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Original Article

JAPANESE QUINCE FRUIT JUICE EXERTS A CARDIOPROTECTIVE EFFECT IN A MODEL OF DIET-INDUCED METABOLIC SYNDROME IN RATS

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Summary

The current study aimed to evaluate the influence of Japanese quince (Chaenomeles japonica) fruit juice (JQFJ) on the myocardium and on the coronary arteries of rats with diet-induced metabolic syndrome (MS). Male Wistar rats (n=50) were divided into 5 groups: Control, MS, MS+JQFJ2.5, MS+JQFJ5 and MS+JQFJ10. MS was induced with a high-fat high-fructose diet for 10 weeks. During that period, all animals were daily orally treated with distilled water (Control and MS groups) or with JQFJ at doses of 2.5 ml/kg, 5 ml/kg and 10 ml/kg (the other three groups, respectively). At the end of the experiment, the myocardium and coronary arteries were examined histopathologically. In group MS, necrotic endothelial cells and exposed basal membrane were observed. JQFJ at 2.5 ml/kg reduced the impairment but activated endothelial cells were still found. JQFJ at 5 ml/kg and 10 ml/kg prevented coronary artery endothelium damage, preserving the normal morphology. The myocardium in MS group presented with cardiomyocyte degeneration and increased distance between the cells. In group MS+JQFJ2.5, the degeneration was decreased. In groups MS+JQFJ5 and MS+JQFJ10, the histology of the myocardium resembled that of the control group. In the current histopathological evaluation, JQFJ prevented the MS-induced impairment of myocardium and coronary arteries.

Keywords: Chaenomeles japonica, Japanese quince, medicinal plants, cardioprotective, metabolic syndrome

Introduction

The average human life expectancy today is as high as it has ever been, all the while disturbed by the exponential growth of non-communicable diseases. A major concern is the prevalence of obesity, hypertension, impaired glucose and lipid homeostasis, all falling under the umbrella of metabolic syndrome (MS). These clustering abnormalities result in an increased risk of diabetes type 2, cardiovascular diseases and

malignant neoplasms.

Despite the multifactorial pathogenesis of MS, two common hallmarks in both the initiation and the progression of the condition are inevitably observed – insulin resistance and low-grade chronic inflammation. Each of them adversely impacts the cardiovascular system, impairing its structure and function. While enhancing endothelial NO production and vasodilation in physiologic conditions, in the state of insulin resistance insulin favours vasoconstriction, smooth muscle cells hypertrophy and accelerated atherosclerosis by mitogen-activated protein kinase (MAPK) activation [1]. Insulin resistance also activates various inflammatory pathways thus increasing the levels of proinflammatory cytokines such as TNF-α, IL-6, IL-1β, PAI-1, MCP-1, leptin, resistin, etc. [1]. When chronically activated and sustained, inflammation constitutes a major determinant of cardiovascular disease.

In the attempt to recreate and better explore the pathogenesis of MS and in search of novel approaches to its prevention and treatment, many experimental animal models have been created. Diet-induced models stand out among the rest with their easier execution from the financial and practical point of view. Frequently used models include the high-fat diet, the high-carbohydrate diet and the combination of them. An example of a high-fat high-carbohydrate diet is the cafeteria diet that is based on highly palatable ultraprocessed foods and closely resembles the feeding pattern in western countries, successfully implementing the etiological factors in humans into the animal model. The cardiovascular changes observed in these models of MS include raised systolic blood pressure, endothelial dysfunction, inflammation, fibrosis, hypertrophy and increased rigidity of the left ventricle in the experimental animals [2].

In recent years personalised nutrition and its significance for the prevention and therapy of non-communicable diseases have gained much scientific interest. Plants with prophylactic and therapeutic applications as well as those that can be used as functional foods – leading to higher benefits for the organism than their expected nutritional value – continue to be a focus of attention. Fruits of the Japanese quince – *Chaenomeles japonica* or *Chaenomeles maulei* as the cultivar introduced in Bulgaria is also known – have been used for centuries in traditional eastern medicine with different applications, including for their positive influence on the cardiovascular system [3]. Along with the propagation of the plant in Europe, its supposed applications have started to be confirmed, but, to our knowledge, there is no data on the cardiovascular effects of the Japanese quince fruit juice yet.

The aim of the current study is to examine the effects of Japanese quince fruit juice on the histology of the myocardium and coronary arteries in rats with diet-induced metabolic syndrome.

Materials and Methods

Japanese quince fruit juice

Japanese quince fruits for the preparation of the juice (JQFJ) were gathered from *Chaenomeles* plants in the region of Troyan, Bulgaria. They were handpicked, grinded, crushed and squeezed. JQFJ was filtered, preserved with potassium sorbate (1.0 g/l) and stored at 0°C.

The contents of phenolic substances (mg/100 ml) were: total phenols 890.0 as gallic acid equivalents; total proanthocyanidins – 2532.9; procyanidin oligomers – 2805.2 as catechin equivalents; phenolic acids mainly presented by vanillic acid – 149.1, caffeic acid – 144.8, chlorogenic acid – 110.0, neochlorogenic acid – 24.4, p-coumaric acid – 15.2, etc.; the flavonoids epicatechin – 55.9, catechin – 52.5, quercetin-3-β-glucoside – 35.8, quercetin – 34.3, rutin: – 27.2, naringin – 14.6, kaemferol – 4.2, etc. [4] The total phenolic content corresponded with a high antioxidant activity as estimated by the oxygen radical absorbance capacity assay (84401.4±1934.2 μmol trolox equivalents/l) and hydroxyl radical averting capacity assay (18167.8±938.8 μmol gallic acid equivalents/l) [4]. The most abundant organic acids (mg/100 ml) were malic acid – 3647 and quinic acid – 1034.0. Carbohydrates (mg/100 ml) were mainly glucose – 1713.0, fructose – 1237.0 and galactose – 320.0 [4].

Experimental animals

Fifty adult male Wistar rats were used for the purposes of the experiment. The animals were divided into 5 groups of 10 rats each:

control (C), MS, MS+JQFJ2.5, MS+JQFJ5 and MS+JQFJ10. For 10 weeks, rats from group C received a standard laboratory diet and tap water ad libitum, while for the induction of MS, the other four groups were given a high-fat high-fructose (HFHF) diet – 17% lard and 17% fructose added to the standard diet as well as 10% fructose solution instead of water (a method, established by Gancheva et al., 2015) [5]. All animals were treated with a flexible orogastric tube on a daily basis. Rats from groups C and MS received destilled water, while the ones from the other 3 groups received JQFJ in increasing doses – 2.5 ml/kg, 5 ml/kg and 10 ml/kg, respectively. At the end of the experiment, all animals were euthanised with diethyl ether. Tissue samples from myocardium and coronary vessels were collected, fixed in 10% neutral buffered formaldehyde solution and included in paraffin. Paraffin blocks were then stained with hematoxylin-eosin.

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 Bulgarian Food Safety.

Results

The wall of the coronary vessel of the control group presented with normal morphology – it was lined with continuous endothelium. In group MS, focal regions of absent endothelium were observed, endothelial cells were necrotic and the basal membrane was left exposed. In group MS+JQFJ2.5, focal regions of absent endothelium were still present and activation of the endothelial cells was observed. In the groups that received the higher doses of JQFJ (MS+JQFJ5 and MS+JQFJ10), endothelial lining of coronary arteries was preserved (Figure 1).

In the control group, a normal structure of the myocardium was observed. In group MS, there was degeneration and increased distance between the cardiomyocytes. In group MS+JQFJ2.5, the impairment was reduced – along with zones of degeneration and increased distance between cardiomyocytes, there were also zones of normal structure. In groups MS+JQFJ5 and MS+JQFJ10 the myocardium did not show any histological alterations (Figure 2).

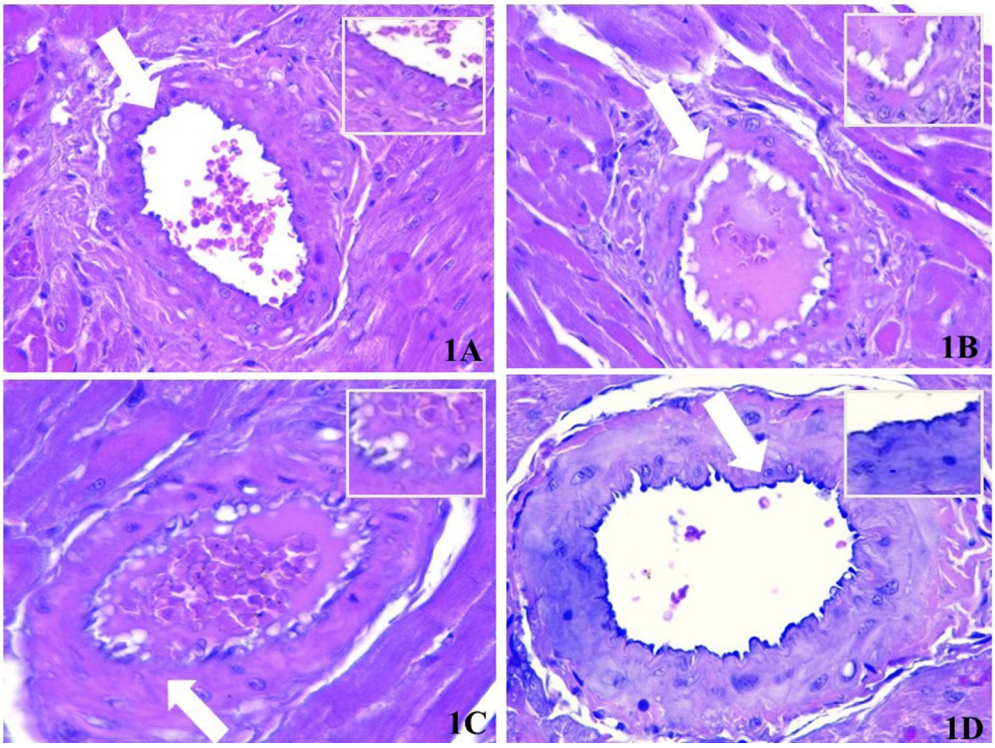

Figure 1. Microscopic appearance of coronary vessels of groups: Control (panel 1A), MS (panel 1B), MS+JQFJ2.5 (panel 1C), and MS+JQFJ10 (panel 1D); Hematoxylin-eosin staining, magnification x 400

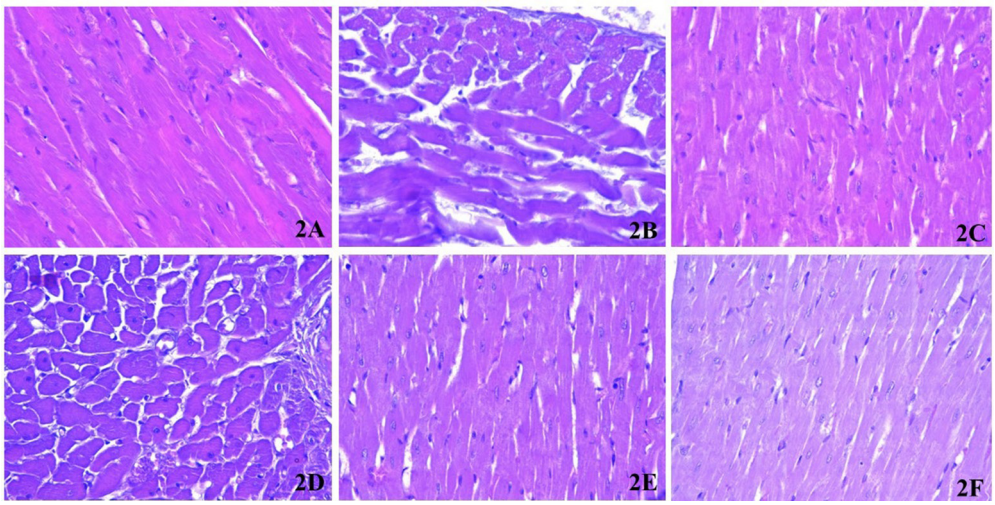

Figure 2. Microscopic appearance of the myocardium of groups: Control (panel 2A), MS (panel 2B), MS+JQFJ2.5 (panels 2C and 2D), MS+JQFJ5 (panel 2E) and MS+JQFJ10 (panel 2F); Hematoxylin-eosin staining, magnification x 200

Discussion

The HFHF diet used in the current experiment led to an impairment of the endothelium. It has been established that endothelial dysfunction is a foundation of impaired regulation of vascular resistance and tissue perfusion and determines the initiation and progression of vascular atherogenesis, developing at later stages of MS [6]. Endothelial dysfunction leads to impairment of the endothelium-dependent vasodilation, further contributing to the complex pathogenesis of the syndrome. Cardiomyocytes also responded with degenerative changes to the HFHF diet. This is in consistence with existing literature on experiments conducted with similar nutritional interventions [7].

JQFJ treatment led to dose-dependent cardio- and vasoprotective effects. Antiatherogenic and cardioprotective effects have also been reported for other fruit juices – black chokeberry [8], pomegranate [9], grapes [10], orange [11] and many others. It has long been known that consumption of plant foods correlates with a lower cardiovascular risk [8-9]. Polyphenols have a significant contribution to these health-beneficial effects. JQFJ is a rich source of polyphenols. MS components and the subsequent cardiovascular consequences are influenced by polyphenols in various ways. Polyphenols improve glycemic control probably by inhibition of α-amylase and α-glucosidase (thus blunting postprandial glucose spikes), sodium-dependent glucose transporter 1 as well as stimulation of insulin secretion and a reduction in hepatic glucose output [12]. Quercetin has been reported to have antioxidant and anti-inflammatory properties, to enhance glucose uptake in muscles and adipocytes and to induce autophagy in the setting of MS and associated disorders [13]. A basic phenomenon in MS is the chronic low-grade inflammation. Inhibition of cyclooxygenase, lipoxygenase, inducible nitric oxide synthase, nuclear factor kappa-B and activating protein-1 (AP-1), as well as MAPK, protein kinase-C and nuclear factor erythroid 2-related factor activation are some of the polyphenols` molecular activities leading to a decrease in inflammation [14]. The anti-inflammatory properties of polyphenols are partially mediated by epigenetic modifications [15]. Another cornerstone in MS pathogenesis is mitochondrial dysfunction. Correct functioning of cellular mitochondria depends on the selective degradation of impaired ones through autophagy – a process, called mitophagy. Mitophagy impairments are an important part of MS genesis and progression and its corresponding conditions, including cardiovascular implications [16]. Polyphenol-mediated mitophagy presents an opportunity for modulation of mitochondrial health through dietary manipulation [17]. Polyphenols also influence mitochondrial biogenesis. They are considered calory restriction mimetics since they activate some of

the molecular mechanisms, stimulated during limited caloric intake. Therefore, they utilize one of the most effective exogenous strategies of improving mitochondrial condition [18]. Polyphenols are also known to modulate gut microbiota, including acting as prebiotics [12]. Another, usually less discussed, mechanism implicated in MS pathogenesis, is circadian dysrhythmia. Polyphenols have been shown to interact with circadian clocks by modulating the transcription and expression of clock genes [19].

Among the polyphenols found in JQFJ, the olygomeric proanthocyanidins [20], vanillic [21], caffeic, chlorogenic [22], p-coumaric [23] and ellagic acid [24] as well as the flavonoids epicatechin, catechin, quercetin, rutin, naringin, kaempferol and myricetin [25] have been reported to have cardio- and/or vasoprotective properties. A serious impediment to scientific progress in the area of dietary polyphenols is the difficulty to transition this knowledge to larger-scale human trials – clinical evidence gathered so far is limited and inconclusive. This might be partly explained by variable polyphenol bioavailability, a lack of appropriate stratification of participants based on inter-individual variations in biological response [26], etc. Therefore, an important direction of future research is further elucidating the significance of polyphenol intake in humans and fruit juices, as a natural part of the diet, offer a suitable approach to do that.

Conclusions

Chronic treatment with Japanese quince fruit juice prevented the development of myocardial and coronary artery impairment in rats with diet-induced metabolic syndrome. The cardio- and vasoprotective effects had a dose-dependent fashion and could possibly be attributed to the high polyphenolic content of the juice. The findings of the current study open up new avenues for research of prevention and treatment of cardiovascular pathologies within the spectrum of metabolic syndrome.

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