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Characterization of dried chokeberry fruit extract and its chronic effects on blood pressure and oxidative stress in spontaneously hypertensive rats

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ABSTRACT

The potential beneficial effects of chronic consumption of dried chokeberry fruit extract (DCE) were evaluated in the present *in vivo* study. The study concentrates on haemodynamic parameters, lipid profile and oxidative stress in spontaneously hypertensive rats. DCE was characterized by HPLC/DAD method and individual anthocyanins, phenolic acids, and flavonoids were determined. Four week administration of DCE, rich in polyphenols, especially anthocyanins, significantly reduced systolic ($P < 0.05$), pulse pressure ($P < 0.05$) compared to control group, probably via significantly increased diuresis ($P < 0.05$). Significant decrease of plasma and erythrocytes TBARS was also found in the treated group. DCE consumption reduced lipid peroxidation through improving plasma FRAP reducing ($P < 0.01$). The activity of SOD in the treated group had been significantly lower ($P < 0.01$) compared to the control group. These results suggest that DCE could exert beneficial effects on cardiovascular health and has a potential as a nutritional supplement in arterial hypertension.

1. Introduction

Hypertension as one of the most frequent chronic cardiovascular diseases is considered a great cause of premature death and disability worldwide, very often associated with oxidative stress and endothelial dysfunction (Schindler, 2007). An increasing amount of research is directed to developing novel antihypertensive agents in order to prevent or treat cardiovascular disorders. This could replace currently available drugs that have many side effects (Endoh & Hori, 2006). High pulse pressure could provoke heart attacks or other cardiovascular disease (Oparil, Zaman & Calhoun, 2003). Polyphenols, secondary plant products are showing high biological activity and health benefits, especially in some chronic diseases (Shahidi & Ambigaipalan, 2015). Chokeberry (*Aronia melanocarpa*) is one of the richest sources of dietary polyphenols with documented health promoting effects, especially for the prevention and treatment of cardiovascular diseases. It also has the greatest antioxidant potential due to the high presence of polyphenols (Kokotkiewicz, Jaremicz & Luczkiewicz, 2010). Anthocyanins, proanthocyanidins, flavan-3-ol and flavonol glycosides and phenolic acids are the main polyphenols present in chokeberries (Rugina et al., 2012; Sueiro et al., 2006). The effects of chokeberry juice and extracts were evaluated earlier in *Wistar* rats with L-NAME induced hypertension (Morosanu, Ciocoiu, Badescu & Badescu, 2011; Ciocoiu, Badescu, Miron & Badescu, 2013), spontaneously hypertensive rats (SHR) (Park & Park, 2011; Hellstrom et al., 2010), healthy humans (Kardum et al., 2014), and those with hypercholesterolemia (Duchnowicz, Nowicka, Koter-Michalak & Broncel, 2012), metabolic syndrome (Broncel et al., 2010), and hypertension type I (Kardum et al., 2015). Numerous studies demonstrate and suggest that chokeberry consumption reduces blood pressure and has a positive influence on other cardiovascular diseases. Nevertheless, the exact mechanism of this phenomenon has not been understood so far, especially the connection with antioxidative effects. Oxidative stress has been involved in many disorders due to the reactive oxygen species (ROS), which play an important role in the development of hypertension by acting on blood pressure increase, myocardial structural changes, and lipid peroxidation (Zhu, Daghini & Chade,

Abbreviations: SHR, spontaneously hypertensive rats; DCE, dried chokeberry fruit extract; ROS, reactive oxygen species; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; PP, pulse pressure; CI, cardiac index; HR, heart rate; TVR, total vascular resistance; CBF, carotid blood flow; CVR, carotid vascular resistance; ABF, aortal blood flow; AVR, aortal vascular resistance, RBF, renal blood flow; RVR, renal vascular resistance; Ccr, creatinine clearance; Cu, urea clearance; Up/cr, urine protein to creatinine ratio; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; CE, catechin equivalents; TE, Trolox equivalent; FRAP, ferric reducing antioxidant power; ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; BHT, butylated hydroxytoluene; HDL, C-high density lipoprotein cholesterol; LDL, C low density lipoprotein cholesterol; TBARS, thiobarbituric acid reactive substances; NO, nitric oxide

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2006). Antioxidant enzymes, as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) represent endogenous defence system which could neutralize ROS. Polyphenols could scavenge free radicals and decrease oxidative damage by possibly donating hydrogen electrons (superoxide anion and hydroxyl radicals) from many hydroxyl groups (Shahidi & Ambigaipalan, 2015).

The aim of this study was to investigate potential effects of a 4 week long polyphenol rich dried chokeberry fruit extract (DCE) treatment of systemic and regional haemodynamics, lipid profile and oxidative status in a model of essential hypertension.

2. Material and methods

2.1. Chemicals

Ethanol, methanol, glacial acetic acid, hydrochloric acid, formic acid, orthophosphoric acid and sodium carbonate were purchased from (Sigma–Aldrich Chemie GmbH, Munich, Germany). HPLC-grade acetonitrile was obtained from Merck (Darmstadt, Germany), and ultrapure water was prepared using a Milli-Q purification system (Millipore, France). The anthocyanin standards cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and flavonoids quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-galactoside (hyperoside) and quercetin-3-*O*-glucoside (isoquercetin) were purchased from Extrasynthese (Cedex, France). Gallic and chlorogenic acids and catechin were obtained from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteu reagent, p-dimethylaminocinnamaldehyde reagent, lithium-heparin, nitrate reductase (NAD[P]H) from *Aspergillus niger*, 4,6-Dihydroxy-2-mercaptopyrimidine (TBA), (N-(1-Naphthyl) ethylenediamine and sulfanilamide) Griess reagent were purchased from Sigma Chemicals Co. (USA). Sodium pentobarbital was from Serva, Heidelberg, Germany. All other reagents were of analytical grade. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.2. Extract preparation

Extract was prepared from dried chokeberry fruits using maceration as the most common technique for extraction. Berries were collected from the plantation located at the mountain Suvobor, Serbia (700 m altitude, 44°08′16.04″N, 20°10′56.28″E) at the end of July 2015., at the fully ripened stage. Soil type was Calcocambisol, loamy-skeletal with rock (< 25%), well drained, with the slightly acidic reaction (pH 6.2). The climate was continental and characterized with average rainfall precipitation of 50 mm and average minimal-maximal temperature range between 3.3 and 27.3 °C during the vegetation period (March–September). The collected berries were dried in the laboratory dryer (Instrumentaria ST 01/02, Zagreb, Croatia) at 40 °C for 48 h. Dried berries (moisture content 10.65 ± 1.39%) were ground by industry mill and obtained particles were separated using a mesh of sieve 0.75 mm (Ćujić et al., 2016) according to Yugoslavian Pharmacopeia (PhYug V, 2000). Maceration was performed on a shaker (Unimax 1010, Heidolph, Germany) with agitation fixed at 170 rpm, at ambient temperature of 25 °C. Ground and sieved berries were shaken in mixture ethanol-water (50%) for 60 min, while solid-solvent ratio was 1:20. These extraction conditions were previously chosen as optimal for the extractions of polyphenols from chokeberry (Ćujić et al., 2016).

2.3. Preparation of the dried chokeberry fruit extract (DCE) for in vivo study

For the purpose of the further *in vivo* study, ethanol was evaporated from the 50% ethanolic extract under the rotary vacuum evaporator. Thus obtained chokeberry extract than was frozen at −80 °C for 1 h before freeze drying, which was carried out at −60 °C (at a pressure of 0.011 mbar) for 24 h and at −60 °C (at pressure of 0.0012 mbar) for an additional hour, in order to remove the capillary water residues (Beta 1–8 Freeze Dryer, Martin Christ, GmbH, Osteroide am Harz, Germany). Extract obtained by lyophilization as the most suitable drying method for sensitive compounds preservation, was labeled as dried chokeberry fruit extract (DCE) and was used for extract characterization and *in vivo* application.

2.4. Determination of total phenolics

The total phenolic content of DCE was determined by a modified Folin-Ciocalteu method (Waterman & Mole, 1994). Briefly, 200 µl of DCE aliquots (0.5 mg/ml) were added to 1 ml of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 800 µl of sodium carbonate (75 g/l) were added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured by a spectrophotometer (Hewlett Packard, 400 N). Gallic acid (0–100 mg/l) was used as a standard for calibration. The calibration curve was ($A = 0.10281 \times C$, $r > 0.99$), and the amounts of total phenolic compounds are presented as milligrams of gallic acid equivalents per gram of DCE (lyophilized extract). Triplicate measurements were taken and mean values were calculated.

2.5. Determination of total anthocyanins

The total anthocyanins content of DCE was investigated according to the procedure described in European Pharmacopoeia 6.0. (2008) with slight modifications. The absorbance of the 200 µl DCE (0.5 mg/ml) was measured at 528 nm, using a 0.1% (v/v) solution of hydrochloric acid in methanol as the compensation liquid. The content of anthocyanins in lyophilized extract, expressed as cyanidin-3-*O*-glucoside chloride, was calculated from the expression: $A \times 5000/718 \times m$ (A = absorbance at 528 nm; 718 = specific absorbance of cyanidin-3-*O*-glucoside chloride at 528 nm; m = mass of DCE to be examined in grams), and results are presented as milligrams of cyanidin-3-*O*-glucoside equivalents per gram of DCE (lyophilized extract). Triplicate measurements were taken and mean values were calculated.

2.6. Determination of total proanthocyanidins

The content of total proanthocyanidin compounds was determined spectrophotometrically using the *p*-dimethylaminocinnamaldehyde (*p*-DMACA) reagents with slight modifications (Li, Tanner & Larkin, 1996). One hundred µl of DCE solution (0.5 mg/ml) were mixed with 80 µl of *p*-DMACA reagent, 2 ml of methanol, and a drop of glycerol. After 7 min, the absorbance was measured at 640 nm. The contents of proanthocyanidins in samples were presented as milligrams of catechin equivalents per gram of DCE (mg CE/g of lyophilized extract).

2.7. Quantitative analysis of individual anthocyanins and flavonoids

For quantification of anthocyanins, flavonoids and phenolic acids, DCE was analysed on Agilent series 1200 RR HPLC instrument (Agilent, Waldbronn, Germany), with DAD detector, on a reverse phase Lichrospher RP-18 (Agilent) analytical column (250 × 4 mm i.d., 5 µm particle size). Anthocyanins were separated by next conditions: the mobile phase consisted of solvent A (10% of formic acid in water) and solvent B (acetonitrile). Samples were separated by gradient elution according to the following scheme: 1% B 0–0.5 min; 1–7% B 0.5–1 min; 7% B 1–4 min; 7–10% B 4–7.5 min; 10–14% B 7.5–11.5 min; 14–25% B 11.5–15.5 min; 25–40% B 15.5–18.5 min; 40–75% B 18.5–22 min; 75% B 22–25 min. Flow was adjusted to 1 ml/min, and detection wavelengths were set at 290, 350 and 520 nm. Quantification was done using calibration curves of anthocyanin standards cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-arabinoside. Flavonoids and chlorogenic acids were separated under conditions: mobile phase consisted of solvent A (1% solution of orthophosphoric acid in water) and