INVESTIGATION OF METAL ELEMENT CONTENT AND REDOX HOMEOSTASIS IN PLANT, ANIMAL, AND HUMAN ORGANISMS

PhD thesis

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1 Introduction

The results of the research in the metabolic processes of metallic compounds in the human organism provide even more comprehensive picture of the physiological effects of individual elements (metallic and non-metallic components and their organic compositions), that are becoming more important in maintaining health due to their immunostimulants, antioxidants and apoptosis-inducing effects.

From the nutritional point of view plants, animals and human organisms are closely linked together because minerals and organic substances present at the lower level of the food chain will come across to the higher levels.

For humans, the most important sources of essential macro- and micro-, metal and non-metal components are foods of plant and animal origin. Metal components influence cell membrane transport processes when binding to proteins thereby they play a significant role in altering the membrane permeability. Furthermore, they play a key role in the endogenous antioxidant enzyme processes in the induction and regulation of transcription factors (activator protein 1 (AP-1) and IκB kinases) due to their role as enzyme constituents (CuZnSOD MnSOD).

However, due to soil and feed contamination, the human body is exposed to indirect health damage through the food chain. In any case of disease, the body's antioxidant defense system weakens, which can be improved with external antioxidants and minerals intake. Antioxidant compounds with low molecular weight and antioxidant enzymes in the organism, as well as natural bioactive substances (vitamins, flavonoids, polyphenols) are responsible for the inactivation of free radicals. Those compounds are considered as antioxidants, that provide protection against the oxidative stress caused by reactive oxygen species (ROS) by reducing them with electron transfer, thereby providing neutralization of the ROS. As a result of this process the non-toxic, i.e. reduced, physiological environment is restored.

However, excessive amounts of antioxidants, vitamins, and trace elements in the body can affect negatively the body's metal ion and redox homeostasis. Excessive amount of antioxidants intake can increase pathological conditions and can weaken the immune system over time, due to their pro-oxidant effect.

In food supplements of natural bioactive agents and medicinal plants, only the concentrations of certain toxic metal components are given by the regulations of the official bodies, however, it would be necessary to determine the presence of other metals due to their role in the redox and metal homeostasis in the human body. Therefore, the research of metal components in food supplements is of high importance.

Little data is available in the literature of flavonoid-flavonoid synergism, antagonism or drug-flavonoid and mineral component interactions. It has been shown that the antioxidant properties of polyphenols in herbal food supplements are greatly influenced by the quality and quantity of the metal components.

Polyphenols can form complexes with metals due to their metal-chelating properties, which have stronger antioxidant properties than the polyphenols themselves. These compounds boost the bioavailability of minerals and bind free metal ions that would catalyze the formation of free radicals. The quantity and quality of the antioxidant and metal element content in herbs and fruits can differ not only between species, but also between different kinds, which is significantly influenced by the environmental factors and the geographical location during their cultivation.

There are few medical publications available to create a meaningful assessment about the molecular biological activity of bioactive substances in plants and plant-based food supplements and their use in adjuvant therapy. The pharmaceutical and medicine sciences are not able to provide sufficient information on the correct use of herbal medicines and food supplements to patients, so a more detailed analytical- and efficacy study of herbs and food supplements has high importance.

The poor dietary habits of the Hungarian population contribute to popular diseases such as obesity, diabetes, hypertension, and the development of tumors and metabolic syndrome. The risk of developing diseases can be prevented and significantly reduced by balanced consumption of natural antioxidant rich foods and dietary supplements. Consumption of berries containing anthocyanins can be promising in the inhibition of inflammatory and lipid peroxidation processes due to their potential antioxidant and lipid-lowering effects.

Mineral intake is also possible by the consumption of food of animal origin. The presence of mycotoxins in food, especially in cereals such as wheat or corn, and in animal feed is a major challenge for the food industry. The human body is exposed to mycotoxicosis through the food chain by the consumption of food of plant and animal origin because mycotoxins, which enter the body of the animals through the mycotoxin-contaminated feed, are also consumed by humans. Mycotoxins have a significant negative effect on the antioxidant defense system as they induce oxidative stress when they get into the human organism. Using herbal active ingredients, that is also necessary for the prevention of animal diseases, against mycotoxin contamination in animal feed, could be a solution.

The role of vitamin D_3 significantly enhanced in the prevention of diseases and various cancerous processes. Besides, theories and recommendations have changed over the past decade regarding vitamin D intake. Based on results of studies of the Hungarian population's vitamin D level, it has been established that a significant part of the population suffers from vitamin D deficiency, therefore in 2012 the Hungarian consensus changed the recommended daily intake of vitamin D from 200 IU to 2000 IU for adults. As a result, there is an increasing rate of consuming higher vitamin D_3 containing supplements. The recommendations are contradictory to determine the daily intake of vitamin D_3 and overdose poses health risks. For this reason, a scientific study of a long-term vitamin D_3 intake under, strict medical control, and its effect on the metal and redox homeostasis in patients with prostate cancer is of particular importance.

2 Objectives

The aim of my research was to find correlations between the metal element content and antioxidant properties of the antioxidant content in plants, and to find relationship between the quantity of antioxidants and metal element concentrations and the metal- and redox homeostasis in animal and human organism.

- My goal was to investigate the metal element composition and antioxidant properties of some dried herbal drugs, powders, tinctures, and dietary supplements.

- I wanted to find a correlation between the antioxidant parameters and the mineral content in plants.
- I investigated whether the average nutritional reference values (NRV) of minerals can be significantly covered by the minerals contained in the recommended daily intake of food supplements.
- In an animal experiment I studied the effect of the active components and the metal element composition of different types of sour cherries in alimentary-induced fatty liver. In the research, I investigated the different bioactive agents and metal components in different types of sour cherry lyophilisates and their effect on the metal element composition and redox homeostasis in certain organs in healthy and fatty liver groups.
- In another animal experiment I studied whether the *Silybum marianum* (SM) seed supplementation in pressed and oil form can prevent the liver damage caused by mycotoxin (fusarium toxins (DON and ZEA)) contaminated feed consumption and what changes would occur in the metal- and redox homeostasis in duck's liver by the SM treatment.
- The physiological importance of vitamin D_3 is undoubtable, especially in patients with prostate cancer. In a human study I examined the effect of a long-term and strictly medical supervised vitamin D_3 treatment on the metal element composition and redox parameters of blood samples from healthy volunteers and patients with various stages of prostate cancer.

3 Methods

3.1 Plant samples

In my research, the element composition and antioxidant properties of herbal and fruit preparations were measured. The drugs and 70% ethyl alcohol extracts (2.5 g/25 ml) of milfoil (*Achillea millefolium* L.) herba, urtica (*Urtica dioica* L.) leaf, hawthorn (*Crataegus monogyna* Jacq.) floral branch, small flower hairy willowherb (*Epilobium parviflorum* Shreb.) flower, calendula (*Calendula officinalis* L.) petal, white mistletoe (*Viscum album* L.) berry and leaf, rosehip (*Rosa canina* L.), lime-tree (*Tilia cordata* Mill.) inflorescence were used. The samples were obtained from Bioextra Zrt. Among the medicinal plants from the

Far East, dried herbal powder, their 70% ethyl alcohol extracts (2.5 g/25 ml), and the food supplements of buckthorn (*Astragalus membranaceus* Schischkin) root, ginseng (*Panax ginseng* L.) root and turmeric (*Curcuma longa* L.) root, Chinese caterpillar mushroom (*Cordyceps sinensis* Berk.) Lingzi mushroom (*Ganoderma lucidum* Fr. Karst) spore, leaf of the ginkgo (*Ginkgo biloba* L.) and the fruit of the milk thistle (*Silybum marianum* L. Gaertn) were used. They were brought to Hungary from various areas of China. The samples were obtained from the Chen Pharmacy.

Different fruit concentrates were also investigated. The fruit concentrates of blueberry (*Vaccinium myrtillus* L.), black currant (*Ribes nigrum* L.), black elderberry (*Sambucus nigra* L.), sour cherry (*Prunus cerasus* L.), sea-buckthorn (*Hippophae rhamnoides* L.) and cranberry (*Vaccinium oxycoccos* L.) were obtained from GPS Powder Kft.

3.2 Animal experiments

The animal experiments were carried out in accordance with the legal framework defined by the legislation of the "1998. year XXVIII Act on the Protection and Welfare of Animals", the "243/1998. (XII. 31.) Government Decree on the Conduct of Animal Experiments", and the " 40/2013. (II. 14.) Government Decree on Animal Experiments".

3.2.1 Changes in the metal element composition and redox homeostasis in rats by sour cherry lyophilisate supplementation

For the rat experiment male Wistar rats obtained from TOXI-COOP Zrt. and lyophilized samples of 3 different sour cherry varieties Pipacs 1 (M1), Fanal (M2) and Újfehértói fürtös (M3) were used.

In a "short-term" experiment, hyperlipidemia and consequent fatty liver were induced in rats by a fatty diet containing 1 m/m% cholesterol, 0.3 m/m% cholic acid and 11 m/m% sunflower mixed with the normal feed. The animals consumed the sour cherry lyophilisate ad libitum in a daily dose of 0.75 g/kg BW.

The rats were divided into 8 groups with 5 animals per group. Group 1 received control food (healthy), group 2 high-fat food (fatty liver), groups 3-5 groups the sour cherry lyophilisate mixed with the control feed (M1, M2, M3), the 6-8 groups received lyophilisate (M1, M2, M3) mixed with the fatty diet. Finally, the rats were anesthetized with ketamine (75 mg/kg BW) and xylazine (7.5 mg/kg BW), and after laparotomy, blood was collected from the abdominal vein then the animals were bled. The rat organs were removed and washed with ice-cold isotonic NaCl solution (0.9 m/m%). The cleaned liver pieces were homogenized in a Potter-Elvehjem apparatus. After that, the samples were stored at -20 °C until the measurements were started. The protein content was determined according to Lowry et al. (1951), our samples were diluted to a concentration of 10 mg/ml for bovine serum albumin with isotonic NaCl solution.

3.2.2 Changes in the metal element composition and redox homeostasis in duck's liver by the consumption of mycotoxin contaminated feed and pressed *Silybum marianum* seed and oil supplementation

In the second animal experiment, 24 Hungarian female white hybrid ducks (Szarvas K94) were used, which were divided into 3 groups with 8 animals per group. The ducks consumed a starter feed for 14 days, followed by a rearing feed (14-47 days). All groups consumed feed containing corn naturally contaminated with mycotoxins (4.9 µg/g DON, 0.66 µg/g ZEA), but two groups' diets also contained *Silybum marianum* (SM) seed extract in different forms. Group 1 (MT) consumed mycotoxins contaminated corn. The mycotoxin feed of group 2 (MT+SM1) contained 0.1% *Silybum marianum* oil; the 3rd group (MT+SM2) contained 0.1% SM oil and 0.5% extruded SM. The ducks were euthanized with carbon dioxide on the 47th day. The ducks' livers were washed with 0.9 m/m% NaCl solution and then chopped. The liver pieces were cleaned from the blood then homogenized in a Potter-Elvehjem apparatus and then stored at -20 °C until the measurements began. The protein content was determined according to Lowry et al. (1951). The samples were diluted to a concentration of 10 mg/ml for bovine serum albumin with isotonic NaCl solution.

3.3 Human study

The study was carried out in accordance with the ethical permissions granted by the Hungarian Scientific and Research Ethics Committee (permit number: TUKEB 167/1997, 15/2004) and the Institute Research Ethics Committee of Semmelweis University (permit number: IKEB 3944/2004).

In the study, we evaluated the data of 42 volunteer patients with an average age of 62.1±15.9, who were classified by the physician into 5 groups: Group 1: BK (Patient Control Group), Group 2: D3+BK (Patient Control group with Vitamin D_3 supplementation), group 3: MKPR (High-risk Prostate Cancer Patients group), group 4: D_3+MKPR (High-risk Prostate Cancer Patients group with Vitamin D_3 supplementation), group 5: D_3+PR (Prostate Cancer patients group treated with Hormone and Vitamin D_3 supplementation). Vitamin D_3 was a generic product available at the pharmacy.

Blood samples were collected in 3.2% sodium citrate anticoagulant Vacutainer tubes (Greiner Bio-One, Hungary, Vacutainer, USA) and stored at 4 °C. Blood samples were prepared using standard routine laboratory methods. The blood separation process was started within 1.5 hours after the blood was collected. The erythrocyte fraction was separated from the blood plasma at 2500 rpm within a settling time of 10 minutes. After that, the "buffy coat" was removed from the erythrocyte fraction and then washed with isotonic (0.9 m/m%) NaCl solution. The crude fraction of the purified erythrocyte (20 µl) was standardized to 1% hemoglobin content with CHR (5 ml) hemoglobin reagent. Until the analysis started, the erythrocytes and blood plasma samples were stored at -20 °C. Then the redox parameters and element content were determined.

3.4 Analysis of samples

The element content of the samples was determined after acid digestion of samples (67% HNO3, 36% HCl and 30% H2O2) using ICP-OES spectrometric method. The total polyphenol content was determined based on the method of Singleton and Rossi (1965), and the results were given in units of gallic acid/g sample. The amount of ascorbic acid in the samples of plant origin was determined based on the VIII. Hungarian Pharmacopoeia (2006).

The H-donor activity was measured with spectrophotometry based on the method of Hatano (1988) and Blois (1957) using the DPPH stable radical. The free radical scavenging capacity was determined according to Blázovics et al. (1999) with luminometry method. The reducing power was measured according to Oyaizu (1986) with spectrophotometry. The concentration of free sulfhydryl groups was determined based on the method of Ellman and Lysko (1967) using the DTNB reagent. The diene conjugate content was measured with spectrophotometry as well based on the AOAC method. The malondialdehyde content was determined based on a modified version of Ottolenghi's method.

3.5 Statistics

For the calculations and statistical analysis Microsoft Office Excel 2016 and Statistics 7 programs (StatSoft Inc., Tulsa, USA) were used. For determining the normality of the data the Shapiro-Wilks and Kolmogorov-Smirnov tests were applied. If ANOVA could not be used due to the normality of the samples or the inhomogeneity of the variances, data was evaluated with Kruskal-Wallis test for more than two independent samples.

Significant difference between groups caused by the treatments were calculated with ANOVA Tukey's post hoc test. If the t-test or the ANOVA Tuckey post-hoc test could not be applied due to the normality of the samples or the inhomogeneity of the variances, the non-parametric Mann-Whitney U-test or the Kruskal-Wallis test were applied. For the comparison of groups of the liver samples from animal experiments, the difference was considered significant at a significance level of $p<0.05$. For the comparison of the results measured in human plasma and erythrocyte the difference was considered significant at a significance level of $p<0.01$. Spearman rank correlation was used to calculate the correlation between groups. Correlations were considered significant at a significance level of $p<0.05$. The tables contain the average and standard deviations ($\bar{x}$±Stdev) of the measurement results.

4 Results

4.1 Evaluation of results of plant samples, extracts and food supplements

In the first part of my research medicinal plants and their extracts were investigated that are used in Europe as part of disease prevention therapies, and in the Far East for healing.

Questions arise about whether herb-based alimentary supplements contain metal elements and antioxidants in a composition or concentration that are harmful to the health, and whether the element content measured in plant-based herbs and food supplements affects their antioxidant properties.

Each of the herbal and fruit preparations in the investigation contains a significant amount of metallic and non-metallic components.

Based on the element composition results measured in medicinal herbs, it can be established that all of the soil metal components (Al, Cr, Fe, Ti) exceeded the average plant concentration (Al>200 µg/g, Cr>0.2 µg/g, Fe>300 µg/g, Ti>2 µg/g) in the samples of urtica leaves (Al: 476.8±6.3 µg/g, Cr: 1.56±0.21 µg/g, Fe: 443.3±32 µg/g , Ti: 32.21±0.30 µg/g), calendula petal (Al: 1558±3 µg/g, Cr: 6.17±0.34 µg/g, Fe: 1401±65 µg/g, Ti : 87.31±2.10 µg/g), buckthorn root (Al: 650±26 µg/g, Cr: 1.72±0.26 µg/g, Fe: 628.0±25.9 µg/g , Ti: 24.24±1.34 µg/g), turmeric root (Al: 502±52 µg/g, Cr: 0.877±0.075 µg/g, Fe: 373.0±34.9 µg/g, Ti : 8.87±1.03µg/g), and ginseng root (Al: 1240±15 µg/g, Cr: 2.52±0.24 µg/g, Fe: 1242±23 µg/g, Ti: 28 .07±0.70 µg/g), which, based on literature data, may indicate soil contamination of the samples or soil acidity.

With the preparation of herbal extracts, the essential and non-essential metal and non-metal components in drugs are both dissolved into the extracts. Their absolute metal element content is usually smaller magnitude compared to the element content of the herbal drugs and the powder preparations. Furthermore, the Ca:Mg ratio tends to shift towards Mg in tinctures in comparison to the results measured in powder preparations, presumably due to the solubility of Ca and Mg compounds in ethyl alcohol. Based on the previous, the tinctures can have a positive effect on the absorption of Mg, which can help alleviate the proinflammatory processes occurring in gastrointestinal diseases.

According to the results the antioxidant properties measured in tinctures of medicinal herbs correlates with the element composition and their active ingredient content in a concentration-dependent manner. The parameters that describe the antioxidant properties (H-donor activity, reducing power, total polyphenol content, antioxidant capacity) are usually related to each other in a directly proportional manner. If this tendency does not exist, it can be attributed to their element content. In higher concentrations a decreasing tendency was observed in the results of the induced free radical level by higher total polyphenol content, but in lower concentrations, the opposite tendency was experienced.

In tinctures the RLU% values above 100% measured in low concentrations indicate the presence of induced free radicals, which is presumably due to the catalytic effect of the high Cu content present in the system.

The free radical scavenging capacity measured in fruit concentrates did not show antioxidant properties at lower concentrations, in the cases where higher RLU% values were measured compared to 100% background intensity (blueberry fruit (2 mg sample/ml): 750.4±20.3 RLU%, sour cherry fruit (2 mg sample /ml): 425.3±18.1). This indicates an increased amount of free radicals in the system, which presumably depends on the catalytic effect of the metal components (Cr and Fe content, e.g. in blueberry fruit concentrate; Cu and Fe content, e.g. in sour cherry fruit concentrate) at lower concentrations. In contrast, the antioxidant property of the fruit concentrate with a low Fe content was outstanding (e.g. in cranberry fruit concentrate).

Furthermore, our results prove that, besides the significant amount of active ingredients, the metal content with potential antioxidant properties (Mg, Mn, Zn) also have a positive effect on the free radical scavenging ability in the case of buckthorn fruit and gingko leaves.

Comparing the amount of metal components that can be consumed with the daily intake of herbs and fruit extracts with the nutrient reference (NRV) values, we can see that Cr (60%), Mn (30%) and Mo (21%) can be covered significantly by the recommended daily dose of certain capsules and fruit concentrates, while the NRV coverage of the metal components dissolved in the tinctures is not significant.

Overall, the examined dried herbs, powder preparations, dietary supplements and extracts comply with the regulations of purity written in the VIII Hungarian Pharmacopoeia and the Food Code. The samples do not contain polluting metal components above the permissible limits, so their daily consumption should not pose any harm on the health.

4.2 Evaluation of the results of animal experiments

4.2.1 Effects of sour cherry lyophilisate consumption on the metal element compositon and redox homeostasis in rat's organs

In a short-term rat experiment, the changes in metal element content and the redox homeostasis were investigated in various tissue samples of healthy individuals and those with alimentary-induced fatty liver by the effect of sour cherry lyophilisates' consumption (Fanal (M1), Pipacs1 (M2), Újfehértói fürtös (M3)).

Based on our results, the element contents in the liver, the heart and the lung show significant differences between the sour cherry lyophilisate supplemented groups and the values measured in the healthy and the fatty liver groups without sour cherry supplementation.

In the results of the element content measured in the fatty liver groups, significantly low values ($p<0.05$) can be observed by most of the metal elements concentration (Ba, Cu, Fe, Li, Mg, Mn, Ni, P, Pb, Sr, Zn) in the group with Újfehértói fürtös (M3) treatment compared to the fatty liver group without sour cherry supplementation.

The concentration of Cu, Mn, Mg, P, Zn is consistently lower both in the sour cherry lyophilisate treated groups (M1, M2, M3) and in the fatty liver group without supplementation compared to the values of the healthy control.

A positive trend can be observed with regard to the antioxidant capacity measured in the fatty liver groups, because the induced free radical level was significantly ($p<0.05$) lower in the groups treated with sour cherry lyophilisate (fatty liver+M1: 81.97±19.46 RLU%, fatty liver+M2: 83.95±15.32 RLU%, fatty liver+M3: 85.18±28.60 RLU%) compared to the average value measured in the untreated fatty liver group (166±55 RLU%). The average H-donor activity results were higher in the M2 and M3 supplemented groups (fatty liver+M2: 54.71±7.79% inhibition, fatty liver+M3: 64.59±15.90% inhibition) compared to the fatty liver group without supplementation (38.81±16.98% inhibition). Both parameters showed simultaneously significant difference ($p<0.05$) between the fatty liver group with M3 sour cherry and the fatty liver group without supplementation. According to that the level of free radicals in the fatty liver was reduced.

The results of the element content measured in the hearts of rats, show that the consumption of Újfehértói fürtös (M3) resulted in a significant ($p<0.05$) decrease of the Cu concentration in the fatty liver group (which is usually significantly higher in diseases). Besides, the Zn concentration was increased significantly ($p<0.05$), which can presumably explain the protective effect of the active ingredients of sour cherry, which activates the immune defense processes by Zn as a secondary messenger. The significant amount of Zn may also be related to the outstanding Zn content of Újfehértó fürtös (M3). It can also be established that the Cu:Zn ratio, as an indicator of inflammation, decreased as a result of the sour cherry lyophilisate treatment in the hearts of both the healthy and fatty liver groups.

The Li concentration was greatly increased in the heart and lung of the healthy groups with the Pipacs1 (M1) supplementation compared to the control group, which can be in correlation with the high Li concentration in Pipacs1 (M1), although the correlation was not significant.

Based on the metal element content and the redox parameter results, it can be concluded that the organic active agents in cherries have a positive effect on the damaged redox homeostasis in rat liver, due to the consumption of the fatty enriched diet. The level of free radicals in rat liver were decreased and the antioxidant protection was increased by the antioxidant content in cherries, which is also supported by the results of the histology results.

There were found significant differences ($p<0.05$) in the element content measured in the organs of animals with fatty liver diet by the treatment of all three types of sour cherries compared to the group consuming only a fatty diet, while the same trend couldn't be observed in the healthy groups with or without sour cherry supplementation.

Based on the results, it can be established that the element content of the liver, the heart and the lung changed in both healthy and fatty liver individuals due to the biologically active agents (polyphenols, metal content) in different types of sour cherries. We can assume that, beside the element content of the sour cherries, the different polyphenol content can also influence the absorption of metal components in different concentrations into the tissues. Furthermore, we can establish that the organs can take up metal components from the circulation in a different way in healthy and fatty organism. Therefore, further examination of the topic is of high importance, especially in metal ion accumulating diseases (porphyria cutanea tarda, Wilson's disease), and in the treatments of diseases that require metal ion supplementation, taking into account the special dietary habits.

4.2.2 Effect of the consumption of mycotoxin contaminated food with pressed and oil form *Sylibum marianum* seed supplement on the metal element content and redox homeostasis in duck's liver

Based on the results of the redox parameters and the element content measured in ducks' livers after the 47 days long treatment, remarkable, sometimes significant differences can be observed between groups with and without SM-supplementation. The free SH-group concentration and H-donor activity in the groups with SM-supplementation are significantly ($p<0.05$) higher than in the group consuming only mycotoxin contaminated forage (MT). The lowest induced free radical level (29.65±4.62 RLU%) was measured in the feed containing MT+*Sylibum marianum* seed oil and pressed extract (SM2), which indicates the good antioxidant properties of that feed. This result correlates well with the induced free radicals measured in the liver of the MT+SM2 group (0.69±0.29 RLU%), as it is significantly lower compared to the result measured in the MT group (1.24±0.57 RLU%). The best reducing power and significantly higher SH group results ($p<0.05$) were also measured in the liver of the MT+SM2 group, which indicates a more reduced environment in the liver tissue, due to the SM supplementation.

From our results, it can be established that during the consumption of SM-supplemented mycotoxin contaminated feed, the antioxidant system of the liver was able to moderate the peroxidative processes induced by mycotoxins, partly as a result of the applied SM doses. Furthermore, the free radical damage caused by the short-term mycotoxin exposure is mitigated by the activity of endogenous antioxidant enzymes (glutathione-redox system) found in the liver.

4.3 Human study

4.3.1 Changes in the metal element content and redox homeostasis by vitamin D_3 treatment in prostate cancer patients

According to the results of the element content and redox parameters, significant differences can be observed between the groups as a result of the vitamin D_3 treatment. Based on that we can assume that the regular controlled intake of vitamin D_3 has an influence on the element content and the redox homeostasis in the patients' bodies.

As a result of the vitamin D_3 supplementation, the concentrations of micro- and macro-metal elements in erythrocytes were increased and the redox values measured in the plasma fractions were better (RLU%, H-donor activity).

In the erythrocytes of prostate cancer patients, vitamin D_3 treatment had a positive effect on the metabolism of essential metal components (Ca, Co, Cr, Mn, Mg, Ni and Zn), as their concentrations were close to the control level. However, the possibility of Li deficiency in prostate cancer patients because of the vitamin D_3 treatment requires further investigation.

The results of the H-donor activity measured in the blood plasma indicate that the vitamin D_3 treatment had a positive effect on the antioxidant system, because the results in the patient control (D_3+BK: 72.0±4.2% inhibition) and prostate cancer groups (D_3+ MKPR: 75.5±12.5 inhibition%, D_3+PR: 69.2±9.9 inhibition%) were consistently better than in the groups without vitamin D_3 treatment (BK: 63.6±12 .5 inhibition%, MKPR: 62.8±14.0 inhibition%). The free radical level was lower in the plasma of the groups with vitamin D_3 treatment (D_3+BK: 105±122 RLU%, D_3+MKPR: 71±40 RLU%, D_3+PR: 71±49 RLU%) than that of without vitamin D_3 treatment (BK: 144±157 RLU%, MKPR: 217±179 RLU%), which indicates a higher antioxidant capacity in the groups treated with vitamin D_3. Significant ($p<0.01$) difference was found between the values of the prostate cancer groups treated with vitamin D_3 and the MKPR group that did not receive vitamin D_3 treatment.

According to the results, it seems that vitamin D_3 treatment can contribute to restoring the balance of redox homeostasis, which can have a positive effect against cancer progression.

Furthermore, vitamin D_3 supplementation can increase the accumulation of Pb, Cr, and Fe in erythrocytes, and can also increase the free radical load on erythrocytes, which is presumably related to the element content.

5 Conclusions

In my thesis, I found correlations between the metal elemental content and redox homeostasis in the plant, animal, and human organisms.

The results of my research help to explore the physiological, health-preserving role of herbs, fruits, and their extracts, as well as dietary supplements, and their safe use in adjuvant therapy.

1. I have found that high Cu content negatively affects the antioxidant properties of some herbal and fruit preparations at low concentrations.
2. I have demonstrated that the daily dosage of certain food supplements from the Far East containing lingzhi mushroom, chinese caterpillar mushroom and horseradish root, as well as blueberry fruit concentrate can exceed the recommended daily amount of certain elements (Cr, Mn, Mo) by over 10 %, hence they can be considered as substantial sources of minerals.
3. In an animal experiment I proved that the polyphenols and mineral content of the applied sour cherry lyophilisate can have different effects on the metal- and the redox-homeostasis measured in animal tissues in healthy and fatty liver groups.
4. In the same animal experiment, I proved that the bioactive compounds (polyphenols, element content) in the applied sour cherry lyophilizate can moderate the damage in the fatty liver of rats due to their antioxidant effect. On the other hand, the excess antioxidant dose can temporarily suppress the endogenous antioxidant system’s activity in healthy individuals.
5. I found reducing tendency of the Ca:Mg ratio in rat liver, as a result of the sour cherry lyophilisate treatment, that correlates well with the free radical level’s reduction in fatty liver.

6. I also found a decreasing tendency of the Cu:Zn ratio in rats' heart with fatty liver, due to the sour cherry lyophilisate treatment, which approached the value of the healthy controls.
7. In a second animal experience I found that the consumption of *Silybum marianum* seed oil and pressed biomass mixed into mycotoxin contaminated feed reduces the tissue damage in duck liver caused by the mycotoxins. The bioactive compounds of *Silybum marianum* have a positive effect on the liver's metal and redox homeostasis.
8. In a human study I found an improving tendency of the redox-homeostasis measured in the plasma fractions of prostate cancer patients, by the effect of vitamin D_3 supplementation. The vitamin D_3 intake also has beneficial effect on the metabolism of the most important essential metal components (Ca, Co, Cr, Mn, Mg, Ni and Zn) in the patients' erythrocytes. However, long-term treatment with vitamin D_3 can cause Fe and Pb accumulation in the erythrocyte, which requires therapeutic consideration.

6 Publications list

6.1 Publications directly related to the topic of the dissertation

1. **Süle K**, Szentmihályi K, Szabó G, Kleiner D, Varga I, Egresi A, May Z, Nyirády P, Mohai M Jr, Blázovics A. (2018) Metal-and redox homeostasis in prostate cancer with vitamin D3 supplementation. **Biomed Pharmacother,** 105: 558-565. Scopus - Medicine (miscellaneous) SJR indikátor: **Q1**, Scopus -Pharmacology SJR indikátor: **Q2**, **IF: 3,743**
2. **Süle K,** Kurucz D, Kajari A, May Z. (2015) Investigation of metal element content of some European and Far Eastern herbs. Európai és távol-keleti gyógynövények és kivonatok fémelemtartalom-vizsgálata. **Orv Hetil**, 156: 1261-1269. Scopus -Medicine (miscellaneous) SJR indikátor: **Q3**, **IF: 0,291, *Dr. Fehér János Díj***
3. **Süle K**, Fehér E, Blázovics A, Fébel H, Papp N, Mátis E, May Z, Stefanovits-Bányai É, Szentmihályi K. (2012) Changes in metal homeostasis in experimentally induced fatty liver by the effect of sour cherry consumption. **Eur Chem Bull** 1: 360-363.
4. **Süle K**, Blázovics A, Fébel H, Papp N, Stefanovics Bányai É, Szentmihályi K. (2020) Changes in metal ion balance in the organs of healthy and fatty liver rats by the

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6.2 Publications not directly related to the topic of the dissertation

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2. Szentmihályi K, Blázovics A, May Z, Mohai Jr M, **Süle K,** Albert M, Szénási G, Sebestyén A, Máthé Cs. (2020) Metal element alteration in the lung by cisplatin and CV247 administration. Biomed Pharmacother, 128: 110-307. IF: 6,530.
3. Szentmihályi K, **Süle K**, Egresi A, Blázovics A, May Z. (2021) Metronidazole does not show direct antioxidant activity in in vitro global systems. Heliyon, 7:4 Paper e06902.
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5. Szentmihályi K, May Z, **Süle K**, Then M. (2013) Mineral element content of some herbs with antiinflammatory effect used in gastrointestinal diseases. Az emésztőrendszer betegségeiben alkalmazható, gyulladáscsökkentő hatással rendelkező néhány gyógynövény és kivonat ásványielem tartalmának jelentősége. Orv Hetil**,** 154: 538-543.
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8. Pham TS, Gönczi K, Kardos G, **Süle K**, Hegedűs L, Kállay M, Kubinyi M, Szabó P, Petneházy I, Tőke L, Jászay Zs, (2013) Cinchona based squaramide catalysed enantioselective Michael addition of α-nitrophosphonates to aryl acrylates: Enantioselective synthesis of quaternary α-aminophosphonates. **Tetrahedron Asymmetry**, 24: 1605-1614. **IF: 2,165**

6.2.1 Awards and achievements related to the topic of the dissertation

1. **Süle K**, Egresi A, Kurucz D, May Z, Blázovics A, Szentmihályi K, The importance of testing essential and conditionally essential metal ion content in commercial herbal drugs and tinctures. Kereskedelmi forgalomban lévő gyógynövénydrogok és tinktúrák esszenciális és feltételesen esszenciális fémiontartalom vizsgálatának jelentősége, Magyar Táplálkozástudományi Társaság és a BGE Kereskedelmi, Vendéglátóipari és Idegenforgalmi Kar közös szervezésében megrendezésre kerülő **"Táplálkozástudományi kutatások " című VIII. PhD konferencia, Budapest, 2018 január 25. II. helyezett.**
2. **Süle K,** Szentmihályi K, Fébel H, Papp N, Fehér E, Hegedűs A, Stefanovics Bányai É, Blázovics A, Different accumulation of metal elements from general diet in different organs of healthy rats. Táplálkozással felvett fémelemek eltérő akkumulációja a különböző szervekben egészséges patkányokban, A Magyar Táplálkozástudományi Társaság és a Szent István Egyetem Élelmiszertudományi kara közös rendezésében, „Táplálkozástudományi kutatások" című VI. PhD Konferenciája, Budapest, 2016. február 2. **Unilever special prize**
3. **Süle K,** Fehér E, Blázovics A, Fébel H, Papp N, Mátis E, Stefanovits-Bányai É, Szentmihályi K, Changes in metal homeostasis in experimentally induced fatty liver by the effect of sour cherry consumption, 4 th International Symposium on Trace Elements in the Food Chain. TEFC, Visegrád, Hungary, 2012 november 15-17, ***Young Research Award***