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Connection between measurement of vitamin B_{12} by (RP)HPLC-ICP-MS hyphenated analytical system and antioxidant capacity

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Vitamins constitute a diverse group of organic compounds essential in trace amounts for the proper growth and maintenance of life. They have different specific roles in metabolism, and their lack or excess can generate serious diseases. When it is necessaryTo ensure the adequate intake of vitamins the human diet can be completed with high range of multivitamin tablets and food products supplemented with vitamins, such as B_{12} fortified energy drinks. Each vitamin protects the human body from oxidizing agents, that's why it is of importance to determine the antioxidant capacity and amount of vitamin in food.

The aim of this study was to develop an (RP)HPLC-ICP-MS method for the determination of cyanocobalamin, the compound used for vitamin B_{12} fortification in food. Moreover we also measured the antioxidant capacity and total phenols in the energy drink sample.

In the first part of the work the reversed-phase HPLC separation was optimized with UV detection. After applying various chromatographic set-ups finally an Agilent Eclipse XDB 4.6 x 250mm (5um particle size) column having a C18 stationary phase was chosen. As mobile phases sodium-acetate (pH = 4.0) - acetonitrile and methanol - 0,05 V/V% trifluoracetic acid/ H_2O were used. We worked with two kinds of ICP-MS configuration, - with oxygen gas and without oxygen gas- and these methods were compared.

After the aqueous extraction and alcohol extraction of the energy drink the antioxidant capacity with FRAP assay and total phenol using reagent of Folin-Ciocalteu with spectrophotometer were determined.

The RP-HPLC-ICP-MS system was compared to HPLC-UV system. The selectivity of HPLC-ICP-MS is better, since the cyanocobalamin measurement based on its Co atom content, similarly the sensitivity of HPLC-ICP-MS is hundredfold better, according to the sensitivity of –UV detection. Moreover HPLC-ICP-MS without oxygen gas is more sensitive, then the other, but HPLC-ICP-MS with oxygen gas is occurred to be a more robust system.

The two extractions had a difference in efficiency, because the value of total phenol of alcohol extraction is twofold, as compared to the value of water extraction. The value of antioxidant capacity was too low according to total phenol, which means, that this assay can't measure the antioxidant effect of each phenol compound.

Both of cianocobalamin detection is good for measurement of B_{12} fortified food with simple matrix and vitamin tablet. But the selectivity of the UV detection wasn't enough in the case of energy drink, because the cianokobalamin coeluated with another compound, accordingly we couldn't determine amount of cianokobalamin properly. The ICP-MS detection is more sensitive, according to the UV detection and it measures the cyanocobalamin based on its Co atom content, so determination is more selective. These attributes allow to measure cianocobalamin in very small range, because amount of B_{12} in food is too low (ng/ml).

Phenolic composition and *in vitro* antioxidant activity correlation in *Sempervivum tectorum* extracts

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Sempervivum tectorum (Crassulaceae) is a widely known herb. In folk medicine its juice and leaves were used against inflammation of the ears. The juice was also applied to herpetic eruptions of the skin, minor burns and wounds. It showed remarkably potent antioxidant activity determined by chemiluminometric and EPR spin trapping methods, and inhibited lipid peroxidation induced both enzymatically and non-enzymatically.

Antioxidative, anti-inflammatory and antinociceptive effects of *Sempervivum* has been previously described, though the mode of action is still unexplained and any compounds has not been attributed to these effects. Phytochemical screening of *Sempervivum* extracts with different polarity proved the presence of notable quantity of polysaccharides, polyphenolic

compounds, flavonoids and organic acids. The purpose of this work is the comprehensive chemical analysis of these extracts, implying their fractionation, and the evaluation of their structure-activity relationship.

Extracts of lyophylized and powdered *Sempervivum* tectorum leaves with different polarity (water, 80% (v/v) methanol, methanol, ethanol, aceton, ethyl-acetate and chloroform) has been studied by LC-ESI-MS/MS. Towards a more detailed phytochemical analysis extracts prepared with methanol and chloroform have been fractionated by column chromatographic methods, and the fractions have been studied by LC-MS. *In vitro* antioxidant activity of extracts and fractions has been determined by spectrophotometry using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethyl-benzothiazolin-6-sulfonic acid) radical scavenging activity assays. Antioxidant activity has been characterized by $IC50^{-1}$ values.

The *Sempervivum* leaf extract, prepared by acetonic extraction and acidic hydrolysis had the highest antioxidant capacity. It contained purely flavonoid aglycones and its antioxidant activity was comparable to the one of kaempferol standard. The extract with the second highest antioxidant activity was prepared by 80% (v/v) methanol. Its LC-MS evaluation proved the presence of rutin, kaempferol-di(rhamno)-hexoside and five other kaempferol-glycoside derivatives. Houseleek extracts affected more potent radical scavenging activity against ABTS, than DPPH. Total polyphenolic content and antioxidant activity of *Sempervivum* extracts showed a significant correlation both ABTS and DPPH radicals (r^2=0,907 and r^2=0,967, respectively).

LC-MS evaluation of *Sempervivum* proved to have a high content of flavonoids and other phenolics, which are assumed to play an important role in the eminent scavenging activity of the extracts. On the basis of correlation between total polyphenol content and antioxidant activity of extracts it was concluded, that the remarkable scavenger activity of *Sempervivum* is due to the synergism of its polyphenolic compounds.

Reducing oxidative stress and leukocyte activation in reperfusion injury with controled reperfusion

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Reperfusion of the limbs after acute and persistent ischaemia is associated with high rates of morbidity and mortality despite complete revascularisation. Reconstruction of blood flow will induce reperfusion injury with oxidative stress and inflammatory responses. There are experimental evidences that modification of the initial reperfusion modalities can minimize this reperfusion injury.

In our study we aimed to confirm in an animal model that controled reperfusion (CR) can reduce oxidative srtess and leukocyte activation in reperfusion injury.

In our work we used 10 yorkshire pigs that were divided in two groups. All of the animals underwent a 4 hours infrarenal aortic occlusion: after anethesia we made median laparatomy and clamped the infrarenal abdominal aortae. In the first group after occlusion we removed the clamp, restored the blood flow and closed te wound. In the second group after ischaemia we made CR. CR consisted of 30-minute infusion of a crystalloid reperfusion solution that was mixed with oxygenated blood (the blood:reperfusion solution ratio was 1:1) distal to the occlusion. After this procedure we restored the normal blood reperfusion.

Blood samples were collected before occlusion, on the end of ischaemic period, and after reperfusion in the15 th minute (from inferior caval vein), in the 1st and 24th hour, and on 7th day (from peripherial vein). To monitore the evoked oxidative stress superoxide-dismutase activity and reduced glutathion concentration were measured. The degree of lipidperoxidation was marked with the quantity of malondialdehyde. The inflammatory respons was marked with the measurement of leukocyte activation. PMA induced free radical production of the leukocytes was measured.

The lipidperoxidation was significantly lower in the early reperfusion in the CR group. CR also lead to a smaller depletion of the antioxidant enzymes. The speed and rate of free radical production of leukocytes were significantly lower in CR group ($p<0,05$).

The results from this study strongly suggest the hypothesis that the results of conventional embolectomy for acute, severe lower-limb ischemia can be improved by CR. The study was supported by OTKA- K67731.

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Effect of T-2 toxin and different selenium compounds on the glutathione redox and lipid peroxide status of broiler chickens

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Fusarium sporotrichoides is a widespread mould worldwide, producing 'type A' trichothecene mycotoxins, e.g. T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, scirpentriol and diacetoxyscirpenol. Trichothecene mycotoxins affect the antioxidant status of animals, primarily due to their pro-oxidant effect. Selenium, as active site of the Se-dependent glutathione peroxidases plays important role in the biological antioxidant system to protect the adverse effect of harmful free radicals.

The objective of this study was to evaluate the effect of T-2 toxin without and with different forms of selenium on the glutathione redox and lipid peroxide status of 21 days old broiler chickens. The birds were divided into four groups, namely control, fed with T-2 toxin contaminated feed (2.05 mg kg^{-1}) without selenium supplementation ('T-2'), fed with T-2 toxin contaminated feed and supplemented with seleno-methionine (Sel-Plex®, Alltech, 0.3 mg Se kg^{-1} feed) ('T-2+ORGSe') and fed with T-2 toxin contaminated feed supplemented with sodium selenite, Sigma, 0.3 mg Se kg^{-1} feed) ('T-2+INORGSe'). T-2 toxin was produced experimentally on maize by Fusarium sporotrichioides strain NRRL 3299. Five animals were exterminated at the start of experiment as absolute control, followed by extermination of 5 birds from each group at days 3, 7 and 14. Blood, liver, kidney and spleen samples were taken, in which reduced glutathione (GSH), malondialdehyde (MDA) concentration and glutathione-peroxidase (GSHPx) activity were measured.

In blood plasma higher ($P<0.05$) GSH concentration were measured in 'T-2' group compared to control (day 14). In red blood cell haemolysate of the 'T-2' and 'T-2+ORGSe' groups lower ($P<0.01$) MDA concentration was measured compared to control (day 3). In line with this at the same time increased GSH concentration were measured in these groups, which was higher ($P<0.01$) in 'T-2+Se' group than the control. T-2 toxin treatment resulted lower ($P<0.01$) GSHPx activity compared to control and both Se-supplemented groups (day 14). In liver homogenate of the treated groups GSH concentrations exceeded during the whole experiment the values of control one, which was significant in 'T-2' (day 7) and 'T-2 + ORGSe' (days 3 and 7) groups. In the 'T-2+INORGSe' group higher ($P<0.05$) GSHPx activity was measured compared to control (day 7). In kidney homogenate – analogously the findings in liver – elevated GSH concentrations were measured in 'T-2' and 'T2-+ORGSe' groups compared to control at each sampling, which was significant in 'T-2' (day 3) and in 'T-2+ORGSe' group (days 7 and 14). GSH concentration of spleen homogenate in 'T-2' (day 14) group and 'T-2+ORGSe' (day 7) groups were lower ($P<0.01$) compared to control.

According to the results consumption of T-2 toxin contaminated feed (2.05 mg T-2 toxin kg^{-1} feed) for two weeks affects the biological antioxidant system of broiler chickens, increasing the amount and activity of glutathione redox system during the first week of mycotoxin exposure, which efficiently protects the lipids from harmful peroxidation processes. However, in the organs which are play important role of metabolism and elimination of T-2 toxin (e.g. liver and kidney), lowered the amount/activity of glutathione redox system was found in the latter half of the T-2 toxin exposure, causing oxidative stress (as measured by the significantly higher MDA concentrations), while Se-supplementation has beneficial effect in the glutathione redox status.

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Antioxidant steroids and the expression of the gene of superoxide dismutase enzyme

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According to our earlier data most steroid end hormones and some intermediate metabolites of the steroidogenesis are able to inhibit the production of free radical superoxide anion.

In our study we intended to investigate, whether gene-expression of the important antioxidant enzyme superoxide dismutase (SOD) changes after incubation with steroidal compounds.

Peripheral blood samples were collected from healthy volunteers (men and women, aged 20-30 years). After the neutrophil cell separation four different steroid treatments (oestradiol, progesterone, testosterone and cortisol; all in 10^8 M concentration, for 2 hours and at 37°C) were performed on 5 million cells. Total RNA was isolated from the treated and control cells, then reverse transcription and real time polymerase chain reaction (RT PCR) were performed on each sample. SYBR Green assays were used for the relative quantification. The SOD_2 gene expression was compared to GAPDH housekeeping gene expression level (incubation with steroidal compounds mentioned above did not alter the expression of this gene in our pilot study).

Upregulated SOD_2 gene expression levels were found after treatment with each steroidal cmpounds. In case of estradiol 14.1fold, progesterone and cortisol 11.3fold increase in average was detected. The largest change (almost twenty –19.7fold rise) was caused by testosterone. The standard deviations of the ddCT values were within one in each treatment.

Based on these data the antioxidant effect of steroid endhormones might be caused at least in part by the enhancement of the SOD gene expression. These results may have innovative pharmacological importance in connection wih free radical mediated disorders.

Metal elements, transmethylating ability and redox homeostasis in tumourous processes

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The receptors, kinases, and nuclear transcription factors affected by metals and metal-induced oxidative stress are associated with cancer growth and spreading. The formaldehyde is in connection with redox homeostasis. HCHO can be formed in transmethylating reactions. Data show the important role of HCHO in proliferative, as well as in apoptotic processes.

Therefore we were interested in studying erythrocyte metal element status, redox homeostasis and transmethylating ability of randomly chosen, operated, middle aged 68 colon and/or prostate tumourous patients and 46 healthy volunteers in both genders.

Tumour markers (CEA, CA 19-9, AFP, PSA) and routine laboratory parameters in sera, redox parameters (scaverger- and reducing ability, SOD, GSHPx) in plasma and erythrocytes, bounded HCHO, HbA1c, protoporphyrin and metal element concentrations in erythrocytes were measured.

We found significant differences in the metal and redox homeostasis between control and operated patients. Significant changes in erythrocyte function can be observed in transmethylating ability and protoporphyrin concentrations as well. The bounded HCHO concentrations were significantly lower in tumourous patients than in healthy controls.

These changes of erythrocytes were similar in operated colon and prostate tumourous patients. We hypothesize that there are similar changes in all other tumour types.

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Diabetes-associated structural and molecular alterations in capillaries supplying the myenteric plexus in rats

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We have recently demonstrated different susceptibilities of nitrergic neurones located in different intestinal segments to diabetic damage. Their different levels of responsiveness to insulin treatment have also been revealed indicating the importance of the neuronal microenvironment in the pathogenesis of diabetic nitrergic neuropathy. Although the myenteric ganglia are not vascularizated, blood vessels closely related to the ganglia play a key role in creating the proper microenvironment for the ganglia.

Recent data confirm that the loss of the modulatory role of the endothelium may be a critical initiating factor in the development of diabetic vascular diseases. The reduction of the endothelium-dependent vasodilatation is mainly induced by a decreased bioavailability of the endothelium-dependent vasodilator nitric oxide and an increase in the activity of toxic oxygen free radical.

To understand the cellular and molecular background of the diabetes related myenteric neuropathy we investigated the capillaries close to the myenteric plexus and raised two main questions; 1. Is there any difference between controls and streptozotocin-induced diabetic rats in the thickness of the basal lamina surrounding these blood vessels? 2. Is there a direct linkage between the quantitative features of Caveolin-1, which is the major negative regulatory protein for endothelial nitric oxide synthase (eNOS), caveolae and eNOS in the endothelium of these vessels.

In this study a streptozotocin-induced chronical diabetic rat model was used. The rats were divided into three groups: controls, streptozotocin-induced diabetics and insulin-treated diabetic rats. Ten weeks after the onset of diabetes the rats were killed by cervical dislocation, and samples of different gut segments were processed for electronmicroscopical investigations. We measured the thickness of basal lamina by the help of electronmicroscopic morphometry. Postembedding immunohistochemistry was used to study the eNOS and Caveolin-1 expression and interaction in capillary endothels in the vicinity of the myenteric plexus. To evaluate the effects of streptozotocin-treatment and insulin replacement statistical analysis was performed, the probability of $P<0.05$ was set as the level of significance.

In diabetic rats, the endothelial basal lamina what plays a key role in permeability and transendothelial transport was significantly thicker than in the controls. The amount of eNOS and its negative regulator protein, Caveolin-1 was increased in diabetic rats. Immediate insulin replacement significantly prevented the thickening of the basal lamina, and overexpression of eNOS and Caveolin-1.

These results indicate a close relationship between vascular dysfunction and diabetic nitrergic neuropathy, suggesting that endothelial dysfunction in the intestine can be a good prognostic factor for developing enteric neuropathy.

Antioxidant properties of home-made fruit concentrate

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Enhanced oxidative stress develops when production and elimination of reactive oxygen derived compounds (ROS) does not balanced. Enhanced ROS production plays role in development of several diseases. In physiological circumstances elevated ROS production can be decreased by enzymes such as superoxide dismutase, catalase, glutathione reductase, and non-enzymatic ways. One part of the non-enzymatic antioxidants are formed in our body – like serum albumin, coruloplasmine, bilirubin, biliverdin etc. – and other part came from the diet.

During the last decades, several clinical and experimental studies were performed to determine the effects of antioxidant supplementation on health and diseases. However, contradiction in the results considering health prevention were found. Moreover, more and more evidences suggest the pro-oxidant or other disadvantageous properties of mega dose antioxidant supplementation. On these basis, one can assume that the most effective sources of antioxidant are natural origin e.g. the diet itself, and consumption of fresh fruit and vegetables to ensure optimal antioxidant, trace element state mostly advised.

In our country consumption of fresh fruit and vegetables are periodic, therefore, effects of conservation on antioxidant content and properties of products must have been studied. In this study we compared antioxidant properties of several conventional home-made fruit concentrates e.g. jams, and some fruit concentrate made by use of gelatin, which shortened preparation time – and was supposed to keep intact antioxidants and vitamins. We determined in water and methanol extracts of jams the total polyphenol, flavonoid and tocopherol content, and measured capability of fruit concentrate to stabilize DPPH radical (H-donor activity), reduce ferric ion, and inhibit xanthin oxidase activity, which produces superoxide anion.

Dry matter content varied between 30-70%, and gelatinized fruit concentrates had 30-50%. Total-phenol content varied between 0.02-0.15 mg/mg in both water and methanol extract, and flavonoid content was between 0.1-0.8 ug/mg, and was significantly higher in methanol than in water extracts. DPPH stabilization was between 0.02-0.8 ug/mg, ferric ion reduction between 0.17-0.34 ug/mg (both expressed in Trolox equivalent). Inhibition of xanthin oxidase activity was negligible in spite of high flavonoid content. Tocopherol concentration varied within a wide range (alpha-toc: 0-5.9 umol/mg; gamma-toc: 0-0.04 umol/mg; delta-toc: 0-0.2 umol/mg). Ascorbic acid content was not determined. Significant relationship was found between total-phenol content and H-donor activity in both water and methanol extracts. The gelatinized products had the lowest values considering all determined parameter, and traditional plum jam proved to be the best.

In conclusion, traditional method for fruit concentrate (jams) preparation requires long time, however, this method preserved antioxidant content and antioxidant properties of jams, since it ensure relative low temperature during the whole process. Artificial gelatinization had disadvantageuos effect in all studied parameter.

Connection between different raising system and antioxidant parameters in geese

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The profitability of animal production largely depends on how efficiently the animals utilize the feedstuffs for maintenance and production. This is markedly influenced by nutritional and keeping systems during the rearing period of animals. The energy and crude protein contents of the diets, and stocking density have great influence on metabolic processes, hormonal status and the redox system of the animals.

The physiological effect of different management techniques used in geese production is scarcely investigated. Several reports have been presented recently describing the biochemical effect of stocking density, however no literature data have been published concerning the influence of stocking density on antioxidant system of geese. The objective of this study was to investigate the connection between different nutritional and management technologies and antioxidant parameters of geese determining two plasma parameters.

540 Gourmaud liver type hybrids (representing both sexes) were included in the experiment, from 1 day to 64 day of age. At the start of the experiment two different stocking densities were used: 2.5 geese/m^2 (12 geese/cage) and 1.5 times higher, 3.8 geese/m^2 (18 geese/cage). Geese were fed with experimental diets with 11, 12 and 13 MJ/kg ME (low, medium and high) each contained 18, 20 and 22% CP in the starter, 16, 17.5 and 19% CP in the grower and 14, 15 and 16% CP in the finisher. Blood samples were taken from wing vein at the end of trial (9 wk of age). The tested plasma parameters for antioxidant status of geese were chemiluminescent intensity (CI) and radical scavenging capacity (RSC). Chemiluminescence assay in plasma was carried out by the method of Blázovics et al. (1999). Radical scavenging capacity of feed samples was determined in the presence of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as described Blois (1958).

The dietary metabolizable energy content had significant effect on antioxidant system of geese. Geese fed diet with highest energy content had increased radical scavenging capacity and the value of chemiluminescent intensity was the best. There was no connection between dietary crude protein content and the measured antioxidant parameters. The redox parameters were similar in male and female geese, there was no sexual differences. The stocking density had significant effect on radical scavenging capacity, however chemiluminescent intensity did not differ. At a stocking density rate of 12 birds per pen plasma radical scavenging capacity was significantly higher, 0.352 mmol/l, compared to 0.299 mmol/l value found in pen with 18 birds.

By measuring these two parameters characterizing the redox status of the organism, significant relation could be revealed between the nutrition and management and the antioxidant system of the geese. For a more detailed interpretation of this effect, further parameters related with main elements of the antioxidant system would need to be analysed. Such data could help to find adequate compounds as feed additives supporting the antioxidant system

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The effect of the fluid resuscitation method and the n-acetylcysteine supplementation on the oxidative stress response after severe burn injury

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The hypovolaemia, followed by burn injury, induces severe oxidative stress in the body. Our previous study has proven that intrathoracic blood volume index (ITBVI) is a better target parameter of fluid resuscitation, than hourly urine output (HUO). There are only few data in the literature regarding to the effectiveness of antioxidant therapy after burn injury. The aim of our study was to analyze the effect of the fluid resuscitation method and the n-acetylcysteine (NAC) supplementation on the oxidative stress response after severe burn injury.

Twenty-seven patients were involved to our study. In Group I (n=8) the fluid resuscitation was guided by the HUO, in Group II (n=8) by the ITBVI. In Group III (n=11) the ITBVI guided fluid replacement was supplemented with NAC administration during the study period. Venous blood samples were taken from patient on admission and on the next 5 consecutive days. We measured the blood-, and oxidative stress parameters (malondialdehyde (MDA), reduced gluthation (GSH), protein sulfhydril (PSH) groups in plasma, the activities of superoxide dismutase (SOD), catalase (CAT) and myeloperoxidase (MPO) enzymes, and PMA induced free radical generating capacity (ROS). Blood samples from healthy volunteers (n=9) served as the control.

There was no significant difference between the groups regarding to age and burned body surface. There was a higher survival in the NAC treated group. White blood cell count normalized by the 3rd day in all groups, but the relative number of granulocytes was significantly ($p<0.05$) higher, the relative number of lymphocytes was significantly ($p<0.05$) lower in the HUO Group. The marked granulocytosis and lymphocytopenia were on the mend in the NAC Group. The MDA level was elevated ($p<0.05$) all along, the ROS from the third day ($p<0.05$) during the observation period compared to the Control Group. MDA in the plasma was lower, the ROS was higher in the NAC Group. The GSH and PSH level, as well as the SOD activity was significantly lower ($p<0.05$), the CAT activity was significantly higher ($p<0.05$) in the HUO and ITBVI Groups compared to the Control Group. There was no significant difference between the patient groups. NAC supplementation significantly increased the PSH levels and the GSH level normalized earlier. The NAC treatment had no effect on the SOD and CAT activity compared to the ITBVI Group.

The ITBVI guided fluid resuscitation has a beneficial effect on the prooxidant state of the body, but it has no effect on the prooxidant parameters. The adjuvant NAC treatment improved the survival of the patients, increased the endogenous, non-enzymatic antioxidant capacity, but didn't reduce the prooxidant parameters and the activation of the white blood cells.

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Biglycan protects cardiomyocytes against simulated ischemia/ reoxygenation injury via an NO-dependent mechanism

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Although biglycan, a proteoglycan component of extracellular matrix, has been suspected to contribute to the development of atherosclerosis, overexpression of biglycan has been shown to induce cardioprotective genes including nitric oxide (NO) synthases in the heart of a transgenic mouse model.

The aim of the present study was to test whether biglycan is cardioprotective against hypoxia/reoxygenation injury in cardiomyocytes and if an NO-dependent mechanism is involved in the cytoprotection.

Therefore, primary cardiomyocytes were prepared from newborn Wistar rats and kept in growing medium (90% DMEM, 10% fetal calf serum) under normoxic conditions (37°C, 5% CO_2). Two days old cultures were treated with 1, 3, 10, 30 and 100 nM biglycan. In separate experiments, biglycan (30 nM) was combined with the NO synthase inhibitor L-nitro-arginin-methyl-ester (L-NAME, 100 μM). After a 20-hour pretreatment, media of the cultures were replaced with a "hypoxic" solution and plates were kept in a hypoxic chamber (gased with 95% N_2 and 5% CO_2 at 37°C) for 150 minutes, which was followed by 120 minutes of reoxygenation. All treatments were continued throughout hypoxia and reoxygenation. Finally, viability tests were done in all groups with Trypane blue staining. In order to check the effect of biglycan on NO synthase (NOS) expression, in separate experiments, normoxic cells were treated with 30 nM biglycan for 20 hours and then mRNA and total protein were isolated.

After simulated ischemia and reoxygenation, 41.8±1.0% of the cells died in control cultures. Biglycan significantly decreased cell death at 3, 10, 30 and 100 nM concentrations. Protection was the strongest at 30 nM (17.3±2.4%). Biglycan enhanced expression of mRNA of endothelial NOS, but not inducible NOS. Endothelial NOS expression at protein level was also significantly elevated after biglycan treatment. The L-NAME abolished the cytoprotective effect of biglycan (36.3±1.6%).

The proteoglycan biglycan exerts a cytoprotective effect against hypoxia/reoxygenation injury via at least in part an NO-dependent mechanism.

Antioxidant characterization of perspective apricot hybrids

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Health-promoting effects of fruits are at least partially attributed to the antioxidant compounds accumulating in fruit flesh. Apricot fruit contains three major types of antioxidant compounds: water-soluble ascorbic acid (vitamin C), lipid-soluble carotenoids and polyphenolics encompassing both hydro- and lipophilic components. To survey the potential health-effects of apricot it is important to know about the variations in quantity of the antioxidant compounds present in fruit. Studies on parents and their progeny may help to shed light on the inheritance of fruit antioxidant properties and to clarify if the increase in fruit antioxidant capacity may be possible in a carefully designed breeding program.

The measurements were carried out on the apricot cultivars maintained in the germplasm collection of the Department of Genetics and Plant Breeding, CUB and 18 hybrids obtained from a breeding program of the Department of Genetics and Plant Breeding, CUB. The following parameters were studied in apricot fresh fruits: colour values (lightness factor, hue angle and chroma colour); ferric reducing ability (FRAP); DPPH-radical scavenging activity; total radical scavenging capacity measured with chemiluminescence methods; as well as total phenol (TPC) and vitamin C contents measured with spectrophotometer and HPLC-DAD, respectively.

The FRAP and TPC assays revealed 22- and 21-fold differences, respectively, between the lowest and the highest values, indicating a great diversity in the antioxidant power of apricot fresh fruits. A perspective hybrid produced outstanding values in all of the antioxidant assays, exceeding 2.5-times the same parameters determined for the best commercial cultivar. The

FRAP values of twelve hybrids resulting from the cross 'Bergeron' ×'Baneasa 4/11' varied between the values determined for the parents ('Bergeron': 3.57 mmolAA/L and 'Baneasa 4/11': 1.12 mmol AA/L). Three hybrids showed FRAP values very similar to that of the 'Baneasa 4/11', while two others almost reached the level measured in 'Bergeron'.

The closest correlation occurred between the FRAP and DPPH-radical scavenging capacity. Close correlations were also obtained between FRAP, TPC, DPPH and vitamin C content data. Colour values did not show significant correlations with any of the measured parameters of water-soluble antioxidants, since colour values were correlated exclusively with the lipid-soluble carotenoids.

Our results indicate that several valuable genotypes can be selected from a progeny obtained from crosses where at least one of the parents is characterized by enhanced fruit antioxidant properties.

This work was co-financed by the Ányos Jedlik programme NKFP06A2-BCETKA06 and the NKTH-OTKA K68921 grant.

Regulation of metal responsive transcription factor MTF-1 expression in common carp

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Metal responsive control of gene expression allows organisms to adjust the concentration of essential metal ions such as Zn^{2+} and Cu^{2+}, within an acceptable range and cope with detoxification of heavy metals (Cd^{2+}, Pb^{2+} and As^{3+}) with no biological function. Metallothioneins (MTs) are widely inducible at transcriptional level by a variety of metals and other stress conditions such as accumulation of reactive oxygen species, hormones and cytokines. Transactivation of metallothionein genes involves the Metal-responsive Transcription Factor (MTF-1) a metal responsive element (MRE) binding, zinc sensitive protein.

In this study we present the first evidence for an *mtf-1* splicing variant (*mtf-1.1a*), originated from the brain of unstressed common carp. We have follow the level of *mtf-1.1a* mRNA in the liver, kidney, heart, muscle and brain of unstressed animals and the effect of heavy metal loading (Cd and As) on the alternative splicing of *mtf-1.1* transcript. For the detection and semiquantitative determination, an *mtf-1.1*-specific primer pair was designed. This primer pair has the potential to amplify a segment from both *mtf-1.1* and *mtf-1.1a* in the same PCR reaction, with a well-distinguishable size difference.

The splice variant of *mtf-1.1* mRNA codes for a truncated MTF-1.1 protein. The lack of a 103 nucleotides internally in the *mtf-1.1a* transcript, between positions 1047-1149, results in a frame shift causing an early termination of translation. The putative MTF-1.1a protein consists of the first 349 amino acids of MTF-1.1 followed by an additional 64 amino acids, which don't resemble at all to the corresponding region of MTF-1.1. The 349 amino acid covers the six Zn-finger DNA binding domains, the nuclear localization (NLS) and the nuclear exporting (NES) signals and the first 12 amino acid of the acidic region. Under unstressed conditions *mtf-1.1a* was detected in all tissues examined, but the liver, with the highest level in the brain. Arsenic alters the level of both *mtf-1.1* and *mtf-1.1a* transcripts in an isoform- and tissue-specific manner. Cadmium had no measurable effect on the alternative splicing of *mtf-1.1* in the liver, while the amount of both *mtf-1.1* transcripts gradually decreased in the brain.

The above observations suggest a tissue- and stressor-specific function of the predicted MTF-1.1a protein. In addition, we have already identified another MTF-1-coding gene, *mtf-1.2*, exclusively expressed in the brain of unstressed animals. The possible presence of 3 MTF-1 protein variants in the brain suggests a crucial role of the MTF-1s in this organ. MTF-1.1a might function as a negative regulator of MRE-controlled expressions. However, it is also possible that the introduction of a new C-terminal domain might result in a new level of regulation by recruiting new proteins to MTF-1.1-controlled promoters.

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Investigation on biological action of Small-flowered Willowherb

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Small-flowered Willowherb (*Epilobium parviflorum* Schreb.) is traditionally used in the prevention and complementary treatment of benign prostatic hyperplasia (BPH). BPH is maintained by complex and still not entirely discovered pathological factors, but age-related hormone imbalance, decreased apoptosis, inflammation and excessive oxidative stress are definitely involved in the patomechamnism. Based on our analysis, willowherb is rich in various structured phenoloids. Its most characteristic compounds are myricetin-, quercetin- and kaempferol-glycosides, as well as the often macrocyclic derivatives of ellagic- and gallic acid (e.g.: oenothein B). Apolar extract of willowherb contains β-sitosterol.

Our workgroup aimed to investigate the mechanism of action of *Epilobium parviflorum* with special regard to its antioxidant activity and anti-inflammatory effect.

H-donor capacity of willowherb was measured by two different spectrophotometric methods (ABTS, DPPH). Inhibitory action on lipidperoxidation was examined with TBA assay, on bovine brain liposomes. Antioxidant cell-protective effect of *Epilobium* was studied on fibroblast cells. Anti-inflammatory effect was investigated on macrophage cells, by examination of COX-enzyme inhibitory action of extract.

Willowherb showed a remarkable H-donor activity (EC_{50}: ABTS 1.71 ± 0.05μg/ml; DPPH 3.01 ± 0.03 μg/ml), comparable to that of ascorbic acid and trolox. In the TBA assay willowherb extract showed concentration-dependent inhibition of lipid peroxidation at doses over 0.20mg/mL (IC_{50}=2.37 ± 0.12mg/mL). *Epilobium* extract exerted steady and concentration-dependent protective effect against oxidative damage generated on fibroblast cells. The protective action was comparable to that of catalase enzyme (250IU/ml). *Epilobium* extract showed concentration-dependent COX-enzyme inhibitory action (IC_{50}=1.4±0.1μg/ml).

Biological action of *Epilobium parviflorum* has been *in vitro* investigated. Based on our results, willowherb possessed high H-donor capacity and antioxidant cell-protective effect. The extract inhibited lipidperoxidation and the activity of COX-enzyme. These results suggest that extract of *Epilobium parviflorum* has antioxidant and anti-inflammatory properties which are likely to contribute to its beneficial effect in BPH. However, for wider, evidence-based application of willowherb further in vitro and in vivo studies are necessary.

Pigment photosensitized reactions make dark-grown pea epicotyls wilt in the light – direct detection of ROS promoting type-I and type-II photochemistry

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Upon illumination, epicotyls of dark grown pea (*Pisum sativum* L.) seedlings loose turgor in their middle section and wilt. Direct detection of singlet oxygen shows the involvement of type-II photoreactions, while co-localization of hydrogen peroxide and protochlorophyllide monomers suggests the contribution of type-I photodynamic pigment reactions as well. Hydroxyl radicals were detectable with spin trapping electron paramagnetic resonance spectroscopy and were also triggered by adding hydrogen peroxide in the dark, demonstrating Fenton chemistry.

In plants, native arrangements of pigment-protein complexes are critical during early plant development. In most angiosperms, various chlorophyllous pigments are safely stored in aggregates (macrodomains), such prolamellar bodies of leaf etioplasts, to prevent photo-oxidation. However, etioplasts in epicotyls or other stem related organs contain only few and small macrodomains and the chlorophyll precursor pigments, such as protochlorophyllide (Pchlide) are predominantly in monomer state in them.

Seven days old pea (*Pisum sativum* L.) epicotyls were germinated and grown in darkness. Singlet oxygen ($^1O_2$) production was visualized with DanePy (3-[*N*-(β-diethylaminoethyl)-*N*-dansyl] aminomethyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole) fluorescence. H_2O_2 was detected by DAB (3,3'-diaminobenzidine) staining or by transition electron microscopy (TEM) using $CeCl_3$. Hydroxyl radicals ($^{\bullet}OH$) were detected with spin trapping EPR spectroscopy using POBN or on the basis of HTPA (hydroxyl-terephthalate) fluorescence. Protochlorophyllide (Pchlide) localization was detected by fluorescence microscopy, its monomers/oligomers were identified by low temperature fluorescence emission spectra.

Illumination caused fast turgor loss and wilting in middle segments of the epicotyls accompanied with accumulation of water in the intercellular cavities. During this process, porphyrin-type pigments were gradually bleached, while $^1O_2$ and lipid peroxidation products were detected suggesting a type-II, porphyrin (Pchlide or Chlide) -photosensitized mechanism.

On the other hand, selective assays showed the presence of three different ROS: $O_2^{-\bullet}$, H_2O_2 and $^{\bullet}OH$ in the illuminated pea epicotyls, preferentially in the mid-sections. H_2O_2 was mainly produced along the radial walls of cells in areas also rich in monomer Pchlide. Although $^{\bullet}OH$ production, which was restricted to the mid-section was light-dependent, the $H_2O_2 \rightarrow {}^{\bullet}OH$ conversion also occurred without illumination, showing the presence of Fenton-catalysts in this region. These data demonstrate that products of a type-I photoreaction also contribute to disordered water status of the epicotyls and their wilting in light.

TIP47 protects of mitochondrial membrane integrity and inhibits oxidative stress-induced cell death

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The intracellular role of tail-interacting protein of 47kDa (TIP47/PP17b) has been controversial, no data are available for its possible role in tumor development, or in regulation of cell death. TIP47 is expressed in almost if not all tissues. During pregnancy, TIP47 serum levels increase; after birth they drop. Although some TIP47 is found on lipid droplets, it is known to be required for the delivery of mannose 6-phosphate receptors from late endosomes to the Golgi, both *in vitro* and in living cells. The protein binds the cytoplasmic domains of the cation-dependent and cation-independent receptors, and is recruited to late endosomes by binding to Rab9 GTPase. The loss of TIP47 destabilizes Rab9 which is also required for proper receptor transport.

The aim of this study was to find an intracellular role of this protein.

The vector containing TIP47, trunceted-TIP47 or the empty pcDNA3.1 vector was transfected into NIH3T3 cells. Cells were treated with H_2O_2 and cell viabilities were measured by MTT-viability assay. TIP47 was silenced by diced-siRNA in Hela cells. Mitochondrial membrane potential was monitored on isolated rat liver mitochondria in vitro by fluorescence of Rh123 or on TIP47 transfected and treated NIH3T3 cells in vivo. Depolarization of mitochondria can be visualized *in vivo* by using the membrane potential sensitive dye, JC-1 by fluorescent microscopy. Ratio of apoptosis and necrosis were evaluated after double staining with fluorescein isothiocyanate (FITC)-labeled annexin V and propidium iodide using flow cytometry and fluorescent microscopy.

TIP47 was over-expressed in a cell line normally not expressing it (NIH3T3), or suppressed by small interfering RNA in a cell line that normally express TIP47 (HeLa) at high extent before exposing cells to oxidative stress. Over-expression of TIP47 prevented hydrogen-peroxide induced cell death and the collapse of mitochondrial membrane potential. Suppression of TIP47 synthesis by small interfering RNA technique sensitized the HeLa cells to hydrogen peroxide induced cell death. We proved with both in vitro and in vivo experiments that TIP47 caused hyperpolarisation of mitochondrial membrane and reduced Ca^{++} induced mitochondrial membrane depolarization. In view of the fact that mitochondria may role in both apoptotic and necrotic cell death, we used flow cytometry to determine the percentage of apoptotic and necrotic cells. The results unambiguously justified protective effects of TIP47 protein.

We provided evidence that TIP47/PP17b can bind to mitochondria and can protect mitochondrial membrane integrity, as well as can prevent oxidative stress induced cell death providing the first evidence the possible oncogenic property of TIP47/PP17b.

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Oxidative stress in Parkinson disease

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Parkinson disease (PD) is a slowly progressive degenerative neuronal disorder comprising combinations of motor problems like bradykinesia, tremor-at-rest and muscle rigidity. Its major characteristic is the selective degeneration of the nigrostriatal pathway. PD is a multifactorial disease; both genetic and environmental factors could play important role in its pathogenesis. From environmental factors the mitochondrial complex I. inhibitor pesticide rotenone has remarkable significance. PD and other chronic neurodegenerative diseases which are characterized by a selective loss of distinct groups of neurons, have a common patomechanism, since oxidative stress and dysregulation of transmitter release play a central, but not initiative role in the development of the disease. One of the most effective therapy target is the monoamin oxidase, besides some compounds with antioxidant properties are seem to be neuroprotective in experimental PD model. The ideal drug decreases the level of pathological free radical production as a monoamin oxidase inhibitor, and via its antioxidant capacity, it also decreases the level of already existing reactive intermediers.

Oour goal was to identify compounds combining these two properties, thus having significantly higher neuroprotective effect than the currently used drugs.

We set an *in vitro* system to screen numerous multitarget drug candidates. PC12 cells were treated with rotenone, the protective effect of the various compounds on cell survival was determined.

From the reference drugs we found significant neuroprotection by deprenyl and rasagiline, and severe neurotoxicity by L-Dopa. Up to this point the majority of the tested compounds did not achieved the level of neuroprotection rendered by deprenyl or rasagiline. However some compounds attained significant neuroprotection in the rotenone model.

The screening of numerous compounds can be realized quickly and dependably with the *in vitro* Parkinson model. It is suitable for identifying drug candidates that have even greater neuroprotective effect, than the currently used drugs.

Eexamination of oxidative stress markers and liver function after open- and transgastric small bowel resection

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In Natural Orifice Transluminal Endoscopic Surgery (NOTES) a flexible endoscope is passed through a natural orifice (transgastric, transvaginal, transanal, transvesical) of the body and intra-abdominal procedures can be performed. Experimental data shows that this new procedure can reduce surgical trauma and inrtaabdominal adhesion formation is minimal. The technique without visible scar launched a new trend in surgery, which is no-scar surgery.

The aim of this study was to compare surgical trauma after open- and transgastric small bowel resection.

Within the framework of EURO-NOTES research program, with co-workers of Markus-Krankenhaus Surgical Clinic (Frankfurt am Main, Germany) transgastric (TG=7) and open small bowel (O=6) resection was performed on pigs. Oxidative stress marker concentrations (malondialdehyde (MDA), glutathione (GSH), SH-groups (SH-), superoxide-dismutase (SOD), liver enzyme (glutarate-oxalacetate-transzaminaze (GOT), glutamate-piruvate-transzaminaze (GPT), laktate-dehidrogenaze (LDH), gamma-GT (GGT), alkalic-phosphatase (ALP)) and total bilirubin (SeBi) concentrations were measured. Blood samples were taken before operation, at the end of operation, on first, third and on seventh postoperative day for biochemical tests.

There were no complications during surgery, all pigs survived. Oxidative stress marker concentrations were increased after operations in both group and decreased postoperatively. GOT, GPT, LDH, SeBi concentrations were increased after operation and decreased postoperatively in both groups. GGT and ALP concentrations were decreased during on monitoring days also in both groups. There was no significant difference between the two groups in concentration.

Transgastric approach means similar surgical stress like open technique. Further examinations are needed with a larger number of pigs and sensitive parameters.

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Comparative analysis of vitamin content of food supplements marketed in Hungary

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According to the Order of Hungarian Minister of Health No. 37/2004. (IV. 26.) food business operators must notify their food supplements at National Institute for Food and Nutrition Science before placing them to the Hungarian market. National authorization before marketing of food supplements ended at first of May 2004. Producers of food supplements have been allowed to use vitamins and minerals in chemical forms exclusively given in supplements I. and II. of the Decree mentioned above. Since 1st of May 2004 more than 4500 food supplements have been notified in Hungary. This number increases daily by four but in the first four months of 2009 nearly nine products per day were included into the list of notified products. More than half of the notified products contain vitamins, solely or in combination with other vitamins, minerals, plant extracts or isolated substances, as well. Most frequently used vitamins are C, E and Bs, including B1, B2, niacin and B6, at smaller frequency other vitamin Bs, vitamin A, D and K are the components of food supplements. Products contain vitamins at different levels but majority of them have vitamins not more than RDA (recommended dietary allowances). Controlling of the real composition of the products before marketing depends only on the decision of the food business operators; the market control authorities screen the products only at limited frequency.

In the frame of PHARE 2005/17/520.01.01 transition facility project National Institute for Food and Nutrition Science was provided with a Thermo Surveyor Plus HPLC-DAD/FLD equipment which could serve as the tool for the screening of the level of vitamins in food supplements sold in Hungary. Validated methods were set out for separating, qualifying and quantifying water and fat soluble vitamins with different chemical structure in food supplements. The most important element of the methods was the sample preparation step (direct extraction, saponification, or acid base solution) of products with different forms as hard and soft gel capsules, tablets, and so on, for obtaining the whole amount of active substances. The preparation step was followed by the separation of different vitamins and chemical forms on a reversed phase chromatography column and finally detection based on UV signal of the molecules. With the use of newly developed methods screening of vitamin content of about fifty food supplements found in Hungarian market was done. It can be stated that most part of the products contain vitamins at level indicated on the label, only in certain cases significantly higher or lower level (20%) of vitamins could be detected. There were some products where declared amount of vitamin indicated in the label did contain only the added vitamin and amount coming from natural source was not summed up. Methods developed are suitable for separation, qualifying, as well as quantifying vitamins with different chemical structure and monitoring the composition of food supplements marketing in Hungary.

Exogenous selenium influences the reactive oxygen radical production and restores intestinal perfusion in a porcine model of cardiac tamponade

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Selenium (Se) is essential for the function of redox regulator enzymes that have major roles in cardiovascular diseases with transient hypoxia, but the clinical value of Se replacement is still controversial.

The aim of our study was to assess the effects of Se treatment on reactive oxygen intermediates (ROI) production and splanchnic circulatory consequences in experimental cardiac tamponade (CT).

Anesthetized, thoracotomized minipigs (n=6) were subjected to acute CT by intrapericardial fluid infusion; the mean arterial pressure was kept at 40 mmHg for 60 min. After removal of the pericardial fluid, macrohemodynamic changes, small intestinal flow and pCO_2 gap (tonometric probe), blood ROI (superoxide and H_2O_2 production, chemiluminometry), plasma

nitrite/nitrate (NOx) level (Griess reaction) were monitored for 180 min. Another group of animals (n=6), received Se infusion (25 μg/kg/h iv) after CT induction.

CT was followed by hemodynamic signs of cardiogenic shock. During resuscitation, the significantly increased intestinal pCO_2 gap, elevated ROI production of the blood referred to prolonged mesenteric ischemia in spite of restored macrohemodynamics. In contrast, superoxide producing capacity of blood, NOx production in plasma, intestinal blood flow and pCO_2 gap were significantly improved by Se treatment.

CT-caused peripheral circulatory derangement could be effectively influenced by Se treatment due to reduced free radical production and improved intestinal microperfusion.

Study on total phenol content and antioxidant capacity (FRAP) of Ginkgo biloba L. leaves from different places

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Blood flow regulating and antioxidant effects of ginkgo (Ginkgo biloba L.) leaves are well known. Products containing standardized ginkgo leaf extracts are among the most popular medicinal goods in Hungary. Also more and more ginkgo teas (crude leaf drugs) are available in the trade flow as monoteas or in mixtures.

Total phenol content and antioxidant capacity (by the FRAP method) were determined from extracts of ginkgo leaves collected in different places.

Places of collection (all in Hungary): Budapest city (Botanical Garden of Eötvös Lóránd University called “Füvészkert”, Botanical Garden of Corvinus University of Budapest - BCU, Margit boulevard in the city centre), Gödöllő city (park of Szent István University), Paks city (city centre) and Székesfehérvár city (city centre). In Füvészkert we collected leaves both from male and female trees . Leaves were dried at 30ºC and than pulverized. Based on prescribes of the Hungarian Pharmacopoeia aqueous and aqueous ethanolic (water/ethanol 80/20, v/v) extracts were made from the prepared leaves. Total phenol content was measured spectrophotometrically (λ = 760 nm) with the use of Folin-Ciocalteu reagent. Antioxidant capacity was determined also spectrophotometrically by the FRAP method.

In case of all samples total phenol content of aqueous extracts was higher than that of aqueous ethanolic extracts. For aqueous ethanolic extracts more pronounced differences were obtained among the samples than for aqueous extracts. In case of aqueous extracts the highest total phenol content (0,132 mg/ml) was detected in the sample from the tree of BCU, while the trees of Füvészkert hed the lowest value (0,079-0,081 mg/ml). In aqueous ethanolic extracts the total phenol content was the highest (0,089 mg/ml) in ginkgo leaves collected in Gödöllő city, statistically signinicantly higher than in the other samples. In case of ethanolic extraction big differences between the sexes could be detected for the phenol content. The lowest value (0,016 mg/ml) was detected from the sample of the female ginkgo tree of Füvészkert.

Total antioxidant capacity determined by the FRAP method was higher in aqueous extracts than in aqueous ethanolic extracts. In case of ethanolic extraction samples of old trees of Füvészkert showed an unexpectedly low antioxidant capacity (0,21-0,28 mmol ascorbic acid/l), significantly lower than the other samples (0,63-1,22 mmol AA/l). In aqueous ethanolic extracts the highest antioxidant capacity (0,81 and 0,73 mmol AA/l) were found for samples of Gödöllő city and of BCU, while the lowest (0,31 mmol AA/l) for the sample of Székesfehérvár city. Antioxidant capacity values of the other samples were about the same. Antioxidant capacity of leaf extracts of male and female trees did not differ from each other.

Both of total phenol content and total antioxidant capacity were higher in aqueous extracts than in aqueous ethanolic extracts. Among the samples collected from different places significant differences were obtained for both of the investigated parameters. Differences in antioxidant capacity did not show connection with the pollution grade of sampling places, these could be caused by age of the ginkgo trees. However the significant differences may worth consideration, as it could have an important role in the therapeautic use of ginkgo leaves. It is also worth to mention, that the antioxidant capacity of ginkgo leaf extracts was much lower than that of products containing standardized extracts, so antioxidant effect of ginkgo teas is lower than that of the standardized extracts.

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Study the structure-activity relationship of silybin analogues using different ROS production sources

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Reactive oxygen species (ROS) formation is indispensable for life. They play role in optimal working of immune system (killing mechanism), in different signal transduction processes, induction of apoptosis or activation of genes. Elimination of ROS requires presence of enzymatic and non-enzymatic antioxidants, in which natural origin antioxidants from the diet are involved. Overproduction of ROS initiates development of diseases; therefore, antioxidant supplementation was suggested for prevention or treatment of those states. However, results of these studies were contradictory, such as the role of Vitamin E in prevention of cardiovascular diseases which was mainly due to the fact that, in most cases, antioxidant requirement e.g. original Vitamin E level, and source(s) of ROS was not determined or took into account.

To certify the importance of ROS source in antioxidant activity of a given molecule, several silybin (1) analogues were synthesized namely, flavanon- (2, 3), flavone-derivatives (4, 5), flavanolignan skeleton (6), dehydrosilybin (7), and hydnocarpin (8), and their effects were compared in inhibition of superoxide anion production in phorbol-ester stimulated human neutrophils, xanthine oxidase activity, H-donor activity, LDL oxidative resistance, and ferric ion reducing capability.

It was found that only silybin and dehydrosilybin possessed measurable H-donor activity. Ferric ion reduction was observed in case of silybin (0.68 teq), dehydrosilybin (0.45 teq) and hydnocarpin (0.28 teq). Xanthine oxidase activity was inhibited by silybin and its flavanon analogues (2, 3) in similar extent (IC50~32 uM), and flavone analogues of silybin (4, 5), dehydrosilybin and hydnocarpin were more effective (IC50~0.2 uM), and silybin skeleton (6) was absolutely ineffective. Oxidative resistance of LDL increased by 3.2 fold by silybin, 2.7 fold by its flavone analogues, while the other compounds had weaker effects (1.2 fold), and silybin skeleton was ineffective. However, phorbol-ester stimulated superoxide anion production was inhibited most effectively by silybin skeleton (58%), followed by hydnocarpin (52%), dehyrosilybin (50%), and flavone analogues of silybin (40%), respectively. Silybin itself and its flavanon analogues were the less effective (15-22%). The inhibition in superoxide anion production was due to inhibition of PKC-alpha activation in neutrophils. On this basis we can conclude that 1. Inhibition of xanthine oxidase activity requires OH groups, and presence of a double bond in C ring enhances the effect; 2. Ferric ion reduction required the intact flavanolignan structure, and double bond in ring C attenuated the effect; 3. Flavanolignan structure independently the presence of OH groups, and double bond had low H-donor activity; 4. Oxidative resistance of LDL was modified by silybin and double bond in ring C attenuated its effect; 5. However, effective inhibition of PKC in human neutrophils was achieved in case of silybin skeleton, e.g. in the absence of OH groups, 2-methoxy-4 hydroxy-phenyl, and 3-hydroxymethyl groups, in the presence of one or more side chain(s) attenuated the effect of silybin skeleton.

In summary, these results clearly show that relationship between structure and antioxidant capacity of a given antioxidant is depend strongly on type(s) of ROS source. Therefore, we suggest the identification of ROS source before initiation of antioxidant supplementation therapy.

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Chromatographic analytical opportunities on a thin film of mobilizable methil-groups of different biological objects under the influence of exogenic treatment

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In case of hyperlipidemic disease caused by alcohol, due to the S-Adenosyl-Methionin (SAM) deficiency, several vital metabolic roads, like protein synthesis, the synthesis of chatecholamins, and the nucleic acids, the methilation of phosphatidil-ethanolamin and phosphatidil-choline, and the activity of the synthesis of glutation, by which lipid peroxidation is hampered, decrease.

It has been proven that SAM is the methyl-donor in the trance-methyl reactions, and that the enzymatic methylation / demethylation processes equally generate HCHO.

Beside the free radicals and H_2O_2, the HCHO plays an important role in living organisms. To better understand the trance-methyl processes, research is being conducted into a compound group of various vegetal origins influencing the natural defensive system of the plants, some members of which have been proven to possibly play a role in human prevention as well.

Published results of different approaches have proved that among several vegetal bioactive molecules the betain (beetroot is an important source of it), and the resveratrol (red wine is one of its important sources) have characteristics blocking free radicals, and an antibiotic effect in charcinogenesis, reducing oxidative stress. The goal of our measurements, based on short term experiments with rats, was the detection of the changes resulting from the exogenic enlargement of mobilizable methyl-groups.

Our measurements were aimed at inherences that could enhance our understanding of the changes due to the quantitative enlargement of the methyl-pool.

During the experiments, male Wistar rats (5 animals per group) were treated for ten days. The control-group was fed with rat-nutriment only. The normal- nutriment -fed groups, treated with red wine and alcohol, received a daily amount of 8 ml per kg of body weight of a10,5 % alcoholic solution. The fat-rich nutriment -fed groups received cholesterol (2%), sunflower-seeds oil (20%) and cholic-acid (0,5%) mixed into the nutriment. The groups consuming beetroot too, received 2 gramm/kg of body weight lyophilized beetroot-powder mixed into the usual nutriment or into the fat-rich nutriment.

At the end of the treatment, we measured, besides the routine- laboratory parameters and the redox- parameters, the methylation- rate in the samples of blood and homogenized liver. We defined the bound endogenous HCHO with dimedone as adduct- forming compound as formaldehide.

We used pre-experiments to adapt the method earlier used for the examination of phytogenous tissues to the planned experiments. After that, we optimized the appropriate model- preparation and the enactable quantitative proportions to the reproducible detection. Finally, we carried out the measurements using the method of thin-layer chromatography, which enables, with the application of the appropriate standard, the simultaneous qualitative and quantitative analysis or comparison of 10 or 12 sample isochrones on a thin film-slab. Further advantages of the method are relatively simple model-preparation and quick and efficient separation.

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Comparative study of oxidative stress parameters in critically ill patients

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Free radical reactions play an important role in the pathohysiological changes in critically ill patients, but there are only few data available regarding to the dynamism of oxidative stress during treatment of critically ill patients. The purpose of this study was to follow and to compare the time course of oxidative stress during treatment of ICU patients.

Patients with burn injury (n=26), sepsis (n=14), polytrauma (PT) (n=13), and acute lung injury (ALI) (n=22) were involved in the study. Blood samples were taken from patient on admission, and on the following 3-5 days. Concentration of malon-dialdehyde (MDA), reduced gluthation (GSH), protein sulfhydril (PSH) groups, the activities of superoxide dismutase (SOD), catalase (CAT) and myeloperoxidase (MPO) enzymes were measured spectrophotometrically. Production of reactive oxygen species (ROS) in whole blood was measured by luminol dependent chemiluminescence following phorbol-myristate-acetate stimulation. Blood samples from healthy volunteers (n=9) served as the control.

While the white blood cell count significantly decreased in burned patients during the treatment, it remained on high level in the other groups. Marked granulocytosis and lymphocytopenia was observed initially in all groups that started to normalize only in burned patients from the day 4. ROS production was significantly elevated in septic and ALI patients from admission, but in burned and PT patients it rose significantly from day 3. Plasma MDA level significantly exceeded the control values, peaking on the days 2 and 3 in all groups. Plasma MPO level was significantly elevated in burned, septic and ALI patients from admission, but in PT patients it rose significantly from day 4. PSH level was significantly reduced in septic patients from admission, and in burned and PT patients from the day 2 and 3. GSH level significantly decreased in burned, PT and ALI patients from the day 2 and 3, while in septic patients it stagnated on a low level during the observation period. SOD enzyme activity was below the level of healthy population in most of the patients group, while catalase enzyme activity significantly exceeded it in all groups.

Significantly elevated levels of pro-oxidant markers with parallel decrease in endogenous antioxidants confirmed the presence of marked oxidative stress in critically ill patients. Time course of changes in oxidative stress parameters diverged markedly in critically ill patients mirroring the pathohysiological changes in different diseases. The significant differences in some oxidative stress parameters in survivor and non-survivor patients may have prognostic value.

The work was supported by the OTKA K060227 grant.

Fatty acid composition of human milk in Hungary with special attention to trans fatty acids

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Survey of fatty acid composition of 66 human milks obtained from 19 counties and Budapest, Hungary was performed in a research project supported by the Scientific Council for Health, Hungary. The selection of pregnant women was met all the requirements of WHO/GEMS Food representative sampling protocol, which was performed by the National Institute for Food and Nutrition Science (NIFNS) with the cooperation of health visitors on the basis of several hundred questionnaires. Human milk samples were collected from mothers below 30 years having first partum and had been living for 5 years in the same place. Country representative samples were collected within 2-8 weeks following the delivery with the help of health visitors and were transported to laboratory of NIFNS. The analysis of fatty acid composition, included trans-fatty acids was performed by gas chromatography.

In Hungary, this kind of monitoring was the first one. The total fat content of the samples was in the range of 0.3-6.2 g/100 g. 10 samples were close to the average fat content (4 g/100g) published in the Hungarian food composition table. Fifty-four samples had lower while two samples had higher fat content. The range of saturated fatty acids (SFA) (C8:0, C10:0, C12:0,

C14:0, C16:0, C17:0, C18:0) in total fatty acids was between 37.5-78.3%. In eleven counties six samples had 2-16% more saturated fatty acids than the average 44% published in Hungarian food composition table.

The percentage of the monounsaturated fatty acids (MUFA) as C14:1, C16:1, and C18:1 was in the range of 13.2 and 43.5. The ratio of polyunsaturated fatty acids (PUFA) including C18:2n-6, C18:3n-3, C18:3n-6, C20:3n-6, and C20:4n-6 was between 6.9 and 25.5%. The level of essential linoleic acid showed a very wide range as 6.9 and 23.7% in total fatty acids while the ratio of linolenic acid was in the range of 0-1.3%.

Among the trans fatty acids (TFA) elaidic acid (C18:1n-9t) originated from the hydrogenated vegetable oils and the linoleic-acid isomers as C18:2t9,t12, C18:2c9,t12, C18:2t9,c12 of ruminant origin could be identified. The ratio of elaidic acid in total fatty acids was 0.07-5.04% that means 0-150 mg elaidic acid in 100 g milk fat calculated on the base on fat content. In one sample 174 mg elaidic acid in 100 g milk was measured. The ratio of C18:2 isomers in total fatty acids was below 0.7%.

During the lactation, the fat composition of the human milk is highly influenced by the fatty acid composition of the diet. Data of this survey shows that the much higher level of SFA, the lower values of MUFA and PUFA, as well as the essential fatty acids in the human milk are due to unhealthy diet. The appearance of TFA in human milk is due to the consumption of foods containing hydrogenated vegetable oils one day before sample collecting. According to a national survey done by NIFNS TFAs present in many industrially produced foodstuffs in Hungary, as well. The TFAs have adverse physiological effects on the development of new-borns; these fatty acids can cause irreversible metabolic changes. The TFAs are able to inhibit the formation of long chain PUFAs as arachidonic and docosahexanoic acids which are inevitable during the brain development of the new-borns, as well as in the metabolic pathway of prostaglandins and thromboxans, the main responsible factors in balancing the blood viscosity and the formation of thrombus.

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What do we know today about lycopene?

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Lycopene is an acyclic carotenoid molecule that does not take part in the synthesis of beta-carotene because of the lack of beta rings. Lycopene is a very powerful antioxidant *in vitro* and *in vivo*, as well. Lycopene shows a marked preventive effect against certain cancer and cardiovascular diseases partly thanking to its antioxidant characteristics. Main dietary sources of lycopene are tomato and foods prepared with tomato, watermelon, red grapefruit and some other exotic fruits. Scientific observations have proved that agricultural practices and food industrial processes significantly determine the lycopene content of fresh or prepared foods. In the frame of a 10 years' cooperation with Szent István University Department of Horticultural Technology the National Institute for Food and Nutrition Science (NIFNS) have measured the lycopene content of at least two dozen tomato varieties, and investigated the effects of horticultural techniques and weather conditions on lycopene level of tomato fruits. It was also studied how food industrial processes and dish preparing techniques determine the lycopene level of food products, finally a functional food with increased lycopene level was prepared with the use of by-products of tomato industry. Dietary lycopene intake was estimated in two small groups of Hungarian population and based on the representative nutrition survey done by NIFNS in 2003-2004, a population based intake was also calculated.

It was proved that lycopene accumulates in the tomato fruit during ripening, the correlation between the colour index and lycopene content can be drawn by a second order equation. Since the optimal temperature for lycopene synthesis is between 16-21°C, significantly lower level of lycopene by 25-30% could be detected in fruits directly exposed to sunlight having higher surface temperature than in that being in the shadow of leaves and having lower surface temperature. Significantly different lycopene levels were observed in different tomato varieties, the highest level (9,55-13,4 mg/100 g) was observed in industrial cultivars, middle values were in fruits of eating varieties harvested in green house (7,0-8,3 mg/100 g), while the lowest levels (4,90-8,02 mg/100 g) could be detected in tomato cultivars for fresh consumption harvested in open air. It was established that several factors including harvesting date or more punctually the weather conditions 5-10 days before the harvesting, the water-stress, the increased CO_2 level, and the grafting significantly modify the lycopene level of berries. Based on the consumption data the lycopene intake was estimated in a children's (n=502) and an adult's (n=205) group as 2.98±4.71 mg/capita/day, and 4.24±8.47 mg/capita/day, respectively. Data showed very big differences among subjects. Using the data

of the representative nutritional survey the population intake was around 2 mg/capita/day, but the lowest and highest levels showed very wide range of intake (0-40 mg/capita/day).

Lycopene has an excellent antioxidant capacity, its preventive and health-promoting properties are well-known and widely proved epidemiologically and experimentally, as well. Climate conditions in Hungary make possible to produce very valuable tomato fruits either economically or nutritionally. Increased consumption of fresh tomato and tomato-based foodstuffs can play an important role in the risk reduction of non-communicable diseases which are in connection of diet and especially increased free radical reactions.

Our preliminary data during the investigation of injuries following suprarenal aortic clamping in rats

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During various surgical interventions (for example: organ transplantations, vascular surgery, tumor removal) it is often necessary to clamp aorta and/or greater arteries for shorter or longer period of time, which disturbs the blood supply of organs/regions and during the following reperfusion as postoperative complications, further injuries can occur. As its most serious complication, systemic inflammatory response syndrome or as part of the syndrome, multiple organ failure can evolve. In our experiments, we aimed to investigate the development of the triggering factors of this condition, and to set up a suitable, appropriate experimental model for also studying the protection of this condition.

Adapting C. J. Shields et al. (2003) aorta occlusion model with small modification, a 30-minute ischemia followed by a 120-minute reperfusion was examined in male Wistar rats. We used 60mg/kgbw of thiopental for anesthesia. After cannulation of femoral artery, blood samples (0.5 ml per each) were taken before ischemia, prior to clip removal, and at the 1^{st}-20^{th}-60^{th}-120^{th} minutes of the reperfusion. The blood-gas analysis and the hematological parameters were immediately measured, and to determine the liver enzymes' levels we stored plasma samples on -70 C° degree until usage. The controls were sham-operated animals.

According to the blood-gas analysis, the pH levels remained within physiological range in both groups (pH = 7,35 – 7,45), the arterial pCO_2 and pO_2 values presented small changes during the experiments. Within hematological parameters the amount of white blood cells significantly increased in the I/R groups compared to controls and the extent of ascent was 50% at the end of the reperfusion. Some important parameters of red blood cells showed slight changes. The liver enzymes, especially the GOT levels increased with 67% towards the end of the reperfusion and the GOT/GPT proportion raised in the I/R groups as well.

In our in situ rat model, according to the examined parameters after I/R systemic inflammation, microcirculatory problems of some organs and results showing hypoperfusion damages were obtained. To determine the multiple organ failure and also for further standardization, we are continuing our researches.

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Antioxidant property of *Grindelia robusta* infusum in the function of steeping time

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Gumplant (*Grindelia robusta* Nutt.) is a perennial plant growing wild in California, cultivated in Europe. *Grindelia robusta* has been found to be especially efficient in spasmodic asthma, giving prompt relief, and cures effectively in cases previously rebellious to medication. Its expectorant effect is also remarkable. Since in the indication fields in which the gumplant used the role of free radicals is proven, the aim of the work was to study the antioxidant properties of plant.

Gumplant was collected from Transylvania, from Botanical Garden of University of Medicine in 2007, Tirgu Mures, Romania. Aquous extracts were made from different parts of the plant (flower, stem and herba) by infusing for different time (5, 10, 30, 60, 120 min). Total scavenger capacity in extracts was determined by a chemiluminescence method. Hydrogen donor ability and reducing power were measured by spectrometric methods.

Hydrogen donor ability and reducing power vary considerably in the function of the steeping time and the concentration applied. The best results were obtained for concentrated extracts in all cases. Hydrogen donor ability and reducing power of teas generally increased with the increasing steeping time. Total scavenger capacity of flower extract also changed similarly, while significant total scavenger capacity of stem and herba was measured in extracts obtained by 5 min steeping time. In summarizing, the highest antioxidant values were obtained after 120 min steeping time in the case of flower extracts, while the optimal(best) steeping time in case of stem and herba extracts vary in large scale of time depending on the kind of antioxidant measurement.

The antioxidant properties of Grindelia robusta extracts depend on several factors, as plant parts, extraction procedure and concentration. In general 30-120 min steeping time proved to gave the highest antioxidant values except for that 5 min steeping time is enough for relevant total scavenger capacity of steam and herba extracts.

Prooxidant effect of trichothecene mycotoxins in poultry

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Fusarium moulds present is most of the temperate climate areas of the world and those are produce trichothecene mycotoxins, such as T-2 toxin, HT-2 toxin, scirpentriol, nivalenol, diacetoxyscirpenol and deoxynivalenol. There are numerous data that trichothecene myco-toxins affect the antioxidant status of animals, primarily due to their pro-oxidant effect. However, not clear whether the pro-oxidant characteristic of these mycotoxins is a direct or indirect effect. Chemical structure of trichothecenes, presence of epoxy group in the trichothe-cene ring, supports the direct effect through their metabolism by the xenobiotic transforming enzyme system.

The objective of our series of studies was to evaluate dose- and time-related effects of the most important trichothecene mycotoxin, T-2 toxin, on glutathione redox and lipid peroxide status of chickens. The birds were fed with diets experimentally contaminated with different doses of T-2 toxin (0.12, 0.4, 1.5, 2.05 or 2.35 mg kg^{-1}) without or with antioxidant supplementation (vitamin E: 10.5 mg + selenium 0.045 mg $animal^{-1}$ day^{-1}) in short-term (14 days) or long-term (39 days) studies. In each experiment five animals were exterminated from each group at days 3, 7 and 14 in short-term and at days 21 and 39 in long-term trials. Blood and liver samples were taken, in which reduced glutathione (GSH), malondialdehyde (MDA) concentration and glutathione-peroxidase (GSHPx) activity were measured.

The results showed that there were not dose-related changes in the parameters investigated, however long-term effect of T-2 toxin was found, mainly in liver. According to the changes of the different parameters in different tissues it can be stated that liver showed the most marked changes which followed by blood plasma and red blood haemolysates. Antioxidant

supplementation of the experimentally T-2 contaminated diet resulted improvement of the antioxidant and moderately in lipid peroxide status.

The possible causes of the lack of dose-relation would be the environmental factors, e.g. temperature and light regimen, also partially different genetic background of the experimental animals, even all of them was the same hybrid. The other possible cause would be the presence or lack of natural metabolites of T-2 toxin or some other not-identified trichothecene mycotoxins, because the experimental contamination was carried out using crude extract from the mainly T-2 toxin producing moulds, *Fusarium sporotrichioides* or *Fusarium tricintum.*

In conclusion it can be stated that T-2 toxin exposure has some pro-oxidant effect also activated or impaired the amount/activity of the glutathione redox system but its effect depends on the duration of the study also some other factors. Additionally, the question, that T-2 toxin has direct or indirect pro-oxidant effect remains open.

Effects of postconditioning on kidney ischemia/reperfusion injury in hypercholesterolemic rats

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Ischemia/reperfusion injury frequently threats the integrity of the organs during surgery. The protective effect of postconditioning (PK), the short repetitive ischemia/reperfusion cycles, applied in the beginning of reperfusion, has been improved the outcome in vital organs. Signaling cascades are induced by PK interfere in several points to preconditioning, which is blocked by metabolic diseases, such as insulin resistance and type 2 diabetes.

The aim of our study was to compare the efficacy of PK after reperfusion injury of both kidneys in metabolically healthy and hypercholesterolemic rats.

Male Wistar rats (N=30) were divided into two groups. Control group of the animals were fed by normal rat chow, the treated group (n=18) was fed with 1.5% cholesterol containing diet for 8 weeks. Both groups of rats were divided to further two subgroups, and were anaesthetized by ketamin: diazepam. One subgroup of rats was subjected to 45 min ischemia and 2 hours reperfusion, in the other subgroups 4x5 min ischemia/reperfusion cycles were applied in the early phase of reperfusion. After 2 hours of reperfusion blood and tissue (kidney, heart, liver, lung) samples were taken. Serum cholesterol, glucose and triglyceride levels were determined by photometric methods. Kidney function was characterized by serum urea, and creatinin levels. Inflammation and oxidative stress were characterized by the measurement of TNF-α and oxLDL concentrations (ELISA) and PMA induced free radical production capacity of whole blood by chemiluminometric method. Tissue injury in kidney was determined by formaline-fixed, paraffin embedded tissue sections (5 μm), stained with PAS and HE. TNF-α levels were also determined by immunohystochemistry.

Serum cholesterol and triglyceride levels were significantly higher in cholesterol fed rats than in control ones. Serum urea and creatinine levels were same in control and hypercholesterolemic groups. A significant elevation was observed in TNF-α level ($p<0.01$), PMA-induced free radical production ($p<0.05$), and in lipid peroxydation (oxLDL; $p<0.05$) after I/R injury in healthy rats, which reduced almost to the normal levels in PK ones. In hypercholesterolemic rats neither the elevation, nor the postconditioning induced reduction were not as significant as in the healthy rats. Surgical intervention caused a great elevation in serum glucose and insulin levels ($p<0.01$). PK caused a further elevation in insulin levels, while the TNF-α concentration and free radical levels were reduced. Tissue TNF-α level, measured in hypoxia sensitive papilla, was significantly higher in cholesterol fed animals, than in control rats, and this high level was not able to change in response to PK. In healthy animals PK caused a significant reduction in tissue TNF-α level, as well.

PK proved to be a very effective defense against I/R in healthy animals, but it was ineffective in hypercholesterolemic ones.

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Serum selenium concentrations of gestational diabetic and control pregnant women in the second trimester of pregnancy

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High serum selenium concentrations are positively associated with the prevalence of type 2 diabetes according to recently published data, whereas in an intervention study, patients who received 200 µg selenium per day as oral supplementation for seven years, had a significantly higher risk of developing type 2 diabetes mellitus compared to the control group. In a previous study, serum selenium levels in gestational diabetic pregnant women were slightly, but significantly higher than in control pregnant women. This finding needed to be confirmed by a larger number of study participants.

To determine serum selenium concentrations and plasma total glutathione peroxidase activity of 31 gestational diabetic and 20 control pregnant women between the 24th and 28th week of pregnancy.

Serum selenium concentrations were measured by hydride generation atomic absorption spectrometry. Plasma glutathione peroxidase activity was determined by an end-point direct assay in the presence of reduced glutathione and cumene-hydroperoxide as co-substrates. Statistical analysis was performed using the Microsoft Excel 7.0 and Statistica™ 4.0 software packages.

Serum selenium concentrations were significantly higher in gestational diabetic (50.4±14.4µg/l) compared to control pregnant women (41.1±7.7µg/l, p=0.004). Plasma total glutathione peroxidase activity did not differ between the two groups of pregnant women (3.30±0.95 E/g protein in case of gestational diabetic and 2.84±0.60 E/g protein in case of control pregnant women). Serum selenium concentrations correlated significantly with plasma glutathione peroxidase activity in control pregnant women. In gestational diabetic study participants serum selenium concentrations correlated inversely with fasting plasma glucose values (p=-0.80).

This study confirmed our previous finding of significantly higher serum selenium concentrations in gestational diabetic compared to control pregnant women. The reasons for this observation are unclear; however, the correlation value shows that serum selenium levels seem to either influence or be influenced by fasting plasma glucose concentrations. Despite higher serum selenium levels in gestational diabetics, selenium-dependent glutathione peroxidase activities are similar in both groups of pregnant women.

Examination of microvascular reactivity in juvenile essential hypertension and haemodialysis

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The relationship of juvenile essential hypertension and impaired microvascular function has not yet been demonstrated. In contrast with hypertension in adulthood, a simultaneous assessment of the markers of oxidative stress and the microvascular reactivity has yet not been performed in adolescent patients with essential hypertension.

To compare the microvascular reactivity and markers of oxidative stress in overweight and lean hypertensive adolescents (OHT, LHT), and young haemodialyzed (HD) patients as positive controls.

Twenty-three OHT adolescents, 10 LHT adolescents, 12 young HD patients and 19 controls were enrolled. Microvascular reactivity of the forearm was assessed by means of laser Doppler flowmetry, measuring alterations of the blood perfusion of the microvasculature. Endothelium-dependent and -independent vasodilation, informative of the endothelium and smooth muscle layers of the vessels, were assessed by means of acetylcholine and sodium nitroprusside iontophoresis, respectively.

Maximal vasodilation was achieved by local heating of the skin to 44°C. The obtained perfusion values were expressed as relative to the basal values. We also determined the ratio of the whole blood oxidized/reduced glutathione (GSSG/GSH), the erythrocyte malondialdehyde levels and the activities of erythrocyte antioxidant enzymes.

Microvascular reactivities in both tests were moderately decreased in the two hypertensive groups, and significantly impaired in the HD group, as compared with healthy controls. Ratios GSSG/GSH were increased in all patient groups, being highest in the HD patients. The erythrocyte malondialdehyde levels and the activities of superoxide dismutase and glutathione peroxidase were significantly elevated in the HD group.

Our results suggest that the impairment of the microvascular reactivity does not precede the development of juvenile essential hypertension: an imparied microvascular reactivity is more likely a consequence and not a cause, being related to the degree of oxidative stress.

Improvement of beer's flavour stability by adding antioxidant vitamins

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Thanks to the growing tendency of the consumer's demands, it is important to find possibilities to improve the shelf life of different foods. The favour stability of a food is an important part of its quality. The flavour changes in beer could be caused by the formation of radicals resulting from the ingress of oxygen.

Vitamin E and C are widely regarded as important dietary antioxidants. These vitamins were added to beer samples at different technological stages using a concentration range between 0 and 40 mg/L for Vitamin C and from 0 to 4 mg/L for Vitamin E. The aim of this study was to determine the flavour stability of these vitamin enriched samples.

The flavour stability of beers can be determined by Electron Spin Resonance (ESR). One of the examined parameter is the lag time. This parameter was determined at packaged sample technological stage in each case. It is in direct connection with the quantity of natural antioxidants found in beer. The formation of hydroxyl radicals will start only after this period of time. The ESR method can be used to analyse the effect of antioxidant vitamins such as vitamin E and C on the flavour stability of beer.

Comparing the different technological stages for vitamin addition, it can be established that the best lag time values were measured when the vitamins were added to the wort after cooling. If vitamin E concentration was higher than 4 mg/L at original pH or vitamin C concentration was higher than 30 mg/L at lower pH, the lag time was higher than 100 minutes. Vitamin addition at the end of fermentation increases the lag time in some cases, but adding vitamin is not recommended in the case of packaged beer.

In the case of original pH ascorbic acid always had a smaller effect on the value of lag time than vitamin E. If these vitamins were added together, their effects were combined so the presence of ascorbic acid reduces the effect of vitamin E.

Conclusions: On the grounds of these facts it can be stated that if the growth of the lag time is the target, individual vitamin addition is the right way to do it. The best results were received when only vitamin E was added to the wort at original pH, or when vitamin C was added to the wort samples separately at a lower pH value.

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Detection of oxidative stress from bronchoalveolar lavage fluid in animal model

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Oxidative stress results from an oxidant/antioxidant imbalance in favour of oxidants. A large number of studies have demonstrated that increased oxidative stress occurs in airways diseases, but there is now substantial evidence that it really plays an important role in the injurious and inflammatory responses in acut lung injury and in asthma.We developed a model that allows the quantification of the airway responsiveness (BHR) and the characterizations of the BAL cellular profile repeatedly within the same rat. The detection of carbonyl proteins (CP) is a wide spread method to detect oxidative stress, where the values are increased. In the present study with the help of our method we examined, whether oxidative stress measured by CP plays a role in the patomechanism of different lung abnormalities, so acut lung injury and asthma in animal model.

We studied 28 male white, Wistar rats (weight range 350–500 g). The animals were housed in a healthy colony and allowed food and water ad libitum. Anesthesia was induced, intubations were performed, muscle relaxation was achieved and mechanical ventilation was used, BALF was collected. The BAL fluid was then centrifuged and CP was detected by spectrophotometry method .Acute lung injury was caused by intraperitoneal (ip) injection of E Coli lipopolisacharide (LPS) and asthma was induced by the combination of ip. and inhalative ovalbumin (OVA).

The acute lung injury caused by LPS was due together with significant oxidative stress which was reflected by the increase of the CP values in BALF. OVA sensitization was connected with a less significant oxidative stress right after the procedure, but the CP values increased further until the next measurements, one month later (12,9 vs 13,9 vs 16,4).

The results confirm the role of oxidative stress in the patomechanism of acute lung injury and asthma in rats. Our method is suitable to detect the oxidative stress in animal model and will be also suitable later to investigate the protective effects of different antioxidants substances.

Examination of biochemical parameters of oxidative stress in childhood asthma

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Increased levels of reactive oxygen intermediates (ROI) and cellular injury have been implicated in many pulmonary diseases including COPD, cystic fibrosis and asthma bronchiale. According to earlier examinations, the production of ROI was elevated in the asthmatic airways due to the action of macrophages, eosinophils and neutrophils, which led to bronchial hyperresponsiveness and caused more severe airway inflammation. ROI also play important roles in the modification of immune response and in the structural alterations of the lung parenchyma.

The aim of our study was to analyse the alterations of several biochemical parameters such as malondialdehyde (MDA), carbonylated proteins, ratio of oxidized/reduced glutathione, vitamin E concentration, total antioxidant capacity and activities of antioxidant enzymes (catalase, glutathione reductase, glutathione peroxidase, superoxide dismutase). The blood samples were obtained from asthmatic children (n=21) and healthy controls (n=12). The clinical state of the patients was compared to the examined biochemical markers.

According to our results, elevated oxidative stress was observed in asthmatic patients even with stable clinical condition. Elevated MDA (mean. 0,86 vs. 0,58 nM/mg prot.) and carbonylated proteins (mean. $4{,}711 \times 10^{-2}$ vs. $3{,}859 \times 10^{-2}$ µg/mg prot.) levels were detected from the blood samples of the patients versus controls.

Our results also proved the presence and importance of oxidative stress in asthma and the possible use of biochemical parameters in the clinical practice. Additionally we set out to examine the expression of the gp91phox subunit of NADPH oxidase enzyme and HO-1 enzyme from blood.

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Genotypic, seasonal and maturity stage variability in antioxidant capacity of stone fruits

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In Hungary, nutrition-related diseases (heart and vascular attacks, different types of cancer) are among the main causes of mortality. Several epidemiological studies suggested that consumption of fruits and vegetables can help in the prevention of these degenerative diseases. However, the statistics show that Hungarian people do not eat enough fruits and vegetables. One of the possible solutions would be the consumption of fruits with enhanced functional properties and higher levels of the required bioactive compounds. Although berry fruits are generally considered to contain outstanding levels of antioxidants, stone fruits are less known from this aspect. The aim of our examinations was to characterize the antioxidant capacity of stone fruits and to clarify the influencing effect of the genotype, ripening status and cultivation plot. In addition, we wanted to assess how the anthocyanin and vitamin C contents contribute to the antioxidant capacity of sour cherries.

In the present study, the antioxidant capacity was measured with ferric reducing ability of plasma (FRAP) and a photochemiluminescence method (ACW) in 11 sour cherry, 19 sweet cherry, 20 Japanese plum, 6 cherry plum and 6 apricot cultivars. In addition, the content of total phenolics (TPC), carbohydrate, vitamin C, monomeric anthocyanins and nutrient elements were also determined.

Cultivar averaged mean values of FRAP and TPC results were the highest in sour cherry, and the lowest in cherry plum. Variations between species were the highest in case of sour cherry and sweet cherry. Most of the sour cherries reached the FRAP values of raspberries, which characteristically contain high antioxidant capacity (5-6 mmol AS/L). A sour cherry cultivar reached the outstanding water-soluble antioxidant capacity value of blackberries and elderberries. This attracts attention to the alluring perspectives of this genotype. Correlations between the FRAP and ACW values were close ($r = 0,78$). In average, the sour cherry cultivars contained the highest amounts from several nutrient elements (e.g. Al, Cu, Fe, Mn etc.). The lowest element quantities were detected for Japanese plum cultivars. Levels of Al and K were outstanding in cherry plums. In case of some neurodegenerative diseases, patients should eat such fruits with lower contents of redox active metals (e.g. Japanese plums), because these patients should avoid these metals. We measured the glucose and fructose contents of sour cherries. The highest values of these two monosaccharides were detected in 'Cigány C404' and in 'Cigány 59' cultivars, while VN-07 contained the lowest levels from these sugars. The highest anthocyanin values were observed in fruits of cultivar candidates. Our analysis revealed a small difference between the lowest and highest vitamin C contents. Genetic background of cultivars forms the decisive factor in determining fruits' antioxidant capacity, although the cultivation plot and season may have also considerable modifying effects. Based on our results we can conclude that functional food products can be established from stone fruits.

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Possible role of reactive oxygen species in the development of immunological tolerance

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Since plasmacytoid dendritic cells (pDCs) are professional antigen-presenting cells, they have an important role in the polarization of the adaptive immune responses toward inflammation or antibody production. As professional interferon type I producing cells, the pDCs also possess a significant antiviral function. Previously, the pDCs were thought to be found only in bone marrow, lymphoid organs and blood; however, recent studies have indicated that they can also be detected in inflamed tissues. The pDCs leaving blood circulation and entering peripheral tissues are affected by - in addition to many other factors - the reactive oxygen species produced by inflammatory reactions. The effects of oxidative stress on the functions of pDCs have not been examined yet.

Our goal was to investigate how oxidative stress can influence the viability, phenotypic characteristics and cytokine production of non-activated pDCs or those activated by Toll-like receptor 9 (TLR9) agonists. We also studied how the experimental oxidative stress conditions, which were used for pDCs, change the viability of other lymphoid and myeloid cells.

Cells were isolated from peripheral blood of healthy donors by the method of magnetic separation. The xanthine oxidase/xanthine (enzyme-substrate) system and hydrogen peroxide were used to create the conditions of oxidative stress. After treatment, the alterations in viability and the phenotypic changes of cells were detected by four-color flow cytometry. The levels of IFN-Ð¸IL-6 and TNF-α cytokines were measured in the supernatants of cell cultures by ELISA.

Our data demonstrate that pDCs are very sensitive to oxidative stress, because exposure to reactive oxygen species significantly decreases their viability, lowers the expression of all examined surface antigens (BDCA-2, HLA-DQ, BDCA-4) and reduces their cytokine production. Our results also indicate that oxidative stress eliminates the activating effects of TLR9 agonists on pDCs. We found that there are significant differences in the sensitivity of lymphoid and myeloid cells to oxidative stress. The lymphoid cells, similarly to pDCs, showed strong responses to oxidative stress, whereas myeloid cells did not.

Lowered expression of cell surface molecules and decreased cytokine production suggest that pDCs exposed to reactive oxygen species produced by inflammatory cells may induce immunological tolerance instead of adaptive immune response upon interaction with naive T-cells. This phenomenon may provide an opportunity for a new, dendritic cell based therapy, in which pDCs treated with reactive oxygen species *in vitro* can be used to create immunological tolerance to a certain antigen *in vivo*, for example in the treatment of autoimmune diseases or severe allergic inflammations.

Measurement of redox parameters in the blood plasma of dogs with renal disease

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Renal disease is common in older dogs and cats, which can lead to cirrhosis and kidney failure. The symptoms appear several months after the onset of the disease when about 66-75% of the kidney tissue is irreversibly damaged. Many old dogs carry some degree of kidney disease, but its progress can be slowed down with an appropriate treatment. Correct diagnosis and therapy require the knowledge of the mechanism of the development of kidney disease and of the parameters, which may play important role during an effective treatment. In addition to previously described medical parameters, little is known about the molecules leading to oxidative stress in canine kidney failure.

Based on literature data, find redox parameters that can be useful during the treatment of kidney diseases. Methods: Blood samples of 60 healthy and 81 dogs with kidney disease (blood plasma creatinine concentration >140µmol/L) were used to determine different redox and routinely measured laboratory parameters. Whole blood stationary free radical concentration was determined using electron spin resonance (ESR) spectroscopy. Malondialdehyde and hydroxynonenal were measured as markers of lipid peroxidation, while protein oxidation was assessed by production of carbonylated proteins. Some antioxidant parameters relevant in kidney disease were also determined: glutathione ratio, enzymatic activity of SOD, as well as FRAP (ferric reducing ability of plasma) and TAS (total antioxidant status).

Free radical concentration of whole blood was significantly higher in samples taken from dogs with kidney disease compared to those taken from healthy animals. Malondialdehyde on its own showed no differences between the two groups, only when measured together with hydroxynonenal, a significant raise in lipid peroxidation was observed in renal disease. Plasma protein carbonylation was significantly higher in the group presenting kidney disease. Within the measured antioxidants reduced glutathione showed differences between the two groups as its levels were higher in diseased dogs compared to their healthy counterparts, and the activity of SOD increased in the same samples as well.

Concentration of free radicals in the blood of dogs with kidney disease is higher than in healthy animals. Lipid peroxidation increased in blood plasma of dogs with kidney disease. Levels of protein carbonyls also increased in blood plasma of dogs with kidney ailment. An induction of the antioxidant mechanism was seen in the blood plasma of sick dogs.

Markers of oxidative stress could be observed in the blood samples of dogs with kidney disease. Question: in addition to an antioxidant rich diet, what other recommendations can be made to slow down the progression of kidney failure and to allow dogs to live as close to normal life as possible under the given circumstances?

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Preventive effect vanadium, zinc and bioflavonoids on the onset of diabetes in BB rats

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Vanadium, other trace elements and bioflavonoids have been shown to be beneficial in the treatment of animal models of type 1 and type 2 diabetes. The aim of the study was to evaluate the preventive effects of vanadate (as ammonium metavanadate), zinc chloride and bioflavonoids in prediabetic BB rats.

80 BB rats were divided into 4 equal groups. Group "V" was treated with ammonium metavanadate (0.1 mmol/l), "Z" with zinc chloride (0.1 mmol/l), and "BF" with Flavin 7® (nutrition additive with bioflavonoids, 0,2 mg/l) in drinking water from 21st day after birth to 171st day of their life, and compared with "C" – control group on pure tap water. In each group food and water intake, urine output and body mass were followed regularly The manifestation of diabetic state was monitored through blood glucose, glycosuria and glycosylated hemoglobin determinations. Antioxidant system activity was estimated through enzyme (red cell superoxide dismutase, red cell catalase, whole blood glutathione peroxidase) as well as total antioxidant status and glutathione assays.

The age of onset of diabetes and its incidence were significantly higher in "BF" and "V" groups as compared to controls ($p<0.001$), and zinc treated group ($p>0.05$). In overtly diabetic rats blood glucose was higher in control group than in "V" and "BF" groups, $p<0.001$. Decrease of parameters of the antioxidant status, at the onset of the treatment as well as immediately after its cessation showed a drop in the treatment groups, but later increased slowly and continuously until the end of the experiment. The activity of antioxidant enzymes increased slowly from the beginning of study up to the point of diabetes manifestation and decreased thereafter. The decline was less evident in rats treated with bioflavonoids.

Both bioflavonoids and vanadate delay the development and lower the manifestation rate of diabetes in BB rats which is not the case in zinc treated animals. The same compounds decrease hyperglycaemia in diabetic rats. Bioflavonoid supplementation could have a beneficial effect on antioxidant status in diabetes mellitus.

Effect of immunonutrition with omega-3 fatty acids on oxidative stress response in polytraumatized patients – Pilot-study

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A state of increased oxidative stress has been recognised in polytraumatic injury that was influenced beneficially by omega-3 fatty acids substitution in patients with type 2 diabetes mellitus. Moreover previous studies have shown that administration of omega-3 fatty acids mixed with other antioxidant substances resulted shorter postoperative periods in the intensive care unit.

We evaluate the effect of nutrition with omega-3 fatty acid on the polytraumatic injury induced oxidative stress.

13 patients were randomised to Intralipid and Omegawen groups, based on their parenteral feeding. There was difference only in omega-3 supplementation between nutrition of the two groups. Blood samples were taken on admission and during the following 5 days. We measured the level of malondialdehid (MDA), glutathion (GSH), plasma SH groups (PSH), the activity of superoxid dismutase (SOD), catalase (KAT), and peroxidase (MPO) enzymes, and the stimulated reactive oxygen species (ROS) production of whole blood. Injury Severity Score (ISS) and Simplified Acute Physiology Score (SAPSII) were calculated on admission. Clinical data, Sequential Organ Failure Assessment Score (SOFA), Multiple Organ Dysfunction Score (MODS) were calculated every day. Primary endpoints were the duration of ICU stay and the number of mechanical ventilated days. For statistical analysis we used Mann-Whitney U test and two-way ANOVA test.

The two groups were similar initially in ISS, SAPS II, MODS, SOFA. The MDA level was significantly higher in both groups compared to the control healthy group ($p<0.05$). We observed an elevating tendency in MPO enzyme activity in both

patients groups that was significantly higher on the 6th day compared to the controls. The induction time of ROS production was longer in the Omegaven group during the examination period than in the control group, and it was significantly longer on the 5th day compared to the Intralipid group. We detected higher catalase activity in Omegaven and in Intralipid group as well, but this activity was significantly lower on the second day in the Omegaven group versus Intralipid group. GSH and PSH levels weren't influenced by the treatment of omega-3 fatty acids.

These data suggest, that polytraumatic injury causes considerable oxidative stress, on which omega-3 fatty acid supplementation has only a moderate effect.

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In vitro toxicity testing of PPI dendrimers

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Dendrimers are a new type of promising synthetic polymers characterized by a dendric branched spherical shape and a high density surface charge. The defined structure of these molecules has led to the interest in dendrimers as substrates for the attachment of antibodies or agents for applications in a number of different areas of biology and medicine. However, information on the mechanisms of dendrimer-induced cytotoxicity and a cell death is still limited. Therefore, it is necessary to undertake studies to determine biological properties of these compounds *in vitro*.

Thus, the aim of our investigation was to compare the effects of poly(propyleneimine) (PPI) dendrimers (PPI with 25% maltotriose units attached to the surface) on cultured human ovarian cancer cells (SK-OV-3) and Chinese hamster ovary cells (CHO). The cells were exposed to various concentrations of dendrimers (ranging from 1 to 300 μM). The toxicity of PPI dendrimers was studied immediately after the incubation with dendrimer (24 h) or 24 h after removing the dendrimer from the medium.

The cytotoxicity of dendrimers was studied by a MTT assay. The morphological features of apoptosis and necrosis were examined by Nomarski DIC combined with a confocal laser scanning microscope (CLSM). The level of reactive oxygen species (ROS) was evaluated with fluorescenct probe: dichlorofluorescein-diacetate (H_2DCFDA) by flow cytometry. Changes in mitochondrial membrane potential were determined using JC-1.

Our studies demonstrated that PPI dendrimers exerted multiple suppressive effects on cancer SK-OV-3 cells, including proliferation inhibition, induction of an apoptotic cell death and a collapse of mitochondrial membrane potential. Most importantly, these compounds were more cytotoxic to cancer cells than to normal CHO cells.

These findings will help to understand the mechanisms of PPI dendrimer cytotoxicity in normal and tumor cells and open the possibility to use them in clinical applications.

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Bioactive compounds in *Alliums* from Vojvodina - antioxidants

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Toughout recorded history *Alliums* especially garlic and onion played rich diverse commercial, culinary, and mystic roles. Today garlic and onion are used for their flavour, aroma and taste, being prepared domestically or forming basic materials for a variety of food manufacturing processes. Onions were among the earliest vegetables to be processed, canned, dried and frozen. Many epidemiological studies have suggested that certain natural foods could prevent the development of different diseases. Garlic and onion are such natural foods. They have a variety of pharmacological effects including tumour cell growth inhibition and chemopreventive activity. Much of the data about human use came from reports of lowered rates and risks of disease (such as cancer) in people with relatively high levels of garlic or other *Alliums* consumption. People also use garlic

and onion to help with several different types of aliments, high cholesterol, high blood pressure, excess blood clotting and coagulation, atherosclerosis, inflammation, bacterial and fungal infections. Garlic is also functional food product composed of numerous macronutrients, vitamins, organosulfur active compounds.

The aim of our study was to investigate different cultivated (*Allium nutans* L., *A. fistulosum* L., *A. vineale* L., *A. pskemense* B. Fedtsch, *A. cepa* L. and *A. sativum* L.) and wild *(A. flavum* L., *A. sphaerocephalum* L., *A. atroviolaceum* Boiss, *A. schoenoprasum* L., *A. vineale* L., *A. ursinum* L., *A. scorodoprasum* L., *A. roseum* L. and *A. subhirsutum* L.), *Allium* species, in order to evaluate their antioxidant properties.

All the antioxidant enzyme activities were determined spectrophotometrically at 25ºC using phosphate buffer (pH 7) plant extracts. The amount of reduced glutathione (GSH) was determined with Ellman reagent, lipid peroxidation (LP) was determined by the thiobarbituric acid (TBA) method. Hydroxyl radical (·OH) was determined by the inhibition of deoxyribose degradation, total flavonoids were estimated according to Marckam and soluble protein content was determined by the method of Bradford. Radical scavenging capacity was determined using 1, 1-diphenyl-2-pycril-hydrasil radical (DPPH) and ESR. Reduction of DPPH radical was determined measuring disappearance of DPPH. Total antioxidant capacity was estimated according to the FRAP. Lipofuscin pigments (LFS), were determined fluorimetricaly.

Our results are one more confirmation that antioxidant and scavenger activities influence the pharmacological activity of garlic and other *Alliums.* In leaves of *Allium fistulosum* L., LFS accumulation was also not observed. As LFS is generated as a product of tissue decay, caused by toxic oxygen species it means it has a high antioxidant capacity. The scavenger activity of *Allium fistulosum* L. was also high; in its presence, generation of the ·OH radical (the most toxic oxygen species) was reduced by 87.09%. Other results concerning *Allium fistulosum* L. support this assessment because the activities of all antioxidant enzymes SOD, CAT, GPX, and GP were high, concentrations of $O_2^{\cdot-}$, ·OH and MDA were low, and the quantity of GSH, flavonoids, vitamin C and soluble proteins were high, as was the carotenoids content.

Presented results indicated that crude extract of *Alliums* from Vojvodina exhibited antioxidant and scavenger abilities in all investigated plant parts especially in leaves. Therefore overground part of *Alliums* could be used as the source of natural antioxidants in the pharmaceutical, cosmetic and food industries for manufacturing antoxic products with potent medicinal and antioxidant activity.

Comparison of antioxidant power in fruits of commercial apple cultivars and cultivar candidates grown in Hungary

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The apple (*Malus domestica* Borkh.) has a privileged role among fruits in the temperate zone. Its consumption is not limited to any particular season because several cultivars are available to cover supply during the whole year. Furthermore, it can be processed, *i.a.* it is an essential components of baby foods. Its valuable inner contents greatly contribute to the wide applicability of this fruit. Apple has remarkable contents of energy and raw fibre and it is also a rich source of vitamins and mineral elements. Apple is one of the most consumed fruits in Hungary, and hence its valuable compounds (vitamins, minerals, polyphenolic compounds) may significantly contribute to the health-promoting effects of human diet.

The aim of this study was to characterize the inner contents, antioxidant power, total phenolic content and mineral nutrient element contents of commercial apple cultivars in comparison with perspective cultivar candidates and estimate their contribution to the coverage of physiological requirements.

Among the main inner content parameters, total phenolic content and antioxidant capacity (FRAP) were measured spectrophotometrically. Mineral element contents in fruits were determined by ICP-OES. Different apple genotypes (well-known commercial cultivars and perspective cultivar candidates) grown under the same conditions were used for the analyses. Antioxidants were compared in different parts (skin and flesh) of the apple samples.

Our results indicate significant differences in all measured parameters among the assayed cultivars and cultivar candidates. Different antioxidant assays revealed 2- to 3-fold differences between the lowest and the highest values in commercial cultivars and cultivar candidates. The antioxidant power of fruits was much influenced by the skin/flesh ratio as smaller fruits

with higher skin/fruit flesh ratios had increased antioxidant capacity compared with larger fruits. It indicates that the antioxidant compounds predominantly accumulate in fruit skin. Considering that all samples were collected in orchards located in the same region, these differences are likely to be explained by the different genetic backgrounds of cultivars and cultivar candidates. Some cultivar candidates were characterized by higher antioxidant capacities and mineral element contents than the main commercial cultivars pointing to the possibility for increasing health-benefits of apple even under constant level of fruit consumption.

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Temporal changes of antioxidant parameters in *Acorus calamus* L.

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Sweet flag (*Acorus calamus* L., Araceae) is widely used medicinal plant as extracts or dried rhizome for several diseases, for external or internal use, as well. Numerous studies performed its antioxidant effects such as decrease of lipid peroxidation in noise-stressed rat brain after application of alcoholic extracts of Acorus. Since, sweet flag is under protection in Hungary and we have relatively little information about antioxidant properties of Hungarian population we decided to estimate some antioxidant parameters and temporal changes of these during vegetation period.

Plant material was collected twice in 2008 (June and October) and after washing with distilled water leaves (L), rhizome with (H) and without bark (HL) were used freshly (homogenate) or as alcoholic and watery extracts made of dried drugs. Parameters measured were FRAP (ferric reducing–antioxidant power), glutathione (GSH) level and free radical scavenging ability using DPPH. Statistical analysis was performed using STATISTICA 8.0 software (analysis of variance and correlation).

Our results showed that homogenate and alcoholic extract of leaves had significantly higher FRAP-values compared to those of watery extracts, in June. Antioxidant capacity in rhizome was usually lower than in leaves. In temporal aspect, a significant decrease (40%) of FRAP appeared in alcoholic samples of leaves, while there were no changes in rhizome. Glutathione (GSH) level was 4-6-fold higher in leaves than in both forms of rhizome and was in significantly positive correlation with FRAP. Fraction of residual DPPH radical (%) was the highest in rhizome with bark (H) which means that it had quite low reducing ability, nevertheless, free radical scavenging capacity of homogenates of leaves and rhizome with bark showed to be significantly higher in October compared to June. According to FRAP we can make a sequence qualifying the three types of samples: homogenate > alcoholic extract > watery extract.

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Table beet and red cabbage, as natural source of antioxidant compounds

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Free radicals derived from oxygen play an important part in the pathomechanism of different illnesses. Living organisms are supplies with an effective defence system against oxygen radicals. The first defence line is composed of antioxidant enzymes but different vitamins and low molecule compounds, such as phenols, thiols and flavonoids, are also effective against radicals. These compounds can be found in high quantities in vegetables. These compounds are mostly of polyphenol type and are able to bind free radicals and protect from the oxidation of biological molecules, membranes and tissues induced by active oxygen and free radicals. In evaluating bioactive content of vegetables an important role is provided to those compounds and are able to bind free radicals and protect from the oxidation of biological molecules, membranes and tissues induced by active oxygen and free radicals. Such are for example phenol type substances whose group includes pigment content as well. The colour materials of table beet and the red cabbage are suitable for natural pigment production and the same time they have favourable nutrition effect too.

During our experiment we measured FRAP and colour content in 4 red cabbage hybrids and 20 beet root varieties. In formation of bioactive substances of vegetables are very important the heritable quality parameters too. In this way we examined not only the different species, and the roil of varieties belong to them.

The red pigments were evaluated from diluted samples. A spectrophotometer was used to determine the absorbance of pigments: λ=538 nm for red pigments and λ=476 nm for yellow ones. For total phenol content the colour reaction to Folin-Denis reagents were evaluated at λ=760nm, by means of a katechin standard (mg katechin/100 ml). Total antioxidant content was expressed in the so-called FRAP values (ferric reducing ability of plasma) in µM/l. The method is based on the ability of antioxidants to reduce Fe(III) ions to Fe(II) ions in buffered sour medium (pH 3,6). The produced Fe (II) can be measured on photometers. Absorbance is proportional to the quantity of the produced Fe(II) ions and the antioxidants, respectively.

Our measurements showed more than threefold differences in total antioxidant activity among varieties, the lowest value being 171.13 µM/l and highest 702.57 µM/l. The corresponding betanin (17.18 and 57.80 mg/100 ml) and total polyphenol (37.5 and 85.5 mg/100 ml respectively) contents show similar differences. The highest FRAP values was measured in the *Bonel, Pablo* and *Pronto* varieties (506.97; 571.43; 702.57 µM/l). Based on our results it can be stated that varieties of higher betanin and poliphenol contents have higher antioxidant values as well. With the further measurements we concluded that red cabbage varieties greatly vary in pigment. There is a correlation between the pigment and dry matter content and FRAP. According to our data the highest FRAP parameters were measured in *Sandoro* F_1 whose colour intensity also proved to be excellent. Lower parameters were shown by *Rendero* F_1 which also lagged behind regarding dry matter content and pigment content.

Our measurements showed the varieties with higher pigment and poliphenol content have high antioxidant values too. There is a close correlation between red pigments (betanin), total polyphenol contents and FRAP values. The correlation between the quantity of these compounds and the FRAP values (r = 0.7799 and r = 0.7435, respectively).

Accordingly, the two compounds must have a role in the evolution of antioxidant effects.

Biological evaluation of volatile oils and aromatic agents by FRAP method

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Biological (antioxidant) values of volatile oils used in medication and basic components used in flavouring and parfumery have not been known so far. For that reason the authors examined the applicability of a chemical method (FRAP) adapted for plant materials. The aim of the work was to study that the volatile oils and essence of parfume of positive effect to biochemical (allergic) processes in the human body may posses any antioxidant properties.

Volatile oils, fragrance compositions (know and unknown combinations, but known tendency) were studied. The measurements were made in 1% solutions. The components of volatile oils and fragrance compositions were identified by GC-MS and FRAP method was used for measurement of antioxidant property.

The identified and frequently occuring basic-, aromatic- and perfumed compounds may be characterized by the following FRAP values: methyl salicylate 303±1 µmol/L, Þamyl acetate 144±2 µmol/L, borneol 284±1 µmol/L, camphor 286±1 µmol/L, carvon 164±2 µmol/L, menthol 1.86±0.23 µmol/L, menthon 1.88±1.10 µmol/L, thymol 284±1 µmol/L, linalol 299±1 µmol/L, linalyl acetate 221±4 µmol/L, limonene 445±3 µmol/L, terpineol 142±1 µmol/L, cinnamic aldehyde 303±1 µmol/L, anethol 509±2 µmol/L, while the FRAP values of volatile oils were the followings: lemon oil 198±1 µmol/L, geranium oil 152±2 µmol/l, sage oil 313±3 µmol/L, pine oil 255±1 µmol/L, muscate sage oil 484±2 µmol/L, patchuli oil 178±2 µmol/L, petitgrain oil 174±3 µmol/L, dill seed oil 323±1 µmol/L, eukalyptus oil 14±2 µmol/L, clove oil 197±1 µmol/L, peppermint oil 167±1µmol/L, rosemary oil 35 ±1 µmol/L. The FRAP values of essence-compositions varied between 250-1000 µmol/L (female 251±8 µmol/L, male 409±13 µmol/L, kid 1093±7 µmol/L). At the T1-T15 style tendency with unknown compositions the values were between 3000-9000 µmol/L in 10% solutions, while smaller values characterize the american unisex essences (P-18, P-19, P-20): 243±2 µmol/L, 252±4 µmol/L and 520±1 µmol/L, respectively.

It has been stated that the FRAP method is suitable for measurement of in vitro antioxidant property of complex compositions.

The FRAP values of volatile oils of the knowledge of main componenst help to estimate the quality of volatile oil with 20-30% deviation. In the case of fragrance compositions with known essence style tendency, the values show the reducing power and they do not contain any other information.

It is recommended to determine the antioxidant values of all aromatic agents, volatile oils and essence-compositions beside physico-chemical characteristics.

Modification of fully activated NADPH oxidase activity by antioxidants

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The first reactive oxygen-derived substance is the superoxide anion produced by NADPH oxidase. This is a multicomponents enzyme containing several cytosolic (phox proteins) and membrane-bound (Cytochrome b558) parts. NADPH oxidase can be activated by receptor-mediated and non-receptor-mediated ways. During the activation the cytosolic components are phosphorylated and translocated to membrane, where they are combined with the membrane-bound part of Cytochome b558. Cytochrome b558 consists of two parts, gp91phox and p22phox proteins. During the last few years it became clear that this complex is not able to produce superoxide anion, it requires coupling of a Rac 1 or 2 GTPase G protein to complex. When coupling of Rac 1/2 is inhibited the NADPH oxidase enzyme could not produce superoxide anion. Now, it is well demonstrated that NADPH oxidase can be found not only in phagocytes such as neutrophils, monocytes, but many other cells as well, they are called to NOX enzymes. NOX was identified in uterus, renal cells, hepatocytes, endothelial cells, lymphocytes, smooth muscle cells etc. Those non-phagocyte NADPH oxidases (NOX1, NOX3, NOX4) differ from phagocyte one (NOX2) in the structure of gp91 phox subunit. This difference results in that non-phagocyte NADPH oxidase produces smaller amounts of superoxide anion and its activation does not require activation of PKC.

During the last years, our group studied the effects of several natural and synthetic antioxidants on superoxide anion production and activation of PKC in human neutrophils. These examinations involved the study the effects of antioxidants on superoxide anion production by fully activated NADPH oxidase (NOX2) as well. During experiments we have found that some antioxidants can decrease it, while others have not such effects. It was also demonstrated that the effects of antioxidants on fully activated NADPH oxidase was independent on PKC inhibiton; and as a consequence, independent from the modification of superoxide anion production in intact neutrophils induced by antioxidants. These differences were the most pronounced in case of tocopherols and their water soluble metabolites (CEHC). Both tocopherols and CEHC-compounds inhibited PKC and superoxide anion production in phorbol-ester stimulated neutrophils, and CEHC were more effective inhibitors as parent tocopherols. In contrast, superoxide anion production by fully activated NADPH oxidase was only decreased by lipid-soluble tocopherols.

On the basis of our observations, we suggest the following mechanism for the action of antioxidants on superoxide anion production by fully activated NADPH oxidase: the bound between the enzyme complex and the small Rac 1/2 protein - which is necessary for superoxide anion production by NOX – might be slack or broken by antioxidants, which is due to the counteraction of lipid-soluble antioxidants and cell membrane. This observation might be useful in those clinical states when activation of NADPH oxidase occurs - in either phagocyte or non-phagocyte cells-, since in these cases we can choose antioxidants which are able to decrease superoxide anion production by fully activated NADPH oxidase(s) (NOX) and prevents development of oxidative stress.

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Biological evaluation of alcoholic extracts of medicinal plants by the application of FRAP method

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Alcohol is often applied as a polar solvent for extraction of bioactive agents of medicinal plants. There are hardly any data on biological values, antioxidant properties of alcoholic extracts, therefore the aim of the work was to determine the antioxidant property/reducing ability of these kind of extracts. For the uniform dosage of agents, they could be hold on as a stock of family doctors, since the alcoholic extracts of herbs choosen are official in the Hungarian Pharmacopoeia (Ph.Hg.VII). Alternative medication, e.g. homeopathy, also often use tictures of alkaloid containing, when the diluted alcoholic solution is applied for symptomatic treatment. The data according to the examination may help to estimate the dose used and the effect in both modern and alternative medication point of view.

The following alcoholic extracts were prepared by the description of Hungarian Pharmacopoeia: Tinctura Amara, Tinctura Chamomillae, Tinctura Chinae, Tinctura Ipecacuanhae, Tinctura Ratanhiae, Tinctura Saponariae, Tinctura Strychni, Tinctura Thymi, Tinct Stramonii. For examination, the extracts of 40% concentration were used.

The measurement of biological active components in extracts was performed according to the Hungarian Pharmacopoeia (Ph. Hg VII) and the determination of antioxidant values was realized by FRAP method. The method is based on the transformation of Fe^{3+} into Fe^{2+} at low pH value. Formation of Fe^{2+}-TPTZ (2,4,6-tripyridyl-S-triazine) complex is traceable by spectrometry based on color reaction at 593 nm.

The quality and quantity characteristics of extracts are agreed with the description of Ph.Hg. VII. The FRAP values of alcoholic extracts are the followings: Tinctura Amara 768.5 µmol/L, Tinctura Chamomillae 741.9 µmol/L, Tinctura Chinae 642.9 µmol/L, Tinctura Ipecacuanhae 281.8 µmol/L, Tinctura Ratanhiae 427.0 µmol/L, Tinctura Saponariae 686.1 µmol/L, Tinctura Strychni 482.9 µmol/L, Tinctura. Thymi 177.7 µmol/L, Tinctura Stramonii 832.3 µmol/L.

It is allocated that this method can be apply as the expression of vegetable alcohol-water extract of biological values.

It has been stated that the FRAP method is suitable for evaluation of biological values of alcoholic-aqueous extracts.