EFFECT OF *ARONIA MELANOCARPA* FRUIT JUICE ON THE ANTIOXIDANT DEFENSE SYSTEM IN RATS WITH DIET-INDUCED METABOLIC SYNDROME

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ABSTRACT

INTRODUCTION: The role of oxidative stress in the development of metabolic syndrome (MS) has been well established. *Aronia melanocarpa* fruits are very rich in polyphenols, which possess an antioxidant effect.

AIM: The current study aimed to evaluate the effect of *Aronia melanocarpa* fruit juice (AMFJ) on the antioxidant defense system in rats with diet-induced MS.

MATERIALS AND METHODS: Fifty male Wistar rats were allocated into 5 groups: control, MS, MS+AMFJ$_{2.5}$, MS+AMFJ$_{5}$ and MS+AMFJ$_{10.}$ For 10 weeks, the control group received regular diet and the other groups—high-fat high-fructose diet (HFHF). During this period, the control group and MS group were treated daily orally with 10 mL/kg distilled water and the other groups—with increasing volume (2.5mL/kg, 5mL/kg, and 10mL/kg) of AMFJ. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were measured in the serum using commercial colorimetric kits.

RESULTS: A significantly higher SOD level was observed in MS group (0.0069±0.0007 U/mL) compared to the control group (0.0051±0.0003 U/mL) ($p<0.01$). AMFJ treatment returned the level of SOD to the control values, with the effect being significant in MS+AMFJ$_{2.5}$ (0.0053±0.0003 U/mL) and MS+AMFJ$_{5}$ (0.0053±0.0003U/mL) groups ($p<0.05$ vs. MS). No significant difference was detected in the activity of GPx in all groups.

CONCLUSION: HFHF diet-induced MS might be associated with superoxide production and compensatory activation of SOD. Due to its antioxidant properties, AMFJ counteracted these processes in the treated groups. Neither the HFHF diet, nor the AMFJ treatment affected the activity of GPx.

Keywords: *Aronia melanocarpa, metabolic syndrome, rats, antioxidant enzymes*

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INTRODUCTION

Oxidative stress (OxS) is a term used to describe the disequilibrium between reactive oxygen species (ROS) production and the ability of the biological systems to neutralize these reactive products. ROS overproduction disrupts cellular and enzyme systems and irreversibly damages proteins, lipids and nucleic acids. In the recent years, their role in the development

of a number of chronic diseases, such as cancer, diabetes, metabolic syndrome (MS), atherosclerotic cardiovascular disease (ASCVD), inflammatory, and neurodegenerative diseases, has been well established (1–5). Endogenous antioxidant protection is mediated by enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx). Superoxide dismutase is a metalloenzyme, which catalyzes the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and plays an essential role in cellular antioxidant protection (6). Glutathione peroxidase catalyzes the reduction of hydroperoxides, including hydrogen peroxide, using reduced glutathione and functions to protect the cell from oxidative damage (7).

Metabolic syndrome is characterized by visceral adiposity, atherogenic dyslipidemia, hyperglycemia and hypertension, with a global prevalence range of 20–25% in adults (8,9). It is associated with an increased risk of ASCVD and type 2 diabetes (10). Consumption of energy-dense foods and physical inactivity are the main culprits of this condition. In the last years, accumulating body of evidence emphasizes on the role of oxidative stress in the development of MS. The disequilibrium between decreased antioxidant capacity and increased ROS production, observed in MS, produces a pro-oxidant state (11). Due to the significant impact of MS on human health, animal models are used to study the pathogenesis and therapy of this condition. In experimental settings, rats are often preferred and MS is induced by feeding them with diet, rich in fat, or fructose, or both. One of the diets, able to reproduce all aspects of MS, is the high-fat high-fructose (HFHF) diet (12).

Polyphenols are natural plant-derived compounds with numerous health benefits. Their antioxidant properties have been widely studied (13). The high dietary intake of polyphenols is associated with lower risk of diabetes, obesity, and MS (14). *Aronia melanocarpa* fruits are characterized by a very high content of polyphenolic compounds especially anthocyanins. A large body of evidence suggest that *Aronia*-derived phenols possess a number of antioxidant activities such as suppression of lipid peroxidation and ROS production as well as radical scavenging and increase of endogenous antioxidant defenses (15–17, 20–23). As MS is considered a pro-oxidant state, it is valuable to assess the effect of antioxidant-rich foods and supplements on the antioxidant defense. It might be expected that *Aronia melanocarpa* fruit juice (AMFJ) would affect the oxidative status in MS. Currently, there are no data about the effect of AMFJ on the antioxidant enzymes in MS.

AIM

The aim of this study was to evaluate the effect of polyphenol-rich AMFJ on the antioxidant defense enzymes, SOD, and GPx in rats with diet-induced MS.

MATERIALS AND METHODS

Aronia melanocarpa Fruit Juice—Preparation and Polyphenolic Content

Aronia melanocarpa fruit juice was prepared by grinding, pressing and squeezing the fresh fruits grown in the Balkan Mountains, Bulgaria. The obtained juice was filtered, preserved with potassium sorbate (1.0 g/L), and stored at room temperature until the experiment, as described previously by Kuzmanova *et al.* (17). The content of investigated polyphenols in AMFJ and the methods of detection are presented in Table 1.

Table 1. Polyphenolic content of Aronia melanocarpa fruit juice; GAE—gallic acid equivalents; HPLC—high-performance liquid chromatography

Ingredients	Content	Method of Detection
Total phenols	5461 GAE/L	Spectrophotometrically (18)
Total proanthocyanidins	3122.5 mg/L	Gravimetrically (19)
Cyanidin 3-galactoside	143.7 mg/L	HPLC
Cyanidin 3-arabinoside	61.7 mg/L	HPLC
Cyanidin 3-glucoside	4.4 mg/L	HPLC
Cyanidin 3-xyloside	11.6 mg/L	HPLC
Chlorogenic acid	585 mg/L	HPLC
Neochlorogenic acid	830 mg/L	HPLC

Experimental Animals

Fifty male Wistar rats with initial body weight of 180–280 g were included in the experiment. They were housed in plastic cages, at an ambient temperature 20–25°C, under 12-hour light/dark cycle. They had free access to food and drinking water.

The rats were allocated into 5 groups (10 rats per group): a control group, receiving regular laboratory rat chow diet and tap water, and four metabolic syndrome groups: MS, MS+$AMFJ_{2.5,}$ MS+$AMFJ_5$ and MS+$AMFJ_{10}$.

Metabolic syndrome was induced by feeding the rats HFHF diet as described by Gancheva *et al.* (12). It was prepared by enriching the regular rat chow with 17% lard and 17% fructose. Additionally, the rats from the MS groups received 10% fructose solution instead of drinking water. The caloric intake of HFHF diet was 405 kcal/100 g, where lard provided 38% of the energy intake and fructose—17%. The fructose solution accounted for additional 40 kcal/100 mL caloric intake.

The MS+$AMFJ_{2.5,}$ MS+$AMFJ_5$, and MS + $AMFJ_{10}$ groups were treated with an increasing volume of AMFJ—2.5 mL/kg, 5.0 mL/kg, and 10 mL/kg body weight, respectively. The doses of 2.5 mL/kg and 5 mL/kg were diluted with distilled water to a total volume of 10 mL/kg. The juice was administered daily through an orogastric probe. The control and MS group received distilled water at the same time and route of administration in a volume of 10 mL/kg body weight. The duration of the study was 10 weeks.

All procedures concerning animal treatment and experimentation were conducted in conformity with the national and international laws and policies (EU Directive 2010/63/EU for animal experiments) and were approved by the Bulgarian Food Safety Agency (Document 177/07.07.2017).

Determination of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) Activity in Serum

At the end of the experimental period, the animals were anesthetized with diethyl ether and blood samples were collected from the sublingual veins for biochemical assays. Superoxide dismutase and GPx activities were estimated colorimetrically by using commercial colorimetric kits purchased from Calbiochem (Germany), according to the instructions of the producer.

Determination of SOD

The kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The SOD activity is presented as international units per ml (IU/mL).

Determination of GPx

The method is based on the measurement of GPx activity indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione, produced upon reduction of hydroperoxide by glutathione peroxidase, is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which glutathione peroxidase activity is rate limiting, the rate of decrease in the absorbance is directly proportional to the GPx activity in the sample. The enzyme activity is presented as nmol/min/mL.

Statistical Analysis

The results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison post-test. GraphPad Prism 5.00 statistical software was used. The data are presented as means ±SEM and $p<0.05$ was considered significant.

RESULTS

SOD activity in rat serum

SOD activity is presented on Fig. 1. One-way ANOVA revealed a difference between the groups ($p=0.0174$) and Dunnett's post-test showed a significantly higher activity of the enzyme in MS group (0.0069±0.0007 U/mL) compared to the control

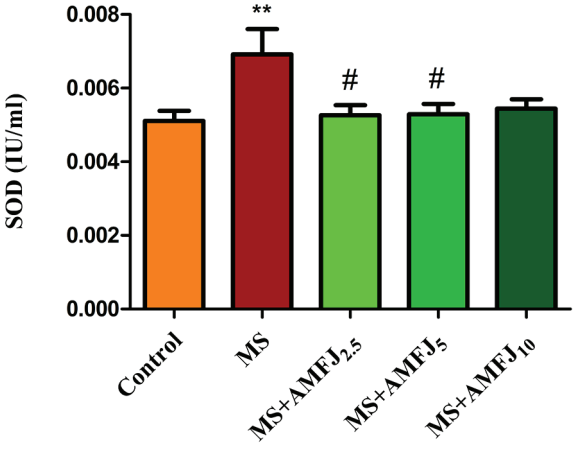

*Fig. 1. Serum superoxide dismutase (SOD) activity in rats with metabolic syndrome (MS) treated with Aronia melanocarpa fruit juice (AMFJ) at doses of 2.5mL/kg, 5mL/kg, and 10mL/kg; **p<0.01 vs. Control; #p<0.05 vs. MS.*

one (0.0051±0.0003 U/mL) ($p<0.001$). AMFJ treatment reduced the activity of SOD compared to the MS group, with the effect being significant in the MS+AMFJ$_{2.5}$ (0.0053±0.0003 U/mL) and MS + AMFJ$_5$ (0.0053±0.0003 U/mL) groups ($p<0.05$ vs. MS). The activity of SOD in all AMFJ-treated groups was similar to that of the control animals.

GPx Activity in Rat Serum

There was no statistically significant difference in the activity of the enzyme GPx among all the tested groups ($p=0.7067$) (Fig. 2).

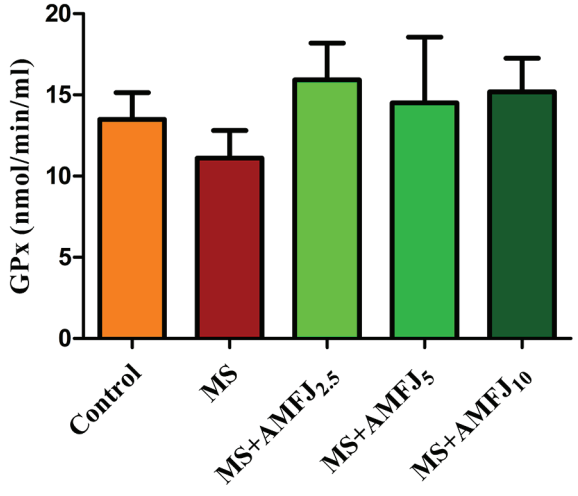

Fig. 2. Serum glutathione peroxidase (GPx) activity in rats with metabolic syndrome (MS) treated with Aronia melanocarpa fruit juice (AMFJ) at doses of 2.5 mL/kg, 5mL/kg, and 10mL/kg activity in rat serum

DISCUSSION

Metabolic syndrome has been recognized as an important cardiometabolic risk factor (10). The pathophysiology of MS is complex and involves multiple related factors. Many experimental and clinical studies reveal that insulin resistance, inflammation, and oxidative stress play a significant role in the pathogenesis of MS. The visceral adipose tissue is an important source of ROS and contributes to the development of low-grade inflammation in MS. Oxidative stress is associated with inflammation activated by the up-regulation of nuclear factor-kappa B (NF-κB) which is implicated in the transcription of genes regulating the production of pro-inflammatory molecules—cytokines and adhesion molecules (4,24). On the other hand, the inflammatory process induces oxidative stress. Pro-inflammatory cytokines, including tumor necrosis factor-alpha, interleukin (IL)-1β, and IL-6, that are secreted by the excessive adipose tissue, aggravate OxS through binding of specific receptors and promoting NF-κB signaling, thus inducing ROS generation (4). In addition, studies have also shown that the concentration of endogenous antioxidants and activity of antioxidant enzymes are generally reduced in MS (25–27).

Our previous study with HFHF diet-induced MS demonstrated that it produced OxS and increased lipid peroxidation (measured by TBARS levels) (28). Therefore, in the present study we aimed to determine the activity of the endogenous antioxidant enzymes SOD and GPx in MS rats. We found that SOD activity was higher in MS group compared to the other groups while no difference was observed in the activity of GPx. These results are in accordance with the study of Vavrova *et al.*, who have found, similarly, increased SOD activity and unaltered GPx activity in patients with MS (29). Interestingly, Yubero-Serrano *et al.* have reported an association between the number of MS components and SOD activity and suggested that it could be used as a predictive tool to determine the degree of the underlying OxS in MS (30). Other experimental and clinical studies also have revealed an association between obesity and increased activity of SOD (31,32). Under physiological conditions, small amounts of superoxide radicals are produced during glucose autoxidation and cellular respiration (mitochondria). Enzymes such as NADPH oxidase, xanthine oxidase, cyclooxygenase and lipoxigenase can additionally contribute to superoxide production. The radicals are inactivated by SOD to hydrogen peroxide which is further converted to water by catalase, GPx or thioredoxin (5). It is known that nutrient excess (especially high-fat diet) induces up-regulation of NADPH oxidase (33). We could build up a hypothesis that the HFHF diet, administered to the MS animals in our experiment, induced superoxide production and increased compensatory the activity of SOD, aiming to inactivate the radical.

Polyphenols are a large group of naturally occurring chemical compounds, bearing one or more phenolic ring. The powerful antioxidant action of these compounds is due to their ability to neutralize free radicals by donating an electron or hydro-

gen atom (13). *Aronia melanocarpa* fruits contain a large amount of polyphenols and, therefore, exhibit strong anti-oxidant properties. Different *in vitro* assays demonstrate the ability of *Aronia* to scavenge a variety of active radicals, including superoxide anion, hydroxyl radical, and nitrogen radicals. In addition, *Aronia* polyphenols are potent inhibitors of lipid peroxidation and suppress the formation of reactive oxygen and nitrogen species (15–17, 34). A number of experimental studies have demonstrated the ability of AMFJ to reduce OxS and ameliorate pathological conditions associated with it (35–38).

In the current study, we aimed to assess the effect of AMFJ on the activity of antioxidant defense enzymes in animals with diet-induced MS. The animals receiving HFHF diet, which were treated with AMFJ, demonstrated lower activity of SOD compared to the untreated rats with MS, the effect being significant in the MS + $AMFJ_{2.5}$ and MS + $AMFJ_5$ groups. In general, the SOD activity in all AMFJ-treated animals was similar to that of the control rats, receiving regular laboratory diet. We can explain our results with the antioxidant activity of the polyphenols present in the fruits. We suggest that AMFJ counteracted the OxS caused by the HFHF diet and prevented the superoxide production and the consequent compensatory activation of SOD in AMFJ-treated rats.

The GPx activity remained unaltered in our study. Neither the HFHF diet, nor the AMFJ treatment affected the activity of this enzyme. Possible explanation of the observed result could be found in the relation between the GPx function and oxidative stress, associated with MS. Glutathione peroxidase acts as an antioxidant enzyme, scavenging lipid peroxidation products. Measurement and quantification of lipid peroxidation could be achieved by malondialdehyde (MDA) or thiobarbituric acid-reactive substrates (TBARS) assays (39). Although these markers are not tested in this experiment, there are studies revealing that elevation of lipid peroxidation is not mandatory in high-fat-induced obesity and diabetes (40, 41) (and presumably MS) and the levels of MDA, TBARS or isoprostanes are low. This fact might be explained with the presence of vitamin E in the fats used as a component of high-fat diets for induction of these pathological conditions (42) as vitamin E can interact with the serum GPx activity.

The current study has some limitations. Only several flavonoids were measured in AMFJ. The content of other antioxidants presented in *Aronia* fruits and juice, such as quercetin and other hydroxycinnamic acids, were not investigated. The effect of the food preservative potassium sorbate was not taken into account. There is a study showing that the compound could induce oxidative stress and decrease the activity of the antioxidant enzyme catalase in female rats (43). However, the content of potassium sorbate in AMFJ was considerably lower (0.1%) compared to the content of potassium sorbate (5%/10% in the diet) in the cited study (43). We could suppose that at this low concentration potassium sorbate would not affect the activity of the antioxidant enzyme. The markers for lipid peroxidation were not investigated. Measuring MDA, TBARS or isoprostanes could clarify the effect of AMFJ on lipid peroxidation and the activity of GPx in MS.

CONCLUSION

The results from our study suggest that HFHF diet-induced MS might be associated with superoxide production and compensatory activation of SOD. AMFJ counteracted the MS-induced elevation of SOD level in the treated groups and had no effect on GPx activity. These findings might encourage the use of AMFJ as a functional food in MS individuals.

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