

ANTI-ANXIETY EFFECT OF *ARONIA MELANOCARPA* FRUIT JUICE ADMINISTERED SUBCHRONICALLY TO RATS

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Abstract

The aim of the present study was to investigate the effect of *Aronia melanocarpa* fruit juice (AMFJ) on anxiety in male Wistar rats. AMFJ was administered orally for periods of 7, 14, 21 and 30 days at doses of 2.5, 5 and 10 mL/kg. The state of anxiety was evaluated using the elevated plus-maze test. For the periods of 7 and 14 days, AMFJ induced a dose-dependent tendency to increase the number of entries into the open arms, the time spent there, the ratio of open arm entries/total entries, and the ratio of time spent in the open arms/total time. For the 21- and 30-day treatment periods, the anti-anxiety effect of AMFJ at doses of 2.5 and 5 mL/kg was not statistically significant, while the dose of 10 mL/kg significantly increased the number of entries into the open arms, the time spent there and the ratio of open/total arm entries, and decreased the time spent in the closed arms in comparison with the saline-treated controls. The findings from the present study suggested a dose- and time-dependent anti-anxiety effect of AMFJ. As polyphenols are the main bioactive substances in the juice, we could conclude that most probably they are responsible for the anti-anxiety effect of AMFJ applied subchronically to rats.

Rezumat

Scopul acestui studiu a fost de a investiga efectul sucului fructelor de *Aronia melanocarpa* (AMFJ) asupra anxietății la șobolani masculi Wistar. AMFJ a fost administrat oral pentru perioade de 7, 14, 21 și 30 de zile la doze de 2,5, 5 și 10 mL/kg. Starea de anxietate a fost evaluată folosind testul labirintului suspendat „elevated plus-maze”. După 7 și 14 zile, AMFJ a determinat un efect dependent de doză prin creșterea numărului de intrări în brațe deschise, prin timpul petrecut acolo, raportul dintre intrările în brațe deschise/intrări totale și raportul dintre timp petrecut în brațe deschise/timp total. După 21 și 30 de zile, efectul anxiolitic al AMFJ la doze de 2,5 și 5 mL/kg nu a fost statistic semnificativ în timp ce la doze de 10 mL/kg a crescut semnificativ numărul de intrări în brațe deschise, timpul petrecut acolo și raportul dintre deschideri/intrări totale în brațe și a scăzut timpul de petrecut în brațe închise în comparație cu martorii tratați cu ser fiziologic. Rezultatele din studiul de față sugerează o dependență de doză și de timp a efectului anxiolitic al AMFJ. Deoarece polifenolii sunt principalele substanțe bioactive din suc, am putea concluziona că, cel mai probabil, aceștia sunt responsabili pentru efectul anxiolitic al AMFJ administrat subcronic la șobolani.

Keywords: anti-anxiety effect, plus-maze, *Aronia melanocarpa* fruit juice, rats

Introduction

Aronia melanocarpa (Michx) Elliot (black chokeberry) is a woody shrub of the *Rosaceae* family. Its fruits are extremely rich in phenolic substances. The procyanidins are the phenolic compound group with the highest concentration in chokeberry fruits [6]. The anthocyanins are the second phenolic compound group in *Aronia melanocarpa* fruits. The chokeberry fruits contain also two phenolic acids: chlorogenic and neochlorogenic acids [6] and a mixture of five quercetin glycosides [19].

Recently, research has been conducted to investigate effective natural anxiolytic (anti-

anxiety) treatments with a low risk of adverse effects [3, 10, 13]. From the studies of CNS-active chemical constituents of medicinal plants, polyphenolic substances such as flavonoids [22] and chlorogenic acid [5] have been demonstrated to possess anxiolytic-like effects. Our previous experiments showed that *Aronia melanocarpa* fruit juice administered as a single dose had an anxiolytic-like effect in rats which was investigated in the social interaction test [21].

The aim of the present study was to investigate whether subchronic oral treatment with *Aronia melanocarpa* fruit juice (AMFJ) could affect anxiety in rats using the elevated plus-maze test.

Materials and Methods

AMFJ was produced from *Aronia melanocarpa* (Michx.) Elliot fruits grown in the Balkan Mountains, Bulgaria, in the region of Troyan. They were handpicked in September, crushed and squeezed. The juice was filtered, pasteurized at 80°C for 10 min and stored at 0°C till the experiment was performed. The contents of phenolic substances in 100 mL AMFJ were: total phenolics, 709.3 ± 28.1 mg as gallic acid equivalents, determined spectrophotometrically according to the Folin-Ciocalteu procedure [18]; total flavonoids, 189.4 ± 8.6 mg as catechin equivalents, measured by a colorimetric assay developed by Zhishen and co-workers [25]; total anthocyanins, 106.8 ± 6.2 mg as cyanidin-3-glucoside equivalents, determined by pH-differential spectrophotometry at pH 1.0 and pH 4.5 [8]; quercetin, 11.8 ± 0.8 mg, measured by a high-performance liquid chromatography method [9]. The values were the mean of duplicate determinations of three samples.

Animals and treatment. Male Wistar rats (200-240 g at the beginning of the experiments) were housed in polypropylene boxes, with free access to food and drinking water. The experiments were carried out according to the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences, in compliance with the national policies and the EEC Directive of 1986 (86/609/EEC).

The animals were treated orally through an oro-gastric cannula during 7, 14, 21 and 30 days of the performed experiment. Four independent groups of 10 rats were used for each treatment period. The groups were treated as follows: Control – distilled water (10 mL/kg), AMFJ_{2.5} – AMFJ at a dose of 2.5 mL/kg diluted with distilled water to a total volume of 10 mL/kg, AMFJ₅ – AMFJ at a dose of 5 mL/kg diluted with distilled water to a total volume of 10 mL/kg, AMFJ₁₀ – AMFJ at a dose of 10 mL/kg. The last dose of AMFJ was administered 30 min before the test session. In the experiment was not used a group treated with anxiolytic drugs (positive control).

Elevated plus-maze. The experiments for the state of anxiety were carried out according to the method described by Pellow *et al.* [17]. The wooden elevated plus-maze consisted of two open arms (50 x 10 cm) facing each other, and two closed arms (50 x 50 x 10 cm) with an open roof. The apparatus was elevated 50 cm above the floor and was illuminated by a 40 W bulb, positioned 50 cm above the apparatus. Each rat was then placed in the centre of the plus-maze facing one of the open arms. The following measurements were taken by an observer during a 5-min test period after the rat had been placed in the centre of the maze: the number of entries into the open arms (EOA) and the time spent in the open arms (TOA), the number of entries into the closed

arms (ECA) and the time spent into the closed arms (TCA), the total number of arm entries (the entries into the open arms and the entries into the closed arms) (TE), the ratio of the number of entries into the open arms/ total number of entries (EOA/TE) and the ratio of time spent in the open arms/total time (TOA/TT). Before each test, the maze was wiped off with a cleaning solution and dried. Experiments were performed between 9.00 h and 13.00 h.

Statistical analysis. Separate one way ANOVA was used to analyse the experimental data for each treatment period. ANOVA data were further analysed by Dunnett's multiple comparison post hoc test. All analyses were performed using GraphPad Prism statistical software.

Results and Discussion

The elevated plus-maze data showed that AMFJ for the periods of 7 and 14 days dose-dependently tended to increase the number of open arm entries (Figure 1), the time spent in the open arms (Figure 2), the ratio of the number of entries into the open arms/total number of arm entries and the ratio of time spent in the open arms/total time (Table I). These effects were not statistically significant.

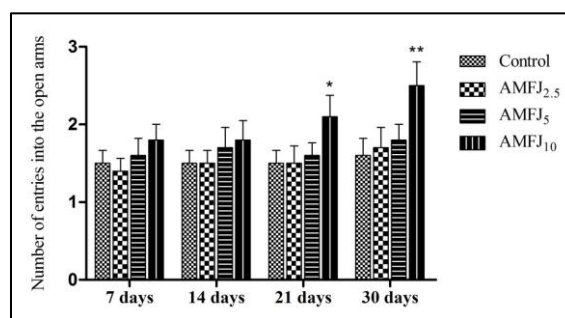


Figure 1.

Effect of AMFJ administered for 7, 14, 21 and 30 days at doses of 2.5 mL/kg (AMFJ_{2.5}), 5 mL/kg (AMFJ₅) and 10 mL/kg (AMFJ₁₀) on the number of entries into the open arms of the elevated plus-maze.

Results are mean ± SEM, n = 10. *p ≤ 0.05 vs. Control; **p ≤ 0.01 vs. Control

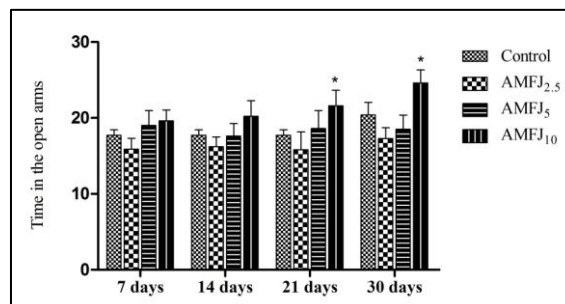


Figure 2.

Effect of AMFJ administered for 7, 14, 21 and 30 days at doses of 2.5 mL/kg (AMFJ_{2.5}), 5 mL/kg (AMFJ₅) and 10 mL/kg (AMFJ₁₀) on the time spent in the open arms of the elevated plus-maze.

Results are mean ± SEM, n = 10. *p ≤ 0.05 vs. Control

Table I

Behaviour variables of rats treated for 7 and 14 days with AMFJ at doses of 2.5 mL/kg (AMFJ_{2.5}), 5 mL/kg (AMFJ₅) and 10 mL/kg (AMFJ₁₀). Values are mean \pm SEM, n = 10.

Days	Variable	Control	AMFJ _{2.5}	AMFJ ₅	AMFJ ₁₀
7	ECA	4.90 \pm 0.55	4.60 \pm 0.56	4.70 \pm 0.42	4.00 \pm 0.47
	TCA (sec)	282.3 \pm 0.73	284.1 \pm 1.40	281.0 \pm 1.97	280.4 \pm 1.43
	TE	6.4 \pm 0.83	6.0 \pm 0.79	6.3 \pm 0.81	5.80 \pm 0.77
	EOA/TE	0.25 \pm 0.03	0.25 \pm 0.04	0.26 \pm 0.03	0.32 \pm 0.04
	TOA/TT	0.06 \pm 0.002	0.05 \pm 0.005	0.06 \pm 0.007	0.07 \pm 0.005
14	ECA	4.9 \pm 0.55	4.7 \pm 0.56	4.3 \pm 0.58	3.9 \pm 0.31
	TCA (sec)	282.3 \pm 0.73	283.8 \pm 1.30	282.4 \pm 1.66	279.8 \pm 2.09
	TE	6.4 \pm 0.83	6.20 \pm 0.85	6.0 \pm 0.83	5.70 \pm 0.68
	EOA/TE	0.25 \pm 0.03	0.26 \pm 0.03	0.30 \pm 0.05	0.32 \pm 0.04
	TOA/TT	0.06 \pm 0.002	0.05 \pm 0.4	0.06 \pm 0.006	0.07 \pm 0.007

ECA – number of entries into the closed arms; TCA – time spent in the closed arms; TE – total number of arm entries; EOA/TE – the ratio of the number of entries into the open arms/total number of entries; TOA/TT – the ratio of time spent in the open arms/total time.

Administered for 21 days at doses of 2.5 mL/kg and 5 mL/kg, AMFJ had no significant effect on anxiety. The dose of 10 mL/kg applied for 21 days significantly increased the number of entries into the open arms ($t = 1.857$, $p \leq 0.04$) (Figure 1), prolonged the time spent in the open arms ($t = 1.787$, $p \leq 0.04$) (Figure 2), increased the ratio of the number of entries into the open arms/total number of entries ($t = 1.949$, $p \leq 0.03$) (Table II), and did not significantly influence the time spent in the closed arms, the number of entries into the closed arms as well as the total number of arm entries in comparison with the saline-treated controls (Table II).

ANOVA analysis of the elevated plus-maze data on the 30th day revealed that the factor dose of AMFJ was significant for the number of entries into the open arms ($F_{3,39} = 2.659$, $p \leq 0.05$) (Figure 1), the time spent in the open arms ($F_{3,39} = 3.649$, $p \leq 0.02$), the time spent in the closed arms ($F_{3,39} = 3.649$, $p \leq 0.02$) (Table II), and was not significant for the number of

entries into the closed arms ($F_{3,39} = 0.910$, $p \leq 0.445$), the total number of entries ($F_{3,39} = 0.906$, $p \leq 0.447$), and the ratio of the number of entries into the open arms/total number of arm entries ($F_{3,39} = 1.766$, $p \leq 0.171$) (Table II).

On the 30th day, the post-hoc comparisons showed that the doses of 2.5 and 5 mL/kg were without a significant effect on the state of anxiety. The dose of 10 mL/kg on the 30th day significantly increased the number of entries into the open arms ($t = 2.377$, $p \leq 0.01$) (Figure 1), prolonged the time spent in the open arms ($t = 1.753$, $p \leq 0.04$) (Figure 2), increased the ratio of the number of entries into the open arms/total number of entries ($t = 1.701$, $p \leq 0.05$), shortened the time spent in the closed arms ($t = 1.753$, $p \leq 0.04$) (Table II), and did not significantly affect the number of entries into the closed arms as well as the total number of arm entries in comparison with the saline-treated controls (Table II).

Table II

The behaviour variables of rats treated for 21 and 30 days with AMFJ at doses of 2.5 mL/kg (AMFJ_{2.5}), 5 mL/kg (AMFJ₅) and 10 mL/kg (AMFJ₁₀). Values are mean \pm SEM, n = 10. * $p \leq 0.05$ vs. Control; ** $p \leq 0.01$ vs. Control.

Days	Variable	Control	AMFJ _{2.5}	AMFJ ₅	AMFJ ₁₀
21	ECA	4.9 \pm 0.55	4.8 \pm 0.39	4.6 \pm 0.62	3.9 \pm 0.57
	TCA (sec)	282.3 \pm 0.73	284.2 \pm 2.36	281.4 \pm 2.37	278.4 \pm 2.06
	TE	6.4 \pm 0.83	6.3 \pm 0.77	6.2 \pm 0.88	6.0 \pm 0.83
	EOA/TE	0.25 \pm 0.03	0.27 \pm 0.04	0.28 \pm 0.03	0.37 \pm 0.05*
	TOA/TT	0.06 \pm 0.002	0.06 \pm 0.008	0.06 \pm 0.008	0.07 \pm 0.007
30	ECA	3.9 \pm 0.31	4.8 \pm 0.49	4.8 \pm 0.70	4.1 \pm 0.38
	TCA (sec)	279.6 \pm 1.66	282.7 \pm 1.41	281.5 \pm 1.87	275.4 \pm 1.73*
	TE	5.5 \pm 0.67	6.5 \pm 0.85	6.6 \pm 0.99	6.6 \pm 0.86
	EOA/TE	0.29 \pm 0.04	0.30 \pm 0.04	0.29 \pm 0.03	0.38 \pm 0.03*
	TOA/TT	0.07 \pm 0.006	0.06 \pm 0.005	0.06 \pm 0.006	0.08 \pm 0.006

ECA – number of entries into the closed arms; TCA – time spent in the closed arms; TE – total number of arm entries; EOA/TE – the ratio of the number of entries into the open arms/total number of entries; TOA/TT – the ratio of time spent in the open arms/total time.

The elevated plus-maze is the most frequently used animal test for studying anxiety. Most anxiolytic-like drugs increase the exploration in the open arms thus, reflected by increased percentage of entries into the open arms and time spent there, at doses that do not affect locomotor activity measured in

the plus-maze as the sum of the total number of arm entries [14].

The results from the present study showed a dose- and time-dependent anti-anxiety effect of AMFJ in rats when exposed to the elevated plus-maze test. The effect was manifested by an increased

exploration of the open arms (increased number of entries into the open arms, time spent there, and ratio of the number of entries into the open arms/total number of entries).

AMFJ is rich in flavonoids, mainly anthocyanins. *Aronia melanocarpa* anthocyanins are cyanidin 3-galactoside, cyanidin 3-glucoside and cyanidin 3-arabinoside [11, 16]. Andres-Lacueva *et al.* [2] have shown that these anthocyanins cross the blood-brain barrier and localize in various brain regions such as cerebellum, cortex, hippocampus or striatum and like other flavonoids, they can act centrally. There are no data in literature about any unwanted and toxic effects of *Aronia melanocarpa* fruits, juice and extracts.

In this experiment, AMFJ showed an anti-anxiety effect in rats which was probably due to its active ingredients such as flavonoids (including anthocyanins and quercetin) and other polyphenols. The most pronounced effect was achieved for the 30-day treatment period with the dose of 10 mL/kg. This finding is consistent with the observation of Willis *et al.* [24] that flavonoids and polyphenols from berries do accumulate in the brain following long-term consumption. The anti-anxiety effect of AMFJ in this study is in accordance with other authors' investigations which have demonstrated anxiolytic effects of plant extracts rich in anthocyanins [4, 12]. Quercetin and chlorogenic acid which are other constituents of AMFJ have also been demonstrated to possess anxiolytic-like effects [1, 5]. The flavonoids have been shown to exert a benzodiazepine-like pharmacological activity due to binding affinity to GABA-A receptors [7, 15, 23]. The potential advantage of these natural compounds over benzodiazepine-type anxiolytics is that they rarely have any side effects [20].

Conclusions

The results from the present study demonstrate that *Aronia melanocarpa* fruit juice applied sub-chronically to rats exerts a dose- and time-dependent anti-anxiety effect which might be due to its polyphenolic ingredients.

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