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ORIGINAL ARTICLE

THE EFFECT OF REGULAR CHOKEBERRY JUICE CONSUMPTION ON ANTHROPOMETRIC AND LIPID PARAMETERS IN WOMEN WITH OVERWEIGHT OR OBESITY

Jana Mrázová, Jana Kopčeková, Marta Habánová, Martina Gažarová, Petra Lenártová

Slovak University of Agriculture, Faculty of Agrobiology and Food Resources,
Institute of Nutrition and Genomics, Nitra, Slovakia

ABSTRACT

Background. *Aronia melanocarpa* is nowadays valued for its high content of biologically active substances, the main group of which are polyphenols, which include anthocyanins, flavonols, flavanols, proanthocyanidins and phenolic acids. From the available sources, we can conclude that extracts and juices from black chokeberry have a great potential in human nutrition and influence on their health.

Objective. The research was to evaluate the effect of regular consumption of 100% organic chokeberry juice on selected anthropometric and lipid parameters of overweight or obese women.

Material and Methods. A clinical study consisted of 19 women with overweight and obesity, age from 44 to 63. The probands consumed 50 ml of chokeberry juice daily for 8 weeks as part of their regular diet. Body composition and biochemical indicators were monitored before consumption, after 4 and 8 weeks of nutritional intervention. Body composition was determined using multifrequency bioelectrical impedance analysis (MFBIA) – InBody 720. Biochemical analyzes of blood serum were performed using standard methods in an accredited laboratory using automatic biochemical analyzer a BioMajesty JCA-BM6010/C.

Results. The monitored group of probands is characterized by menopausale and postmenopausale women, overweight or obese women with hypercholesterolemia without pharmacological treatment. Statistically significant differences ($p<0.05$) were observed when evaluating the amount of body fat (BFM) of the probands before the start of consumption and after the consumption of chokeberry juice. We noted a statistically significant reduction especially in the assessment of visceral fat (VFA) ($p<0.001$). There were no fundamentally significant changes in the lipid profile of women in this intervention study. With short-term consumption of chokeberry juice (after 4 weeks), we recorded an average reduction in total cholesterol and LDL-cholesterol, but without statistical significant. We also focused on the evaluation of the inflammatory marker CRP and noted a significant beneficial reduction of CRP ($p<0.05$).

Conclusions. In the research, we evaluated the effect of 8 weeks consumption of 100% chokeberry juice on selected anthropometric parameters, focusing on changes in visceral fat and total fat in overweight and obese women. In conclusion, we can state that the regular consumption of chokeberry juice has a beneficial effect on fat tissue in women of reproductive age, which can reduce the risk of cardiovascular diseases.

Keywords: *black chokeberry, body composition, lipid parameters, inflammatory marker*

INTRODUCTION

Aronia melanocarpa (Michx.) Elliot, black chokeberry, belongs to the rose family (*Rosaceae*) and the apple subfamily (*Pomodieae*). It comes from the eastern parts of North America and eastern Canada. Aronia reached Europe through Russia in the 19th century, where it was cultivated in Siberia and later spread throughout Russia and other European countries [19]. Aronia, thanks to its resistance to cold, can be grown in mild climatic conditions, but it can withstand temperatures below -35°C [6]. In the past, the nutritional value of aronia was not sufficiently appreciated, but the constantly emerging evidence of its health-promoting properties is increasing the interest in this crop. Fresh and unprocessed chokeberry berries are often not consumed due to their bitter taste, therefore chokeberry fruits are used in the production of juices, nectars, syrups, jams, fruit desserts, jellies, wines and various liqueurs, but also as a food coloring [15, 28]. The composition and quality of individual components of chokeberry fruit depends on many factors, e.g. on the variety, maturity, environment, climatic conditions, soil, harvesting

Corresponding author: Jana Kopčeková, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic; phone: +421 37 641 4225; e-mail: jana.kopcekova@uniag.sk

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method, etc. Aronia became known thanks to its high biological activity, which is ensured by polyphenols. Berries are one of the richest sources of polyphenols, which include anthocyanins, flavonols, flavanols, proanthocyanidins and phenolic acids. Furthermore, aronia is considered an excellent source of minerals such as potassium, calcium, phosphorus, magnesium, sodium, iron and zinc [22]. *Aronia melanocarpa* has the highest antioxidant activity among all berry varieties, which is related to the high content of polyphenolic compounds, especially procyanidins and anthocyanins [11]. The content of total phenolic substances in chokeberry fruits ranges from 0.69 to 2.56 $mg \cdot g^{-1}$ of fresh weight and from 3.44 to 7.85 $mg \cdot g^{-1}$ of dry weight. The mechanisms of *in vivo* antioxidant activity of chokeberry phenolic substances include scavenging of free radicals, suppression of reactive oxygen species (ROS) formation, prooxidant inhibition and restoration of antioxidant enzymes, and probably restoration of cellular signaling responsible for regulating the level of antioxidant compounds and enzymes [26].

Obesity is reaching epidemic proportions and has therefore become a major global public health problem. The condition is the result of excessive accumulation of adipocytes, which store and regulate energy in the form of triacylglycerols or free fatty acids. The pathogenesis of obesity-related complications is thought to involve abnormal adipokine production by adipocytes, although the exact mechanism has not been established. Oxidative stress and obesity are thought to be linked, and oxidative stress has also been found in obese individuals. In a study conducted by Kim et al. [7], an inhibitory activity on adipocyte differentiation was proven by chokeberry extract, which contained substances such as chlorogenic acid, methyl-3-O-caffeoylquinic acid, dihydroxycinnamoylcyclopenta-2,3-diol, cyanidin-3-glucoside, cyanidin-3-xyloside. The most abundant anthocyanin in chokeberry, cyanidin-3-O-galactoside, significantly reduced the weight of rats in studies by Jiao et al. [5], also reduced lipid parameters, levels of triacylglycerols, total cholesterol, LDL cholesterol, and on the contrary increased levels of HDL cholesterol.

The lipid-lowering effect of flavonoids has been demonstrated in many animal and human studies [8]. Aronia juice has been shown to have beneficial effects on total cholesterol and lipid levels [27]. Significant anti-inflammatory effects of chokeberry have been demonstrated in various *in vivo* and *in vitro* studies. Oral administration of chokeberry extract inhibits the production of prostaglandin E2 and reduces levels of nitric oxide, production of interleukin-6 and tumor necrosis factor (TNFα) in macrophages and microglia, increases production of interleukin 10. These anti-inflammatory effects were primarily caused by specific polyphenols (cyanidin-3-arabinoside and quercetin), which are represented as a minor component of total chokeberry polyphenols [4].

The aim of the research was to evaluate the effect of regular consumption of 100% organic chokeberry juice on selected anthropometric and lipid parameters of overweight or obese women.

MATERIALS AND METHODS

The research was carried out at the Institute of Nutrition and Genomics of the Faculty of Agrobiology and Food Resources of Slovak University of Agriculture in Nitra (SUA), with the focus on monitoring the effect of regular consumption of 100% organic chokeberry juice (product of a company from Slovakia) on selected anthropometric and biochemical parameters of the probands. The clinical study involved 30 women and we selected 19 women with overweight and obesity, age from 44 to 63 (mean age 51.21±4.63 years). The probands consumed 50 ml of chokeberry juice daily for 8 weeks and they were instructed to maintain their normal eating habits during the study, to refrain from consuming any drugs and dietary supplements and not to modify their physical activity. We used the 24-h dietary records, where respondents provides information about the type and quantity of all food and beverages consumed during the period. We evaluated the data using Planeat. This software is designed for a complete analysis of food, meals, and recipes based on the composition of the raw ingredients. Body composition and biochemical indicators were monitored before consumption, after 4 and 8 weeks of nutritional intervention. Participants were their own controls and the changes in their parameters were evaluated. The study was approved by the Ethics Committee at the Specialized Hospital of St. Zoerardus in Nitra, Slovak Republic (protocol number 3/101921/2021) and study was conducted between March and May 2022. The monitored group of probands consisted of volunteers without health problems and pathological changes in the basic biochemical parameters in the blood. The condition of participation in the research was the informed consent of the participants to the conditions of the study and the planned examinations. Body composition (body weight – BW, body fat mass – BFM, visceral fat area – VFA, skeletal muscle mass – SMM, fat free mass – FFM, body mass index – BMI, waist to hip ratio – WHR) was determined using multifrequency bioelectrical impedance analysis (MFBIA) – InBody 720 (Biospace Co. Ltd., Seoul, Korea).

Biochemical analyzes: total cholesterol, high density cholesterol (HDL cholesterol), low density cholesterol (LDL cholesterol), triacylglycerols and inflammatory marker) of blood serum were performed using standard methods in an accredited laboratory using a BioMajesty JCA-BM6010/C automatic biochemical analyzer. The level of lipid and inflammatory parameters was measured using commercial DiaSys kits. Low-density lipoprotein cholesterol was calculated using the Friedewald equation.

The chokeberry juice was made by cold pressing from organic black chokeberry without additives, stabilized only by pasteurization.

The content of nutrients and some important bioactive substances, as well as the juice antioxidant activity were quantified in the Institute of Food Sciences of SUA. The total phenolic content (TPC) was determined by the spectrophotometric method [9] according to the Folin-Ciocalteu method using spectrophotometer Shimadzu UV/VIS-1800 and expressed in mg gallic acid equivalents (GAE)/g. Phenolic compounds were determined by HPLC Agilent 1260 Infinity II (Agilent Technologies GmbH, Waldbronn, Germany) with quaternary solvent manager coupled with degasser, sample manager, column thermostat and DAD detector by slightly modified method according by Gabriele et al. [3]. The antioxidant activity of chokeberry juice was measured using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the results were expressed as percentage of inhibition of the radical [1]. Vitamin C content was determined by HPLC system Waters Separations Module 2695 with UV detector 2996.

Statistical analysis

We used the Shapiro-Wilk test to determine whether the variables were normally distributed and the parametric variables were compared by the paired t-test using software Statistica Cz version 14. All data are expressed as the mean ± standard deviation (SD) and p-values of 0.05 or less were considered significant.

RESULTS AND DISCUSSION

Many studies have shown the positive effect of small berries in the prevention of important metabolic disorders associated with obesity, oxidative stress and chronic inflammation [14, 23]. The beneficial effects of berries are related to the antioxidant and anti-inflammatory properties of polyphenols [12]. The effects of chokeberry extracts have been demonstrated both *in vitro* and *in vivo*, especially in cardiovascular diseases [18].

In this interventional clinical trial, women consumed 50 ml of 100% chokeberry juice every day for 8 weeks. The concentration of bioactive compounds and antioxidant activity of chokeberry juice were as follows: total phenolic content 4.90±0.51 mg gallic acid equivalents, ferulic acid 100.37±0.45 $mg{\cdot}l^{-1}$, rutin 238.69±0.25 $mg{\cdot}l^{-1}$, benzoic acid 204.71±1.09 $mg{\cdot}l^{-1}$, neochlorogenic acid 372.36±1.89 $mg{\cdot}l^{-1}$, chlorogenic acid 497.86±0.52 $mg{\cdot}l^{-1}$, vitamin C 12.60±0.08 $mg{\cdot}100\ g^{-1}$ and antioxidant activity 83.18±0.51%.

Basic characteristics of the probands before intervention are presented in Table 1. The monitored group of probands is characterized by: menopausal and postmenopausal women (from 44 to 63 years old), these are overweight or obese women (BMI from 25.97 to 36.36 $kg{\cdot}m^{-2}$) with hypercholesterolemia.

Consumption was well tolerated by volunteers and showed no changes in liver and kidney function in blood serum during the intake of chokeberry juice (Table 2).

Table 1. Basic characteristic of probands before intervention

Parameter	Mean ± SD	Min. - Max.
Age (year)	51.21±4.63	44.00-63.00
Body weight (kg)	80.54±9.34	59.50-97.80
Body mass index ($kg{\cdot}m^{-2}$)	29.65±2.42	25.97-36.36
T-CH ($mmol{\cdot}l^{-1}$)	6.32±1.05	4.79-8.24
HDL-CH ($mmol{\cdot}l^{-1}$)	1.61±0.34	0.94-2.27
LDL-CH ($mmol{\cdot}l^{-1}$)	4.10±0.95	2.68-5.80
TG ($mmol{\cdot}l^{-1}$)	1.34±0.60	0.63-2.96
ALT ($\mu kat{\cdot}l^{-1}$)	0.33±0.12	0.16-0.63
AST ($\mu kat{\cdot}l^{-1}$)	0.36±0.12	0.24-0.62
GGT ($\mu kat{\cdot}l^{-1}$)	0.36±0.13	0.22-0.75
Urea ($mmol{\cdot}l^{-1}$)	5.12±1.84	2.5-8.3
Creatinine ($\mu mol{\cdot}l^{-1}$)	67.55±9.95	51.8-85.2
Uric acid ($\mu mol{\cdot}l^{-1}$)	315.42±65.03	222-488

values are means ± standard deviation (SD); Min. – minimum; Max. – maximum

Table 2. Metabolic, liver and kidney markers of the subjects before and after chokeberry juice consumption

Parameter	Baseline Mean ± SD	Week 8 Mean ± SD	p-value
ALT (µkat·l^{-1})	0.33±0.12	0.32±0.11	ns
AST (µkat·l^{-1})	0.36±0.12	0.37±0.11	ns
GGT (µkat·l^{-1})	0.36±0.13	0.40±0.36	ns
Bilirubin (µmol·l^{-1})	10.18±3.19	9.25±2.84	ns
Urea (mmol·l^{-1})	4.93±1.69	5.28±1.82	ns
Creatinine (µmol·l^{-1})	66.57±9.24	67.71±8.90	ns
Uric acid (µmol·l^{-1})	305.83±51.26	306.94±52.78	ns
Albumin (g·l^{-1})	48.94±1.81	48.77±1.45	ns

ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma glutamyl transferase; values are means ± standard deviation (SD); ns – non significant

Visceral adipose tissue is a hormonally active component of total body fat that affects several processes in the human body. Abnormally high deposition of visceral adipose tissue is known as visceral or abdominal obesity. Visceral fat surrounds internal organs and its increased amount causes health problems, such as glucose metabolism disorders, cardiovascular diseases (ischemic heart disease, hypertension), prostate, breast and colorectal cancer [21].

Jurendić and Ščetar [6] proved that in obese mice treated with aronia extract melanocarpa with a high fat content there was a significant reduction in body weight and improved insulin sensitivity in 46 compared to controls. The study reports the inhibitory activity of polyphenols, in particular proanthocyanidins, against pancreatic lipase, as regards the suppression of fat absorption from foods and strategies against overweight and obesity.

According to the WHO assessment and classification, we recorded 43.3% of overweight probands (25-29.9 kg·m^{-2}), 16.7% of probands with a BMI of 30-34.9 kg·m^{-2} with the obesity of the first degree and 3.3% had obesity II. degree. Regular consumption of chokeberry juice had a statistically significant effect on the reduction of body weight after 8 weeks ($p<0.01$). Weight reduction, however, did not affect skeletal muscle mass, we did not record statistical evidence. Statistically significant differences ($p<0.05$) were observed in the comparison of the average value of the amount of body fat of the probands before the start of consumption and after the end of consumption of chokeberry juice. We noticed a statistically significant reduction especially in the assessment of visceral fat of women during consumption as well as after consumption, the average value decreased from the initial from 126.87 cm^2 to 122.52 cm^2 ($p<0.001$). The effects of chokeberry juice consumption on body composition are shown in Table 3. In this clinical study, we aimed to evaluate the impact of regular consumption of 100% chokeberry

Table 3. Effect of regular consumption of chokeberry juice on body composition of probands

Parameter	Baseline Mean ± SD	4 Weeks Mean ± SD	8 Weeks Mean ± SD	p-value
BW (kg)	80.54±9.34	79.86±9.62	79.75±9.52	<0.01^b
BFM (kg)	31.73±5.74	31.26±6.11	30.99±6.17	<0.05^b
BFM (%)	39.24±3.78	38,91±4.03	38.63±4.10	ns
BMI (kg·m^{-2})	29.65±2.42	29.39±2.48	29.36±2.54	<0.01^b
SMM (kg)	26.85±3.14	26.72±3.09	26.82±3.09	ns
FFM (kg)	48.81±5.26	48.58±5.19	48.76±5.05	ns
VFA (cm^2)	126.87±20.96	124.01±21.27	122.52±20.92	<0.05^a <0.001^b
TBW (kg)	35.77±3.88	35.59±3.81	35.73±3.71	ns
ICW (kg)	22.11±2.41	22.02±2.37	22.11±2.32	ns
ECW (kg)	13.67±1.48	13.57±1.46	13.63±1.40	ns
WHR	0.98±0.05	0.97±0.06	0.96±0.05	ns

values are means ± standard deviation (SD); [a]significant difference between weeks 4 and weeks 8; [b]significant difference between baseline and week 8; ns – non significant juice on selected anthropometric and lipid parameters of overweight and obese women with untreated hypercholesterolemia. Based on anthropometric measurements, we recorded a statistically significant decrease in visceral fat in the monitored group of probands. WHR index ratio waist and hips higher than 0.85 means a risk for the development of cardiovascular diseases. When evaluating the WHR index, we found that the average value of the participants was 0.98 at the beginning of the study, which decreased to 0.96 after stopping the consumption of chokeberry juice without statistical evidence. Our results confirm the results of a clinical study by Kim et al. [7] by the inhibitory activity on adipocyte differentiation by chokeberry extract. The results of the study by Jiao et al. [5] confirmed hypotheses in the prevention of high-fat obesity, focusing on the potential of cyanidin-3-O-galactoside from *Aronia melanocarpa*. Cyanidin-3-O-galactoside attenuated high-fat diet-induced obesity in rats by inhibiting adenosine 5'-phosphorylation monophosphate (AMP) activated protein kinase (AMPK) and inhibited induced inflammation by promoting signal transducer and activator of transcription 3 (STAT3) phosphorylation and suppressing nuclear factor kappa-B p65 (NF-κB p65) in the nucleus. The potential mode of action describes Lim et al. [10] which examined the anti-adipogenic effect of cyanidin-3-O-galactoside *Aronia melanocarpa* extract and its underlying mechanisms were investigated in an in vivo system during an 8-week high-fat diet on body weight and body fat mass in mice. Consequently, increases in body weight and fat mass and subsequent inhibition by supplementation have been reported. White adipose tissue mass (epididymal fat, retroperitoneal fat, mesenteric fat, and inguinal fat) was dose-dependently decreased by chokeberry in mice.

The effect of the consumption of chokeberry juice on the lipid profile is presented in Table 4. The probands of the studied group suffer from hypercholesterolemia and are not pharmacologically treated. In hypercholesterolemia, LDL cholesterol is the main transport form of endogenous cholesterol. Its level effects cholesterol level in the body and increases the risk of cardiovascular and cerebrovascular diseases. HDL cholesterol reverse transports cholesterol from tissue to the liver and breaks it down, which can protect the intimate o vessels and the higher level of HDL cholesterol, the lower the risk cerebral thrombosis and atherosclerosis [17]. In this interventional study, there were no fundamental significant changes in the lipid profile of women. With the short-term consumption of 100% chokeberry juice (after 4 weeks), we recorded an average reduction in total cholesterol and LDL-cholesterol without statistical evidence. We hypothesize that longer-term regular consumption of chokeberry juice may affect the lipid profile, especially risk factors of cardiovascular diseases, similar to what many clinical studies have shown.

Skoczyńska et al. [24] observed the effect of regular consumption of chokeberry juice on the lipid parameters of men with mild hypercholesterolemia, while the clinical study showed a statistically significant reduction in total cholesterol ($p<0.001$) and LDL cholesterol ($p<0.01$) and triglycerides ($p<0.001$) and increase in HDL cholesterol ($p<0.001$) in the blood of the probands. Broncel et al. [2] evaluated the effect of consuming 100 mg chokeberry extract three times a day for 8 weeks. After the end of the study, they confirmed a statistically significant reduction in cholesterol, LDL cholesterol and triacylglycerols in probands with metabolic syndrome. Chokeberry extract contains a significant amount of niacin, which can alleviate cardiovascular disease by reducing lipid activity.

Lipid parameters were also evaluated in a study by Tasic et al. [25], which confirmed after 4 weeks of consumption of chokeberry juice, a statistically

Table 4. Effect of regular consumption of chokeberry juice on lipid profile of probands

Parameter	Baseline Mean ± SD	4 Weeks Mean ± SD	8 Weeks Mean ± SD	p-value
Total cholesterol (mmol·l^{-1})	6.32±1.05	6.18±1.06	6.47±0.89	ns
HDLcholesterol (mmol·l^{-1})	1.61±0.34	1.67±0.29	1.68±0.26	<0.05^{b}
LDLcholesterol (mmol·l^{-1})	4.10±0.95	3.91±1.05	4.17±0.89	ns
Triacylglycerols (mmol·l^{-1})	1.34±0.60	1.32±0.59	1.36±0.59	ns
LDL/HDL cholesterol ratio	2.62±0.64	2.40±0.71	2.53±0.59	ns

values are means ± standard deviation (SD); ^{a}significant difference between weeks 4 and weeks 8; ^{b}significant difference between baseline and week 8; ns – non significant

Table 5. Effect of regular consumption of chokeberry juice on inflammation markers of probands

Parameter	Baseline Mean ± SD	Week 8 Mean ± SD	p-value
CRP ($mg \cdot l^{-1}$)	5.38±1.46	4.3±1.18	<0.01
IL-6 ($ng \cdot l^{-1}$)	7.15±1.07	6.52±1.25	<0.01
ORM ($g \cdot l^{-1}$)	0.91±0.19	0.66±0.22	<0.001

values are means ± standard deviation (SD); CRP – C-reactive protein; IL-6 – interleukin-6; ORM – orosomucoid

demonstrable decrease in total cholesterol, LDL cholesterol and a favorable increase in HDL cholesterol in women with metabolic syndrome. Many studies have shown that regular consumption of chokeberry products has a positive effect on the lipid profile [2, 16, 24]. However, there were no fundamentally significant changes in the lipid profile of women in this intervention study. With short-term consumption of 100% chokeberry juice (after 4 weeks), we noted a reduction in total cholesterol and LDL cholesterol, but without statistical significant.

Anti-inflammatory activity of chokeberry fruits is primarily associated with the prevention of cardiovascular diseases, diabetes and disorders of the immune system [20]. In a study by Zhang et al. [28] chokeberry juice showed greater anti-inflammatory effects such as rutin or a rutin-magnesium complex. In view of the fact that chokeberry flavonoids reduce the severity of inflammation, regardless of statins, they can be used clinically for secondary prevention of ischaemic heart disease [13]. In the monitored clinical study, we also focused on inflammatory markers at the baseline and after finish of chokeberry juice consumption: C-reactive protein (CRP), interleukin-6 (IL-6) and orosomucoid (ORM). After the chokeberry juice consumption, significant reduction of CRP, IL-6 ($p<0.01$) and ORM ($p<0.001$) were found (Table 5).

CONCLUSIONS

Black chokeberry is a highly biologically valuable plant, suitable for maintaining health and preventing civilization diseases. It is available on the market in various forms, recent attention has focused on the use of fruit juices as a concentrated source of antioxidants. Juice consumption is an effective method to promote fruit and vegetable consumption and is very popular in many countries. In the research, we evaluated the effect of 8-week consumption of 100% chokeberry juice on selected anthropometric and biochemical parameters, focusing on changes in visceral fat and total fat in overweight and obese women. In conclusion, we can conclude that regular consumption of chokeberry extract affects the fat tissue of women of reproductive age. However, further studies with a modified methodology regarding the duration of the intervention and amount of daily intake are needed to more thoroughly examine the effect of consumption of 100% chokeberry juice in the prevention and treatment of CVD.

Conflict of interest

The authors declare no conflict of interest.

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