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ARONIA MELANOCARPA FRUIT JUICE AMELIORATES THE HISTOPATHOLOGICAL CHANGES IN THE MYOCARDIUM AND CORONARY ARTERIES IN A RAT METABOLIC SYNDROME MODEL

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Abstract. Aim: *The purpose of this study was to examine the effect of Aronia melanocarpa fruit juice (AMFJ) on the histology of the myocardium and coronary arteries in metabolic syndrome (MS) rats.* ***Materials and methods:*** *The animals were divided into five groups: control, MS, MS+AMFJ2.5, MS+AMFJ5, and MS+AMFJ10. For 10 weeks, the control group received regular rat chaw and tap water, the other groups – high-fat, high-fructose diet and 10% fructose in their drinking water to induce MS. Throughout the experiment, rats received a daily oral treatment: the control and MS groups – 10 ml/kg distilled water, the other groups – AMFJ at doses of 2.5, 5.0, and 10.0 ml/kg, respectively. At the end of the experimental period, the animals were sacrificed and tissue samples from the myocardium and coronary arteries were evaluated histologically.* ***Results:*** *In the MS group, degeneration of the cardiomyocytes and endothelial cell necrosis were detected. In the MS+AMFJ2.5 group, cardiomyocyte damage was decreased, the endothelium was damaged and the preserved cells were activated. In MS+AMFJ5 and MS+AMFJ10 groups, the myocardium and the endothelial layer of the coronary arteries were preserved.* ***Conclusion:*** *These results indicate cardio- and vasoprotective effects of the juice.*

Key words: *Aronia melanocarpa, histology, myocardium, coronary artery, metabolic syndrome*

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INTRODUCTION

Metabolic syndrome (MS) has been linked to an increased risk of cardiovascular diseases. Each MS component is an individual risk factor for the development of coronary calcifications, coronary dysfunction, coronary artery disease, myocardial dysfunction, acute myocardial infarction, and/or heart failure [1]. Imaging and pathoanatomic studies have identified the pathogenetic substrates of these MS complications. Myocardial triglyceride accumulation was increased in MS and diabetic patients, and was positively correlated with myocardial deformity and perfusion disorders, according to magnetic resonance spectroscopy [2]. Histopathological analysis of the myocardium of patients with myo-

cardial infarction and advanced MS showed inflammation and fibrosis [3]. By using virtual histological methodology, Zheng et al. discovered that fibro-atheromatous plaques were more common and larger in the coronary arteries of patients with diabetes and/or MS than in those without MS [4]. Because of the enormous social and medical burden of these complications, new approaches to lowering cardiovascular risk in MS patients are being investigated.

Aronia melanocarpa fruits are characterized by a very high content of biologically active substances, called polyphenols (PPs). Numerous studies have found Aronia PPs exert a number of beneficial effects, including cardioprotective and vasoprotective ones [5]. However, currently there are no available studies analyzing the effect of Aronia melanocarpa fruit juice (AMFJ) on the histology of the myocardium and coronary arteries in rats with experimental model of MS.

The purpose of this study was to assess the effect of AMFJ supplementation on the histology of the myocardium and the coronary arteries in rats with diet-induced MS.

MATERIALS AND METHODS

Composition and polyphenolic content of AMFJ

AMFJ was prepared by grinding, pressing, and squeezing the fresh aronia fruits grown in the Balkan Mountains, Bulgaria. The juice was filtered, preserved with potassium sorbate (1.0 g/l) and stored at room temperature. The polyphenolic content of AMFJ was as follows [6]: total phenols – 5461 gallic acid equivalents/l, total proanthocyanidins – 3122.5 mg/l, 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. Total phenols were determined spectrophotometrically, total proanthocyanidins – gravimetrically, cyanidin glycosides and phenolic acids – by high-performance liquid chromatography.

Experimental protocol

Fifty male Wistar rats with average weight 180-280 g were used in this experiment. Standard environmental conditions were used to house the experimental animals – in plastic cages, at an ambient temperature of 20-25ºC, under 12-h light/dark cycle. They had free access to food and drinking fluids.

In each experimental group, 10 rats were included. The groups were: control, MS, MS+AMFJ$_{2.5}$, MS+AMFJ$_5$, and MS+AMFJ$_{10}$. The control rats were fed a regular rat chaw and had tap water for drinking. To induce MS, the rest of the groups were on a high-fat, high-fructose (HFHF) diet and had 10% fructose solution for drinking. The HFHF consisted of 17% lard and 17% fructose added to the regular rat chow. The caloric value of the regular rat chaw was approximately 279 kcal/100 g. The HFHF diet had a caloric value of 405 kcal/100 g, with lard accounting for 38% of energy intake and fructose accounting for 17%. The fructose in the drinking fluid added 40 kcal per 100 ml solution.

Treatment of the experimental animals was carried out on a daily basis for a period of 10 weeks by using an orogastric tube. The control and MS group were treated with distilled water in a total volume of 10 ml/kg body weight. AMFJ at doses of 2.5, 5.0 and 10.0 ml/kg body weight was used to treat MS+AMFJ$_{2.5}$, MS+AMFJ$_5$ and MS+AMFJ$_{10}$ groups, respectively. The doses of 2.5 and 5.0 ml/kg of AMFJ were diluted with distilled water to a total volume of 10.0 ml/lkg.

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

Histological evaluation of the myocardium and the coronary arteries

At the end of the 10th week, the rats were anesthetized with diethyl ether and sacrificed. Tissue samples of myocardium and coronary arteries were fixed in 10% neutral buffered formalin and after appropriate treatment were embedded in paraffin. Sections 5 μm thick were standardly stained with hematoxylin-eosin to assess the histological changes.

RESULTS

The histological findings of the myocardium samples are shown in Fig.1.

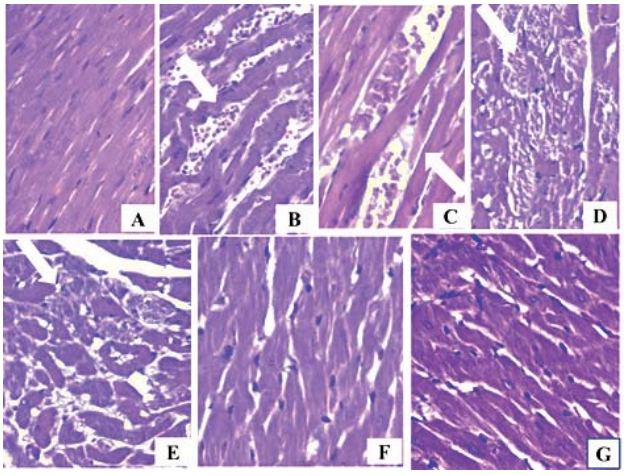


Fig. 1. Myocardium of rats from the control group (panel A), MS group (panels B, C, D), MS+AMFJ$_{2.5}$ group (panel E), MS+AMFJ$_5$ (panel F) and MS+AMFJ$_{10}$ group (panel G); arrows – cardiomyocyte degeneration. H&E x 200

In the control group, the cardiomyocytes were closely located next to each other. They had vesicular nuclei and eosinophilic cytoplasm (Fig. 1A). In the MS group, cardiomyocytes had degenerative changes, with loss of transverse striation, often wavy (Fig. 1B). Focal hemorrhages (Fig. 1C) and focal necrosis (Fig. 1D) were detected. The low dose of AMFJ (2.5 mL kg^{-1}) reduced the cardiomyocyte damage compared to the MS group, but degenerative changes were still found in the parenchymal cells, which were often distant from each other (Fig. 1E). In MS+$AMFJ_5$ and MS+$AMFJ_{10}$ groups, the myocardium showed no histological abnormalities (Fig. 1F, Fig. 1G).

The histological findings in the coronary arteries are shown in Fig. 2.

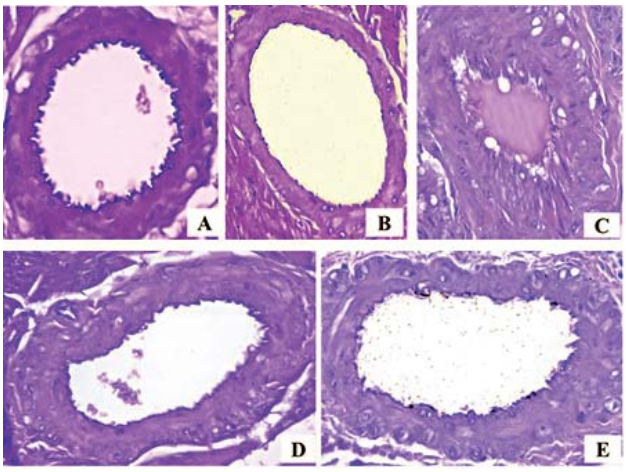


Fig. 2. Coronary arteries of rats from the control group (panel A), MS group (panel B), MS+$AMFJ_{2.5}$ group (panel C), MS+$AMFJ_5$ group (panel D) and MS+$AMFJ_{10}$ group (panel E). H&E x 200

Coronary arteries in rats from the control group showed an intact endothelial layer and no structural changes were found in their wall (Fig. 2A). In MS group, the endothelium was damaged, there was necrosis of endothelial cells and the basement membrane was exposed (Fig. 2B). In MS+$AMFJ_{2.5}$ group, the endothelium was also damaged and the preserved cells showed signs of activity – the nuclei of endothelial cells were located perpendicular to the basement membrane (Fig. 2C). In MS+$AMFJ_5$ and MS+$AMFJ_{10}$ groups, endothelial layer of the coronary arteries was intact (Fig. 2D, Fig. 1E).

DISCUSSION

Several pathogenetic factors contribute to myocardial and vascular damage in MS. The sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) are both activated by oxidative stress (OxS). These systems, in turn, induce OxS, indicating that the relationship is bidirectional [7]. The SNS and RAAS both stimulate the cardiovascular system, causing positive tropic effects in the myocardium as well as vasoconstriction and endothelial damage in the blood vessels [8, 9]. Increased adipocytokine production by abdominal adipose tissue, in conjunction with the previously mentioned regulatory systems, contributes to the increase in heart rate, circulating blood volume, end-diastolic volume in the ventricles, cardiac output, and vascular resistance. These changes result in impaired energy metabolism, abnormal oxygen demand, diastolic dysfunction, and concentric myocardial hypertrophy [1]. The endothelial activation observed histologically in this study is a pro-inflammatory response [10]. We suppose it is a presentation of endothelial dysfunction in MS.

The arterial vascular structure (endothelial layer and basement membrane) is damaged in MS, pre-diabetic conditions, and diabetes, and these changes are associated with significant micro- and macrovascular complications. Endothelial dysfunction has been identified as a major contributor to vascular complications in MS. Reduced nitric oxide (NO) production, as well as increased release of vasoconstrictors and reactive oxygen species (ROS), lead to blood vessel damage, resulting in an accelerated atherosclerotic process and increased blood pressure – changes seen in MS [11]. Furthermore, it has long been known that adverse metabolic effects (hyperglycemia) activate matrix metalloproteinases, which degrade and damage type IV collagen of endothelial basement membrane [12].

The current study aimed to evaluate the histology of myocardium and coronary vessels in rats with diet-induced MS, as well as the effect of AMFJ on the structure of these organs. In MS rats, the high-calorie diet caused cardiomyocyte degeneration and disrupted cellular microarchitectonics. MS induced endothelial cell activation, endothelial necrosis, and basement membrane damage in the coronary arteries. AMFJ treatment improved the histological findings and reduced the pathological changes in myocardium and coronary blood vessels, particularly at the higher doses (5.0 and 10.0 mL kg^{-1}).

Although studies on the effect of *Aronia melanocarpa* on cardiovascular structure are scarce, available publications show beneficial effects. A study conducted by Daskalova et al. showed that treating aging rats with Aronia juice slowed down the age-related accumulation of collagen fibers in the aortic wall. Moreover, the level of the atherogenic LDL-cholesterol was reduced [13]. A recent study elucidated that AMFJ supplementation in aging rats increased the

expression of angiotensin-converting enzyme type 2 (it antagonizes angiotensin II-mediated effects) in coronary tunica media and slowed the age-related myocardial remodeling [14].

The cytoprotective effects of AMFJ in the present study could be related to the antioxidant effects of the PPs. Nuclear factor erythroid-2-related factor 2 (Nrf2) is a transcription factor that regulates the cellular response to OxS and increases the expression of the antioxidant enzymes superoxide dismutase, glutathione peroxidase and NAD(P)H quinone oxidoreductase. Under normal conditions (without free radical production) Nrf2 is coupled with ECH-associated protein 1 (KEAP 1). Keap1 induces proteasome-mediated lysis of Nrf2 and terminates its effects [15]. When these proteins dissociate from each other in OxS, Nrf2 enters the cell and induces the expression of genes encoding the aforementioned antioxidant enzymes. This is thought to be the primary mechanism underlying the antioxidant activity of PPs. Thus, antioxidant protection is likely to prevent SNS and RAAS overactivation and protect the myocardium. In a study of H9c2 cardiomyoblasts, antioxidant-mediated cardioprotection was described for the flavonol catechin as well as for the anthocyanidins cyanidin and delphinidin [16]. In another study, cranberry extract rich in cyanidin-3-galactoside, cyanidin-3-arabinoside, and cyanidin-3-glucoside inhibited the activation of apoptotic pathways in cardiomyocytes by preventing OxS [17]. These flavonoids are abundant in AMFJ used in this study.

Some flavonols have also been shown to produce a direct sympatholytic effect. In a study of Louis et al., anthocyanin-rich aqueous bilberry extract prevented norepinephrine-mediated cardiomyocyte hypertrophy and apoptosis *in vitro* [18]. Another study found that resveratrol exerted an antihypertrophic effect on the myocardium, which was attributed to increased NO and AMP-activated protein kinase expression [19].

RAAS-inhibition is a potential mechanism that could protect the myocardium from damage in MS. Suppressing this system would reduce pathological remodeling of the myocardium and production of ROS, both of which are involved in MS-related myocardial disorders. Parichatikanond et al. (2012) investigated the effects of the flavonol quercetin and the anthocyanins cyanidin and delfinidin on human embryonic kidney (HEK)-293 cells after dexamethasone treatment. The corticosteroid increased ACE activity while the previously mentioned PPs decreased it, regardless of dexamethasone treatment [20]. By modulating local (renal) or systemic RAAS activity, catechin, epicatechin, and polymeric proanthocyanidin-rich acai (Euterpe oleracea) seed extract reduced the inflammatory response and OxS in MS patients [21]. Although these studies were conducted on other tissues, we can expect the same effects to occur in myocardial tissue as well.

The vasoprotective effect described in this study is most likely due to an improvement of endothelial function mediated by the PPs in the juice. There are several studies demonstrating such an effect of PPs (including those derived from *Aronia melanocarpa*). Kim et al., for instance, reported that *Aronia melanocarpa* juice could induce endothelium-dependent relaxations in porcine coronary arteries via the redox-sensitive activation of endothelial nitric oxide synthase [22]. In another study, anthocyanin-rich chokeberry extract inhibited TNF-alpha-mediated expression of vascular adhesion molecule-1 (VCAM-1). This mechanism is thought to be responsible for the anti-atherogenic effect of *Aronia melanocarpa* [23]. Zapolska-Downar et al. found that chokeberry extract produced an anti-inflammatory effect on human aortic endothelial cells by suppressing the expression of VCAM-1, intercellular adhesion molecule-1 (ICAM-1) and NF-κB [24].

CONCLUSION

Metabolic syndrome is associated with myocardial and coronary damage. Aronia melanocarpa fruit juice reduced the histopathological changes in the myocardium and the coronary vessels in rats with diet-induced metabolic syndrome. These results indicated the cardioprotective and vasoprotective effects of the juice.

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