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EFFECT OF *ARONIA MELANOCARPA* FRUIT JUICE ON BEHAVIOR OF RATS EXPOSED TO SOCIAL ISOLATION

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ABSTRACT
PURPOSE: The present study investigated the effects of *Aronia melanocarpa* fruit juice (AMFJ) on the behavior of male Wistar rats exposed to social isolation (SI). MATERIAL AND METHODS: Experimental rats were divided into 5 groups (n = 12): Control, SI, SI+AMFJ$_5$, SI+AMFJ$_{10}$, SI+D. Control rats were housed in groups of 6 while the isolated animals were in individual cages. After the first 4 weeks of isolation, for the next 4 weeks the animals were treated daily orally with water (Control and SI), or AMFJ at a dose of 5 ml/kg (SI+AMFJ$_5$) and 10 ml/kg (SI+AMFJ$_{10}$), or Diazepam 0.1 mg/kg (SI+D). After the 8 weeks experimental period, open field test (OFT), social interaction test (SIT) and forced swim test (FST) were carried out. RESULTS: SI caused a tendency to decrease locomotor activity in the OFT and the treatment with AMFJ and Diazepam did not significantly affect this tendency. In the SIT, the interaction time was significantly longer between animals exposed to SI while the behavior of isolated rats treated either with AMFJ or Diazepam was similar to that of the controls. In the FST, SI caused a tendency to increase immobility time. AMFJ and Diazepam prevented that effect and the immobility time of SI+AMFJ$_{10}$ was significantly lower than that of SI group. CONCLUSION: AMFJ showed an antidepressant-like effect in socially isolated rats.

Key words: *Aronia melanocarpa*, behavior, social isolation, rats

INTRODUCTION

Mammalians form social organizations and communication between members is very important for their survival. Isolation may be a powerful stressor for rodents. Numerous studies have demonstrated that in rodents, isolation rearing remarkably impairs cognitive and emotional functions (1, 2) and induces anxiety and depression-like behavior (3).

Aronia melanocarpa (black chokeberry) fruits are used for human consumption as juice, syrup, jam, and wine. They are extremely rich in polyphenolic substances – proanthocyanidins, phenolic acids and flavonoids from the subclass of anthocyanins. Previous studies have shown that *Aronia melanocarpa* fruit juice has anxiolytic and antidepressant-like effects in rats (4, 5).

The purpose of the present study was to investigate the effects of *Aronia melanocarpa* fruit juice (AMFJ) on the behavior of male Wistar rats exposed to social isolation.

MATERIALS AND METHODS

Experimental substances

AMFJ was produced from *Aronia melanocarpa* Elliot fruits using a juice centrifuge. The juice was filtered, sterilized for 10 min and stored at 0 °C till the experiment.

The contents of phenolic substances in 100 ml AMFJ were: total phenolics, 546.1 mg as gallic acid equivalents, determined spectrophotometrically according to the Folin-Ciocalteu procedure (6); total proanthocyanidins, 312.3 mg, determined by gravimetric isolation

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according to the procedure described by Howell et al. (7); phenolic acids (chlorogenic – 58.5 mg, neochlorogenic – 83.0 mg) determined by a high-performance liquid chromatography (HPLC) method at wavelength of λ=280 nm; anthocyanins (cyanidin-3-galactoside – 14.4 mg, cyanidin-3-arabinoside – 6.2 mg, cyanidin-3-xyloside – 1.2 mg and cyanidin-3-glucoside – 0.4 mg) determined by HPLC at wavelength of λ=520 nm. Agilent 1220 HPLC system (Agilent Technology, Palo Alto, Ca) was used.

Diazepam solution 5 mg/ml (Sopharma, Bulgaria) was used in the experiment.

Animals

Male Wistar rats (200 ± 20 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. They had access to food and drinking water ad libitum. All procedures concerning animal treatment and experimentation were conducted in compliance with National and International laws and policies (EEC Council Directive 86/609).

Experimental procedure

The rats were divided into 5 groups of 12 animals each: Control, SI (socially isolated), $SI+AMFJ_5$, $SI+AMFJ_{10}$, SI+D. For a period of 8 weeks, control rats (Control) were housed in groups of 6 while the isolated animals were in individual cages. After the first 4 weeks, for the next 4 weeks the animals were treated daily orally through an orogastric cannula. Rats from Control and SI groups received distilled water (10 ml/kg). Animals from $SI+AMFJ_5$ and $SI+AMFJ_{10}$ were treated with AMFJ at doses of 5 ml/kg (diluted with water to a total volume of 10 ml/kg) and 10 ml/kg, respectively. Diazepam 0.1 mg/kg was administered as a water solution with a volume of 10 ml/kg (SI+D). After the experimental period, behavioral tests were carried out one after the other.

Open field test (OFT)

OFT is a common measure of exploratory behavior and general activity in rodents (8). It was performed for 5 min in an arena (100 × 100 × 40 cm) painted white except for 6 mm blue lines that divided the floor into 25 equal size (20 × 20 cm) squares. Behaviors recorded were: crossings (the number of lines crossed with the four paws) and rearings (the number of times the animal stood on its hind limbs).

Social interaction test (SIT)

Rats were tested according to the method developed by Sandra and Hyde (9) under conditions of high light, unfamiliar arena and unknown test partner to create a high level of anxiety. The two partners were matched by weight (difference of no more than 10 g). 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. Passive contact (sitting or lying next to each other) was not considered as social interaction. The longer time for social contacts showed lower degree of anxiety.

Forced swim test (FST)

The method of Porsolt et al. (10) was used to asses the immobility of the rats as a measure of their depressive-like behavior. Each rat was placed in a glass cylinder pool (17 cm in diameter and 60 cm in height) for 5 min. The cylinder was filled with 30 cm water (21±1°C) to ensure that the animal could not touch the bottom of the cylinder with its hind paws or its tail. The test was performed in two sessions with a 24 h interval. The results from the second session were recorded.

Statistical analysis

Results are presented as mean±S.E.M. The data were tested by one-way ANOVA, followed by Dunnett's multiple comparison post test to identify significant difference. All analyses were performed using GraphPad Prism statistical software. A level of $p<0.05$ was considered significant.

RESULTS

Open field test (OFT)

SI caused a tendency to decrease horizontal **(Figure 1A)** and vertical **(Figure 1B)** locomotor activity: from 50.7±8.6 and 22.2±2.9 for the control group to 35.7±6.8 (70.4%) and 17.1±2.1 (77.7%) for the SI group, respectively. In AMFJ- and Diazepam-treated animals, locomotor activity was further slightly decreased but it was not significantly different from that of the SI group and from the control group with the exception of the vertical activity of SI+D rats which was significantly lower than that of the control group ($p<0.05$).

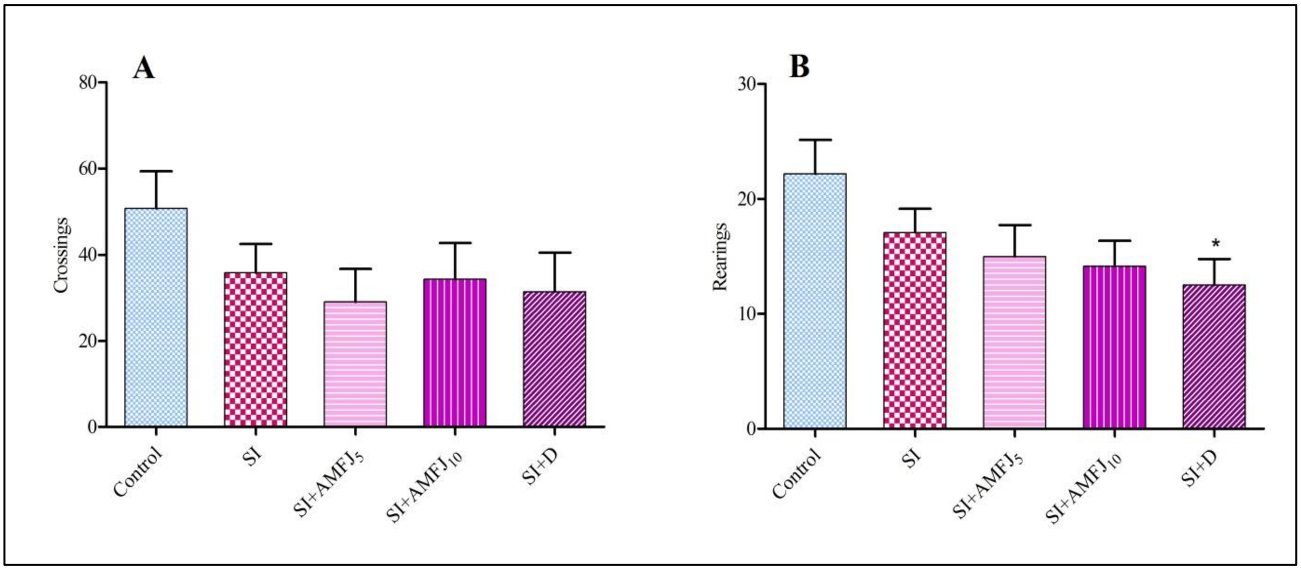

Figure 1. Effect of AMFJ and Diazepam on horizontal (panel A) and vertical (panel B) locomotor activity in the open field test in rats exposed to social isolation; *p<0.05 vs. Control

Social interaction test (SIT)

In the SIT, the time of social contacts between animals from SI group (24.7±4.5 sec) was significantly higher (p<0.01) than the control time (9.3±1.3 sec). The behavior of isolated rats treated either with AMFJ or Diazepam was similar to that of the controls and thus, the social interaction time in these groups (17.7 for SI+AMFJ$_5$; 11.1±2.1 sec for SI+AMFJ$_{10}$; 9.9 ±2.9 sec for SI+D) was not significantly different from the control one **(Figure 2A)**.

Forced swim test (FST)

Social isolation caused a tendency to increase immobility time from 51.7±6.7 sec for the control animals to 62.9±6.6 sec for the SI group. AMFJ and Diazepam treatment antagonized that tendency and the immobility time of SI+AMFJ$_{10}$ (38.9±4.0 sec) was significantly lower (p<0.05) than that of the SI group **(Figure 2B).**

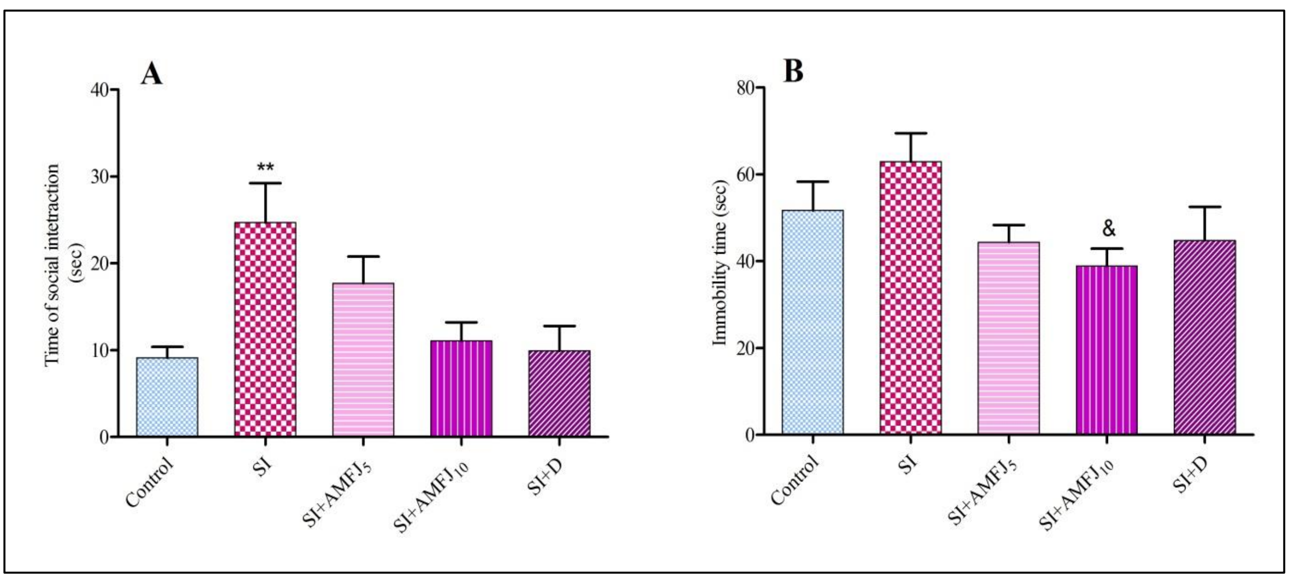

Figure 2. Effect of AMFJ and Diazepam on time spent in social interaction in the social interaction test (panel A) and on the immobility time in the forced swim test (panel B); **p<0,01 vs. Control, $^{\&}$p<0,05 vs. SI

DISCUSSION

AMFJ is a rich source of polyphenols. There are literature data that polyphenols from berries are able to traverse the blood-brain barrier and accumulate in the brain after long-term consumption (11).

The OFT showed a tendency of locomotor activity reduction in isolated animals. In AMFJ- and Diazepam-treated animals, locomotor activity was further slightly decreased, the effect being more pronounced with Diazepam which can be attributed to the sedative effect of the drug.

As social isolation is a kind of chronic stress we could expect an increased state of anxiety and respectively reduced social interaction time between the isolated animals. Contrary to this, in the present experiment social isolation caused a significant increase in the social contacts between the test partners from the SI group. We could suppose that the continuous isolation of animals increased the interest to the test partner. Results from SIT suggest that this test might not be appropriate to study anxiety behavior in isolated rats. In the SIT the rats treated either with AMFJ or Diazepam behaved like the control animals. So, the treatments counteracted the effects of social isolation.

Consistent with the results of Djorjevic et al. (3), in the present experiment social isolation induced a tendency to increase the immobility time in the FST. Treatment with AMFJ and Diazepam counteracted that effect of social isolation on the immobility time. The highest was the effect of AMFJ at the dose of 10 ml/kg. On the basis of these results, we could suppose that AMFJ possessed antidepressant-like effect in isolated rats. This effect of AMFJ could be attributed to its polyphenolic ingredients. Studies in animal models of depression have established that polyphenols reduce oxidative stress in the brain, modulate monoaminergic neurotransmission and reduce the non-adaptive responses to stress.

CONCLUSION

In the present study, the subchronic administration of AMFJ resulted in an antidepressant-like effect in socially isolated rats. This effect might be due to the polyphenolic ingredients of the juice.

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