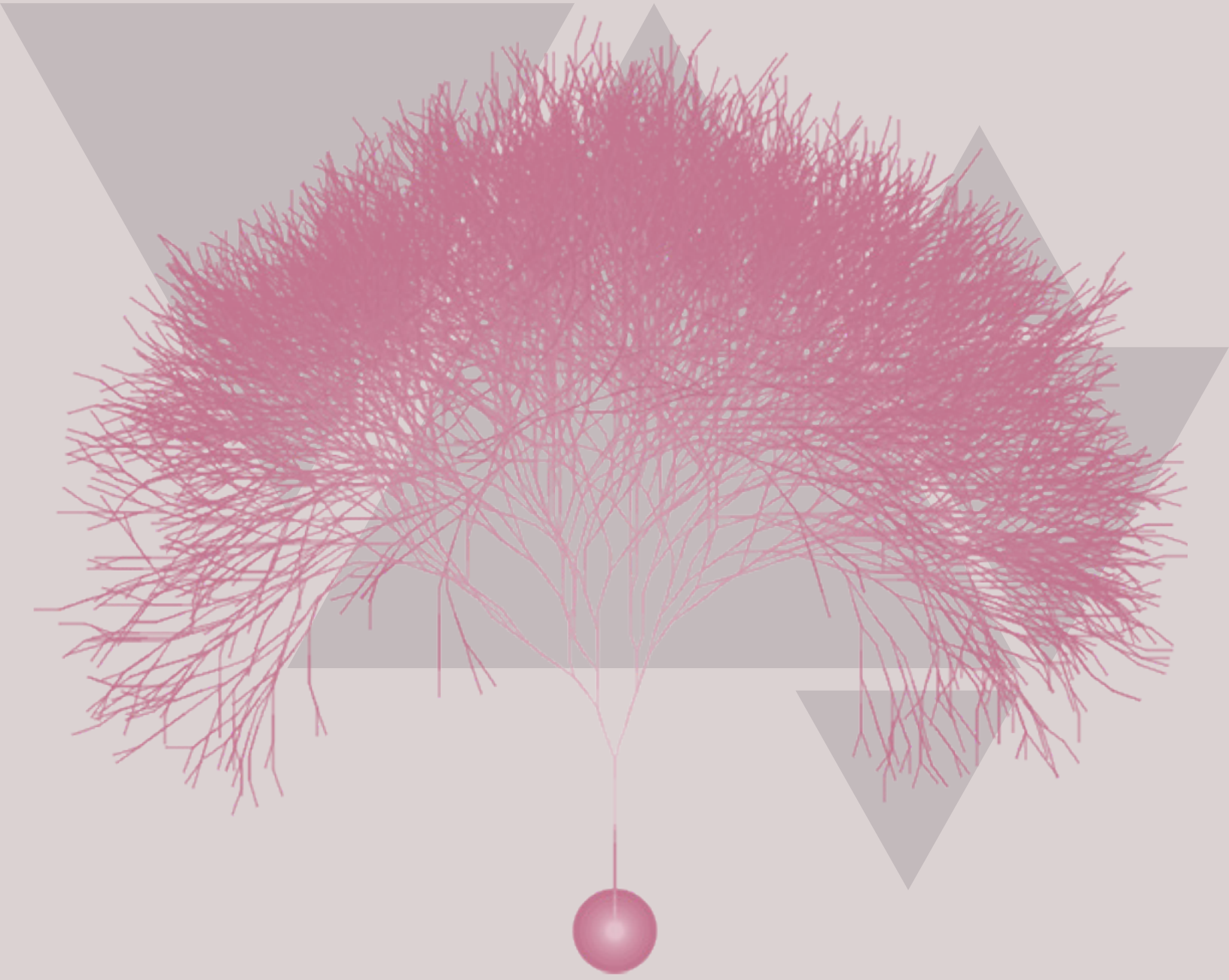
Serbian Society for Mitochondrial and Free Radical Physiology
Third Congress

REDOX MEDICINE

REACTIVE SPECIES SIGNALING, ANALYTICAL METHODS, PHYTOPHARMACY, MOLECULAR MECHANISMS OF DISEASE



Book of Abstracts

Belgrade, September 25-26, 2015.

Serbian society for mitochondrial and free-radical physiology

BOOK OF ABSTRACTS

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Reactive Species Signaling, Analytical Methods, Phytopharmacy, Molecular Mechanisms of Disease

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PLENARY LECTURES

NITRIC OXIDE AND OXYGEN: ACTIONS AND INTERACTIONS IN HEALTH AND DISEASE

Salvador Moncada

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Nitric oxide (NO) inhibits cell respiration reversibly and in competition with O_2 through the inhibition of the mitochondrial cytochrome c oxidase (Complex IV). At concentrations lower than those required to inhibit respiration, endogenous NO enhances the reduction of the electron transport chain, thus enabling cells to maintain their O_2 consumption. This facilitates the release of superoxide anion, which initiates the transcriptional activation of NF-kB as an early signal of a stress response. Through free radical formation, long-term inhibition of mitochondrial respiration by NO leads to persistent inhibition of Complex I. This is dependent on the S-nitrosylation of a specific thiol in the active form of this protein. S-nitrosylation of Complex I might indicate the early stages of a pathological process. Inhibition of mitochondrial respiration by low concentrations of NO at critical O_2 concentrations also leads to prevention of the stabilization of hypoxia-inducible factor-1α (HIF-1α) due to the redistribution of O_2 towards non-respiratory O_2-dependent targets. This prevents the cell from registering a state of hypoxia at low O_2 concentrations. On the other hand, at higher concentrations NO increases the expression of HIF-1α by an action most probably involving a free radical mechanism. Full inhibition of respiration by NO leads to a situation in which in spite of O_2 being present cells are unable to use it. We have labeled this state, “Metabolic Hypoxia”. Cells respond to metabolic hypoxia by activating glycolysis as a defense mechanism or by initiating apoptotic death when unable to initiate glycolysis as it happens in neurons.

UNRAVELING THE MECHANISM OF SELENIUM CATALYSIS IN GPx4: HOW REDOX CHEMISTRY IMPACTS ON LIFE, FROM SPERMATOGENESIS TO CELL DEATH

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Glutathione peroxidases (GPxs) build a family of enzymes that reduce hydroperoxides by thiols. Today we know 8 members of the vertebrate family and GPx4 is the sole monomeric selenium dependent enzyme. The limited specificity of monomeric enzymes for GSH is a functional feature of GPx4, which becomes competent, when GSH is permissively low, for the oxidation of protein thiols needed to build up the structure of mature spermatozoa. In cellular membranes, the reduction of lipid hydroperoxides (ROOH) catalyzed by GPx4 in the presence of GSH, is the most efficient peroxidation-inhibiting mechanism. The peroxidase activity integrates the antioxidant reaction of tocopherol and prevents activation of lipoxygenases. Seemingly due to this reaction, GPx4 is indispensable for cell survival. The experimental model of GPx4 silencing, indeed, is seen as the paradigm of necroptotic cell death pathway. However, in front to the relevance of GPx4, the mechanistic problems of fast catalysis and interaction with membranes had remained largely unanswered so far. We addressed these issues by combining: quantum mechanics-based DFT calculations; high resolution MS spectroscopy; Surface Plasmon Resonance (SPR); molecular modeling, dynamics and docking. Oxidation of selenium by ROOH is primed by a critical charge-separation in the active site leading to a complex decay without any energy barrier. This fast decay forms the ROH as ideal leaving group and selenenic acid. The latter reacts either with the thiol substrate or, in absence of it, with a downstream peptide nitrogen, thus forming a cyclic selenenylamide. The formation of this species prevents the irreversible over-oxidation of selenium and β-cleavage of Sec. The SPR studies on the interaction between GPx4 and membranes showed that a cationic area on the GPx surface binds to polar heads of phospholipids by a strong electrostatic interaction and molecular dynamics indicates that this also drives the precise orientation of the hydroperoxidic group on the fatty acid toward the catalytic redox center. This evidence is evocative of a “surfing” on the anionic surface of the membrane of the enzyme competent for the removal of lipid hydroperoxides.

TARGETING MITOCHONDRIA IN MALE (IN)FERTILITY. A NEW THERAPEUTIC APPROACH

Vesna Otašević

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In spermatozoa, mitochondria play an important role in maturation and function, and its functionality is positively correlated with human sperm fertilization ability and quality. Conversely, mitochondria represent major production sites and targets for reactive oxygen and nitrogen species (ROS and RNS, respectively). Recent studies suggest the importance of ROS/RNS and mitochondrial activity in the acquisition of sperm fertilizing potential; however, the underlying molecular mechanisms are still unknown. Thus, we examined here whether and how changes in sperm redox milieu affect the sperm functionality. For modulation of sperm cells redox state we have used superoxide dismutase (SOD) mimic M40403. Compared with the control, incubation in Tyrode's medium for 3 h, under noncapacitating conditions, decreased sperm motility, the amount of nitric oxide ($^{\bullet}$NO), the number of MitoTracker® Green FM positive mitochondria, and the expression of complex I and complex IV of the mitochondrial respiratory chain. In turn, SOD mimic M40403 treatment restored/increased these parameters, as well as the expression of endothelial nitric oxide synthase, MnSOD, and catalase. These results showed, that the SOD mimic, M40403, might improve a molecular basis of sperm mitochondrial function, as well as increase sperm motility, and, thus, have positive effects on the functionality of spermatozoa. Increase in the $^{\bullet}$NO level in spermatozoa, caused by M40403, implies $^{\bullet}$NO involvement in the observed effects of the drug. This leads to the hypothesis that the utilization of a redox modulator, M40403, is a promising pharmacological approach for the improvement of sperm function during assisted fertilization and for the treatment of infertile states accompanied by mitochondrial impairments and/or disturbed sperm redox state.

ROS-INDUCED ROS - IMPLICATIONS FOR MITOCHONDRIAL ROS ON VASCULAR FUNCTION

Andreas Daiber

2nd Medical Department – Molecular Cardiology, Medical Center of the Johannes Gutenberg University, Mainz, Germany

Oxidative stress is a well-established hallmark of cardiovascular disease and there is strong evidence for a causal role of reactive oxygen and nitrogen species (RONS) therein. Improvement of cardiovascular complications by genetic deletion of RONS producing enzymes and overexpression of RONS degrading enzymes proved the involvement of these species in cardiovascular disease at a molecular level. Vice versa, overexpression of RONS producing enzymes as well as deletion of antioxidant enzymes was demonstrated to aggravate cardiovascular complications. With the present overview we present and discuss different pathways how mitochondrial RONS interact (crosstalk) with other sources of oxidative stress, namely NADPH oxidases, xanthine oxidase and an uncoupled nitric oxide synthase. The potential mechanisms of how this crosstalk proceeds are discussed in detail. Several examples from the literature are summarized (e.g. hypoxia, angiotensin II mediated vascular dysfunction, cellular starvation, nitrate tolerance, aging, hyperglycemia, b-amyloid stress) and the underlying mechanisms are put together to a more general concept of redox-based activation of different sources of RONS via enzyme-specific ‘‘redox switches’’. Mitochondria play a key role in this concept providing redox triggers for oxidative damage in the cardiovascular system but also act as amplifiers to increase the burden of oxidative stress and associated complications such as vascular dysfunction. Based on these considerations, the characterization of the role of mitochondrial RONS formation in cardiac disease as well as inflammatory processes but also the role of mitochondria as potential therapeutic targets in these pathophysiological states should be addressed in more detail in the future. *Antioxid. Redox Signal.* 20, 308-324.

ROS GENERATION BY FLAVIN DEHYDROGENASES OF THE MITOCHONDRIAL RESPIRATORY CHAIN

Mráček T, Holzerová E, Drahota Z, Kovářová N, Vrbacký M, Ješina P, Kľučková K, Rohlena J, Neuzil J, Houštěk J

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Overproduction of reactive oxygen species (ROS) has been implicated in a range of pathologies. Mitochondrial flavin dehydrogenases glycerol-3-phosphate dehydrogenase (mGPDH) and succinate dehydrogenase (SDH) represent important ROS source, but the mechanism of electron leak is still poorly understood. We focused on two aspects of their biology. First, using models of isolated solubilized dehydrogenases and site directed mutagenesis of ubiquinone-binding Qp site in SDH we explored the mechanisms of ROS production by these enzymes. We implicate flavin as the most likely source of electron leak in SDH under in vivo conditions, while we propose coenzyme Q as the site of ROS production in the case of mGPDH. Distinct mechanism of ROS production by the two dehydrogenases is also apparent from induction of ROS generation by ferricyanide which is unique for mGPDH. Second, using native electrophoretic systems we studied the native membrane organisation of both dehydrogenases and found mGPDH to be associated into homooligomers and SDH to form high molecular weight complexes not involving other OXPHOS complexes. By this approach, we also directly demonstrated that isolated mGPDH itself as well as its supramolecular assemblies are all capable of ROS production.

MITOCHONDRIAL FUNCTION AND INSULIN SENSITIVITY

Tomas Jelenik

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Impaired mitochondrial function, i.e. decreased respiratory capacity and increased production of reactive oxygen species (ROS), has been linked to the development of insulin resistance, obesity and type 2 diabetes mellitus (T2DM). Indeed, suppressed substrate oxidation can lead to disturbed glucose homeostasis as well as accumulation of specific lipid metabolites (diacylglycerols, ceramides), which activate pathways leading to inhibited insulin signaling. Furthermore, decreased ATP synthesis can restrict energy-consuming processes such as insulin-stimulated glucose uptake and insulin secretion from β-cells. While ROS produced within physiological levels positively regulate insulin signaling, chronically increased levels of ROS can lead to impaired insulin signaling by activating stress-sensitive and inflammatory pathways. Moreover, accumulation of oxidatively damaged bio-macromolecules can contribute to the onset of diabetes and its complications. In principle, these mechanisms can operate in all tissues, however their regulation under pathophysiological conditions of impaired glucose homeostasis is tissue-specific. For example, we have shown that mitochondrial respiration is decreased in the muscle of obese insulin resistant patients as well as in patients with T2DM. This is associated with accumulation of diacylglycerols and impaired insulin signaling. On the other hand, mitochondrial respiration is increased in the liver of obese with hepatic steatosis, despite insulin resistance. This adaptation to increased substrate fluxes is lost in steatohepatitis and associates with increased ROS. We observed similar pattern of events in mouse models with insulin resistance, hepatic steatosis and diabetes. Despite a large body of evidence of association between impaired mitochondrial function and insulin resistance, there are studies showing that both abnormalities are not always related. These contradictions could be partially attributed to differences in the employed methods and protocols but also to multifactorial alterations underlying the heterogeneous conditions of T2DM in specific tissues. In conclusion, mitochondrial function often decreases with insulin resistance in the muscle, while it increases during early stages of insulin resistance in the liver. The mechanisms and casual relationships between both abnormalities could be better understood by using animal models with genetic modification of specific mitochondrial genes.

KEYNOTE TALKS

SELENIUM-DERIVED COMPOUNDS AS PHARMACOLOGICAL TOOLS TO TARGET THE REDOX SIGNALLING AND DETOXIFICATION FUNCTION OF GSTP

Desiree Bartolini, Marta Piroddi, Loredana Incipini, Luca Sancineto, Vanessa Pieri, Claudio Santi, Francesco Galli

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Glutathione S-transferase π (GSTP), a phase II gene downstream of the Nrf2-ARE/EpRE transcription pathway, plays a key role in both the detoxification and signalling response to a number of electrophilic agents. Se-organic molecules with thiol peroxidase activity are an example of these agents with proven influence on GSTP signalling. Recently, starting from the structure of the lead compound (PhSe)2, we developed a series of diselenides with mitigated thiol peroxidase activity and increased selectivity of action on GSTP signalling. The Nrf2 activation response and hormetic activity of these compounds have been explored. The new diselenides, and particularly the form in which carboxylic acid was present as coordinating group nearby to the selenium atom of (PhSe)2 (DSBA), behave as mild thiol peroxidases leading to a moderate generation of H_2O_2 and NOx, and signalling of stress-activated and survival-promoting MAPKs, which ultimately control the mitochondrial pathway of apoptosis. Used in murine embryonic fibroblasts (MEFs) and HepG2 human hepatocarcinoma cells to produce sub-maximal conditions of stress, the diselenide compounds stimulated Nrf2 nuclear translocation and the transcription of the same Nrf2 gene as well as of GSTP and other phase II genes. This resulted in a higher degree of protection against H_2O_2 cytotoxicity (hormetic effect). Diselenide toxicity increased in GSTP knockout MEFs by a higher generation of NOx and SAPK-JNK activation. A lowered hormetic potential of these cells was observed in association with an abnormal expression and nuclear translocation of Nrf2 protein. Immunoprecipitation and affinity purification experiments revealed the existence of a Nrf2/GSTP complex in MEFs and HepG2 cells. Covalent oligomers of GSTP subunits were observed in DSBA treated cells. In conclusion, GSTP gene expression influences the Nrf2-dependent response to hormetic diselenides. Mechanistic interpretation for this GSTP-dependent effect may include a direct and redox-sensitive interaction of GSTP with Nrf2 protein.

GLUTATHIONE TRANSFERASE POLYMORPHISM IN NON-MALIGNANT AND MALIGNANT KIDNEY DISEASES

Tatjana Simić

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Cytosolic glutathione transferases (GSTs) are a family of enzymes that protect cells by detoxifying various xenobiotics and also possess antioxidant activity. Almost all members of cytosolic GST classes exhibit genetic polymorphism, resulting in complete lack or lowering of enzyme activity. As a result of polymorphic GST expression, great inter-individual differences in GST isoenzyme profiles exist in renal parenchyma, affecting both the capacity for biotransformation and protection from free radicals in renal tissue. Recent studies showed that GST polymorphism is implicated in several non-malignant and malignant kidney diseases, such as end-stage renal disease (ESRD), Balkan endemic nephropathy (BEN) and renal cell carcinoma (RCC). Thus in ESRD, a typical oxidative disease, individual GST polymorphisms influence vulnerability to both protein and lipid oxidation, with *GSTM1-null* genotype having the most pronounced effect. Moreover, combined *GSTM1-null/GSTA1-active* genotype might be considered as genetic marker for cardiovascular death risk in ESRD patients. In this line, ESRD patients may be stratified according to the GST genotype in terms of the level of oxidative stress and prognosis, hence potentially enable optimization of antioxidant treatment. Concerning the role of GSTs in BEN, *GSTA1* polymorphism was associated with increased risk of this endemic disease, which leads to progressive reduction of renal parenchyme. Furthermore, based on *in silico* simulation, we suggested that GSTA1, being the most promiscuous GST isoenzyme, might be involved in conjugation of ochratoxin A, a potential cause of BEN. Finally, GSTs are involved in the biotransformation of several compounds recognized as risk factors for RCC. In this line, we have shown that *GSTM1*, *GSTT1*, *GSTA1* and *GSTP1* polymorphisms might be associated with the risk of RCC, with special emphasis on *GSTM1-null* and *GSTP1-variant* genotypes. Furthermore, combined *GSTM1-null*, *GSTT1-active*, *GSTA1-low activity* and *GSTP1-variant* genotypes might be considered as "risk-carrying genotype combination" in clear cell RCC. Taken together, these results imply important role of GST polymorphisms in oxidative phenotype and efficiency of detoxification, which may affect susceptibility or prognosis of several malignant and non-malignant kidney diseases.

MODULATION OF MACROPHAGE REACTIVE OXYGEN AND NITROGEN SPECIES PRODUCTION BY NEUROPEPTIDE Y

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Neuropeptide Y (NPY), containing 36 amino acids, is one of the most evolutionary conserved peptides. Due to a very wide tissue distribution of NPY and its significance for the human and animal physiology, peptides with high structural homology to NPY, e.g., peptide YY (PYY), pancreatic polypeptide (PP) and their truncated forms NPY2-36, NPY3-36 and PYY3-36, are specified as members of NPY family. Beside effects on the central nervous system, including regulation of food intake, energy balance, nociception, anxiety and sexual behavior, NPY in the periphery has a potent mitogenic activity, stimulates angiogenesis and is chemotactic for vascular smooth muscle cells. NPY-related peptides are potent inhibitors of intestinal fluid secretion and motility. However, the presence of NPY in the cells of the immune system, together with the expression of specific Y-receptor subtypes (Y1, Y2 and Y5) on immune cells, suggested ability of autocrine/paracrine immune regulation by endogenous NPY and related peptides. Experimental evidences revealed that NPY in vitro stimulated rat macrophage reactive oxygen species production via Y1 and Y2 receptors, whereas activation of Y5 receptors suppressed oxidative burst in these cells. Quite the opposite, NPY in vitro considerably decreased rat granulocyte peroxide production, and this effect was mediated by Y2 and Y5 receptors. Regarding the production of NO by LPS-activated macrophages, NPY-induced augmentation involved Y1 and Y2, and excluded mediation via Y5 receptors. Furthermore, NPY enhanced NO production in macrophages from young rats, whereas it was ineffective in this respect in macrophages from aged rats. As a whole, direct effect of NPY on reactive oxygen and nitrogen species production is dependent on cell type, engagement of specific NPY receptors and age.

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GSTP1 GENETIC POLYMORPHISM, RS1695, IS ASSOCIATED WITH THE CHRONIC HEART FAILURE RISK

Ana Savić-Radojević[1,5], Dejan Simeunović[2,5], Natalija Odanović[1], Tanja Radić[1], Slavica Radovanović[3], Vesna Ćorić[1,5], Marija Plješa-Ercegovac[1,5], Marija Matić[1,5], Dragan Simić[4,5], Tatjana Simić[1,5]

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The role of genetic polymorphism of glutathione transferases (GSTs), superfamily of enzymes involved in antioxidant defense and regulation of apoptotic signaling pathways, in the chronic heart failure (CHF) syndrome has emerged recently. In our pilot case-control study the distribution of common GSTs polymorphisms in CHF patients with idiopathic dilated cardiomyopathy (IDC) and coronary artery disease (CAD) was investigated. *GSTA1* (-69C>T), *GSTM1*, *GSTP1* (Ile105Val) and *GSTT1* genotypes were determined in 194 CHF patients (109 of CAD and 85 of IDC) and 274 age- and gender-matched controls. No significant association was demonstrated for *GSTA1*, *GSTM1* and *GSTT1* genotypes with CHF occurrence due to either CAD or IDC. Carriers of at least one variant *GSTP1**Val allele were at 1.7-fold higher CHF risk than *GSTP1**Ile/Ile carriers (p=0.031), which was even higher when combined with variant *GSTA1**B allele (OR=2.2; 95%CI=1.1-4.4; p=0.034). In CHF patients stratified to the underlying disease, similar association was observed only in CAD (OR=2.8; 95%CI=1.1-7.3; p=0.033). In addition, CAD patients, carriers of combined *GSTP1/GSTA1* risk alleles had significantly decreased left ventricular end systolic diameter compared to *GSTA1**AA/*GSTP1**IleIle carriers (p=0.021). We concluded that among common GST polymorphisms analyzed, only variant *GSTP1**Val allele has shown a significant association with CHF, regardless of specific cause.

TARGETING THE NITRIC OXIDE/SUPEROXIDE RATIO IN DIABETIC RAT TISSUES

Aleksandra Janković

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Diabetes mellitus (DM) is a chronic metabolic disease characterized by low insulin synthesis and/or action with the consequent gluco- and lipotoxic damages in different tissues. Up to 70% of all diabetics suffer from pathologic skin changes during the course of disease. Results of our previous studies revealed that precursor of nitric oxide (NO) synthesis L-arginine restituted insulin immunopositivity in the pancreas and normalized plasma insulin levels in alloxan-induced DM. Beneficial effects of L-arginine in DM are attributed to the restitution of the NO-cyclic guanosine monophosphate signaling in the pancreas. Similarly, increased superoxide anion radical ($O_2^{\bullet-}$) production secondary to hyperglycemia and impairment of antioxidant defense are suggested to play a role in the etiology of different tissues damage in DM, partly by influencing NO-mediated signaling. Thus we tested the effects of synzymes with superoxide dismutase (SOD)-like catalytic activity (manganese macrocyclic complexes: Mn(pyane)Cl_2 and M40403) on the redox status, antioxidant defense and NO bioeffects in the pancreas and the skin of alloxan-induced diabetic rats. The results showed that SOD mimics support the physiological effects of NO on β-cell regeneration and the restitution of blood insulin level, by upregulation of antioxidant enzymes, neuronal and endothelial nitric oxide synthases (nNOS and eNOS) and increase in NO concentration. Also, M40403 in diabetic skin induced eNOS and heme oxygenase-1 expression (that were down-regulated in DM), reinforced endogenous antioxidant defense, by increasing the expression of MnSOD and catalase, and finally, normalized peroxynitrite production, which was increased in diabetic skin. Thus, L-arginine and SOD mimics could be used as a powerful molecular tools for manipulation the disturbed pancreatic and skin redox state in DM, by removing $O_2^{\bullet-}$ and stimulating NO production, i.e. increasing the bioavailability of NO.

HOW TO USE AMINOXYL RADICALS TO EXAMINE BBB PERMEABILITY IN ALS RATS. *IN VIVO* EPR STUDY

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Amyotrophic lateral sclerosis (ALS) is neurodegenerative disease characterized by progressive degeneration of motor neurons in the primary motor cortex, corticospinal tracts, brainstem and the spinal cord, leading to muscle weakness and atrophy. Using magnetic resonance imaging (MRI) technique, we showed the presence of iron deposits in the motor cortex and other brain regions of ALS patients, which indicated the potential leakage of iron to CSF through compromised blood-brain barrier (BBB). To confirm this assumption, *in vivo* L-band electron paramagnetic resonance (EPR) spectroscopy using aminoxyl spin-probes has been applied. For experiments, control and transgenic rats of Sprague-Dawley breed with a larger number of copies of the human SOD1 gene with inserted G93A mutation into the genome, have been used. Spin-probes with diverse ability to pass through the cell membrane and BBB were selected and injected through the tail vein. *In vivo* EPR signal intensities of spin-probes were measured in the head region as a function of time. The results showed significantly different reduction kinetics of spin-probes in ALS animals in respect to controls. To gain comprehensive insight into the level of involvement of various sections in modified redox status, as well as to investigate in which extent the BBB is compromised, the pharmacokinetic model was developed. Using this model, the contribution of specific compartments in the reduction of spin-probes could be quantified exposing higher BBB permeability in ALS compared to control animals. Accordingly, this unique combined experimental and theoretical approach has shown to be a promising tool for evaluation of redox status of small animals *in vivo*.

SHORT PRESENTATIONS

PARAOXONASE 1 STATUS AND REDOX DISBALANCE IN PREGNANCY

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Pregnancy is a physiological condition with increased susceptibility to oxidative stress. The purpose of the present study was to determine changes in plasma paraoxonase-1 (PON1) activity (an indicator of paraoxonase phenotype) throughout normal pregnancy and its relationship with maternal oxidative stress status. We recruited 43 healthy pregnant Caucasians at their first ante-natal hospital appointment at "Narodni Front" Gynecology and Obstetrics Clinic in Belgrade. Blood was sampled towards the end of each trimester, before delivery (at the 38th gestational week) and more than 4 weeks postpartum. Fasting glucose, uric acid and lipid status parameters [total cholesterol (t-C), LDL-C, HDL-C, TG, Apo A-I and apolipoprotein B (Apo B)] were measured in serum. The frequencies of the PON1 phenotype in the studied population were determined using a two-substrate (paraoxon/diazoxon) activity method. We measured the prooxidant-antioxidant balance (PAB), total anti-oxidative status (TOS), total antioxidant capacity (TAC) and concentration of sulphydryl (SH) groups in serum, as parameters of oxidative stress status. PON1 activity significantly decreased at gestational week 32 ($p<0,001$). In addition, the lipid profile was more atherogenic. PAB was significantly increased across gestational weeks and significantly decreased after delivery, even below that found during the 1st trimester [365,4 (321,8-467,7) vs 642,6 (617,5-739,9) $p< 0,001$]. We noticed also significant increase in the TOS concentrations during pregnancy. However, TOS concentrations after delivery were still higher compared to the 1st trimester (not quite statistically significant). A statistically significant increase in concentration of both the SH group in 3rd trimester and after delivery and the TAC in the 2nd and in the 3rd trimesters, before delivery and after delivery compared with the 1st trimester were observed. There were independent direct associations between maternal smoking habits before pregnancy, glucose concentrations and PAB with PON 1 activity in the third trimester. Changes in lipid profile, body composition, intensive oxidative stress cause changes in PON1 activities throughout pregnancy, particularly during later stages of pregnancy. PON 1 activity was lower and the antioxidant capacity of HDL particles was less influential. Therefore, according to our results, the change in PON1 activity could be an additional reason for the probable pregnancy-related increased risk of CVD in later life. Multiple regression analysis revealed influences of maternal smoking, glucose metabolism switching and oxidative stress burden on PON1's decreased antioxidative capability. There are only a few reports concerning PON 1 activities throughout pregnancy and the results are not uniform. Also, according with our results, we propose that the PAB could be a new marker of oxidative status during pregnancy.

MESOPOROUS NANOSTRUCTURED MATERIAL SBA-15 AS A CARRIER FOR TARGETED DRUG DELIVERY IN THE MODEL OF MELANOMA *IN VITRO*

Mirna Bulatović[1], Goran Kaluđerović[2], Aleksandra Korać[3], Đorđe Miljković[1], Igor Golić[3], Sanja Mijatović[1], Danijela Maksimović-Ivanić[1]

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Drug loaded mesoporous silica nanoparticles represent a promising modality for enhancing efficacy of poorly soluble cancer therapeutics. We have already demonstrated the strong therapeutic potential of the functionalized SBA-15 nanostructured material (SBA-15p) loaded with an organotin (IV) compound (SBA-15pSn) in the mouse model of melanoma. In this study we evaluated this experimental therapeutic in a highly invasive, therapy-resistant human A375 melanoma cell line. The mechanism of cellular uptake was determined by electron microscopy. The cell viability was detected using crystal violet and mitochondrial dehydrogenase activity assays. Different types of cell death, cellular senescence and transdifferentiation of melanoma cells were analyzed by flow cytometry. Imunoblot analysis was done for the expression of key signaling pathways. Obtained data revealed that SBA-15pSn nanoparticles were internalized by macropinocytosis and nonspecific cellular uptake. Organotin (IV) compound grafted on SBA-15p strongly inhibited the growth of A375 cells *in vitro*, triggering caspase dependent apoptosis and premature cellular senescence. The expression of low affinity nerve growth factor receptor indicated that subpopulation of senescent clones differentiated into Schwann-like cells upon the treatment with SBA-15pSn. Morphological transformation of SBA-15pSn treated melanoma cells indicated that these cells possessed lower metastatic potential in comparison to untreated control. Our findings demonstrated that grafting of chemotherapy drugs in mesoporus silica materials can be a novel strategy for targeted delivery of highly toxic and hydrophobic drugs in cancer therapy.

Supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No. 173013).

POSTERS

P1

AGING HAS THE OPPOSITE EFFECT ON CIRCADIAN VARIATIONS OF cAMP AND NO-cGMP SIGNALING ELEMENTS IN RAT LEYDIG CELLS

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Reproductive physiology is profoundly influenced by circadian rhythms. However, the circadian dynamic of signaling pathways in Leydig cells (LCs) – the main testosterone producers in males, is still unknown. The present work shows circadian aspect of two important signaling pathways for LCs adrogenesis, cAMP and nitric oxide (NO) – cGMP signaling pathway, as well as changes that occur in aging. All measurements were performed on 3- and 24-month-old rats in six time points during the day and analyzed by cosinor method. RIA analysis showed that despite unchanged level of serum LH (measured by ELISA), testosterone was decreased in aging, retaining low amplitude circadian rhythm. Further, content of intracellular cAMP and cGMP was measured by ELISA. Similar to testosterone, the amount of cAMP in LCs from both groups showed 24-h oscillation but was lowered in LCs from 24-month-old rats. The peak of cAMP oscillations happened a few hours before the peak of testosterone – around ZT5. cGMP also displayed circadian oscillations but this time aging led to concentration incensement in the most measured time points. Peak of the cGMP rhythmic pattern was around the midday period. Gene expression analysis of cAMP signaling elements showed that *Prkaca* and *Prkar2a* lost rhythmicity in aging. Most of the cAMP-specific Pdes (*Pde4a, Pde4d, Pde7b, Pde8a*) completely changed the expression patterns, since *Pde8b* showed no rhythmicity in both groups but expression was significantly lower in aging. Considering NO-cGMP signaling pathway, NO producers, *Nos2* and *Nos3*, displayed changes in circadian expression between the groups. In accordance with cGMP rise in aging, *Gucy1a3* and *Gucy1b3*, enzymes involved in cGMP production, were also significantly increased, as well as *Prkg1*, the main effector molecule of this signaling pathway. Further, cGMP-specific Pdes, like *Pde5a, Pde6a, Pde6h* and Pde9*a*, had rhythmic expression which in some of them (*Pde5a, Pde6a, Pde6h*) was also elevated in the aging group in all or at least one time point. In conclusion, the results indicate the opposite effects of aging on cAMP and cGMP circadian dynamic in LCs.

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P2

LOCALIZATION OF ZINC OR "ZINCOSOMES" IN HUMAN OOCYTE USING CONFOCAL AND ELECTRON MICROSCOPY

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Understanding of basic role of zinc in maturating of oocytes is very important for accurately driving procedures of *in vitro* fertilization, especially for patients for whom the only applicable technique is *in vitro* maturation, for example for cancer treated patients, and for gonadotropin unresponsive patients. Oocytes of these patients grow in sequential culture media, and it is very important to know in which stadium of maturation is zinc usable, and when it may be harmful or cause apoptosis. Zinc homeostasis in eukaryotic cells is controlled on the levels of uptake, intracellular sequestration in zinc storing vesicles ('zincosomes'), nucleocytoplasmic distribution and elimination. Therefore, we the aim of this study was to examine subcellular presence and localization of the zinc in human oocyte during *in vitro* maturation. Cells were taken from IVF patients treated with short-term protocol and recombinant FSH or purified gonadotrophin, Menopure and Cetrotide. Cells were collected, in agreement of patients, as genetic incompetent cells (GV and MI) or as genetic competent cells in pairs where spermatozoa were absent on the day of aspiration of oocyte. All oocytes were denuded two hours after collection with hyaluronidase (SynVitro®Hyadase, Origio), and washed three times in IVF medium beyond oil. Further, oocytes were incubated with FluoZin3 and observed by Leica TP5 confocal microscopy. After sequential imaging, oocytes were routinely prepared for transmission electron microscopy in order to correlatively check the observed zinc localization at ultrastructural level. Our results with FluoZin-3 show that this zinc sensor is able to reveal the vesicular nature of Zn(II) in oocytes. Also, confocal microscopy revealed the presence of zincosomes in oocyte cytoplasm. Observed zincosomes were of different sizes, some were larger and highly brilliant, while others were smaller, less brilliant, and more abundant. For light and electron microscope correlations, we used the topographical correlation of selected fluorescent zincosomes on semi-fine sections. With this approach, we found the presence of numerous vesicles of different size near the cell membrane and throughout oocyte cytoplasm which clearly correspond to FluoZin3-stained zincosomes.

P3

AN INSIGHT INTO POLYPHENOLIC COMPOUNDS OF WINES OBTAINED FROM THREE CABERNET FRANC CLONE CANDIDATES

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Phenolic compounds exhibit strong antioxidant activities associated with their abilities to scavenge free radicals, donate hydrogen, chelate metals, break radical chain reactions, and quench singlet oxygen *in vitro* and *in vivo.* As a result, many foods which contain abundant phenolic compounds have attracted increased consumer attention in recent years. Grapes and wines are one of the most important sources of phenolic compounds. Wines are recommended for moderate consumption as an alcoholic beverage according to the reports on the so-called French Paradox. The quality of wine largely depends on the composition and contents of its phenolic compounds, so phenolic analysis can be used as an effective tool in characterizing different wines. This work aimed to evaluate the content of total polyphenolic compounds (the Riberau-Gayon-Maurié procedure), anthocyanins (by spectrophotometric procedure, using pH differential method), tanins (the Nègre procedure) and Folin-Ciocalteu index (by spectrophotometric procedure, using Folin-Ciocalteu reagent), respectively in the wines produced from three Cabernet Franc clone candidates obtained in the perennial clonal selection. The aforementioned chemical parameters were determined in the relevant wine samples covering the period 2008-2012. In comparison with both the standard Cabernet Franc wine (originating from mother vine) and the wines obtained from other two candidate clones, the Cabernet Franc wine of the clone candidate No. 010 was found to have the highest content of total polyphenolics (1.85 ± 0.02 g/L) and anthocyanins (178.55 ± 3.75 mg/L) as well as Folin-Ciocalteu index 36.86 ± 0.12. The wine of clone candidate 02 had the highest concentration of tanins (1.15 ± 0.02 g/L). The improved content of phenolic compounds that is tightly linked with cardiovascular physiology may contribute to the medicinal properties of the Cabernet Franc wine originating from the clone candidate No. 010.

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P4

EVALUATION OF POLYPHENOLIC COMPOUNDS IN WINES OBTAINED FROM MERLOT CLONE CANDIDATES

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Medicinal plants have a historical role as a source of molecules with a broad spectrum of therapeutic effects. *Vitis vinifera* variety is one of the oldest agricultural crops and a rich source of different polyphenols. Some of them like quercetin and resveratrol are already in clinical trials for many health problems. Polyphenols mainly contribute to certain organoleptic characteristics of wines, such as astringency, bitterness and particularly color. In addition, interest in wine phenolics is still increasing due to their antioxidant and free radical-scavenging properties, supported by the health benefits resulting from moderate wine consumption with respect to cardiovascular diseases, cancer, diabetes and others. The aim of this work was to evaluate the content of total polyphenolic compounds and anthocyanins as well as the Folin-Ciocalteu index of the wines produced from eleven clone candidates obtained in the clonal selection of Merlot variety (*Vitis vinifera* L.). The contents of total polyphenolic and anthocyanins were determined by the Riberau-Gayon-Maurié method and spectrophotometric procedure (pH differential method), respectively. The spectrophotometric procedure using Folin-Ciocalteu reagent (FCR) was employed for determination of Folin-Ciocalteu index. The wines of eleven clone candidates of Black Merlot cultivar had the content of total polyphenol substances from 1.49 g/L (No. 028) to 2.06 g/L (No. 031). The highest/lowest Folin-Ciocalteu index was found for varieties No. 025 and No. 028. The content of antocyanins ranged from 172.27 to 313.42 mg/L for wines of varieties No. 025 and No. 034. To obtain a more comprehensive picture of the quality of the wines further research work will be directed towards determination of the content of particular phenolics and antocyanins in all wine samples.

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P5

POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *THYMUS SERPYLLUM* EXTRACTS DEPENDING ON EXTRACTION CONDITIONS

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Polyphenols are secondary plant metabolites that are involved in a wide range of specialized physiological and biochemical processes, not only in plants, but also in animals and humans. Phenolic compounds possess antioxidative properties acting as radical scavengers and chain-breaking antioxidants. The inactivation of reactive oxigen species or the prevention of their cellular formation is consider to be a practical approach to reducing the risk of cancer, cardiovascular and neurodegenerative disorders. Because of these reasons, plant which is rich in polyphenols represents a great challenge for food and pharmaceutical industry. The purpose of this study was to optimize a method for extracting antioxidant polyphenols compounds from *Thymus serpyllum* (*Lamiaceae*). Optimization of the extraction was carried out through varying the type of solvent (water and 50% ethanol) and three different extraction processes: a) at room temperature, b) at 60 °C and c) at room temperature with the assistance of ultrasound water bath, while using solid:solvent ratio 1:30, particle size 0.3 mm and extraction time 15 min. The content of total polyphenols (TPC) in obtained extracts was determined spectrophotometrically using Folin-Ciocalteu's reagent. In order to determine the antioxidant capacity of extracts, the 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenil-1-picrylhydrazyl (DPPH) radical scavenging assays were applied. The highest total polyphenols content and antioxidant activity values (ABTS and DPPH methods) were recorded in 50% ethanol extract, at 60 °C (31.27 mg/L GA, 17.89 μmol/g Trolox and IC_{50} 1.86 mg/mL, respectively). No significant differences were observed between total polyphenols and antioxidant activity in ethanol extracts obtained at room temperature and in ultrasound bath ($p > 0,05$). The lowest total phenols content and antioxidant activity were obtained in water extract at room temperature in ultrasound water bath (19.34 mg/L GA, 10.46 μmol/g Trolox and IC_{50} 4.42 mg/mL, respectively). This study found that both total polyphenols and antioxidant activities determined were significantly affected by the type of solvent and temperature. Also, the levels of total polyphenolic compounds were correlated with free radical scavenging activities against ABTS and DPPH radicals ($r^2 = 0.9326$ and $r^2 = 0.7962$, respectively). In conclusion, ethanol extracts obtained at high temperature represent a good source of the compounds with significant antioxidant activity.

P6

IN VITRO ANTIOXIDANT ACTIVITY OF THE SELECTED MUSHROOM SPECIES OF THE FAMILY MORCHELLACEAE (ASCOMYCOTA)

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Mushrooms have become attractive for human nutrition in the last decades as a source of physiologically beneficial bioactive compounds making them functional foods. The aim of this work was to study anti-DPPH radical activity and ferric reducing potential (FRAP) of crude ethanolic extracts of three wild-growing species of the family Morchellaceae (Ascomycota): *Verpa bohemica*, *Morchella conica* and *Morchella elata*, collected in Eastern Serbia (Sikole, near Negotin) during spring 2013, in relation to total phenolic content measured colorimetrically by the Folin – Ciocalteu assay. The highest anti-DPPH radical activity and ferric reducing potential was observed for *M. elata* (IC_{50}59.11 μg/ml and 59.63 mg AAEq/g d.w., respectively). In addition, these results showed that ethanolic extracts of *V. bohemica* contained more total phenolics (30.08 mg GAEq/g d.w.) than screened *Morchella* species. The correlations obtained between anti-DPPH radical activity and total phenolic content (r^2 = 0.92) indicated potential impact of phenolic compounds only for *V. bohemica*. On the other hand, the observed ferric reducing potential of *M. elata* is likely to be linked to some other classes of organic compounds. Taken all together, the selected fungal species may actually inspire further experiments with antioxidant design, aiming to offer powerful antioxidants with a broad range of uses.

P7

EFFECTS OF EXTRACT OF *Teucrium* SPP. ON VIABILITY, MIGRATION POTENTIAL AND REDOX STATUS OF COLON CANCER (SW-480) AND BREAST CANCER (MDA-MB-231) CELL LINES

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This study was carried out to further understand the cytotoxic, antimigratory, and pro/antioxidative potential of the methanol extracts of *Teucrium polium* and *Teucrium montanum* on colon cancer (SW-480) and breast cancer (MDA-MB-231) cell lines. For this purpose, the cell viability and migration potential analysis were performed in real-time by using a real time cell analyzer (RTCA xCELLigence system). The analysis of parameters of redox status include determination of superoxide anion radical, $O_2^{\cdot -}$ by NBT assay, nitrites by Griess assay and reduced glutathione by GSH assay. Results showed that *T. montanum* induced significant cytotoxic effect on MDA-MB-231 cells, while on SW-480 cells cytotoxicity was observed only in the highest concentrations. *T. montanum* extract significantly decreased migratory potential (compared to control) of both cell lines. *T. montanum* showed greater increasing of $O_2^{\cdot -}$ and nitrites on MDA-MB-231 cells, while GSH content increased only on SW-480 cells. *T. polium* induced, in our opinion, an effect opposite to the effects recorded with *T. montanum*. *T. polium* showed cytotoxic effect on both cell lines (especially on SW-480 cells). Also, *T. polium* exhibited greater antimigratory effect on both cell lines in comparison to *T. montanum*. Investigation of redox status parameters revealed that *T. polium* on MDA-MB-231 cells induced statistically significant and dose dependent increasing of $O_2^{\cdot -}$. On SW-480 cells it was observed an acute increasing of $O_2^{\cdot -}$. Also, *T. polium* induced decreasing of nitrites and GSH on both cell lines. Both, *T. montanum* and *T. polium* extracts showed prooxidative character, which could be related to their cytotoxic effects on both cell lines. These extracts, in non-toxic concentrations, showed also antimigratory potential. We concluded that *T. montanum* mainly affected MDA-MB-231 breast cancer cells, while *T. polium* primarily affected SW-480 colon cancer cells. From our results it was concluded that SW-480 cells are more sensitive than MDA-MB-231 cells to *Teucrium Spp.* treatment.

P8

ANTIPROLIFERATIVE AND ANTIOXIDATIVE EFFECTS OF 3-(4-SUBSTITUTED BENZYL)-5-ISOPROPYL-5-PHENYLHYDANTOINS ON HUMAN COLON CANCER CELL LINE HCT-116

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Hydantoin derivatives are mainly used in medicine as anticonvulsants for the treatment of epilepsy and cardiac arrhythmias and, more recently, tumors. Cancer is one of the most lethal diseases today, so the majority of studies are focused on the research of hydantoin derivatives as potential anticancer drugs. In this study, a series of seven isopropyl hydantoin derivatives have been synthesized and their anti-proliferative effect on human colon cancer cell line, HCT-116, and effect on antioxidant activity of these cells were investigated. Anti-proliferative and antioxidant effects of new seven derivates of hydantoin, at concentrations 0.01, 0.1, 1, 10, 50 and 100 μM on human colon cancer cell line HCT-116 were determined spectrophotometrically, 24h after treatment. Investigated hydantoin derivates in 50 and 100 μM concentration express statistically significant anti-proliferative activity on HCT-116 cells. The derivative with the substituted benzyl group at the N3 position showed the best anti-proliferative activity (for all concentrations). Treatment with all investigated compounds induced decreasing of $O_2^{\cdot -}$ and increasing of NO_2^- concentrations in supernatants of treated HCT-116 cells. The treatment with all compounds resulted in an increase of glutathione that indicates a change in redox homeostasis. Based on the obtained results, it can be concluded that the investigated hydantoins act as antioxidants, because they decrease the production of superoxide anion radical and increase concentrations of glutathione, but they also induce the increase of production of nitrites. Our further research will be based on a different series of hydantoin compounds, but only those containing a benzyl group, because they induced significant anti-proliferative effects, a significant decrease of superoxide anion radical and a significant increase of production of nitrites. Based on the results, we will evaluate specific signalling pathways of these compounds.

P9

METABOLIC RECRUITMENT OF SKELETAL MUSCLE DURING COLD ACCLIMATION: REGULATORY ROLE OF PGC-1α/PPAR SIGNALING

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Skeletal muscle plays an important role in maintaining of the energy homeostasis and body temperature in homeothermic animals. In contrast to the clear role in skeletal muscle shivering thermogenesis in thermoregulation early in cold acclimation, its role during prolonged cold exposure, when non-shivering is activated, is not clear. The aim of this study was to examine the metabolic recruitment of skeletal muscle during 45-days cold acclimation in terms of its regulatory bioenergetics pathways. To this end, rats were exposed to cold (4±1°C) for periods of 1, 3, 7, 12, 21 and 45 days. Animals kept at room temperature (22±1°C) were considered as control. The expressional profile/activity of peroxisome proliferator-activated receptors (PPARs) isoforms and proliferator-activated receptor-γ coactivator-1α (PGC-1α), along with OXPHOS components, and key enzymes in glucose (glyceraldehyde-3-phosphate dehydrogenase, glycogen phosphorylase, hexokinase, lactate dehydrogenase) and lipid (acyl CoA dehydrogenase and succinyl-CoA-synthetase) metabolism, were examined. Compared with controls there was an initial increase in the protein level of 5′- AMP-activated protein kinase α (day 1), followed by an increase in PGC-1α and PPARs: PPARα and PPARγ from day 1 and PPARδ from day 7 of cold acclimation. Activation of the PGC-1α/PPAR transcription program was accompanied by increased protein expression of the key metabolic enzymes in β-oxidation, the tricarboxylic acid cycle and oxidative phosphorylation, with the exceptions in complex I (no changes) and ATP synthase (decreased at day 1). Cold did not affect hexokinase and GAPDH protein levels, but increased lactate dehydrogenase activity compared with controls (1–45 days). It seems likely that upregulation of the PGC-1α/PPAR transcription program early during cold acclimation triggers the molecular recruitment of skeletal muscle underlying the shift to more oxidative metabolism during prolonged cold acclimation.

P10

EFFECTS OF STRAWBERRY EXTRACT *FRAGARIA ANANASSA*, DUTCH VAR. ALBA ON HYDROGEN PEROXIDE INDUCED DNA DAMAGE

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Strawberries (*Fragaria ananassa*, Dutch var. alba) are fruits rich in antioxidant phytochemicals that have been identified as efficient agents against increased level of oxidative stress. DNA is a sensitive biomarker of oxidative stress and oxidative DNA damage has been implicated in the development of chronic diseases and cancer. The objective of the work is to investigate the effect of strawberry extract in attenuation of DNA damage induced by hydrogen peroxide in human leucocytes, and to determine the optimal concentrations for its effects. The metanolic, polyphenol-rich strawberry extract was obtained from Marche Polytechnic University, Ancona, Italy, and was diluted in phosphate buffered saline (PBS) at five final concentrations: 25, 50, 100, 150 and 200 μg/mL. The genotoxic potential of the strawberry extract was tested by *in vitro* exposure of peripheral blood leukocytes from six young and healthy volunteers to different concentrations of the extract for 30 minutes at 37 ^{o}C before measuring the level of DNA damages in treated cells and compared to values found in controls (exposed only to PBS). The samples of leukocytes were exposed to 50 μM hydrogen peroxide for 20 minutes at 4 ^{o}C before the incubation with the strawberry extract. The positive control was treated only with hydrogen peroxide under the same conditions. The level of DNA damage was measured after the treatments by Comet assay. The investigation of potential genotoxicity of the strawberry extract showed that the extract does not increase the level of DNA damage under described conditions. All concentrations were shown to be non-genotoxic and produced no DNA damage to the cells. On the other hand, the evaluation of its antigenotoxic properties showed that the strawberry extract significantly attenuated hydrogen peroxide-induced DNA damage at all used concentrations. A concentration dependant decline of DNA damage could be observed with the rise of extract concentration. Although all investigated concentrations of strawberry extract decreased the level of DNA damage, the concentration of 0.1 mg/mL was the lowest that showed a statistically significant effect. Obtained results confirm that the strawberry extract does have antigenotoxic properties, and its effects are concentration dependant. A detected beneficial properties make it recommendable for further research on the molecular mechanisms of action.

P11

DYNAMICS OF MITOCHONDRIAL MEMBRANE POTENTIAL CHANGES IN CULTURED MURINE ASTROCYTES UNDER HYPOXIA AND GLUCOSE DEPRIVATION

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When faced with long periods of nutrient deprivation (ND) or oxygen-glucose deprivation (OGD) astrocytes are capable of utilizing alternative sources of energy in order to maintain their viability. Here we investigated the interconnection of the alternative energy metabolism with the maintenance of mitochondrial membrane potential (MMP). The changes in MMP were visualized as the change in the fluorescence of JC-1 in murine astrocytes *in vitro* in two experimental setups: 1) after 6 or 8 h of OGD within one hour after reoxygenation and 2) during ND along with the inhibition of either autophagy using chloroquine or lipolysis using orlistat. We showed that astrocytes were resilient to extended periods of OGD, which had a negligible effect on MMP during reperfusion, whereas ND contributed to a more negative MMP. In addition, hypoxia further enhanced mitochondrial hyperpolarization when applied several hours after the beginning of ND. Results regarding the application of the respiratory inhibitor sodium azide indicated a decreased dissipation and an increased buildup of mitochondrial membrane potential in aforementioned conditions. Furthermore, we observed that early inhibition of autophagy (8 h) makes astrocytes vulnerable to subsequent ND. The inhibition of lipolysis also provoked cell death, but it emerged much later (24 h) compared to the effects of autophagy inhibition. Moreover, the application of these inhibitors prevented ND-related hyperpolarization of mitochondrial membrane. These results clearly show that autophagy and lipolysis are essential for astrocytes survival under these stress conditions, which may add to their role as neuron-supporting cells.

P12

EFFECTS OF MELATONIN ON AUTOPHAGIC PROCESSES IN THE LIVER OF DIABETIC RATS

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Autophagy is a cellular process that involves lysosomal degradation and recycling of intracellular organelles and proteins. By removing damaged and dysfunctional cellular components in order to maintain energy homeostasis during cellular stress, autophagy can serve as a cytoprotective mechanism. Also, it could lead to cell death if it's overactive or defective. Molecular mechanisms responsible for the two faces of autophagy are still partially known. Therefore, for the development of therapy based on autophagy modulation, it's necessary to fully define these processes. This study investigated the role of oxidative stress on autophagic processes in the liver of diabetic rats and effects of melatonin, as an antioxidant, on autophagy initiation/modulation. The liver, as one of the main target organs of insulin, takes an important role in regulation of glucose homeostasis. In diabetes, hypoinsulinemia followed by hyperglycemia increases mitochondrial proton gradient within the cells. In this state organelles become the source of reactive oxidative species leading to macromolecule damage which may cause necrotic, apoptotic or autophagic cell death. In the liver of diabetic rats obtained four weeks after diabetes induction with streptozotocin (65 mg/kg, i.p.), light and electron transmission microscopy showed significant changes in the structure of the cells and a large number of necrotic cells. By using Western blot, immunoprecipitation and confocal microscopy analyses, autophagy in diabetic liver was confirmed by increased expression of proteins required for autophagosome formation, LC3B and Beclin1, and by the presence of Beclin1 interactions with its activator HMGB1. In the state of oxidative stress HMGB1 is relocated from the nucleus to the cytoplasm. Continuous melatonin treatment of diabetic rats (2mg/kg/daily, i.p.) leads to significant reduction of liver damage, presumably through elevated mitochondrial autophagy. Melatonin additionally contributes to elevated expression of LC3B and Beclin1, HMGB1-Beclin1 interactions and autophagosome formation. Thus, it seems that melatonin protects the liver from diabetes induced damage by favoring autophagy as a protective mechanism.

P13

ARONIA MELANOCARPA JUICE IMPROVES PLASMA FATTY ACID STATUS IN HUMAN ADULTS WITH LOW CARDIOVASCULAR RISK

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Imbalanced dietary polyunsaturated fatty acids (PUFA) intake is associated with increased risk of cardiovascular (CV) disease. High arachidonic acid (AA)/eicosapentaenoic acid (EPA) ratio, and reduced tissue and/or blood levels of omega-3 fatty acids, as reflected in the tissue EPA plus docosahexaenoic acid(DPA) level (ie, the omega-3 index), are associated with increased risk for coronary heart disease. We investigated the associations between *Aronia melanocarpa* juice (AMJ) supplementation and plasma fatty acid (FA) status upon 4-week intervention in human adults with low CV risk factors. Randomized, parallel, placebo-controlled study was conducted in 109 participants, divided in three groups. Group NUT (n=41) was drinking AMJ with full dose of polyphenols, group CEN (n=42) consumed AMJ with 1/4 dose of polyphenols, and control group (BAC, n=26) was drinking placebo with exact chemical composition as NUT, but without bioactive polyphenols. Plasma phospholipid FA status was determined using GC chromatograph, as a percentage of individual FA in total pool. Analyses were performed at the begining and at the end of the study. Two-way ANOVA interaction (treatment*time) and Tukey post-hoc test were used for statistical analysis of the data. Our preliminary data indicate that both NUT and CEN significantly increased EPA (p=0.009, and p=0.024, respectively) and decreased AA/EPA ratio (p= 0.002 and p=0.021, respectively), upon 4 week treatment. NUT significantly decreased AA (p=0.034), and the AA/DHA (p=0.058) ratio in comparasion to the pre-treatment. The results of our study offer an indication that AMJ have dose-dependent, beneficial effects on CV health, by improving plasma phospholipid FA status, as an intermediary metabolite involved.

P14

ACTIVITY OF XANTHINE OXIDASE AND MYELOPEROXIDASE IN PATIENTS WITH AGE-RELATED CATARACT AND HYPERTENSION

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Not all the mechanisms responsible for the occurrence of hypertension are known to date, but many research results indicate the importance of reactive oxygen species in the pathogenesis and development of this disease. The study included 130 patients with age-related cataract, 69 of whom were diagnosed with hypertension, 20 patients with hypertension (HT) and type 2 diabetes mellitus (DM), and 41 subjects in addition to age-related cataract had an accompanying diagnosis. In the plasma of the examinees the following was measured: products of lipid peroxidation (LP), malondialdehyde (MDA) and lipofuscin-like fluorophores (LLF), activity of prooxidative enzymes xanthine oxidase (XO) and myeloperoxidase (MPO), antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx), the concentration of thiol (-SH) groups and ferric reducing activity of plasma (FRAP). Significantly higher concentration of LP products was measured in the plasma of patients with age-related cataract and hypertension (LLF, 79.7±11.0 RFU/ml; MDA, 6.0±0.5 μmol/L), and in the plasma of patients with DM and HT (LLF, 92.3±4.5 RFU/ml; MDA, 8.0±0.6 μmol/L) compared to the patients with only age-related cataract (LLF, 48.4±5.2 RFU/ml, MDA, 4.6±0.8 μmol/L) ($p<0.01$). Activity of prooxidative enzymes XO and MPO was significantly higher in the plasma of patients with HT (XO, 9.0±1.2 U/L; MPO, 77.3±8.4 U/L) and with HT and DM (XO, 11.9±0.9 U/L; MPO, 89.5±5.0 U/L) compared to patients with age-related cataract (XO, 6.2±0.9 U/L; MPO, 52.4±6.3 U/L) ($p<0.01$). Our research has shown that patients with age-related cataract and hypertension were exposed to increased oxidative damage of biomolecules, based on the increased plasma LLF and MDA content and decreased levels of thiol (-SH) groups. Oxidative changes of biomolecules in these patients were associated with increased activity of the XO, MPO and GPx enzymes and a lower extracellular SOD activity and total ferric reductive ability of plasma.

P15

ANTIOXIDATIVE ACTIVITY, TOTAL POLYPHENOLS, TANNINS AND ANTHOCYANINS CONTENT OF BLACKCURRANT JUICES (*Ribes nigrum* L.), VARIETY BEN SAREK

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A wide range of nutrients (vitamins, carbohydrates, minerals and organic acids), especially polyphenols, make black currants, *Ribes nigrum* L., one of the most investigated species in the berry kingdom. These constituents have been known to take part in many biological activities as well as to improve health and prevent diseases such as chronical noncommunicable diseases. The objective of the research was to determine the contents of total polyphenols, tannins and anthocyanins and to evaluate the antioxidative activities of the juices from blackcurrants, variety Ben Sarek. The juices were prepared from fresh, undamaged samples of berries, collected during 2008, 2009 and 2010. The total phenolic and tannins contents were determined using Folin-Ciocalteu method and the evaluation of the total amount of anthocyanins was conducted according to European Pharmacopeia 6.0. The antioxidant capacity was assessed using two complementary *in vitro* tests - DPPH (2,2-Diphenyl-1-picrylhydrazylý) and *â*-caroten/linoleic acid model. The highest amount of total anthocyanins and tannins were found in Ben Sarek juice from 2008, and juice from 2010 was the richest in polyphenols. The juice from 2009 was the most powerful in inhibition of lipid peroxidation in *β*-carotene-linoleic acid, and in inhibition of free radicals, in DPPH system. Due to high antioxidative activity blackcurrent juices, variety Ben Sarek, may have considerable role in prevention of diseases related to oxidative stress. All the presented facts may contribute greatly in related further research.

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P16

MAGNESIUM DIMINISHED EFFECT OF CADMIUM ON THE ACTIVITY OF SUPEROXIDE DISMUTASE IN KIDNEY BUT NOT IN LIVER OF MICE EXPOSED TO ACUTE CADMIUM INTOXICATION

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The study was designed to investigate the role of magnesium (Mg) pretreatment on the activity of superoxide dismutase (SOD) and the content of cadmium (Cd) in kidney and liver of mice exposed to acute Cd intoxication. Animals were divided into three groups: I - control group - not treated animals, II - Cd group: mice given single oral dose of 20 mg Cd/kg b.w. as aqueous solution of $CdCl_2$, and III - Mg+Cd group: animals given orally 40 mg Mg/kg b.w. as aqueous solution of $Mg(CH_3COO)_2$, 1h before Cd treatment. Activity of SOD and Cd content were determined in investigated organs after 4, 6, 12, 24, and 48 h. The obtained results show that acute Cd intoxication significantly decreased SOD activity in both organs: in all investigated times in liver and after 6, 12 and 24 h in kidney. In mice pretreated with Mg, SOD activities were decreased after 6, 12, 24, and 48 h in liver, but were not altered in kidney if compared with control group. These results imply a positive role of Mg pretreatment on SOD activity in the kidney, but not in liver of mice exposed to acute Cd intoxication. This could be explained by the fact that under the same experimental conditions Mg induced reduction of Cd content in the kidney of mice and thus had beneficial effect on the activity of SOD in this organ, while Mg pretreatment had no effect on hepatic Cd content if compared with animals given Cd only.

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P17

EFFECTS OF DIFFERENT *Teucrium* SPECIES ON MECHANISM OF APOPTOSIS IN COLON AND BREAST CANCER CELL LINES

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The aim of this study is to investigate the cytotoxic effects of methanolic extracts from *Teucrium polium* L. and *Teucrium montanum* L. and their effects on the mechanisms of induced apoptosis in colon (SW-480) and breast (MDA-MB-231) cancer cell lines. Cytotoxic activity was determined by MTT assay, type of cell death by acridine orange / ethidium bromide assay on fluorescent microscope, protein expression of Fas receptor on cell membrane by immunofluorescence staining, and activity of caspase 8 and 9 by colorimetric assay. Results showed that extracts significantly decreased viability of tested cells and caused medium cytotoxic activity, with lower IC_{50} values in SW-480 than in MDA-MB-231 cells. Also, the tested plant species induced apoptosis in different percentage according to concentration of extract, type of cell line and time of cell exposure to extracts. A dominant type of cell death is apotosis. Treatments influenced apoptosis biomarkers, leading to Fas protein overexpression in MDA-MB-231 cells and activation of caspase 8, which suggest that these extracts trigger apoptosis via an external apoptosis pathway. In SW-480 cells expression of Fas receptors was unchanged, as well as caspase 8 activity in comparison to control cells. Caspase 9 activity in the MDA-MB-231 cell line was increased in treated cells, while in SW-480 cells this activity was the same as in the control cells. Increased activity of caspase 9 in MDA-MB-231 cells also indicates the activation of the apoptotic internal pathway. Finally, we concluded that the examined species of plants induced apoptosis in various ways and that is prominent in MDA-MB-231 cell line. When compared two tested plant species, *Teucrium montanum* shows a better cytotoxic activity. Based on the results we can conclude that the investigated plants can be considered as a potential source of natural bioactive substances in potential tumor therapy.

P18

EXTRACELLULAR MYELOPEROXIDASE ACTIVITY AND BLOOD PRESSURE IN MAINTENANCE HEMODIALYSIS PATIENTS

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Inflammation and oxidative stress play critical roles in atherosclerosis and hypertension (HTA) in general population, being significant risk factors of increased mortality rates among patients with end-stage renal disease (ESRD) on maintenance hemodialysis (HD). Evidences suggest that a phagocyte-derived pro-oxidant enzyme myeloperoxidase (MPO) can initiate and propagate oxidative damage, contributing to the development of atherosclerotic plaque. Given that MPO is released in vascular compartment at variable extent during each HD session we investigated its relationship with systolic (SBP) and diastolic blood pressure (DBP) in ESRD patients. Beside routine biochemical analyses, serum MPO activity, C-reactive protein (hsCRP) and markers of oxidative damage, total hydroperoxides and advanced oxidation protein products (AOPP), were measured in 28 adult ESRD patients, before and after completion of a single HD session. HTA was defined as on antihypertensive medication, or as having pre-HD SBP ≥140 mmHg, so patients were considered as normotensive (non-HTA; n=11), or hypertensive (HTA; n=17). In comparison to non-HTA, patients with HTA were younger (p=0.006), with slightly better nutritive risk index (p=0.081). Other variables, like gender distribution, HD vintage, index of atherosclerosis, HD-induced blood volume change, pre-HD leukocyte count, hsCRP, and AOPP did not differ between groups. Pre-HD MPO activity (18.9 vs. 11.7 U/L; p=0.016) and hydroperoxides (9.08 vs. 7.57 μmol/L; p=0.041) were higher in HTA than in non-HTA group. Pre-HD MPO activity significantly correlated with hydroperoxides (rho=0.435; p=0.024), AOPP (rho=0.424; p=0.030), and SBP (rho=0.452; p=0.019), but not with DBP (rho=0.185; p=0.337). The ROC analysis showed that pre-HD MPO > 17.9 U/L was associated with HTA criterion (AUC=0.773; p=0.0020). Interestingly however, post-HD MPO activity was poorly correlated with either SBP or DBP. These results suggest that interdialyic rather than intradialytic activity of extracellular MPO can be implicated in regulation of SBP among ESRD patients on maintenance HD.

P19

ANTIOXIDATIVE ACTIVITY OF SECONDARY METABOLITES FROM FERTILE AND STERILE STEMS OF *EQUISETUM TELMATEIA* ERHART

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Equisetum telmateia Erhart - great horsetail, (genus *Equisetum*, family Eqiusetaceae) is a dimorphic species that inhabits damp and wet areas generally near streams, rivers and wetlands of Europe, western Asia, northwest Africa and north America. It is herbaceous perennial plant with separate green sterile stems and spore bearing fertile stems. The leaves, nodes, internodes and branch buds are initiated in the fall but elongate after a period of rest the following spring. Fertile stems are straight and unbranched, and end in a single fusiform cone. Vegetative shoots bear photosynthetic, verticillate branches. In this study comparative analyzes of total phenolic content, flavonoid concentration and *in vitro* antioxidant activity of methanolic extracts from fertile stems, spore bearing strobilus and sterile stems were performed. Obtained values for the total phenolic content, expressed in terms of gallic acid equivalent per ml of crude extract, were 73.81 mg GA/ml for sterile stems, 66.89 mg GA/ml for fertile stems and 66.11 mg GA/ml for strobilus, respectively. The results obtained for the flavonoid concentration, expressed in terms of rutin equivalent per ml of crude extract, were 70.57 mg RU/ml and 24.86 for sterile and fertile stems, as well as 20.26 mg RU/ml for strobilus. The antioxidant activity were examined in ten different concentrations (from 100% to 0.195%) using DPPH reagent. Obtained values for sterile stems are ranged between 5.18% and 98.05% of inhibition, for fertile stems between 4.08% and 85.71% while for strobilus values were between 4.44% and 85.402% inhibition. Comparative analysis of investigated plant parts showed significant differences in the amount of secondary metabolites. When compared with the values for antioxidant activity, it was found that extracts of sterile stems, besides largest concentration of secondary metabolites, possess the highest antioxidant activity.

P20

MITOCHONDRIAL PEROXIDATIVE DAMAGE INDUCED BY nC60 NANOAGREGATES AND CHLOROMETANES IN *DAPHNIA MAGNA* MIDGUT CELLS

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Adsorption of non-polar compounds by suspended fullerene nanoaggregates may enhance their toxicity and affect the fate, transformation, and transport of non-polar compounds in the environment. The potential of stable fullerene nanoaggregates as contaminant carriers in aqueous systems, and impact of chloromethanes (CH_2Cl_2, $CHCl_3$ and CCl_4), were studied in midgut epithelial cells of *Daphnia magna* by light and electron microscopy. The most prominent effects of chloromethane in synergy with nC_{60} were observed in enterocyte mitochondria. Transmission electron microscopy analysis revealed the cell injuries that led to their loss. Enterocytes showed extensive cytoplasmic vacuolization with disruption and loss of specific subcellular organelles (lipid droplets, microvilli). All signs of mitochondrial peroxidative damage could be noted: crenellation of the outer membrane coupled to its dissociation, followed by mitochondrial swelling and vesiculation of the inner membrane. Our observations indicate that cellular damages occurred after nC_{60}/chloromethane treatments were more likely chloromethane toxic-related, than nanosize-related adverse effects. A high lipophilicity of nC_{60} molecules points to the ability of nC_{60} to pass the cellular barrier and enter the subcellular structures, particularly mitochondria.

P21

CYTOTOXIC, PROAPOTOTIC, PROOXIDANT AND ANTIMIGRATORY EFFECTS OF TWO NEWLY SYNTHESIZED Pt(IV) COMPLEXES AND THEIR RESPECTIVE LIGANDS ON COLON CANCER CELL LINES

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It is known that platinum complexes are used in medicine for various types of cancers disorders including colorectal cancers, in single and co-treatments with different known drugs or medicines isolated from natural sources. Considering that induction of apoptosis and suppressed cell motility are one of the main strategies in cancer therapy, we investigated cytotoxic action, type of cell death, antimigratory potential and production of reactive oxygen and nitrogen species of two newly synthesized platinum(IV) complexes with some *O,O'*-dialkyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-propanoic acid ([$PtCl_4$(dbu-*S,S*-eddp)] (C1) and [$PtCl_4$(dpe-*S,S*-eddp)] (C2)) and their respective ligands on HCT-116 and SW-480 cell lines. The cytotoxic activity was measured by MTT assay. The antimigratory activity of extracts was determined by Transwell assay. Prooxidant/antioxidant status was followed by monitoring superoxide anion radical ($O_2^{\bullet-}$), nitrites and reduced glutathione (GSH) levels. Our results showed that the highest cytotoxic effects were caused by treatment with C2 complex on SW-480 cells (IC_{50}=36.54±1.50 μM) after 24 h, while its ligand had the highest effects on HCT-116 cell line (IC_{50}=67.72±1.71 μM) after 72 h. All treatments induced oxidative stress in tested cells with significant increase of $O_2^{\bullet-}$ and nitrite levels, as well as decreased GSH content. The highest cytotoxic activity was followed by strong proapototic and significant prooxidant activities. All drugs tested on HCT-116 cells demonstrated significant antimigratory effects which correlated with the increased production of $O_2^{\bullet-}$, while the same effect on SW-480 cells was showed only by ligands. In conclusion, tested drugs (C2 complex and its ligand) had significant cytotoxic, proapoptotic and prooxidative activities on both colon cancer cell lines, while all complexes had significant anti migratory potential on HCT-116 cells.

P22

HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES IN THE OVARY OF PIGS TREATED WITH SELENOPYRAN

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Selenium is important to male fertility, but the information how this element could be involved in healthy reproduction of female is limited. Due to better biological utilization, the interest to the organic selenium increasing during the last years. The present work aimed to study the effect of injective application of selenopyran on the ultrastructure of the ovary of pigs. The experiment was conducted with 18 gilts of Danube white breed, ages between 120-228 days, divided into two groups. The animals received equal basal diets without selenium additives. The content of Se in basal diet was 0.15 mg per kg of forage. The experimental gilts (n=9) were injected intramuscularly with oil solution of selenopyran (9-phenyl-symmetrical octahydroselenoxanthene) once per month with dose 0.1 mg Se/kg body weight. This organic source of selenium contains Se and has a lot of advantages: the possibility for precise dosage of the compound; the toxity of it is lower than sodium selenite (LD50=1600 mg/kg against LD50=3.25 mg/kg). With regards to its chemical structure, selenopyran plays a role as selenium storage and liberates the selenium slowly according the needs of the organisms. After slaughtering, one ovary from each animal was used for the histological analysis under light and electron microscope. The second ovary was used for the estimation of the selenium content in ovarian tissue by the atomic absorption spectroscopy method using apparatus SpectrAA 220Z „Varian“. The histological analysis of ovaries had shown the more active folliculogenesis in pigs treated with selenopyran. The full divisions of the developing follicles and the presence of corpora lutea were detected as a confirmation of the ovulating process. The finding of the corpus albica is evidence that some of selenopyrane treated pigs passed through the second wave of ovulation. In control animals only a few tertiary and ovulatory follicles were found. Also, the atretic follicles were observed in the ovaries of control animals, but not in experimental. At ultrastructural level no signs of aberration within all three types follicle were observed. Hence, our results suggest that treatment with selenopyran has beneficial effect on pigs folliculogenesis resulting in more healthy follicles going to ovulation.

P23

A COMPUTATIONAL INSIGHT INTO ANTIRADICAL ACTIVITY OF THE MARINE NATURAL PRODUCT AVAROL

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Alzheimer's disease (AD) can be significantly related to oxidative stress. It is a progressive and irreversible neurodegenerative disease characterized by dysfunction of intracellular and extracellular biochemical processes leading to neuronal death. Oxidative damage is one of the earliest cytopathological markers of neuronal dysfunction involving AD. Avarol belongs to the most significant secondary metabolites of the marine world, having a wide spectrum of biological activities and low toxicity. It can pass through the blood-brain barrier, a common obstacle in the AD drug development. The antiradical activity of this marine natural product originates from two hydroxyl groups in the hydroquinone part of the molecule. For the purpose of computational prediction of avarol antiradical activity, the reactions of hydrogen abstraction (from its hydroxyl groups) were analyzed. All calculations were carried out using Gaussian 09 software package. The structures of the reactants and reaction products were optimized with the BP86 functional and 6-311+G(d) basis set. The solvent (ethanol) was modelled with the polarizable continuum model. The data analysis has shown that most of the reactions are exergonic, with the sterically less protected hydroxyl group being more reactive towards free radical species. However, the differences in Gibbs free energy for the hydroxyl groups were small (1.04 - 1.74 kcal/mol). The greatest changes in Gibbs free energy were found for the reaction with hydroxyl radical. On the contrary, these changes were rather small for ascorbyl and DPPH radicals. All reactions of nitric-oxide and ascorbyl radicals with the sterically more protected hydroxyl group were endergonic. The computed data obtained for the ascorbyl radical are in good agreement with the experimental one including ethanol as a solvent. Taken all together, these results encourage further research work directed towards the possible application of avarol and/or its semi-synthetic tio- and amino-derivative(s) in AD treatment.

P24

THE CORRELATION BETWEEN TOTAL POLYPHENOLIC CONTENT AND ANTI-DPPH RADICAL ACTIVITY OF SELECTED *VRANAC* RED WINE SAMPLES

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Red wine polyphenolic compounds, which consist of various powerful antioxidants such as flavonoids and stilbenes, possess well known protective effects on the cardiovascular system, as well as antitumour, antiviral and antiallergic properties. These natural products are actually believed to be health-promoting. Herein the total polyphenolic contents of selected *Vranac* red wine samples (originating from FYR Macedonia) were correlated to their anti-DPPH radical activities. The polyphenolic content was determined by the Folin-Ciocalteu method, while the anti-DPPH radical activity was evaluated by standardised spectroscopic methods (EPR and UV-VIS). All wine samples contained a notable quantity of polyphenolic compounds, ranging from 1508 to 2513 mg/L. On the other hand, their anti-DPPH radical activities varied in the ranges 25-33 % and 25-41 % using EPR and UV-VIS, respectively. A positive correlation was observed between the total polyphenolic content and anti-DPPH radical activity. Furthermore, the experimental data obtained for anti-DPPH radical activity (applying two different instrumental techniques) were consistent with each other. Further research work will be directed towards determination of the content of particular polyphenolic compounds in these wine samples, using UPLC/MS chromatography with TQ analyser.

P25

THE CHALLENGE OF VISUALIZING INSULIN-INDUCED MITOCHONDRIAL INNER MEMBRANE REMODELING IN RAT BROWN ADIPOCYTES

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Brown adipose tissue (BAT) is an important organ involved in the process of non-shivering thermogenesis induced by various physiological stimuli. To fulfill this role, brown adipocytes have numerous mitochondria with uncoupling protein 1 (UCP1) localized in inner mitochondrial membrane. Many studies revealed that the organization of the inner mitochondrial membrane, *e.g.* cristae, can be remodeled under specific metabolic needs of the cells. Insulin regulates BAT activity, improves mitochondrial function and is important for efficient ATP production. In this study, we investigated the effect of insulin on mitochondrial cristae remodeling in brown adipocytes. Two month old Wistar rats were fed with standard pelleted food *ad libitum*. The rats were divided into three groups, each consisting of six animals. The first two groups were treated with low (0.4 IU/kg) or high (4 IU/kg) doses of insulin i.p. (Mixtard® 30, NovoNordisk, Denmark) over three days, while the group treated with a 0.9% saline solution i.p. served as a control. The interscapular portion of BAT was isolated and used for mitochondrial isolation. Mitochondria were isolated using modified protocol after Cannon and Lindberg (1979). Resulting mitochondria enriched fractions were fixed in 2.5% glutaraldehyde in 0.1M Sørensen phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in increasing concentrations of ethanol and embedded in Araldite. 200 nm thin sections, mounted on formvar coated nickel grids, were used for immunogold localization of complex III and complex IV of the electron transport chain. These gold particles were used as fiducial markers for tomographic reconstruction. A single tilt series of 91 images were taken every 1º from -45º to +45º using Philips/FEI CM12 transmission electron microscope equipped with SIS MegaView III. Alignment of these micrographs by cross-correlation and reconstruction was made using IMOD eTomo software (http://bio3d.colorado.edu/imod/).

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OPPOSITE EXPRESSION OF MITOCHONDRIAL BIOGENESIS MARKERS IN STEROID-PRODUCING CELLS OF ADRENAL GLAND AND TESTES FROM STRESSED ADULT RATS

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Steroid-producing cells of the adrenal cortex as well as Leydig cells of testes require functional mitochondria, since these organelles are an essential part and the key control point for steroid hormones biosynthesis and regulation. The aim of this study was to determine the transcriptional profile of molecular markers of mitochondrial biogenesis in steroid-producing cells of the adrenal cortex and Leydig cells of testes by applying *in vivo* and *in vitro* studies. Immobilization stress (IMO) was performed for 2 hours daily for one (1xIMO), two (2xIMO) or ten (10xIMO) consecutive days. Real time PCR results showed that the transcription of the main regulators of mitochondrial biogenesis and integrator of environmental signals, *Ppargc1a* and *Ppargc1b,* significantly decreased in the adrenal cortex of 10xIMO rats. Oppositely, a significant increase of the same transcript was registered in Leydig cells from the same rats. In parallel, transcription of *Ucp3*, the mediator of regulated proton leak, decreased in adrenal cortex cells, but increased in Leydig cells of the same group of rats. Incubation of primary cultures of purified Leydig cells isolated from undisturbed rats, with the stress hormone adrenaline, increased transcription of the main markers of mitochondrial biogenesis (*Ppargc1a*, *Ppargc1b*, *Nrf1* and *Nrf2a*). The results also showed that propranolol, as a nonselective β-ADRs-blocker, attenuated this effect. Administration of prazosin, the selective α1-ADRs antagonist, in combination with adrenaline did not change the adrenaline-induced stimulation of *Ppargc1a, Ppargc1b, Nrf1* and *Nrf2a* transcription in Leydig cells. On the other hand, prazosin alone significantly increased the transcriptional level of *Nrf2a* in Leydig cells. The results indicate that the expression of *Ppargc1*, master regulator of mitochondrial biogenesis, is stimulated by β-adrenergic receptors, not by α1-ADRs. *In vitro* stimulation of Leydig cells with corticosterone, another stress hormone, gave less striking effects. In summary, the results of *in vivo* experiments suggest that reduction of transcription of mitochondrial biogenesis markers could be a possible mechanism that protects the body from excessive glucocorticoid production from adrenal glands in stress conditions, while at the same time stimulation of mitochondrial biogenesis markers transcription in Leydig cells could serve as mechanism to preserve testosterone production.

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SCREENING OF ANTIOXIDANT ACTIVITY OF SOME *N*-ALKYLARYL-3- OR 4-SUBSTITUTED GLUTARIMIDE DERIVATIVES

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Natural products, as constituents of plants, have long been sources of drugs. The development of new therapeutic agents and drug discovery are focused on natural products due to their large diversity in nature and their widespread biological activities. Both naturally occurring and synthetic cyclic imides, especially five- and six-membered systems, are important groups of bioactive molecules. 9-methylstreptimidone (9-MS), a derivative of natural glutarimide streptimidone exerts significant inhibitory activity toward nuclear factor-κB (Nf-κB). Nf-κB is involved in cancer and inflammation. Recent findings indicate that some of ethyl 2,6-dioxo-*N*-aryl-piperid-3-ene-4-carboxylates showed promising free radical scavenging ability. Here we report the results of radical scavenging activity of five glutarimide derivatives, synthesized by tandem process, which involves a base-catalyzed Michael addition of active methylene compounds to secondary acrylamides or crotonamides, followed by intramolecular *N*-acylation of the carboxamido group. To the best of our knowledge, there is no literature data on antioxidative activity of those compounds. The antioxidant profile of compounds was examined by *in vitro* protocol, involving their interaction with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable free radical (method of Bilos). Ascorbic acid, BHT, catechin and á-tocopherol were used as the positive control. The obtained results show that compound No.4 (*Tert-pentyl-2,6-dioxo-1-phenethylpiperidine-3-carboxylate*) have very weak activity (2.3-3.9 %), while the other compounds tested had no scavenging ability of DPPH radical in the examined concentration range (0.05-3 mg/mL). The radical scavenging ability of the positive controls BHT, ascorbic acid, á-tocopherol and catechin were between 0-21.0%, 79.8-69.6%, 84.3-83.3% and 13.9-77.8%, respectively. This derivative will be a starting point for further structural modifications in order to obtain compounds with a better antioxidative profile.

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THE EFFECTS OF *N*-ACETYL-L-CYSTEINE ON DECIDUOUS TEETH DENTAL PULP STEM CELLS ANTIOXIDATIVE PROTECTION

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Deciduous teeth dental pulp stem cells (DTSC) could be easily obtained, efficiently cryopreserved and seemed to be more primitive than those isolated from bone marrow and adipose tissue. *N*-acetyl-L-cysteine (NAC) is amino-thiol compound which can act both as a precursor of reduced glutathione, and as a direct ROS scavenger, regulating cell antioxidative capacity and preventing oxidative damage, an important role when considering the DTSC *in vitro* expansion at 20% of oxygen. Since NAC could potentially be used for stem cells *in vitro* expansion protocols, the aim of this study was to investigate the effects of its different concentrations on DTSC antioxidative protection: superoxide-dismutase (SOD) and catalase (CAT) activity, total thiol groups content as well as the level of oxidative damage of lipids and proteins. Twenty-four hours after treatment with 0.1 mM and 1 mM NAC, a significant reduction in the content of carbonyl groups as well as the degree of lipid peroxidation was found. Although NAC is being considered as a significant source of thiol groups and a cysteine donor for the synthesis of glutathione, the effect of NAC treatment on the content of thiol groups 24h after treatment was variable, and only NAC concentration of 0.1 mM significantly increased content of thiol groups. The activities of SOD and CAT 24h after NAC treatment were variably reduced, depending on the NAC concentration. Namely, total SOD and CAT activity significantly decreased after treatment with 0.1 mM and 1 mM NAC. Adding NAC to DTSC culture after 48 h reduced levels of MDA. Concentration of 0.1 mM significantly reduced levels of MDA, but 1 and 2 mM NAC induced reduction did not reach significance. After 48h NAC treatment did not influence the concentration of total thiol groups as well as the content of carbonyl derivatives of proteins. The activity of SOD and CAT was significantly decreased by 0.1 mM NAC. The lowest NAC dose exerted significant positive effect on DTSC proliferation as well as antioxidative protection creating beneficial environment for stem cells *in vitro* cultivation.

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DIARYLHEPTANOIDS FROM *ALNUS VIRIDIS* DECREASE MITOCHONDRIAL TRANSMEMBRANE POTENTIAL AND PROMOTE SUPEROXIDE ANION PRODUCTION IN NCI-H460 AND HACAT CELLS

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A comparative study was performed on diarylheptanoids isolated from the barks of black alder (*A. glutinosa*) and green alder (*A. viridis*) to address their biological effect on cancer and normal cells and determine their structure-activity relationship. Diarylheptanoids actions were studied in human non-small cell lung carcinoma (NCI-H460) and normal keratinocytes (HaCaT). Citotoxicity was examined by the sulforhodamine B assay. Cell cycle distribution and cell death detection were determined by flow-cytometry following propidium iodide (PI) and Annexin-V-FITC/PI labeling, respectively. Superoxide anion generation was detected by dihydroethidium staining and assessed by flow-cytometry and fluorescence microscopy. Mitochondrial transmembrane potential ($\Delta\Psi_m$) was assessed by flow-cytometric measurement of JC-1 fluorescence as well as live imaging. The structure comparison in *A. viridis*/*A. glutinosa* analog pairs, **1/3** and **2/4**, revealed that 3’ and 3”-OH groups in *A. glutinosa* compounds significantly decrease their cytotoxic activity. **3** and **4** did not cause considerable cell death observed after **1** and **2** treatment in both cell lines. **1** and **2** notably perturbed cell cycle kinetics and decreased the percentage of NCI-H460 and HaCaT cells in G_1 phase with major increase in dead cells (G_0 phase). **1** and **2** treatment triggered significant intracellular accumulation of superoxide anion compared to *A. glutinosa* diarylheptanoids. Same compounds also notably decreased $\Delta\Psi_m$ in NCI-H460 and HaCaT cells after 24 h treatment. The obtained results indicate that disturbance of $\Delta\Psi_m$ and superoxide anion production represent important mechanisms of pro-apoptotic action of investigated compounds. However, minor differences in the chemical structure can considerably influence pro-oxidant activity of diarylheptanoids.

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PROTECTIVE EFFECTS OF QUERCETIN AND (-)-EPICATECHIN AGAINST COPPER INDUCED OXIDATIVE STRESS IN RAT LIVER

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Copper (Cu) is an essential trace element that plays an important role in biological systems, however, increased concentration may cause toxic effects. Flavonoids are natural plant polyphenol compounds which exhibit a wide spectrum of biological activity and may exert antioxidant effects through different mechanisms. The effects of flavonoid quercetin (QE), and (-)-epicatechin (EC) under subchronic exposure to sublethal doses of Cu on the oxidative-antioxidative status in rats liver were studied. Male *Wistar* albino rats (n=28) were divided into 4 experimental groups: (1) control, (2) Cu-treated (as $CuCl_2$, at a concentration of 560 mg/L through drinking water for 5 weeks); (3) QE+EC-treated (40 mg/kg body weight each, *i.p.*, every third day for the last 3 weeks); and (4) Cu+QE+EC-treated, at concentrations and manner previously described. After subchronic Cu intoxication activities of cytotoxicity liver markers AST, ALT, ALP, GGT, and LDH in the serum were elevated, while presence of QE and EC with Cu maintained the levels of those parameters closer to normal values. Cu-increased lipid peroxidation (LPO) and decreased concentrations of reduced glutathione (GSH) and vitamin C contribute to oxidative damage in liver tissue. Changes in the activities of the antioxidant enzymes after subchronic intoxication with Cu also indicated the occurrence of oxidative stress in liver tissue. Treatment with QE and EC reversed Cu-induced alterations of antioxidant defense system and significantly prevented Cu-induced liver damage. Flavonoids QE and EC significantly increase the concentration of non-enzymatic antioxidants GSH and vitamin C in cells thus contributing to an increase in intracellular antioxidant capacity. In conclusion, Cu intoxication induced oxidative stress and altered the antioxidant defense system, resulting in oxidative damage to rat liver. Flavonoids QE and EC exhibited antioxidant capacity and, cooperatively with endogenous antioxidants, diminished toxic effects of Cu in rat liver.

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ANTIOXIDANT ACTIVITY OF HESPERIDIN ISOLATED FROM ORANGE PEEL

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The flavonoid hesperidin is a bioflavonoid, *i.e.* flavanone glycoside consisting of flavanone (a class of flavonoids) hesperitin and the disaccharide rutinose. It is predominantly found in lemons and oranges. The peel and membranous parts of these fruits have the highest hesperidin concentrations. There are many reports regarding its antioxidant, antiinflammatory, antiallergenic, antihypertensive, antimicrobial, hypolipidemic, anticarcinogenic and vasodilatory properties. The isolation method of hesperidin involved extraction of the dried citrus peel successively with petroleum ether followed by methanol. The petroleum ether removes the lipophilic fraction in the peel and the methanol extracts the glycoside (hesperidin). The obtained methanol extract was evaporated at the rotary evaporator until a syrup consistency was reached. The residue was then mixed with 6% acetic acid - the precipitated solid was the crude hesperidine. It was then sucked off with a Buchner funnel, washed with 6% acetic acid and dried at 60 °C until its constant weight. For recrystallization, a 5% solution of the crude product in dimethyl sulfoxide was produced under stirring and heated to 60–80°C. Afterwards the same amount of water was added slowly whilst stirring. Cooling to room temperature precipitates hesperidin. It was sucked off, washed with a little warm water first, then with *iso*-propanol and finally dried in the desiccators until its constant weight. The characterization of the isolated hesperidin was carried out using UV-VIS, FT-IR and HPLC methods. The antioxidant activity of hesperidin was investigated spectrophotometrically using DPPH, FRAP and TBA-MDA methods. The degree of DPPH radical neutralization depends on the hesperidin concentration and incubation time applied. The best antioxidant activity has been shown by hesperidin incubated for 30 minutes. Hesperidin concentrations needed for 50% of initial DPPH radical concentration neutralization were 0.509 mg/ml (without incubation), 0.256 mg/ml (after 20 minutes) and 0.173 mg/ml (after 30 minutes incubation with radical). Inhibition of lipid peroxidation of 55% was achieved by hesperidin concentration of 0.1 mg/ml, while the FRAP value was 3.37 mmolFe^{2+}/g of hesperidin. Presented results clearly demonstrate that the isolated hesperidin is a potential natural antioxidant.

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GLUTATHIONE DEPLETION INCREASES OXPHOS CAPACITY OF THE RETROPERITONEAL ADIPOSE TISSUE IN RATS

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The prooxidative redox milieu mostly reflects metabolic activity of the tissue. Notably, cold-induced adrenergic stimulation decreases relative fat mass, intensifies lipolysis, glucose and fatty acid oxidation and increases the expression of uncoupling protein 1 (UCP1) in white adipose tissue (WAT). This is accompanied by depletion in the tissue glutathione (GSH) content. The aim of this study was to investigate whether the redox state *per se*, impact the metabolic activity, in particular oxidative capacity of fat tissue. For this purpose, rats with basal metabolic activity (kept at room temperature) and intense metabolic activity (exposed to cold for 1, 3, 7 or 21 days), were treated by GSH-depleting agent buthionine sulfoximine (BSO). Marked shrinkage of lipid droplets and an increase in the size and number of mitochondria characterized adipocytes in retroperitoneal white adipose tissue (rpWAT) of BSO-treated cold-exposed rats, as compared to untreated, room temperature maintained (control) rats. In parallel, protein levels of the components of oxidative phosphorylation (OXPHOS) Complex II, Complex III, cytochrome *c*, Complex IV and ATP synthase as well as the UCP1 were increased in BSO-treated cold-exposed rats. Remarkably, compared to untreated rats, long-term BSO treatment at room temperature also increased expression levels of UCP1, as well as the OXPHOS components, and induced structural remodeling indicating of lipolytic/oxidative mode of rpWAT function. Results suggest that BSO treatment potentiates the cold-exposure induced increase in oxidative capacity of rpWAT, and mimics the effects of adrenergic stimulation. Thus, GSH depletion may be a promising target for curbing obesity development and treating obesity-associated insulin resistance.

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FREE RADICAL SCAVENGING ACTIVITY AND TOTAL PHENOL CONTENT OF SUBMERGED MYCELIUM EXTRACTS OF THE SPECIES *COPRINUS COMATUS* (O.F. Müll.) Pers. (1797) AND *COPRINELLUS TRUNCORUM* (Scop.) Redhead, Vilgalys & Moncalvo (2001)

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Nowadays fungi are recognized as functional food both due to their high nutritive and medicinal properties. The present study aimed to investigate antioxidant potential of two autochthonous terricolous fungal species, *Coprinus comatus* (O.F. Müll.) Pers. (1797) and *Coprinellus truncorum* (Scop.) Redhead, Vilgalys & Moncalvo (2001). Their anti-DPPH radical activities (using hot water extracts, obtained from submerged mycelia) were correlated with the total phenolic (TP) contents. More precisely, two types of hot water extracts were performed using lyophilized biological material: submerged mycelial biomass (MB) and submerged broth (SB). The highest anti-DPPH radical activity was found for *C. comatus* SB extract (IC_{50} 5.06 µg/mL): actually, its MB extract reached a lower IC_{50} value (13.27 µg/mL). In comparison, *C. truncorum* showed lower anti-DPPH values with IC_{50} of 42.39 and 7.52 for SB and MB extracts, respectively. On the other hand, the TP contents of MB extracts were found to be higher for both fungal species: 81.95±2.62 µg/mL (*C. comatus*) and 81.64±2.26 µg/mL (*C. truncorum*), respectively. Statistically significant positive correlations of *C. truncorum* SB (r^2 =0.97) and MB (r^2=0.91) extracts are worth noting. Therefore, the identification of chemical profiles of both *C. truncorum* hot water extracts is currently in progress in our labs.

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HYDROXYL RADICAL SCAVENGING ACTIVITY VERSUS TOTAL PHENOL CONTENT OF AUTOCHTONOUS FUNGAL SPECIES *COPRINUS COMATUS* (O.F. Müll.) Pers. (1797) AND *COPRINELLUS TRUNCORUM* (Scop.) Redhead, Vilgalys & Moncalvo (2001)

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Hydroxyl radicals (•OH) are short-lived and highly reactive free radical species involved in the pathology of numerous oxidative stress related diseases. Since many lignicolous fungi have been recently recognised as promising sources of natural antioxidant substances, the aim of the present study was to investigate the capacity of crude extracts of two terricolous autochtonous fungi from the region of Sremski Karlovci (Vojvodina, Serbia), namely *Coprinus comatus* (edible) and *Coprinellus truncorum* (conditionally edible), to scavenge free •OH and evaluate their total phenolic contents (TPs). The scavenging capacities (RSC) of methanol (100% MeOH - M) and ethanol (80% EtOH - E) fungal extracts were determined using deoxiribose based assay, while the TPs were evaluated by the Folin Ciocalteu assay. The highest/lowest RSCs were found for *C. truncorum* M (IC_{50} 1.73 µg/mL) and *C. comatus* M (IC_{50} 8.34 µg/mL) extracts, respectively. The E extracts of both fungal species reached similar RSC values (IC_{50} 2.08 and 2.52 µg/mL, *C. comatus* and *C. truncorum*, respectively). In addition, the obtained TP contents were shown to be higher for the E extracts (*C. comatus* >*C. truncorum*, 69.47±2.83 and 54.74±5.75 mg GAEq/g d.w., respectively). As for the correlations between RSCs and TPs, the statistically significant one was found only for the fungus *C. comatus* M (r^2=0,90), indicating phenolic compounds as its possible key antioxidants. Further research work will be directed towards other physiologically relevant free radicals including both ROS and RNS species.

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THE EFFECTS OF LONG-TERM SUCROSE OVERFEEDING ON RAT BROWN ADIPOSE TISSUE

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Brown adipose tissue (BAT) is specialized to dissipate chemical energy in the form of heat through the expression of uncoupling protein 1 (UCP1) incorporated in mitochondrial cristae. The physiological role of BAT is to protect body temperature during cold exposure, especially in small mammals. BAT activity can also be stimulated by some aspects of diet (diet-induced thermogenesis, DIT), which makes it attractive as possible target for therapy of obesity. BAT thermogenesis is a complex process followed by intensified lipolysis, increased UCP1 expression and mitochondriogenesis. In this study, male albino Wistar rats were divided into two equal groups and fed with commercial rat food. Animals from experimental group were offered to drink 10% sucrose solution instead of tap water for 3 weeks. Interscapular portion of BAT was prepared for light and transmission electron microscopy (TEM), complemented with stereological and immunohistochemical analysis. Long-term sucrose-overfeeding led to variety of morphological changes in brown adipocytes, of particular mitochondria. Volume density of mitochondria and cristae were increased, as well as mitochondrial profile area and number per cell profile. Stereological analysis of mitochondrial cristae number demonstrated a shift to higher values after sucrose treatment. UCP1-immunopositive brown adipocytes were observed more frequently than in the control group, and reaction was stronger. In conclusion, morphological changes accompanied with increased UCP1 expression indicate increased BAT thermogenic capacity after sucrose treatment. These findings are particularly relevant in the view of the recent observation that functionally responsive BAT is present in adult humans.

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IN VITRO EVALUATION OF ANTIOXIDANT POTENTIAL OF THE FUNGUS *STEREUM SUBTOMENTOSUM* POUZAR (1964)

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In spite of the fact that fungi have been attracting much research attention in the last decade, the family Stereaceae remains insufficiently examined. Therefore, the aim of this study was to preliminarily evaluate total phenolic contents (TPCs) and antiradical activities (against DPPH and •OH radicals) of ethanolic (EtOH) and methanolic (MeOH) extracts of the white rot fungus *Stereum subtomentosum* Pouzar (1964). The analysed biological material was collected on mountain Fruška Gora (Vojvodina, Serbia). The highest antiradical activity was observed for MeOH extract (DPPH•, IC_{50} 4.91 µg/mL). The same extract was also found to be richer in phenolic compounds (87.02 versus 28.63 mg GAEq/g d.w.). It's noteworthy to mention that extracts reacted in a different extent towards the screened free radicals ($p<0.01$). High correlation factor ($r^2=0.95$) for TPC and anti-DPPH radical activity of its MeOH extract suggests the relevance of phenolics which need to be isolated and chemically characterised. Novel antioxidants of natural origin may be identified.

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THE FUNGUS *PLEUROCYBELLA PORRIGENS* (PERS.) SINGER MAY OFFER NOVEL ANTIOXIDANTS OF NATURAL ORIGIN

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Edible lignicolous mushroom species *Pleurocybella porrigens* (Pers.) Singer (1947) (belonging to Marasmiaceae) contain high amount of dietary antioxidants in their fruiting bodies. Since the antiradical (AR) activity may depend on the solvent used and extraction conditions (such as time duration and temperature applied), the aim of this study was to analyse AR activity (DPPH$^{\bullet}$ and $^{\bullet}$OH, using common spectrophotometric methods) of ethanol (EtOH) and methanol (MeOH) extracts of autochthonous sample collected on Tara Mountain (Serbia). The obtained results for anti-DPPH radical activity were significantly different ($p<0.01$; IC_{50} 23.48 and 41.59 μg/mL, EtOH and MeOH extracts, respectively). However, both extracts exhibited rather high anti-hydroxyl radical activity with no statistically important difference (IC_{50} 2.96 and 5.14 μg/mL, EtOH and MeoH extracts, respectively). High positive correlation between total phenolic content (TPC) and anti-hydroxyl radical activity of the EtOH extract ($r^2=0.98$), coupled with its promising TPC (72.96 mg versus 30.47 mg GAEq/g d.w.), clearly indicate the need for further studies. According to the experimental data obtained, it may be suspected that both EtOH and more polar extracts of *P. porrigens* (including the water one) could offer novel antioxidants of natural origin with improved nutritional value.

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DAILY TREADMILL RUNNING DECREASE STRESS-INDUCED OXIDATIVE STRESS IN THE SPLEEN OF RATS

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Daily physical exercise has been widely used in recent years with therapeutic and preventive purposes in a series of pathophysiological conditions. Chronic individual housing of rats, which represents a very strong social stressor, has been shown to downregulate the cellular immune response. In this study we wanted to investigate whether daily treadmill running decreases oxidative stress in the spleen of chronically psychosocially stressed rats. We applied a combined model of chronic social isolation and long-term daily treadmill running (CSITR) in rats. CSITR treatment was achieved by exposing the individually housed rats to the daily treadmill running for a period of 12 weeks. The duration and speed of running was gradually increased from week to week, from the initial 10 minutes-10m/min up to 20 minutes-20m/min at 0 $^{\circ}$C incline. In this study we investigated how daily treadmill running affected the concentration of malondialdehyde (MDA) and activity of antioxidant enzymes (SOD, CAT and GPx) in the spleen of chronically psychosocially stressed rats. Our results show that chronic individual housing of rats increased concentration of MDA by 21 %, while exposure of chronically stressed rats to daily treadmill running showed unchanged level of MDA compared with control animals. In addition, we found that CSITR decreased the enzyme activities of total SOD by 36% and GPx by 30%, while CAT activities remained unchanged in the spleen. These results confirm that the daily treadmill running induces adaptations that decrease stress-induced oxidative stress. These adaptations result from the cumulative effects of chronic exercise of sufficient intensity and duration. This confirm that daily exercise has protective role against stress-induced oxidative stress in the spleen of rats.

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TESTOSTERONE-ENANTHATE, THE WIDELY USED AND ABUSED ANABOLIC ANDROGENIC STEROID, DISRUPT MITOCHONDRIAL MEMBRANE POTENTIAL AND MITOCHONDRIAL PROTEINS INVOLVED IN STEROIDOGENIC FUNCTION OF ADULT RAT LEYDIG CELLS

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Steroidogenic function of Leydig cells depends on mitochondrial membrane potential and mitochondrial transport machinery (transduceosome) which ensures cholesterol import into the mitochondria and initiates steroid biosynthesis. Although testosterone-enanthate (TE) is the widely used/abused anabolic androgenic steroid (AAS) that disturbs regular production of androgens in Leydig cells, most molecular events during disturbed testosterone homeostasis are complex and largely unknown. The purpose of this investigation was to determine the effects of disturbed testosterone homeostasis on mitochondrial membrane potential, transduceosome elements and consequently, steroidogenic function of Leydig cells from adult rats. Performed *in vivo* experiments revealed that mitochondrial membrane potential, measured by etramethylrhodamine, ethyl ester (TMRE), and expression of transduceosome components, *Star*/StAR, *Tspo, Prkar1a* and *Cyp11a1* (analyzed by RQ-PCR and Western blot) were decreased in the TE-treated group. In line with these results, androgen levels in Leydig cells and medium were decreased, which indicate reduced ability of Leydig cells to produce androgens. cAMP-PRKA pathway represents the main signaling pathway involved in regulation of steroidogenesis at all levels, so we measured cAMP level in Leydig cells by EIA, and noticed TE-induced inhibition. All these effects were diminished with application of androgen receptor (AR) blocker (Androcur). Results from *in vitro* studies revealed that some of the effects depend on testosterone-AR, while others could be due to disturbed LH and/or other signals. Presented data confirmed that functional mitochondria are essential support for the process of steroidogenesis and we demonstrated, to the best of our knowledge for the first time, that a systemic blockade of AR prevents TE-induced disruption of mitochondrial membrane potential and steroidogenic function of Leydig cells.

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THYROID HORMONES REGULATE MITOCHONDRIA-PEROXISOMES CROSSTALK IN RAT BROWN ADIPOCYTES

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Brown adipose tissue (BAT) is a thermogenic tissue specializing in the conversion of lipids into heat. Brown adipocytes (BA) contain numerous mitochondria and lipid droplets. Previous studies have shown that the thyroid hormone has a central role in BAT adipogenesis *in vitro* and BAT development in mouse embryos. In hypothyroidism bioenergetic and thermogenic capacity of BAT are suppressed. As a consequence of reduced mitochondrial activity, peroxisomes take more role in fatty acid β-oxidation in BA. Peroxisomes are dynamic organelles adapting their morphology, number, enzyme content and metabolic functions in response to physiological stimuli. The most important functions of peroxisomes are fatty acid oxidation and hydrogen peroxide degradation. The aim of this study was to analyze mitochondria and peroxisomes ultrastuctural remodeling in BAT induced by hypothyroidism using antithyroid drug methimazole (MMI). Two month old male Wistar rats were fed with standard pelleted food *ad libitum*. The rats were divided into four groups. Three groups were treated with 0.04% MMI solution in drinking water for 7, 15, 21 days respectively; untreated animals in the fourth group served as control. The interscapular portion of BAT was isolated and fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer, postfixed in 1% osmium tetroxide, routinely dehydrated and embedded in Araldite. Ultrathin sections were mounted on copper grids and examined on aCM12 transmission electron microscope. Compared to control, in MMI-treated groups mitochondria are enlarged, swelled with partially degraded cristae. We also observed increased number of peroxisomes in MMI-treated groups related to treatment duration. Besides, in MMI-treated groups, a large number of lysosomes was noticed - especially in the MMI group treated for 15 days. Hence, our results showed that hypothyroidism causes changes in mitochondrial structure and peroxisome number in BA, suggesting that thyroid hormones play a central role in their maintaining and crosstalk.

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THE EFFECT OF ALS IgGs ON HYDROGEN PEROXIDE PRODUCTION IN BV-2 CELLS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder with a very fast progression, no diagnostic tool for the presymptomatic phase, and still no effective treatment of the disease. Only 10% of all ALS patients have a hereditary component, and the vast majority (around 90%) of all cases are sporadic, with unknown aetiology. Although ALS affects motor neurons, the overall pathophysiological condition points out to the non-cell autonomous mechanisms, where astrocytes and microglia play crucial roles in the disease progression. Since oxidative stress is one of the many components contributing to the disease, we decided to examine the acute effect of immunoglobulin G (IgG) from sera of ALS patients on hydrogen peroxide production in the cytosol of the BV-2 microglial cell line. BV-2 cells were transfected with HyPer, fluorescent sensor capable of detecting intracellular hydrogen peroxide, with submicromolar affinity. Since HyPer is sensitive to pH changes, control experiments with cells transfected with SypHer, pH-sensitive construct, were done in parallel. Time-lapse images were recorded 24 h post-transfection on a Zeiss Axio Observer 1 inverted microscope equipped with EMCCD camera using FITC settings. After establishing the baseline, IgGs (0.1 mg/ml, from 9 ALS patients, 3 healthy and 2 disease controls) were delivered on top of the recording cells, and their effect followed for 5 minutes. IgGs from 3 ALS patients induced slow exponential rise of HyPer intensity, with maximal normalized fluorescence 0.214-0.491, also inducing similar increase of SypHer intensity, but with maxima at least 0.1 units smaller than with HyPer. None of the control IgGs induced changes with neither of the indicators. Our results show the generation of hydrogen peroxide combined with pH changes in the cytosol of BV-2 cells following exposure to IgGs from one third of ALS patients tested. The observed phenomena could potentially trigger and/or influence the activation of microglia, known to occur in later stages of ALS. Therefore, revealing the ALS IgG signalling cascade in microglial cells could offer a valuable molecular biomarker and/or a potential therapeutic target.

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CHENOPODIUM MURALE L. HAIRY ROOT EXUDATES INDUCE ULTRASTRUCTURAL CHANGES IN WHEAT (*TRITICUM AESTIVUM* L.) SEEDLINGS

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Hairy root exudates of allelopathic weed *Chenopodium murale* L. have been previously shown to exert allelopathic activity against test plant species lettuce, wheat and *Arabidopsis*, inhibiting germination and seedling development of these plants. In the present study we used transmission electron microscopy (TEM) to investigate the effect of different concentrations of *Chenopodium murale* hairy root exudates, applied as phytotoxic media (PM), on ultrastructural changes of meristematic cells in *Triticum aestivum* L. roots and leaves. TEM studies of root cells treated with lower concetration of PM, showed irregular shaped cell walls, numerous distributed organelles in cytoplasm, prominent nucleus and distinct nucleolus. Exposure to higer concentration of PM showed that the mitochondria and chloroplasts were removed with the plasmalemma into the cell interior. TEM observation of treated leaves cells showed undulating cell walls, dense cytoplasm with numerous organelles and large nucleus with three nucleolus. In some cells the chloroplasts were removed and grouped near the cell wall, while in some other cells the increased number of mitochondria were presented. In conclusion, ultrastructural analysis of treated seedlings showed significant ultrastructural changes. Removing of organelles with the plasmalemma into the cell interior and reorganization of the vacuolar compartment was probably occurred in order to protect the cell if the tonoplast was damaged by toxicity of *C. murale root exudate.* The increased number of mitochondria could be connected with increased ATP generation as an adaptation process against *C. murale* allelochemicals.

P43

GSTA1, M1 AND T1 POLYMORPHISMS MODIFY THE RISK OF BLADDER CANCER IN INDIVIDUALS ORIGINATING FROM BALKAN ENDEMIC NEPHROPATHY REGION

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Increased incidence of urinary tract tumors in the Balkan endemic nephropathy (BEN) areas is well-known phenomenon. However, it is still unclear whether there is a specific genetic predisposition leading to a highly increased bladder cancer (BC) risk in patients with BEN. We examined the associations between glutathione S-transferases (GST) gene polymorphisms and BC risk in a Serbian group of BC patients from BEN areas. A hospital-based case-control study included a total of 201 BC cases, 67 from BEN region, and 122 controls. GSTM1 and GSTT1 polymorphism were identified by PCR, while GSTA1 and GSTP1 were identified by RFLP-PCR method. Individuals with *GSTA1/AB+BB* genotype exhibited a 2.6 times higher BC risk compared to those with *GSTA1-AA* genotype who were from non-BEN region (OR=2.60, p=0.015). Carriers of *active-GSTM1* genotype had a 2.9 times greater risk of developing BC compared to those with *active-GSTM1* genotype but were from non-BEN region (OR=2.90, p= 0.010). *Active-GSTT1* genotype carriers from BEN region exhibited 2.1 times greater risk of developing BC compared to those from non-BEN region with active-GSTT1 (OR=2.10, p=0.027). Our results may contribute to understanding the role of GST polymorphism on bladder cancer risk in BEN region residents.

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PINEAL IS INVOLVED IN SHAPING OF 24-H RHYTHMIC ACTIVITY OF NO-cGMP SIGNALING IN ADULT RAT LEYDIG CELLS

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The pineal hormones play a key role mediating the influence of photoperiod on the reproduction of many species. However, pineal effects on oscillators in reproductive organs are poorly understood, especially their influences on 24-h rhythm of signaling pathways that regulate sex-steroids production. The present study was designed to clarify pineal impact on the activity and expression of the elements of nitric oxide (NO)-cGMP signaling pathway in androgen-secreting Leydig cells. We used the pinealectomized rats as a model where hormones that convey information about environmental lighting were abolished and sham-operated rats as a control. To follow a circadian rhythm of Leydig cell activity *in vivo* experiments were performed at 6 time points during 24h (light regiment: 12h of light and 12h of dark). The pineal removal was confirmed by measuring the serum melatonin level and rhythm analysis of all obtained parameters were done by cosinor method. RQ-PCR analysis, in Leydig cells from control rats, revealed a 24-h rhythmic expression of genes responsible for NO production (*Nos3* and *Nos2*), but no significant rhythm in expression of genes responsible for cGMP production (*Gucy1a3* and *Gucy1b3*), cGMP degradation (*Pde5a*), and for the main effector in this signaling pathway (*Prkg1*). Pinealectomy moved forward the peak of *Nos3* rhythmic expression (from ZT7.3 in control to ZT2.3 in pinealectomised rats), completely canceled the rhythm of *Nos2*, initiated a cyclic pattern of *Gucy1b3* and *Pde5a* transcription but without effect on *Gucy1a3* and *Prkg1*. Moreover, a rhythmic daily variation of Leydig cell cGMP levels was changed with a significant phase shift in pinealectomized rats (from ZT7.8 in control to ZT15.7 in pinealectomized group). At the same time the serum testosterone levels were increased in pinealectomised rats even though low-amplitude diurnal rhythm was preserved. Taking together obtained results suggest that pineal hormones are involved in shaping of 24-h rhythmic activity of NO-cGMP signaling pathway in adult Leydig cells.

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P45

ACETAMINOPHEN-INDUCED CHANGES OF HAEMATO-BIOCHEMICAL AND OXIDATIVE STRESS PARAMETERS IN RAT BLOOD: PROTECTIVE ROLE OF VITAMIN C AND β-GLUCAN

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Acetaminophen (APAP, paracetamol) is widely used as an over-the-counter analgesic and antipyretic drug. The aim of this study was to investigate the possible protective effects of vitamin C (Vit C, 100 mg/kg/day i.p.) and β-glucan (40 mg/kg/day i.p.) on altered hematological, biochemical and oxidative stress parameters in the blood of rats treated with APAP (100 mg /kg/day i.p.) for 3 days. Exposure of rats to APAP caused changes of some haematological parameters (RBCs count, Hb concentration, Ht value and WBCs count), suggesting that the APAP induced haematotoxicity. APAP reduced serum total protein (TP), albumin and globulin, while it increased alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities compared to the control. The results indicate that APAP led to a significant decrease in the concentrations of Na^+ and K^+ and an increase of Ca^{2+} in the serum compared to the control. Coadministration of Vit C and β-glucan with APAP reversed these changes of haematological and biochemical parameters and diminished the toxic effects of APAP. The obtained results indicate that the concentration of LPO in erythrocytes significantly increased, while the concentration of GSH significantly decreased in the group treated with APAP compared to control group. Intoxication of rats with APAP was followed by decreased activity of antioxidant enzymes (SOD, CAT and GSH-Px). Coadministration of Vit C and β-glucan with APAP reversed APAP-induced alterations in these oxidative stress parameters. This study suggests that APAP has significant prooxidative effects and may disrupt oxidant/antioxidant balance in erythrocytes. Furthermore, coadministration with Vit C and β-glucan has a protective effects on APAP-induced oxidative damage and haematotoxicity.

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THE ROLE OF GLUTATHIONE S-TRANSFERASES IN SUSCEPTIBILITY TO PROGRESSIVE MYOCLONUS EPILEPSY

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Oxidative stress is recognized as an important factor in progressive myoclonus epilepsy (PME). Genetic polymorphism of glutathione S-transferases (GSTs), which are involved in both protection from oxidative damage and detoxification, might alter the capacity for protecting tissues from exogenous and endogenous oxidants. We aimed to assess a possible association between GST polymorphism and PME, as well as, correlation between GST genotypes and oxidative phenotype in PME patients. *GSTA1*, *GSTM1*, *GSTP1* and *GSTT1* genotypes were determined in 26 patients with PME and 66 controls. Byproducts of protein oxidative damage (thiol groups (P-SH) and nitrotyrosine), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities were determined. The frequency of *GSTA1*, *GSTM1* and *GSTP1* genotypes was not significantly different between PME patients and controls, while individuals with *GSTT1-null* genotype were at 5.44-fold higher risk of PME than carriers of *GSTT1-active* genotype. Moreover, significant risk of PME was obtained in carriers of both *GSTT1-null* and *GSTM1-null* genotypes. Carriers of combined *GSTA1- active* and *GSTT1-null* genotype were at highest, 7.55-fold increased risk of PME. Byproducts of protein damage did not reach statistical significance, while SOD and GPX activities were significantly higher in PME patients then in controls. When stratified according to GST genotype, P-SH groups were significantly lower only in patients with *GSTT1-null* genotype in comparison to carriers of *active* genotype. Only SOD activity was increased in *GSTT1-null* when compared to corresponding *active* genotype. Based on the results, we concluded that *GSTT1-null* genotype might be associated with the increased risk and enhanced susceptibility to oxidative stress in PME patients.

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OXIDATIVE STATUS OF RAT INTESTINE CHANGES DUE TO D,L-HOMOCYSTEINE THIOLACTONE ADMINISTRATION

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Numerous studies have shown that hyperhomocysteinemia might be an independent risk factor for gastrointestinal diseases. The relation between hyperhomocysteinemia and inflammatory bowel disease (IBD), such as Chron's disease and ulcerative colitis is also proven. Oxidative stress appears to play a role in the pathogenesis of several inflammatory gastrointestinal diseases. The aim of this study was to examine the influence of D,L-homocysteine thiolactone on antioxidant status of rat intestine. The activity of catalase (CAT), levels of thiobarbituric acid reactive substances (TBARS) and total antioxidant status (TAS), were investigated in the isolated gut of young male rats in control group (8 rats) and after 3-hour incubation in high doses of D, L-homocysteine thionolactone (Hcy) (10μmol/L) (8 rats). Samples of duodenum, ileum and colon were homogenized in sodium phosphate buffer (1:10). Homogenates were centrifuged at 4 ˚C for 10 min at 10000g and the supernatant was taken to biochemical assays. Our results showed that high D, L-homocysteine thionolactone concentration reduced enzymatic catalase activity in homogenates of the isolated segments of duodenum (27.04%) $p<0.01$; ileum (37, 27%) and colon (34, 17%) $p<0.001$. Exposition to high D,L-homocysteine thiolactone concentration significantly increased TBARS levels in duodenum (106.05%), ileum (47.24%) and colon (112.75%) ($p<0.01$). Homocysteine also modified the total antioxidant status of homogenates from the duodenum, ileum and colon increasing by 20.68% (duodenum), 24.74% (ileum) and 14.88% (colon) ($p<0.001$). Homocysteine induced consistent oxidative stress in rat intestine (reduced activity of catalase and increased level of TBARS), but the elevated activity of TAS in our experiments could be explained as an adaptive response to generated free radicals which indicates the failure of the total antioxidant defense mechanism to protect the tissues from damage caused by homocysteine.

P48

INFLUENCE OF MIXTURE OF DECABROMINATED DIPHENYL ETHER (BDE-209) AND (Cd) ON ANTIOXIDATIVE DEFENCE SYSTEM i.e. TOTAL –SH GROUPS CONTENT IN LIVER AND KIDNEY OF RATS

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The aim of this study was to examine the influence of decabrominated diphenyl ether (BDE-209) on cadmium (Cd) effects on the antioxidative defence system *i.e.* the total –SH groups content in liver and kidney of rats. The combination of chemicals was chosen based on the data that BDE-209 and Cd are the most abundant pollutants in environmental and human samples. Male *Wistar* rats (~200g), were divided into eight groups: C – control group, DMSO control group, Cd groups: exposed orally to 2.5, 7.5 or 15 mg Cd/kg bw/day as solution in DMSO and Cd + BDE209 groups: exposed orally to 1000 mg BDE-209/kg bw/day in combination with all doses of Cd. Liver and kidney were removed after 28 day period of exposure, homogenised and concentration of total –SH groups was determined by Ellman method. Results show that subacute exposure to Cd significantly increased, in dose-dependent manner, total –SH groups concentration in both organs. Calculated Benchmark dose of 5% ($BMDL_5$) was 0.78 mg Cd/kg bw/day. After subacute exposure to the Cd combined with 1000 mg BDE-209/kg bw/day, concentration of –SH groups was significantly decreased in liver, but only slightly in kidney. A dose dependent manner was observed when the mixture was applied, however $BMDL_5$ for Cd and BDE-209 as covariance could not be derived. The explanation is ratio between Benchmark dose and $BMDL_5$ higher than 10, what is requirement for effects assessment. Obtained results imply that polyhalogenated chemicals may increase Cd influence on –SH groups when applied as mixture. Even though, BDE-209 by itself does not interfere with –SH groups, it could contribute to reduction of antioxidant capacity in liver and kidney caused by Cd.

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EFFECTS OF DIFFERENT PLANTS USED IN TRADITIONAL MEDICINE FOR DIGESTIVE DISORDERS ON METABOLIC ENZYMES IN COLON CANCER CELL LINES

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The aim of research is to examine effects of different plants, traditionally used for treatment of digestive disorders, on gene expression of metabolic enzymes involved in detoxification, drug metabolism and drug resistance in HCT-116 and SW480 cell lines. Investigated plants, *Teucrium chamaedrys* L., *Gentiana punctata* L., *Centaurium erythrea* Rafn. and *Ligustrum vulgare* L., were from Serbian flora. Gene expression was investigated for genes from different phases of anticancer drug metabolism: phase I which involved CYP protein family (*CYP1A1*), glutathione S transferase (*GSTP1*) which forming conjugates with glutathione in phase II and ABC membrane transporters (*MRP2*) which transported this conjugates out of the cell in phase III. Gene expression was determined by PCR method. This enzymes and transporters play a crucial role in protecting the healthy cells from the harmful effects of carcinogens, however in cancer cells it can lead to drug resistance. Because of that, possible treatment of cancer involves the invention or synthesis drugs which inhibit their activity. Targeting of these enzymes by substances from natural origin is potential success in prevention and cancer therapy. Observed results generally showed ability of investigated plant extracts (concentration of 50 μg/ml) to inhibit gene expression of these enzymes in compare to control cells, with some exceptions. All of investigated plants inhibited *CYP1A1* gene expression in both cell lines. Gene expression of *GSTP1* and *MRP2* were also inhibited in all treatments in HCT-116 cells. There are some differences in effects of *L. vulgare* on SW480 cells, which increased *GSTP1* and *MRP2* gene expression, suggesting on possible conjugation of bioactive constituents from this plant with glutathione by GSTP1 enzyme and transport conjugates by transporter protein through cell membrane. *C. erythrea* also increased *GSTP1* gene expression, because of possible metabolism of their active compound with glutathione. This investigation has great impact on investigation of new anticancer substances from natural source, in order to reduce the resistance of malignant cells, as one of the greatest problems in the tumor therapy.

P50

SYNERGISTIC ANTICANCER ACTION OF LYSOSOMAL MEMBRANE PERMEABILIZATION AND GLYCOLYSIS INHIBITION

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We investigated the *in vitro* anticancer effect of combining lysosomal membrane permeabilization (LMP)-inducing agent N-dodecylimidazole (NDI) with glycolytic inhibitor 2-deoxy-D-glucose (2DG). Cell viability was measured by MTT and LDH tests. Oxidative stress, lysosomal permeabilization, mitochondrial depolarization and apoptosis/necrosis were analyzed by flow cytometry. Cell morphology was examined by electron microscopy. Intracellular ATP content was measured by bioluminescence assay. NDI-triggered LMP and 2DG-mediated glycolysis block synergized in inducing rapid ATP depletion, mitochondrial damage, and reactive oxygen species (ROS) production, eventually leading to necrotic death of U251 glioma cells, but not primary astrocytes. NDI/2DG-induced death of glioma cells was partly prevented by lysosomal cathepsin inhibitor E64 and antioxidant α-tocopherol, indicating the involvement of LMP and oxidative stress in the observed cytotoxicity. LMP-inducing agents chloroquine and NH_4Cl also displayed synergistic anticancer effect with 2DG, while glycolytic inhibitors iodoacetate and sodium fluoride synergistically cooperated with NDI, thus confirming that the anticancer effect of NDI/2DG combination was indeed due to LMP and glycolysis block, respectively. Based on these results, we propose that NDI-triggered LMP causes initial mitochondrial damage that is further increased by 2DG due to the lack of glycolytic ATP required to maintain mitochondrial health. This leads to a positive feedback cycle of mitochondrial dysfunction, ATP loss, and ROS production, culminating in necrotic cell death. Therefore, the combination of LMP-inducing agents and glycolysis inhibitors seems worthy of further exploration as an anticancer strategy.

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ANTIOXIDANT EFFECTS AND POLYPHENOLIC CONTENTS OF WILD GROWING AND CULTURED *Ruta graveolens* L.

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Ruta graveolens L., rue, is a shrub-like perennial aromatic plant which preferably grows wildly in Mediterranean region, although it is also cultivated in gardens worldwide. Rue is highly used in the traditional medicine in many countries for treating a variety of diseases and also as a spice and flavor. The objective of the study was to determine and compare antioxidant effects and phenolic contents of methanolic and ethanolic wild growing, collected at the beginning and at the end of the flowering season (Sićevačka gorge, Sićevo, Serbia), and cultured rue (Novo Selo, Niš, Serbia) extracts. The content of total flavonoids in the extracts was determined spectrophotometrically using aluminium chloride reagent and the total polyphenolic and tannins contents were assessed by the Folin-Ciocalteu colorimetric procedure. Antioxidant capacities were investigated in two complementary test systems: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and *â*-carotene–linoleic acid test. The total phenolic content of the tested samples varied from 57.90 to 166.91 mg of catechin equivalent (CE)/g of extract and the amount of total tannins ranged from 29.36 to 102.69 mg CE/g while the total flavonoids content ranged from 4.18 to 27.72 mg of rutin equivalent/g. All the extracts exhibited significant antioxidant potential in free radicals scavenging (DPPH) and antilipoperoxidant assays. As expected, the extracts from wild growing rue, containing the highest amounts of polyphenols, tannins and flavonoids, demonstrated the strongest antioxidant activity in all tested systems. The study also revealed the relevant fact that the rue should be collected at the beginning of blossoming stage in order to accomplish the maximal quantity of secondary metabolites and favorable pharmacological effects.

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POLYPHENOL-RICH POMEGRANATE JUICE EXERTS ANTIOXIDATIVE EFFECT THROUGH DECREASE IN GPx ACTIVITY IN METABOLIC SYNDROME SUBJECTS

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Metabolic syndrome (MS) represents a cluster of cardiovascular risk factors encompassing 25% of the general population, and is followed by overall oxidative disbalance. Regular consumption of fruits, vegetables and other foods rich in bioactive substances could be beneficial in the prevention and treatment of disorders associated with oxidative stress. Pomegranate juice (PJ) exerts beneficial health properties attributed to high polyphenol content and its anti-oxidative properties. The aim of this study was to investigate the biological effect of pomegranate juice on oxidized low-density lipoproteins (oxLDL) plasma status, and erythrocyte glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in subject with MS. A six week, randomized controlled trial was conducted in 29 subjects (MS was diagnosted according to NCEP ATP III) receiving daily either 300 mL of PJ or no treatment. Plasma oxLDL level was measured by ELISA, while the activity of GPx and SOD were measured by commercial Ransel kits (Randox Laboratories, UK). We observed a significant decrease ($p<0.05$) in the activity of GPx after consumption of PJ, in comparison with control groups. Six week consumption of PJ exerted a favorable effect in terms of decreasing total plasma oxLDL and SOD activity in erythrocytes, but without statistical significance. Our results indicate that consumption of pomegranate juice may change oxidative status in subjects with MS through decrease in activity of some of the enzymes of anti-oxidative defense, primarily GPx.

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THE MARINE SESQUITERPENOID HYDROQUINONE AVAROL DOES EXHIBIT MODERATE ANTI-ASCORBYL RADICAL ACTIVITY

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The marine sesquiterpenoid hydroquinone avarol is a main secondary metabolite of the Mediterranean sponge *Dysidea avara* (Schmidt, 1862). Previous studies have shown that avarol has a rather good antioxidant potential. Herein we report for the very first time its moderate anti-ascorbyl radical activity (40%) evaluated by electron paramagnetic resonance spectroscopy (EPR) at *in vitro* conditions. According to the experimental data obtained, both avarol hydroxyl groups are likely to take part in the relevant chemical reaction, but in a varying degree. Further perspectives of this intriguing research work will target simplified chemical structures (inspired by the avarol scaffold) coupled with *ex vivo*/*in vivo* antiradical screens of wide range. As a consequence, new commercially available therapeutics may be developed.

P54

ANTIOXIDATIVE RESPONSE OF BROWN ADIPOCYTES TO ERYTHROPHAGOCYTOSIS IN INSULIN-TREATED RATS

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The increased blood flow and capillaries permeability and/or adipogenic/angiogenic activity in brown adipose tissue (BAT) upon thermogenic stimuli, can produce many red blood cells (RBCs) in extracellular space. We already revealed the brown adipocytes' (BA) ability to engulf, intake and degrade those extravasated RBCs by phagocytosis. Moreover, after insulin-induced massive RBCs extravasation and phagocytosis in BAT we demonstrated a toxic effect of released iron on engaged adipocytes. Lipid peroxidation, mitochondrial swelling, increased autophagy and lipofuscin accumulation were among the observed cell injuries. Despite the increased cell damage, the following apoptosis or necrosis within this erythrophagocytic adipocytes (EPAs) were not detected. Hence, we aimed this study to examine the antioxidant defense (AD) enzymes expression in EPAs after experimentally induced hyperinsulinemia. Male Wistar rats were treated acutely (1 day) or chronically (3 days) with low (0.4 IU/kg bm) or high (4 IU/kg bm) dose of insulin. Saline-treated animals served as controls. The interscapular BAT depot was removed and prepared for the microscopic examinations. Immunohistochemically, increased expression of AD enzymes, including catalase and superoxide dismutase (SOD) isoforms, MnSOD and CuZnSOD, was shown in EPAs of both insulin-treated and control rats. As it was expected, SOD isoforms were localized in (MnSOD) and around mitochondria (CuZnSOD), with heterogeneity among these organelles even in the same cell. This is in line with the observed variations in the level of mitochondrial damage in single EPAs. Observed increase of AD enzymes expression could be considered as a compensatory response to increased erythrophagocytosis-induced oxidative stress as it was demonstrated *in vitro* with other phagocytic cell types. Free iron, a product of hemoglobin oxidation, is a potent causer of lipid peroxidation and ROS production, activating the systems of its removal in EPAs, including herein demonstrated increased ferritin accumulation and increased immunoexpression of heme oxygenase-1. In conclusion, erytrophagocytosis could be considered as physiological activity of BAs since it was demonstrated in BAT of both insulin-treated and control rats. To fight increased iron accumulation and ROS production, EPAs engage different systems of enzymatic and non-enzymatic AD.

P55

ANTIOXIDANT ACTIVITY OF *Thymus glabrescens* Willd. METHANOLIC EXTRACTS

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Most of species from the genus *Thymus* L. have been used as carminative, stomachic, choleretic, spasmolytic, antitussive, expectorant, antioxidant, antibacterial, antifungal, antiviral, anthelmintic and astringent agents. The aim of this research was to determine the content of total polyphenols and tannins in *Thymus glabrescens* methanolic extracts and to evaluate of their antioxidant activity. The above-ground parts of *T. glabrescens* were collected at full flowering stage in June 2010, 2011 and 2012 in Kunovica, near Niš. Extracts were prepared with the ultrasonic method using absolute methanol in 1:10 ratio. The total polyphenolic and tannins contents were determined using Folin-Ciocalteu method and the antioxidant activities were assessed by *in vitro* test in 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) system, with ascorbic acid as a positive control. All extracts expressed strong antioxidant effects with high contents of total polyphenols and tannins. The highest amount of total polyphenols was determined in the extract from 2011 and the extract from 2010 was the richest in tannins. The highest antioxidant potential was determined in methanolic extract from 2010 which indicated that the tannins, as a special type of polyphenols, highly contributed to the antioxidative activity of the extract. The methanolic extracts from *T. glabrescens* Willd. exhibited strong antioxidant activity. Due to the ability to neutralize free radicals extracts can play a significant role in the prevention of diseases related to oxidative stress. It is also necessary to investigate the safety and toxicity of the extracts to find the right application in pharmacy and medicine.

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NICOTINE TOXICITY AND CHANGES OF REDOX STATUS IN THE BLOOD OF RATS: PROTECTIVE EFFECTS OF QUERCETIN AND VITAMIN C

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Cigarette smoking is one of the most serious public health problems in both developed and developing countries, which adversely affects the quality of life. The primary addictive component of cigarette smoke is nicotine, a water-soluble alkaloid found in *Nicotiana tabacum*. The current study was designed to investigate the protective effect of quercetin (QN) and vitamin C against nicotine-induced prooxidant and antioxidant imbalance in circulation of experimental rats. Male albino rats of Wistar stain were injected with nicotine (1 mg/kg/day, i.p.) or saline. Quercetin (40 mg/kg/day i.p.) and vitamin C (100 mg/kg/day i.p.) was administered along with nicotine injections for 10 days. The levels of superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and lipid peroxide (LPO) in circulation were significantly increased when compared to normal levels and were brought down to near normal in QN and vitamin C co-treated group. Intoxication of rats with nicotine was followed by significantly decreased concentration of reduced glutathione (GSH) and activities of examined antioxidant defense enzymes (superoxide dismutase (SOD) and catalase (CAT)) compared to the control. Activities and levels of these antioxidants were significantly raised in nicotine + QN + Vit C treated animals when compared to nicotine group. Obtained results demonstrated that nicotine has significant prooxidative effects and may disrupt redox status in rat blood, while combining QN and vitamin C provided antioxidant defense with strong haematoprotective activity against nicotine-induced toxicity.

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THE ROLE OF NEUROPEPTIDE Y IN OXIDATIVE/ANTIOXIDATIVE BALANCE IN HUMAN TROPHOBLAST CELL LINE

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Trophoblast migration is an essential step in implantation and placenta formation. Aberrant trophoblast invasion of the uterine spiral arteries results in poor placental perfusion and placental hypoxia/reoxygenation, which could result in development of oxidative stress that contributes to endothelial dysfunction and clinical manifestation of several pregnancy disorders. Neuropeptide Y (NPY) is a sympathetic co-transmitter released during stress and is involved in several physiological processes such as food intake, angiogenesis and vasoconstriction. The levels of NPY are significantly higher in circulation of preeclamptic women compared to healthy ones. The aim of this study was to determine the effects of NPY on the oxidative/antioxidative disbalance of human trophoblast cell line which could contribute to altered cell migration. Cells were cultivated for 72h in a cell culture medium which contained NPY in non-cytotoxic concentration (10^{-9} mol/l), corresponding to elevated physiological levels. Intracellular concentrations of superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), nitrite (NO_2^-), reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined. Results of this study show that the concentrations of H_2O_2, $O_2^{\bullet-}$, GSH and GSSG were significantly increased compared to non treated cells. NPY treatment of human trophoblasts induces decrease in the levels of NO_2^-, an NO indicator, a signal molecule that could affect the adhesive state of cadherine on the cell membrane. In general, the results of our study show that NPY is significant trigger of oxidative stress in human trophoblast cell lines and that these alterations in biomarkers of oxidative stress could be one of the mechanisms which diminish trophoblasts migratory capacity leading to pathogenesis of several pregnancy complications.

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IN VITRO HYDROXYL RADICAL SCAVENGING CAPACITY OF SELECTED CRUDE EXTRACTS OF *SCHIZOPHYLLUM COMMUNE* Fr. 1815 ORIGINATED FROM DIFFERENT TREE HOST SPECIES

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Schizophyllum commune Fr. 1815 is a lignicolous species with a cosmopolitan distribution that can be found on every continent, except Antarctica; mostly saprotrophic or parasitic on deciduous tree hosts such as *Platanus*, *Fraxinus* and *Betula*, causing white rot. This study reports the anti-hydroxyl radical activity of methanolic and water extracts of the *Sch. commune* samples collected from several tree host species (*Fagus*, *Tilia*, *Salix* and *Celtis*) grown in urban sites of Novi Sad (Vojvodina, Serbia). It was hypothesised that the difference in supstrate could influence the aforementioned antiradical activity. The experimental data obtained pointed out higher hydroxyl radical scavenging capacities of its methanolic extracts, as followed: IC_{50} 0.02 µg/mL (*Tilia*) > IC_{50} 0.53 µg/mL (*Celtis*) > IC_{50} 0.78 µg/mL (*Salix*) > IC_{50} 4.10 µg/mL (*Fagus*). In comparison, the relevant IC_{50} values of *Sch. commune* water extracts ranged from 0.42 (*Celtis*) to 5.35 µg/mL (*Tilia*). *Sch. commune* methanolic extract (*Tilia*) was 205 times more active than the same one made originating from a *Fagus* tree. In addition, this extract was approx. 268 times more effective compared with the respective (*Tilia*) water extract. Taken all together, the genus *Tilia* (as the fungal supstrate) may offer novel and powerful antioxidants with moderate polarity targeting the most reactive free radical species (hydroxil radicals) at physiological conditions. Therefore, future studies will be focussed on a broad range screening of the respective *Sch. commune* samples aiming to seasonally identify the trend for anti-hydroxyl radical activity.

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PHENOLOGICAL, PLANT PART AND INTERPOPULATION VARIABILITY OF SECONDARY METABOLITES CONTENT AND ACTIVITY OF *INULA HELENIUM* L.

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The purpose of this study is the determination of the total content of phenolics, flavonoids as well as antioxidant activity of the ethanol extracts from different plant parts of *Inula helenium* L. (Asteraceae) sampled from the different populations as well as during the different phenological stages. The plant material was sampled from the same population during the different phases of the vegetation period during and after the flowering. The obtained amounts for the total phenolic content (expressed in terms of gallic acid equivalent – mg GA/g extract) ranged from 16.73 to 89.85 mg GA/g. The concentration of flavonoids (expressed in terms of rutin equivalent – mg RU/g extract) varied from 9.32 to 376.22 mg Ru/g. The IC_{50} values of antioxidant activity determined using DPPH free radical method varied from 161.60 to 1563.02 μg/ml. The ethanol extracts from flowers and roots contained the highest concentration of phenolic compounds, but the ethanol extracts from leaves contain the highest concentration of flavonoids, whereas the root ethanol extract showed the highest antioxidant activity level. Based on the obtained results, the quantity of the phenolics, as well as antioxidant activity, significantly varied among the different populations, which stemmed from the different impacts of environmental factors. The results demonstrated that the higher value of these compounds was measured in plant organs during the flowering in relation to the after flowering phase. The difference in the synthesis of the biologically active substances during the vegetation period indicated that the intensity of the activity of these compounds lessened after the flowering. This research showed that the plant organs of the species *Inula helenium* represent the abundant source of bioactive substances, and that the quantity of these compounds greatly differs among the different populations as well as in the same populations during the different phenological stages.

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RADICAL SCAVENGING ACTIVITY OF PUFFBALLS: PHENOLS VS GLUCANS

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Radical scavenging ability of mushrooms *Handkea utriformis* (HU), *H. excipuliformis* (HE) and *Vascellum pratense* (VP) methanol extracts was assessed by DPPH (Dudonné et al., 2009) and ABTS assays (Re et al., 1999). Antioxidant compounds were estimated to establish the connection between them and scavenging ability. Phenolics were determined according to Skotti et al. (2014) and sugars according to DuBois et al. (1956). Glucans were determined by Megazyme β-glucan assay kit. The extracts showed high scavenging ability and different order of activity in assays; DPPH neutralization (%) of the extracts at 20 mg/mL was 88.6% for HU, 73.2% for HE and 80.3% for VP. At the same concentration, HU, HE and VP showed ABTS scavenging activity as 0.92, 0.70 and 0.91 mM of Trolox, respectively. Phenolic content (HU=19.8, HE=14.9 and VP=20.3 mg/g) shows excellent correlation with ABTS scavenging ability of the extracts, but not with DPPH. Sugars/β-glucans may have greater role in DPPH neutralization assay; Sugars/β-glucan content (%) in HU (19.6/16.7) was higher comparing to VP (9.0/8.2) and HE (9.5/7.3). Better DPPH scavenging ability of HU may be due to higher sugar/glucan content, as its phenolic content is identical to VP; on the other hand, VP and HE have almost the same carbohydrate/glucan content, but VP has a higher amount of phenolics and better activity. Glucans poor solubility in ethanol, used as a solvent in ABTS assay prevents them from acting as "scavengers" of ABTS radicals; however, their contribution to the extracts activity is evident in DPPH assay, in which methanol is used as a solvent.

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CHLOROPHYLL AND CHLOROPHYLLIN DEGRADATION INDUCED BY AAPH

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The degradation of two pigments, chlorophyll and chlorophyllin, induced by a free radical initiator was investigated in this study. The thermal hydrophilic 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH) - free radical initiator is widely used for research of oxidation reactions kinetics. Oxidation process by AAPH is usually followed by formation of numerous radicals such as ˙OH, ˙OOH, as well as keto radicals, that continue oxidation reactions, at temperatures higher than 37 °C. Chlorophyll and chlorophyllin degradation were monitored *in vitro*, in aqueous medium, in the presence of a thermal initiator at 40°C. The final concentration of thermal initiator was adjusted to $3.2x10^{-3}$M, and the pigment concentrations were set at four values, $2.5x10^{-6}$, $5.0x10^{-6}$, $1.0x10^{-5}$ and $2.5x10^{-5}$ M, in the reaction mixture, in order to investigate possible concentration effect on the pigment stability. The reaction was followed with a UV-VIS spectrophotometer in spectral range between 300 and 800 nm, in a reaction period of 0-100 min. Degradation is more intense during the first 40 min of the reaction, and then reaches a constant value after about 100 min of reaction, for both pigments. Differences in degradation reactions dynamics between chlorophyll and chlorophyllin, induced by thermal hydrophilic initiaton AAPH, were clearly observed. Chlorophyllin is more stable than chlorophyll, for the highest $2.5x10^{-5}$ M concentration (degradation percentages were 50 and 70%, respectively, for 100 min). An increasing trend of degradation for chlorophyll with its increasing concentrations was observed. Such an effect is not observed for chlorophyllin: the degradation percentage of all tested concentrations was between 50 and 62%, for a period of 100 min. The results showed the effect of concentration on chlorophyll degradation in reactions induced by AAPH radical. On the other hand, chlorophyllin degradation seems not to be concentration dependent.

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OXIDATIVE PHOSPHORYLATION AND ANTIOXIDATIVE DEFENCE IN THE PANCREAS OF GROUND SQUIRRELS DURING COLD ACCLIMATION AND HIBERNATION

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Mammalian hibernators actively undergo numerous biochemical adaptations that enable them to spend the winter in a state of greatly reduced whole-body metabolism. Among several tissues significant for the regulation of overall energy homeostasis in states of altered metabolic demand, the role of the pancreas, in terms of endocrine control of glucose and lipid metabolism, is very important. As a result, this study focused on investigating the molecular basis of mitochondrial energy-producing pathways along with their regulating mechanisms, as well as the (re)organization of the antioxidative defence in the pancreas during the prehibernatory period and the hibernating state. To this end, male *Spermophilus citellus* were divided into two groups, one kept at room temperature (control group) and the other exposed to low temperature (cold exposed group). Active animals from the cold exposed group were sacrificed after 1, 3, 7, 12, and 21 days; animals that entered hibernation were sacrificed after 2-5 days of torpor. Pancreatic protein levels of oxidative phosphorylation components, metabolic regulators (AMP activated protein kinase α - AMPKα and nuclear respiratory factor 1 - NRF1) and antioxidative defence enzymes were determined by Western blotting. Our results showed that the protein levels of respiratory complexes I, II, III, IV and cytochrome *c* were increased in response to prolonged cold exposure and that such expression profiles were maintained during hibernation. In parallel, AMPKα and NRF-1 were shown to be upregulated. Moreover, prolonged cold exposure and hibernation induced an increase in the protein expression of antioxidative defence enzymes, especially copper-zinc superoxide dismutase (CuZnSOD) and glutathione peroxidase (GSH-Px). However, the level of ATP synthase showed a slight decrease. In conclusion, these results point to a controlled metabolic remodeling in the pancreas of ground squirrels during prolonged cold exposure and in hibernation, which includes an improvement of mitochondrial oxidative capacity along with a proportional upregulation of antioxidative defence.

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MITOCHONDRIAL BIOGENESIS IS POSSIBLE ADAPTIVE RESPONSE OF TESTICULAR LEYDIG CELLS FROM STRESSED ADULT RATS

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Mitochondria are the starting point for steroid biosynthesis in steroid producing cells as well as the most important component of stress response in all cells. Nevertheless, there are no data about generation of new mitochondria in steroidogenic cells, including Leydig cells from testes. Here we investigated the parameters of mitochondrial biogenesis in testosterone-producing Leydig cells from rats exposed to the psychophysical stress by immobilization (IMO). IMO stress was applied for 2 h daily for one (1xIMO), two (2xIMO) or ten (10xIMO) days. Proof that IMO was an effective stressor were elevated serum levels of adrenaline and corticosterone as well as decreased serum testosterone level in all stressed groups. Quantification of TMRE fluorescence in Leydig cells from all experimental groups revealed reduced mitochondrial membrane potential ($\Delta\psi_m$) in 1x and 2xIMO groups, while $\Delta\psi_m$ was restored in 10xIMO rats. There was positive correlation between $\Delta\psi_m$ of Leydig cells and androgens production of Leydig cells also reduced in all stressed rats but partially recovered in 10xIMO group. The increased mitochondrial mass in Leydig cells from the 10xIMO group was detected by quantitative analysis of MitoTracker-Green fluorescence as well as relative intensity of fluorescence. In line with this were results of transmission electron microscopy showing that acute and two times repeated stress altered architecture of mitochondrial cristae, while 10xIMO increased number of mitochondria and recovered mitochondrial architecture. Results of RQ-PCR and Western blot analyses revealed a significant increase in the expression of the all markers of mitochondrial biogenesis in Leydig cells from 10xIMO rats. All types of IMO increased level of *Ppargc1b* transcript in Leydig cells, while *Ppargc1a* increased only after 10×IMO. In parallel, repeated stress increased expression of *Nrf1*/*NRF1* and *Nrf2a* (downstream targets of PGC1) as well as TFAM and TFB2M (downstream targets of both NRF1 and NRF2). In the same cells, a similar pattern was observed for essential kinases related to regulation of steroidogenesis and PGC1 activation. Our results support the conclusion that stress, a constant factor in life of humans, induces mitochondrial biogenesis in testosterone-producing Leydig cells, probably to protect basal steroid production in stress conditions.

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FOOD ENRICHED WITH FISH FLOUR DECREASE n-6/n-3 RATIO IN LIVER PHOSPHOLIPIDS IN MALE WISTAR RATS

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Dietary intake of different food influences fatty acid composition of lipid fractions, especially phospholipids as the main constituents of cell membranes. The polyunsaturated fatty acids (PUFA) intake is more and more associated with the prevention and development of chronic diseases with an inflammatory component. The liver has an important role in the synthesis and metabolism of phospholipids. Liver phospholipids build the structure of the hepatocyte membrane, take part in metabolic activities and repairing after metabolic disturbances. Our aim was to investigate the effects of food enriched with fish flour and/or food enriched with milk powder on fatty acid composition of liver lipids in rats treated for 4 weeks. Male Wistar rats were randomly assigned into three experimental groups (n=10, 375g±5). The control group recieved standard food (Veterinarski zavod, Subotica). Group II was fed a diet with fish flour and III received a diet with milk powder. The animals were sacrificed by decapitation and part of the liver was frozen at –80 °C. The fatty acid composition of liver phospholipids was detrmined by Gas Chromatography (Shimadzu GC). Our results showed that food enriched with fish flour significantly increased liver phospholipid concentrations of docosahexaenoic acid (22:6), PUFA and n-3 and decreased n-6/n-3 ratio compared to control. Food enriched with milk powder increased arachidonic acid (20:4), MUFA, PUFA, n-6 and n-6/n-3 ratio while decreased eicosapentaenoic acid (20:5), (22:6) and n-3 compared to control. Fish oil/powder is a well known antioxidant. Food enriched with fish flour is more efficient due to decrease of n-6/n-3 ratio (as a risk factor) and our future examinations will address it.

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CHANGES IN ANTIOXIDATIVE CAPACITY DURING DEVELOPMENT OF DRUG RESISTANCE IN RAT C6 GLIOMA CELLS

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Glioblastoma (GBM) is the most common type of brain primary tumors displaying therapy resistance and great invasion potential. Cellular response to anticancer drugs depends on the oxidative status and changes in antioxidative systems. There is a strong need to understand the mechanism that underlies the phenomenon of chemoresistance. To that end, we induced drug resistance by exposing rat C6 glioma cells to increasing concentrations of 3-bis (2-chloroethyl)-1-nitrosourea (BCNU). Sulforhodamin B test was used to determine cytotoxic effect of BCNU, cisplatin (CPt) and temozolomide (TMZ) as well as the effect of H_2O_2 on sensitive and newly established resistant C6 cell line (RC6). Detection of reactive oxygen species (ROS) was performed by flow cytometric analysis of dihydroethidium (DHE) fluorescence intensity and fluorescent microscopy in C6 and RC6 cells. The expression of enzymes involved in oxidative stress defense was analyzed on mRNA level by RT-PCR and qRT-PCR. Invasive potential of C6 and RC6 cells was analyzed with gelatin degradation assay *in vitro* and in an animal study using Fast Blue fluorescently stained C6 and RC6 cells inoculated into the brain of male Wistar rats. C6 cells continuously treated with BCNU developed significant resistance to this drug and to other DNA damaging agents, CPt and TMZ. The development of drug resistance was followed by a significant increase in ROS production and decreased sensitivity to H_2O_2. The mRNA expression levels of *mnsod*, *inos* and *gpx* were increased and *hif1a* decreased in RC6 cells compared to C6 cells which is in line with obtained changes in ROS content and increased antioxidative capacity of RC6 cells. Resistant cells were more invasive and aggressive according to gelatin degradation assay and animal studies. In conclusion, continuous BCNU treatment induced upregulation of antioxidant capacity in RC6 rat glioma cells. During adaptation to oxidative stress, RC6 cells developed a more aggressive phenotype. Therefore, our orthotopic RC6 glioma allograft can be used as a valuable tool in preclinical studies for the examination of potential therapeutic strategies for overcoming chemoresistance and suppression of glioma invasion.

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PROTEIN AND LIPID OXIDATIVE DAMAGE BIOMARKERS AS PROGNOSTIC FACTORS OF FIVE-YEAR SURVIVAL IN HEMODIALYSIS PATIENTS

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Oxidative stress in end-stage renal disease (ESRD) patients is further potentiated during hemodialysis contributing to poor cardiovascular and overall outcome of these patients. Among other causes, susceptibility to oxidative stress in ESRD is influenced by the genetic polymorphism in antioxidant and detoxifying enzyme glutathione transferases (GST). Therefore, we assessed the prognostic significance of oxidative stress biomarkers in ESRD patients stratified according to GST genotype. A total of 199 hemodialysis patients and 199 age- and gender matched controls were included in the study. GSTA1, GSTM1, GSTP1 and GSTT1 genotypes were determined by PCR and RFLP-PCR method. Markers of oxidative protein (AOPP; carbonyl groups) and lipid (MDA; MDA adducts) damage were determined spectrophotometrically and by ELISA. Prooxidant-antioxidant balance (PAB) has also been measured. A 5-year all-cause mortality and cardiovascular mortality were prospectively registered. Elevated protein and lipid markers of oxidative damage are significantly associated with investigated GST-null/low activity genotypes, predominantly with GSTM1-null genotype (carbonyl groups: p=0.005; AOPP: p=0.001, MDA adducts: p=0.001). The level of oxidative stress is even more pronounced in the patients with all null or low activity GST genotypes. Cox regression analysis demonstrated that stratified values of MDA (HR=1.5, p=0.049, 95%CI=1.00-2.28) and PAB (HR=2.22, p=0.001, 95%CI=1.39-3.52) were independent all-cause mortality predictors. AOPP has shown to be cardiovascular mortality predictor (HR=2.32, p=0.006, 95%CI=1.27-4.24) together with stratified values of MDA (HR=1.89, p=0.021, 95%CI=1.1.-3.25) and PAB (HR=2.5, p=0.003, 95%CI=1.36-4.47). Regarding GST genotype, only GSTM1-null genotype was independent predictor of all-cause mortality (HR=1.79, p=0.009, 95%CI=1.15-2.77). It may be concluded that null/low GST genotypes are associated with enhanced susceptibility to oxidative stress in ESRD patients. Moreover, GSTM1-null genotype might be considered as a genetic marker of overall death risk. These results also suggest that AOPP, MDA and PAB could contribute to risk prediction of overall and cardiovascular death over known indicators which could improve attempts towards individualization in antioxidant therapy.

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EX VIVO AND *IN VIVO* STUDIES OF THE BRAIN OXIDATIVE STATUS IN THE RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by loss of neurons in the upper and lower motor pathways of the cortex, brainstem and spinal cord, resulting in muscle atrophy, paralysis, and death within 3 to 6 years after disease onset. Majority of ALS cases are sporadic, while only around 10% are familial with a known genetic background. Among the latter, approximately 20% are caused by dominantly inherited mutations in the Cu/Zn superoxide dismutase (SOD1) gene, which are considered to lead to a toxic gain of function ending in elevated reactive oxygen and nitrogen species production, neuroinflammation and death of motor neurons. The most studied and best characterized models of ALS are the transgenic mice and rat models overexpressing the human SOD1 gene carrying the G93A mutation. In order to asses the timeline of redox status changes of the brain in ALS, oxidative stress parameters were examined in the brainstem and hippocampus tissue homogenates of presymptomatic (preALS) and symptomatic (ALS) hSOD1 G93A ALS rats and nontransgenic controls by spectrophotometrical biochemical assays, which was followed by *in vivo* studies of the brain oxidative status in these animals by Electron Paramagnetic Resonance Spectroscopy (EPRS). Biochemical analyses revealed increased superoxide radical production, nitrite content and index of lipide peroxidation, as well as decresed SOD1 and increased SOD2 activity in both investigated brain regions of preALS and ALS animals. EPRS measurements revealed different reduction kinetics of the 3CP nitroxide redox probe between preALS and ALS animals compared to nontransgenic controls. Fitting the 3CP decay curves in these animals to a mathematical two-compartment model showed increased blood-brain barrier permeabilty and intracellular reduction of the spin probe in preALS and ALS rats, while the drainage of the spin probe by bloodstream was increased only in the latter group. This study brings new insight into the redox status of the brain in ALS, and indicates increased oxidative stress and blood-brain barrier permeability as potential early biomarkers of the disease.

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INFLUENCE OF CHOLESTEROL HOMEOSTASIS ON LIPID PEROXIDATION

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Cholesterol homeostasis represents the balance between cholesterol synthesis and absorption. Increased cholesterol synthesis leads to reduced absorption and vice versa, in order to maintain homeostasis. Levels of cholesterol absorption markers (campesterol, stigmasterol and β-sitosterol) and synthesis markers (desmosterol and lathosterol) are disturbed in coronary artery disease (CAD) patients, causing dyslipidemia and contributing to the progression of atherosclerosis and subsequent development of CAD. We sought to investigate whether cholesterol metabolism varies in different physiological and pathological conditions and to examine their impact on lipid peroxidation. This study included 79 patients with presenting symptoms of CVD (47 were not receiving statin treatment and the remaining 32 patients were on statin therapy). Control group comprised of 31 subjects. Non-cholesterol sterols (NCS) concentrations were quantified using a gas chromatography–flame ionization detector method (GC-FID). Measurement of the malondialdehyde (MDA) by thiobarbituric acid reactive substances (TBARS) assay was used for assessing lipoprotein peroxidation. Our results have shown that both groups of patients had higher levels of synthesis markers and lower absorption markers levels. MDA levels were higher in both patient groups. Each group was then divided into four subgroups, according to synthesis and absorption efficiency (1.poor synthesizers and poor absorbers; 2. poor synthesizers and good absorbers; 3.good synthesizers and poor absorbers; 4.good synthesizers and good absorbers). Among healthy subjects, MDA values were the lowest (0.40±0.150 μmol/L and 0.37±0.224 μmol/L, respectively) in the subgroups with preserved cholesterol homeostasis (subgroups 2. and 3.), while the subgroup 1 had the highest MDA values (0.75±0.381 μmol/L) ($p<0.05$). Subgroup 4 had lower MDA values (0.51±0.156 μmol/L) comparing to subgroup 1, presumably due to protective effect of absorbed phytosterols. In CAD patients on effective statin therapy (subgroup 1), MDA values tended to be lower due to reduced synthesis. In CAD patients receiving no statin treatment with disturbed cholesterol homeostasis no statistically significant difference in MDA levels between subgroups was observed. Determination of NCS may indicate the existence of different cholesterol metabolic profiles and present a potentially useful tool for more accurate screening of individual propensity towards optimal therapy.

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ASSOCIATIONS OF APGAR SCORE AND SIZE AT BIRTH WITH LIPOPROTEIN SUBCLASSES IN JUVENILE OBESITY

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The growing trend of childhood obesity represents a major health concern worldwide. Juvenile obesity is associated with several metabolic abnormalities, one of them being atherogenic dyslipidemia. However, even if cardiovascular disease complications usually become manifested in adulthood, the process of atherogenesis might start much earlier. Suboptimal fetal growth is associated with obesity risk in childhood, but also with increased rate of metabolic diseases in later life. This study investigates associations of neonatal data (Apgar score, birth weight and birth length) with low-density lipoprotein (LDL) and high-density lipoprotein (HDL) subclasses in the group of obese children, as well as a possible impact of breastfeeding duration on obesity-associated lipoprotein subclasses distributions. The study included 42 obese children, aged 14.2±2.1 years. LDL and HDL subclasses were separated by gradient gel electrophoresis. Concentrations of glucose, total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C were measured by routine laboratory methods. Compared with obese children with Apgar ≥ 9, the group with Apgar < 9 had a significantly higher proportion of small, dense LDL particles (P<0.05), due to reduction of LDL I (P<0.01) and increase of LDL III subclasses (P<0.05). Birth weight was positively associated with the proportions of LDL I particles (P<0.001), whereas birth height was positively associated with the proportion of HDL 2b subclasses (P<0.05). The group of never or less than 3 months breastfed children had significantly smaller LDL size (P<0.01) and reduced proportion of HDL 2a particles (P<0.05) than their ≥ 3 months breastfed peers. The results of the current study have shown significant associations of neonatal characteristics with LDL and HDL particles distributions in obese children. In addition, our results point toward positive aspects of longer breastfeeding duration on lipoprotein particles distributions in obese children.

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ANTITUMOR POTENTIAL OF ISOXANTHOHUMOL: SENSITIZATION TO PACLITAXEL *IN VIVO*

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Isoxanthohumol (IXN), a natural prenylflavonoid from the hops (*Humulus lupulus L*.), possesses diverse biological properties like antiinflammatory, antioxidant, antiangiogenic and antitumor. The aim of this study was to evaluate the direct effect of IXN against melanoma cells, as well as its potential to increase the effectiveness of conventional chemotherapy *in vitro* and *in vivo*. The study was performed on two different melanoma cell lines, B16 and A375, and on syngeneic mouse melanoma model *in vivo*. Results revealed a dose-dependent decrease of cell viability upon the treatment with IXN. The observed effect was followed by remarkable morphological transformation and loss of dividing potential in both tested cell lines, indicating their phenotypical alterations. While elevated tyrosinase activity without enhancement of melanin content indicated that modified B16 cells underwent the process of non-classic differentiation, the loss of pluripotent characteristics of A375 cells was confirmed by the inhibition of Notch 1, β-catenin and Oct-3/4. In parallel, a certain percentage of subpopulations in both cell cultures was subjected to programmed cell death in a caspase independent manner. Cell differentiation and/or death could be related to IXN mediated reactive oxygen (ROS) and nitrogen species (RNS) scavenging properties. At the intracellular level, IXN promoted different modifications in the upper part of the PI3K/Akt and MEK-ERK signaling pathways in B16 and A375 cells. Nonetheless, it was estimated that, after transient activation, the expression of p70S6K and its target S6 ribosomal protein was inhibited in both types of melanoma cells. In addition to direct anticancer effect of IXN, this study shows for the first time its remarkable capacity to sensitize melanoma cells to a subtoxic dose of paclitaxel *in vivo* and *in vitro*. In summary, these data indicate that IXN is worthy of further investigation in the field of experimental oncology.

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CORRELATION BETWEEN OXIDATIVE STRESS AND FATTY ACIDS PROFILES IN PLASMA AND LIVER PHOSPHOLIPIDS IN AGED MALE RATS

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Unsaturated fatty acids are important in prevention and protection in many diseases. In all these patologies role of redox active species (oxigen and nitric) is crucial. Our aim was to correlate parameters of oxidative stress and fatty acids profiles in phospholipides of plasma and liver in aged male rats. Experiments were performed on male Wistar rats (22 months, b.w. 370g). The animals received standard laboratory food and water ad libitum. The animals were sacrified, plasma and erythrocytes were taken while liver was frozen (-80 ˚C) until experiment were done. Parameters of oxidative stress (malonyl dialdehyde-MDA, catalase-CAT, superoxide dismutase-SOD, nitrites, SH groups), in erythrocytes, plasma and liver, were measured with standard laboratory kits on UV/VIS spectrophotometer and ELISA. Fatty acids profiles were determined by gas chromatography after samples preparation standard procedure. Correlations beetween parameters were done (Graph Pad Prisma 5). Results showed positive correlations: MDA/22:6 , SOD/18:1, n-9 in plasma and MDA/CAT in liver. There was a negative correlation between SOD/22:6 in plasma and SH/20:5 in liver. DHA (22:6) has oposite correlation with MDA and SOD in plasma while EPA has negative correlation with tiol groups in liver.

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COMBINED GSTO1/GSTO2 GENOTYPE INCREASES RISK OF CLEAR RENAL CELL CARCINOMA

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Clear cell RCC (cRCC) is the most common and probably the most aggressive RCC subtype, characterized by the highest rate of local invasion, metastasis and mortality. Although glutathione S-transferases, GSTO1 and GSTO2, exhibit a unique range of different activities involved in regulation of inflammation, apoptosis and cellular redox homeostasis, no study has explored the association between GSTO1 and GSTO2 polymorphisms and cRCC. *GSTO1* (rs4925) and *GSTO2* (rs156697) genotyping was performed in 189 cRCC patients and 231 age- and gender-matched controls by PCR-RFLP. Another *GSTO2* polymorphism, rs2297235 was determined by Taqman based real time-PCR. We found that neither of the GSTO polymorphisms contributed independently towards the risk of cRCC. However, carriers of the combined variant *GSTO1**AspAsp/*GSTO2**Asp allele (rs156697) were at 2.2-fold increased cRCC risk compared to carriers of the G*STO1**Ala allele and *GSTO2**Asn/Asn (wild type) genotype (p=0.049). Moreover, a significant modifying effect on cRCC risk conferred by hypertension and smoking, as recognized risk factors, had been found in individuals with aforementioned risk genotypes (OR=7.4, 95%CI=1.7-31.9, p=0.007; OR=3.3, 95%CI=1.1-9.9, p=0.029 respectively). Based on our findings regarding association of combined gene variants of *GSTO1* and *GSTO2* with cRCC risk, it can be assumed that in terms of their role in inflammatory response and cellular signaling these polymorphisms might modulate risk for cRCC development.

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GSTO1-1 OVEREXPRESSION IN TRANSITIONAL CELL CARCINOMA

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Several studies implicate glutathione transferase omega 1-1 overexpression (GSTO1-1) in the onset of drug resistance of cancer cells. It has been shown that GSTO1-1 is involved in the activation of IL-1β which is critical mediator of chronic inflammation in cancer, thus regulating expression of different sets of cytokines, including IL-8. Beside the association of high IL-8 expression with the disease-specific transitional cell carcinoma (TCC) survival, recent data also indicate its role as biomarker of TCC recurrence. The aim of this study was to determine expression profile of GSTO1-1 and quantify IL-8 in transitional cell carcinoma (TCC) patients. GSTO1-1 expression and IL-8 concentrations were determined in 35 TCC tumor and corresponding non-tumor specimens. GSTO1-1 thioltransferase activity was determined spectrophotometrically. Tissue concentrations of IL-8 were estimated by enzyme linked immunosorbent assay (ELISA). GSTO1-1 expression was determined by Western blot and real time-polymerase chain reaction. GSTO1-1 activity was significantly higher in tumor compared to surrounding non-tumor tissue (12.21 ± 6.27 vs. 5.54 ± 3.99 mU/mg of protein; p= 0.004). The increased GSTO1 expression in tumor tissue was also confirmed on protein and mRNA level, while its thioltransferase activity showed clear correlation with tumor grade and invasivness. In tumor samples of TCC patients we also found significantly higher IL-8 levels compared to corresponding non-tumor specimens (22.94 ± 3.67 vs. 6.49 ± 1.43 pg/mg of protein; p=0.000). Tissue level of IL-8 increased proportionally along with tumor grade (p=0.054) and invasiveness (p=0.016). Our results provide additional support for the role of inflammation in TCC and indicate that GSTO1-1 could be further validated in association with other already used markers for early detection and prognosis of TCC.

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TARGETING THE SURVIVAL PATHWAYS OF CANCER CELLS WITH METFORMIN AND THYMOQUINONE: ROLE OF MITOCHONDRIA AND ROS FORMATION

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Tumor cells undergo metabolic transformations which are essential for their development and progression. Transformed cells show high demand for glycolytic intermediates. There are examples where dietary restriction has shown anti-cancer effects. Metformin is a compound that acts through a similar mechanism and it is known as the most prescribed anti-diabetic drug in the world with a proven safety profile. It belongs to the group of biguanides which accumulate in mammalian mitochondria, affect oxidative phosphorylation and stimulate reactive oxygen species (ROS) production. Another group of biologically active molecules with potential to target tumor cells are quinones. They are undergoing facile reduction-oxidation. Two main processes involved in antitumor activity of these compounds are redox cycling and direct reaction with cellular nucleophiles such as protein and non-protein sulfhydryls. Thymoquinone (TQ) is phytochemical with quinone moiety showing promising antitumor effects. Objective of this study was to evaluate effects of metformin and TQ on viability and survival of DHL-4 and K562 human leukemic cell lines. WST-1 and trypan blue assays were used. We found that both, metformin and TQ, show inhibitory effects on the survival of studied cancer cell lines. Further data about their intracellular targets, synergistic potential and exact mechanisms of their action are warranted.

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GSTM1-NULL, GSTT1-ACTIVE AND GSTA1 LOW-ACTIVITY AND GSTP1-VARIANT ARE RISK-CARRYING GENOTYPES FOR CLEAR CELL RENAL CELL CARCINOMA

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As a result of polymorphic expression of glutathione transferases (GST), great inter-individual differences in GST isoenzyme profiles exist in renal parenchyma, affecting both the capacity for biotransformation and protection from free radicals in renal tissue. We aimed to determine the association of established risk factors and specific GST gene variants in patients with clear renal cell carcinoma (cRCC), as well as to discern whether phenotype changes reflect genotype-associated risk. *GSTA1, GSTM1, GSTP1* and *GSTT1* genotypes were determined in 199 patients with cRCC and 274 matched controls. BPDE-DNA adducts were determined in all DNA samples obtained from cRCC smokers. Significant association between GST genotype and risk of cRCC development was found only for the *GSTM1-null* and *GSTP1-variant* genotype (OR=2.07, p=0.02 and OR=3.14, p<0.001, respectively). Moreover, 22% of all recruited cRCC patients were carriers of combined *GSTM1-null, GSTT1-active, GSTA1-low activity* and *GSTP1-variant* genotypes and exhibited 8.49-fold elevated RCC risk in comparison to controls (p=0.09). Significant modifying effect on cRCC risk, conferred by hypertension, was present in individuals with GSTM1-*null, GSTT1-active, GSTA1 low-activity* or *GSTP1-variant* genotype. Smoking contributed significantly to cRCC risk only in carriers of *GSTP1-variant* genotype (OR=3.70, p=0.001). However, the *GSTM1-null/GSTP1-variant/GSTA1 low-activity* genotype combination was present in 94% of smokers with cRCC, increasing the risk of cRCC up to 7.57 (p=0.02). Furthermore, smokers with *GSTM1-null* genotype had significantly higher concentration of BPDE-DNA adducts (2.74ng/ml (1.64-17.93)) in comparison to *GSTM1-active* smokers (2.13ng/ml(1,39–5.22), p=0.05). Combined *GSTM1-null, GSTT1-active, GSTA1-low activity* and *GSTP1-variant* genotypes might be considered as "risk-carrying genotype combination" in cRCC patients, with special emphasis on *GSTM1-null* and *GSTP1-variant* genotypes.

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SOD ACTIVITY AND LIPID PEROXIDATION IN PATIENTS AFFECTED BY CELIAC DISEASE

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Celiac disease (CD) is an autoimmune intestinal disorder provoked by gliadin, a component of gluten, which is characterized by a severe damage to the mucosal surface. In order to explain the role of oxidative stress in the pathogenesis of CD, we measured the activity and the protein levels of the copper, zinc superoxide dismutase (CuZnSOD) and manganese SOD (MnSOD) in the intestinal biopsy specimens of 69 children with CD. The concentration of lipid hydroperoxides (LOOH) was also assessed in the same samples. Based on the histological findings, patients were divided into following groups: Marsh 0: mucosa with no signs of inflammation (n = 31); Marsh 1: mucosa with intraepithelial lymphocytosis (n = 5); Marsh 2: intraepithelial lymphocytosis accompanied by crypt hyperplasia (n = 4); Marsh 3a: mucosa with partial villous atrophy (n = 20); Marsh 3b: mucosa with subtotal villous atrophy (n = 9). For the statistical purposes groups Marsh 1 and Marsh 2 were treated as one (Marsh 1+2, n = 9). In the intestinal mucosa MnSOD activity was significantly elevated in Marsh 3a group ($P < 0.01$), in comparison to Marsh 0, while CuZnSOD activity remained unchanged. Significant increase in LOOH concentration was found in both groups with villous atrophy (Marsh 3a: $P < 0.001$; Marsh 3b: $P < 0.01$), comparing to Marsh 0. Relative CuZnSOD and MnSOD protein levels in the intestinal mucosa did not vary significantly between the analyzed groups. Positive correlations were found between the severity of mucosal lesion and CuZnSOD activity ($r_s = 0.28$, $P < 0.05$), MnSOD activity ($r_s = 0.27$, $P < 0.05$), as well as LOOH concentration ($r_s = 0.55$, $P < 0.001$). Our results demonstrate that a significant disturbance in antioxidative status occurs in patients affected by CD, especially those with the advanced mucosal damage. Changes in antioxidative enzyme activities and LOOH concentration significantly correlate with the severity of histological damage. A seriously impaired antioxidative capacity for degradation of LOOH may persist even after several years of gluten free diet. A diet rich in natural antioxidants as well as appropriate dietary supplements could be beneficial for full mucosal healing of celiac patients.

AUTHOR INDEX

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