

EFFECT OF ARONIA MELANOCARPA FRUIT JUICE ON CARRAGEENAN-INDUCED PAW EDEMA IN METABOLIC SYNDROME RATS

Mehmed Abtulov¹, Vasilena Kuzmanova², Atanas Kuzmanov², Simeon Todorov², Danail Pavlov³, Krasimir Kuzmanov⁴, Milena Todorova¹, Miroslav Eftimov¹, Silvia Gancheva¹, Maria Zhelyazkova-Savova¹, Stefka Valcheva-Kuzmanova¹

¹Department of Pharmacology and Clinical Pharmacology and Therapeutics, Faculty of Medicine, Medical University of Varna

²Student, Faculty of Dental Medicine, Medical University of Varna

³Department of Biochemistry, Molecular Medicine and Nutrigenomics, Faculty of Pharmacy, Medical University of Varna

⁴Vivarium, Medical University of Varna

ABSTRACT

INTRODUCTION: Chronic, low-grade inflammation plays a critical role in the pathogenesis of the metabolic syndrome (MS). *Aronia melanocarpa* fruits are rich in biologically active compounds—polyphenols, which possess a variety of health benefits including an anti-inflammatory effect.

AIM: The aim of this article is to evaluate the effect of polyphenol-rich *Aronia melanocarpa* fruit juice (AMFJ) on carrageenan-induced acute inflammation in rats with diet-induced MS.

MATERIALS AND METHODS: Forty male Wistar rats were included in the experiment. They were allocated into 4 groups: MS, MS+AMFJ_{2.5}, MS+AMFJ₅, and MS+AMFJ₁₀, all receiving high-fat high-fructose diet and 10% fructose in the drinking water for 10 weeks. The MS group served as a control and was treated daily with distilled water orally, while the other groups received AMFJ at doses of 2.5 mL/kg, 5 mL/kg, and 10 mL/kg, respectively. At the end of the experiment, carrageenan was injected in the left hind paw in order to induce acute inflammation. Paw edema was evaluated with plethysmometer on the 30th min and 1st, 2nd, 3rd, 4th, and 5th hour after the injection.

RESULTS: In the MS group, the carrageenan-induced paw edema increased gradually with time reaching the highest value on the 5th hour. A decrease throughout the whole 5-hour period was observed in groups treated with AMFJ, the effect being most pronounced and statistically significant in MS+AMFJ₅ group on the 2nd and 3rd hour.

CONCLUSION: *Aronia melanocarpa* fruit juice treatment in rats with MS resulted in a decrease in the carrageenan-induced paw inflammation. The anti-inflammatory effect might be attributed to the polyphenols in AMFJ.

Address for correspondence:

Mehmed Abtulov
Faculty of Medicine
Medical University of Varna
55 Marin Drinov St
9002 Varna
e-mail: Mehmed.Abtulov@mu-varna.bg

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INTRODUCTION

Metabolic syndrome (MS) is a clinical condition affecting a significant percentage of the global population. It is a constellation of biochemical and clinical abnormalities (impaired levels of fasting glucose, triglycerides and HDL, central obesity, insulin resistance, elevated blood pressure), which increase the risk of developing atherosclerotic cardiovascular disease, and type 2 diabetes (1). Studies have shown that chronic, low-grade inflammation plays a critical role in the pathogenesis of the metabolic syndrome (2).

Carrageenan is a high molecular weight seaweed-derived sulfated polysaccharide commonly used as emulsifying agent in infant formulas, dairy products, and milk alternatives (almond milk) (3). It has been documented that carrageenan triggers an acute inflammatory response when injected subcutaneously. Carrageenan-induced rat paw edema is an experimental model used in preclinical studies in order to explore the anti-inflammatory potential of different molecules.

Aronia melanocarpa (black chokeberry) is an ornamental plant from the *Rosaceae* family, which was introduced in Europe in the early twentieth century. Due to the astringent taste, fresh fruits are usually used for the production of juice, nectar, wine, jam, dessert, tea, and herbal supplements (4). *Aronia melanocarpa* fruits are rich in polyphenolic compounds. Highest are the amounts of proanthocyanidins and anthocyanins (5). Polyphenols have been shown to possess a variety of health benefits due to their antioxidant, antiproliferative, and anti-inflammatory effects (6).

AIM

The purpose of the present study was to evaluate the effect of polyphenol-rich *Aronia melanocarpa* fruit juice (AMFJ) on carrageenan-induced acute inflammation in metabolic syndrome rats.

MATERIALS AND METHODS

AMFJ Preparation and Determination of the Content of Biologically Active Substances

The juice was prepared by grinding, pressing, and squeezing the fresh fruits grown in the Balkan Mountains, Bulgaria. It was filtered and preserved with potassium sorbate (1.0 g/L) (7). The amount

of total phenols, determined spectrophotometrically according to the procedure described by Folin-Ciocalteu (8) and presented as gallic acid equivalents (GAE), was 5461 GAE/L. The amount of proanthocyanidins was 3122.5 mg/L and it was evaluated using the gravimetric method described by Howell *et al.* (9). The content of cyanidin glycosides and phenolic acids was determined by high-performance liquid chromatography and their contents were: cyanidin 3-galactoside 143.7 mg/L, cyanidin 3-arabinoside 61.7 mg/L, cyanidin 3-glucoside: 4.4 mg/L, cyanidin 3-xyloside: 11.6 mg/L; chlorogenic acid 585 mg/L, and neochlorogenic acid: 830 mg/L.

Animals and Experimental Protocol

Forty male Wistar rats (initial body weight 160–280 g) were included in the experiment. The animals were kept under 12/12 light-dark cycle, at an average ambient temperature of 20–25°C and had access to food and drinking water *ad libitum*. The animals were allocated into 4 groups (10 rats in each group): MS, MS+AMFJ_{2.5}, MS+AMFJ₅, and MS+AMFJ₁₀. To induce MS, in the course of 10 weeks they were fed high-fat high-fructose (HFHF) diet consisting of lard (17%) and fructose (17%) with regular rat chow and received 10% fructose in the drinking water (10). During the whole experimental period, the MS+AMFJ_{2.5}, MS+AMFJ₅, and MS+AMFJ₁₀ groups were respectively treated daily with AMFJ orally at doses of 2.5, 5 and 10 mL/kg. The MS group served as a control and received distilled water (10 mL/kg). The experimental protocol is shown in Table 1.

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)

Table 1. Experimental protocol

Group	Diet	Treatment
MS	HFHF	10 mL/kg distilled water
MS+AMFJ _{2.5}	HFHF	2.5 mL/kg AMFJ diluted with distilled water to a total volume of 10 mL/kg
MS+AMFJ ₅	HFHF	5 mL/kg AMFJ diluted with distilled water to a total volume of 10 mL/kg
MS+AMFJ ₁₀	HFHF	10 mL/kg AMFJ

Table 2. Values of the paw edema (mL) on the subsequent time intervals; * $p < 0.05$ vs. MS group

Group	Paw Edema (30 th minute)	Paw Edema (1 st hour)	Paw Edema (2 nd hour)	Paw Edema (3 rd hour)	Paw Edema (4 th hour)	Paw Edema (5 th hour)
MS	0.16 ± 0.07	0.37 ± 0.08	0.57 ± 0.07	0.71 ± 0.07	0.75 ± 0.10	0.87 ± 0.09
MS+AMFJ _{2.5}	0.12 ± 0.05	0.17 ± 0.06	0.53 ± 0.04	0.65 ± 0.04	0.78 ± 0.06	0.70 ± 0.12
MS+AMFJ ₅	0.20 ± 0.01	0.24 ± 0.03	0.36 ± 0.03*	0.40 ± 0.06*	0.62 ± 0.08	0.70 ± 0.09
MS+AMFJ ₁₀	0.14 ± 0.06	0.19 ± 0.06	0.41 ± 0.06*	0.40 ± 0.10	0.56 ± 0.09	0.73 ± 0.07

and were approved by the Bulgarian Food Safety Agency (Document 177/07.07.2017).

Induction of Paw Edema

At the end of the treatment period, carrageenan at a dose of 1 mg as 0.1 mL of freshly prepared solution in 0.9% saline was injected into the plantar surface of the left hind paw of the rats to induce acute inflammatory response. The paw volumes (mL) were evaluated initially (before injection), on the 30th minute and on the 1st, 2nd, 3rd, 4th, and 5th hour after the injection by a digital plethysmometer LE7500 Panlab, Barcelona. The difference between the paw volumes before and after the injection correlated with the intensity of the inflammation induced.

The paw edema (mL) was calculated using the following formula:

$$\text{Paw edema} = V_s - V_0,$$

where: V_s —the paw volume measured at the six time intervals after the carrageenan injection, V_0 —the initial paw volume.

Statistical Analysis

GraphPad Prism 5.00 statistical software was used. The results were analyzed by one-way ANOVA and followed by Dunnett's multiple comparisons test. They were presented as mean ± SEM and $p < 0.05$ was considered to indicate statistical significance.

RESULTS

Sub-plantar carrageenan injection induced an acute inflammation in all experimental groups. The values of the paw edema (mL) after the injection are presented in Table 2. In the MS group, carrageenan-induced paw edema increased gradually with time reaching the value of 0.87 ± 0.09 mL on the 5th hour. A decrease of the paw edema throughout the whole 5-hour period was observed in groups treated with AMFJ, the effect being most pronounced and statistically significant in the MS+AMFJ₅ group on the 2nd and 3rd hour and in the MS+AMFJ₁₀ group on the 2nd hour (Fig. 1).

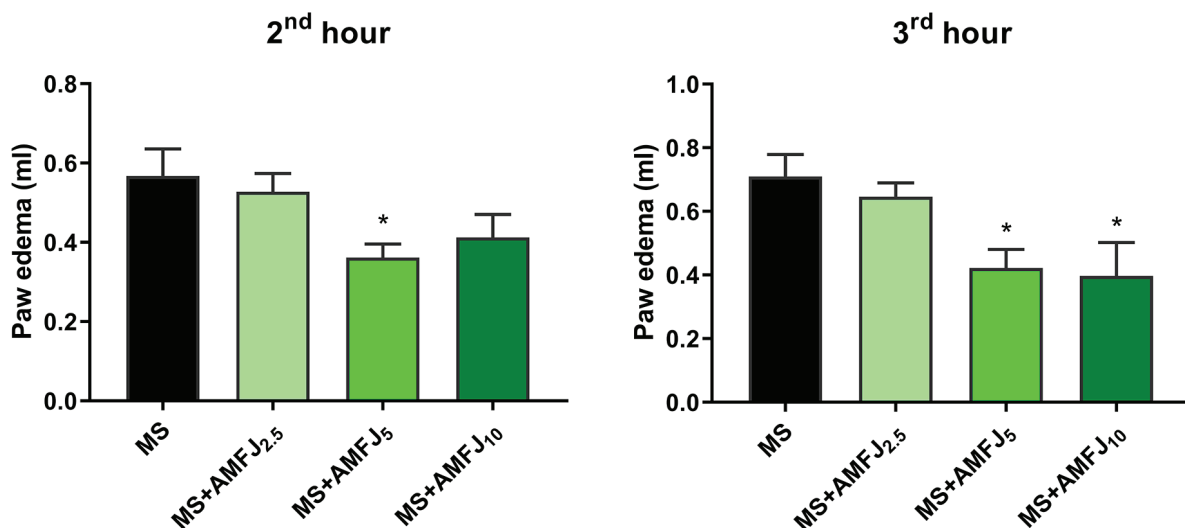


Fig. 1. Rat paw edema (mL) on the 2nd (left) and 3rd (right) hour after carrageenan injection; * $p < 0.05$ vs. MS

DISCUSSION

Metabolic syndrome is considered as a low-grade inflammatory state. This is associated with the visceral adipose tissue, which is a source of a number of cytokines, chemokines, hormonal elements, and various proteins. Some of these products have an anti-inflammatory activity (adiponectin), others—a pronounced pro-inflammatory (leptin, resistin, PAI-1, CRP, IL-1, IL-6, TNF- α , fibrinogen, serum amyloid A). In MS, the levels of pro-inflammatory mediators are elevated and those of anti-inflammatory mediators are decreased (11). Inflammatory molecules produced by visceral adipose tissue lead to a reduction in the insulin sensitivity, oxidative stress, endothelial dysfunction, and hypercoagulability. Taken together, all of these mechanisms contribute to the development of hypertension, hyperglycemia, and dyslipidemia, as well as other disorders associated with metabolic syndrome.

Carrageenan-induced paw edema is a commonly used model of acute inflammation. It consists of 2 phases: first phase (1–2 h after carrageenan injection) characterized by an increased release of serotonin, bradykinin, histamine from mast cells, and second phase (3–6 h after carrageenan injection) characterized by neutrophil infiltration, production of reactive oxygen species (ROS), as well as release of arachidonate metabolites such as prostaglandins, leukotrienes, and cytokine release (IL-1 β , IL-6, IL-10, and TNF- α) (12,13).

Aronia melanocarpa fruits are among the plant sources with the highest content of polyphenolic ingredients. Currently there are no data about the effects of *Aronia melanocarpa* fruit juice on carrageenan-induced edema in an experimental model of MS. This study examined the anti-inflammatory potential of polyphenol-rich AMFJ on carrageenan-induced paw inflammation in rats with MS. *Aronia melanocarpa* fruit juice caused a suppression in the acute inflammatory response during the six time intervals, having its highest values on the 2nd (in MS+AMFJ₅) and 3rd hour (in MS+AMFJ₁₀ group) after carrageenan injection.

The effect on the 2nd hour might be attributed to the ability of AMFJ to antagonize the effects of histamine, serotonin, and bradykinins. Such an effect of AMFJ has been well documented in a model of his-

tamine-induced and serotonin-induced rat paw inflammation (14). In this study, the anti-inflammatory effect remained high during the 3rd hour and was less pronounced thereafter. During the second phase of carrageenan-induced inflammation, AMFJ antagonized the effect of arachidonate metabolites and various cytokines. Such activities of Aronia polyphenols have been demonstrated in other studies. In a rat model of amiodarone-induced pulmonary toxicity, AMFJ administration decreased IL-6 (15). Dry *Aronia melanocarpa* extract (containing at least 25% anthocyanins) was found to inhibit markers of inflammation (IL-1 β , TNF- α) and lipid peroxidation (malondialdehyde, MDA) in lipopolysaccharide-stimulated RAW 264 cells (16). Similarly, in a model of MS in rats induced by fructose-rich diet, *Aronia* extract supplementation resulted in a decrease of pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) and increase of adiponectin (17). Athletes, supplemented with chokeberry juice, showed a decrease in the level of TNF- α and higher total antioxidant capacity (18). Black chokeberry supplementation in patients after myocardial infarction resulted in a decrease in the level of monocyte-chemoattractant protein-1 (MCP-1), C-reactive protein (CRP), IL-6, ICAM, and VCAM and an increase of the level of the anti-inflammatory adiponectin (19).

As described, carrageenan-induced paw inflammation is associated with oxidative stress. Based on that fact, we could assume that substances having an antioxidant effect would be beneficial. There are number of studies which demonstrate the antioxidant activity of *Aronia melanocarpa* polyphenols. Valcheva-Kuzmanova *et al.* found that AMFJ and its polyphenolic substances have catalase-like and superoxide dismutase-like effects and radical scavenging activity (20, 21). The juice used in this experiment was demonstrated to possess a high oxygen radical absorbance capacity (ORAC) and hydroxyl radical averting capacity (HORAC) *in vitro* (7). Polyphenol-rich *Aronia melanocarpa* extracts significantly and dose-dependently inhibited the superoxide radical formation in patients at high cardiovascular risk (arterial hypertension, hypercholesterolemia, smoking, and diabetes mellitus) (22). In a model of 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis, AMFJ improved the macroscopic and microscopic signs of colitis and prevented the increase in the con-

centrations of thiobarbituric acid reactive substances (TBARS; marker for oxidative stress) in the colon (23). In a study by Coiociu *et al.* the antioxidant activity of polyphenol-rich *Aronia melanocarpa* extract was examined in an L-NAME-induced experimental model of arterial hypertension. A significant increase in the glutathione-peroxidase activity, total antioxidant capacity, as well as a significant decrease in the serum level of MDA was observed in *Aronia*-treated groups (24).

CONCLUSION

In rats with MS, AMFJ alleviated the carrageenan-induced paw inflammation. This effect might be attributed to the anti-inflammatory and antioxidant effects of the polyphenolic ingredients of the juice.

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