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ACTIVITY OF PARAOXONASE 1 AND LIPID PROFILE IN RATS FED CORNELIAN CHERRY OR CHOKEBERRY IN DIFFERENT TYPES OF DIET

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Abstract: The study investigates effects of addition of cornelian cherry fruits or chokeberry juice on: activity of paraoxonase 1, lipid profile as well as essential elements in 3 types of diet: control, fructose, high-fat in Wistar rats. For 5 weeks 9 groups of male rats were fed control, fructose, high-fat diets as well control, fructose and high-fat diets enriched with cornelian cherry fruits or chokeberry juice: Activity of paraoxonase 1 was marked both in plasma and liver. Lipid parameters, calcium and magnesium were determined in plasma.Chokeberry juice better than cornelian cherry influenced on activity of paraoxonase 1 and lipid profile in conjunction with different type of diet. Protective effect of antioxidant products such an cornelian cherry or chokeberry depends on type of diet in which is used.

Keywords: fructose diet, high-fat diet, cornelian cherry, chokeberry, paraoxonase 1

Paraoxonase 1 (PON1) is an enzyme involved in protection against oxidation, among others, two fractions of cholesterol: low-density lipoprotein (LDL) and high-density lipoprotein (HDL) by hydrolyzing activated products of phospholipids and lipid peroxides. Nevertheless, beside having an antioxidant function, paraoxonase may participate in bioactivation of drugs or cell proliferation and apoptosis (1, 2). Paraoxonase is a calcium-dependent enzyme, therefore supply of this element may indirectly effect on lipid profile (3). Similarly, magnesium by increasing excretion of fatty acids in feces as well lowering level of enzymes involved in lipogenesis, may regulate lipid metabolism too (4, 5).

Expression of genes for PON1 occurs primarily in blood and liver. Activity of this enzyme may be adjust by factors like age, state of health. For instance, in postmenopausal women activity of PON1 is reduced (6). A similar dependence was observed at cardiovascular disease (7), insulin resistance (8) or acute infection. Diet plays also no less important role in PON1 regulation. Too much supply of fructose (9) or fat (10) also lowering this enzyme. In turn, vitamin C and E, quercetin as well other antioxidant compounds contained in green tea, red wine, grapes, pomegranates, blueberries (11), cumin (12) cause opposite effect by significantly increasing activity of PON1.

Cornelian cherry and chokeberry are rich sources of antioxidants. Fruits of cornelian cherry contain among others: ascorbic acid, anthocyanins, phenols (13) in turn, chokeberry: large amount of polyphenols, such as flavonoids, anthocyanins, phenolic acids and quercetins (14). Therefore, we can suppose that consumption of these fruits can also increase PON1 activity.

In this study, we checked the influence of different type of diet such as: control, fructose, high-fat as well as addition of cornelian cherry fruits or chokeberry juice to them on activity of paraoxonase 1 (PON1) both in plasma and liver of rats. Besides, in plasma of these rats we determined changes among lipid parameters like: level of total cholesterol, HDL, triglycerides as well activity of hepatic enzymes: alanine aminotransferase (ALAT); asparagine aminotransferase (AST). We analyzed

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also changes in concentration of calcium and magnesium in plasma too. In this research model we determined the effect of food supplements rich in antioxidants (cornelian cherry fruits and chokeberry juice) at high supply of monosaccharides or saturated fatty acids as a potential factors disturbing activity of PON1 and lipid profile.

MATERIALS AND METHODS

In this experiment, male Wistar rats aged twelve-weeks and weighing 250 ± 15 g were divided into 9 groups of 6 animals each. All animals were acclimatized for 1 week before the main feeding experiment. The rats from each group were fed the following diets for 5 weeks: control feed, fructose feed, high-fat feed. The feeds from control, fructose and high-fat diet were also enriched with lyophilized fruits of cornelian cherry in amount of 10% of daily feed ration or chokeberry juice mixed with water in a volume ratio of 2 : 1. The composition of diets is shown in Table 1.

All animals had free access to feed and water. The rats were kept in a room with a constant: temperature (23^{o}C), humidity (50-60%) and 12-hour day/night cycle. At the end of the experiment after a 16-hour fast, all animals were euthanized by an intraperitoneal injection of sodium thiopental (60 mg/kg). This experiment was conducted with an approval of the I Local Ethics Committee for Animal Experiments of Jagiellonian University in Cracow, the approval number 80/2009 17.09.2009. Blood samples were taken from aorta into heparinized tubes and then centrifuged (at 2500 × g for 15 min at 4^{o}C) to obtain plasma which was kept frozen (at -80^{o}C) until further analyses. In turn, the liver was rapidly removed and immediately frozen in liquid nitrogen, and stored at -80^{o}C until further analyses. Then, before the analysis, liver tissue was homogenized in 0,15 M phosphate buffer (pH 7.4) to 5% as final concentration, using homogenizer Ultra Turrax T25 basic ultraspeed tissue grinder (12000 rotation/minute). All procedures were performed on ice. After that, homogenized tissues were centrifuged at 3500 × g for 10 min at 0-4^{o}C.

Analytical procedure

Paraoxonase 1 (PON1) enzyme activity was determined by a modified Eckerson method (15). A mixture of 0.25 M Tris buffer (pH 8) and 0.1 M paraoxon in a volume ratio of 19 : 1 (v/v) was added to homogenate samples. Then, absorbance was measured at 412 nm during 2 min. PON1 activity was estimated based on changes in concentration of substrate. Other parameters were analyzed using biochemical analyzer Alizé with standard kits (total cholesterol (TCHOL); high-density lipoprotein (HDL); triglyceride (TG); alanine aminotransferase (ALAT); asparagine aminotransferase (AST); elements: calcium (Ca) and magnesium (Mg)) from Biomerieux. Thus obtained results were compared with Control Serum 1, ODC0003 and Control Serum 2, ODC0004 (OLYMPUS). All reagents were of analytical grade and were purchased in Sigma Aldrich Chemical Company (Steinheim, Germany).

Table 1. The composition of experimental diets (percentage content of individual components).

Components	Control diet (CN) %	Fructose diet (FN) %	High-fat diet (AN) %
Starch	62	32	32
Casein	20	20	20
Oil	5.0	5.0	5.0
Lard	0	0	30
Fructose	0	30	0
Calcium carbonate	2.8	2.8	2.8
$Ca_3(PO_4)_2$	2.9	2.9	2.9
Lecithin	1.0	1.0	1.0
NaCl	0.3	0.3	0.3
Cellulose	4.7	4.7	4.7
Minerals and vitamins mix.	1.0	1.0	1.0
MgO	0.07	0.07	0.07
K_2SO_4	0.23	0.23	0.23

Table 2. Biochemical parameters.

X1-diet	X2-supplement	PON1_P [U/mg protein]	PON1_L [U/mg protein]	Ca [mM/L]	Mg [mM/L]	TCHOL [mM/L]	HDL [mg/dL]	TG [mM/L]	ALAT [U/L]	AST [U/L]
C	**N**	343.93 ± 67^{AB}	3797.8 ± 1064^{AB}	3.13 ± 0.27^{AB}	0.83 ± 0.15^{A}	1.99 ± 0.48^{A}	33.1 ± 6.6^{AB}	1.28 ± 0.54^{CD}	37.12 ± 7.04^{ABCD}	121.07 ± 56^{A}
F	**N**	386.56 ± 23^{AC}	6156.3 ± 2404^{B}	3.48 ± 0.13^{B}	1.15 ± 0.06^{BC}	1.90 ± 0.31^{A}	37.5 ± 5.9^{B}	1.75 ± 0.24^{C}	30.49 ± 8.13^{ABC}	160.72 ± 17^{A}
A	**N**	245.79 ± 63^{B}	3670.5 ± 1225^{AB}	3.03 ± 0.11^{AB}	0.81 ± 0.07^{A}	1.76 ± 0.04^{A}	25.6 ± 9.0^{B}	0.63 ± 0.20^{A}	47.29 ± 9.89^{BCD}	153.70 ± 59^{A}
C	**D**	385.59 ± 36^{AC}	3647.5 ± 1206^{AB}	3.16 ± 0.08^{AB}	0.93 ± 0.13^{AB}	1.70 ± 0.17^{A}	31.9 ± 0.7^{AB}	1.51 ± 0.27^{ABC}	31.22 ± 7.01^{ABC}	156.78 ± 27^{A}
F	**D**	504.29 ± 45^{CD}	4525.1 ± 2629^{AB}	3.37 ± 0.36^{AB}	0.95 ± 0.07^{ABC}	2.07 ± 0.43^{A}	37.5 ± 3.6^{AB}	1.82 ± 0.34^{C}	51.41 ± 23.93^{CD}	134.27 ± 57^{A}
A	**D**	247.37 ± 45^{B}	3962.5 ± 3315^{AB}	3.13 ± 0.22^{AB}	0.82 ± 0.13^{A}	1.73 ± 0.08^{A}	31.6 ± 1.7^{AB}	0.73 ± 0.27^{A}	55.38 ± 13.89^{D}	178.72 ± 40^{A}
C	**J**	613.07 ± 129^{D}	3818.9 ± 2269^{AB}	3.14 ± 0.3^{AB}	0.93 ± 0.10^{AB}	1.67 ± 0.17^{A}	40.4 ± 6.3^{A}	0.89 ± 0.21^{AB}	27.54 ± 5.18^{AB}	177.50 ± 21^{A}
F	**J**	326.56 ± 50^{AB}	1256.2 ± 207^{A}	3.27 ± 0.17^{AB}	1.10 ± 0.07^{BC}	1.69 ± 0.26^{A}	37.4 ± 5.1^{AB}	1.15 ± 0.17^{ABD}	16.93 ± 4.48^{A}	111.71 ± 50^{A}
A	**J**	409.28 ± 107^{AC}	5216.4 ± 2014^{AB}	2.98 ± 0.19^{A}	1.17 ± 0.21^{C}	2.20 ± 0.33^{A}	43.3 ± 10.6^{A}	0.78 ± 0.12^{AB}	37.07 ± 2.77^{ABCD}	166.50 ± 28^{A}

PON1_P – activity of paraoxonase 1 in plasma; PON1_L – activity of paraoxonase 1 in liver; Ca – calcium; Mg – magnesium, TCHOL – total cholesterol; TG – triglycerides; HDL – high-density lipoprotein; ALAT – alanine aminotransferase; AST – asparagine aminotransferase. (CN) – control diet without additives; (CD) – fruits of cornelian cherry with control diet; (CJ) – chokeberry juice with control diet; (FN) – fructose diet without additives; (FD) – fruits of cornelian cherry with fructose diet; (FJ) – chokeberry juice with fructose diet; (AN) – high-fat diet without additives; (AD) – fruits of cornelian cherry with high-fat diet; (AJ) – chokeberry juice with high-fat diet. Data are presented as means from independent measurements ± standard deviation (SD). Different letters in the same columns indicate significant differences according to Tukey's test ($p < 0.05$).

Statistical analysis

The results in this study were presented as mean values ± standard deviations (SD). The Shapiro-Wilks test was used to check statistical evaluations of all parameters. Statistical differences between X1-diet (control diet; fructose diet; and high-fat diet) and X2-supplement (cornelian cherry; chokeberry) were analyzed by a "two-way ANOVA" test with a biochemical parameters difference as 14 dependent variables and X1-diet, X2-supplement and X1-diet × X2-supplement. The critical significance level was set as $p < 0.05$. The "Tukey's honestly significant difference" (HSD) test was applied to assess significant differences ($p < 0.05$) between samples. The statistical analysis was conducted using the STATISTICA 10 PL software (StatSoft, Inc.).

RESULTS

The results of analyzed parameters are gathered in Table 2. The addition of cornelian cherry fruits to control diet did not statistically affect changes in analyzed parameters. In contrast, addition of chokeberry juice to that diet contributed to a significant increase in activity of PON1 in plasma and decrease of TG level.

Cornelian cherry fruits in fructose diet did not significantly affect changes in analyzed parameters. On the other hand, addition of chokeberry juice to that diet contributed to a significant decrease in activity of PON1 in liver and level of TG in plasma. The addition of cornelian cherry fruits to high-fat diet did not statistically affect changes in analyzed parameters. In contrast, chokeberry juice in that diet contributed to a significant increase in activity of PON1 in plasma as well as level of magnesium and HDL fraction.

Comparing effect of type of diet on analyzed biochemical parameters, we observed that fructose diet significantly increased concentration of magnesium compared to control diet. High-fat diet statistically decreased level of TG as compared to control diet. In case of ALAT and AST we did not observe any effect of fructose and high-fat diet as well as used supplements (cornelian cherry fruits, chokeberry juice).

DISCUSSION AND CONCLUSIONS

Inadequate diet is associated with free radicals. In turn, free radicals may lead to decrease

in activity of paraoxonase 1 (16, 17). For example, Hedrick et al. (18) and Forte et al. (19) observed that high-fat diet significantly decreased activity of this enzyme in plasma. Therefore, numerous experiments were conducted to study natural nutrients that may intensify activity of this enzyme (18-20).

In our study chokeberry juice in conjunction with high-fat as well control diet significantly increased activity of PON1 in plasma. In contrast, fruits of cornelian cherry did not significantly affect change in activity of this parameter, regardless of type of diet (Table 2). Based on our results, activity of PON1 is dependent on type of diet and as well as on natural supplement.

The liver is a central organ of detoxication. Nevertheless, oxidative stress leads to structural and functional failure of antioxidant enzymes contained in hepatocytes. Inadequate diet may be one of causes of oxidative stress. Al-Rejaie et al. (21) applied for this purpose high-cholesterol diet. Enriching that diet with rutin resulted in decreased activity of PON in this organ. Authors explain that oxidative stress has been limited in that model and was manifested in lowering activity of it (21). Similarly in our study, in rats fed fructose diet enriched with chokeberry juice, we noted a significant decrease of activity of hepatic PON1 relative to fructose diet. In case of fruits of cornelian cherry there was no significant effect on that enzyme in applied diets (Table 2). Therefore, effect may depend on doses and types of delivered antioxidants.

de Oliveira et al. demonstrated that administration of extracts from cocoa (*Theobroma cacao* L.) and cupuassu (*Theobroma grandiflorum*) which are rich in polyphenols did not modify concentration of total cholesterol in rats fed high-fat diet (22). In turn, using with that diet different types of wines which are also considered a source of polyphenols decreased level of this parameter (23). In our study, addition of fruits of cornelian cherry or chokeberry juice to different types of diet had no effect on TCHOL in plasma. However, chokeberry juice in conjunction with a high-fat diet significantly increased level of HDL compared to high-fat diet. The enrichment of fructose as well as control diets with chokeberry juice significantly decreased concentration of TG in plasma (Table 2). Suh et al. observed a similar effect when adding polyphenols as dealcoholized wines to high-fat diet (23). Supplementing rutin to high-cholesterol diet also reduced level of this parameter (21). This again indicates that a potential beneficial effect of antioxidant compounds on lipid metabolism is dependent on type of diet in which are used.

Magnesium deficiency is recognized as a factor that may additionally intensify negative effects of high-fat diet on health e.g., by increasing risk of insulin resistance (24). Insufficient concentration of this element may also disturb lipid metabolism (4, 5). In our study, enriching high-fat diet with chokeberry juice significantly increased level of Mg in plasma (Table 2). It should be also noticed that diet containing high amounts of saturated fat overloads the cardiovascular system. In turn, chokeberry is known as a natural agent strengthen function of this system (25). What is more, we noted that an excessive amount of fructose in diet significantly increased concentration of this macroelement in plasma (Table 2) and it should be aim of further research to find explanation. However, magnesium is mostly intracellular cation hence, our observation does not necessarily reflect real impact of fructose on homeostasis of Mg in cell. In case of calcium, we did not observed any change in concentration (Table 2). In activity of ALAT and ASP we did not obtain too any significant changes, what is a proof of lack of hepatotoxicity in this research model (Table 2).

Based on our results chokeberry juice effectively affects analyzed parameters compared to fruits of cornelian cherry in our study. Chokeberry juice regulates activity of PON1 and lipid profile in conjunction with different types of diet like fructose or high-fat.

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