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ARTICLE

Long-Term Effects of Three Commercial Cranberry Products on the Antioxidative Status in Rats: A Pilot Study

Irena Palikova,[†] Jitka Vostalova,*[,†] Adela Zdarilova,[†] Alena Svobodova,[†] Pavel Kosina,[†] Rostislav Vecera,[‡] David Stejskal,[#] Jitka Proskova,[#] Jan Hrbac,[∥] Petr Bednar,[⊥] Vitezslav Maier,[⊥] Drahomira Cernochova,[¶] Vilim Simanek,[†] and Jitka Ulrichova[†]

[†]Department of Medical Chemistry and Biochemistry, Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic, [‡]Department of Pharmacology, Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic, [#]Department of Laboratory Medicine, Central Moravian Hospital County Inc., Prostejov Hospital, Prostejov, Czech Republic, [∥]Department of Physical Chemistry, Faculty of Science, Palacky University, tr. 17. listopadu 1192/12, 771 46 Olomouc, Czech Republic, [⊥]Department of Analytical Chemistry, Faculty of Science, Palacky University, tr. 17. listopadu 1192/12, 771 46 Olomouc, Czech Republic, and [¶]Department of Histology, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic

Cranberry (*Vaccinium macrocarpon* Ait. Ericaceae) fruits and juice are widely used for their antiadherence and antioxidative properties. Little is known however about their effects on clinical chemistry markers after long-term consumption. This study was conducted to evaluate the effect of three commercial cranberry products, NUTRICRAN90S, HI-PAC 4.0, and PACRAN on the antioxidative status of rodents, divided into three experimental groups. The products were given as dietary admixtures (1500 mg of product/kg of stock feed) for 14 weeks to male Wistar rats (Groups 2−4) and a control Group 1 which received only stock feed. There were no significant cranberry treatment-related effects on oxidative stress parameters, catalase, glutathione peroxidase, glutathione reductase, glutathione transferase, superoxide dismutase, total antioxidant capacity, thiobarbituric acid reactive substances, advanced oxidation protein products, total SH-groups, or any other measured clinical chemistry markers. Hematological parameters, body weight, and food consumption were also unaffected by intake of cranberries. Only liver glutathione reductase activity and glutathione levels were significantly lower in Group 4 than in Group 1. Plasma alkaline phosphatase alone was significantly decreased in Group 2. No gross pathology, effects on organ weights, or histopathology were observed. No genotoxicity was found, and total cytochrome P450 level in liver was unaffected in all groups. The levels of hippuric acid and several phenolic acids were significantly increased in plasma and urine in Groups 2−4. The concentration of anthocyanins was under the detection threshold. The dietary addition of cranberry powders for 14 weeks was well tolerated, but it did not improve the antioxidative status in rats.

KEYWORDS: *Vaccinium macrocarpon*; powdered juice; powdered fruits; phenolic acids; flavonoids; anthocyanins; rat; urine; hippuric acid; antioxidative status

1. INTRODUCTION

Fruits of *Vaccinium macrocarpon* Ait. Ericaceae (cranberry) are of growing public interest as a functional food owing to the potential health benefits of their components. Cranberries contain mainly dietary fiber, glucose and fructose, vitamin C, organic acids, phenolic acids, flavonols, anthocyanins, and proanthocyanidins (*1*, *2*). Cranberry juice and/or fruits have long been consumed for the prevention of urinary tract infection (UTI) and as a natural antioxidant (*3*). Cranberry components inhibit the adhesion of pathogenic species of *Escherichia coli* to uroepithelial cells. According to the literature, cranberry A type proanthocyanidin trimers are responsible for the antiadherence effect (*4*, *5*) whereas flavonols and anthocyanins exhibit antioxidative and anti-inflammatory properties (*6*, *7*). The latter is also relevant to the prevention of chronic conditions such as diabetes mellitus (*8*), cardiovascular diseases (*9*), and cancer (*3*). Recently, we demonstrated in a clinical trial with healthy women that a daily dose of 1200 mg of dried cranberry juice over 8 weeks resulted in a statistically significant decrease in serum levels of advanced oxidation protein products (*2*). Advanced oxidation protein products (AOPP) are defined as a novel marker of oxidative damage and considered reliable for estimating the degree of oxidant-mediated protein damage (*10*). However, few

*Corresponding author. Tel: +420 585 632 316; fax: +420585 632 302; e-mail: j.psotova@email.cz.

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recent studies have evaluated the effect of the long-term consumption of cranberry products on antioxidative status and metabolism in healthy mammals.

The objective of this study was to assess if 14 weeks cranberry consumption through the feed could improve the antioxidative status of rats and affect plasma AOPP concentration. Three cranberry products were used with varying content of phenolics. Body and organ weights, histopathology of organs, oxidative stress parameters in blood and liver, clinical chemical and hematological markers, lymphocyte DNA damage, and total cytochrome P450 in liver were evaluated. Metabolites of phenolics in plasma, urine, liver, and feces were measured.

2. MATERIALS AND METHODS

2.1. Test Substances and Chemicals. Decas Botanical Synergies (USA) was chosen as the supplier of commercial cranberry powders (*11*). We tested NUTRICRAN90S, a water-soluble spray-dried cranberry concentrate fruit juice standardized to a minimum of 90% cranberry fruit solids and 1.4% proanthocyanidins; HI-PAC 4.0, a spray-dried cranberry fruit juice, standardized to 90% cranberry fruit solids and 4.75% proanthocyanidins; and PACRAN, a cranberry powder consisting of 100% cranberry solids, standardized to 1.5% proanthocyanidins. Our analysis of commercial cranberry products is given in **Table 1**.

2.2. Diets. The commercial diet contained wheat, soybean extract, barley, wheat bran, fishmeal, powdered yeast Vitex, powdered whey, rape expeller, Na_2CO_3, $CaHPO_4 \cdot H_2O$, NaCl, DL-methionine, H_3SiO_4, etoxiquin, citric acid, propyl gallate, vitamin A, D_3, E, $CuSO_4 \cdot 5H_2O$; declared composition per 1 kg: N-substances (210 g), fiber (42 g), fat (27 g); sugar (75 g), ash (56 g), vitamin A (18000 IU), vitamin D_3 (2000 IU), vitamin E (60 mg), Cu (18 mg) (KrMimo Tetcice, Czech Republic).

Control Diet (*Group 1*). The powdered commercial diet (800 g) was blended with microcrystalline cellulose (190 g) and magnesium stearate (10 g).

Experimental Diet 1 (*Group 2*). A dose of 1500 mg of NUTRICRAN90S was blended with the powdered commercial diet (798.5 g), microcrystalline cellulose (190 g), and magnesium stearate (10 g) to make the feed pellets.

Experimental Diet 2 (*Group 3*). A dose of 1500 mg HI-PAC 4.0 was blended with the powdered commercial diet (798.5 g), microcrystalline cellulose (190 g), and magnesium stearate (10 g) to make the feed pellets.

Experimental Diet 3 (*Group 4*). A dose of 1500 mg of PACRAN was blended with the powdered commercial diet (798.5 g), microcrystalline cellulose (190 g), and magnesium stearate (10 g) to make the feed pellets.

The diets were prepared monthly and analyzed by LC/MS periodically to confirm concentration, homogeneity, and stability of the cranberry powders in the diets. The pellets were stored in paper bags and kept dry.

2.3. Animals. The study was approved by the Ethics Committee, Ministry of Education, Czech Republic and conducted in compliance with the Experimental Animal Protection Act No. 167/1993 L.C. Male Wistar rats (= 24; 210 ± 10 g bw) were purchased from BioTest Ltd., Konarovice, Czech Republic. The rats were acclimatized one week before the experiment. They were kept in plastic cages containing dust-free sawdust, two animals per cage. On the day of treatment, the animals were 10 weeks old with a mean body weight (234 ± 2 g; n = 24). They were randomized to four groups (n = 6 per group); control - Group 1 (232 ± 18 g bw); experimental diet 1 - Group 2 (235 ± 12 g bw); experimental diet 2 - Group 3 (236 ± 10 g bw) and experimental diet 3 - Group 4 (234 ± 11 g).

During the acclimatization period and during testing, the conditions in the animal room were as follows: temperature 23 ± 2 °C (checked daily); relative humidity 30−70%; light/dark cycle: 12 h/12 h. All animals had free access to their respective diets and water. The feed consumption was checked twice a week. The health of the animals was checked daily and the body weight was monitored twice a week and prior to sacrifice.

Administration of Cranberry Powder. The animals consumed ad libitum either the control diet or the experimental diets containing 1500 ppm of cranberry powders for 100 days.

Sample Collection and Preparation. The animals were deprived of food 12 h before terminal i.m. anesthesia by fentanyl (4 μg/100 g bw), medetomidin (20 μg/100 g bw), and diazepam (0.5 mg/100 g bw). After opening the abdominal cavity, macroscopic examination of the main organs (GIT, heart, kidneys, liver, lungs and other organs) was done. The urine was collected from the animal's bladder (to prevent urine components from oxidative processes and contamination by feces) and stored at −80 °C for analysis of phenolic acids, flavonoid and anthocyanin content. The blood was collected from the aortic bifurcation into Na_2EDTA-tubes (Sarstedt, Germany) and heparin-lithium-tubes (Sarstedt, Germany). A part of the Na_2EDTA-blood was used for lymphocyte isolation for genotoxicity assay and for blood count (0.5 mL). The rest of the Na_2EDTA-blood was centrifuged at 2500*g* for 10 min at 4 °C to obtain the plasma. The plasma aliquots were stored at −80 °C for determination of phenolic and anthocyanin content and parameters of antioxidant capacity. Heparin-lithium-blood was centrifuged at 2500*g* for 10 min at 4 °C to obtain the plasma for the clinical chemistry. Na_2EDTA-erythrocytes were washed with phosphate buffered saline and stored at −80 °C for determination of oxidative stress parameters. Liver, muscle, kidney, heart, and intestine were removed, washed in cold phosphate buffered saline and the selected organs and tissues were weighted and stored at −80 °C. Livers used for detection of oxidative stress parameters and phenolic acids, flavonoid, and anthocyanin content were frozen and stored at −80 °C. Histological examination of liver, intestine, kidney, and heart was done. Fecal samples were collected three times during the study once per month for estimation of phenolic acids, flavonoid and anthocyanin content.

Table 1. Determination of Organic and Phenolic Acids, Anthocyanins, and Flavonoids in the Cranberry Powders[a]

	NUTRICRAN90S	HI-PAC 4.0	PACRAN
Organic/phenolic acids (%)	**7.342**	**6.586**	**0.598**
malonic	0.177	0.200	n.d.
citric	1.980	1.954	n.d.
malic	1.892	1.600	0.263
quinic	2.570	2.085	n.d.
protocatechuic	0.065	0.077	0.052
gentisic	0.008	0.023	n.d.
caffeic	0.010	0.014	0.006
dihydrocaffeic	0.121	0.129	0.053
chlorogenic	0.025	0.035	n.d.
p-coumaric	0.079	0.075	0.047
vanillic	0.021	0.052	n.d.
benzoic	0.372	0.311	0.160
Flavonoids (%)	**1.352**	**2.179**	**0.680**
quercetin	1.352	2.179	0.680
Anthocyanins/anthocyanidins (%)	**0.440**	**0.610**	**0.105**
cyanidin-3-arabinoside	0.024	0.070	0.012
cyanidin-3-galactoside	0.054	0.081	0.021
cyanidin-3-glucoside	0.031	0.010	0.003
cyanidin-3-pentoside	0.013	n.d.	0.001
cyanidin-3,5-dihexoside	n.d.	0.05	n.d.
delphinidin	0.008	n.d.	0.003
peonidin-3-arabinoside	0.025	0.109	0.016
peonidin-3-galactoside	0.135	0.220	0.041
peonidin-3,5-digalactoside	0.106	0.050	0.002
peonidin-3-glucoside	0.045	0.030	0.006

[a] n.d. - not determined.

2.4. Determination of Organic Acids and Phenolics in Cranberry Powders, Diet, Feces, Tissues, and Plasma. *2.4.1. Determination of Aliphatic Organic Acids*. Aliphatic organic acids were determined by capillary electrophoresis method employed with indirect spectrophotometric detection in covalently coated fused silica capillary. The choose of the background electrolyte resulted from previously published work (*12*), where the salicylic acid, as UV absorbing co-ion, was used. The analyses were carried out on the capillary electrophoresis HP 3D Agilent (Waldbronn, Germany) equipped with on-column diode array detector.

Capillary coating procedure according to the previously published work was used (*13*). The detection wavelength was set at 238 nm. Separation was done at −30 kV, the capillary was thermostatted at 25 °C. Running electrolytes were prepared by dissolving appropriate amount of salicylic acid in deionized water (18 MΩ·cm^{-1}, Millipore, MA, USA) and titrating to pH 3.5 by triethylamine.

50 mg of a each cranberry powder was dissolved in 50 mL of methanol. The sample was filtered through a 45 μm microfilter (Millipore, MA, USA) and degassed by sonication and directly injected to capillary. A mixture of standards (malic, malonic, citric, succinic, and quinic acid) at six different concentrations (50−200 mg/L) was used for calibration. The calibration dependences were linear range ($R^2 \geq 0.99$). Limit of detection was 5.3 mg/L and limit of quantification was 8.2 mg/L.

2.4.2. Determination of Phenolics and Anthocyanins

Sample Preparation. Diet, feces, and liver (0.25 g) were homogenized with a mechanical homogenizer in 2 mL of 0.12% (v/v) trifluoroacetic acid in methanol. The homogenate was vortexed vigorously for 1 min and centrifuged at 3000*g* for 10 min at 4 °C and the supernatant (1.5 mL) was evaporated under N_2 at 35 °C.

Urine (0.25 mL) was mixed with appropriate mobile phase A (0.25 mL), vortexed vigorously for 1 min and centrifuged at 13000*g* for 10 min at 4 °C. The supernatant was applied onto an HPLC column (Gemini C18, 5 μm, 110 Å, 150 × 2 mm, Phenomenex, Torrance, CA) for direct analysis.

Determination of Phenolics. Cranberry powders (44 mg) were diluted in 50 mM phosphate buffer, pH 7.0 (1.5 mL) and centrifuged (5 min, 3500*g*). Plasma was diluted with phosphate buffer (1:1). The evaporated samples of diet, feces, and liver were dissolved in a phosphate buffer (1 mL). The solutions of all samples were filtered through a Teflon membrane microfilter (porosity 0.45 μm). The sample (1 mL) was then applied on an SPE column (mixed sorbent RP/anex, Strata Screen A, 200 mg of sorbent/3 mL reservoir, Phenomenex, Torrance, CA, USA), conditioned previously with methanol and phosphate buffer. The column was washed with 3 mL of deionized water and eluted with 3 mL of methanol and subsequently with 3 mL of 1% HCl in methanol. Both eluates (methanol and 1% HCl in methanol) were united and evaporated using a N_2 stream at 35 °C.

The solid residue was dissolved in the mobile phase A (10 mM acetic acid and 5% (v/v) acetonitrile in water; volume 0.25 mL) and the solution was analyzed by LC/MS (Gemini C18, 5 μm, 110 Å column, Phenomenex, Torrance, CA) using gradient elution: mobile phase A, 10 mM acetic acid and 5% acetonitrile (v/v); mobile phase B, acetonitrile; gradient, 0−5 min, 10% (v/v) B; 5−25 min, 10−90% (v/v) B; 25−40 min, 90% (v/v) B; 40−45 min, 90−10% (v/v) B; and 45−50 min, 10% (v/v) B. The setup of ion source and ion optics was tuned using a methanolic solution of 4-hydroxybenzoic acid. A mixture of standards (protocatechuic, gentisic, 3-hydroxy-3-phenylpropionic, 3-hydroxy-4-phenylpropionic, 4-hydroxybenzoic, ellagic, 2-hydroxyphenylacetic, 3-hydroxyphenylacetic, 4-hydroxyphenylacetic, hippuric, benzoic, salicylic, rosmarinic, ferulic, caffeic, dihydrocaffeic, chlorogenic, gallic, 3-hydroxycinnamic, coumaric, vanillic, and syringic acids, quercetin, rutin, catechin, and epicatechin) at four different concentrations (0.01−10 mg/mL) was used for calibration. The calibration dependences were linear ($R^2 \geq 0.99$). Limit of detection was 1.5 μg/mL and limit of quantification was 10 μg/mL.

Determination of Anthocyanins. Cranberry powders (44 mg) were diluted in 0.01% HCl (1.5 mL) and centrifuged (5 min, 3500*g*). The plasma was diluted in 0.01% HCl (1:1). Evaporated samples of diet, feces, and liver were dissolved in 1 mL of 0.01% HCl. The solutions were applied (0.5 mL) on Strata SDB-L column for solid phase extraction (styrene-divinylbenzene copolymer, 500 mg of sorbent/3 mL reservoir, Phenomenex, CA, USA), conditioned previously with methanol and 0.01% (v/v) aqueous HCl. The column was subsequently washed with 3 mL of 0.01% HCl, and the anthocyanin dyes were eluted using 3 mL of 0.01% (v/v) HCl in methanol. The eluates were evaporated using a gentle stream of N_2 at 35 °C.

The solid residues were dissolved in the mobile phase A (0.12% trifluoroacetic acid, 5% acetonitrile) and analyzed by LC/MS using gradient elution: mobile phase A, 0.12% trifluoroacetic acid and 5% acetonitrile (v/v); mobile phase B, 0.12% trifluoroacetic acid in acetonitrile (v/v); gradient, 0−35 min, 10−90% B; 35−40 min, 90% B. Quantification of anthocyanins was done using standard solutions of cyanidine (aglycone) and malvidine-3-glucoside in a concentration range of 1−100 mg/mL. The calibration dependences were linear ($R^2 = 0.994$). The response factors of individual pigments found in cranberry powders were neglected. Limit of detection was 1.5 μg/mL and limit of quantification was 10 μg/mL.

2.5. Clinical Chemistry. On day 100 following sacrifice, sodium, potassium, chlorides, bilirubin, cholesterol, urea, creatinine, and activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholinesterase (CHE), and total protein were determined in plasma on an Advia analyzer 1650 (Bayer, USA).

2.6. Hematology. Hemoglobin, hematocrit, erythrocytes, mean volume of erythrocytes, thrombocytes, leukocytes, and differential leukocyte count were analyzed in Na_2EDTA-blood.

2.7. Parameters of Oxidative Stress. Lipid peroxidation was assessed by measuring the presence of thiobarbituric acid reactive substances (TBARS) in plasma, erythrocytes, or liver homogenates (*14*). The level of glutathione (GSH) in erythrocytes and liver homogenate was determined according to Sedlak and Lindsay (*15*) using Ellman's reagent. The plasma level of total SH-groups was determined according to Hu (*16*). An indirect spectrophotometric method used for the assessment of superoxide dismutase (SOD) activity in erythrocytes and liver homogenate was based on the generation of $O_2^{\bullet -}$ by a mixture of nitro blue tetrazolium, NADH, and phenazine methosulfate (*17*). Glutathione peroxidase (GPx) activity in erythrocytes and liver homogenate was assayed using a modification of the Tappel method (*18*). Catalase activity in erythrocytes and liver was determined according to Beers and Sizer (*19*), a method based on measuring the quantity of decomposed hydrogen peroxide over time. Advanced oxidation protein products (AOPP) in plasma were assessed spectrophotometrically at 340 nm (*20*). The plasma and liver homogenate total antioxidant capacity (TAC) was detected by cyclic voltammetry (*21*). The protein concentration was determined by the Bradford method and hemoglobin according to Evelyn (*22*).

The protein level of superoxide dismutase (SOD1/2), glutathione peroxidase (GPx), and glutathione reductase (GSHred) was detected by immunoblotting (*23*) after SDS−PAGE electrophoresis using a primary antibody against SOD1/2 (rabbit polyclonal SOD1/2; dilution 1/500), GPx (rabbit polyclonal GPx; dilution 1/500) and GSHred (rabbit polyclonal GSHred; dilution 1/500) and a secondary antibody conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Inc., Santa Cruz, USA) using chemiluminescence detection.

2.8. Total and Specific 1A1/2 Content of Cytochrome P450. Liver microsomes were isolated by ultracentrifugation and the cytochrome P450 content was measured according to Omura and Sato (*24*). The microsomes protein concentration was evaluated according to Lowry. The level of cytochrome P450 1A1/1A2 was assessed in pooled microsomal samples by immunoblotting (*23*) after SDS−PAGE electrophoresis using a primary antibody against Cyp1A1/2 (goat polyclonal IgG; dilution 1/500) and a secondary antibody conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Inc.; Santa Cruz, USA) using chemiluminescence detection.

2.9. Histopathology. Liver, heart, kidney, and ileum specimens were fixed in Baker mixture, embedded in paraffin and 7 μm thick sections were cut on a rotary microtome. Sets of histological sections were stained with hematoxylin-eosin and PAS. The histological evaluation was performed on an Olympus BX 40 light microscope (Olympus C&S s.r.o., Prague, Czech Republic).

2.10. Detection of Single-Stranded DNA Breaks. DNA breaks were measured in peripheral lymphocytes using an alkaline version of Comet assay (*25*). Lymphocytes were isolated on Histopaque 1077 gradients. DNA damage was analyzed after staining with ethidium bromide on an Olympus IX70 fluorescence microscope (Olympus C&S s.r.o., Prague, Czech Republic). One hundred cells per slide were analyzed and divided into four classes of DNA damage standard scale. Total DNA damage was calculated: (number of cells in class 0 × 0 + number of cells in class 1 × 1 + number of cells in class 2 × 2 + number of cells in class 3 × 3 + number of cells in class 4 × 4)/100. The maximal damage was set at value of 400.

2.11. Statistical Analysis. All values were expressed as a mean ± SD and ANOVA was used to analyze the data ($p < 0.05$). A regression analysis (logarithmic regression model) was used to test the significance of differences in weight gain in the four groups of rats ($p < 0.05$).

3. RESULTS

3.1. Cranberry Powder Analysis. The organic acids, flavonoids, and anthocyanins concentrations in commercial cranberry powders NUTRICRAN90S, HI-PAC 4.0, and PACRAN were analyzed by LC/MS and capillary electrophoresis (**Table 1**). Anthocyanin/anthocyanidin content was the highest in HI-PAC 4.0.

3.2. Diet Analysis. The concentration, homogeneity, and stability of phenolic acids, flavonoids, and anthocyanins in the prepared diets were periodically analyzed by LC/MS. The sum of phenolic acids in diets was 0.3 ± 0.4 μg/g (Group 1), 5.5 ± 0.9 μg/g (Group 2), 8.2 ± 0.5 μg/g (Group 3), 6.9 ± 0.2 μg/g (Group 4), respectively. The benzoic acid concentration in diets was 1.0 ± 0.5 μg/g (Group 1), 2.0 ± 0.9 μg/g (Group 2), 5.0 ± 1.0 μg/g (Group 3), 3.5 ± 1.1 μg/g (Group 4), respectively. Flavonoids quercetin, rutin, catechin, and epicatechin were found only in traces. The concentration of anthocyanins in diet was 6.6 ± 1.6 μg/g (Group 2), 9.2 ± 2.5 μg/g (Group 3), or 1.5 ± 0.5 μg/g (Group 4).

3.3. Feed Consumption. The daily diet consumption was 72 g per cage (two animals) on average. The daily dose of phenolic acids for Groups 1−4, respectively, changed from 0.05 (Group 1), 0.81 (Group 2), 1.23 (Group 3), and 1.0 mg/kg/day (Group 4) at the beginning of the experiment to 0.02 (Group 1), 0.36 (Group 2), 0.54 (Group 3), and 0.45 mg/kg/day (Group 4) at the end of the experiment; that is, average doses over 100 days were 0.03 (Group 1), 0.47 (Group 2), 0.72 (Group 3), and 0.60 mg/kg/day (Group 4), respectively.

The daily dose of anthocyanins for Groups 2−4, respectively, fell from 0.97 (Group 2), 1.37 (Group 3), and 0.16 mg/kg/day (Group 4) at the beginning to 0.44 (Group 2), 0.61 (Group 3), and 0.07 mg/kg/day (Group 4) at the end of the experiment; that is, average doses over 100 days were 0.57 (Group 2), 0.81 (Group 3), and 0.09 mg/kg/day (Group 4), respectively. No significant alteration in feed consumption was found in any group.

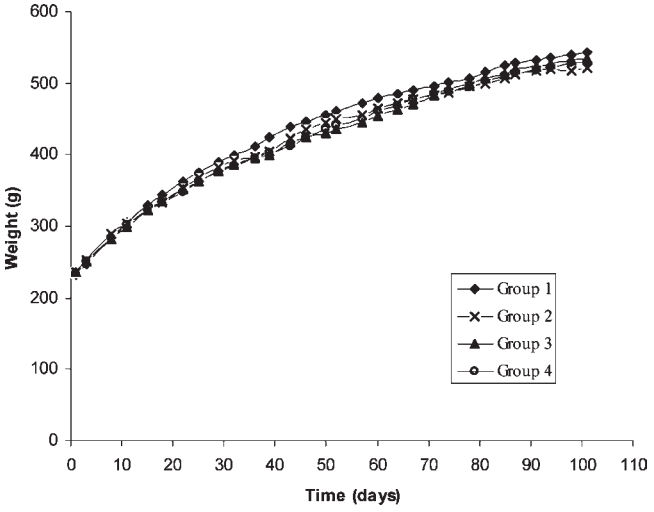

Figure 1. Effect of cranberry powders on body weight of rats. The animals consumed ad libitum either standard diet (Group 1), or experimental diets containing 1500 ppm cranberry powders (Group 2−4), for 100 days as described in Section 2. Body weights were monitored twice per week and prior the sacrifice.

3.4. Body and Organ Weights. The weight gain curves displayed very similar courses for all groups (**Figure 1**). There was no significant difference in weights of liver, right or left kidney, or heart for any of the groups (**Table 2**). No health impairments were found.

3.5. Clinical Chemistry Parameters. The following parameters were investigated: sodium, potassium, chloride, bilirubin, cholesterol, urea, creatinine, ALT, AST, ALP, CHE, and total protein (**Table 3**). The ALP level of Group 2 was significantly lower than control group (Group 1) ALP level.

3.6. Hematological Parameters. No effect of experimental diets was found on monitored hematological parameters, that is, hemoglobin, hematocrit, erythrocytes, mean erythrocyte volume, thrombocytes, leukocytes, or differential leukocyte count (**Table 4**).

3.7. Oxidative Stress Parameters and Content of Cytochrome P450. Some significant effects of tested substances on the selected parameters of oxidative stress in blood and liver were found. In Group 4 liver GSHred activity and glutathione level was significantly lower than Group 1 (**Table 5**). AOPP level in plasma was same at the beginning and the end of experiment in all groups of rats. The experimental diets had no effect on liver protein level of SOD1/2, GPx, or GSHred or on microsome levels of CYP1A1/2 proteins (data not shown).

3.8. Genotoxicity. To examine the genotoxicity of cranberry powders, formation of single-stranded DNA breaks in peripheral lymphocytes was analyzed by Comet assay. There were no significant differences between groups. The values for Group 1−4 were 3.8 ± 5.2, 4.4 ± 4.0, 2.3 ± 3.4, and 9.7 ± 10.2, respectively.

3.9. Microstructure of Heart, Ileum, Kidney and Liver. *Heart*: Heart tissue was free of pathological findings in all groups.

Ileum: There was no evidence of the infiltrate in any of the specimens. The ilea in all animals were similar.

Kidney: Changes in the number of mitoses in Lieberkühn's crypts were not recorded. No edema or dilatation of lymphatic capillaries were noted in the samples.

Table 2. Rat Body and Organ Weights on Day 100[a]

weight (g)	Group 1	Group 2	Group 3	Group 4
body	542.8 ± 45.9	521.3 ± 25.6	533.7 ± 28.1	529.5 ± 25.2
liver	17.33 ± 2.14	15.58 ± 1.66	16.59 ± 1.61	14.74 ± 1.29
right kidney	1.51 ± 0.12	1.48 ± 0.11	1.57 ± 0.11	1.38 ± 0.12
left kidney	1.54 ± 0.09	1.50 ± 0.10	1.57 ± 0.10	1.37 ± 0.11
heart	1.23 ± 0.14	1.18 ± 0.14	1.27 ± 0.12	1.28 ± 0.15

organ weight/body weight (%)	Group 1	Group 2	Group 3	Group 4
liver	3.21 ± 0.41	2.99 ± 0.32	3.10 ± 0.17	2.78 ± 0.15
right kidney	0.28 ± 0.02	0.28 ± 0.03	0.29 ± 0.01	0.26 ± 0.02
left kidney	0.29 ± 0.02	0.29 ± 0.03	0.29 ± 0.01	0.26 ± 0.02
heart	0.23 ± 0.02	0.23 ± 0.02	0.24 ± 0.02	0.24 ± 0.03

[a] The animals were sacrificed on day 100 as described in Section 2. Body and organ weights were determined and are expressed as means ± SD of six animals per group. Organ weights were normalized for body weight (lower part of **Table 2**).

Liver: No differences in microstructure were revealed.

Selected microphotographs of the heart, ileum, kidney, and liver of all tested groups of animals are shown in **Figure 2**. In all tissues of experimental animals morphological findings corresponded to those of control animals.

3.10. Distribution of Phenolic Acids, Flavonoids, and Anthocyanins. At the end of the 100-day toxicity experiment, feces, plasma, urine, and liver were analyzed for the phenolic acid and anthocyanin content (**Table 6**). Animals from Groups 2, 3, and 4 had significantly higher concentrations of phenolic acids in plasma, urine, feces, and liver than controls (Group 1). The levels of anthocyanins in plasma, feces, urine, and liver were under the limit of quantification (10 μg/mL; **Table 6**). The main metabolite of phenolic acids, benzoic acid, flavonoids, anthocyanins, and probably proanthocyanidins seems to be hippuric acid which was significantly elevated in the urine (**Figure 3**).

Table 3. Clinical Chemistry Parameters in Plasma[a]

parameter	unit	Group 1	Group 2	Group 3	Group 4
sodium	mmol/L	142 ± 1	142 ± 2	141 ± 1	142 ± 2
potassium	mmol/L	3.7 ± 0.3	3.8 ± 0.2	3.9 ± 0.1	3.8 ± 0.2
chloride	mmol/L	101 ± 2	100 ± 2	100 ± 1	100 ± 2
bilirubin	μmol/L	1.3 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.3 ± 0.3
cholesterol	μmol/L	2.0 ± 0.8	1.8 ± 0.3	1.7 ± 0.4	1.8 ± 0.1
urea	mmol/L	6.5 ± 0.8	6.7 ± 0.7	6.6 ± 0.7	6.8 ± 1.0
creatinine	μmol/L	61.0 ± 2.5	62.5 ± 5.5	67.8 ± 21.3	60.7 ± 2.9
ALT	μkat/L	1.2 ± 0.3	1.3 ± 0.4	1.5 ± 0.5	0.9 ± 0.8
AST	μkat/L	1.3 ± 0.2	1.4 ± 0.4	1.7 ± 0.5	1.1 ± 0.1
ALP	μkat/L	1.9 ± 0.2	1.5 ± 0.3[b]	2.1 ± 0.4	1.6 ± 0.3
CHE	μkat/L	4.2 ± 1.2	4.8 ± 1.2	4.7 ± 0.8	3.2 ± 0.8
total protein	g/L	73.0 ± 2.1	71.7 ± 4.2	71.8 ± 2.1	73.2 ± 3.4

[a] On day 100 animals were sacrificed. Values are expressed as means ± SD of six animals per group. [b] The value is significantly different from control ($p^* < 0.05$).

Table 4. Hematological Parameters[a]

parameter	unit	Group 1	Group 2	Group 3	Group 4
hematocrit	l	0.44 ± 0.04	0.44 ± 0.03	0.44 ± 0.03	0.43 ± 0.03
erythrocytes	T/l	8.7 ± 0.7	8.6 ± 0.7	8.5 ± 0.6	8.4 ± 0.7
mean volume of erythrocytes	fl	51.3 ± 1.8	52.2 ± 1.2	51.8 ± 1.0	51.3 ± 1.2
leukocytes	G/l	7.6 ± 2.4	7.3 ± 1.9	7.0 ± 1.4	5.9 ± 1.7
neutroph segment	l	0.17 ± 0.07	0.17 ± 0.08	0.14 ± 0.03	0.15 ± 0.06
lymphocytes	l	0.79 ± 0.07	0.80 ± 0.08	0.84 ± 0.08	0.83 ± 0.06
thrombocytes	l	868 ± 265	1064 ± 158	854 ± 223	795 ± 225
hemoglobin	g/l	157 ± 12	157 ± 7	159 ± 9	150 ± 10

[a] On day 100 the animals were sacrificed. The following parameters listed in the table were investigated. Values are expressed as means ± SD of six animals per group.

4. DISCUSSION

A major goal of the present study was to assess the effect of three commercial cranberry powders on the antioxidative status in rats and if the AOPP plasma level is affected. We used a cranberry powder daily dose (mg/kg/day) 10 times higher than in our study in healthy women (*2*). All animals remained healthy during the 100-day study. The daily consumption of feed (36 ± 4 g) was comparable in all groups. No treatment diet related changes to body or organ weight were found (**Figure 1**, **Table 2**). No histopathological changes were manifested in selected organs (**Figure 2**). The experimental diets contained much higher amounts of phenolic acids, benzoic acid and flavonoids than the control diet. Of phenolic acids, ferulic, caffeic, dihydrocaffeic, and benzoic acid predominated. Protocatechuic, gentisic, 2-hydroxyphenylacetic, salicylic, rosmarinic, chlorogenic, gallic, 3-hydroxycinnamic, and vanillic acids were found in traces. Phenolic acids in diet are known to be absorbed from GIT in stomach or intestine, free or in glycoside form. In the organism, the phenolic acids are transformed into more soluble derivatives and excreted in urine. Some dietary phenolic acids can be metabolized by gut microflora and other phenolic acids are formed, mainly benzoic acid derivatives that can be absorbed and found in plasma (*26*, *27*). Phenylpropionic, benzoic or their hydroxy-derivatives and above all hippuric acid are the main phenolic acid metabolites in the body. Phenolic acids and their metabolites were found in liver, urine, and feces in different quantities but no accumulation was observed. In liver, higher amounts of hippuric, caffeic, and benzoic acid and only traces of rosmarinic, dihydrocaffeic, chlorogenic, and gallic acid were detected. Plasma contained mainly hippuric acid and chlorogenic acid and traces of benzoic acid. In the feces of experimental animals, hippuric, dihydrocaffeic, 4-hydroxyphenylacetic, ferulic, gallic, and benzoic acid predominated, and protocatechuic, gentisic, ellagic, 2-hydroxyphenylacetic, salicylic, 4-hydroxybenzoic, rosmarinic, caffeic, chlorogenic, 3-hydroxycinnamic, and

Table 5. Oxidative Stress Parameters and Cytochrome P450 in Plasma, Erythrocytes, and Liver[a]

parameter	unit	Group 1	Group 2	Group 3	Group 4
TAC[a]	μA	5.76 ± 1.42	5.96 ± 1.89	4.17 ± 1.28	5.28 ± 2.34
SH-groups[a]	mmol/g[d]	3.27 ± 0.57	3.79 ± 3.53	3.68 ± 1.77	3.32 ± 0.88
TBARS[a]	nmol/g[d]	127.7 ± 7.7	130.3 ± 20.4	131.9 ± 13.4	136.4 ± 12.0
AOPP[a]	mmol/g[d]	0.61 ± 0.13	0.51 ± 0.14	0.56 ± 0.19	0.67 ± 0.09
GSH[b]	μmol/g[e]	13.11 ± 2.31	12.99 ± 4.01	11.90 ± 1.6	14.44 ± 4.29
TBARS[b]	nmol/g[e]	0.49 ± 0.08	0.49 ± 0.06	0.42 ± 0.04	0.41 ± 0.08
GPx[b]	μmol/min/g[e]	130.6 ± 20.2	121.8 ± 29.0	129.7 ± 37.6	113.5 ± 24.4
GST	μmol/min/g[e]	9.57 ± 1.77	8.38 ± 1.64	7.93 ± 1.62	8.75 ± 1.23
SOD[b]	U/g[e]	2.34 ± 0.49	1.97 ± 0.65	1.98 ± 0.50	2.08 ± 0.34
catalase[b]	μmol/min/g	77.8 ± 30.7	89.5 ± 36.6	74.6 ± 20.5	85.6 ± 45.8
TAC[c]	μA	4.11 ± 0.40	3.91 ± 0.22	3.63 ± 0.68	3.73 ± 0.84
GSH[c]	μmol/g[d]	22.9 ± 3.7	19.3 ± 4.8	24.4 ± 7.2	14.6 ± 6.1[b]
TBARS[c]	nmol/g[d]	80.8 ± 17.7	79.4 ± 19.8	100.7 ± 19.2	79.0 ± 29.0
GPx[c]	μmol/min/g[d]	237.9 ± 77.9	248.1 ± 45.9	247.6 ± 112	237.2 ± 30.5
GSHred[c]	μmol/min/g[d]	600.1 ± 82.2	588.4 ± 67.5	624.5 ± 112.0	506.1 ± 50.0[b]
catalase[c]	μmol/min/g[d]	39.8 ± 2.4	42.7 ± 2.9	44.4 ± 6.0	36.9 ± 2.5
SOD[c]	U/g[d]	8.08 ± 1.28	7.56 ± 1.69	8.17 ± 1.63	7.66 ± 1.61
cytochrome P450[c]	μmol/g[d]	0.36 ± 0.21	0.43 ± 0.21	0.39 ± 0.18	0.44 ± 0.17

[a] On day 100 the animals were sacrificed. [a]Plasma. [b]Erythrocytes. [c]Liver. [d]The value was expressed per 1 gram of protein; [e]The value was expressed per 1 gram of hemoglobin. Values are expressed as means ± SD of six animals per group. [b] The value is significantly different from animals treated with standard diet ($p^* < 0.05$).

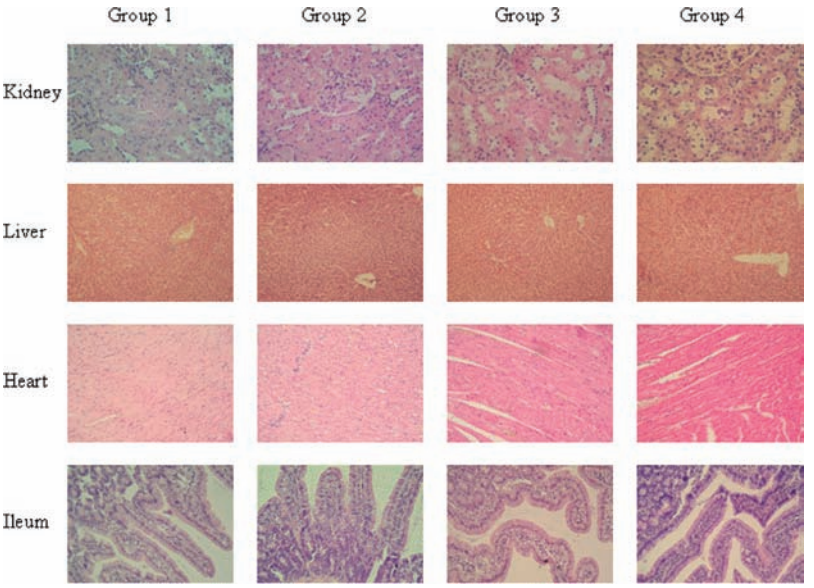

Figure 2. Representative microphotographs of the liver, kidney, ileum, and heart of all groups. Magnification factor (60×).

Table 6. Determination of Phenolics in Plasma, Urine, Feces, and Liver[a]

sample	Group 1	Group 2	Group 3	Group 4
		Phenolic Acids		
plasma[a]	n.d.	0.53 ± 0.03[b]	0.55 ± 0.08[b]	0.59 ± 0.10[b]
urine[a]	n.d.	2.66 ± 1.60[b]	2.57 ± 2.46[b]	2.66 ± 1.71[b]
feces[b]	1.39 ± 0.13	4.66 ± 1.06[b]	6.04 ± 1.51[b]	5.43 ± 0.87[b]
liver[b]	n.d.	0.63 ± 0.09[b]	1.22 ± 0.71	1.24 ± 0.46[b]
		Flavonoids		
plasma[a]	n.d.	n.d.	n.d.	n.d.
urine[a]	n.d.	n.d.	n.d.	n.d.
feces[b]	n.d.	n.d.	n.d.	traces
liver[b]	n.d.	n.d.	traces	n.d.
		Hippuric Acid		
plasma[a]	2.70 ± 0.99	5.74 ± 1.65	6.08 ± 2.05	6.17 ± 2.23
urine[a]	29.99 ± 12.14	139.44 ± 42.96[b]	127.54 ± 22.94[b]	262.30 ± 69.59[b]
feces[b]	1.58 ± 0.18	4.46 ± 0.48[b]	2.40 ± 1.95	2.80 ± 0.46
liver[b]	0.68 ± 0.09	0.95 ± 0.08	1.54 ± 0.63	1.77 ± 0.70
		Benzoic Acid		
plasma[a]	traces	traces	traces	0.12 ± 0.01[b]
urine[a]	n.d.	traces	0.21 ± 0.07[b]	0.26 ± 0.17
feces[b]	2.16 ± 0.40	1.70 ± 0.08	1.58 ± 0.33	1.66 ± 0.48
liver[b]	0.91 ± 0.45	0.50 ± 0.19	0.71 ± 0.41	0.55 ± 0.32

[a] n.d. - not determined. [a] μg/mL; [b] μg/g; values are expressed as means ± SD of six animals per group. [b] The value is significantly different from animals treated with standard diet ($p^* < 0.05$).

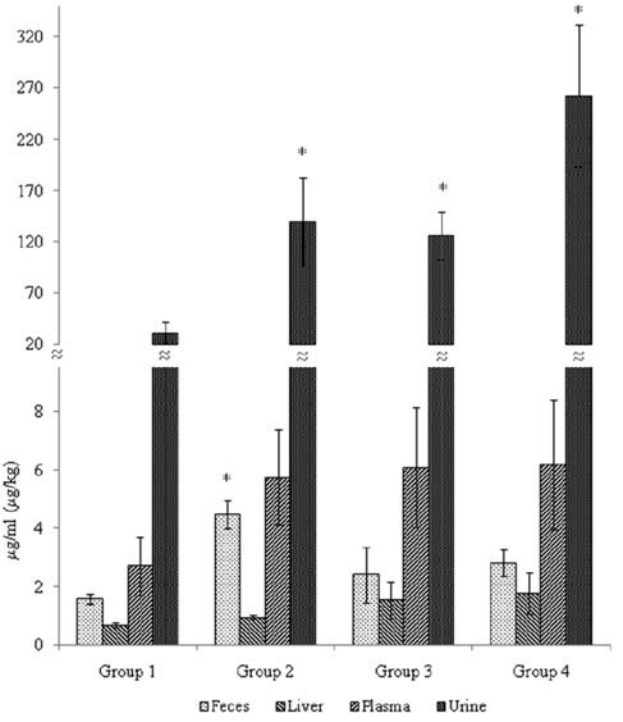

Figure 3. Content of hippuric acid in feces, liver, plasma, and urine. The animals consumed ad libitum either standard diet (Group 1), or experimental diets containing 1500 ppm cranberry powders (Groups 2−4), for 100 days as described in Section 2. The content of hippuric acid in was determined by LC/MS.

salicyluric acids were found in traces. The urine contained huge concentrations of hippuric acid but lower levels of other acids, such as 3-hydroxy-3-phenylpropionic, ellagic, 2-hydroxyphenylacetic, 3-hydroxyphenylacetic, 4-hydroxyphenylacetic, 4-hydroxybenzoic, ferulic, caffeic, dihydrocaffeic, benzoic, and salicyluric. The presence of hippuric acid as a putative main metabolite of benzoic acid, phenolic acids, flavonoids and also anthocyanins and proanthocyanidins, in urine may explain the effect of cranberry products in the prevention of urinary tract infections (*5*), **Figure 3**. The urine concentration of hippuric acid corresponded well with increased phenolics intake in feed in Groups 2−4 and this is in accordance with our previous results in humans (*2*). Traces of intact anthocyanin pigments (under the limit of quantification in all samples) can also be explained by their cleavage and conversion to hippuric acid (**Table 6**).

A 100-day consumption of cranberry powders had no effect on the clinical chemistry and hematological parameters tested (**Tables 3** and **4**). Only the level of plasma alkaline phosphatase was significantly decreased in Group 2. Likewise, the level of total liver cytochrome P450 was not affected. After 100-day oral administration of cranberry in rats, there was no lymphocyte DNA damage. The experiment showed that in rats, over 3 months feeding with a diet containing 1500 ppm cranberry powder,

no effects on monitored parameters associated with oxidative stress were found (**Table 5**). Contrary to the finding of Valentova et al. (*2*) in human, AOPP concentrations in plasma were same at the beginning and the end of experiment in all groups in rodents. Only GSHred activity and GSH levels were significantly decreased in Group 4 compared to the control group. These changes in GSH antioxidant system were observed mainly in the liver and they might be explained by the metabolization of feed constituents here or by the formation of metabolites with glutathione (*28*). This effect on GSH/GSHred was not confirmed after a 180-day consumption in humans (*29*).

The tested commercial cranberry products (dry powdered juices and fruits) were well-tolerated by rats. The components and metabolites of cranberry did not accumulate in the tissues and were excreted through the urine mainly as hippuric acid. Even though cranberry powders contained different amounts of polyphenolics, none provided evidence for significant modulation of antioxidative status, oxidative stress parameters, and AOPP in rat.

ABBREVIATIONS USED

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AOPP, advanced oxidation protein products; AST, aspartate aminotransferase; CYP, cytochrome P450; CHE, cholinesterase; GIT, gastrointestinal tract; GPx, glutathione peroxidase; GSH, glutathione; GSHred, glutathione reductase; GST, glutathione transferase; LC-MS, liquid chromatography with mass spectrometry detection; ROS, reactive oxygen species; SOD, superoxide dismutase; SPE, solid phase extraction; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; UTI, urinary tract infection.

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