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Behavioral effects of the subchronic *Chaenomeles maulei* fruit juice administration to healthy male rats

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Polyphenols, known for their behavioral effects, are the main pharmacological entity found in the fruits of *Chaenomeles japonica var. maulei (Mast.) Lavall,e (Chaenomeles maulei).* The present study aimed to investigate the effects of *Chaenomeles maulei* fruit juice (CMFJ) subchronic administration in tests for locomotion, anxiety and depression in healthy rats.

The experimental animals were 32 male healthy Wistar rats divided into four groups of 8 animals: Control, $CMFJ_{2.5}$, $CMFJ_5$ and $CMFJ_{10}$. After 30 days treatment period, the open field test (OFT), social interaction test (SIT) and forced swim test (FST) were carried out. OFT was used for evaluation of locomotor activity – horizontal and vertical. The central time in the OFT and the social interaction time in the SIT were recorded as measures of anxiety. The immobility time in the FST served as a measure of behavioral despair.

All doses of CMFJ produced an elevation in the horizontal activity in comparison with the Control group ($p<0.05$). The vertical activity of CMFJ-treated animals was also increased but not significantly. The administration of CMFJ did not affect significantly the time spent in the central area of the open field and did not produce any significant changes in the social interaction time. In the FST, CMFJ caused a slight, insignificant decrease in the immobility time.

In conclusion, the subchronic CMFJ administration to rats improved the locomotor activity, probably due to its biologically active ingredients. Polyphenols have been shown to modulate the monoaminergic neurotransmission leading to psychomotor activation.

Key words: *Chaenomeles maulei,* polyphenols, behavioral effects, locomotor activity, monoaminergic

INTRODUCTION

Recently the investigational efforts have been directed towards complementary and alternative medicine as sources of better-tolerated components for the treatment of psychiatric disorders. Phytotherapy is the oldest form of treatment. Nowadays, plants are subjects to numerous studies as an alternative of the conventional therapy, exerting a better safety profile. Unützer et al.[1] have shown high rates of use of complementary and alternative medicine among the individuals with mental illnesses.

Of these, depression and neurodegenerative diseases are highly prevalent with a significant impact on the quality of life [2], with oxidative stress suggested as a part of their pathophysiology [3, 4]. Antioxidant effects of polyphenols are well studied. Polyphenols are known to reach sufficient concentrations in the brain and modulate behavioral manifestations, exerting anxiolytic, antidepressant and psychostimulant activity [5]. Polyphenols are the main pharmacological entity found in the fruits of *Chaenomeles japonica var. maulei (Mast.) Lavall,e (Chaenomeles maulei).* The plant has been cultivated in Bulgaria for the last 40 years. It is a shrub, belonging to the *Chaenomeles* genus, originally distributed in China and Japan. The fruits of *Chaenomeles* are traditionally used for their health-promoting and nutritional value. In a comparative study between the *Chaenomeles* species, *Chaenomeles maulei* fruits have shown one of the highest flavonoid concentration and a high total reducing power, indicating an antioxidant capacity [6].

The present study aimed to investigate the effects of *Chaenomeles maulei* fruit juice (CMFJ) administration in tests for locomotion, anxiety and depression in healthy rats.

EXPERIMENTAL

Chaenomeles maulei fruit juice

Fruits from *Chaenomeles maulei* were grown in the Balkan Mountains, Bulgaria, in the region of Troyan. After handpicking, fresh fruits were grinded, crushed and squeezed. The juice was filtered, preserved with potassium sorbate (1.0 g/l) and stored at 0 °C till the experiments. The total content of phenolic compounds was determined by the spectrophotometric Folin-Ciocalteu assay [7]. Absorbance was read at 760 nm. Gallic acid was used as a standard. The spectrophotometric assay showed a very high content of phenolic compounds – 8900.00 mg gallic acid equivalents per liter of juice. The high content of polyphenols was

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confirmed by an HPLC analysis that revealed the presence of phenolic acids and flavonoids. The phenolic acids were presented in the highest concentration by vanillic acid, caffeic acid and chlorogenic acid. From the flavonoids, the most abundant were epicatechin, catechin and quercetin [**8**].

Animals

In the current study, male Wistar rats with a mean weight of 250±30 g were used. The animals were housed in plastic cages in a well ventilated room maintained at 22±1 °C and on a 12/12 light/dark cycle, with a free access to food and drinking water. 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 Agency (Document 141/23.06.2016).

Experimental procedure

The experiments were performed on a total of 32 animals. They were divided in 4 groups of 8 animals each and were treated orally through an orogastric cannula for 30 days. The groups were designated as Control, $CMFJ_{2.5}$, $CMFJ_5$ and $CMFJ_{10}$. Control rats were treated with distilled water (10 ml/kg) once daily. CMFJ was also applied once daily at doses of 2.5 ml/kg diluted to a total volume of 10 ml/kg ($CMFJ_{2.5}$ group), 5 ml/kg diluted to a total volume of 10 ml/kg ($CMFJ_5$ group) and 10 ml/kg ($CMFJ_{10}$ group). After 30 days treatment period, the open field test (OFT), social interaction test (SIT) and forced swim test (FST) were carried out on consecutive days.

Open field test (OFT)

A common method used for assessment of rodent behavior and locomotion is the open field test (OFT) [**9**]. The test was performed on the 30th day, one hour after animal treatment. The equipment for the test included a wooden arena (100 × 100 × 40 cm) painted white with blue lines that divided the floor into 25 equal squares of 20 × 20 cm. The inner squares formed the central area. Each animal was placed in the centre of the open field and was observed for 5 min. The measure of the horizontal activity was the number of lines crossed with the four paws (crossings). The measure of the vertical activity was the number of times the animal stood on its hind limbs (rearings). The increased time spent in the central area (the central time) was an index of reduced anxiety.

Social interaction test (SIT)

The social interaction test was conducted on the 31st day, one hour after animal treatment. According to the method developed by File and Hyde [**10**], all animals were placed under conditions of high light, unfamiliar arena and unknown test partner. The two partners were matched by weight (difference of no more than 10 g). As an experimental equipment, the square arena (100 × 100 × 40 cm) of the open field apparatus was used. Each rat was gently put at the opposite corner of the arena towards the partner. During a period of 5 minutes, the following behaviors were observed and recorded: sniffing, nipping, grooming, following, mounting, kicking, jumping on, and crawling under or over the partner (active interaction). Passive contact (sitting or lying next to each other) was not recorded as social interaction. The prolonged time spent in social interaction showed reduction of anxiety.

Forced swim test (FST)

Developed by Porsolt [**11**], the forced swim test was used to evaluate the immobility of rats as a measure of depressive-like behavior. The procedure was performed one hour after animal treatment on the 32nd and 33rd days in two sessions – test session and experimental session, with a 24 h interval. According to the protocol, each rat was placed in a glass cylinder for 5 min. The cylinder was filled with water (21±1°C), making sure that the animal could not escape or touch the bottom of the cylinder with its hind paws and tail. The immobility time in the experimental session was recorded as the minimal movement required to keep a rat's head above the water. The increased immobility time was a measure of behavioral despair.

Statistical analysis

The results obtained were expressed as mean ± SEM. The data were tested by one-way ANOVA, followed by Dunnett's multiple comparison post test. A level of $p < 0.05$ was considered significant. All analyses were performed using GraphPad Prism statistical software.

RESULTS

Open field test (OFT)

All doses of CMFJ produced a significant elevation in the horizontal activity of rats in comparison with the Control group ($p<0.05$). The number of crossings for the control animals were 27.4±7.2, while those for $CMFJ_{2.5}$ were 55.7±10.7, for $CMFJ_5$ – 68.9±11.7 and for $CMFJ_{10}$ – 53.4±6.8 (Fig.1A).

The vertical activity of CMFJ-treated animals was slightly but not significantly increased in comparison with the control results (Fig.1B).

The administration of CMFJ at different doses did not affect significantly the time spent in the central area of the open firld when compared to the Control (Fig.1C).

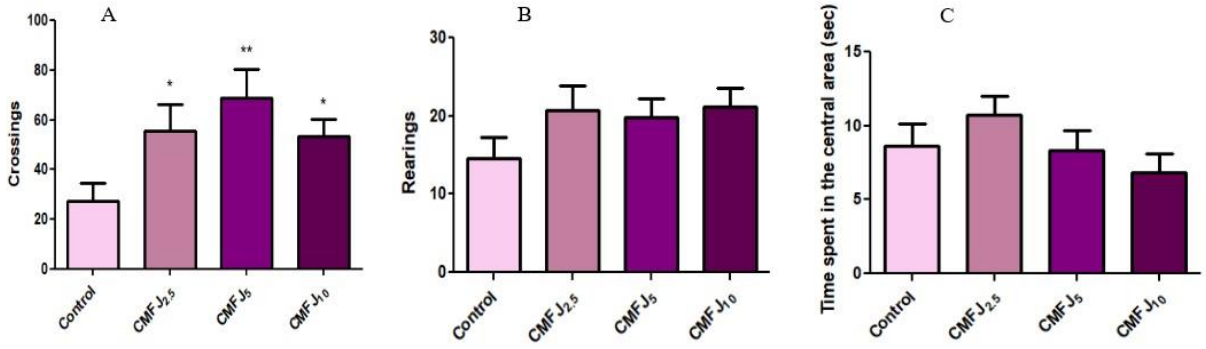

Fig. 1. Effect of subchronic CMFJ administration at doses 2.5, 5 and 10 ml/kg on horizontal (plot A), vertical (plot B) locomotor activity and time spent in the central area (plot C) in the open field test in rats; **p<0.01, *p<0.05 vs. Control.

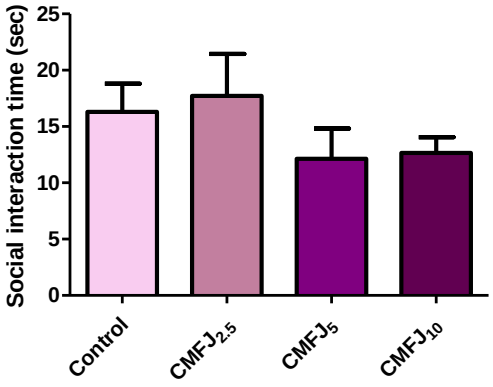

Fig. 2. Effect of subchronic CMFJ administration at doses 2.5, 5 and 10 ml/kg on the social interaction time in rats

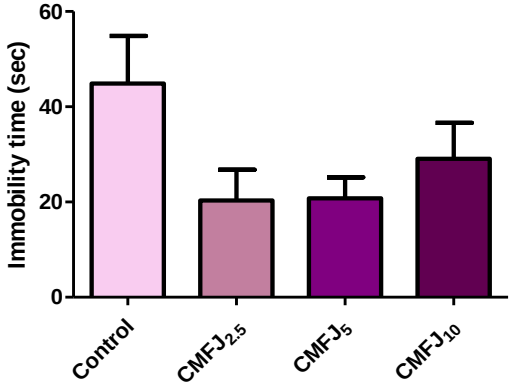

Fig. 3. Effect of subchronic CMFJ administration at doses 2.5, 5 and 10 ml/kg on the immobility time in the forced swim test in rats

Social interaction test (SIT)

In the SIT, the administration of CMFJ did not produce any significant changes in the time spent in social interaction between the partners (Fig.2).

Forced swim test (FST)

In the FST, the doses of CMFJ 2.5 ml/kg and CMFJ 5 ml/kg caused a decrease in the immobility time. For $CMFJ_{2.5}$ group the immobility time was 20.3±6.5 sec and for $CMFJ_5$ group it was 20.8±4.4 sec, while in the control group the results were 44.9±10.0 sec. However, these results did not reach statistical significance in comparison with the Control.

DISCUSSION

The fruit of *Chaenomeles maulei* are a promising source of vitamin C and polyphenolic compounds. The *Chaenomeles* fruits have been traditionally used as an energizing food supplement with a lot of health promoting properties. There is a strong correlation between the phenolic content of a plant and the antioxidant activity [12].

The results from the current investigation showed that CMFJ after subchronic administration to rats significantly increased the horizontal activity in the OFT, especially at the dose of 5 ml/kg. The vertical activity was also increased to a certain extent. This effect might be due to a psychostimulant effect of the juice possibly due to effects on monoamine transporters as target molecules for psychostimulants [13]. This is in agreement with the finding that *Chaenomeles* fruits had a positive effect in rats with Parkinson's disease, which was due to inhibition of the dopamine transporter [14]. *Chaenomeles* extracts as antioxidants were also shown to reduce neuronal cell loss and to possess a neuroprotective effect. The latter was due to

inhibition of toxic ligands transport in the nerve cells [15].

The time spent in the central area of the open field as well as the time spent in social interaction between the test partners in the SIT were not significantly affected by CMFJ which suggested the lack of an anxiolytic effect. In the FST, the immobility time was decreased but not significantly by CMFJ. This result showed that CMFJ did not exert an antidepressant effect. The decrease in the immobility time might be due to the increased locomotor activity, as demonstrated in the OFT.

In conclusion, the administration of CMFJ improved the locomotor activity in rats after subchronic administration, probably due to the interaction with the monoaminergic system leading to psychomotor activation.

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