EFFECTS OF *ARONIA MELANOCARPA* FRUIT JUICE ON OXIDATIVE STRESS, ENERGY HOMEOSTASIS, AND LIVER FUNCTION IN OVERWEIGHT AND HEALTHY-WEIGHT INDIVIDUALS

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ABSTRACT

INTRODUCTION: Overweight and obesity are disorders of energy metabolism, associated with oxidative stress and chronic low-grade inflammation. *Aronia melanocarpa* fruits are rich in polyphenolic compounds with antioxidant properties. The fruit juice exhibits lipid-lowering, antihyperglycemic, and hepatoprotective activities in experimental settings.

AIM: The study aimed to examine the effects of *Aronia melanocarpa* fruit juice on oxidative stress, energy homeostasis, and liver function in overweight and healthy-weight volunteers.

MATERIALS AND METHODS: The study included 11 overweight and 11 healthy-weight individuals. The participants consumed 50 mL of 100% *Aronia melanocarpa* fruit juice 3 times daily for 3 months. Blood samples were obtained at the baseline and the end of the study. Oxidative stress was evaluated by the serum activity of superoxide dismutase (SOD) and catalase (CAT), and the concentration of thiobarbituric acid reactive substances (TBARS). Energy metabolism and liver function were assessed by standard biochemical tests. C-reactive protein was measured as a non-specific inflammatory marker.

RESULTS: The fruit juice increased the activity of SOD and reduced the serum level of TBARS in the overweight group. The CAT activity was insignificantly increased in both groups. Aronia decreased the level of gamma-glutamyl transferase and reduced slightly the C-reactive protein, fasting blood glucose, and glycated hemoglobin levels in the overweight group. The liver function tests and the lipid profile were not affected.

CONCLUSION: *Aronia melanocarpa* fruit juice improved the parameters of oxidative status. The present pilot study confirmed the beneficial effects of Aronia fruits on human metabolic health.

Keywords: *Aronia melanocarpa, antioxidant, energy metabolism, liver function, overweight, pilot study*

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Received: December 10, 2021
Accepted: December 13, 2021

INTRODUCTION

Overweight and obesity are the most common disorders of energy metabolism. They are defined as abnormal or excessive fat accumulation. According to the World Health Organization (WHO) the worldwide prevalence of obesity nearly tripled between 1975 and 2016. In 2016, 39% of adults were overweight, and 13% were obese.

Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity. The waist-to-height ratio is a useful additional measure. As an anthropometric index for central adiposity, it reflects the distribution of body fat (1).

Overweight and obesity induce severe consequences on human health. Visceral adiposity is a well-known risk factor for the development of cardiovascular diseases, non-alcoholic fatty liver disease, and type 2 diabetes (2–4). In addition, obesity is associated with an increased prevalence of depression, anxiety disorders, and cognitive impairment (5–7). The low-grade chronic inflammation and oxidative stress are the recognized pathological mechanisms linking visceral adiposity with the development of these non-communicable diseases (8).

Aronia melanocarpa (Michx) Elliot (black chokeberry) is a woody shrub of the *Rosaceae* family. Its fruits are commonly used for the production of juice, syrup, jellies, tea, and wine. They are extremely rich in phenolic compounds. The procyanidins are the group with the highest concentration in the fruits (9,10). Other phenolic substances at high concentrations are flavonoids (proanthocyanidins, anthocyanins, quercetin glycosides) and phenolic acids (chlorogenic and neochlorogenic) (10). The numerous biological activities of *Aronia melanocarpa* fruit juice have been widely studied in experimental settings. Polyphenols of *Aronia* fruits possess complex antioxidant and anti-inflammatory activities (11,12). The fruit juice improves the glucose and lipid metabolism in diabetic and high-cholesterol fed rats (13,14). It exerts hepatoprotective effects in experimental models of liver damage (15,16). *Aronia melanocarpa* fruit juice affects also animal behavior, demonstrating anti-anxiety and antidepressant-like activities and improvement of memory and learning in rats (17–19).

AIM

The current study aimed to examine the effects of *Aronia melanocarpa* fruit juice on oxidative stress, energy homeostasis, and liver function in overweight and healthy-weight volunteers.

MATERIALS AND METHODS

Participants and Study Design

The study included 22 adults (13 females and 9 males) who were classified as either overweight (mean age of 51.9±3.9 years) or healthy-weight subjects (mean age of 41.1±4.4 years), according to their BMI and waist-to-height ratio. The group of overweight participants included 11 subjects with a BMI equal to or greater than 25 and less than 30 and a waist-to-height ratio equal to or greater than 0.49 for females and 0.53 for males. The group of healthy-weight participants included 11 subjects with values of BMI between 18 and 25 and/or waist-to-height ratio in the range of 0.42–0.48 for females and 0.43–0.52 for males. *Aronia melanocarpa* fruit juice 100% was purchased from Aronia Alive Agriculture Ltd (Bulgaria). The participants were instructed to consume 50 mL of the juice 3 times daily before meal for 3 months. Blood samples for biochemical analyses were obtained at the baseline and the end of the study. All participants gave written informed consent prior to the enrolment. The study was approved by the local Ethical Committee of the Medical University of Varna (№ 61/30.03.2017 and № 80/24.01.2019).

Evaluation of Oxidative Status

Oxidative stress was evaluated by determining the serum activity of superoxide dismutase (SOD) and catalase (CAT), and the concentration of thiobarbituric acid reactive substances (TBARS).

SOD and CAT Activity

SOD and CAT activity were determined in serum by using commercial colorimetric assay kits (Calbiochem®) following the instructions of the producer.

The SOD assay kit utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. SOD activity was measured in U/mL. One unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Standards with increasing SOD activity were prepared

according to the producer's instructions. The absorbance of standards and the samples was measured by using an ELISA reader LKB 5060-006 (LKB Instruments, Australia) at 450 nm. The absorbance of the standard with activity 0 U/mL was divided by itself and by all the other standards and sample absorbances to yield the linearized rate. The linearized SOD standard rate was plotted as a function of final SOD activity. The SOD activity of the samples was calculated using the equation obtained from the linear regression of the standard curve substituting the linearized rate for each sample.

The CAT assay kit utilizes the peroxidation function of CAT for the determination of enzyme activity. The method is based on the reaction of the enzyme with methanol. The formaldehyde produced is measured after the addition of a chromogen. Standards with increasing formaldehyde concentration were prepared according to the producer's instructions. The absorbance of each standard and sample was measured by using an ELISA reader LKB 5060-006 (LKB Instruments, Australia) at 540 nm. The absorbance of the standard containing 0 μM formaldehyde was subtracted from itself and all other standards and samples. The corrected absorbance of standards was plotted as a function of final formaldehyde concentration. The formaldehyde concentration of the samples was calculated using the equation obtained from the linear regression of the standard curve substituting corrected absorbance values for each sample. The CAT activity of the samples was calculated using the following equation:

CAT activity = (μM of sample / 20 min) x Sample dilution = nmol/min/mL

One unit was defined as the amount of enzyme that would cause the formation of 1.0 nmol formaldehyde per min at 25°C.

TBARS Concentration

TBARS were determined colorimetrically at 532 nm by using the method of Ohkawa et al. (20). The method measures the absorbance of the color produced as a result of the reaction of thiobarbituric acid with the lipid peroxides. Aurius 2021 UV-VIS spectrophotometer (Cecil Instruments Ltd, UK) was used. Malondialdehyde, the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids, was used as a standard.

Evaluation of Energy Homeostasis, Liver Function, and Inflammation

Energy homeostasis was assessed by the levels of fasting blood glucose (FBG) and glycated hemoglobin (HbA1c), aiming to evaluate the carbohydrate metabolism, and by the lipid profile (triglycerides, LDL-cholesterol, HDL-cholesterol, and total cholesterol) to characterize the lipid metabolism. The liver function was assessed by the serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT). The level of C-reactive protein was determined as a non-specific marker of inflammation. All analyses were performed immediately after blood sampling by routine laboratory methods in the clinical laboratory of St. Marina University Hospital, Varna, Bulgaria.

Statistics

The results are presented as means ± standard error of the mean (SEM). The differences between the groups were compared at the baseline and the end of the study by the unpaired Student's two-tailed t-test. The changes in the parameters of each individual, resulting from the *Aronia melanocarpa* fruit juice consumption, were analyzed by the paired Student's two-tailed t-test. Differences were considered significant at $p < 0.05$. The statistical software GraphPad Prism 5 was used (GraphPad Software, Inc.).

RESULTS

Effects of Aronia melanocarpa Fruit Juice on Oxidative Stress and Inflammation

The effect of *Aronia melanocarpa* fruit juice on the activity of the antioxidant enzymes is presented in Fig.1. The SOD activity (Fig. 1A) in the serum of overweight participants was insignificantly lower at the baseline (0.078±0.011 vs. 0.046±0.010 U/mL). *Aronia melanocarpa* fruit juice increased the activity of the enzyme in this group (0.046±0.010 vs. 0.071±012 U/mL; p=0.0172). At the end of the period of Aronia consumption, there were no differences in this parameter between overweight and healthy-weight subjects. The effect of the fruit juice on CAT activity (Fig. 1B) was less pronounced. The juice increased the activity of the enzyme similarly in both

groups and at the end of the consumption period it was 34.7% and 34.9%, greater than the one measured at the baseline in healthy-weight and in overweight subjects, correspondingly. However, the effects were statistically insignificant.

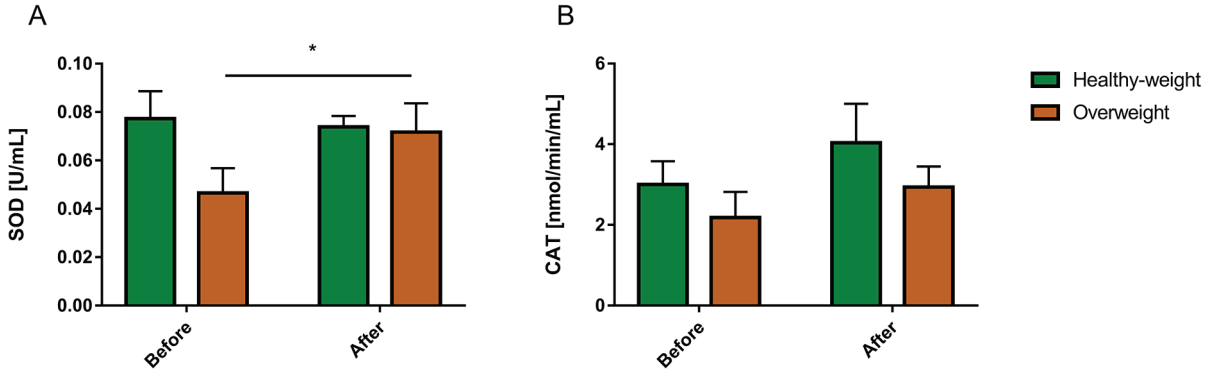

Fig. 1. Effects of Aronia melanocarpa fruit juice on antioxidant enzymes in healthy-weight and overweight individuals.
***A.** Activity of superoxide dismutase (SOD) at the baseline and the end of the consumption period.*
***B.** Activity of catalase (CAT) at the baseline and the end of the consumption period; * p<0.05 between the groups*

The effect of *Aronia melanocarpa* fruit juice on lipid peroxidation, as assessed by TBARS serum concentration, is presented in Fig. 2. The lipid peroxidation at the baseline was insignificantly greater in the overweight individuals compared to the healthy-weight participants (26.17±2.88 vs. 32.85±2.16 nmol/mL). The fruit juice reduced the TBARS serum concentration in both groups, but the difference was significant only in the overweight group (32.85±2.16 vs. 23.24±2.62 nmol/mL, p<0.0001). At the end of the study, overweight individuals had the same level of lipid peroxidation as the healthy-weight participants.

The changes in C-reactive protein concentrations are presented in Fig. 3. At the baseline, overweight individuals demonstrated insignificantly higher values of C-reactive protein compared to healthy-weight subjects (2.38±1.1 vs. 4.1±1.1 mg/L). At the end of the consumption period, the C-reactive protein level was slightly reduced (3.06±0.76 vs. 4.1±1.1 mg/L) in the overweight group. The fruit juice did not affect this parameter in the healthy-weight group.

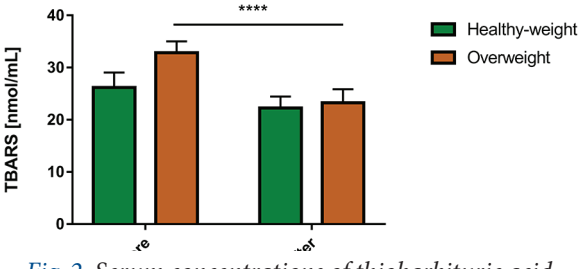

Fig. 2. Serum concentrations of thiobarbituric acid reactive substances (TBARS) in healthy-weight and overweight individuals before and after the consumption of Aronia melanocarpa fruit juice;
*****p<0.0001 between the overweight groups*

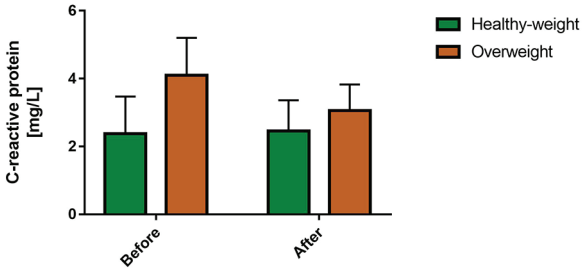

Fig. 3. C-reactive protein concentrations in healthy-weight and overweight individuals before and after the consumption of Aronia melanocarpa fruit juice

Effects of Aronia melanocarpa Fruit Juice on Energy Metabolism and Liver Function

The parameters of energy metabolism and liver function are presented in Table 1. The initial levels of FBG and HbA1c of overweight individuals were elevated compared with those of healthy-weight participants (p=0.0318 for FBG and p=0.0078 for HbA1c). *Aronia melanocarpa* fruit juice reduced the FBG and HbA1c levels in the overweight group with 0.21 mmol/L and 0.23% respectively. At the end of the

study, the FBG values of overweight individuals were similar to these of healthy-weight participants. Nevertheless, the difference in HbA1c levels between the groups remained significant (p=0.0319).

At the baseline, the serum levels of triglycerides (TG) were higher in the overweight compared to the healthy-weight group (p=0.0028). The differences in serum levels of total cholesterol (TC), LDL-cholesterol (LDL-C), and HDL-cholesterol (HDL-C) were insignificant. The lipid profile was only slightly affected by the Aronia juice consumption. The TG levels were insignificantly increased in both groups. TC and LDL-C were not affected by the juice, and the HDL-C level was elevated by 13.3% compared to the initial value in the healthy-weight individuals.

At the baseline, the AST level was greater in overweight compared to healthy-weight individuals (p=0.0171) and GGT was insignificantly elevated. The fruit juice did not affect significantly AST and ALT serum levels. However, the GGT concentration was reduced at the end of the consumption period in the overweight group (p=0.0358).

DISCUSSION

Overweight and obesity have grown to epidemic proportions in the last 30 years. Visceral adiposity is the main pathological characteristic of these metabolic disturbances. The expansion of visceral fat stores is associated with the generation of reactive oxygen species (ROS) and increased expression and secretion of inflammatory cytokines by adipocytes, such as tumor necrosis factor-alpha and many others. They stimulate the generation of ROS by activating NF-κB signaling. In its turn, the increased level of ROS induces a further release of pro-inflammatory cytokines and the expression of adhesion molecules and growth factors through redox-sensitive transcription factors. In this way, the visceral fat accumulation provokes a positive reinforcing cycle between a low-grade chronic inflammation and ROS generation. The state of long-term oxidative stress depletes the antioxidant defense system and results in decreased activity of antioxidant enzymes, which increases the susceptibility of cells to oxidative damage. Oxidative stress leads to insulin resistance within adipose tissue as well as in peripheral tissues, thus promoting lipogenesis and worsening of the reinforcing cycle. Insulin resistance is one of the hallmarks of obesity and accounts for many of its comorbidities, including type 2 diabetes, metabolic syndrome, cardiovascular diseases, and neuropsychiatric disorders (8).

Aronia melanocarpa fruits contain a high amount of polyphenolic ingredients and possess one of the highest *in vitro* antioxidant activities among fruits. Different *in vitro* assays have demonstrated the ability of Aronia and its products to scavenge active species, such as superoxide anion, hydroxyl radical, and nitrogen radicals. Aronia polyphenols are potent inhibitors of lipid peroxidation. In addition to the radical scavenging activity, Aronia polyphenols suppress the formation of reactive oxygen and nitro-

*Table 1. Parameters of glucose and lipid metabolism and liver function in healthy-weight and overweight subjects before and after the consumption of Aronia melanocarpa fruit juice. *p<0.05, ** p<0.01 vs the corresponding healthy-weight group; # p<0.05 vs the overweight group at the baseline*

	Baseline Values		Final Values	
	Healthy-Weight	Overweight	Healthy-Weight	Overweight
FBG [mmol/L]	5.28 ± 0.18	5.95 ± 0.23*	5.41 ± 0.22	5.74 ± 0.16
HbA1c [%]	5.18 ± 0.09	5.70 ± 0.15**	5.21 ± 0.08	5.47 ± 0.08*
TG [mmol/L]	0.92 ± 0.08	1.56 ± 0.17**	1.16 ± 0.09	1.86 ± 0.43
TC [mmol/L]	5.28 ± 0.31	5.61 ± 0.36	5.57 ± 0.33	5.55 ± 0.34
LDL-C [mmol/L]	3.20 ± 0.21	3.55 ± 0.28	3.16 ± 0.23	3.52 ± 0.32
HDL-C [mmol/L]	1.65 ± 0.24	1.34 ± 0.09	1.87 ± 0.30	1.26 ± 0.07
AST [U/L]	22 ± 1.12	26.6 ± 1.34*	22.4 ± 2.04	26 ± 2.77
ALT [U/L]	25 ± 5.22	25.4 ± 1.95	29.2 ± 5.06	28.4 ± 4.17
GGT [U/L]	23.5 ± 5.24	33.9 ± 3.20	27.9 ± 5.66	29.3 ± 3.12 #

gen species and restore the activity of antioxidant enzymes (9–11).

The beneficial effects of *Aronia melanocarpa* fruit juice on metabolic and liver parameters of experimental animals in different disease models, prompted us to examine it also in humans, expecting that its powerful antioxidant properties could be translated into clinically relevant effects. In this study, we aimed to study the impact of consuming *Aronia melanocarpa* fruit juice on oxidative status, glucose and lipid metabolism and liver function in healthy individuals and participants with impaired energy metabolism.

According to the WHO, BMI serves as the main parameter to define overweight and obesity, as it is a simple measure suitable for screening the population for metabolic disturbances. However, BMI can be inaccurate and misleading because it does not take into account the muscle mass, bone density, and overall body composition. To determine more precisely the participants with visceral adiposity, we used as an additional parameter the waist-to-height ratio. It reflects the distribution of body fat and correlates better with the risk of cardiovascular diseases and diabetes than BMI does (1).

At the baseline, the average values of the biochemical parameters in the group of overweight participants were in the reference ranges. However, the markers of glucose metabolism, TG, and AST were significantly higher than those of the healthy-weight group. The rest of the parameters, including the level of lipid peroxidation, the activity of antioxidant enzymes, and C-reactive protein, were also unfavorably shifted, though insignificantly. The differences between healthy-weight and overweight participants in our study at the baseline indicated that the increased body weight was associated with impairment of oxidative status, energy metabolism, and liver function.

The fruit juice consumption affected beneficially many of the parameters in the overweight group. Aronia fruit juice increased the activity of SOD, thus improving the antioxidant defense system, and strongly reduced the concentration of TBARS, demonstrating a powerful effect on lipid peroxidation in this group. At the end of the study, the markers of oxidative stress showed no difference between the healthy-weight and overweight individuals, reflecting a reversal of the impaired oxidative status by the fruit juice. Thus, the current study confirmed the complex antioxidant properties of *Aronia melanocarpa* fruit juice. Similar results on the parameters of oxidative status with inhibition of lipid peroxidation and activation of SOD have been reported in several other small clinical trials, focused on the beneficial effects of Aronia in healthy people (21–23) and in patients with hypercholesterolemia and metabolic syndrome (24, 25). In addition to its antioxidant properties, Aronia has been shown to improve lipid metabolism, blood pressure, and the overall potential for clot formation in studies involving patients with clinically manifested disturbances of energy metabolism and cardiovascular disorders (24–26). In our study, Aronia fruit juice consumption affected slightly and insignificantly the parameters of glucose and lipid metabolism, probably because of the normal values of these parameters in the overweight group at the baseline. Studies, investigating the effect of Aronia on healthy subjects, have reported, similarly to us, no changes in the blood lipid profile (23). It should be stressed, however, that some of the biochemical parameters, elevated in the overweight compared to the healthy-weight group at the baseline, such as the FBG and TG, were reduced to levels similar to those of the healthy-weight subjects at the end of our study, suggesting nevertheless some beneficial effect of Aronia juice on glucose and lipid homeostasis.

Overweight and obesity are the main risk factors for liver damage. The production of ROS and secretion of inflammatory cytokines by adipocytes induce insulin resistance in the liver, resulting in stimulation of *de novo* hepatic lipogenesis with a resultant increase of fatty acids in the liver (3). *Aronia melanocarpa* fruit juice has demonstrated its hepatoprotective properties in different experimental models of liver damage. The beneficial effects have been explained by the antioxidant activity of the fruits (15,27). In addition, *Aronia melanocarpa* probably ameliorates hepatic lipid metabolism through PPARγ2 downregulation (28). The potential hepatoprotective effects of *Aronia melanocarpa* in humans have not yet been well established. In our study, drinking Aronia juice reduced significantly GGT serum levels in the overweight individuals. Our results suggest that the beneficial effects of Aronia on

liver function found in experimental animals might be present also in humans.

CONCLUSION

The current pilot clinical study demonstrated the positive effects of *Aronia melanocarpa* fruit juice on parameters of the oxidative status. The fruit juice improved the activity of superoxide dismutase, and inhibited the process of lipid peroxidation in the overweight participants. It also affected in a favorable manner some of the parameters of energy metabolism and liver function in these individuals. Our study provides initial confirmation of the beneficial effects of *Aronia melanocarpa* fruit juice on human metabolic health in a limited sample of volunteers and warrants the conduction of a larger clinical investigation, allowing to achieve more convincing and generalizable results.

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