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Influence of chokeberry juice on arterial blood pressure and lipid parameters in men with mild hypercholesterolemia

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Abstract:
Aronia melanocarpa is a common plant in Eastern Europe and in the North America. The fruit contains a lot of polyphenols, including anthocyanins, caffeic acid, and its derivatives also present in chokeberry at relatively high concentrations. Anthocyanins have antioxidant and anti-inflammatory properties and, therefore, may be potentially used to prevent oxidative stress, frequently associated with cardiovascular diseases.
The aim of the study was to estimate the influence of anthocyanins contained in chokeberry juice on arterial blood pressure, lipid parameters, inflammatory state parameters and concentrations of antioxidant vitamins in men with mild hypercholesterolemia.
Fifty eight healthy men with the diagnosed mild hypercholesterolemia without pharmacological treatment were enrolled to the study in 2006. In all men biochemical measurements were carried out 4 times: at the beginning, after 6 weeks of regular chokeberry juice drinking, after 6 weeks without the juice drinking, then repeated after 6 weeks of chokeberry juice drinking. Laboratory tests included: total, low density lipoprotein (LDL), and high density lipoprotein (HDL) cholesterol and its subfractions: HDL_2 and HDL_3, triglycerides, lipid peroxides (LPO), C-reactive high sensitivity protein (hsCRP), homocysteine, fibrinogen, glucose and antioxidant vitamins.
Regular chokeberry juice drinking resulted in reduction of total cholesterol level ($p < 0.001$) and LDL cholesterol ($p < 0.01$) and triglycerides ($p < 0.001$), and increased HDL_2 cholesterol ($p < 0.001$) level. Moderate but significant decreases in the serum glucose ($p < 0.05$), homocysteine ($p < 0.001$) and fibrinogen ($p < 0.01$) concentrations were also observed. These beneficial metabolic changes were associated with significant hypotensive effect of chokeberry juice drinking. Our studies showed that drinking of *Aronia melanocarpa* fruit juice may have a beneficial effect on reduction of cardiovascular risk.

Key words:
chokeberry juice, serum lipids, cardiovascular risk

Introduction

Aronia melanocarpa, black chokeberry is a common plant in Eastern Europe and in the North America. In many countries the strongly colored and pungently flavored juice is widely used for juice and wine production. The pea-sized, violet-black berries are harvested in autumn and have a strong, stable natural color with a dry and sour flavor. The fruits contain a lot of polyphenols, especially anthocyanins, caffeic acid, and its derivatives are also present in chokeberry at relatively high concentrations. Chlorogenic acid, peonidin-3-galactoside, cyaniding-3-galactoside, and cyaniding-3-glucoside are the most important antioxidants in blueberries, cranberries, wild chokeberries, and lingonberries [18]. Phenolics of chokeberries, like quercetin and cyanidin, with 3',4'-dihydroxy substituents have highly effective radical scavenging structures [19]. Anthocyanins are one of the most important water-soluble plant pigments. They belong to flavonoids and are derivatives of 2-phenyl-benzo-gamma pyrene.

Anthocyanins have antioxidant and anti-inflammatory properties and, therefore, may be potentially used to prevent oxidative stress, associated with cardiovascular and renal diseases [9, 11]. Anthocyanins from chokeberry decrease lipid peroxidation which may be potentially used to combat oxidative stress. They significantly reduced the content of thiol protein groups and non-significantly increased glutathione peroxidase activity, total content of antioxidants and nitrite concentration in animal studies [3]. Recent studies have shown that chronic treatment with flavonoids improves vascular function and cardiovascular remodeling by decreasing superoxide anion production as well as by increasing NO release from endothelial cells [15]. *Aronia melanocarpa* fruit juice showed an antihyperlipidemic effect in rats with hyperlipidemia and could be valuable in reducing this factor of cardiovascular risk [15]. This fruit juice is very rich in polyphenolic substances (40–70 mg/g dry weight) and most of them are anthocyanins (over 50%): cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-arabinoside and cyanidin-3-xylozyde [16, 17]. Chokeberry flavonoids reduce the severity of inflammation, and independently of statins, can be used clinically for secondary prevention of ischemic heart disease [4, 5]. Some other studies showed that 30-day administration of 240 mg of anthocyanins from *Aronia melanocarpa* (Aronox produced by Agropharm) a day, caused a substantial increase in glutathione peroxidase and catalase activities and decrease in the lead, aluminium and copper concentration, while zinc concentration in red blood cells was increased [3]. Pilaczyńska et al. reported that an increased intake of anthocyanins limited the exercise-induced oxidative damage to red blood cells, most probably by enhancing the endogenous antioxidant defense system [8]. Anthocyanins, contained in chokeberry juice influenced the redox parameters in rowers performing physical exercise during a 1-month training camp. The athletes were randomly assigned to receive 150 ml of chokeberry juice daily, containing 23 mg/100 ml of anthocyanins. Thus, antioxidant and chelating properties of *Aronia melanocarpa* fruit juice were earlier observed.

The aim of this study was to estimate the influence of anthocyanins contained in chokeberries juice on arterial blood pressure, lipid parameters, inflammatory proteins and concentration of antioxidant vitamins in men with mild hypercholesterolemia.

Materials and Methods

A group of 58 men with the diagnosed mild hypercholesterolemia without pharmacological treatment (with the total serum cholesterol level above 200 mg/dl) were enrolled to the study. The average volunteers' age was 54.1 ± 5.6 years, mean body mass index (BMI) was 27.7 ± 2.9 kg/m^2. All men were occupationally active, in general had healthy lifestyle with regular physical effort, incidentally smoked cigarettes (from one to ten cigarettes per day) and/or drank alcohol (four volunteers). Obesity (BMI over 30 kg/m^2) was diagnosed in five persons, mild arterial hypertension (the I degree) was observed in twenty two men. One men had impaired glucose tolerance. Summarizing, the studied group comprised 58 men with moderate degree of risk factors for atherosclerosis.

Biochemical characteristics of the study group are shown in Table 1.

Tab. 1. Characteristics of the study group (n = 58 men)

	Studied population
Age [years]	54.1 ± 5.6
Height [cm]	176.7 ± 6.3
Body mass [kg]	86.5 ± 10.2
BMI [kg/m^2]	27.7 ± 2.9
Total cholesterol [mg/dl]	250.7 ± 52.6
LDL cholesterol [mg/dl]	163.2 ± 46.2
HDL cholesterol [mg/dl]	49.9 ± 10.6
Triglycerides [mg/dl]	188.2 ± 112.0

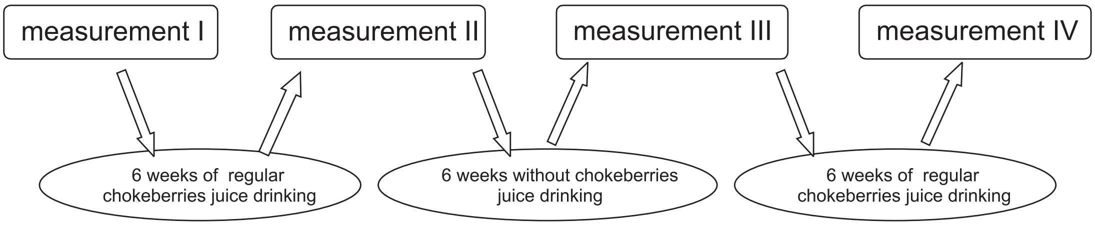

Fig. 1. Protocol of the study

In all men physical examination and biochemical blood tests were carried out four times: at the beginning (I), after 6 weeks of regular chokeberry juice drinking (II), after 6 weeks without the juice drinking (III), then repeated after 6 weeks of chokeberry juice drinking (IV). Volunteers did not change their lifestyle nor the diet during the study. Protocol of the study is shown in Figure 1.

The written informed consent was obtained from all volunteers taking part in the study. The study was approved by the Local Ethics Committee (license no. 1016/2003).

The volunteers drunk 100% chokeberry juice obtained naturally from the ecological farm Dzieciolowo (A. M. Lech, Poland) at amount of 250 ml per 24 h. The content of phenolics in this juice (mg/100 ml) was [7]:

Neochlorogenic acid	49.21
Chlorogenic acid	45.50
(–)Epicatechin	1.48
p-Coumaric acid derivative	0.40
Polymeric procyanidins	293.38
Quercetin 3-rutinoside	1.68
Quercetin 3-galactoside	2.83
Quercetin 3-glucoside	2.25
Quercetin 3-vicianoside	1.15
Quercetin 3-robinobioside	1.17
Eriodietyol 3-7-glucouronide	7.86
Cyanidin 3-galactoside	12.49
Cyanidin 3-glucoside	0.71
Cyanidin 3-arabinoside	5.12
Cyanidin 3-xyloside	0.59
Cyanidin	0.22
Total	426.04

Volunteers enrolled into the study, drinking black chokeberry juice did not declare any other products rich in vitamins A and E in their diet.

Laboratory tests from fasting venous blood were carried out four times. Blood lipid levels were assessed with the use of routine methods. Concentrations of total cholesterol, LDL cholesterol and triglycerides were measured using enzymatic assay (Spinreact). For determination of HDL_2 and HDL_3 cholesterol, precipitation test QUANTOLIP (Technoclone GmbH) was applied. Serum level of lipid peroxides was measured colorimetrically according to Satoh method [10], homocysteine was assayed using immunoenzymatic method (Abbott). Fibrinogen (by turbidimetric method), glucose (colorimetrically) and C-reactive high sensitivity protein (by nephelometric method) were measured using DADE Behringer Diagnostics assays (Mannheim). Vitamins A and E were determined simultaneously using high-performance liquid chromatography with fluorescence detection [13].

Statistical analysis

Statistical analysis was performed with the use of „Statistica PL 6.0" program (Stat Soft, Poland). Means (x) and standard deviations (SD) were given. The distribution of the variables was checked with W-Shapiro-Wilk test. ANOVA Friedman test was then used because variables had non-parametric distribution. Statistically significant differences between the means were estimated with *post-hoc* test. Values of $p < 0.05$ were accepted as statistically significant.

Results

During the study, significant decreases in serum total cholesterol (I *vs*. III: $p < 0.001$; I *vs*. IV: $p < 0.001$; II *vs*. IV: $p < 0.05$) and LDL cholesterol (I *vs*. III: $p < 0.01$; I *vs*. IV: $p < 0.01$; II *vs*. IV: $p < 0.05$) levels were observed. HDL cholesterol concentration was similar in all measurements, however, analysis of HDL cholesterol subfractions showed a significant increase in total HDL_2 cholesterol level (I *vs*. III: $p < 0.001$; I *vs*. IV: $p < 0.001$; II *vs*. III: $p < 0.05$; II *vs*. IV: $p < 0.01$). HDL_3 cholesterol and lipid peroxides levels were similar in all measurements (Tab. 2, 3).

Tab. 2. Serum lipid concentration in the study group (n = 58 men) at the beginning of the study (I), after 6 weeks of regular chokeberry juice drinking (II), then after 6 weeks without the juice drinking (III) and after 6 weeks of repetition of chokeberry juice drinking (IV)

	Total cholesterol [mg/dl]	LDL cholesterol [mg/dl]	HDL cholesterol [mg/dl]	Triglycerides [mg/dl]
I	250.7 ± 52.6	163.2 ± 46.2	49.9 ± 10.6	188.2 ± 112.0
II	238.1 ± 33.1	152.1 ± 43.4	47.3 ± 9.4	163.2 ± 73.5
III	222.7 ± 48.7	139.4 ± 54.5	50.5 ± 10.9	140.2 ± 56.9
IV	218.3 ± 37.4	135.4 ± 45.3	50.5 ± 10.9	138.6 ± 61.9
p	I *vs.* III: p < 0.001 I *vs.* IV: p < 0.001 II *vs.* IV: p < 0.05	I *vs.* III: p < 0.01 I *vs.* IV: p < 0.01 II *vs.* IV: p < 0.05	ns	I *vs.* III: p < 0.01 I *vs.* IV: p < 0.001

Tab. 3. HDL cholesterol subfractions and lipid peroxides in the study group (n = 58 men)

	HDL_2 cholesterol [mg/dl]	HDL_3 cholesterol [mg/dl]	Lipid peroxides [nM/ml]
I	5.69 ± 3.73	42.79 ± 8.24	0.72 ± 0.53
II	7.12 ± 5.08	41.42 ± 7.75	0.74 ± 0.19
III	9.33 ± 4.69	40.23 ± 9.89	0.80 ± 0.27
IV	9.90 ± 4.24	40.85 ± 7.88	0.83 ± 0.21
p	I *vs.* III: p < 0.001 I *vs.* IV: p < 0.001 II *vs.* III: p < 0.05 II *vs.* IV: p < 0.01	ns	ns

During the study, significant decrease in glucose (I *vs*. II: $p < 0.05$; I *vs*. IV: $p < 0.05$), homocysteine (I *vs*. IV: $p < 0.001$) and fibrinogen (I *vs*. IV: $p < 0.01$; II *vs*. IV: $p < 0.05$) levels were observed. Levels of uric acid and C-reactive protein were similar in all measurements (Tab. 4, 5). Analysis of vitamin levels showed a significant increase in vitamin A concentration (I *vs*. IV: $p < 0.001$; II *vs*. IV: $p < 0.001$; III *vs*. IV: $p < 0.001$) during the study whereas the vitamin E level was similar in all measurements (Tab. 6).

Tab. 4. Serum glucose, uric acid and homocysteine levels in the study group (n = 58 men)

	Glucose [mg/dl]	Uric acid [mg/dl]	Homocysteine [μM/l]
I	99.3 ± 14.5	5.3 ± 1.1	9.4 ± 1.9
II	91.8 ± 14.3	5.03 ± 0.96	8.9 ± 1.6
III	94.7 ± 17.4	5.21 ± 1.15	8.9 ± 1.6
IV	91.6 ± 17.1	5.14 ± 1.17	8.8 ± 2.3
p	I *vs.* II: p < 0.05 I *vs.* IV: p < 0.05	ns	I *vs.* IV: p < 0.001

Tab. 5. Serum high sensitive C-reactive protein and fibrinogen concentration in the study group (n = 58 men)

	hs C-reactive protein [mg/l]	Fibrinogen [mg/dl]
I	1.75 ± 1.32	335.4 ± 75.5
II	2.00 ± 2.15	324.2 ± 90.7
III	1.94 ± 2.31	298.8 ± 59.8
IV	2.01 ± 2.34	280.8 ± 101.8
p	ns	I *vs.* IV: p < 0.01 II *vs.* IV: p < 0.05

Tab. 6. Vitamin levels in the study group (n = 58 men)

	Vitamin A [μM/l]	Vitamin E [μM/l]
I	2.48 ± 0.60	28.88 ± 10.85
II	2.48 ± 0.65	29.82 ± 11.19
III	2.32 ± 0.50	28.91 ± 5.79
IV	2.90 ± 0.72	30.66 ± 6.21
p	I *vs.* IV: p < 0.001 II *vs.* IV: p < 0.001 III *vs.* IV: p < 0.001	ns

A statistically significant decrease in arterial blood pressure was noted in systolic blood pressure (I *vs*. IV: $p < 0.001$; III *vs*. IV: $p < 0.05$) and in diastolic blood pressure (I *vs*. II: $p < 0.05$; I *vs*. IV: $p < 0.01$; III *vs*. IV: $p < 0.05$) (Tab. 7).

Tab. 7. Arterial systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the study group (n = 58 men)

	SBP [mmHg]	DBP [mmHg]
I	138.6 ± 19.1	89.0 ± 10.9
II	130.4 ± 10.9	82.9 ± 6.2
III	133.6 ± 12.9	87.9 ± 10.3
IV	125.1 ± 14.3	82.0 ± 9.0
p	I *vs.* IV: p < 0.001 III *vs.* IV: p < 0.05	I *vs.* II: p < 0.05 I *vs.* IV: p < 0.01 III *vs.* IV: p < 0.05

Discussion

The prevention of cardiovascular diseases in men with mild hypercholesterolemia (with slightly increased cholesterol level over 200 g/dl) depends on the coexistence of the other risk factors. The choice between pharmacological or non-pharmacological treatment in these patients is usually a problem. In practice, recommendations concerning appropriate diet (i.e. DASH) are often not respected by patients because of their habits, especially when they are asked to modify the whole diet. On the other hand, there is a significant lack of easily accessible and cheap food products, which are effective in reduction of mild hyperlipidemia. *Aronia melanocarpa* fruit juice seems to be a valuable natural product in the anti-atherosclerotic diet.

In this study, men with mild hypercholesterolemia were not treated with pharmacotherapy and they formerly kept appropriate diet. In these conditions, drinking chokeberry juice for 6 weeks and than, after 6-week pause, another 6-week round of juice drinking, caused significant changes in metabolism of lipids, glucose and some proteins. Significant decline in the serum total and LDL cholesterol levels as well as triglycerides, took place after the second treatment. In comparison to basal values, these lipid concentrations remained lower after 6-week break in drinking juice. The involvement of LDL cholesterol and triglycerides in the development and progression of atherosclerosis is one of the best proven cases in modern medicine. A positive correlation between LDL cholesterol or triglycerides and the risk of cardiovascular events has been observed in many large-scale population studies. The advantages of reduction of these lipids level were proven in many intervention trials. Hypolipemic effect of chokeberry juice was gradual and relatively long-lasting. It was appreciable after the first 6 weeks of drinking and significant after the next 6-week pause.

Although the chokeberry juice drinking did not change the total HDL cholesterol level, it increased the HDL_2 subfraction of cholesterol ($p < 0.01$). This subfraction is involved in reverse cholesterol transport [1], postprandial lipid metabolism mediated by lipoprotein lipase [14] and takes part in coagulation as a cofactor of proteins C and S [2]. In normolipemic men with coronary atherosclerosis, decreases in HDL_2 and HDL_3 levels were observed [12]. The beneficial increase in HDL_2 cholesterol in serum of men treated with chokeberry juice could be connected with reduction of cholesterol content in arterial wall as well as increased antithrombotic activity. Moreover, this potential antithrombotic effect of *Aronia* juice is more probable because this juice reduced the level of fibrinogen ($p < 0.01$) and homocysteine ($p < 0.001$) at the end of the study.

Similarly to the impact of the chokeberry juice on the lipid metabolism, the influence of this juice on serum fasting glucose level was long-lasting. The decrease in glucose concentration, was strictly depended on the juice intake.

Metabolic changes observed in men with lipid disturbances were associated with reduction in systolic and diastolic blood pressure by a mean of 13 and 7 mmHg, respectively. Similar hypotensive effect of flavonoids-rich extract from chokeberry fruits was lately observed by Naruszewicz et al. in patients after myocardial infraction, treated simultaneously with statins [5].

The common mechanism of the advantageous metabolic and functional changes could be significant and may result from strong antioxidant effect of flavonoids contained in chokeberry juice. It is known that *Aronia melanocarpa* fruit juice is rich in anthocyanins having high oxygen radical absorbing capacity [6, 7, 19]. This property can explain anti-inflammatory potential and antithrombotic activity, as well as hypotensive effect of the tested juice.

In conclusion, regular drinking of chokeberry juice has the beneficial effect on some lipid and other metabolic parameters, and on arterial blood pressure in men with mild hypercholesterolemia. Our studies showed the crucial advantage of drinking *Aronia melanocarpa* fruit juice in the reduction of the cardiovascular risk.

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