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RESEARCH ARTICLE

Possible protective role of elderberry fruit lyophilizate against selected effects of cadmium and lead intoxication in Wistar rats

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Abstract The objective of this study was the investigation whether the administration of the elderberry fruit lyophilizate under exposure to cadmium(Cd) and (Pb) lead may protect against some effects of their toxic action in Wistar rats. Rats were fed with diets containing Cd (Cd 0.025 mg/kg b.m.) or Pb (Pb 0.025 mg /kg b.m.) with the addition of the freeze-dried elderberry fruits (BEF) in the amount of 5 %. BEF added to the diet with Cd significantly decreased the activity of AST and ALT compared to the rats fed with the control diet with Cd (C + Cd). Activity of glutathione peroxidase was significantly higher in the blood of rats fed with BEF diet compared with animals fed with BEF + Cd, BEF + Pb, and C + Pb diets. Addition of BEF to the diets with Cd or Pb significantly decreased the uric acid concentration compared to the level of this parameter in the serum of animals fed with control diets containing Cd or Pb. The level of the Cd significantly decreased in the livers of rodents fed with BEF + Cd diet as compared to the concentration of this metal in the livers of rats fed with C + Cd diet. Elderberry fruit lyophilizate did not protect against the increased concentration of Cd or Pb in kidneys and bones of experimental rats; however, it improved the function of livers and kidneys, especially of rats intoxicated with Cd.

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Abbreviations

TWI	Tolerable weekly intake
PTWI	Provisional Tolerable Weekly Intake
BEF	Elderberry fruits
C-group	fed the AIN-93G diet
C + Cd group	fed the AIN-93G diet with cadmium
C + Pb group	fed the AIN-93G diet with lead
BEF group	fed diet containing 5 % of freeze-dried elderberry fruit
BEF group	fed diet containing 5 % of freeze dry elderberry fruit and cadmium
BEF + Pb group	fed diet containing 5 % of freeze dry elderberry fruit and lead
WBC	White blood cells
RBC	Red blood cells
Ht	Hematocrit
Hb	Hemoglobin
TC	Total cholesterol
HDL	HDL-cholesterol
TAG	Triacylglycerides
LDL	LDL-cholesterol
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
TBARSs	Tiobarbituric acid reactive substances
GR	Glutathione reductase
GPX	Glutathione peroxidase
HO-1	Heme oxygenase-1
DMT1	Metal transporter protein 1
MTP1	Metal transporter protein 1

Introduction

Lead (Pb) and cadmium (Cd) are heavy metals which are nephro- and hepatotoxic in the human body; they disturb the mineral balance in an organism, as well as the activity of several enzymes. They are accumulated in the body over the years, which may affect various health problems. In many food products, the maximum content of these metals is high. According to the European Food Safety Authority (EFSA) report, the major source of Cd in daily diets are grains and grain products (bread and rolls), vegetables, vegetable products, starchy roots, and tubers (leafy vegetables, brassica vegetables, potatoes), meat and its products, fish, seafood, as well as milk and dairy products (European Food Safety Authority 2012). In the case of Pb, the major sources in diets are the following: milk and dairy products, meat and meat products, and vegetables, especially leafy vegetables (Engström et al. 2012; Martorell et al. 2011; Beccaloni et al. 2013; Martí-Cid et al. 2008; Munõza et al. 2005; Flora et al. 2003).

Cd occurs naturally in small amounts, as a natural component of the earth's crust, and the growth of its contents is due to the volcanic activity, erosion of rocks, and human activities. About 80 % of Cd emission is associated with the production of zinc. Only 10 % of global emission of cadmium to the environment is from natural sources. This heavy metal accumulates in water, especially in the sediments, as well as in soil. From these sources, it might get into the plants mainly by roots and by food chain to the human body (Beccaloni et al. 2013; Järup and Åkesson 2009; Martí-Cid et al. 2008). In 2009, the European Food Safety Agency CONTAM Panel established the tolerable weekly intake (TWI) of cadmium at the level of 2.5 μg/kg b.m. In 2011 this value was also approved by Join Experts Committee on Food Additives (www.efsa.europa.eu). Additionally in 2010, FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable monthly intake of cadmium at 25 μg/kg body weight (WHO 2010).

Cadmium migrates to the human body through the respiratory system and gastrointestinal tract and is accumulated in human body all lifelong and collected, especially in the liver and kidneys. It can be removed with urine and feces (Järup and Åkesson 2009; Julin et al. 2011; Engström et al. 2012). The International Agency for Research on Cancer (IARC) has classified cadmium, as well as cadmium compounds as carcinogenic for people (WHO 2010).

Pb is a heavy metal, widely distributed in the nature and is harmful to humans and animals at low concentrations. The contact of human organism with Pb depends mainly on environmental and occupational exposure. The main source of its emission into the environment is the steel, electrical, chemical, and energy industry. Pb gets into the organism mainly by the respiratory system, skin, and in lower amount by gastrointestinal tract with food, especially milk, cereals, vegetables, and water (Järup and Åkesson 2009; Ociepa-Kubicka and Ociepa 2012; WHO 2010). According to the EFSA CONTAM Panel, the contamination of food with Pb is low, and toxicity of it is also low or negligible in humans. For this reason, the provisional tolerable weekly intake (PTWI) is not given (www.efsa.europa.eu).

In the human body, Pb may cause dysfunction of many enzymes, in blood binds with red blood cells and with plasma proteins. It is accumulated mainly in the bones and is also dangerous for the proper development of the central nervous system in fetus and children (Ahmed et al. 2005; Flora et al. 2003).

Protein, vitamins, selected minerals, dietary fiber, and polyphenols as well as other substances presented in food, may limit the absorption of heavy metals from gastrointestinal tract or reduce their harmful effects on the body. Black elderberry fruits are particularly rich in some of these abovementioned compounds (Dawidowicz et al. 2006; Thole et al. 2006; Mikulic-Petkovsek et al. 2014). Especially, they contain anthocyanins (for example cyanidin-3-sambubioside and cyanidin-3-glucoside), flavonoids, vitamin C, pectins, as well as organic acids. The fruits may give antianelgetic, diaphoretic, and slightly laxative effect (Dawidowicz et al. 2006; Thole et al. 2006; Mikulic-Petkovsek et al. 2014; Bryła et al. 2014). In food industry, the fruits are the source of natural pigment which is used for dyeing juice, vine, and jams (Bryła et al. 2014; Kołodziej and Drożdżal 2011). In some studies, it has been reported that polyphenolic compounds, including anthocyanins or extracts from fruits rich in these substances, reduce harmful effect of Cd or Pb in animal studies (Kowalczyk et al. 2003; Liu et al. 2010; Liu et al. 2012; Gong et al. 2014; Brzóska et al. 2015a). To our best knowledge, in the available literature there is no data concerning the effect of elderberry fruits on Cd or Pb intoxication in laboratory animals.

The objective of this study was the investigation whether the administration of the elderberry fruit lyophilizate under exposure to the cadmium and Pb may protect against some effects of their toxic action in Wistar rats.

Based on the abovementioned objective, the following research hypothesis was formulated: the addition to the experimental diets of black elderberry fruit lyophilizate may protect from the unfavorable effect of Cd and Pb action in rats.

Material and methods

Elderberry fruits have been obtained from countryside areas of Malopolska, Poland. Fresh samples of black elderberry fruits were frozen and freeze-dried with lyophilizer (Christ Alpha 1-4, Gefriertrocknungsanlangen, Germany). In these prepared samples, the basic chemical composition was measured to properly balance experimental diets (Table 1). Total proteins, raw fat, total dietary fiber, and ash were measured according to

Table 1 Chemical composition of black elderberry fruit

Ingredient	g/100 g d.w.
Protein	16.20 ± 0.07
Crude fat	11.37 ± 0.29
Carbohydrates	24.05 ± 0.14
Dietary fiber	42.83 ± 0.24
Ash	5.54 ± 0.006
Polyphenolic compounds	3395.94 ± 12.80
Total anthocyanins	2049.77 ± 12.99
Cd mg/kg s.m	0.190 ± 0.00
Pb mg/kg s.m	1.065 ± 0.00

the AOAC (2006) methods (procedures no. 950.36, 935.38, 991.43, 930.05, respectively). The content of total polyphenols was determined by the Folin-Ciocalteu reagent in methanolic extract (methanol-HCl 2 % (95/5 *v*/*v*) (Sigma-Aldrich, st. Louis, MO, USA) (Benvenuti et al. 2004). The level of total anthocyanins was measured by pH differentiation method (Giusti and Wrolstad 2001).

Animal study

Six-week-old Wistar rats were purchased from Animal Husbandry Brwinów, Mazowsze, Poland. Experimental procedures were complied with the Polish Ethical Standards and approved by the I Local Ethic Committee in Kraków, Poland. Before experiment, rodents were acclimatized for 1 week on standard rodent chow. After it, animals ($n = 36$) were randomly divided into six experimental groups and fed diets based on AIN-93G diets (Reeves 1997). Group I was fed with control AIN-93G diet-C diet; group II AIN-93G diet containing 5 % of freeze-dried black elderberry fruit (BEF); group III diet containing 5 % black elderberry fruit and Cd in amount 0.025 mg Cd/kg b.m. (BEF + Cd); Group IV was fed with diet containing Cd in amount 0.025 mg Cd/kg b.m. (C + Cd); group V diet with 5 % black elderberry fruit and Pd in amount 0.025 mg Pb/kg b.m. (BEF + Pb); and group VI diet containing Pb in amount 0.025 mg Pb/kg b.m. (C + Pb;. Table 2). Elderberry fruits lyophilizate was incorporated in diets in the form of a powder. Cd (cadmium chloride, POCH, Gliwice, Poland) and Pb (lead acetate, POCH, Gliwice, Poland) were added to diets after dissolving in the distilled, deionized water. In both cases, the dose of 0.025 mg/kg b.m. was calculated for metals. The doses of heavy metals were selected based on literature (Nampoothiri and Gupta 2008; Wang et al. 2009; Pandya et al. 2010). The dose of Cd or Pb used in this study was described as the observed low toxic effect (LOEL) (Pandya et al. 2010).

During the experiment, rodents were kept individually in metabolic cages. Animals had free access to distilled, deionized water and intake of diets was in amount 10 % of body mass. Diet was given every day to each animal and the intake of it and leftover were assessed. Metals were added directly to the portions of diets. The body weight gain was recorded during the whole experiment on a weekly basis and the level of diet was increased as well as the addition of heavy metals to diets to keep the dose of heavy metals 0.025 mg/kg b.w. Urine and feces were collected in last week of the experiment (every day) to determinate the excretion of Cd, Pb, and selected trace

Table 2 Composition of experimental diets [g/kg]

Ingredient	C diet	BEF	BEF + Cd	C + Cd	BEF + Pb	C + Pb
Corn starch	532.486	502.2	502.2	532.486	502.2	532.486
Saccharose	100	100	100	100	100	100
Casein	200	200	200	200	200	200
Soybean oil	70	70	70	70	70	70
Fiber	50	32.8	32.8	50	32.8	50
Mineral mix[a]	35	35	35	35	35	35
Vitamin mix[a]	10	10	10	10	10	10
Choline chloride	2.5	2.5	2.5	2.5	2.5	2.5
TBHQ[b]	0.014	0.014	0.014	0.014	0.014	0.014
Black elderberry fruit	0	50	50	0	50	0
Cadmium [mg/kg b.m][c]	0	0	0.025	0.025	0	0
Lead [mg/kg b.m][c]	0	0	0	0	0.025	0.025

C diet AIN-93G diet, *BEF* diet containing 5 % of freeze dry elderberry fruit, *BEF + Cd* diet containing 5 % of freeze dry elderberry fruit and cadmium, *C + Cd* AIN-93G diet with cadmium, BEF + Pb diet containing 5% of freeze dry elderberry fruit and lead, *C + Pb* the AIN-93G diet with lead

[a] according to AIN-93G

[b] *tert*-butylhydroquinone

[c] Cadmium and lead were added to the portion of diets before feeding rats

elements (copper, iron, zinc). Collected samples were stored at −20 °C until the analysis.

At the end of the experiment (after 5 weeks), fasted rats ($n = 36$) were sacrificed. Blood was obtained by heart puncture and collected in test tubes. Parts of the blood samples were collected in heparinized tubes for measurements of the white blood cells (WBC), red blood cells (RBC), hematocrit (Ht), hemoglobin (Hb), thrombocytes as well as activity of glutathione peroxidase (GPX). Other parts of blood samples were collected to obtain serum by centrifugation ($1500 \times g$, 15 min.). The livers, kidneys, hearts, and femoral bones were dissected, washed in 0.9 % sodium chloride, dried with laboratory tissue paper, and weighed. Serum and tissue samples were kept frozen at −80 °C until the analysis.

Analyses in serum and blood

Selected hematological parameters, i.e., WBC, RBC, Hb, Ht as well as thrombocytes were measured with an auto analyzer (Sysmex K-4500, Sysmex Europe GmbH, Norderstedt, Germany).

Serum was analyzed for the concentration of total cholesterol-TC (cat no. Liquick Cor-CHOL60 2-204; PZ Cormay S.A. Lublin, Poland), HDL-cholesterol (cat no. HDL 2-052, PZ Cormay S.A. Lublin, Poland), and triacylglycerides-TAG (cat no. Liquick Cor-TG60 2-253, PZ Cormay S.A. Lublin, Poland). The differences between TC and HDL were used for calculations of LDL level (Friedewald et al. 1972).

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum was measured using the Alpha Diagnostic kits (Alpha Diagnostic, Warsaw, Poland; cat no. A6661-050, A6624–050, respectively). Creatinine concentration was determined with a kit (cat no. 2-232; PZ Cormay, Lublin, Poland). The level of glucose was measured in whole blood with glucometer (Accu-chek, Roche Diagnostic, Mannheim, Germany).

The concentration of tiobarbituric acid reactive substances (TBARSs) was measured with OxiTekTBARS kit (cat no. 850-287-KI01, Zeptometrix, Bufallo, NY, USA). Total antioxidant status was measured with the IMAnOx (TAS/TAC) kit (cat no KC 5200 Kit Immunodiagnostic AG, Bensheim, Germany. Uric acid concentration was measured with commercially available kit (cat no. 2-208; PZ Cormay, Lublin, Poland).

Activity of glutathione reductase (GR) and glutathione peroxidase (GPX) were measured in the serum of rats with a kit (cat no. GR 2368, RS 505, respectively; Randox Laboratories Ltd.,UK). The activity of heme oxygenase-1 (HO-1) was measured with commercial available kit (cat. no. 2-245; PZ Cormay, Lublin, Poland) for bilirubin concentration.

Crude lipid concentration in selected organs

The crude fat content was determined by the Soxhlet method with a Soxtec Avanti 2050 Auto Extraction Unit (Tecator Foss, Hillerød, Sweden), (Fortuna et al. 2003). Samples were used for measurements of crude lipids content according to the application note of the Tecator Foss (ASN 3131) with slight modification concerning drying method (Kopeć et al. 2013). Petroleum ether was used to extract fat. The livers, kidneys, and hearts were freeze-dried in lyophilizer (Christ Alpha 1–4, Gefriertrocknungsanlangen, Germany). After freeze-drying, organs were weighed, grounded, and used for analysis.

Determination of trace elements in the tested materials

After freeze-drying, about 500 mg of tested rat materials such as liver, kidney, bones, diets, and feces were digested with 6 mL of 65 % HNO_3 (65 %, Suprapure, Merck Company, Darmstad, Germany) and 1 mL H_2O_2 (30 %, Merck Company, Darmstad, Germany). The approach to the urine digestion was as follows: 25 ml of urine was dried for dry mass digestion vessel in the condition 105 °C by 24 h and then the same volume of reagents as it was in the organs were added to each single sample. Samples were digested with the use a Multiwave 3000 Microwave Digestion of Perkin Elmer according to the recommended mineralization program. The optimum microwave digestion conditions were as follows: a 10-min recovery time for the liver, kidney, foods, feces, and urine materials and a 15-min recovery time for the bones. After that step, all tested materials (samples) were held 15 min at the power 1400 W for proper digestion. After digestation, samples were transferred to 10-mL volumetric flasks using 1 % of nitric acid solution (Suprapure, 65 %).

In a prepared solution, the content of studied trace elements were determined by using atomic emission spectrometer Optima 7300 Dual View Perkin Elmer. Each sample was analyzed in two replications. If the results of the analysis of these replications differed from one another by more than 5 %, another two analyses of that sample were conducted. The validity of the analytical method was verified using a certified reference material from the Veterinary and Food Sciences in Giessen, Germany. The results obtained in this way was verified using a reference material and were within the certified values.

Gene expression

Messenger RNA (mRNA) was isolated from the livers of rats with a commercially available kit (cat no. 036-100, Total RNA Mini Plus A&A Biotechnology, Gdynia, Poland). The concentration of mRNA was measured by a spectrophotometer at an absorbance 260 and 280 nm (Multiscan Go, Thermo Scientific Electron Corporation, Wilmington, Delaware, USA). For complementary DNA (cDNA) synthesis, mRNA

was reverse transcribed with the TranScriba cDNA Synthesis Kit, (cat no. 4000-100 A&A Biotechnology, Gdynia, Poland). cDNA was subjected to real-time PCR in a reaction of a mixture containing TaqMan Gene Expression Master mix and primers with fluorescent marked starters (cat no.4369016, Life Technologies, Carlsbad, CA, USA) and starters for the following genes: glutathione reductase (Gsr), glutathione peroxidase (Gpx), heme oxygenase-1 (Hmox1), and superoxide dismutase (Sod) with fluorescent marked starters (Life Technologies, Carlsbad, CA, USA). The thermal profile of the PCR reaction included initial denaturation (15 min at 95 °C), 40 amplification cycles of denaturation (1 s at 95 °C), annealing (20 s at 60 °C), and elongation (20 s at 72 °C) with the use of the following equipment: the CFX96 Touch™ Deep Well Real-Time PCR Detection System (Bio Rad, Hercules, CA, USA). The expression rates were calculated as the normalized threshold cycle (C_T) difference between a control and a sample with the adjustment for the amplification efficiency relative to the expression level of the housekeeping gene *Sp1*.

Statistical analysis

The data was presented as mean ± SD. Two-way analysis of variance (ANOVA/MANOVA) (Statistica v. 10.0, StatSoft, Inc., Tulsa, OK, USA) was applied for testing the differences between experimental treatments. The post hoc Tukey test was used for the identification of statistically significant differences at a level of $P < 0.05$.

Results

Body gain and selected organs weight

Elderberry fruits lyophilizate had no impact on the body gain, liver, heart, and kidney weights in rats intoxicated with Cd as compared to the rats fed with C + Cd diet. Additionally, these parameters were lower in rats fed with C + Cd and BEF + Cd diets compared with the rodents fed with other experimental diets ($P < 0.05$). Rats fed with the BEF + Pb had lowest body gain compared to the rodents fed with the C diet (Table 3).

Selected biochemical parameters in blood and serum as well as gene expression

The amount of the WBC and thrombocytes were not affected by various dietary treatments ($P > 0.05$). The level of RBC was not affected by addition of the BEF to the diet containing Cd and Pb as compared to the rats fed with C + Cd and C + Pb diets. RBC significantly increased in the blood of rats fed with the BEF diet compared with the blood of animals fed with the C diet ($P < 0.05$). The lowest Hb and Ht were measured in the blood of rats fed with the BEF + Cd and BEF + Pb diets compared to rodents fed with the BEF diets (Table 4).

Lipid profile was not affected by various dietary treatments (data not shown). Activity of AST and ALT in the serum of rats fed with BEF + Cd significantly decreased compared to the activity of these enzymes in the serum of rats fed with C + Cd diet ($P < 0.05$). Creatine concentration, in the serum of rats, was not affected by various dietary treatments. Glucose level significantly decreased in the blood of rats fed with BEF + Pb diet compared to the blood of rats fed with C + Pb diet. On the other hand, glucose level was significantly higher in the blood of rats fed with the C diet in comparison to the blood of animals fed with C + Cd diet ($P < 0.05$).

TBARS and TAS level in the serum of rats was not affected by various dietary treatments. The uric acid concentration significantly decreased in the serum of rats fed with BEF + Cd and BEF + Pb compared to the level of this parameter in the serum of animals fed with the C + Cd and C + Pb diets, respectively. GR activity was significantly lower in the serum of rats fed with BEF + Cd diet as compared to rodents fed with C + Cd. GPX activity was significantly higher in the blood of rats fed with the BEF diet compared to the animals fed with the BEF + Cd, BEF + Pb, and C + Pb diets. HO-1 activity was not affected by various dietary treatments (Table 4).

The expression of Gsr, Gpx, Hmox1, and Sod genes was not affected by various dietary treatments (Table 4).

Table 3 Body gain and selected organs weight of experimental rats [g]

Treatment	C diet	BEF	BEF + Cd	C + Cd	BEF + Pb	C + Pb
Body gain	157 ± 12b	144 ± 8ab	24 ± 15c	30 ± 29c	126 ± 14a	139 ± 12ab
Liver	9.72 ± 0.72a	8.65 ± 0.34a	5.91 ± 0.81b	5.86 ± 1.62b	9.85 ± 0.74a	10.20 ± 0.79a
Heart	0.92 ± 0.09a	0.87 ± 0.09a	0.60 ± 0.13b	0.59 ± 0.15b	0.92 ± 0.11a	0.96 ± 0.08a
Kidneys[a]	2.17 ± 0.13 ac	1.88 ± 0.20c	1.25 ± 0.14b	1.27 ± 0.28b	2.50 ± 0.19a	2.33 ± 0.43a

C-group fed the AIN-93G diet, *BEF group* fed diet containing 5 % of freeze dry elderberry fruit, *BEF + Cd group* fed diet containing 5 % of freeze dry elderberry fruit and cadmium, *C + Cd group* fed the AIN-93G diet with cadmium, *BEF + Pb group* fed diet containing 5 % of freeze dry elderberry fruit and lead, *C + Pb group* fed the AIN-93G diet with lead

Values in rows with different letters (a, b, c) are significantly different, $P \leq 0.05$

[a] Weight of both kidneys

Table 4 Selected biochemical parameters level in the blood, the serum, and selected relative gene expression in the liver of experimental rats

Treatment	C diet	BEF	BEF + Cd	C + Cd	BEF + Pb	C + Pb
WBC [10^9/L]	8.32 ± 3.13a	6.98 ± 1.09a	11.37 ± 7.90a	6.72 ± 1.60a	10.73 ± 5.85a	8.63 ± 1.77a
RBC [10^12/L]	7.44 ± 0.24a	8.13 ± 0.37b	7.60 ± 0.28ab	7.65 ± 0.59ab	7.67 ± 0.27ab	7.61 ± 0.49ab
Hemoglobin [g/L]	14.71 ± 0.66b	15.28 ± 0.65b	12.37 ± 1.28a	14.52 ± 1.36b	12.35 ± 0.93a	12.45 ± 1.03a
Hematocrit [%]	42.82 ± 1.35 ac	44.97 ± 2.07c	37.67 ± 2.52b	42.77 ± 4.62 ac	38.45 ± 2.46ab	38.67 ± 2.07ab
Thrombocytes [10^9/L]	1133 ± 130a	1211 ± 140a	1215 ± 100a	1153 ± 284a	1205 ± 153a	1183 ± 106a
AST [u/L]	59.07 ± 5.96a	79.15 ± 15.36a	103.60 ± 37.32a	208.21 ± 111.30b	71.44 ± 31.25a	52.96 ± 16.32a
ALT [u/L]	21.97 ± 4.18a	17.02 ± 2.07a	31.72 ± 8.98a	56.27 ± 19.76b	29.68 ± 14.30a	24.44 ± 9.71a
Creatine [mmol/L]	61.88 ± 3.52a	59.00 ± 6.50a	61.88 ± 6.50a	53.24 ± 6.50a	59.00 ± 3.52a	60.44 ± 5.46a
Glucose [mg/dL]	121 ± 10bc	114 ± 9abc	109 ± 5ab	102 ± 15a	112 ± 15ab	132 ± 2c
TBARS [nmol/mL]	40.79 ± 5.01a	38.36 ± 19.83a	31.09 ± 11.10a	58.22 ± 47.14a	26.26 ± 6.87a	34.84 ± 4.48a
TAS [μmol/L]	368 ± 27a	390 ± 33a	427 ± 65a	418 ± 35a	359 ± 53a	365 ± 30a
Uric acid [mmol/L]	220 ± 37ab	283 ± 56abc	186 ± 41a	328 ± 97c	179 ± 60a	309 ± 49bc
GR [U/L]	139.52 ± 10.92a	148.66 ± 23.46a	180.22 ± 45.72a	248.32 ± 38.65b	139.52 ± 13.74a	134.54 ± 8.91a
GPX [U/L]	9039 ± 2734ab	10467 ± 1167b	4800 ± 1250a	7144 ± 1635ab	5346 ± 512a	5050 ± 750a
HO-1 [mmol/L]	1.74 ± 0.62a	1.86 ± 0.47a	3.02 ± 0.22a	2.50 ± 0.26a	2.15 ± 0.89a	2.85 ± 1.45a
Relative genes expression in liver [%]						
Gsr	2.22 ± 0.13a	2.13 ± 0.11a	1.99 ± 0.13a	2.04 ± 0.16a	2.01 ± 0.14a	2.06 ± 0.21a
Gpx	1.65 ± 0.10a	1.77 ± 0.08a	1.71 ± 0.11a	1.65 ± 0.12a	1.59 ± 0.10a	1.57 ± 0.18a
Hmox1	2.17 ± 0.15a	2.15 ± 0.11a	1.94 ± 0.12a	1.98 ± 0.20a	1.92 ± 0.12a	2.00 ± 0.24a
Sod	1.65 ± 0.10a	1.67 ± 0.06a	1.54 ± 0.09a	1.49 ± 0.13a	1.49 ± 0.08a	1.53 ± 0.17a

C-group fed the AIN-93G diet, *BEF group* fed diet containing 5 % of freeze dry elderberry fruit, *BEF + Cd group* fed diet containing 5 % of freeze dry elderberry fruit and cadmium, *C + Cd group* fed the AIN-93G diet with cadmium, *BEF+ Pb group* fed diet containing 5 % of freeze dry elderberry fruit and lead, *C + Pb group* fed the AIN-93G diet with lead

Values in rows with different letters (a, b, c) are significantly different, $P \leq 0.05$

[a] Weight of both kidneys

Crude lipids content in selected organs

The highest content of crude lipids was measured in the liver of rats fed with all experimental diets compared with the C diet ($P < 0.05$). Crude lipids level in the kidneys was not affected by various dietary treatments. The highest content of crude lipids in the hearts was measured in groups fed with the BEF + Cd and C + Pb diets compared with the hearts of animals fed the C + Cd diet (Table 5).

Concentration of Cd, Pb, and selected minerals in urine and feces

Elderberry fruit lyophilizate did not increase the excretion of Cd or Pb in urine of rats fed with diets containing these heavy metals. The level of Cu significantly decreased in the urine of rats fed with BEF + Cd and C + Cd diets compared to the concentration of this metal in urine of rodents fed with BEF diet. Additionally, Cu concentration was significantly lower in

Table 5 Crude lipids content in selected organs [% per d.w.]

Treatment	C diet	BEF	BEF + Cd	C + Cd	BEF + Pb	C + Pb
Liver	2.33 ± 0.89c	11.04 ± 3.67b	9.68 ± 2.58ab	8.94 ± 1.94ab	9.33 ± 0.92ab	6.99 ± 1.40a
Kidneys[a]	12.82 ± 7.09a	10.98. ± 0.49a	6.57 ± 303a	8.31 ± 3.92a	9.18 ± 1.86a	8.05 ± 3.28a
Heart	9.45 ± 2.54ab	5.95 ± 0.99ab	11.15 ± 5.99a	5.46 ± 2.54b	8.12 ± 1.81ab	11.22 ± 2.49a

Values in rows with different letters (a, b, c) are significantly different, $P \leq 0.05$

[a] Weight of both kidneys

C-group fed the AIN-93G diet, *BEF group* fed diet containing 5 % of freeze dry elderberry fruit, *BEF + Cd group* fed diet containing 5 % of freeze dry elderberry fruit and cadmium, *C + Cd group* fed the AIN-93G diet with cadmium, *BEF+ Pb group* fed diet containing 5 % of freeze dry elderberry fruit and lead, *C + Pb group* fed the AIN-93G diet with lead

urine of rats from all experimental groups in comparison to the rodents fed with the C diet. The concentration of iron and zinc in urine was not affected by various dietary treatments (Table 6).

The level of Cd and Pb in feces of rats fed with BEF + Cd and BEF + Pb diets significantly decreased compared to the rodents fed with C + Cd and C + Pb diets, respectively. The concentration of copper in feces was not affected by various dietary treatments. The significantly lowest excretion of iron with the feces was measured in all experimental groups with the exception of C + Pb group as compared to rats fed with the C diet. The concentration of zinc was significantly lower in feces of rats fed with the BEF + Cd diet compared with the animals fed with C + Cd diet ($P < 0.05$).

Concentration of Cd, Pb, and selected minerals in the liver, kidney, and femoral bone

Animals fed with BEF + Cd diet had lower concentration of Cd in the liver as compared to the rodents fed with C + Cd diet. The lowest concentration of copper was measured in the livers of rats fed with the BEF + Cd and C + Cd diets compared with the other experimental groups ($P < 0.05$). The lowest level of iron was measured in the livers of rats fed with the BEF + Cd and C + Pb diets as compared to the livers of animals fed with the C, C + Cd, and BEF + Pb diets ($P < 0.05$). The concentration of zinc significantly decreased in the livers of rats fed with BEF + Cd and C + Cd diets as compared to the other experimental diets ($P < 0.05$).

Table 6 The concentration of cadmium, lead, and selected trace elements in urine, feces, and selected organs in rats [mg/kg/d.m]

Treatment	C diet	BEF	BEF + Cd	C + Cd	BEF + Pb	C + Pb
Urine						
Cd	0.0027 + 0.003a	0.00 + 0.00a	0.491 + 0.041b	0.506 + 0.36b	0.0032 + 0.003a	0.00 + 0.00a
Pb	0.093 + 0.03a	0.055 + 0.024a	0.094 + 0.0001a	0.167 + 0.028a	0.915 + 0.29b	0.593 + 0.25b
Cu	0.276 + 0.04c	0.199 + 0.07a	0.018 + 0.001b	0.022 + 0.0008b	0.16 + 0.05a	0.125 + 0.05a
Fe	6.03 + 2.43a	2.25 + 1.70a	1.05 + 0.61a	0.83 + 0.48a	3.16 + 0.55a	4.41 + 2.27a
Zn	1.78 + 0.91a	1.09 + 0.44a	0.76 + 0.58a	0.76 + 0.51a	2.24 + 1.39a	1.92 + 0.90a
Feces						
Cd	0.433 + 0.32a	0.55 + 0.24a	342 + 251a	1597 + 430b	6.63 + 2.49a	4.924 + 4.82a
Pb	8.84 + 2.94a	9.45 + 5.78a	240 + 176a	14.86 + 8.93a	1390 + 983b	2524 + 192c
Cu	55.22 + 14.73a	29.17 + 11.61a	54.07 + 28.41a	133.22 + 87.11a	37.49 + 19.13a	47.62 + 8.83a
Fe	464 + 66c	250 + 101ab	63.85 + 51a	236 + 157ab	246 + 177ab	390 + 62bc
Zn	433 + 61b	274 + 97abc	112 + 60c	381 + 125ab	232 + 160 ac	395 + 67ab
Liver						
Cd	0.0125 + 0.03a	0.0348 + 0.028a	126.53 + 39.78b	192.39 + 57.16c	0.044 + 0.02a	0.0518 + 0.008a
Pb	0.423 + 0.24a	0.33 + 0.09a	0.39 + 0.12a	1.31 + 0.80a	24.69 + 8.14b	17.86 + 6.75b
Cu	14.68 + 1.95a	13.44 + 1.19a	6.51 + 1.93b	5.94 + 1.69b	11.71 + 1.94a	12.18 + 1.41a
Fe	269 + 67bc	239 + 63ab	122 + 44a	381 + 27c	345 + 65bc	124 + 95a
Zn	88.55 + 4.68a	87.23 + 5.64a	167.13 + 47.02b	161.57 + 23.06b	85.14 + 8.54a	85.24 + 5.63a
Kidney						
Cd	0.00 + 0.00a	0.041 + 0.004a	162 + 48b	163 + 28b	0.050 + 0.032a	0.069 + 0.03a
Pb	2.04 + 1.12a	2.29 + 0.59a	5.05 + 2.39a	6.39 + 2.41a	74.63 + 10.69b	80.20 + 18.99b
Cu	11.34 + 1.32a	11.74 + 1.47a	7.91 + 1.46b	8.39 + 1.80bc	11.21 + 1.09 ac	13.45 + 2.33a
Fe	205 + 31a	229 + 45a	213 + 65a	262 + 43a	169 + 39a	235 + 52a
Zn	67.72 + 4.92a	80.77 + 45.95ab	117.27 + 27.74c	95.75 + 16.28bc	69.53 + 3.15ab	75.57 + 15.37ab
Bone						
Cd	0.00 + .00a	0.00 + 0.00a	1.52 + 0.51b	2.00 + 0.41b	0.00 + 0.00a	0.00 + 0.00a
Pb	2.23 + 1.33a	1.17 + 0.68a	1.26 + 0.50a	15.01 + 1.64a	318.77 + 17.65b	250.47 + 82.65b
Fe	41.51 + 6.02a	51.62 + 4.46a	41.96 + 4.39a	43.82 + 17.46a	41.79 + 5.49a	40.49 + 9.50a
Zn	117.96 + 4.09a	114.62 + 9.12a	101.79 + 16.88b	82.03 + 4.53b	110.63 + 10.46a	117.14 + 12.69a

C-group fed the AIN-93G diet, *BEF group* fed diet containing 5 % of freeze dry elderberry fruit, *BEF + Cd group* fed diet containing 5 % of freeze dry elderberry fruit and cadmium, *C + Cd group* fed the AIN-93G diet with cadmium, *BEF + Pb group* fed diet containing 5 % of freeze dry elderberry fruit and lead, *C + Pb group* fed the AIN-93G diet with lead

Values in rows with different letters (a, b, c) are significantly different, $P \leq 0.05$

Elderberry fruits lyophilizate did not decrease the level of Cd or Pb in the kidney and femoral bone of rats compared to the rodents fed with C + Cd or C + Pb diets, respectively (Table 6). The concentration of Cu significantly decreased in the kidney of rats fed with BEF + Cd diet as compared to its concentration in the kidney of rodents fed with BEF diet. The level of iron in the kidney and femoral bone was not affected by various dietary treatments. The highest level of zinc was measured in the kidney of rats fed with the BEF + Cd as well as with C + Cd diets as compared to the rodents fed with C diet ($P < 0.05$).

Copper content was not detected in femoral bones of all the experimental rats (data not shown). The concentration of zinc was significantly lower in the femoral bones of rats fed with the BEF + Cd and C + Cd diets as compared to other experimental diets.

Discussion

In this study we have found that freeze-dried elderberry fruits did not affect the body mass gain as well as selected organ weight of the rats intoxicated with Cd (Table 3). Cd posses a wide range of negative, toxic effect in the organism (Enli et al. 2010; Engström et al. 2012). Its toxic properties could cause the lower body mass gain and organ weights in rats. It has been reported that Cd can inactivate amino acids and protein (Ociepa-Kubicka and Ociepa 2012), and probably, it was the major reason of low body mass gain and lower mass of selected organs of animals during the experimental period. The elderberry fruits are rich in polyphenolic compounds, especially flavonols and anthocyanins (rutin, isoquercitin, cyanidin-3-sambubioside, cyanidin-3-glucoside). These compounds have strong antioxidant activity and may protect against the harmful effect of various toxins including heavy metals (Kowalczyk et al. 2003; Thole et al. 2006; Glińska et al. 2007). This protective effect of elderberry fruits was not found in the case of body mass gain and selected organs weight. In the available literature, there are no data concerning the effect of various plants as the rich source of anthocyanins on the body mass gain of experimental animals intoxicated with Cd. Results obtained in this study are similar to data published by Enli et al. (2010). These authors reported that intoxication of pregnant rats with Cd administrated as the $CdCl_2$ in drinking water (70 mg/L per 21 days of pregnancy) significantly decreased fetus weight and their livers as well as kidney weight compared with the control animals.

The presence of elderberry fruits in the diet containing Pb contributed to significant decrease of the body gain of experimental rats as compared to rodents fed with the C diet but this parameter was not different in rodents fed with BEF diet. It can be suggested that Pb and bioactive compounds of elderberry fruits (polyphenols, dietary fiber) could interact with nutrients and decreased bioavailability or the absorption of nutrients. Additionally, the presence of Pb could cause the higher energy expenditure and lower body mass gain. Faulk et al. (2014) have reported that body mass of mice intoxicated with Pb depends on gender and dose of this pollutant in the diet. These authors have found that male mice had higher body mass with increased content of Pb in diets compared to female mice. It has been also reported that presence of Pb in food products may decrease body mass of infants and children growth (Hernandez-Avila et al. 2002; Piomelli 2002; Little et al. 2009).

Addition of Cd and Pb to the diets containing BEF significantly decreased Hb and Ht, although iron excretion in feces in the groups fed with BEF + Cd and BEF + Pb were not changed (Table 6). On the other hand, the level of iron in the liver of rats fed diets with BEF + Cd was lower. It can be suggested that the iron has been used in the synthesis of catalase to reduce the oxidative stress caused by heavy metals or was bound by phenolic compounds. Piomelli (2002) showed that Pb affects the metabolism of iron, mainly by inhibition of some enzymes involved in the heme synthesis. In our study, a lower level of the hemoglobin and hematocrit in groups treated with Pb was shown as compared to the BEF group. We also have found that the level of Hb and Ht in rats fed with C + Cd diet was not affected as compared to animals fed with the C diet. It has been reported that presence of Cd in a diet may decrease the absorption of Fe and other bivalent metals by connection with divalent metal ion transporter 1 (DMT1) or with metal transporter protein 1 (MTP1) in enterocytes (Park et al. 2002). It gives lower accumulation of this trace element in the organism. It has been reported that Cd may affect the level of iron especially in women. In the case of men, it should be evaluated (Julin et al. 2011; Kippler et al. 2007). Results obtained in this study are different to data published by Kowalczyk et al. (2003). These authors have shown that Hb and Ht in the plasma of rats were not affected by the presence of anthocyanins and cadmium in the experimental diets. Albeit, these authors performed experiment on rats weighing 210 g at the beginning of experiment.

In contrast to the findings of other authors, we have not found any changes in lipid profile. In some studies it has been suggested that presence of products rich in anthocyanins in the experimental diets contributed to decreased lipid profile (Xia et al. 2003; Kim et al. 2013). Additionally, in the case of rodents (rats, mice) usually the atherogenic diet was used. Probably it was the major reason why in this study, lipid profile was not affected by presence of elderberry fruit in the diets. On the other hand, in this study the content of crude fat in the liver was significantly higher in the BEF group as compared to the rats fed with the C and C + Pb diets. It can be suggested that flavons and anthocyanins presented in the BEF diet could affect the lipid metabolism in the liver and more lipids were accumulated in this organ. Another possible

mechanism is connected with presence of Cd in elderberry fruits. Cadmium could also affect the lipids metabolism, and indeed, it contributed to the higher level of lipid in the liver of rats. Our results differed from data published by other authors. Seymour et al. (2008) reported that cherry tart, rich in anthocyanins, decreased the liver steatosis. Also Prangthip et al. (2013) have reported that anthocyanins rich black rice improved hyperlipidemia in streptozotocin rats.

The concentration of crude lipids was higher in the hearts of rats fed the BEF + Cd and C + Cd diet as compared to hearts of rodents fed the C diet. It can be suggested that BEF did not decrease this adverse effect.

BEF added to the diet containing Cd decreased the activity of AST and ALT, which is an important finding of our study. It could be also suggested that bioactive compounds from elderberry fruits, especially polyphenolic compounds could protect hepatocytes from damage and oxidative stress caused by Cd and increase the activity of AST as well as ALT. Additionally, polyphenolic compounds could chelate Cd and decreased its toxic effect in the liver. Similar results were published by Gong et al. (2014). These authors reported that activity of AST and ALT significantly decreased in mice intoxicated with Cd in the presence of blubbery extract in a dose-dependent manner. Claudio et al. (2016) reported that purple carrot extract (rich source of polyphenolic compounds) improved the hepatocytes degeneration caused by harmful effect of Cd in adult Wistar rats. These authors did not measure activity of AST and ALT in the serum.

There was no effect of freeze-dried elderberry fruits in the presence of Pb on activity of AST and ALT. Our results are opposite to data published by Liu et al. (2012). These authors reported that in the serum of rats intoxicated with Pb, the activity of AST and ALT significantly increased compared with the control animals. Additionally, these authors treated animals with puerarin-isoflavone which decreased this effect. In their study, rats received Pb in water solution (500 mg Pb/L), but authors did not report the amount of drank solution by rat per day.

In addition to the experimental diets, freeze-dried elderberry fruits in the presence of Cd or Pb did not affect the creatine content in the serum of rats. A high level of this biochemical parameter in the serum is the effect of kidney damage especially in acute kidney damage (Liu et al. 2010; Liu et al. 2012). On the other hand, some authors suggested that creatine is not a specific biochemical parameter in assessment of acute kidney damage and sometimes did not change (Bonventre 2007; Schrier et al. 2004). Dkhil et al. (2014) reported that the creatinine content significantly decreased in rats intoxicated with Cd ($CdCl_2$) by intraperitoneal injection in the presence of extract from *Physalis peruviana* L., which is a good source of phenolic compounds.

We did find that the level of uric acid significantly decreased in the serum of rats fed with BEF + Cd and BEF + Pb diets compared with the animals fed the with C + Cd diet as well as C + Pb diets, which is also an important finding of our studies. It can be suggested that antioxidants from BEF provide protection from oxidative stress caused by presence of Cd or Pb and synthesis of the uric acid, as the antioxidant, was lower. The high level of this biochemical can be detected also upon kidney damage (Kanellis and Kang 2005; So and Thorens 2010). Cd and Pb have strong nephrotoxic effect. They can damage renal tubules and increase oxidative stress in these organs. Cd is mainly retained in the kidneys where it can cause faster damage of renal tubules than when it is deposited in the liver. Pb may also cause acute or chronic kidney damage, but it depends on the dose of this heavy metal (Flora et al. 2003; Flora et al. 2012; Järup and Åkesson 2009). For the best knowledge of authors, in available literature there are not information concerning the effect of freeze-dried whole fruits or vegetables rich in polyphenolic compounds on Cd or Pb intoxication in animal model; usually, the extracts rich in polyphenolic compounds including anthocyanins, flavonoids, (obtained from aronia, blueberry fruits, *Physalis peruviana* L. fruit), quercitin or puerarin were used. Dkhil et al. (2014) have shown the protective effect of extract from *Physalis peruviana* L. on kidney damage caused by cadmium intoxication. Also, Liu et al. (2010) reported that the concentration of uric acid significantly decreased in rats intoxicated with Pb in the presence of quercetin. Flora et al. (2003) reported that rats intoxicated with Pb (0.1 % lead acetate administrated in drinking water) in the presence of the various antioxidants (vitamin C, vitamin E) decreased oxidative stress, and kidney damage was lower compared to the animals intoxicated with Pb.

In addition to the experimental diets, freeze-dried elderberry fruits in the presence of Cd or Pb did not affect the concentration of TBARS and TAS in the serum of rats. On the other hand, intoxication with Cd significantly increased GR activity in the serum of rats. Addition of BEF to the diet containing Cd significantly decreased GR activity and tended to decrease GPX activity. These results were not found in case of Pb intoxication in presence or absence of BEF (Table 4). It is well known that GR is necessary for regeneration of glutathione. GR and GPX with glutathione protect organism against oxidative stress caused by free radicals. It can be suggested that Cd and Pb contributed to the lower activity of GPX by interaction with selenium, inhibition of the thiol group of enzymes, or inactivation of the synthesis of these enzymes (Kozłowska et al. 2015; Ociepa-Kubicka and Ociepa 2012). It can also be suggested, that Pb did not change the level of glutathione, but Cd can inhibit thiol groups in glutathione. In addition to the experimental diets, BEF could cause regeneration of glutathione inhibited by Cd without involving GR and GPX. However, this mechanism cannot be suggested for Pb action in experimental rats. These findings were also confirmed by the analysis of the expression of antioxidant enzyme genes

(Gsr, Gpx, Hmox1, and Sod), which was not affected by the presence of elderberry fruits in diets containing heavy metals. HO-1 level tended to be higher in rats fed with the C + Cd, C + Pb as well as BEF + Cd diets. HO-1 is an enzyme which is now accepted as the mediator of cyto- and tissue protection against harmful substances, i.e., free radicals, UV, heavy metals, heme, nitric oxide, and pro-inflammatory cytokines (Paine et al. 2010; Gozzelino et al. 2010).

The addition of BEF, to the diet containing Cd, decreased the concentration of this heavy metal in feces and the liver as compared to animals fed with C + Cd diet. Additionally, the concentration of Pb in feces of rats fed with BEF + Pb diet was also lower as compared to the rats fed with C + Pb diet. The lower concentration of Cd and Pb in feces in the presence of BRF can be explained by the higher absorption of these heavy metals in the small intestine especially in the duodenum. It has been reported that Cd is absorbed in the duodenum (Park et al. 2002). It can be suggested that Cd or Pb were chelated by phenolic compounds or bound to DMT1 and MTP1 proteins in enterocytes and it caused the observed changes. It can be also suggested that dietary fiber from BRE can be fermented to short-chain fatty acids. Lower pH in the small and large intestine could be caused by higher absorption of Cd or Pb and consequently lower concentration of them in feces of animals intoxicated with these metals in presence of BRE. Albeit, more research should be performed to find the mechanism of absorption of Cd and Pb in presence of BRE. These results are different from data published by Brzóska et al. (2015b), which reported that aqueous extract of polyphenolic compounds from aronia reduced Cd absorption (in dose 5 mg/kg) from the gastrointestinal tract and concentration in the blood and kidneys. What is more, Gong et al. (2014), which used extract from blueberries as a rich source of anthocyanins, reported that concentration of Cd in the liver decreased with higher content of anthocyanins in diets of mice. Our results are similar to data published by Enli et al. (2010). These authors reported that the concentration of Cd in livers as well as in kidneys of dams and fetus intoxicated during pregnancy with this heavy metal was significantly higher compared with the control animals.

We found that the concentration of copper significantly decreased in the urine, liver, and kidneys in groups of rats fed the BEF + Cd and C + Cd diets. Copper is an essential trace element which plays important role in defense of oxidative stress. It can be suggested that copper was absorbed from intestine in lower amount in the presence of Cd. The DMT1 and MTP1 are also necessary for proper absorption of Cu. These proteins probably bind Cd in enterocytes and it could cause lower concentration of Cu in the liver, kidney as well as in urine.

In our study, we have found that the concentration of zinc significantly increased in the liver and kidneys of rats fed with the BEF + Cd and C + Cd diets as compared with rodents fed with the C diet. These results are opposite to the concentration of copper. Probably the harmful effect of Cd caused these changes. It has been reported that metallothionein, a protein which has a strong capacity to bind some metals especially Zn, Cu, and Fe to keep homeostasis of these trace elements in the organism is produced in higher amount, especially in the liver and kidney during intoxication with Cd (Park et al. 2001; Wang et al. 2009). Higher content of Zn in the liver, kidney could be the effect of higher production of metallothionein. This could also explain higher content of iron in the liver of rats fed the C + Cd diet. On the opposite, metallothionein can bind heavy metals including Cd and Pb. These compounds are toxic for cells and can cause changes in metabolism (Vásak and Hasler 2000; Park et al. 2001).

In conclusion, elderberry fruit lyophilizate did not protect against the increased concentration of Cd or Pb in kidneys and bones of experimental rats; however, it improved the function of livers and kidneys, especially of rats intoxicated with Cd.

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