D modelling exhibited the presence of aromatic cage, made up of tyrosines and including a tryptophan amino acid. Since functional aromatic cage structure was reported for the mammalian and human ion channels, the role of the aromatic cage structure of the toxin awaits to be further investigated. To the best of our knowledge our results are the first report for the presence of aromatic cage of scorpion toxins.

Keywords: *Androctonus crassicauda*, scorpion, toxins.

▶ Poster No. 136

Anti-inflammatory effect of thymol on cotton pellet granuloma test

Ayça Çakmak¹, Suleyman Aydın²

¹Department of Pharmacology, Faculty of Medicine, Bozok University, Yozgat, Turkey

²Department of Pharmacology, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

Labiatae plant family is a rich source of essential oils containing thymol which are used for various diseases including inflammation since antiquity in the Mediterranean countries. Thymol is one of the volatile oxygenated monoterpenes found in various natural sources including Labiatae plants which are shown to interact by transient receptor potential (TRP) ion channels. The aim of the present study was to investigate the effect of thymol on proliferative phase using cotton pellet method on rats.

Three different doses (10, 50 and 100 mg/kg) of commercially purchased thymol diluted in DMSO was used as test substance and ruthenium red as TRP blocker, acetylsalicylic acid and indomethacine as standard anti-inflammatory drugs. Adult Sprague

Dawley rats of either sex were anesthetized by propofol prior to the surgically implantation of sterilized and dried cotton pellets into the subcutaneous parts of scapular regions. After the application of the test substances and standard drugs for 7 days intraperitoneally, pellets were removed, dried and weighed. Anova and Tukey HSD were used for statistical evaluation by the help of packages of R programming language for the statistical evaluation.

The inflammation was inhibited by doses of thymol at 10, 50 and 100 mg/kg. Ruthenium red was observed not to block the anti-inflammatory action of thymol.

In conclusion, since there are 27 TRP members in mammalian systems, our results suggest a complex interaction of the thymol molecule on TRP cation channels.

Keywords: TRP Channels; Labiatae plant; Thymol.

▶ Poster No. 137

Evaluation of serum levels of progranulin and bone morphogenetic protein-4 in patients with osteoarthritis

Serdar Hira, Cüneyt Tamam, Uğur Demirpek, Mehmet Gem

Department of Biochemistry, Tatvan Military Hospital, Bitlis, Turkey.

Osteoarthritis (OA) is a chronic, slowly progressive disease of the joints and is one of the most common causes of pain and disability in middle-aged and older people. The etiology and pathogenesis underlying this disease are poorly understood. Progranulin (PGRN), a secreted glycoprotein expressed in many cell types, has been linked to wide variety of biological processes including oxidative stress (1). In recent years, increasing evidence suggests that PRGN stimulates chondrocyte proliferation and is considered an essential regulator of cartilage metabolism (2). Bone morphogenetic protein-4 (BMP-4), a member of transforming growth factor- β superfamily of proteins, is involved in bone and cartilage development and induces chondrogenesis (3).

This study aimed to investigate serum BMP-4 and PRGN levels in patients with OA and present a new evidence of pathogenesis OA disease. The study included 38 female osteoarthritis patients and 38 female healthy volunteers. Serum PRGN and BMP-4 concentrations were measured using enzyme-linked immunosorbent assay. We also measured body mass index and erythrocyte sedimentation rate (ESR), white blood cells (WBC) and neutrophil lymphocyte ratio (NLR). Mean BMP-4 levels were significantly lower in OA women compared to controls ($29,66 \pm 13,61$ vs $72,81 \pm 44,06$ ng/mL, $p < 0.001$). Mean PRGN levels were found to be significantly lower in OA women compared to controls ($71,93 \pm 33,83$ vs. $268,33 \pm 180,45$ ng/mL, $p < 0.001$). There were no significant differences in WBC and NLR levels between two groups ($p = 0.763$, $p = 0.925$, respectively). ESR values was significantly higher in patients group than controls group ($p = 0,022$). There was a significant positive correlation between serum BMP-4 levels and serum PRGN levels in patients with OA. In conclusion, BMP-4 and PRGN levels may play a role in the pathogenesis of OA and could be a useful biomarker of OA.

Keywords: Osteoarthritis; BMP-4; Progranulin; oxidative stress.

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► Poster No. 138

Effects of some food additives on DNA damage in human lymphocytes

Dilek Aşçı Çelik, Vehbi Atahan Toğay, Nurten Özçelik

Department of Medical Biology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Food additives are not a natural constituent of a food,

which are added to foods in processing, preparation, implementation, packaging, transport and storage phases for technological purposes and inconsumable as a food whether or not nutritious. In recent years, this has been shown in several studies that food additives play a role in the development of cancer, liver-kidney failure and other diseases via their mutagenic effects (1). Some of the food additives have been tested in different organisms and these studies have been reported genotoxic and carcinogenic effects of some food additives (1-3). Purpose of the current study was to investigate effects of citric acid (E330,CA), ascorbic acid (E300,AA) and sodium citrate (E331,SC) on DNA in human lymphocytes via comet assay. CA, AA and SC are used as food additives with the purpose of pH regulator, flavor enhancer, preservative, antioxidant, stabilizer in drinks, jellies, baked goods, jams, marmalades, candies, canned fruits and vegetables, dairy products, meat products and baby foods (1-4). Human lymphocytes were incubated with CA, AA and SC in different concentrations (50,150,300,600 µg/mL) for 1 hour at 37 °C. After that tail length, tail moment and % tail DNA parameters have been evaluated. In conclusion, the results indicate that there is a significant increase in the DNA damage in lymphocytes after 1h of *in vitro* exposure to CA 600 µg/mL dose when the values compared to control group and other doses of CA. However, any statistically significant difference could be found in the other doses of CA and any doses of AA and SC.

Keywords: Food additives, DNA damage, comet assay

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Protective effects of silibinin on DNA in diabetes induced rats

Tuğba Semerci Sevimli¹, Murat Sevimli², Vehbi Atahan Toğay¹, Dilek Aşçı Çelik¹, Nurten Özçelik¹

¹Department of Medical Biology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

²Department of Histology and Embryology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Diabetes mellitus is a disease that occurs as a result of the lack of insulin and/or insulin resistance and characterized by the defects in carbohydrate, fat and protein metabolism. Metabolic disorders in diabetes is observed due to insulin is a significant anabolic hormone. Lack of insulin or insulin resistance is responsible from abnormalities that occur on insulin receptors, signal transduction, effector enzyme and genes especially in fat and muscle tissues (1). It might be chromosomal changes, DNA strand breaks, DNA replication-transcription-repair defects in diabetes through affecting DNA by advanced glycosylation products. Comet assay which is a simple, rapid and sensitive technique for analyzing and quantifying DNA damage in individual cells (2). Silibinin is a flavonoid which has anti-inflammatory, anticarcinogenic, antioxidant and cyto-protective effects and derived from a plant called *Silybum marianum* (3,4). Purpose of current study was to investigate protective effect of silibinin against diabetic DNA damage in streptozotocin (STZ) induced diabetic rat lymphocytes via comet assay. There were four groups including control group, diabetic group (STZ, 65 mg/kg as intraperitoneal and single dose), silibinin treatment group (STZ+100 mg/kg silibinin) and silibinin group (100 mg/kg silibinin). In conclusion, results indicate that there is a statistically significant increase in the DNA damage in lymphocytes of diabetic group compared to the control group and this damage is significantly reduced in silibinin treatment group. There is no statistically significant damage in silibinin group compared to the control group. In this case it can be interpreted as silibinin can prevent or reduce diabetes induced DNA damage.

Keywords: Diabetes, silibinin, comet assay, DNA damage.

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Principal Contact

Prof. Dr. Mustafa NAZIROGLU / Editor in Chief
Suleyman Demirel University, Faculty of Medicine, Department of Biophysics
32260 Cunur - Isparta / TURKEY
Phone: +90 246 2113641 Fax: +90 246 2371165
mustafanaziroglu@sdu.edu.tr
biophysics@sdu.edu.tr