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# Altered element homeostasis and transmethylation ability in short-term polyphenol rich supplementation in hyperlipidemic animal model

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## ORIGINAL RESEARCH PAPER

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**ABSTRACT**

Non-alcoholic fatty liver disease is one of the most common chronic liver diseases with unclarified pathomechanism and without evidence-proven therapy. Dietary polyphenols, targeting oxidative stress, are at the center of investigations. Our aim was to examine the effects of a polyphenol rich extract on metal element homeostasis and transmethylation ability in non-alcoholic fatty liver model. A ten-day rat model

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was used (control group, hyperlipidemic group with fat-rich diet, hyperlipidemic group with fat-rich diet and polyphenol supplementation, $N = 8$ in each group). The hyperlipidemic diet increased the concentration of the majority of the elements with significantly higher contents of B, Co, Cu, Fe, Mg, Mn, Na, Ni, P, Se, Si, and Zn in the liver. Further elevation of Al, Pb, and Sn concentrations could be observed in polyphenol supplemented animals. The polyphenol supplement unexpectedly decreased the transmethylation ability of the liver (132.00 vs. 114.15 vs. 92.25 HCHO μg $g^{-1}$) further. The results emphasize the possible role of altered metal and non-metal element concentrations and decreased transmethylation ability in the pathomechanism of fatty liver disease. Dietary supplementation with natural compounds may have undesirable effect as well, there is the necessity to improve the efficacy of polyphenol formulations because of their low oral bioavailability.

**KEYWORDS**

**hyperlipidemia, fatty liver, metal and non-metal elements, transmethylation ability, redox homeostasis, polyphenol dietary supplement**

# 1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common type of chronic liver disorder with an increased risk of progression to cirrhosis, hepatocellular carcinoma formation, and end-stage liver disease (Chen and Yeh, 2021).

The earliest event is „lipotoxicity" with the liver taking a central role with the cytokine, growth factor, and adipokine regulation. In fatty liver, a decrease in transmethylation processes, supporting the optimal functioning of the glutathione redox system, can also be observed (Petroni et al., 2021).

Metal ion changes can start pathological processes; however, these pathways may also lead to alterations in metal ion concentration (Gáspár et al., 2020).

Current NAFLD therapies are narrow, so much attention has been focused on the identification of dietary substances to provide a new strategy. Polyphenols, the most significant antioxidant compounds in the human diet, can prevent oxidative stress, promote fatty acid beta-oxidation, modulate insulin resistance and *de novo* lipogenesis by acting on the activity of lipogenic enzymes and improving the expression of lipolytic proteins (Abenavoli et al., 2021).

Our aim was to examine the effect of polyphenol-containing vegetable and fruit compound concentrate on short-term hyperlipidemic animal model, with special regard to metal and non-metal element alterations and transmethylation ability contributing to redox homeostasis.

# 2. MATERIALS AND METHODS

## 2.1. Materials

Nitric acid originated from Carlo Erba (65%, Chaussée du Vexin, France), hydrogen peroxide was obtained from Lachner (30%, Beržų, Lithuania) of analytical grade. Standard solutions were ICP multi-element standards (100 mg $L^{-1}$, CPA-Chem Product, Stara Zagora, Bulgaria)

and EPA30 multielement standard (5–100 mg $L^{-1}$, Bernd Kraft GmbH, Duisburg, Germany). All other reagents were purchased from Reanal Chemical Co. (Budapest, Hungary).

Food supplement was jam-like "Vegetable and fruit colourful compound concentrates" with permission number: OÉTI 45/É that is commercially available in Hungary. Declared component composition by manufacturer: *Sambucus nigra* (berry), *Vaccinium mirtillus* (extract), *Rubus nigra* (berry), *Beta vulgaris* var. *rubra* (tuber), *Vitis vinifera* (berry), *Hippophae rhamnoides* (fruit oil), *Hippophae rhamnoides* (juice), *Hippophae rhamnoides* (berry juice), *Aronia rotundifolia* (extract), and *Hibiscus sabdariffa* (extract). Antioxidant content per 100 g of dietary supplement: total polyphenols: 2,100 mg, from this anthocyanidins 330 mg and flavonoids 30 mg, vitamin C is 48 mg and carotenoids are 2.2 mg. Carbohydrate content is 69.3%. The element content of the supplement and the percentage daily element intake by the daily consumption of rats (1 g $kg^{-1}$) calculated for a person (70 kg) in relation to Nutritional Reference Value (NRV, Yates, 2007) is demonstrated in Table 1.

The jam-like dietary supplement was mixed into the fat rich food (described below) and dried in plug form at room temperature.

## 2.2. Rat experiment

Young male Wistar albino rats ($N = 24$, 150 ± 10 g body weight) were used in this 10-day experiment. Animal house conditions were 23 °C, 12 h/12 h light/dark, 50% humidity, and *ad libitum* access to food and water. The animals were divided into three groups. The control group animals were kept on a normal diet obtained from BIOFARM PROMT Kft (BFP; Gödöllő, Hungary). In the hyperlipidemic group the animals were fed with a fat rich control diet (cholesterol 2.0%, sunflower oil 20%, cholic acid 0.5%). The hyperlipidemic+polyphenol treatment group animals were kept on a hyperlipidemic diet and treated with jam-like dietary supplement (1 g $kg^{-1}$ body weight). At the end of the 10-day diets, the animals were anesthetised with Nembutal (35 mg/body weight kg). After laparotomy, blood was collected from the abdominal vein, then the animals were exsanguinated.

Blood was collected in Vacutainer blood collection tube (Fisher Scientific, Waltham, MA, USA) with 3.2% sodium citrate. The samples were stored at 4 °C, and separeted on the same day.

*Table 1.* The element content of dietary supplement (μg $g^{-1}$) and the percentage daily element intake by the daily consumption of rats (1 g $kg^{-1}$) calculated for a person (70 kg) in relation to the Nutritional Reference Value (NRV)

| Element | Element content (μg $g^{-1}$) and element intake (%) in parenthesis | Element | Element content (μg $g^{-1}$) and element intake (%) in parenthesis |
|---|---|---|---|
| Al | 21.6 ± 1.2 (48.8) | | |
| B | 6.88 ± 0.26 (48.2) | Mg | 472 ± 12 (8.8) |
| Ba | 1.85 ± 0.05 (25.9) | Mn | 18.71 ± 0.87 (65.5) |
| Ca | 662.6 ± 8.9 (5.8) | Na | 325.4 ± 9.1 (1.1) |
| Cr | 1.01 ± 0.02 (176) | P | 501.9 ± 15.7 (5.0) |
| Cu | 1.71 ± 0.06 (11.9) | Si | 63.72 ± 2.24 (31.9) |
| Fe | 24.24 ± 1.51 (12.1) | Sr* | 3.82 ± 0.14 |
| K | 505 ± 10 (10.1) | Zn | 5.98 ± 0.19 (4.2) |

*: there is no NRV value for this element

Collected blood was centrifuged (2,500 r.p.m. for 10 min) and the supernatant was removed. Plasma was frozen at –80 °C.

The liver was removed, shredded, and washed in isotonic KCL solution, then homogenised in isotonic KCl solution (30 v/v %) in Potter-Elvehjem instrument for appr. 20 sec in cold ice surrounding. The protein content of the liver homogenate was set at 10 mg ml $^{-1}$ by the Lowry method (Lowry et al., 1951).

The experimental procedures were approved by the Government Office of Pest County, Food Chain Safety, Plant Protection and Soil Conservation Directorate, Budapest, Hungary (number of permission: 22.1/2237/003/2009).

## 2.3. Enzyme activity and lipid profile determinations from blood

Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol (CHOL), LDL-cholesterol (LDL-CHOL), and triglyceride (TG) parameters were determined by spectrophotometry on Beckman Coulter automated chemical analyser (USA).

## 2.4. Elemental analyses

Wet liver homogenate samples (0.5 g) were digested in the mixture of 3 ml $HNO_3$ (65%) and 2 ml $H_2O_2$ (30%) in an open block digestion system with 200 °C heating, then it was transferred to a 10 ml volumetric flask and filled up with high purity water (18.5 MΩ). Measurements were carried out for Al, B, Ba, Ca, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Si, Sn, Sr, and Zn with a Spectro Genesis simultaneous ICP–OES spectrometer (Spectro Analytic Instruments GmbH, Kleve, Germany) (Szentmihályi et al., 2014). Because of the use of isotonic KCl solution for the preparation, potassium concentration could not be measured.

For the selenium determination, a computer-guided TraceLab 50 type polarographic analyser (Radiometer Analytical SAS, Loveland, Colorado, USA) was used by stripping voltammetric measurement on hanging mercury (working) electrode and in the presence of reference electrode (silver/silver chloride) and counter electrode (platinum) (Szentmihályi et al., 2009).

## 2.5. Examination of transmethylation ability

The liver homogenates were treated with dimedone solution (0.05% dimedone in methanol) for 24 h, therafter this suspension was centrifuged at 1,500 *g* for 10 min at 4 °C. The clear supernatants were used for chromatographic separations, which were carried out on Silica gel 60 F254 precoated chromatoplates (Merck Co., Darmstadt, Germany) using a chloroform-methylene chloride mixture (35:65, V/V) for formaldemethone determination. Calibration curves were made by means of authentic substances at $\lambda$=265 nm for formaldemethone (Sárdi and Tyihák, 1998).

## 2.6. Statistical analysis

One way ANOVA followed by the Tukey's post-hoc test and Kruskal-Wallis ANOVA were performed to evaluate significant differences among the groups using TIBCO Statistica as a software package (Version 13.5.0.17).

## 3. RESULTS AND DISCUSSION

NAFLD is the leading etiological factor of chronic liver disorders. Obesity, impaired glucose homeostasis, and hyperlipidemia are the main risk factors, the role of oxidative stress response, mitochondrial damage, endoplasmic reticulum stress, and inflammatory cytokines are proven. Physical exercise with a Mediterranean diet are the cornerstones of the therapy together with the control of glucose homeostasis and lipid level lowering, however, evidence-based medication is missing (Abdelmalek, 2021).

In this "short term" experiment, feeding a high-fat diet for 10 days resulted in a significant increase in ALP activities of plasma liver enzymes. As a result of hyperlipidemia, significantly higher cholesterol and LDL-cholesterol concentrations were measured. The polyphenol treatment non-significantly decreased the elevated cholesterol level, however, elevated the triglyceride concentration to the control group values (Table 2).

The atherogenic diet decreased the liver concentration of Sn, however, increased concentration was detected for the majority of the measured elements, with a significant change for B, Co, Cu, Fe, Mg, Mn, Na, Ni, P, Se, Si, and Zn compared to the control group. The addition of the polyphenol supplement to the atherogenic diet significantly decreased the concentration of B, Na, and Si. Further elevation of Al, Pb, and significantly Sn concentrations could be observed in polyphenol supplemented animals compared to the ones fed atherogenic diet. The polyphenol treatment could not alter the elevated Zn, Cu, Mg, Mn, and Se concentrations in the hyperlipidemic group (Table 3).

The concentrations of most important elements in the antioxidant defense system, as Cu, Fe, Mn, Se, and Zn, increased in the liver of the hyperlipidemic group, assumably as the result of compensatory increased antioxidant enzyme defense, provoked by the atherogenic diet causing oxidative stress. However, mucosal injury and altered gut permeability was proven in fatty liver, resulting in changed element absorption and compensatory accumulation in the liver (Blázovics et al., 2020). The elevation of the Cu, Co, and Fe concentrations could further enhance the free radical production via the Fenton and Fenton-like reactions (Szentmihályi, 2019). However, they can be situated in the active center of antioxidant enzymes. The supplementation with polyphenols would have been expected to reduce their concentrations, however, their values remained almost unchanged. Since the preparation does not contain large amounts of these elements (Table 3) and despite the high dose, the intake of these elements (except for Mn) is not significant. In the case of rats consuming 1 g $kg^{-1}$ of product per day, from these elements only the Mn intake reached and even exceeded 15% of the Nutritional Reference Value (NRV, Yates, 2007) when converted to humans, signing that the product is a significant element source for Mn. Natural food supplements may contain significant amounts of carbohydrates, as the carbohydrate content of our preparation used was over 60%. Natural carbohydrates can help to increase the absorption of minerals (Holbrook et al., 1989). Similar results were found earlier in a rat experiment that applied a high-fat diet and *Cichorium intybus* extract (Kocsis et al., 2004).

The concentrations of Al, Pb, and Sn were still elevated in the dietary supplement group in comparison with the hyperlipidemic animals, indicating the existence of a provoking condition, although the high Sn concentration returned to the control value. However, the further elevation of Al and Pb concentrations in the polyphenol treated group brought unexpected results. Although a higher dose of Al could be ingested by the dietary

*Table 2.* Plasma routine parameters (liver enzyme activities and lipid profiles) in the animal groups

| Groups | ALP (U $L^{-1}$) | ALT (U $L^{-1}$) | AST (U $L^{-1}$) | CHOL (mmol $L^{-1}$) | LDL-CHOL (mmol $L^{-1}$) | TG (mmol $L^{-1}$) |
|---|---|---|---|---|---|---|
| Control | 432 ± 80[a] | 40.01 ± 7.03[a] | 84.51 ± 22.86[a] | 1.54 ± 0.02[a] | 0.106 ± 0.025[a] | 1.146 ± 0.513[a] |
| Hyperlipidemic | 908 ± 213[b] | 35.93 ± 9.14[a] | 88.29 ± 16.47[a] | 4.96 ± 1.504[b] | 0.537 ± 0.122[b] | 0.464 ± 0.136[b] |
| Hyperlipidemic + polyphenol treatment | 925 ± 177[b] | 42.33± 13.65[a] | 109.4 ± 21.86[a] | 4.42 ± 1.17[b] | 0.531 ± 0.182[b] | 1.012 ± 0.667[ab] |

Values marked with different letters mean significant differences between the groups.

*Table 3.* Element concentrations (μg $g^{-1}$ wet weight) in the wet liver homogenates of the experimental animal groups

| Elements | Control | Hyperlipidemic | Hyperlipidemic +polyphenol treatment |
|---|---|---|---|
| Al | 3.25 ± 1.09[a] | 3.41 ± 0.68[a] | 3.78 ± 0.67[a] |
| B | 15.16 ± 3.92[a] | 20.12 ±1.58[b] | 17.28 ± 1.77[a] |
| Ba | 0.019 ± 0.005[a] | 0.024 ± 0.006[a] | 0.023 ± 0.005[a] |
| Ca | 5.69 ± 1.86[a] | 7.60 ± 1.45[a] | 6.81 ± 1.87[a] |
| Co | 0.004 ± 0.001[a] | 0.007 ± 0.002[b] | 0.007 ± 0.003[ab] |
| Cr | 0.115 ± 0.044[a] | 0.242 ± 0.197[a] | 0.196 ± 0.243[a] |
| Cu | 0.213 ± 0.047[a] | 0.277 ± 0.036[b] | 0.283 ± 0.046[b] |
| Fe | 6.25 ± 2.83[a] | 8.96 ± 1.42[b] | 8.05 ± 2.55[a] |
| Li | <0.03[a] | 0.056 ± 0.006[a] | <0.03[a] |
| Mg | 6.92 ± 1.22[a] | 9.69 ± 1.12[b] | 9.71 ± 2.01[b] |
| Mn | 0.091 ± 0.019[a] | 0.140 ± 0.034[b] | 0.135 ± 0.032[b] |
| Mo | 0.109 ± 0.087[a] | 0.202 ± 0.191[a] | 0.109 ± 0.026[a] |
| Na | 24.80 ± 6.63[a] | 42.46 ± 8.74[b] | 31.68 ± 8.72[a] |
| Ni | 0.195 ± 0.049[a] | 0.447 ± 0.223[b] | 0.301 ± 0.083[b] |
| P | 127.5 ± 19.6[a] | 197.7 ± 29.8[b] | 188.6 ± 46.2[b] |
| Pb | 0.205 ± 0.073[a] | 0.242 ± 0.062[a] | 0.383 ± 0.233[a] |
| Se | 0.011 ± 0.003[a] | 0.033 ± 0.012[b] | 0.033 ± 0.011[b] |
| Si | 31.19 ± 6.30[a] | 49.69 ± 15.71[b] | 37.97 ± 3.01[c] |
| Sn | 0.195 ± 0.049[a] | 0.153 ± 0.052[b] | 0.194 ± 0.048[a] |
| Sr | 0.019 ± 0.006[a] | 0.023 ± 0.010[a] | 0.023 ± 0.007[a] |
| Zn | 1.35 ± 0.29[a] | 1.74 ± 0.36[b] | 1.70 ± 0.28[b] |

Values marked with different letters mean significant differences between the groups

supplement with a higher intake than the 15% of NRV (Table 1), the concentration of Pb was below the detection limit in the product. Likely, the elevated concentration was also due to the effect of carbohydrates. High Al content is not favourable, since its prooxidant activity was described, especially in neurodegenerative disorders. It facilitates both superoxide- and iron-driven biological oxidation. It also competes with, and substitutes Mg, Fe, and Ca in or on proteins and their co-factors, interfering with Ca-signaling (Walton, 2012). Similarly, the higher Pb level is not favourable either, as it may cause liver damage. Lead is considered a major toxic metal causing haematological, neurological, immunological, hepatic, and renal dysfunctions. Lead also leads to disruption of the anti-oxidative enzyme system, organ function, and lipid membranes of the cell causing oxidative stress (Matović et al., 2015; Amin et al., 2021).

In the case of rats consuming the dietary supplement, the element intake, converted to humans, exceeds 15% of the NRV (Yates, 2007) for other elements, as B, Ba, Cr, Si, beside Mn. Although the Cr intake is relatively high at 176% of the NRV, it does not reach the Tolerable Upper Intake Level and apparently does not affect the hepatic Cr level as in the case of B, Ba, and Si, since their concentration decreased in the hyperlipidemic group.

The role of endogenous methylation and demethylation has been confirmed in the epigenetic regulation, in posttranslational modification of proteins. Adequate dietary intake of methyl

donor groups and transmethylations is very important in different biological systems (Sárdi et al., 2009), although the methyl balance and transmethylation fluxes are not known in detail in many cellular pathways in humans (Blázovics and Sárdi, 2018).

Various epigenetic mechanisms mediated gene-environment interactions take part in the pathomechanism of NAFLD. Aberrant histone methylation profile has been proven in the process (Abdelmalek, 2021).

In our short-term hyperlipidemia animal experiment the significantly decreased HCHO values demonstrate lower transmethylation ability. The addition of dietary supplementation unexpectedly further decreased the transmethylation ability of the liver, which may be related to the unchanged concentration of high essential elements (Cu, Fe, Mn, Ni, Se, and Zn) and the unexpected Pb, Sn, and Al accumulation in the liver because of the impairment of antioxidative defense system (Fig. 1). It became increasingly evident that there is a formaldehyde cycle in biological systems in which the formation of the methyl group of L-methionine takes place through formaldehyde, and the formation of formaldehyde from S-adenosyl-L-methionine is linked to different enzymatic transmethylation reactions (Blázovics and Sárdi, 2018) (Fig. 2).

The dietary factors, polyphenols, modulate numerous physiological processes (cellular redox potential, enzymatic activity, cell proliferation, and signaling transduction pathways). However, polyphenols have low oral bioavailability, affected by the type of bioactive compounds, polarity, molecular mass, plant matrix, their solid state, the metabolic processes mediated by the liver (phase I and II metabolism), intestine, and microbiota (Ozdal et al., 2016; Gáspár et al., 2020; Luca et al., 2020; Di Lorenzo et al., 2021).

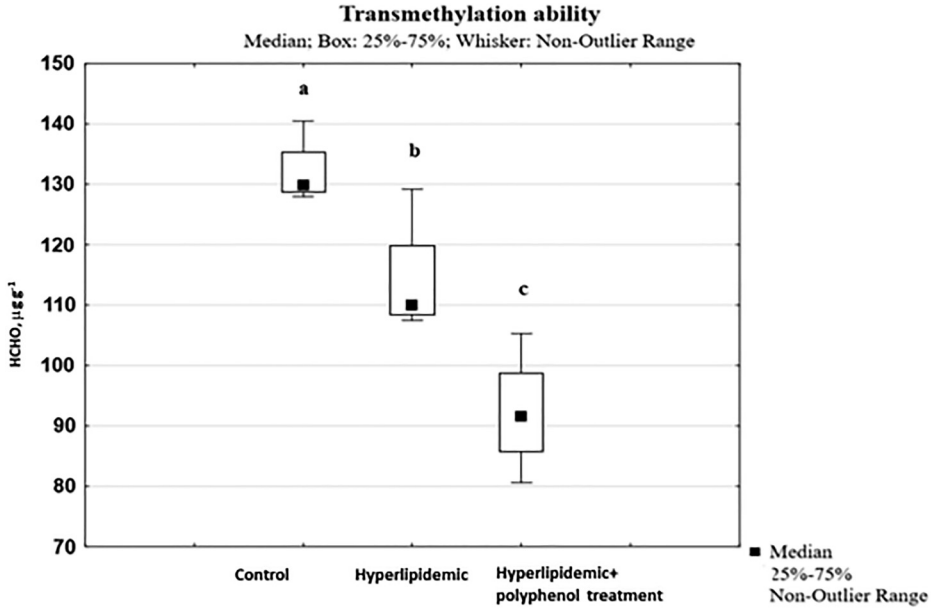


*Fig. 1.* Liver transmethylation ability in liver homogenates treated with dimedone solution in the animal groups. Values marked with different letters mean significant differences between the groups

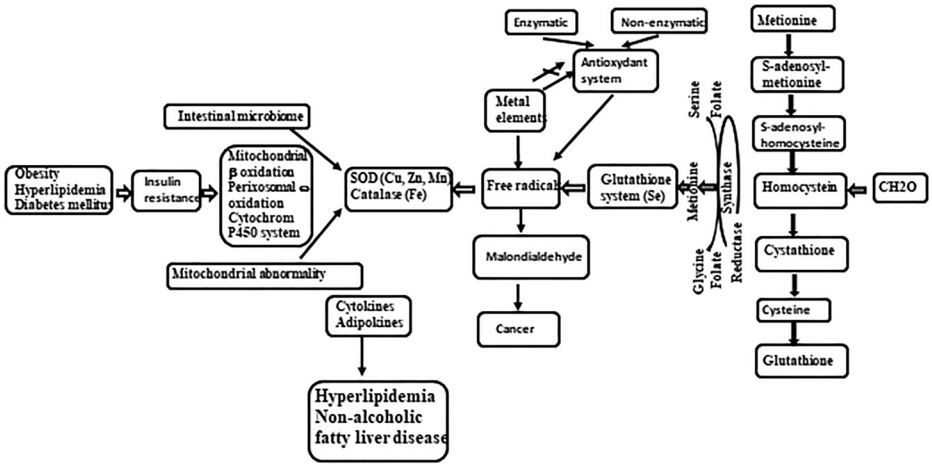


*Fig. 2.* The role of metal elements and transmethylation in maintaining redox homeostasis (Tyihák et al., 1998; Blázovics and Sárdi, 2018)

## 4. CONCLUSIONS

Our short-term hyperlipidemic animal model results emphasize the possible role of altered metal and non-metal element concentrations, as well as the decreased transmethylation ability, and the modified redox homeostasis taking part in the pathomechanism of fatty liver disease. Although the current knowledge suggests that natural dietary supplementation could help the NAFLD prevention and treatment, our short-term animal experiment results emphazise the need of further experiments to examine the applicability and roles of these dietary natural compounds as chemopreventive agents of NAFLD.

## REFERENCES

Abdelmalek, M.F. (2021). Nonalcoholic fatty liver disease: another leap forward. *Nature Reviews Gastroenterology & Hepatology*, 18(2): 85–86.

Abenavoli, L., Larussa, T., Corea, A., Procopio, A.C., Boccuto, L., Dallio, M., Federico, A., and Luzza, F. (2021). Dietary polyphenols and nonalcoholic fatty liver disease. *Nutrients*, 13(2): 494.

Amin, I., Hussain, I., Rehman, M.U., Mir, B.A., Ganaiem S.A., Ahmad, S.B., Mir, M.U.R., Shanaz, S., Muzamil, S., Arafah, A., and Ahmad, P. (2021). Zingerone prevents lead-induced toxicity in liver and kidney tissues by regulating the oxidative damage in Wistar rats. *Journal of Food Biochemistry*, 45(3): e13241.

Blázovics, A. and Sárdi, É. (2018). Methodological repertoire development to study the effect of dietary supplementation in cancer therapy. *Microchemical Journal*, 136: 121–127.

Blázovics, A., Sipos, P., Kocsis, I., Fébel, H., Kleiner, D., Szentmihályi, K., and Fehér, E. (2020). A zsírdús étrend indukálta steatosis és a bél–máj tengely változásai. (Fat rich diet-induced steatosis and changes in the gut–liver axis). *Orvosi Hetilap*, 161(35): 1456–1465.

Chen, Y.Y. and Yeh, M.M. (2021). Non-alcoholic fatty liver disease: a review with clinical and pathological correlation. *Journal of the Formosan Medical Association*, 120(1 Pt 1): 68–77.

Di Lorenzo, C., Colombo. F., Biella, S., Stockley, C., and Restani, P. (2021). Polyphenols and human health: the role of bioavailability. *Nutrients*, 13(1): 273.

Gáspár, Z., Egresi, A., Lengyel, G., Blázovics, A., Tőke, J., and Hagymási, K. (2020). Az endogén fémionok koncentrációinak változása különböző kórokú, idült májbetegségekben. (Changes in endogenous metal element concentrations in various aetiologies of chronic liver disease). *Orvosi Hetilap*, 161(22): 917–923.

Holbrook, J.T., Smith, J.C., and Reiser, S. (1989). Dietary fructose or starch: effects on copper, zinc, iron, manganese, calcium, and magnesium balances in humans. *The American Journal of Clinical Nutrition*, 49(6): 1290–1294.

Kocsis, I., Szentmihályi, K., Rapavi, R., Hubay, M., Kéry, Á., Stefanovits-Bányai, É., Szőke, É., and Blázovics, A. (2004). Effect of *Cichorium intybus* (L.) extract on the redox balance and metal ion content of fatty liver in hyperlipidemic rats. *Trace Elements and Electrolytes*, 21(4): 262–268.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1): 265–275.

Luca, S.V., Macovei, I., Bujor, A., Miron, A., Skalicka-Woźniak, K., Aprotosoaie, A.C., and Trifan, A. (2020). Bioactivity of dietary polyphenols: the role of metabolites. *Critical Reviews in Food Science and Nutrition*, 60(4): 626–659.

Matović, V., Buha, A., Đukić-Ćosić, D., and Bulat, Z. (2015). Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. *Food and Chemical Toxicology*, 78: 130–140.

Ozdal, T., Da, S., Xiao, J., Boyacioglu, D., Chen, F., and Capanoglu E. (2016). The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. *Nutrients*, 8(2): 78.

Petroni, M.L., Brodosi, L., Bugianesi, E., and Marchesini, G. (2021). Management of nonalcoholic fatty liver disease. *British Medical Journal*, 372: m4747.

Sárdi, É., Stefanovits-Bányai, É., Kocsis, I., Takács-Hájos, M., Fébel, H., and Blázovics, A. (2009). Effect of bioactive compounds of table beet cultivars on alimentary induced fatty livers of rats. *Acta Alimentaria*, 38(3), 267–280.

Sárdi, É. and Tyihák E. (1998). Change of biotransformation steps of formaldehyde cycle in watermelon plants after infection with *Fusarium oxysporum*. *Acta Biologica Hungarica*, 49(2–4), 353–362.

Szentmihályi, K. (2019). Fémelem-homeosztázis és oxidatív stressz patológiás folyamatokban. (Metal element homeostasis and oxidative stress in pathological processes). *Orvosi Hetilap*, 160(36): 1407–1416.

Szentmihályi, K., Bekő, G., Székely, E., May, Z., and Blázovics, A. (2009). New method for selenium determination in blood samples of patients with PCT and Wilson's diseases by cathodic stripping voltammetry. *Clinical Chemistry and Laboratory Medicine*, 47: S1–S409, W-B-073.

Szentmihályi, K., May, Z., Szénási, G., Máthé, C., Sebestény, A., Albert, M., and Blázovics, A. (2014). Cisplatin administration influences on toxic and non-essential element metabolism in rats. *Journal of Trace Elements in Medicine and Biology*, 28(3), 317–321.

Tyihák, E., Trézl, L., and Szende, B. (1998). Formaldehyde cycle and the phases of stress syndrome. *Annals of the New York Academy of Sciences*, 851(1): 259–270.

Walton, J.R. (2012). Aluminum disruption of calcium homeostasis and signal transduction resembles change that occurs in aging and Alzheimer's disease. *Journal of Alzheimer's Disease*, 29(2): 255–273.

Yates, A.A. (2007). Establishing new principles for nutrient reference values (NRVs) for food labeling purposes. *Nutrition Research and Practice*, 1(2): 89–93.