

## HYPOTHENSIVE AND ANTIOXIDANT EFFECTS INDUCED BY POLYPHENOL RICH BLACK CHOKEBERRY (*ARONIA MELANOCARPA* [MICHX.] ELLIOTT) JUICE

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Chokeberry (*Aronia melanocarpa* (Michx.) Elliott) has been traditionally used for centuries in the herbal medicine of the Native North Americans considering the numerous pharmacological activities. The aim of this research was to analyze the effects of the chokeberry juice on the cardiovascular activity, in order to authenticate the use of *Aronia melanocarpa* as a functional food. Nevertheless, the antioxidative properties of chokeberry juice were determined by DPPH method and the  $\beta$ -carotene/linoleic acid model system, to prove the estimated activity of the juice. The significant antioxidative activity was confirmed. The concentration of chokeberry that inhibited 50 % of DPPH free radicals (IC<sub>50</sub>) was  $1.25 \pm 0.08$  mg/ml. In  $\beta$ -carotene/linoleic acid model system IC<sub>50</sub> was achieved by concentration of  $1.73 \pm 0.07$  mg/ml. The effects of chokeberry juice on the blood pressure and heart rate in anaesthetized rabbits were performed. The results demonstrated the reduction of the blood pressure (EC<sub>50</sub> value of  $195.63 \pm 14.45$  mg/kg, the concentration which elicited 50 % of maximal response) and heart rate (EC<sub>50</sub> value of  $171.71 \pm 11.21$  mg/kg) in the animals. The administration of the chokeberry juice could produce hypotension and negative chronotropic effects. However, it is necessary to conduct the study in human population to confirm those findings.

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**Key words:** *Aronia melanocarpa* (Michx.) Elliott, chokeberry juice, antioxidant effects, blood pressure, heart rate

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### Introduction

The berries of *Aronia melanocarpa* (Michx.) Elliott (black chokeberry, aronia, aronia berry), a woody shrub of the Rosaceae family, have been used in the Native North American herbal medicine for centuries. In the last decades, the interest of food and medicinal science has been focused on chokeberry's secondary metabolites with highly expressed antioxidant activity and their possible use in

the prevention and treatment of many diseases (1, 2).

The chemical and nutritive composition of *A. melanocarpa* berries have been determined by previous research. Chokeberries are the rich source of polyphenolic compounds, as phenolic acids, proanthocyanidins, anthocyanins, flavonols, and flavanones (2-5). Anthocyanins are the dominating flavonoids in chokeberry, representing about 25 % of total polyphenols (6-9). Studies have also confirmed the presence of dietary fiber in *A. melanocarpa* berries (10) and several other constituents such as microcrystalline cellulose, pectins, lignins, cutin-like polymers and condensed tannins (11). The content of fat and proteins in fruits was analyzed, and there was found to be 0.14 g/100 g FW of fat and 0.7 g/100 g FW of proteins in fresh chokeberries (10). Fresh chokeberries also contain 16-18% of reducing sugar, which is often used in a diet as a weak laxative (9). The mineral content (ash values) of fresh chokeberry has also been confirmed and it was 440 mg/100 g 13 and 580 mg/100 g. During processing, the mineral content of juices was between 300 and 640 mg/100 mL (12). Chokeberry fruits contain relatively high average amounts of K and Zn. Nevertheless, they contain small amounts of Na, Ca, Mg and Fe (10). In fresh pressed juice vitamins B1, B2,

B6, C, pantothenic acid and niacin (13) were found. Some studies have confirmed the presence of carotenoids,  $\beta$ -carotene and  $\beta$ -cryptoxanthin, in comparatively high amounts of chokeberry fruits (10, 14).

*A. melanocarpa* fruits contain mostly cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, cyanidin-3-O-xyloside and cyanidin-3-O-glucoside, among anthocyanins (15). The high content of phenolic compounds, especially anthocyanins with their highly expressed antioxidant activity, correlates with the biological activity of these berries and their use in health promoting and well-being purposes (16-18). Anthocyanins have an active role in cardio and neuroprotection, and as anticancer, hepatoprotective, gastroprotective, anti-inflammatory, normolipidemic and normo-glycemic agents and intracellular antioxidants (19-22).

We performed this study to examine the effects of chokeberry juice on the blood pressure and heart rate in anaesthetized rabbits, in addition to its antioxidant properties.

## Materials and methods

### *Plant material and juice preparation*

Fruits of black chokeberry were collected from a plantation field on the mountain Suvobor (750 m.a.s.l.), Serbia, in August 2011. The berries were stored at +5°C for 24 h. Fresh berries were crushed and squeezed. The yield of the juice with respect to the weight of the fresh fruits was 73 %. The juice was filtered, pasteurized at 80°C for 10 min and stored at 0°C. For the experiment the juice was diluted with distilled water to the appropriate concentration.

### *Determination of antioxidant capacity*

The free radical scavenging activity of the juice on the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was carried out according to the procedure described previously (23), with slight modifications. The juice sample (2 g) was diluted with distilled water up to 10 ml, centrifuged (2500 $\times$  g; 10 min) and a supernatant was used for analyses. The antiradical capacity of the sample was evaluated using a dilutions series, in order to obtain a large spectrum of sample concentrations. The juice (100  $\mu$ l) was mixed with 1400  $\mu$ l of 80 mM methanol solution of DPPH. The absorbance at 540 nm was measured after 20 min. The percentage of inhibition was calculated using the equation:

Inhibition % =  $[(A_0 - A_i) / A_0] \times 100$ , where  $A_0$  is absorbance of the control and  $A_i$  is absorbance of the sample. The concentration of the juice that inhibited 50% of DPPH radicals ( $IC_{50}$ ) was calculated from the concentration/% inhibition curve. Trolox was used as a positive control.

The assessment of the lipid peroxidation inhibitory activity was carried out according to the  $\beta$ -carotene/linoleic acid model system described by Koleva and others (2002) (24), with modifications. The juice sample (2 g) was diluted with distilled water up to 10 mL, centrifuged (2500 $\times$  g; 10 min)

and supernatant was used for analyses. A solution of  $\beta$ -carotene was prepared by dissolving 2 mg of crystalline  $\beta$ -carotene in 10 ml of chloroform. One millilitre of the solution, 180 mg of Tween 20 and 25  $\mu$ l of linoleic acid were pipetted into a round-bottom flask. After all chloroform had evaporated, 50 ml of oxidized distilled water were added to the flask with gentle shaking. Aliquots (200  $\mu$ l) of this aqueous emulsion were pipetted into each of the wells of 96-well microtiter plates containing 40  $\mu$ l of chokeberry juice in different concentrations. The microtiter plates had been shortly shaken before zero time absorbance was measured by Elisa reader at 450 nm ( $A_0$ ) (ELISA microplate reader Multiskan Ascent No354 (Thermo Labsystems, Finland).

Microtiter plates were then incubated at 55°C for 2 hours and absorbances were read again ( $A_{120}$ ). A blank, deprived of  $\beta$ -carotene, was prepared as a negative control. Antioxidant activity was calculated using the following equation (25):

$$\text{Inhibition \%} = (A_{120} / A_0) \times 100.$$

The concentration of the juice that provided protection of 50 %  $\beta$ -carotene ( $IC_{50}$ ) was calculated from the concentration / % inhibition curve. Trolox was used as a positive control.

### *Blood pressure and heart rate measurement*

Artery blood pressure in the anaesthetized rabbits was measured as described previously (26). The rabbits were anesthetized intravenously with urethane (750 mg/kg). The catheter filled with heparinized saline (60 IU ml<sup>-1</sup>) was implanted into left carotid artery. This catheter was connected to a blood pressure transducer (P-1000-A) coupled with a Narcophysograph (NARCO Bio system, Houston, USA) for measurement arterial pressure. Arterial blood pressure was expressed in mmHg, as systolic and diastolic blood pressure, and then the mean arterial blood pressure was calculated using the following formula:

$$\text{Mean arterial pressure} = \text{diastolic pressure} + (\text{systolic pressure} - \text{diastolic pressure}) / 3$$

The measurements of the blood pressure and heart rate were made before and after the administration of the chokeberry juice. Arterial pressure was allowed to return to the resting level between injections. Changes in blood pressure were recorded as the difference between the steady state values before and after the injection.

Animals were treated with 0.2 ml of black chokeberry juice, which was administered in rising concentrations (0.45 - 150 mg/kg) at intervals of 15 - 20 min. The effects of the black chokeberry were followed during 10 minutes of continuous registration of the arterial blood pressure. All results were based on the data obtained in six different sets of experiment. All experimental procedures with animals were in compliance with the European Union Directive (2010/63/EU) for animal experiments and were also approved by the Animal Ethics Board of the Medical Faculty in Nis (number 01-206-7).

### Statistical analysis

The experimental results were presented as mean  $\pm$  SD of the mean of 6 determinations. Statistical evaluation was performed using the Student's t-test. A probability value of  $p < 0.05$  was considered to be significant. The effective concentrations  $EC_{50}$ , that is the concentration which elicited 50 % of maximal response, were established by regression analysis. Statistical analyses were performed using SPSS statistical software package (ver. 20.0; Chicago, IL, USA).

## Results

### Antioxidant assessment

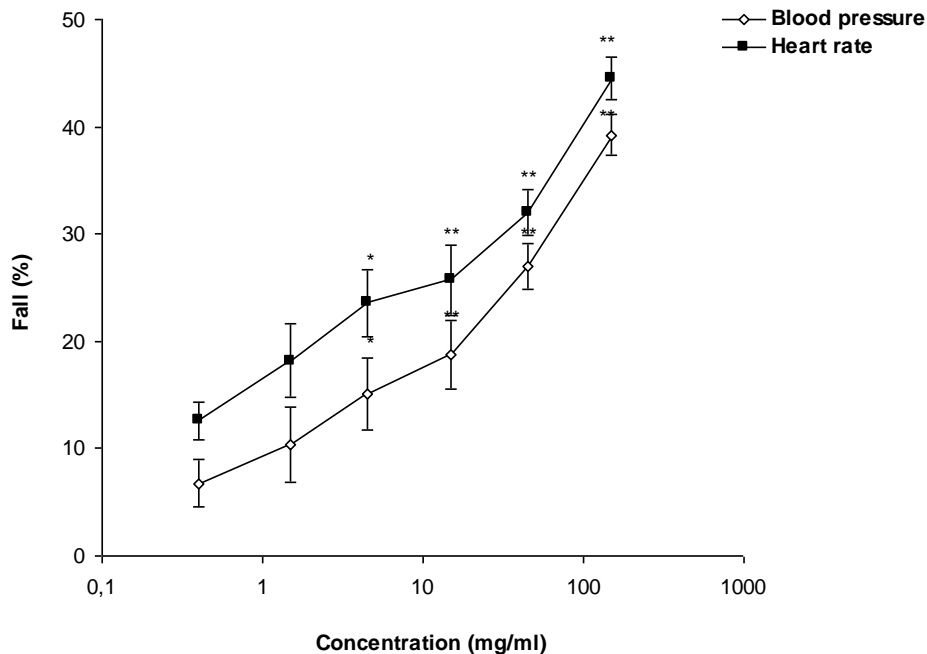
The assessment of antioxidant potency was conducted by two in vitro complementary methods. The concentration of chokeberry juice that inhibited 50% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals ( $IC_{50}$ ) was  $1.25 \pm 0.08$  mg/ml. In  $\beta$ -carotene/linoleic acid model system  $IC_{50}$  was achieved by concentration of  $1.73 \pm 0.07$  mg/ml. Trolox show-

ed better activity in  $\beta$ -carotene/linoleic acid model system than in DPPH ( $1.69 \pm 0.11$  and  $6.15 \pm 0.64$  mg/ml, respectively).

### Hypotensive and bradycardic effects of black chokeberry juice

The intravenous injection of the chokeberry juice, which was administered in rising concentrations, immediately induced significantly and dose-dependent decrease of the systolic, diastolic and mean arterial blood pressure and heart rate of rabbits. The chokeberry juice at doses of 150 mg/kg decreased arterial blood pressure of rabbits for  $39.23 \pm 4.58$  % ( $p < 0.01$ ), with  $EC_{50}$  value of  $195.63 \pm 14.45$  mg/kg.

*In vivo* intravenous application of juice at doses of 150 mg/kg (Graph 1) caused a significant decrease in heart rate in anesthetized rabbits for  $44.48 \pm 5.02$  % ( $p < 0.01$ ), with  $EC_{50}$  value of  $171.71 \pm 11.21$  mg/kg. After the hypotensive peak, the blood pressure and heart rate increased progressively and reached the basal value in about 3-5 min.



values shown are mean  $\pm$  standard deviation of six separate experiments  
\* $p < 0.05$  and \*\*  $p < 0.01$  compared to baseline values before treatment

**Graph 1.** Dose-dependent effects of intravenous injection of the black chokeberry (*Aronia melanocarpa* [Michx.] Elliott) juice on the mean arterial blood pressure and heart rate of anaesthetized rabbits

## Discussion

Black chokeberry fruits are the one of the richest plant sources of phenolic substances, mainly anthocyanins-glucosides of cyanidin (27). Numerous studies have shown that the polyphenolic compounds present in fruit and vegetable exhibit a wide range of biological effects. Well-known are their role as free radical scavengers and their potentially significant interactions with biological systems (28). Anthocyanins exhibit lipid-lowering and anti-aggregative action in patients with metabolic syndrome (29). Yang et al. (2011) (30) showed that a dietary supplementation with the blueberry extracts and cyanidin-3-O-galactoside from blueberry in the aged mice might induce changes of the endogenous plasma and brain metabolic profiles which improves cognitive impairment and neurodegenerative diseases. Previous studies showed that chokeberry juice and products demonstrate strong antioxidative activity (31, 32) and that was confirmed by our results.

Strong antioxidant activities of chokeberry products qualify them to be used in prevention and treatment of numerous non-communicable diseases, such as cardiovascular diseases (33–38). The present study also showed that black chokeberry juice produced significant decrease in blood pressure. Blood pressure is determined by cardiac output and total peripheral resistance; hence chokeberry juice was investigated on heart rate for its possible inhibitory effects. Our study also confirms that the chokeberry juice has hypotensive and bradycardic effects on cardiovascular system. Intravenous injection of the black chokeberry juice induced the short-term and dose-dependent hypotensive and negative chronotropic effects in anesthetized rabbits. Results showing that the chokeberry juice caused a decrease of blood pressure with bradycardic effect indicate that the chokeberry juice might have effect at the cardiovascular regulation region of the central nervous system and/or effect at the heart. These findings are consistent with the literature data. The blood pressure-lowering properties of the juice used in our study are in accordance to those obtained by Hellstrom et al. (2010) (39) with a lyophilized chokeberry juice (*Aronia mitchurinii*) and polyphenols in spontaneously hypertensive rats. Dietary supple-

mentation with extract of the black chokeberry reduced systolic and diastolic blood pressure in patients after myocardial infarction (38), as well as in patients with metabolic syndrome (40, 41).

*In vivo* studies in animal models, as well as in humans, have confirmed that consumption of the chokeberry preparations could improve the blood pressure and lipid status. (35, 38, 42–44). It can be explained by the inhibition of the activity of angiotensin I-converting enzyme by the chokeberry extracts (39, 45). It is known that the chokeberry preparations could protect the coronary arteries (46) and heart muscle (38) from the oxidative damage. Literature data confirmed its anti-inflammatory properties which are connected with the protection of the human aorta and aortic endothelial cells *in vitro* (34, 47).

## Conclusion

The present study demonstrated that black chokeberry juice (*Aronia melanocarpa*) was able to reduce the blood pressure and heart rate in anesthetized rabbits. Intravenous administration of the chokeberry juice could produce hypotension and negative chronotropic effects. Hence, the chokeberry fruit could be used as a functional food ingredient for the control functions of the cardiovascular system. However, it would be necessary to carry out clinical studies to demonstrate its hypotensive effects in humans.

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Aronija (*Aronia melanocarpa* (Michx.) Elliott) se vekovima tradicionalno koristila u biljnoj medicini drevnih naroda Severne Amerike, s obzirom na brojne farmakološke aktivnosti koje ispoljava. Cilj ovog istraživanja bilo je ispitivanje efekata soka ploda aronije na aktivnost kardiovaskularnog sistema, kako bi se potvrdila upotreba soka kao funkcionalne hrane. Antioksidativna aktivnost soka procenjena je pomoću DPPH metode i  $\beta$ -karoten / linolna kiselina model sistema. Potvrđena je značajna antioksidativna aktivnost soka aronije. Koncentracija soka aronije koja je inhibirala 50 % DPPH radikala ( $IC_{50}$ ) bila je  $1,25 \pm 0,08$  mg/ml. U  $\beta$ -karoten / linolna kiselina model sistemu  $IC_{50}$  vrednost iznosila je  $1,73 \pm 0,07$  mg/ml. Efekti soka na krvni pritisak i frekvenciju srčanog rada ispitivani su na anesteziranim kunićima. Rezultati su pokazali smanjenje krvnog pritiska ( $EC_{50}$  vrednost bila je  $195,63 \pm 14,45$  mg/kg, koncentracija koja je izazvala 50 % maksimalnog odgovora) i frekvencije srca ( $EC_{50}$  vrednost bila je  $171,71 \pm 11,21$  mg/kg) kod kunića. Primena soka aronije može izazvati hipotenzivni i negativni hronotropni efekat. Neophodno je sprovesti studije na ljudima kako bi se ovi efekti i potvrdili.

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**Ključne reči:** *Aronia melanocarpa* (Michx.) Elliott, sok aronije, antioksidativna aktivnost, krvni pritisak, frekvencija srčanog rada