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Effect of Chokeberry Juice Consumption on Antioxidant Capacity, Lipids Profile and Endothelial Function in Healthy People: a Pilot Study

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Abstract

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Many studies show that the consumption of juices rich in polyphenols may increase serum antioxidant capacity, improve blood lipid profile, and endothelial function. The relation between the consumption of chokeberry juice and changes in the antioxidant capacity, blood lipid profile, and endothelial function as important indicators in the assessment of cardiovascular risk were determined. The study was conducted on a group of 11 healthy volunteers, who consumed chokeberry juice for three weeks. The research included determination of serum antioxidant capacity, blood lipid profile analysis, and measurements of endothelial function. The 3-week consumption of chokeberry juice significantly increased serum antioxidant capacity, and the best results were observed just after one week of the experiment. However, there was no significant change in the blood lipid profile, except for the persons with a higher level of triglycerides, in whom the consumption of chokeberry juice reduced these compounds to normal values. The endothelial function was normal in all patients and did not significantly change during the study.

Keywords: antioxidant; chokeberry; cardiovascular risk; polyphenols; cardiovascular risk

A diet rich in fruit and vegetables is considered to be an excellent source of natural antioxidants. Antioxidants supplied with food may play an important role in scavenging free radicals, which are responsible for aging and many civilisation diseases (Huang *et al.* 2012). Numerous population studies show that regular consumption of fruit and vegetables rich in antioxidants may prevent cardiovascular disease (Dauchet *et al.* 2006; He *et al.* 2007; Heiss *et al.* 2010). Oral application of fruit juices can increase the serum antioxidant capacity and improve the blood lipid profile. Chrzczanowicz *et al.* (2008) have shown that the consumption of 1 l of apple juice increased the blood serum antioxidant capacity of patients in just an hour, but over the next 4 h the value decreased to the baseline. Better results can be achieved by the consumption of berries rich in polyphenols (Howard *et al.* 2012) and their products, especially juices (Piljac-Zegarac *et al.* 2009). Chokeberry juice, for example, has a high content of bioactive compounds and high antioxidant capacity (Kapci *et al.* 2013). Therefore, polyphenolic-rich products from chokeberries possess cardioprotective, hepatoprotective, antidiabetic, and anticarcinogenic properties (Kulling & Rawel 2008; Broncel *et al.* 2010; Denev *et al.* 2012). *Aronia melanocarpa* fruits contain a lot of polyphenols, especially anthocyanins (mainly cyanidin glycosides), phenolic acids (chlorogenic and neochlorogenic acid), and flavonols (quercetin derivatives) (Wu *et al.* 2004;

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Oszmiański & Wojdyło 2005; Skoczyńska *et al.* 2007; Nowak *et al.* 2015). Moreover, chokeberries are also rich in proanthocyanidins (PACs), which affect the astringent taste of fruit (Wilkes *et al.* 2014).

Various methods have been proposed to determine the antioxidant capacity. The DPPH method is frequently used to analyse the antioxidant capacity of fruit and vegetable juices (Vinson *et al.* 1998, 2001; Seeram *et al.* 2008; Kiss *et al.* 2014). Recently, efforts have been made to use it for the determination of the antioxidant activity of blood plasma of humans or animals, e.g. the horse (Janaszewska & Bartosz 2002; Molyneux 2004; Martinez *et al.* 2006). This method is relatively simple, inexpensive, and fast. Compared to other methods, it gives a linear correlation between the content of flavonoids and the antioxidant capacity (Chrzczanowicz *et al.* 2008; Apak *et al.* 2013; Kapci *et al.* 2013).

A diet rich in polyphenols may also contribute to an improvement of the blood lipid profile. Research conducted on a small group of patients with mild hypercholesterolemia (total cholesterol over 200 mg/dl) showed that chokeberry juice consumption contributed to reduced levels of triglycerides (TG), total cholesterol (TC) and LDL cholesterol (LDL-C) as well as increases levels of HDL cholesterol (HDL-C) (Skoczyńska *et al.* 2007). These results may suggest that the intake of chokeberry juice may aid the prevention of cardiovascular disease, but this therapeutic use requires further research. It would be helpful to perform an assessment of the endothelial function, which ensures an adequate tension of the walls of blood vessels, inhibits the activity of platelets and prevents the adhesion of leukocytes. Classical risk factors for atherosclerosis cause *inter alia* endothelial dysfunction (Stacker & Keaney 2004; Munzel *et al.* 2010). It is believed that the primary mechanism common to all known risk factors for atherosclerosis is oxidative stress. Oxidative stress issues from an imbalance between the production of oxidants (free radicals of oxygen and nitrogen) and antioxidant defence (superoxide dismutase, catalase, glutathione peroxidase). Overproduction of free radicals, both oxygen species (ROS) and nitrogen species (RNS), may be a result (Stacker & Keaney 2004). Among changes caused by ROS is the uncoupling of endothelial nitric oxide synthase (eNOS), which begins to produce RNS. As a result, oxidative stress causes the reduction in NO bioavailability. Besides, RNS can cause direct structural damage to cells and subsequent overproduction of free radicals (Stacker & Keaney 2004; Förstermann 2010; Munzel *et al.* 2010; Tousoulis *et al.* 2011). The improvement of the endothelial function is a target of both primary prevention of atherosclerosis in patients with risk factors and treatment of diseases such as cardiovascular atherosclerosis. Various clinical studies have made attempts to assess the impact of antioxidants (such as vitamins C and E, coenzyme Q10, and polyphenols) on the endothelial function. Major research projects (e.g. Heart Protection Study, Primary Prevention Project) have not confirmed the beneficial effect of antioxidant supplementation, but a number of smaller studies revealed some beneficial effects (Stacker & Keaney 2004; Thomas *et al.* 2008; Zych & Krzepiłko 2010).

The aim of the study was to determine the relation between the consumption of chokeberry juice and changes in the antioxidant capacity, blood lipid profile and endothelial function as important indicators in the assessment of cardiovascular risk.

This pilot study was approved by the Bioethical Committee of Collegium Medicum in Bydgoszcz (No. KB336/2013).

MATERIAL AND METHODS

Subjects. Participants were recruited from the clinic of cardiology associated with the University Hospital of Collegium Medicum in Bydgoszcz. The experimental protocol was approved by the Bioethical Committee. All subjects signed an informed consent after reading the purpose and schedule. Inclusion criteria of participants were: age 18–75 years, sex: men or women, uniform diet excluding other sources of antioxidants and supplements (polyphenolic-rich extracts, vitamins C, A or E, etc.). Subjects were professionally active, with moderate physical effort. They did not change either their lifestyle or the diet during the experiment. Exclusion criteria were: alcohol abuse, chronic inflammatory diseases, chronic organ disorders, diagnosed neoplastic disease, uncontrolled diabetes, diseases which require hormonal treatment, diagnosed kidney disorders, hepatic deficiency, cirrhosis, and other chronic hepatic diseases. The study was conducted on a group of 11 healthy, non-smoking volunteers aged between 23 to 55 years (mean 41.9 years, 70% women), with correct BMI (mean 23.7). Finally one person was excluded because of the intolerance to chokeberry juice.

Chokeberry juice. The study used organic chokeberry juice, produced by traditional methods from

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chokeberry fruits – *Aronia melanocarpa* (Michx.) Elliott cv. Aronia, which belongs to the family *Rosaceae*, native to eastern North America, is a 2–3 m high shrub. The black berries are borne in clusters and ripen in early autumn in Poland. Chokeberry fruits originated from an ecological plantation (organic farm with EKO certificate) located in the south-western parts of Poland and were collected between September and October. The juice was produced by traditional methods, using wicker hydraulic presses and mild flow pasteurisation (temperature no more than 90°C). From one kilogram of fruit about 0.6–0.7 l of juice is generally obtained, without any additives. Chokeberry juice was selected during our previous research, consisting of an analysis of selected fruit juices and nectars rich in polyphenols. This product was characterised by a high polyphenol content (approximately 560 mg GAE/100 ml) determined by the Folin-Ciocalteu assay (Singleton *et al.* 1999). Moreover, its antioxidant capacity determined by DPPH method was several-fold higher than in commercial juices (approximately 400 mg Trolox/100 ml) (Nowak *et al.* 2015).

Experimental protocol. All subjects consumed chokeberry juice between meals (250 ml per day) for three weeks. An exception was Day 7 and 14, when they consumed juice at fasting in the morning, one hour before blood collection. At Day 1 of experiment (before drinking the juice), the starting fasting serum antioxidant capacity was determined in each subject. Next, they drank chokeberry juice in the amount of 250 ml in about 10–15 minutes. Then, 1 h after the application of juice, blood samples were collected for determinations of antioxidant capacity, total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglyceride levels (TG). Blood samples were drawn from the antecubital vein into polyethylene tubes, and the serum was obtained by centrifugation at 3000 rev/min for 10 minutes. All analyses were repeated cyclically each week, i.e. at Day 7, 14, and 21, according to the schedule of experiment.

Additionally, the endothelial function was assessed (EndoPAT 2000 device; Itamar Medical, Ltd., Caesarea, Israel) in all subjects on the first day (before the application of juice), and after 7, 21, and 28 days of the experiment, i.e. after 1 and 3 weeks of drinking the chokeberry juice and one week after its administration. The endothelial function was not assessed in the first hour after the consumption of juice. Data from literature show that an EndoPAT device is unsuitable for assessment of an endothelial response caused by acute noxious or therapeutic factors (Moerland *et al.* 2012).

This research is a pilot study before the administration of chokeberry juice to populations of patients with incident cardiovascular disease and with a high risk of developing atherosclerosis.

Analytical methods. The serum antioxidant capacity was determined by a modified spectrophotometric method developed by Yen and Chen, using 1,1-diphenyl-2-picrylhydrazyl (DPPH – Sigma-Aldrich, Steinheim, Germany) stable radical cation (Yen & Chen 1995; Molyneux 2004). Absorbance was measured on a Hitachi U-3900 spectrophotometer (Hitachi, Tokyo, Japan) at the 517 nm wavelength. Then, the results were converted into the percentage of DPPH inhibition (% DPPH).

The biochemical analysis of blood was determined by standard methods in an accredited laboratory of the University Hospital. Concentrations of TC, HDL-C, and TC were measured using an immunoenzymatic assay (ELISA – Cell Biolabs, Inc., San Diego, USA). The level of LDL-C was calculated by the Friedewald formula: LDL = TC – HDL – TG/5 (mg/dl) (Friedewald *et al.* 1972; Chatterjee & Mendez 2011).

The assessment of the endothelial function was performed on an EndoPAT 2000 device . This apparatus contains plethysmographic sensors, which assess changes in the volume of fingertip caused by passive congestion after an earlier occlusion oppression of the brachial artery (5-min press by sphygmomanometer cuff to a pressure of 60 mmHg over the systolic blood pressure) (Moerland *et al.* 2012). The results are automatically calculated as a ratio of RHI (reactive hyperaemia index). According to the specification of the device, the correct RHI index is above 1.67. This method is simple, fast and non-invasive, having a recommendation for such determinations (Axtell *et al.* 2010; Moerland *et al.* 2012).

Statistical analysis. A software package Statistica Version 10.0, (StatSoft, Krakow, Poland) was used for statistical analysis. Because the conditions of normality and constant variance were not satisfied for some variables, non-parametric tests were performed. In order to compare the related groups the Friedman test was used. Pairwise comparisons were carried out using the Wilcoxon signed rank test with the Bonferroni correction. The median was reported as a measure of the central tendency and an interquartile range (IQR) indicated the spread of

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Table 1. Medians (IQR) of lipid profile in three weeks of observation

Week	TC	LDL-C	HDL-C	TG
	(mg/dl)			
0	195 (168–222)	128 (101–149)	53.0 (45.0–60.0)	112.0 (84.0–121.0)
1	180 (165–226)	105 (95–156)	48.0 (42.0–53.0)	99.0 (68.0–115.0)
2	190 (171–222)	115 (102–143)	48.0 (46.0–55.0)	96.0 (85.0–127.0)
3	195 (176–226)	120 (108–148)	56.0 (46.0–62.0)	102.0 (63.0–119.0)
P-value*	0.045	0.023	0.009	0.62

*Friedman test; IQR – interquartile range; week 0 – analysis done before application of chokeberry juice; week 1, 2, 3 – analysis done after 1, 2, 3 week of experiment (e.i. at 7, 14 and 21 day); values are presented as madian and interquartile range: TC – total cholesterol, LDL-C – LDL cholesterol, HDL-C – HDL cholesterol, TG – triglycerides; *P*-value – probability value evaluated by Friedman test

the data. *P*-values lower than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Antioxidant capacity. The influence of chokeberry juice consumption on changes in the serum antioxidant capacity was determined. The median (IQR) of the antioxidant capacity (before application of the first portion of juice) expressed as a percentage of the DPPH inhibition (% DPPH) was 5.47 (4.03–7.19). It was observed that the consumption of chokeberry juice significantly increased the serum antioxidant capacity in all participants ($P < 0.001$). After a week of drinking the juice, the median level rose to 23.94 (22.08–26.67). Further administration of juice resulted in sustaining the antioxidant capacity on an equally high level of 20.06 (18.76–25.35), without significant differences between the first and second week of drinking the juice (Figure 1). After the third week, a high serum antioxidant capacity persisted, compared to the baseline (before drinking the juice), but the median value (12.82) for all the participants was lower than after two weeks of drinking the juice. This could be due to the action of bioactive compounds in the juice and the maximum reduction of free radicals after 1–2 weeks of its consumption. However, these conclusions require confirmation by further studies on a larger group of people.

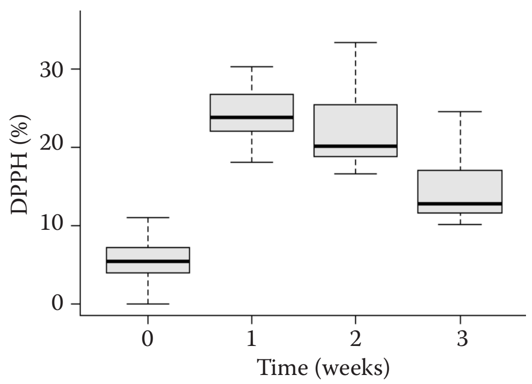

Figure 1. Box-and-whisker plots of the serum antioxidant capacity after the consumption of chokeberry juice

%DPPH – percentage of the DPPH inhibition (median value, interquartile range, min & max); week 0 – analysis done before the application of chokeberry juice; week 1, 2, 3 – analysis done after 1, 2, 3 weeks of experiment (i.e. at day 7, 14 and 21).

Other researchers also reported a significant increase in the serum antioxidant capacity after the consumption of juice. Chrzczanowicz *et al.* (2008) showed a significant increase in the serum antioxidant capacity in 12 subjects during 1 h after the consumption of 1 l of apple juice. Yuan *et al.* (2011) also confirmed an increase in the total antioxidant capacity after 2 weeks of drinking fruit juices rich in polyphenols (grape juice and apple juice).

Blood lipid profile. The influence of chokeberry juice consumption on the blood lipid profile, in particular the level of TG, TC, HDL-C, and LDL.C, was analysed in parallel. The result of the Friedman test indicated some significant fluctuations for TC, HDL-C, and LDL-C fractions (Table 1). The value of the median of HDL-C after a week of drinking the juice was 48.0 (42.0–53.0 mg/dl) and remained on a similar level for another week of the experiment. After 3 weeks, there was a significant increase in HDL-C from 48.0 mg/dl to 56.0 mg/dl (46.0–62.0). However, the multiple comparisons tests did not show any significant differences between the repeated measurements for TC, HDL-C, and LDL-C.

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The conclusions of post-hoc analysis and the Friedman test are inconsistent probably due to the small sample size of our study.

Other researchers have also reported insignificant changes in these values after the long-term consumption of juices (Skoczyńska *et al.* 2007; Flammer *et al.* 2013). Skoczyńska *et al.* (2007) in their study concerning the 6-week consumption of chokeberry juice by 58 men with mild hypercholesterolemia (TC > 200 mg/dl) did not reveal any significant effects for TC, HDL-C, and LDL-C. Furthermore, in the study by Flammer *et al.* (2013), patients with peripheral endothelial dysfunction and cardiovascular risk factors consumed cranberry juice (2 × 230 ml) over a period of four months. In this case, no significant changes in TC, HDL-C, and LDL-C were observed either.

The final parameter considered in our experiment was the level of triglycerides. At the onset of the experiment, the median was 112.0 mg/dl, but went down to 102.0 mg/dl after three weeks of consuming the chokeberry juice. The decrease was not significant because of the small group of people participating in this study. In order to confirm the above relations, further studies are needed on a larger group of participants. However, among the subjects with a higher level of triglycerides (n = 4, mean TG = 201.0 mg/dl), a reduction in these compounds was observed. In this sub-group, the levels of triglycerides decreased by over 12% after 1 week, and by 31% after 2 weeks of drinking the juice, compared to the baseline. Skoczyńska *et al.* (2007) also reported a decrease in triglycerides by 13% during 6 weeks of

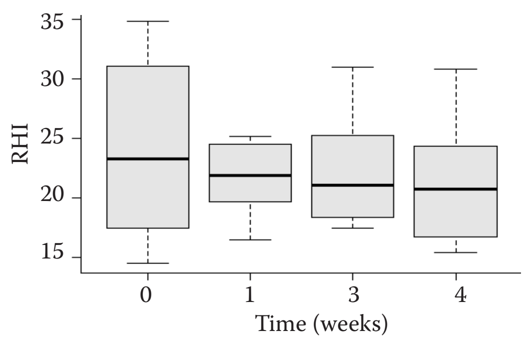

Figure 2. Box-and-whisker plots for the RHI

RHI – reactive hyperaemia index (automatically calculated on an EndoPAT 2000 device); week 0 – analysis done before the application of chokeberry juice; week 1, 3 – analysis done after 1 and 3 weeks of drinking the chokeberry juice (i.e. at Day 7 and 21); week 4 – analysis done one week after finishing the consumption of juice (i.e. at Day 28); the endothelial function was not assessed in the 2nd week

an experiment involving patients with mild hypercholesterolemia.

Endothelial function. An independently evaluated effect of drinking the chokeberry juice on the endothelial function yielded the following findings. The results were assessed by an automatic RHI (reactive hyperaemia index) indicator determined on an EndoPAT 2000 device. There was no significant difference between the medians of RHI determined before serving the juice and during its consumption (P = 0.52, Figure 2). The initial value of the RHI was expressed by a median of 2.33 (1.75–3.11). After 7 days of consuming the chokeberry juice, the median RHI was 2.18 (1.97–2.45) and after 3 weeks, the median RHI fell down to 2.10 (1.84–2.52). One week after the administration of the juice finished, the endothelial function was again evaluated in all the persons with the median RHI of 2.07 (1.67–2.43).

Similar results were obtained by Flammer *et al.* (2013) in their study on the consumption of cranberry juice for 4 months. These authors did not observe any significant changes in the endothelial function, defined by the RHI on an EndoPAT. Literature implies the beneficial effect of a diet rich in polyphenols such as an improvement in the endothelial function, particularly in persons with the RHI below norm. Patients with advanced cardiovascular diseases who consumed red orange juice in the amount of 500 ml per day (2 × 250 ml) for one week were observed to present a significant improvement in the endothelial function defined by the FMD (flow-mediated dilation) parameter measured on the brachial artery by an ultrasound Sonoline G50 device (Siemens, Malvern, USA) (Buscemi *et al.* 2012). In another study, participants with early atherosclerosis consumed olive oil and olive oil supplemented with catechins (EGCG – epigallocatechin-3-gallate) in an amount of 30 ml per day for 4 months and were observed to achieve a significant improvement in the RHI value from 1.38 ± 0.15 to 1.60 ± 0.30. However, among patients with the correct RHI, the consumption of olive oil did not contribute to any improvement of this parameter (Widmer *et al.* 2013). Another study showed that in patients with the coronary arterial disease the endothelial function improved in 2 h after drinking the cranberry juice (480 ml) but this effect was not found in long-term research (4 weeks) (Dohadwala *et al.* 2011).

According to our research results and a review of results available in literature, it should be underlined that the consumption of chokeberry juice may improve

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the endothelial function, especially in people with its dysfunction, which is essential for the primary prevention of atherosclerosis.

CONCLUSION

The consumption of chokeberry juice in doses of 250 ml per day increases the serum antioxidant capacity to the maximum level in just one week of administration. However, there was no significant change in the blood lipid profile, in particular in TC, HDL-C, LDL-C, and TG. On the other hand, among the subjects with a higher baseline of triglycerides, a decrease in these compounds to the normal range occurred after three weeks of consuming the chokeberry juice, but this effect requires confirmation by further studies on a larger group of people. For all subjects, the endothelial function assessed as the RHI value measured on an EndoPAT 2000 was correct and did not change significantly after consuming the chokeberry juice. The results of this pilot study are encouraging to undertake further clinical research considering the use of chokeberry juice in the prevention and treatment of cardiovascular diseases. After obtaining the funding sources we are planning further studies on a large group of patients who will consume chokeberry juice including the control group (placebo, without juice).

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