

ASSESSMENT OF CHAENOMELES MAULEI FRUIT JUICE EFFECTS IN TESTS FOR DEPRESSION AND ANXIETY

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Abstract. *Depression and anxiety are prevalent chronic psychiatric disorders affecting many people worldwide. Over the past decades, much attention has been drawn to herbal psychopharmacology, offering fewer adverse reactions. The main bioactive compounds in Chaenomeles maulei fruits are polyphenols known for their behavioral effects. The objective of the present study was to assess Chaenomeles maulei fruit juice (CMFJ) effects in tests for anxiety and depression. The animals used were 32 male healthy Wistar rats treated orally with CMFJ for 16 days. They were divided in four groups of 8 animals. The control group received distilled water and the rest of the groups were treated with CMFJ at 2.5, 5 and 10 ml/kg doses. We assessed the social interaction time as a measure of anxiety and the immobility time in the forced swim test as a measure of behavioral despair. In the social interaction test, the 14 days administration of CMFJ did not produce any significant changes in the time spent in social interaction. After 16 days of administration all doses of CMFJ significantly decreased the immobility time of the rats ($p < 0.05$) in comparison to the control group. These results allow making the conclusion that CMFJ lacked anxiolytic activity, but showed an antidepressant-like effect.*

Key words: *anxiety, depression, forced swim test, Chaenomeles maulei, polyphenols*

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INTRODUCTION

Depression is a chronic psychiatric disorder manifesting with lowered pleasure, mood and interest. It is a common and recurring disease, affecting negatively a person's lifestyle. Depression is considered one of the main reasons of disability worldwide and is highly resistant to treatment [1]. Anxiety is a common symptom of many psychiatric disorders as well as other medical conditions [2]. Worldwide, one in five people at least once in their lives have met with clinical criteria of anxiety disorder [3]. By the year

2030, depression is predicted to account for the second greatest increase in morbidity after cardiovascular diseases. Depression can be diagnosed as "major depression" which comprises numerous symptoms including anxiety. Important mechanisms in the pathogenesis of depression are impairment of the monoamine transporters, decrease in their production or secondary messenger systems dysfunction. Recently, some attention has been drawn to the role of hypothalamus-pituitary-adrenal dysfunctions, changes in glutamatergic transmission, impaired opioid function and abnormal circadian rhythm [4, 5].

Although there are many highly effective antidepressants available, there is an increasing interest in natural antidepressants with fewer side effects. Most of the phytodrugs are “over-the-counter” products with a complex mechanism of action, involving a multitude of biological responses. In the majority of cases, the medicinal plants with antidepressant action affect the reuptake mechanism and receptor binding of monoamines, in addition to some endocrine, antioxidant, neuro-immunological modulation [6]. Some herbal drugs with antidepressant activity, which have undergone preclinical trials, contain mainly polyphenols and triterpenes as components exerting pharmacological effects [7].

Chaenomeles is a genus of shrubs from the *Rosaceae* family. Native to Japan, Korea, China, Bhutan and Burma, the plants are related to the quince (*Cydonia oblonga*) and the Chinese quince (*Pseudocydonia sinensis*). The plants are widely applied in the traditional medicine of those countries due to their diverse effects. An investigation, aiming to elucidate the main bioactive principles of *Chaenomeles* species, showed that polyphenols, especially flavan-3-ols, were highly abundant. It has been observed that the antioxidant activity of the *Chaenomeles* fruits correlated to their total polyphenol contents [8]. *Chaenomeles maulei* has recently been introduced to the Bulgarian market in the form of a bio juice. The main bioactive compounds in *Chaenomeles maulei* fruits are polyphenols, such as flavonoids and phenolic acids, but also high amounts of vitamin C [9].

Polyphenols have been investigated and proven protective against a wide range of diseases including psychiatric disorders. Some of them cross the blood-brain barrier in concentrations high enough to exert an effect. Their brain penetration depends on their lipophilicity, interactions with transport proteins, like P-glycoprotein and stereochemistry [10]. Animal studies have demonstrated that polyphenols cross the blood-brain barrier independently of their route of administration [11]. Polyphenols have been shown to induce behavioral effects such as improved working memory, mood and attention [12]. The neuroprotective effects of CMFJ have not been studied yet.

Ginkgo biloba extract, which is rich in flavonoids, improved brain activity, short-term memory, learning and concentration in animals suffering from cognitive impairment [13]. *Aronia melanocarpa* fruit juice, containing polyphenols as bioactive components, dose-dependently decreased the immobility time in the forced swim test [14]. This suggested an antidepressant-like effect. Some of the mechanisms, related to the positive effects of polyphenols on mood and cognition, include a reduction in the levels of free radicals [15],

increase in cerebral blood flow, interaction with receptors, ion channels and growth hormones, increase in the levels of norepinephrine, dopamine and 5-HT in prefrontal cortex, hypothalamus and striatum [16].

The neurobehavioral properties of polyphenols are well-studied, but the biological activity of CMFJ has not been examined yet.

AIM OF THE STUDY

The aim of the present study was to investigate the effect of *Chaenomeles maulei* fruit juice on rats' behavior in the forced swim test and social interaction test.

MATERIAL AND METHODS

Animals

The experimental animals were healthy male Wistar rats with a mean weight of 250 ± 30 g. During the experiment, 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. They had 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).

Chaenomeles maulei fruit juice (CMFJ)

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 [17]. Absorbance was read at 760 nm. Gallic acid was used as a standard. The total content of phenolic compounds was 8900.00 mg gallic acid equivalents per liter of juice.

Experimental procedure

The experiments were carried out on 32 male healthy Wistar rats. They were divided in 4 groups of 8 animals each and were treated orally through an orogastric cannula. The animals were allocated in the following groups: Control, CMFJ_{2.5}, CMFJ₅ and CMFJ₁₀. The Control group was treated with distilled water (10 ml/kg). The rest of the groups received CMFJ at doses of 2.5, 5 and 10 ml/kg once a day. The doses of 2.5 and 5 ml/kg were diluted with distilled water to a total volume of 10 ml/kg. The doses were chosen on the basis of

previous experiments which showed that *Aronia melanocarpa* fruit juice at these doses produced relevant central nervous system effects in rats [14, 18]. The administration of the juice lasted for 16 days.

Social interaction test

The social interaction test is a well-known test to study the state of anxiety [19]. In this test, the dependent variable is the time spent in social interaction between partners. Each rat was tested for social interaction with an unknown test partner that did not differ by more than 10 g in weight. Both members of a pair had the same drug treatment. The square arena (100 × 100 × 40 cm) of the open field apparatus was used as a test box. The rats were gently placed at the opposite corners of the arena. The following behaviors were observed and scored during a 5 min session: sniffing, nipping, grooming, following, mounting, kicking, jumping on and crawling under or over the partner. The passive contact (sitting or lying with bodies in contact) was not included in the social interaction score [19]. A decrease in social interaction, without a concomitant decrease in locomotor activity, is defined as a specific anxiogenic effect; an increase in social interaction, without an increase in locomotor activity, is defined as a specific anxiolytic effect [20]. The test was performed on the 14th day, 60 min after CMFJ administration.

Forced swim test

We assessed the immobility time of rats as a measure of behavioral despair in the forced swim test [21]. It is considered an adequate test for screening of antidepressants. It produces a mental condition of hopelessness, reflecting the human depressed state, in which the immobility is a direct reaction to the test itself and does not persist outside the test situation. The animals are tested one by one in a transparent plexiglas cylinder (17 cm in diameter and 60 cm in height) containing water (22 ± 1 °C). The cylinders were filled so that there was left space at the top, not allowing the animals to escape. The test was performed in two sessions (training and test) separated by 24 hours. In the training session, on the 15th experimental day, one hour after CMFJ treatment, an animal was dropped down in water and its activity was documented for 5 min. The test

session was conducted on the 16th day, 60 min after the CMFJ administration and immobility time was recorded. It was the time spent floating passively or just making those movements necessary to maintain the animal's head above water [22].

Statistical analysis

Results are presented as mean ± S.E.M. The data were tested by one-way ANOVA, followed by a Dunnett's Multiple Comparison Post Test. GraphPad Prism statistical software was used to perform all analyses. A level of $p < 0.05$ was considered significant.

RESULTS

Social interaction test (SIT)

In the SIT, the time spent in social contacts between the partners was not significantly affected after 14 days of CMFJ administration. The social interaction time of the CMFJ_{2.5} group was slightly decreased compared to the Control group and elevated in CMFJ₅ and CMFJ₁₀ groups (Table 1). The analysis of the results showed a dose-dependent trend towards increasing the interaction time (Fig. 1).

Table 1. Effect of CMFJ on the time spent in social interaction in the social interaction test (SIT) and the immobility time in the forced swim test (FST) in rats; * $p < 0,05$ vs. Control

| Test \ Group | Control group | CMFJ _{2.5} | CMFJ ₅ | CMFJ ₁₀ |
|---|---------------|---------------------|-------------------|--------------------|
| Time spent in social interaction in the SIT (sec) | 17.89 ± 2.3 | 15.71 ± 1.31 | 17.58 ± 2.75 | 18.66 ± 3.19 |
| Immobility time in the FST (sec) | 66.38 ± 8.43 | 41.00 ± 5.17* | 37.88 ± 8.34* | 39.5 ± 5.61* |

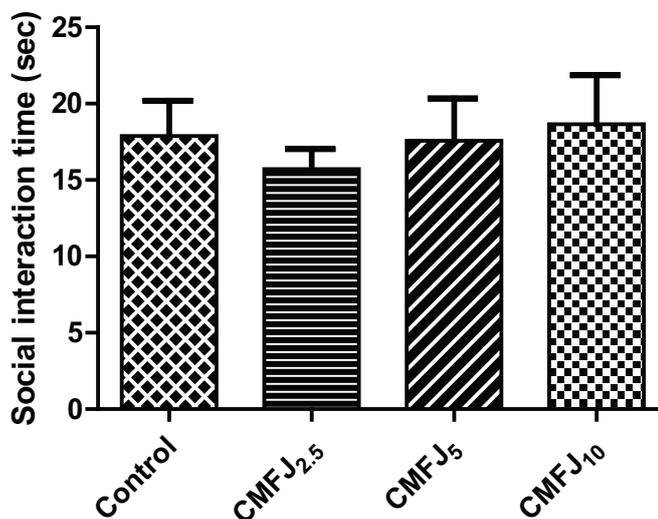


Fig. 1. Effect of CMFJ administered for 14 days on the time spent in social interaction of rats. Results are presented as mean ± S.E.M.; n = 8

Forced swim test (FST)

After 16 days of administration, all doses of CMFJ significantly decreased ($p < 0.05$) the immobility time of the treated rats in comparison with the Control group. The immobility time durations of the CMFJ_{2.5} group, CMFJ₅ group and CMFJ₁₀ group were respectively 61.7% of the control time, 57.0% of the control time and 59.5% of the control time (Table 1). Thus, applied for 16 days, CMFJ at all treatment doses showed a significant decrease in the immobility time in the FST (Fig. 2).

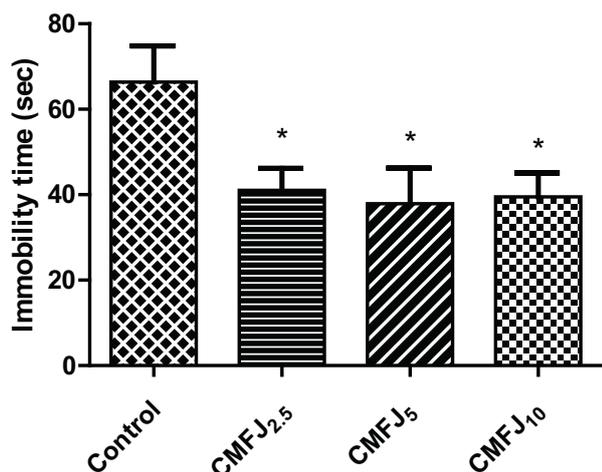


Fig. 2. Effect of CMFJ administered for 16 days on the immobility time of rats in the FST. Results are presented as mean \pm S.E.M.; $n = 8$, * $p < 0,05$ vs. control

DISCUSSION

Depression and anxiety are the predominant neuropsychiatric disorders. The SIT, developed by File and Hyde [19], relies on a rodent's natural tendency to avoid bright light and unfamiliar surroundings. Animals placed in a novel or brightly lit chamber exhibit decreased social interaction [23]. Anxiolytic agents increase and anxiogenic agents decrease the social contact between two unfamiliar rats in a new and unfamiliar environment [24]. In the SIT, the 14 days administration of CMFJ did not produce any significant changes in the time spent in social interaction. This could be interpreted as a lack of an anxiolytic effect.

FST, developed by Porsolt and colleagues [21], is established as one of the most widely used tests for assessing antidepressant-like activity in rats, due to its reliability, specificity and ease of use [25]. The forced swim test is based on the assumption that immobility is a measure of behavioral despair [26]. The results from the present study showed that 16 days of administration of CMFJ at all doses shortened significantly the immobility time in the FST. These re-

sults are consistent with the statements that some polyphenol-rich plants produce antidepressant-like effects. This may be due to their antioxidant activity and altered monoaminergic responses [27].

Antidepressants and stimulants may produce similar effects in the FST. The shortening of the immobility time might be due to either an antidepressant effect, or an increased locomotor activity. In a previous study (unpublished data), we found that CMFJ at a dose of 5 ml/kg significantly increased both the horizontal and vertical movements in the open field test, suggesting both CNS and locomotor stimulation. The dose of CMFJ 10 ml/kg did not affect significantly the locomotor activity. At the same time in the FST that dose decreased significantly the immobility time. This finding allows making the conclusion that CMFJ showed an antidepressant-like effect in the present experiment.

In the treatment of depression and anxiety, the use of naturally occurring antioxidants like polyphenols may be highly beneficial. They are the main bioactive compounds found in *Chaenomeles maulei* fruits. They are capable of crossing the blood-brain barrier and exert antidepressant effects [28]. Some bioactive substances from plant origin are capable of alleviating depression-like behavior by restoring the levels of serotonin, norepinephrine and dopamine in the brain [29]. Some antidepressants potentiate the responses produced by dopamine receptors stimulation, just like reward-related behaviors. Zhao et al. [30] established that *Chaenomeles speciosa* fruits dose-dependently inhibited the transporters for dopamine and norepinephrine reuptake, which possibly mediated antiparkinsonian-like effects. The inhibition of norepinephrine and dopamine reuptake might account for the antidepressant-like effects of CMFJ in this experiment. Polyphenols were shown to delay the initiation of and/or slow the progression of neurodegenerative disorders, including a potential to inhibit neuronal apoptosis triggered by neurotoxic species (e.g., oxidative stress and neuroinflammation) [31]. They possess a potent antioxidant capacity [32]. These findings might also be a part of the complex mechanism of action of CMFJ components.

CONCLUSION

In conclusion, CMFJ decreased the immobility time in the FST after 16 days of treatment and did not change the time spent in social interaction in the SIT. This might be interpreted as a probable antidepressant-like effect and a lack of an anxiolytic activity.

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Conflict of interest

The authors have no conflict of interest to declare.

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