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BEHAVIORAL EFFECTS OF CHRONIC JAPANESE QUINCE FRUIT JUICE ADMINISTRATION TO RATS WITH DIET-INDUCED METABOLIC SYNDROME

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Abstract. Introduction: *Metabolic syndrome (MS) is often associated with anxiety and depression. Chaenomeles japonica (Thunb.) Lindl, also known as Chaenomeles maulei or Japanese quince, is a medicinal plant with a long history of use for its health-promoting properties.* ***Aim:*** *The aim of this study was to investigate the effects of Japanese quince fruit juice (JQFJ) administration on locomotor activity, anxiety and depressive behavior in rats with diet-induced MS.* ***Materials and methods:*** *Forty adult male Wistar rats were divided into 4 groups: MS, MS+JQFJ2.5, MS+JQFJ5 and MS+JQFJ10. All groups received a high-fat high-fructose diet for the induction of MS. MS animals were daily orally treated with distilled water and the other groups with JQFJ at doses of 2.5 ml/kg, 5 ml/kg and 10 ml/kg, respectively. During the 10th week of the experiment, behavioral tests were carried out.* ***Results:*** *In the open field test, no effect of JQFJ treatment on locomotor activity was observed. In the elevated plus maze test, a dose-dependent increase in the time spent in the open arms (OA) of the apparatus and in the ratio time spent in OA vs. total time spent in any of the arms was interpreted as an anxiolytic effect. The immobility time in the forced swim test did not differ significantly among the groups, which demonstrated a lack of antidepressant action.* ***Conclusions:*** *Chronic Japanese quince fruit juice administration produced a dose-dependent anxiolytic-like effect in rats with diet-induced MS, probably due to its high content of polyphenols.*

Key words: *anxiety, Chaenomeles japonica, depression, medicinal plants*

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INTRODUCTION

The processing, memory and expression of fear- and anxiety-related behaviors are mediated by several brain regions, including the amygdala and its connections to the thalamus, the medial prefrontal cortex, the hippocampus, the hypothalamus, the bed nucleus of the stria terminalis, the brainstem and the periaqueductal grey [1]. Metabolic syndrome (MS) is often associated with anxiety [2] and depression along with other cognitive and neuropsychiatric disorders. The relationship is bidirectional since anxiety and depression might also interfere with a patient`s likelihood to adhere to pharmacological and lifestyle recommendations in the management of MS as well as lead to poor eating habits and low

physical activity. The coexistence of MS and anxiety gets even more complicated when we consider that the first-line drugs for long-term therapy for anxiety, the selective serotonin reuptake inhibitors, are able to induce various metabolic disturbances. The escape from the vicious circle has been searched lately among substances with natural origin such as medicinal plants that offer balanced, easily accessible interventions. Moreover, their use has been associated with higher patient's adherence.

A proper candidate might be *Chaenomeles japonica* (*Thunb.*) *Lindl*, known as Maule's quince, or *Chaenomeles maulei* as the cultivar introduced in Europe is referred to. It is also known as Japanese quince. It is a shrub belonging to the family Rosaceae [3] used for centuries in traditional eastern medicine for its various biological properties, including antioxidant, anti-inflammatory and neuroprotective properties. Indeed, due to its unique properties, Japanese quince fruit juice has recently gained the attention of the scientific community. Previous studies have considered the behavioral effects of Japanese quince fruit juice (JQFJ) in healthy rats [4] and in a model of mild stress [5], but there is no data regarding its effects on animals with diet-induced MS. JQFJ is an exceptional source of polyphenols such as procyanidin oligomers, phenolic acids and flavonoids [6].

Polyphenols are secondary metabolites produced by plants with protective actions against harmful environmental conditions such as ultraviolet radiation or microbial pathogens. Oxidative stress is a mechanism implicated in the pathophysiology of many neuropshychiatric diseases, including anxiety and depression. Health-promoting effects of a polyphenol-rich diet are due not only to the well-established antioxidant activity of polyphenols, but also to their ability to modulate various signaling pathways.

AIM

The aim of the present study was to investigate the effects of chronic JQFJ administration on locomotor activity, anxiety and depressive behavior in rats with diet-induced MS.

MATERIALS AND METHODS

Japanese quince fruit juice

Japanese quince fruit juice was produced from plants grown in the region of Troyan in the Balkan Mountains in Bulgaria. Fruits were handpicked when ripe and 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, kaempferol 4.2, etc. [6] The total phenolic content corresponded with a high antioxidant activity as estimated by the oxygen radical absorbance capacity (84401.4 ± 1934.2 μmol trolox equivalents/l) and hydroxyl radical averting capacity (18167.8 ± 938.8 μmol gallic acid equivalents/l) assays [6]. 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 [6].

Experimental animals, induction of MS and treatment protocol

Forty adult male Wistar rats bred in the Animal Centre of Medical University of Varna were used in the experiment. They were housed in plastic cages with 5 animals in each cage and were kept at 22 ± 2 °C and a 12h light/dark cycle. Animals were divided into four groups of ten rats each: MS, MS+JQFJ2.5, MS+JQFJ5 and MS+JQFJ10. All the groups were given a high-fat high-fructose diet (17% of lard and 17% fructose added to the standard diet as well as 10% fructose solution instead of drinking water) for ten weeks to induce MS. The animals were treated orally on a daily basis with a flexible orogastric tube. The MS group received distilled water, while the other three groups MS+JQFJ2.5, MS+JQFJ5 and MS+JQFJ10 were treated with JQFJ at increasing doses (2.5 ml/kg, 5 ml/kg and 10 ml/kg, respectively).

Open field test (OFT)

The open field test is a common method for assessing locomotion. The OFT apparatus was a wooden arena (100 x 100 cm) with walls 40 cm high. Its floor was divided into 25 squares of equal size. Each rat was placed individually in the center of the arena and its behavior was observed for 5 minutes. The number of squares crossed (horizontal movements) and the number of rearings (vertical movements) were registered in order to assess the locomotor activity. After each test session the apparatus was thoroughly cleaned and allowed to dry in order to remove the scent of the previous animal and to prevent interference with the spontaneous behavior of the next one.

Elevated plus maze (EPM) test

The elevated plus maze apparatus consisted of two open arms and two closed arms, connected to a central platform. The maze was elevated at 50 cm above the floor level. During the experiment each rat was individually placed in the central compartment facing one of the open arms and its activity was tracked for a period of 5 minutes. The number of entries into the open arms (OA), time spent in the OA, number of entries in closed arms (CA) and time spent in CA were registered. The ratio of entries in the OA vs. total arm (TA) entries and the ratio of time spent in OA vs. total time spent in any of the arms were calculated. Increased exploration time of the open arms, including time spent in OA and number of entries in OA, are interpreted as an anxiolytic effect.

Forced swim test (FST)

The forced swim test apparatus was a glass cylinder with a diameter of 17 cm and a height of 50 cm. It was filled with warm (30°C) water up to 30 cm as to prevent the animal from touching the bottom with his hind paws or tail and to make sure it is forced to swim. Two test sessions were performed on two consecutive days. Each animal was individually placed into the cylinder for a period of 5 minutes and the total time spent in immobility was measured. The FST is a model of behavioral despair. The immobility time is considered an index of depression with decreased immobility time being interpreted as an antidepressant effect.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software. Data was expressed as Means ± SEM (standard error of the mean) and analyzed with one-way ANOVA followed by Dunnet`s post-test for each test and a post test for linear trend for EPM test. A p-value less than 0.05 was considered statistically significant.

RESULTS

Open-field test

The results from the OFT are presented in Table 1. The number of horizontal movements was slightly increased in group MS+JQ2.5, but the differences did not reach statistical significance. The number of vertical movements was not affected by the treatment. Thus, no effect on locomotor activity was registered in the OFT.

Elevated plus-maze test

The time spent in the OA of MS rats was 8.89 ± 3.05 sec. JQFJ treatment caused a tendency of increased time (sec) in the OA: 24.77 ± 7.54 for MS+JQFJ2.5, 33.14 ± 7.77 for MS+JQFJ5 and 33.06 ± 11.32 for MS+JQFJ10. One-way ANOVA with a post-test for linear trend of the time spent in OA showed a positive linear trend (p = 0.0354), as seen in Fig.1. There was no statistically significant difference in the number of OA entries among the groups – 1.30 ± 0.67 for MS, 1.50 ± 0.45 for MS+JQFJ2.5, 1.70 ± 0.4 for MS+JQFJ5 and 1.50 ± 0.48 for MS+JQFJ10.

The ratio time spent in OA over time spent in any of the arms in MS group was 0.04 ± 0.01. JQFJ treatment caused a tendency of ratio increase: 0.10 ± 0.03 for MS+JQFJ2.5, 0.13 ± 0.03 for MS+JQFJ5 and 0.14 ± 0.05 for MS+JQFJ10. The post-test for

Table 1. Results from the open field test

Group	MS	MS+JQFJ2.5	MS+JQFJ5	MS+JQFJ10
Horizontal movements	85.3 ± 8.1	116.2 ± 7.72	92.6 ± 13.85	84.4 ± 11.66
Vertical movements	22.4 ± 1.37	23.6 ± 2.71	22.8 ± 2.94	22.3 ± 3.30

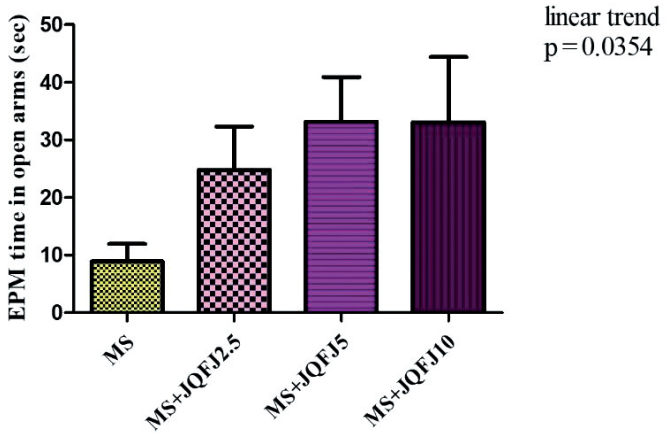

Fig. 1. Time spent in OA in the EPM test. Positive linear trend: p < 0.05

linear trend of the ratio showed a positive linear trend (p = 0.0335), as seen in Fig. 2.

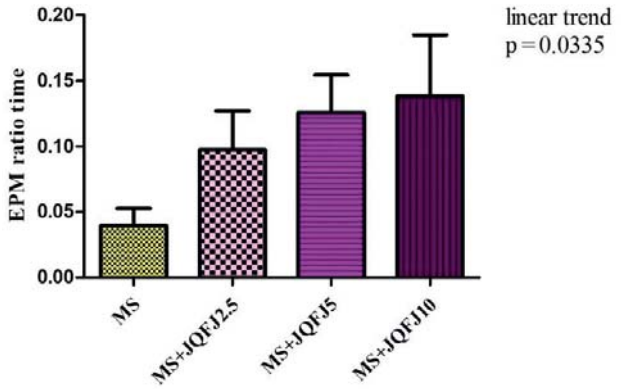

Fig. 2. Ratio time spent in OA vs. total time spent in OA+CA in the EPM test. Positive linear trend: $p < 0.05$

The ratio of entries in the OA vs. total arm entries in MS group was 0.07 ± 0.02. The same was increased but not significantly in all the groups receiving JQFJ – 0.12 ± 0.04 for MS+JQFJ2.5, 0.15 ± 0.04 for MS+JQFJ5 and 0.14 ± 0.04 for MS+JQFJ10.

Locomotion measured by the total number of entries in any of the arms did not differ significantly among the groups – 11.60 ± 1.39 for MS, 9.44 ± 1.53 for MS+JQFJ2.5, 9.20 ± 1.3 for MS+JQFJ5 and 10.30 ± 1.4 for MS+JQFJ10.

Forced swim test

The results from the FST are presented in Table 2. The immobility time was slightly increased in all three groups treated with JQFJ but without reaching statistical significance. Therefore, a lack of antidepressant effect of the juice was observed.

Table 2. Results from the forced swim test

Group	MS	MS+JQFJ2.5	MS+JQFJ5	MS+JQFJ10
Immobility time	170.2 ± 13.21	198.8 ± 12.70	183.6 ± 5.237	185.8 ± 11.06

DISCUSSION

The model used for induction of MS has been verified by the presence of visceral adiposity, elevated serum triglycerides and insulin resistance in the experimental animals [7]. The present study demonstrated no change in the locomotor activity of rats with MS following the treatment with JQFJ as revealed by the open field test.

In the forced swim test no antidepressant action of the juice was observed. This data resembles the findings in healthy male rats [8].

Elevated plus-maze is a well-established unconditioned test for measuring anxiety levels in rodents. It is based on the natural aversive behavior of rats in novel open spaces in combination with fear of balancing on a narrow, raised platform as opposed to the spontaneous exploratory behavior of the animal. The present study demonstrated that chronic administration of JQFJ in rats with MS caused an increase in the time spent in the open arms in a dose-dependent manner which indicated an anxiolytic-like effect. These results correspond with the anxiolytic effect reported for JQFJ administered to healthy rats [4], as well as to rats subjected to mild stress induced by impaired circadian rhythm [5]. In both mentioned experiments with subchronic administration of the juice no dose-dependency was established while with the chronic treatment in the present study a clear dose-dependent effect was evident.

A growing amount of scientific evidence outlines anxiety as a gut-brain-axis disorder [9]. Polyphenols have been known to modulate the composition of gut microbiota as to increase the beneficial bacteria and to inhibit the pathogenic strains [10]. The possibility of gut microbiota modulation directly affecting brain processes might offer an explanation to some long-standing discrepancies between low oral bioavailability of some polyphenols and their significant biological effects observed in epidemiological studies. Research shows that gut dysbiosis might even affect blood brain barrier permeability [9].

An important feature that currently effective pharmacological (such as selective serotonin reuptake inhibitors) and non-pharmacological (cognitive-behavioral therapy) treatments of anxiety share, is the ability to modulate synaptic plasticity [1]. Studies show that dietary flavonoids are able to promote the expression of neuroplasticity-related genes and proteins. Adult hippocampal neurogenesis has been outlined as at least partly responsible for the beneficial effects of polyphenols on anxiety and depression [11]. Quercetin has been reported to increase neurogenesis and synaptogenesis [12].

Inflammation is considered one of the possible links between anxiety and metabolic syndrome. Higher level of pro-inflammatory cytokines is observed in patients with anxiety [9]. Since in humans it is more difficult to directly measure neuroinflammation, often peripheral inflammation and coagulation markers are evaluated. Large human cohort studies reveal a positive correlation between anxiety and C-reactive protein, interleukin-6, homocysteine, fibrinogen, tumor necrosis factor-alpha and white blood cell counts [13,14]. Polyphenols exert anti-inflammatory actions by a variety of mechanisms, including modulation of

immune cell populations, cytokine production and the expression of inflammatory genes [15].

Many of the phenolic substances found in JQFJ have extensive neuropsychiatric impact. Anxiolytic effect has been reported for vanillic [16], caffeic [17], chlorogenic [18] and p-coumaric acid [19]. Reported possible mechanisms of action include a GABA-A interaction. Some of the flavonols present in the juice, such as quercetin, likely share the same mechanism [20], while others like kaempferol have been described to inhibit the fatty acid amide hydrolase in vitro, therefore affecting the endocannabinoid system [21]. However, whether this is the mechanism responsible for the in vivo anxiolytic effect of this flavonol remains to be established. Evidence suggests that complex genomic modifications in the hippocampus of high-fat diet-induced obese mice underlie the anxiolytic effect of epicatechin [22].

CONCLUSION

Chronic Japanese quince fruit juice administration produced a dose-dependent anxiolytic-like effect in rats with diet-induced metabolic syndrome probably due to its high content of polyphenols and their ability to exert antioxidant and anti-inflammatory actions, modulate gut microbiota, affect neurotransmitter systems and influence synaptic plasticity.

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Ethical statement: *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 (Document177/07.07.2017).*

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