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Effect of bilberry extract (*Vaccinium myrtillus* L.) on drug-metabolizing enzymes in rats

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ABSTRACT

Vaccinium myrtillus L. (bilberry) fruit is a blue-colored berry with a high content of anthocyanins. These bioactive secondary metabolites are considered to play a major role in the health-promoting properties of bilberries. Our *in vivo* study was designed to assess the possible influence of bilberry extract on drug-metabolizing enzymes (DMEs). Rats were exposed to bilberry extract in drinking water at two concentrations (0.15 and 1.5 g/L). Selected DMEs were determined (mRNA expression and enzymatic activity) after 29 and 58 days in rat liver. In addition, a panel of antioxidant, physiological, biochemical and hematological parameters was studied; these parameters did not demonstrate any impact of bilberry extract on the health status of rats. A significant increase in activity was observed in cytochrome P450 (CYP) 2C11 (131% of control) and CYP2E1 (122% of control) after a 29-day administration, while the consumption of a higher concentration for a longer time led to a mild activity decrease. Slight changes were observed in some other DMEs, but they remained insignificant from a physiological perspective. According to our results, we conclude that the consumption of bilberries as a food supplement should not pose a risk of interacting with co-administered drugs based on their metabolism.

1. Introduction

Vaccinium myrtillus L. (bilberry) is a low-branched, deciduous shrub belonging to the Ericaceae family. Bilberry, often referred to as the European blueberry, contains a variety of phenolic compounds, including flavonols (quercetin, myricetin or isorhamnetin), tannins, ellagitannins, phenolic acids and anthocyanins (Chu et al., 2011).

The most abundant bioactive compounds in bilberries are anthocyanins, the water-soluble pigments that are responsible for the bluish-black color of bilberry fruit (Chu et al., 2011). These secondary metabolites are composed of anthocyanidins (aglycones of anthocyanins with a flavonoid structure), sugar moiety(ies) and in many cases acyl group(s). The sugars occurring in these glycosides with the highest frequency are glucose, galactose, arabinose and rhamnose (Bártíková et al., 2013; Chu et al., 2011). The physiological functions of the anthocyanin pigments in fruits and seeds are probably to attract pollinators and seed dispersers, while foliar anthocyanins provide protection against ultraviolet (UV) radiation and pathogen attack (Silvan et al., 2016; Winkel-Shirley, 2001).

The biological properties of individual anthocyanins have been studied in detail, which is not true for whole bilberry extract. However, according to recent opinion, a wide range of observed biological properties are a result of complementary, additive, and/or synergistic effects produced by multiple phytochemicals found in berries rather than being an effect of a single constituent (Seeram, 2008). In line with this idea, we decided to focus our study on the bilberry extract.

Bilberries have been known for several of their health-promoting properties, such as the improvement of ocular disorders, spatial working memory, lowering of blood glucose, anti-inflammatory and lipid-lowering effects, enhancement of antioxidant defense and lowering of oxidative stress (Chu et al., 2011; Lee et al., 2017; Williams et al., 2008). Because of their many positive effects on human health, there is increasing production and consumption of dietary supplements with a high content of bilberry extract. However, high doses of bilberry extract could exhibit undesired effects, including possible interactions with concurrently and subsequently administered drugs. Although the interactions of drugs with many dietary supplements have been

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recently shown to cause a significant effect on pharmacotherapy (Chen et al., 2015; Stage et al., 2016), no drug interactions with a chronic or overdose intake of bilberry extract have been reported to date.

Bilberry extract could affect drug behavior and efficacy mainly via changing the activity of DMEs. These enzymes catalyze the conversion of lipophilic drugs and other xenobiotic compounds into polar products to facilitate their elimination from the body. However, many xenobiotics, including dietary supplements, can affect the expression and/or activity of DMEs, mostly via binding to their regulatory nuclear receptor, most often causing an induction of the respective DMEs, or can interact directly with the active site of a DME (often inhibiting its activity). Both the induction and inhibition of DMEs represent the principle mechanisms of dietary supplement-drug interactions (Anzenbacher and Zanger, 2012; Prakash et al., 2015).

As these interactions could be risky to patients, our *in vivo* study was designed to evaluate the possibility of drug interactions with bilberry extract. The aim of this study was to determine whether there is an effect of bilberry extract on selected DMEs (CYPs, carbonyl-reducing and the main conjugating enzymes) and their corresponding nuclear receptors. Rats were used as a model species. In addition to studying the expression and activity of selected hepatic DMEs, the effects of bilberry extract on antioxidants were assessed. In addition, the welfare of rats (weight, water and food consumption), and their biochemical and hematological parameters were also monitored.

2. Materials and methods

2.1. Materials

Extract of dried ripe bilberry fruit, *Vaccinium myrtillus* (L.) *fructus siccus*, was purchased from Walmark (Třinec, Czech Republic) in the form of powder. The powder was mixed daily with fresh drinking (tap) water in order to prepare two solutions – one with a higher (1.5 g/L) concentration and another with a lower (0.15 g/L) concentration of the lyophilisate. Fresh solutions were prepared daily as a result of a stability study of applied bilberry extract, that was conducted before the animal study – the decrease in anthocyanins concentration after 24 h was less than 20%. These solutions of bilberry extract were given to the rats to drink.

The higher concentration reflected the NOAEL (no observable adverse effect level) of 1500 ppm, as determined by Palikova et al. (2010) for lyophilized cranberry (*Vaccinium macrocarpon* Ait.) juice in rats. The anthocyanin concentration of this solution was determined using the standardized methodology (Hu et al., 2016) and equaled ca. 400 μg/mL. The relative representation of individual anthocyanins was as follows: Delphinidin-3-*O*-arabinoside 30.8%, petunidin-3-*O*-galactoside 15.9%, cyanidin-3-*O*-galactoside 12.9%, peonidin-3-*O*-glucoside 8.9%, cyanidin-3-*O*-arabinoside 7.6%, delphinidin-3-*O*-glucoside 6.2%, delphinidin-3-*O*-galactoside 5.3%, malvidin-3-*O*-glucoside 4.7%, petunidin-3-O-glucoside 4.1%, malvidin-3-*O*-galactoside 3.6%.

The calculation of the lower extract concentration was based on the estimated minimum human exposure to anthocyanins – important bioactive components of the fruit. The intake of bilberries in an average 80 kg human was approximated to be 20–60 g/d (dry weight, ESCOP (2003)) or 20–60 g/d (unprocessed fruit, Gruenwald (2000)) and the total anthocyanin content to be 2–4% dry weight (Latti et al., 2008) or 0.3–0.7% fresh ripe fruit (Prior et al., 1998). The daily human exposure to anthocyanins may vary between 60 and 420 mg (from fresh fruit) or between 400 and 2400 mg (from dry fruit), i.e. from 0.75 to 30 mg/kg, with a median close to 4 mg/kg body weight. If the daily rat consumption of fluids oscillates around 100 mL/kg body weight, the concentration of anthocyanins in drinking water reflecting an average human intake may approach 40 μg of anthocyanins (*i.e.* 0.15 mg of the aforementioned powder) per mL of solution.

2.2. Ethical issues

All protocols and experimental procedures of the study were approved by the Ethics Committee of the Ministry of Education, Youth and Sports (Protocol MSMT-30145/2016–4). All animals received humane care in compliance with the Experimental Animals Protection Act No. 167/1993 L.C.

2.3. Animals

36 male rats of the Wistar strain, specific pathogen free (initial body weight 200–225 g), originally from Harlan, Italy (supplied by Anlab, Prague, Czech Republic), were used to avoid the possible hormonal fluctuations that occur in females. During the acclimatization period (7–10 days before experiment), the animals were housed in animal quarters in standard plastic cages with dust-free sawdust, 2 rats per cage, under standard and controlled conditions with free access to drinking water and standard rat chow (ssniff® R/M-H, 10 mm pellets containing 87.8% dry matter, 19% crude protein, 3.3% crude fat, 4.9% crude fiber, 6.4% crude ash, 54.2% nitrogen-free extracts, 36.6% starch, 4.7% sugar and 16.3 MJ/kg gross energy, ssniff Spezialdiäten, Soest, Germany).

2.4. Experimental design

The whole experiment lasted for four or eight weeks with food and drink provided ad libitum. Rats were randomly divided into six groups and treated as shown in Table 1. Each group was divided into two subgroups so that three animals could be housed per cage. The rats in their respective cages were identified by earlobe perforation. Cages and sawdust were changed once per week. After 29 days, the groups D29_C, D29_0.15 and D29_1.5 were weighed and anesthetized by the intramuscular injection of a mixture of fentanyl, dexmedetomidin and diazepam (4, 8.4 and 500 μg per 100 g of body weight, resp.). The abdominal cavity was opened and arterial blood was withdrawn from the aortal bifurcation to sacrifice the animals. The liver was immediately removed and washed with homogenization buffer (0.25 M sucrose; 1 mM EDTA; pH 7.4); a small piece of tissue was placed in RNAlater (Qiagen, Hilden, Germany) for RNA analysis; the rest of the liver was frozen immediately in liquid nitrogen (Linde-gas, Munich, Germany). Blood aliquots were immediately transferred to EDTA or heparin-containing tubes. Whole blood was analyzed without delay for the basic blood count using an ABX ABC Vet Hematology Analyzer (Horiba,

Table 1
Definitions of individual groups. Designations D29 and D58 reflect the duration of the experiment, i.e. 29 or 58 days; 0.15 and 1.5 indicate the administered dose, i.e. 0.15 or 1.5 g/L and C is for the control (tap water).

Group	Characteristics	No. of rats	Duration	Drinking (ad libitum)	Feed (ad libitum)
D29_C	Control	6	4 weeks	Water	Standard pellets
D58_C	Control	6	8 weeks	Water	Standard pellets
D29_0.15	Experimental	6	4 weeks	Bilberry extract (0.15 g/L)	Standard pellets
D58_0.15	Experimental	6	8 weeks	Bilberry extract (0.15 g/L)	Standard pellets
D29_1.5	Experimental	6	4 weeks	Bilberry extract (1.5 g/L)	Standard pellets
D58_1.5	Experimental	6	8 weeks	Bilberry extract (1.5 g/L)	Standard pellets

Kyoto, Japan) according to the manufacturer's instructions. Heparinized or EDTA-treated blood was centrifuged (for 15 min at 4000 g and 4 °C), to obtain plasma that was stored at −80 °C until analysis using a routine clinical chemistry platform, an Aution Max analyzer (Arkray Inc., Kyoto, Japan). The selection of routine laboratory tests was principally based on, but not necessarily confined to, the recommendations described elsewhere (Weingand et al., 1996).

The experiment was terminated on day 58 by the sacrifice of the remaining animals (*i.e.* groups D58_C, D58_0.15 and D58_1.5) using the procedures described above.

2.5. Health status observation

The daily routine included the recording of the individual clinical state (including the animal's behavior), examination of stool appearance and consistency (assessed qualitatively for the respective cage), the supply of fresh feed and drink and the determination of daily feed consumption and fluid intake relative to cumulative body weight, and expressed per cage. The measurement of individual body weight was performed as part of the health status observation twice a week (data for the remaining days were computed from the known values using linear interpolation).

2.6. Subcellular fractions preparation

Livers from animals inhabiting the same cage (n = 3) were pooled together. Hence two samples for each group were obtained. Pooled livers were homogenized in the homogenization buffer, pH 7.4 (0.25 M sucrose, 1 mM EDTA), with an ULTRA-TURRAX T 25 basic instrument (IKA-Werke, Staufen, Germany). The liver homogenate was subjected to differential centrifugation to obtain the subcellular fractions as mentioned elsewhere (Prokop et al., 2018). The cytosolic and microsomal fractions obtained were stored at −80 °C. The Bicinchoninic acid (BCA) method was used for the determination of protein concentration. Total CYP content was determined in the microsomal fraction according to the standard protocol (Phillips and Shephard, 2006).

2.7. Phase I enzyme activity assays

2.7.1. CYPs activity assays

Drug-metabolizing enzymes in rats were identified by a comparison of literature data, mainly the Cytochrome P450 Knowledgebase (http://cpd.ibmh.msk.su) (Lisitsa et al. 2001) and the PharmVar database (https://www.pharmvar.org/) (Gaedigk et al., 2018). The activities of enzymes (orthologues of human liver drug-metabolizing CYP forms) were evaluated in the microsomal fractions of rat liver. Each experiment was performed in three repeated measurements with three replications where not stated otherwise. The amount of organic solvents was no higher than 0.2% (v/v) in the reaction mixture.

The activities of selected rat CYP enzymes were measured by methods reviewed in Cytochrome P450 Protocols (Phillips and Shephard, 2006), and 7alpha-hydroxytestosterone was determined according to the work of Dovrtelova et al. (2015). CYP activities in rat liver microsomes were determined by the following enzymatic reactions: CYP1A1, ethoxyresorufin O-deethylation; CYP1A2, phenacetin O-deethylation; CYP2A1, testosterone 7α-hydroxylation; CYP2B1/2 pentoxyresorufin O-dealkylation; CYP2C6, warfarin 7-hydroxylation; CYP2C11, diclofenac 5-hydroxylation; CYP2D1/2 bufuralol 1′-hydroxylation; CYP2E1, chlorzoxazone 6-hydroxylation; CYP3A1, midazolam 1′-hydroxylation; CYP3A2, diazepam 3-hydroxylation. The NADPH generating system consisted of: 0.5 mM $NADP^+$, 4 mM isocitric acid, 5 mM $MgSO_4$, and 0.2 U/mL isocitrate dehydrogenase. For substrates, metabolites, substrate concentrations, amount of rat microsomes (CYP/volume), incubation times and quenching agents, see Table 2.

Specific substrates were metabolized by individual CYPs to characteristic products. These were measured in a Shimadzu Class VP HPLC system (Shimadzu, Kyoto, Japan) with UV or fluorescence detection. A LiChrospher RP-18 column (5 µm) 250 × 4 mm equipped with a 4 × 4 mm guard column, or Kinetex F5 (2.6 µm) 150 × 4.6 mm equipped with a 2 × 4.6 mm guard column (for 2A1 activity only), were used for distinguishing the metabolites and substrates.

2.7.2. Activities of carbonyl-reducing enzymes

Carbonyl-reducing enzyme activities were assayed in the cytosolic fraction from the liver homogenate. Enzyme assays were performed in three independent repetitions with 4–8 parallel measurements for each sample. Activity determination was based on the spectrophotometric detection of formed product or the detection of decreasing substrate/cofactor levels using a Tecan Infinite M200 microplate reader (Tecan Group Ltd., Männedorf, Switzerland). The calculated activity was normalized to the protein content and expressed as a percentage of the control.

The activities of aldo-keto reductase (AKR) 1A1, AKR1C, carbonyl reductase 1 (CBR1) and NAD(P)H:quinone oxidoreductase 1 (NQO1) were determined in the cytosol according to previously-published methods mentioned elsewhere (Lněničková et al., 2018).

2.8. Phase II enzyme activity assays

The conjugating enzyme activities were assayed in the cytosolic or microsomal fraction in three independent repetitions with 3–6 parallel measurements for each sample. Activity determination was based on spectrophotometric detection of the formed product or the detection of decreasing substrate/cofactor levels using the Tecan Infinite M200 microplate reader or HPLC analysis. The studied conjugating enzymes included glutathione S-transferase (GST), sulfotransferase (SULT), UDP-glucuronosyltransferase (UGT) and catechol-O-methyltransferase (COMT). The calculated activities were normalized to the protein content and expressed as a percentage of the control.

GST, SULT and UGT activities were determined by spectrophotometric

Table 2
Incubation conditions for individual CYP assays used for activity determination.

CYP	Substrate	Substrate conc. (µM)	Metabolite	Amount of CYP (pmol/µL)	Quenching agent	Incubation time (min)
1A1	ETRR	2.6	resorufin	35/100	0.2 mL MeOH	15
1A2	Phenacetin	25	paracetamol	70/200	0.01 mL 70% $HClO_4$	20
2A1	Testosterone	100	7α-hydroxytestosterone	100/500	0.1 mL 1M Na_2CO_3/2M NaCl	20
2B1/2	PTRR	2.6	resorufin	70/100	0.2 mL MeOH	20
2C6	Warfarin	200	7-hydroxywarfarin	250/250	5 µL 70% $HClO_4$	20
2C11	Diclofenac	16	4′-hydroxydiclofenac 5-hydroxydiclofenac	35/200	0.05 mL ACN/CH_3COOH (94:6)	25
2D1/2	Bufuralol	25	1′-hydroxybufuralol	67.3/200	0.02 mL70% $HClO_4$	20
2E1	Chlorzoxazone	50	6-hydroxychlorzoxazone	160/1000	0.05 mL 42.5% H_3PO_4	20
3A1	Midazolam	2.8	1′-hydroxymidazolam	12.56/100	0.1 mL 100% MeOH	8
3A2	Diazepam	100	3-hydroxydiazepam	70/200	0.1 mL 100% ACN	20

PTRR – 7-pentoxyresorufin ETRR – 7-ethoxyresorufin.

Table 3
Biochemical parameters of rat blood plasma.

Analyte (unit)	Experimental group						Post tests (groups)
	29 days			58 days			
	D29_C	D29_0.15	D29_1.5	D58_C	D58_0.15	D58_1.5	D29_C vs. D58_C
Na (mmol/L)	**142** ± 1.6	**140** ± 1.1	**142** ± 2.9	**143** ± 2.0	**143** ± 1.7	**142** ± 1.9	n.s.
K (mmol/L)	**4.4** ± 0.49	**4.1** ± 0.29	**3.8*** ± 0.17	**4.3** ± 0.38	**4.1** ± 0.10	**4.5** ± 0.95	n.s.
Cl (mmol/L)	**101** ± 2.0	**99** ± 1.4	**100** ± 3.3	**99** ± 2.5	**99** ± 3.1	**99** ± 3.2	n.s.
Ca (mmol/L)	**2.8** ± 0.06	**2.7** ± 0.07	**2.8** ± 0.06	**2.7** ± 0.04	**2.7** ± 0.03	**2.6** ± 0.07	†
P (mmol/L)	**2.6** ± 0.11	**2.4** ± 0.14	**2.6** ± 0.14	**2.4** ± 0.42	**2.3** ± 0.20	**2.7** ± 0.57	n.s.
GLU (mmol/L)	**15.6** ± 3.2	**17.1** ± 2.0	**13.7** ± 1.3	**12.0** ± 2.5	**11.6** ± 1.7	**10.5** ± 1.9	n.s.
UREA (mmol/L)	**9.2** ± 1.4	**7.7*** ± 0.50	**7.7** * ± 0.80	**7.2** ± 1.0	**6.7*** ± 0.61	**7.3** ± 0.79	†
CREA (μmol/L)	**26.5** ± 3.6	**23.3** ± 1.6	**23.8** ± 2.8	**26.8** ± 3.6	**26.2** ± 3.5	**27.3** ± 4.5	n.s.
UA (μmol/L)	**36.0** ± 5.7	**33.5** ± 20	**18.3** ± 6.0	**36.4** ± 16	**22.2** ± 5.1	**53.3** ± 48	n.s.
TBIL (μmol/L)	**1.22** ± 0.89	**0.72** ± 0.19	**0.40** ± 0.14	**0.94** ± 0.62	**0.85** ± 0.23	**0.75** ± 0.39	n.s.
ALT (μkat/L)	**0.89** ± 0.08	**0.90** ± 0.27	**0.70** ± 0.09	**0.83** ± 0.10	**0.76** ± 0.08	**0.69** ± 0.11	n.s.
AST (μkat/L)	**4.03** ± 0.72	**2.98** ± 1.30	**2.04** ** ± 0.48	**3.04** ± 1.45	**2.07** ± 0.39	**2.56** ± 0.87	n.s.
ALP (μkat/L)	**1.85** ± 0.23	**1.79** ± 0.36	**1.71** ± 0.52	**1.47** ± 0.39	**1.24** ± 0.22	**1.34** ± 0.36	n.s.
AMYL (μkat/L)	**34.0** ± 3.2	**35.4** ± 2.6	**36.3** ± 4.3	**26.8** ± 4.1	**29.9** ± 4.4	**23.4** ± 3.5	†
TP (g/L)	**60.5** ± 2.8	**59.6** ± 2.4	**60.6** ± 0.7	**59.7** ± 2.1	**59.1** ± 2.1	**56.2*** ± 2.0	n.s.
ALB (g/L)	**38.5** ± 2.0	**37.6** ± 2.9	**39.3** ± 1.4	**36.8** ± 1.9	**34.8** ± 4.4	**34.9** ± 3.1	n.s.
GLOB (g/L)	**22.1** ± 2.07	**22.0** ± 2.56	**21.3** ± 1.22	**22.9** ± 1.04	**24.3** ± 2.56	**21.3** ± 3.07	n.s.
A/G	**1.76** ± 0.20	**1.74** ± 0.30	**1.86** ± 0.17	**1.61** ± 0.11	**1.46** ± 0.32	**1.67** ± 0.31	n.s.
CHOL (mmol/L)	**2.0** ± 0.3	**2.5** ± 0.7	**2.2** ± 0.4	**2.6** ± 0.7	**2.2** ± 0.4	**1.8*** ± 0.4	n.s.
HDL (mmol/L)	**1.6** ± 0.2	**1.9** ± 0.3	**1.8** ± 0.3	**2.1** ± 0.6	**1.7** ± 0.3	**1.4*** ± 0.3	n.s.
non-HDL (mmol/L)	**0.44** ± 0.12	**0.67** ± 0.32	**0.35** ± 0.15	**0.58** ± 0.21	**0.49** ± 0.14	**0.40** ± 0.12	n.s.
TRIG (mmol/L)	**0.66** ± 0.16	**0.81** ± 0.23	**0.75** ± 0.35	**0.77** ± 0.38	**0.70** ± 0.33	**0.47** ± 0.07	n.s.
HI (1/100 gHb/L)	**13.8** ± 9.5	**14.3** ± 11.0	**10.8** ± 5.7	**31.0** ± 17.6	**25.5** ± 12.1	**28.0** ± 20.4	n.s.

Data are given as means ± SD. Asterisks *, ** and *** signify $p < 0.05$, < 0.01 and < 0.001 vs. the control group, n.s. indicates no significant differences between 29-days control and 58-days control ($p > 0.05$, ordinary ANOVA). Where $p < 0.05$, Tukey post hoc test was used. † symbols denote $p < 0.05$ for the comparisons between controls.
Abbreviations: GLU – glucose, CREA – creatinine, UA – uric acid, TBIL – total bilirubin, ALT – alanine aminotransferase, AST – aspartate aminotransferase, ALP – alkaline phosphatase, AMYL – alpha amylase, TP – total protein, ALB – albumin, GLOB – globulin, A/G – albumin-to-globulin ratio, CHOL – total cholesterol, HDL – high-density lipoprotein cholesterol, TRIG – triglycerides, HI – index of haemolysis (free haemoglobin).

methods mentioned elsewhere (Lněničková et al., 2016). Cytosolic COMT activity was measured according to Aoyama et al. (2005). During the selection of the method, we evaluated the possibility of utilizing the method of Lee et al. (2001) as well as that of Brunetti et al. (2013), but the method of Aoyama et al., (2005) was selected because of its simplicity. This method is based on the formation of normetanephrine from norepinephrine. The amount of normetanephrine was measured using isocratic HPLC analysis. The mobile phase contained 9.5 mM sodium phosphate buffer (pH 7.0), 40 mM boric acid and 4 mM sodium 1-hexanesulfonate in 5% acetonitrile (v/v), the flow rate was 0.8 ml/min. The analyses were performed in a Shimadzu Class VP HPLC system with fluorescence detection. A LiChroCART® 250-3 HPLC cartridge with LiChrospher® 60 RP-select B (5 μm) chromatographic column (Merck, Kenilworth, NJ, USA) was used for determining the metabolite.

2.9. Determination of oxidative stress parameters in rat blood plasma and erythrocytes

Spectrophotometric measurements were performed in an Infinite M200PRO microplate reader UV-VIS spectrophotometer (Tecan Group Ltd., Männedorf, Switzerland). Hemoglobin determination and oxidative stress factors were measured by the methods described by Palikova et al. (2010). The calculated activities were normalized to the protein content (plasma parameters) or hemoglobin content (erythrocytes parameters) and expressed as a percentage of the control.

The sum of total -SH groups, the ferric reducing antioxidant power (FRAP), the level of thiobarbituric acid reactive substances (TBARS), and the amount of reduced glutathione (GSH) in plasma were measured by the spectrophotometric methods mentioned elsewhere (Prokop

Table 4
Hematological parameters of rats.

Parameter (unit)	Experimental group				ANOVA (groups)		
	D29_C	D29_0.15	D29_1.5	D58_C	D58_0.15	D58_1.5	D29_C vs. D58_C
WBC (10^3/mm^3)	**3.0** ± 0.6	**3.3** ± 0.8	**2.5** ± 0.6	**3.4** ± 0.9	**3.1** ± 0.6	**3.5** ± 2.0	n.s.
RBC (10^6/mm^3)	**7.7** ± 0.3	**7.5** ± 0.5	**7.7** ± 0.5	**8.5** ± 0.3	**8.6** ± 0.5	**8.7** ± 0.4	††
HGB (g/dL)	**15.3** ± 0.6	**15.1** ± 0.6	**15.5** ± 0.7	**16.3** ± 0.8	**16.1** ± 0.8	**16.2** ± 0.5	†
HCT (%)	**45** ± 2	**44** ± 3	**45** ± 3	**50** ± 3	**50** ± 2	**50** ± 2	†
PLT (10^3/mm^3)	**568** ± 73	**630** ± 98	**612** ± 109	**596** ± 105	**638** ± 34	**570** ± 42	n.s.
MCV (μm^3)	**59** ± 0.9	**59** ± 3	**59** ± 1	**59** ± 2	**58** ± 1	**58** ± 1	n.s.
MCH (pg)	**20** ± 0.2	**20** ± 1	**20** ± 1	**19** ± 0.7	**19** ± 0.6	**19** ± 0.5	n.s.
MCHC (g/dL)	**34** ± 0.5	**34** ± 1	**34** ± 1	**33** ± 0.7	**32** ± 0.4	**32** ± 0.5	n.s.

Data are given as means ± SD. Symbols † and †† signify $p < 0.05$ and $p < 0.01$ differences between 4 weeks control and 8 weeks control group. Significance of differences was determined by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons. n.s. stands for not significant differences.
Abbreviations: WBC – white blood cells, RBC – red blood cells, HGB – haemoglobin, HCT – hematocrit, PLT – platelets, MCV – mean (red blood) cell volume, MCH – mean (red blood) cell HGB, MCHC – mean (red blood) cell HGB concentration.

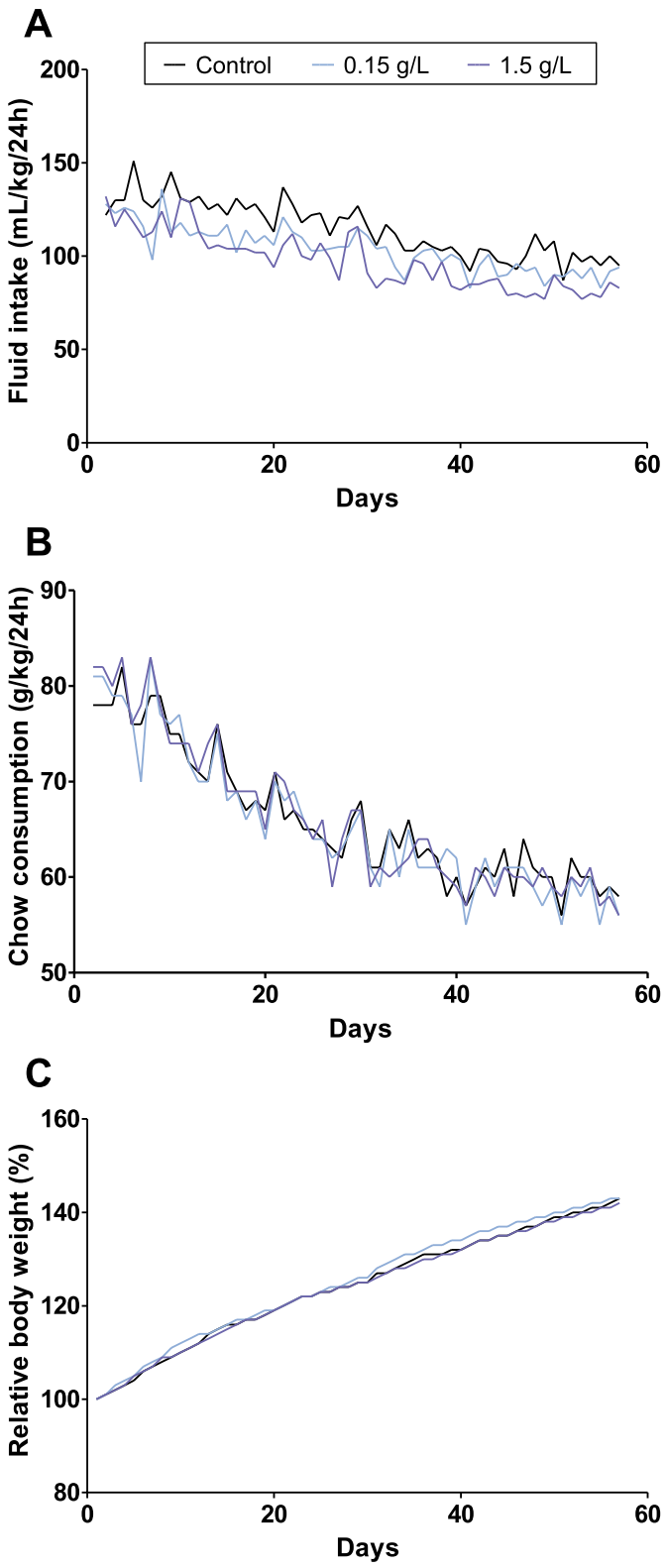

Fig. 1. Comparison of differences in fluid intake (A), chow consumption (B) and relative (C) body weight between control (D29_C + D58_C), 0.15 g/L (D29_0.15 + D58_1.5) and 1.5 g/L (D29_1.5 + D58_1.5).

et al., 2018).

The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GSR) in erythrocytes were measured in the same manner as previously (Prokop et al., 2018). The hemoglobin concentration in the erythrocytes was determined spectrophotometrically by employing Drabkin's reagent.

2.10. RNA isolation and quantitative real-time PCR (qPCR)

The tissue samples of rat livers were stored in RNAlater (Qiagen, Hilden, Germany) at −80 °C. RNA isolation was performed using an RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purity and integrity of RNA was determined spectrophotometrically by the ratio of isolated nucleic acids at 260/280 nm. First-strand cDNA was synthetized from total RNA with the use of a Transcriptor High Fidelity cDNA synthesis kit (Roche, Basel, Switzerland). Real-time PCR and the quantification of mRNA were determined with a LightCycler 1536 Instrument (Roche, Basel, Switzerland). The mRNA was quantified by specific TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA). The 1536-well plates were pipetted with an Automate Labcyte Echo (Dublin, Ireland). Commercially available primers (Life Technologies, Prague, Czech Republic) were used to determine the mRNAs of CYP3A23/3A1, CYP2E1, CYP2D2, CYP2C11, CYP2C6, CYP2B2, CYP2B1, CYP2A1, CYP1A2, AKR1A1, AKR1C1, AKR1C14, CBR1, NQO1, GSTA1, SULT1A1, COMT, UGT1A1, constitutive androstane receptor (CAR), pregnane X receptor (PXR), aryl hydrocarbon receptor (AhR), nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and hypoxanthine phosphoribosyltransferase 1 (HPRT1). Measurements were performed in triplicates.

The obtained data was evaluated by the “Delta-Delta Ct Method” (Livak and Schmittgen, 2001). HPRT1 was used as the reference gene. The results were stated as the fold change in treatment groups relative to the control.

2.11. Statistics

The data were processed using the statistical software GraphPad InStat, version 3.06 (GraphPad Software, San Diego, CA, USA). Descriptive statistics were followed by the Kolmogorov-Smirnov test. Based on the data distribution, the results are expressed, unless otherwise noted, as mean ± standard deviation (SD, for Gaussian data) or median with range (for data with a non-Gaussian distribution). Conventional ANOVA (with Tukey-Kramer multiple comparisons *post hoc* test, where appropriate) was utilized to check for possible differences between groups (for the labeling of groups, see Table 1) D29_C, D29_0.15 and D29_1.5 or between groups D58_C, D58_0.15 and D58_1.5 (clinical chemistry, hematology, DMEs activity and mRNA expression). The unpaired T-test (with Welch correction, where appropriate) was employed to assess the D29_C *vs.* D58_C group (clinical chemistry, hematology). Kruskal-Wallis nonparametric ANOVA with Dunn's multiple comparisons *post hoc* test was selected for the interpretation of differences in feed and fluid intake between cumulative data obtained from the groups D29_C + D58_C *vs.* D29_0.15 + D58_0.15 *vs.* D29_1.5 + D58_1.5. One-way ANOVA was used for the same comparisons regarding (absolute or relative) body weight. The selected level of significance was $p < 0.05$.

3. Results

3.1. Effect of bilberry extract on animal physiology

Post hoc analysis showed significant differences between the amount of consumed tap water (control groups; median 114.5 mL/kg/d) and consumed bilberry extract at the lower (median 103.5 mL/kg/d; $p < 0.01$) and higher (median 96.5 mL/kg/d; $p < 0.001$) concentration (Fig. 1A). However, the type of drink neither affected feed intake nor relative body weight (% of initial weight). See Fig. 1 for details.

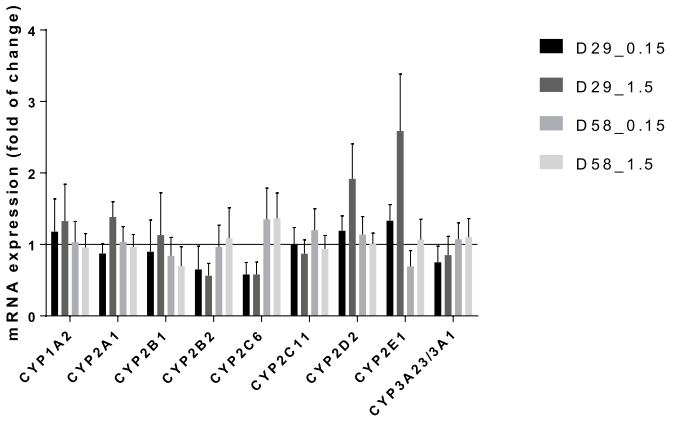

Fig. 2. mRNA expression of cytochromes P450 in liver after 29 and 58 days of bilberry extract administration in drinking water (0.15 or 1.5 g/L). Results are expressed relative to control group and represent means ± SD (n = 3). Significance of differences from the control was determined by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons.

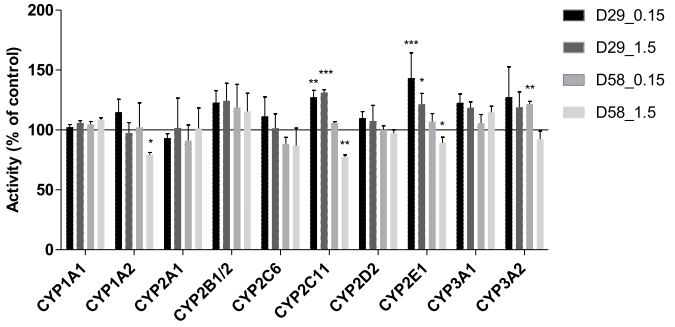

Fig. 3. Activity of cytochromes P450 in liver after 29 and 58 days of bilberry extract administration in drinking water (0.15 or 1.5 g/L). Results are expressed relative to control group and represent means ± SD (n = 3). Significance of differences from the control was determined by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.2. Effect of bilberry extract on biochemical and hematological parameters

The consumption of bilberry extract only caused a significant decrease in a few biochemical parameters (potassium, urea level, aspartate aminotransferase activity, total protein, level of total cholesterol and high-density lipoprotein cholesterol) (see Table 3). Moreover, no parameter exhibited a dependency on dose or time. Calcium, urea level and amylase activity were statistically lower in older control animals (D58_C) than in younger control animals (D29_C). As for hematology, bilberry extract consumption did not affect any hematological parameters in rats. However, the aging of animals changed several parameters (such as red blood cells or hematocrit) regardless of bilberry consumption (See Table 4).

3.3. Effect of bilberry extract on expression and activities of cytochromes P450

The mRNA expression of nine CYPs (CYP1A2, CYP2A1, CYP2B1, CYP2B2, CYP2C6, CYP2C11, CYP2D2, CYP2E1, CYP3A23/3A1) was determined (Fig. 2). We observed a slight increase in CYP2E1 mRNA levels in the liver from D29_1.5 rats. Longer exposure of rats to bilberry extract did not lead to any significant change in the monitored CYP mRNA levels.

The activities of ten CYPs (CYP1A1, CYP1A2, CYP2A1, CYP2B1/2, CYP2C6, CYP2C11, CYP2D2, CYP2E1, CYP3A1, CYP3A2) were assayed in rat liver microsomes (Fig. 3). The 29-day consumption of bilberry extract caused significant increases in CYP2E1 and CYP2C activity. On the other hand, the 58-day consumption of the higher bilberry dose led to a mild decrease in the activities of CYP2E1, 2C and 1A2.

3.4. Effect of bilberry extract on expression and activities of carbonyl-reducing enzymes

AKR1A1, AKR1C1, AKR1C14, CBR1, and NQO1 mRNA levels were assayed in rat liver (Fig. 4A). The NQO1 mRNA level was significantly decreased in rats treated for 58 days with the lower concentration of bilberry extract. The carbonyl-reducing activities of CBR1, AKR1A1, AKR1C and NQO1 (Fig. 4B) were only moderately affected by bilberry extract or not at all.

3.5. Effect of bilberry extract on expression and activities of conjugating enzymes

We determined the mRNA expression of GSTA1, SULT1A1, COMT and UGT1A (Fig. 5A). The mRNA level of COMT in the livers of rats from group D58_0.15 was significantly decreased compared to the controls. No other changes in the mRNA levels of conjugating enzymes induced by bilberry extract consumption were observed.

The activity of conjugating enzymes was measured in the cytosolic (GST, SULT, COMT) or microsomal (UGT) fraction (Fig. 5B). SULT activity was increased in rats treated for 58 days with both concentrations of bilberry extract. GST, COMT and UGT activities exhibited no response to bilberry extract.

3.6. Effect of bilberry extract on expression of nuclear receptors

The expression of CAR, PXR, AhR and Nrf2 receptors was tested, because these receptors play important roles in the regulation of many DMEs (Aleksunes and Klaassen, 2012; Kohle and Bock, 2009) (Fig. 6). Bilberry extract consumption for 29 days mildly elevated the mRNA level of PXR. The 58-day exposure to a higher concentration of bilberry extract caused a slight decrease in CAR and AhR mRNA levels.

3.7. Effect of bilberry extract on parameters of antioxidant systems

Several parameters of antioxidant systems were assayed in rat

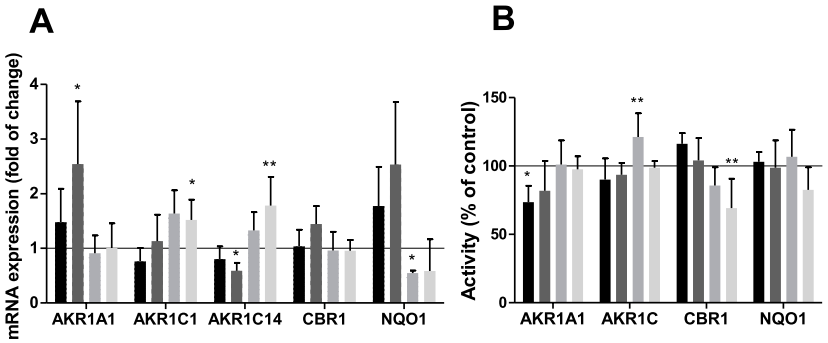

Fig. 4. mRNA expression (A) and activity (B) of reducing enzymes in liver after 29 and 58 days of bilberry extract administration in drinking water (0.15 or 1.5 g/L). Results are expressed relative to control group and represent means ± SD (n = 3). Significance of differences from the control was determined by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons (* $p < 0.05$, ** $p < 0.01$).

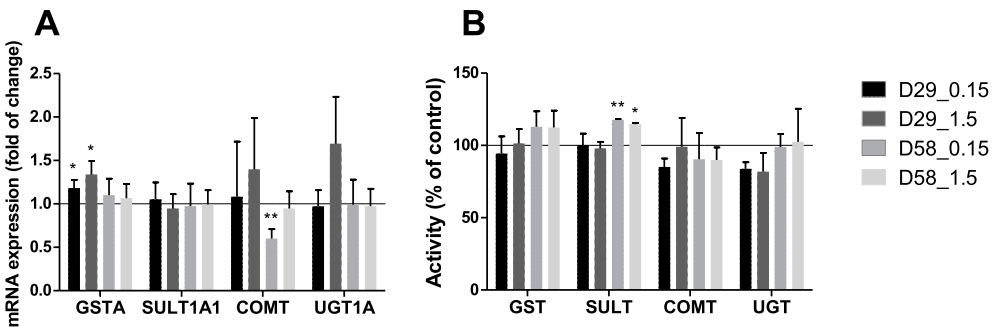

Fig. 5. mRNA expression (A) and activity (B) of conjugating enzymes in liver after 29 and 58 days of bilberry extract administration in drinking water (0.15 or 1.5 g/L). Results are expressed relative to control group and represent means ± SD (n = 3). Significance of differences from the control was determined by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons (* $p < 0.05$, ** $p < 0.01$).

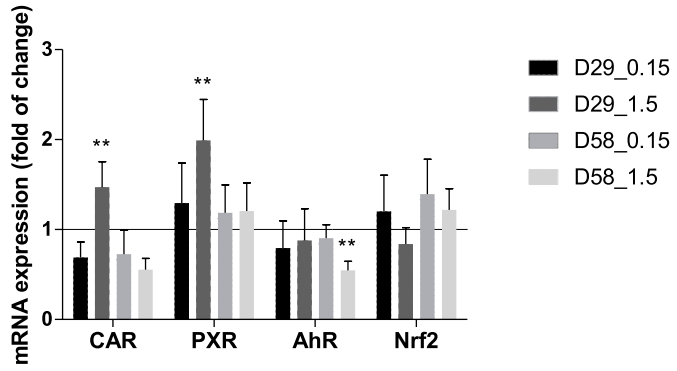

Fig. 6. mRNA expression of nuclear receptors (CAR, PXR and AhR, Nrf2) in liver after 29 and 58 days of bilberry extract administration in drinking water (0.15 or 1.5 g/L). Results are expressed relative to control group and represent means ± SD (n = 3). Significance of differences from the control was determined by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons (** $p < 0.01$).

erythrocytes (TBARS, GSH, SOD, GSR, GST, GPx) and plasma (TBARS, -SH, FRAP). Bilberry extract treatment did not significantly affect any antioxidant systems (Fig. 7).

4. Discussion

Bilberries have several health-promoting effects, but a chronic or overdose intake of bilberry extract (especially in dietary supplements) may lead to interactions with concurrently or subsequently administered drugs. In this study, male rats were treated with bilberry extract in two dosage schemes with the aim of evaluating the effect of a higher or lower concentration of bilberry extract in potable water on DMEs. Simultaneously, physiological, biochemical and hematological parameters were monitored in experimental animals.

As for physiological parameters, bilberry extract seemed to be less appetizing for the animals than pure tap water. However, drinking bilberry extract neither altered the palatability of the standard chow diet nor the weight of animals. As rats were in a standard type of cage, we were unable to monitor the quality or quantity of urine, but the feces were macroscopically normal, suggesting no significant effect of the two-month consumption of bilberry extract on gastrointestinal physiology and appetite.

Bilberry is known for its anti-inflammatory, cardioprotective and hypoglycemic effects (Chu et al., 2011). In our study, bilberry extract consumption only significantly affected a few biochemical parameters. Moreover, the observed changes were mild and without any dependency on dosage or time. In addition, bilberry consumption neither changed hematological parameters, oxidative stress parameters, nor the levels of antioxidants in plasma. In the context of reference values for male Wistar rats (Boehm et al., 2007; Giknis, 2008; Liang Chung-Tiang, 1999), the chronic consumption of bilberries seems to produce neither a conclusive protection, nor a pathology.

However, the main aim of our study was to evaluate the potential effect of bilberry extract on DMEs expression and activities, as this issue has not been fully elucidated yet. In rats that consumed bilberry extract for 29 days, a significant increase in CYP2E1 and CYP2C11 activity was observed. The enhancement of CYP2E1 activity is undesirable, as CYP2E1 produces superoxide and hydrogen peroxides, which increase oxidative stress (Aviram et al., 1999). As for the fact that levels of –SH groups and TBARS in plasma together with GSH and TBARS in erythrocytes remained unchanged in rats consuming bilberry extract; we assume that the bilberry extract did not increase oxidative stress despite the increased CYP2E1 activity. Rat CYP2C11 is structurally and functionally close to human CYP2C9, which is involved in the metabolism of several drugs, e.g. phenytoin, S-warfarin, nonsteroidal anti-inflammatory drugs (NSAIDs), sulfonylureas (antidiabetic drugs) and others (Ibrahim, 2016). The bilberry-induced activity of CYP2C9 could lead to a moderate decrease in the efficacy of the above-mentioned drugs. The potential of anthocyanins to elevate CYP2C11 was described previously in rats (Ibrahim, 2016). On the other hand, the 58-day consumption of the higher bilberry dose led to a mild decrease in CYP2E1 and 2C11 activities, indicating that enzyme inhibition outbalanced the induction when the higher dose was consumed for a longer time. The chronic administration of bilberry extract for 58 days also caused a mild increase in CYP3A2 and decrease in CYP1A2 activity. Considering the fact that all bilberry-induced modulation of CYPs activities was only moderate, we assume a low clinical significance of this effect.

Carbonyl-reducing enzymes have been evolved by organisms to detoxify reactive carbonyl compounds (Matsunaga et al., 2006). In the

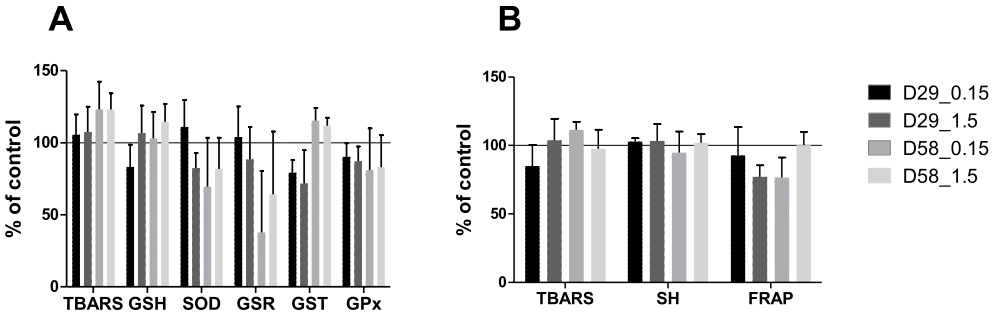

Fig. 7. Antioxidant parameters in erythrocytes (A) and plasma (B) after 29 and 58 days of bilberry extract administration in drinking water (0.15 or 1.5 g/L). Results are expressed relative to control group and represent means ± SD (n = 3). Significance of differences from the control was determined by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons.

literature, there is only limited information about the effect of bilberry extract or anthocyanins on these enzymes. Kropat et al. (2013) described elevated NQO1 mRNA expression after bilberry extract consumption in healthy volunteers (Kropat et al., 2013). In our study, we observed only insignificantly elevated NQO1 mRNA levels after the 29-day consumption of bilberry extract. However, the 58-day consumption led to a reduction in NQO1 mRNA level. The short-term consumption of bilberry extract led to an elevation of AKR1A1 mRNA level, while its activity was mildly reduced. The mRNA levels of AKR1C1 and AKR1C14 were increased after the long-term consumption of bilberry extract and AKR1C activity was also increased, but only in rats with the lower dose of bilberry extract. The expression of CBR1 mRNA was not affected by bilberry extract, but CBR1 activity was decreased in the D58_1.5 group. These data are in accordance with findings (Szotakova et al., 2013) that anthocyanidins exhibit the potential to decrease CBR1 activity in rat and human liver subcellular fractions. However, the observed changes are relatively mild and should not significantly affect the biotransformation of drugs bearing a carbonyl group.

In phase II of biotransformation, xenobiotics or phase I metabolites undergo conjugation reactions with endogenous agents, e.g. glutathione, glucuronic acid, amino acids, sulfates, methyl or acetyl groups, to reduce toxicity and/or increase hydrophilicity. Glucuronidation and methylation were identified as the main metabolic pathways of anthocyanins (Chu et al., 2011). Thus, anthocyanidins in bilberry extract could compete with other UGT and methyltransferase substrates. This effect was already described in rat and human liver subcellular fractions, where all the studied anthocyanidins (pelargonidin, delphinidin, malvidin and cyanidin) decreased UGT activity (Szotakova et al., 2013). (Dvorak et al., 2014) determined the mRNA levels of several isoforms of UGT and GST after 24 and 48 h of exposure to 27 anthocyanins in primary cultures of human hepatocytes. According to their study, anthocyanidins and anthocyanins had no effect on the expression of UGT. The data obtained in our study are in agreement with the above studies. Bilberry extract did not exhibit any significant effect on UGT mRNA level, and the activity was mildly decreased after 29 days of bilberry extract administration. In our study, GST and SULT activity was mildly increased by the long-term administration of bilberry extract. The elevation of GST mRNA and activity caused by bilberry extract, anthocyanins or anthocyanidins was observed previously (Dvorak et al., 2014). In any case, all the observed changes in conjugation enzyme expression and activities were so mild that no significant pharmacological/toxicological consequences can be expected.

In summary, the effects of the chronic consumption of bilberry extract at two concentrations on DMEs and a panel of physiological, biochemical, hematological, and antioxidant parameters were studied and evaluated in rats. The results showed that the chronic intake of bilberries as a food supplement should not have a harmful effect on the rat organism. Moreover, bilberry fruits consumption possesses very low, if any, potential to affect drug metabolism and efficacy.

Declaration-of-competing-interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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Transparency document

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Abbreviations

AhR	Aryl hydrocarbon receptor
AKR	Aldo-keto reductase
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMYL	Alpha amylase
AST	Aspartate aminotransferase
A/G	Albumin-to-globulin ratio
BCA	Bicinchoninic acid
CAR	Constitutive androstane receptor
CBR	Carbonyl reductase
CHOL	Total cholesterol
COMT	Catechol-O-methyltransferase
CREA	Creatinine
CYP	Cytochrome P450
DMEs	Drug-metabolizing enzymes
EDTA	Ethylenediaminetetraacetic acid
FRAP	Ferric reducing antioxidant power
GLOB	Globulin
GLU	Glucose
GPx	Glutathione peroxidase
GSR	Glutathione reductase
GSH	Glutathione
GST	Glutathione S-transferase
HCT	Hematocrit
HDL	High-density lipoprotein
HGB	Haemoglobin
HI	Index of haemolysis
HPLC	High-performance liquid chromatography
HPRT1	Hypoxanthine phosphoribosyltransferase 1
MCH	Mean (red blood) cell HGB
MCHC	Mean (red blood) cell HGB concentration
MCV	Mean (red blood) cell volume
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NSAIDs	Nonsteroidal anti-inflammatory drugs
NQONAD(P)H	NAD(P)Hquinone oxidoreductase
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
PLT	Platelets
PXR	Pregnane X receptor
RBC	Red blood cells
SOD	Superoxide dismutase
SULT	Sulfotransferase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBIL	Total bilirubin
TP	Total protein
TRIG	Triglycerides
UA	Uric acid
UGT	UDP-glucuronosyltransferase
WBC	White blood cells

References

Aleksunes, L.M., Klaassen, C.D., 2012. Coordinated regulation of hepatic phase I and II drug-metabolizing genes and transporters using AhR-, CAR-, PXR-, PPAR alpha-, and nrf2-null mice. Drug Metab. Dispos. 40, 1366–1379. https://doi.org/10.1124/dmd.112.045112.

Anzenbacher, P., Zanger, U.M., 2012. Metabolism of Drugs and Other Xenobiotics, first ed. Wiley-VCH Verlag & Co. KGaA, Weinheim.

Aoyama, N., Tsunoda, M., Imai, K., 2005. Improved assay for catechol-O-methyltransferase activity utilizing norepinephrine as an enzymatic substrate and reversed-phase high-performance liquid chromatography with fluorescence detection. J. Chromatogr. A 1074, 47–51. https://doi.org/10.1016/j.chroma.2005.03.037.

Aviram, M., Kent, U.M., Hollenberg, P.F., 1999. Microsomal cytochromes P450 catalyze the oxidation of low density lipoprotein. Atherosclerosis 143, 253–260. https://doi.org/10.1016/S0021-9150(98)00296-2.

Bártíková, H., Skálová, L., Drsata, J., Bousova, I., 2013. Interaction of anthocyanins with drug-metabolizing and antioxidant enzymes. Curr. Med. Chem. 20, 4665–4679. https://doi.org/10.2174/09298673113209990153.

Boehm, O., Zur, B., Koch, A., Tran, N., Freyenhagen, R., Hartmann, M., Zacharowski, K., 2007. Clinical chemistry reference database for Wistar rats and C57/BL6 mice. Biol. Chem. 388, 547–554. https://doi.org/10.1515/Bc.2007.061.

Brunetti, L., Orlando, G., Ferrante, C., Recinella, L., Leone, S., Chiavaroli, A., Di Nisio, C., Shohreh, R., Manippa, F., Ricciuti, A., Vacca, M., 2013. Orexigenic effects of omentin-1 related to decreased CART and CRH gene expression and increased norepinephrine synthesis and release in the hypothalamus. Peptides 44, 66–74. https://doi.org/10.1016/j.peptides.2013.03.019.

Chen, M.J., Suzuki, A., Borlak, J., Andrade, R.J., Lucena, M.I., 2015. Drug-induced liver injury: interactions between drug properties and host factors. J. Hepatol. 63, 503–514. https://doi.org/DOI 10.1016/j.jhep.2015.04.016.

Chu, W., Cheung, S.C.M., Lau, R.A.W., Benzie, I.F.F., 2011. Bilberry (Vaccinium myrtillus L.). In: Benzie, I.F.F., Wachtel-Galor, S. (Eds.), Herbal Medicine: Biomolecular and Clinical Aspects, Boca Raton (FL).

Dovrtelova, G., Noskova, K., Jurica, J., Turjap, M., Zendulka, O., 2015. Can bioactive compounds of Crocus sativus L. influence the metabolic activity of selected CYP enzymes in the rat? Physiol. Res. 64 (Suppl. 4), S453–S458.

Dvorak, Z., Srovnalova, A., Svecarova, M., Vrzal, R., 2014. The effect of anthocyans on the expression of selected phase II xenobiotic-metabolizing enzymes in primary cultures of human hepatocytes. Food & function 5, 2145–2151. https://doi.org/10.1039/c4fo00347k.

ESCOP, 2003. Myrtilli Fructus – Bilberry Fruit, Monographs, second ed. G. Thieme Verlag, Stuttgart, pp. 345–350.

Gaedigk, A., Ingelman-Sundberg, M., Miller, N.A., Leeder, J.S., Whirl-Carrillo, M., Klein, T.E., Comm, P.S., 2018. The pharmacogene variation (PharmVar) consortium: incorporation of the human cytochrome P450 (CYP) allele nomenclature database. Clin. Pharmacol. Ther. 103, 399–401. https://doi.org/10.1002/cpt.910.

Giknis, M.L.A., Clifford, C.B., 2008. Clinical Laboratory Parameters for Crl:WI (Han). Charles River Laboratories.

Gruenwald, J., Brendler, T., Jaenicke, C., 2000. PDR for Herbal Medicines, second ed. Medical Economics Company, Inc., Montvale.

Hu, Y., Deng, L., Chen, J., Zhou, S., Liu, S., Fu, Y., Yang, C., Liao, Z., Chen, M., 2016. An analytical pipeline to compare and characterise the anthocyanin antioxidant activities of purple sweet potato cultivars. Food Chem. 194, 46–54. https://doi.org/10.1016/j.foodchem.2015.07.133.

Ibrahim, Z.S., El-Shazly, S.A., Ahmed, M.M., Soliman, M.M., 2016. Effects of pomegranate on drug metabolizing cytochrome P450 enzymes expressions in rats. Glob. Vet. 16, 481–490. https://doi.org/10.5829/idosi.gv.2016.16.05.103153.

Kohle, C., Bock, K.W., 2009. Coordinate regulation of human drug-metabolizing enzymes, and conjugate transporters by the Ah receptor, pregnane X receptor and constitutive androstane receptor. Biochem. Pharmacol. 77, 689–699. https://doi.org/10.1016/j.bcp.2008.05.020.

Kropat, C., Mueller, D., Boettler, U., Zimmermann, K., Heiss, E.H., Dirsch, V.M., Rogoll, D., Melcher, R., Richling, E., Marko, D., 2013. Modulation of Nrf2-dependent gene transcription by bilberry anthocyanins in vivo. Mol. Nutr. Food Res. 57, 545–550. https://doi.org/10.1002/mnfr.201200504.

Latti, A.K., Riihinen, K.R., Kainulainen, P.S., 2008. Analysis of anthocyanin variation in wild populations of bilberry (Vaccinium myrtillus L.) in Finland. J. Agric. Food Chem. 56, 190–196. https://doi.org/10.1021/jf072857m.

Lee, J., Chang, C., Liu, I., Chi, T., Yu, H., Cheng, J., 2001. Changes in endogenous monoamines in aged rats. Clin. Exp. Pharmacol. Physiol. 28, 285–289. https://doi.org/10.1046/j.1440-1681.2001.03439.x.

Lee, Y.M., Yoon, Y., Yoon, H., Park, H.M., Song, S., Yeum, K.J., 2017. Dietary anthocyanins against obesity and inflammation. Nutrients 9. https://doi.org/10.3390/nu9101089.

Liang Chung-Tiang, C.M.-H., Chou-Chu, Hong, Huang, Kuen-Jeng, 1999. Establishing the blood chemistry reference values for SPF rats and mice. J. Chin. Soc. Vet. Sci. 25, 55–68.

Lisitsa, A.V., Guseva, S.A., Karuzina, I.I., Archakov, A.I., Koymans, L., 2001. Cytochrome P450 database. SAR QSAR Environ. Res. 12, 359–366. https://doi.org/10.1080/10629360108033244.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Lněničková, K., Procházková, E., Skálová, L., Matoušková, P., Bártíková, H., Souček, P., Szotáková, B., 2016. Catechins variously affect activities of conjugation enzymes in proliferating and differentiated caco-2 cells. Molecules 21, 1186. https://doi.org/10.3390/molecules21091186.

Lněničková, K., Svobodová, H., Skálová, L., Ambrož, M., Novák, F., Matoušková, P., 2018. The impact of sesquiterpenes β-caryophyllene oxide and trans-nerolidol on xenobiotic-metabolizing enzymes in mice in vivo. Xenobiotica 48, 1089–1097. https://doi.org/10.1080/00498254.2017.1398359.

Matsunaga, T., Shintani, S., Hara, A., 2006. Multiplicity of mammalian Reductases for xenobiotic carbonyl compounds. Drug Metab. Pharmacokinet. 21, 1–18. https://doi.org/10.2133/dmpk.21.1.

Palikova, I., Vostalova, J., Zdarilova, A., Svobodova, A., Kosina, P., Vecera, R., Stejskal, D., Proskova, J., Hrbac, J., Bednar, P., Maier, V., Cernochova, D., Simanek, V., Ulrichova, J., 2010. Long-term effects of three commercial cranberry products on the antioxidative status in rats: a pilot study. J. Agric. Food Chem. 58, 1672–1678. https://doi.org/10.1021/jf903710y.

Phillips, I.R., Shephard, E.A., 2006. Cytochrome P450 Protocols, second ed. Humana Press, Totowa.

Prakash, C., Zuniga, B., Song, C.S., Jiang, S., Cropper, J., Park, S., Chatterjee, B., 2015. Nuclear receptors in drug metabolism, drug response and drug interactions. Nucl. Recept. Res. 2. https://doi.org/10.11131/2015/101178.

Prior, R.L., Cao, G.H., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., Mainland, C.M., 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. J. Agric. Food Chem. 46, 2686–2693. https://doi.org/10.1021/Jf980145d.

Prokop, J., Anzenbacher, P., Mrkvicová, E., Pavlata, L., Zapletalová, I., Šťastník, O., Martinek, P., Kosina, P., Anzenbacherová, E., 2018. In vivo evaluation of effect of anthocyanin-rich wheat on rat liver microsomal drug-metabolizing cytochromes P450 and on biochemical and antioxidant parameters in rats. Food Chem. Toxicol. 122, 225–233. https://doi.org/10.1016/j.fct.2018.10.029.

Seeram, N.P., 2008. Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. J. Agric. Food Chem. 56, 627–629. https://doi.org/10.1021/jf071988k.

Silvan, J.M., Reguero, M., de Pascual-Teresa, S., 2016. A protective effect of anthocyanins and xanthophylls on UVB-induced damage in retinal pigment epithelial cells. Food Funct 7, 1067–1076. https://doi.org/10.1039/c5fo01368b.

Stage, T.B., Damkier, P., Christensen, M.M.H., Nielsen, L.B.K., Hojlund, K., Brosen, K., 2016. Impaired glucose tolerance in healthy men treated with st. John's wort. Basic Clin. Pharmacol. 118, 219–224. https://doi.org/10.1111/bcpt.12486.

Szotakova, B., Bartikova, H., Hlavacova, J., Bousova, I., Skalova, L., 2013. Inhibitory effect of anthocyanidins on hepatic glutathione S-transferase, UDP-glucuronosyltransferase and carbonyl reductase activities in rat and human. Xenobiotica 43, 679–685. https://doi.org/10.3109/00498254.2012.756557.

Weingand, K., Brown, G., Hall, R., Davies, D., Gossett, K., Neptun, D., Waner, T., Matsuzawa, T., Salemink, P., Froelke, W., Provost, J.P., DalNegro, G., Batchelor, J., Nomura, M., Groetsch, H., Boink, A., Kimball, J., Woodman, D., York, M., FabiansonJohnson, E., Lupart, M., Melloni, E., 1996. Harmonization of animal clinical pathology testing in toxicity and safety studies. Fundam. Appl. Toxicol. 29, 198–201. https://doi.org/10.1006/faat.1996.0022.

Williams, C.M., El Mohsen, M.A., Vauzour, D., Rendeiro, C., Butler, L.T., Ellis, J.A., Whiteman, M., Spencer, J.P., 2008. Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. Free Radic. Biol. Med. 45, 295–305. https://doi.org/10.1016/j.freeradbiomed.2008.04.008.

Winkel-Shirley, B., 2001. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol. 126, 485–493. .126.2.485. https://doi.org/10.1104/.