ANTIDEPRESSANT-LIKE EFFECT OF ARONIA MELANOCARPA FRUIT JUICE APPLIED SUBCHRONICALLY TO RATS

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ABSTRACT

PURPOSE: Polyphenols are bioactive substances found in many plants. *Aronia melanocarpa* fruits are one of the richest sources of polyphenols, amongst them proanthocyanidins, flavonoids and phenolic acids. Some of the polyphenols are reported to cross the blood-brain barrier and thus they can act centrally. The aim of the present study was to investigate the effect of *Aronia melanocarpa* fruit juice (AMFJ) on depressive-like behavior in subchronically treated (21 and 30 days) male Wistar rats utilizing the forced swim test (FST).

MATERIAL AND METHODS: AMFJ was applied orally through an orogastric cannula once daily at doses of 2,5 ml/kg, 5 ml/kg and 10 ml/kg for periods of 21 and 30 days to the respective experimental groups. The FST was carried out 1 hour after the treatment on the 21st and 30th day.

RESULTS: AMFJ applied for 21 days decreased significantly ($p < 0,05$) the immobility time in the FST at the doses of 2,5 ml/kg and 10 ml/kg. In rats treated with AMFJ for 30 days, the immobility time was dose-dependently decreased and at the dose of 10 ml/kg it was significantly lower ($p < 0,05$) than the control one.

CONCLUSION: The decreased immobility time in the FST suggests an antidepressant-like effect of AMFJ in rats which could be due to the polyphenolic ingredients of the juice.

Key words: *Aronia melanocarpa, forced swim test, behavior, antidepressant, male rats*

INTRODUCTION

Depression is a common mental disorder in which sufferers exhibit depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth and disturbed sleep or appetite. It is estimated that at least 20% of all individuals suffer from a depressive episode at least once in their lifetime (4). Anxiety may coexist with depression, known as an anxiodepressive syndrome that makes coping more difficult. A dysregulation of the central nervous system (CNS) involving the neurotransmitters norepinephrine (noradrenaline), serotonin (5-hydroxytriptamine, 5-HT) and dopamine has been suggested to play a role in the pathogenesis of depression and the mainstream of research in depression has principally focused on noradrenergic and serotonergic systems. The current antidepressants in use, including tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, all exert their antidepressant effect by increasing the levels of monoamines serotonin and/or norepinephrine. However, heterogeneity of clinical response to antidepressants and susceptibility to adverse effects are major clinical problems. Recently, the use of tradi-

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tional herbs has provided a promising alternative in the treatment of depression because of better compliance and lower side effects (9,21,20). *Hipericum perforatum* (St John's Wort) is one of the most popular herbal drugs and is the only herbal alternative to the classic synthetic antidepressants in the therapy of mild to moderate depression and less likely to cause side effects than commonly prescribed antidepressants (7). According to the actual state of scientific knowledge the total herbal extract has to be considered as the active substance. However the data suggest that the flavonoid quercetin rutinoside (rutin) is essential for the antidepressant activity of *Hypericum perforatum* extract (10). Studies of other plant extracts such as *Cecropia glazioui* Sneth rich in catechins, procyanidins and flavonoids (15) or *Mentha aquatica* containing the flavonoid naringenin have also demonstrated antidepressant activities (11). Individual flavonoids also have effects in depressive conditions. Studies with quercetin have indicated its antidepressant activity in streptozotocin-induced diabetic mice (2).

Aronia melanocarpa Elliot (black chokeberry) is a woody shrub of the Rosaceae family, genus Aronia, native to the eastern North America, now commonly planted in Eastern Europe. *Aronia melanocarpa* 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.

The aim of the present study was to assess the antidepressant-like activity of *Aronia melanocarpa* fruit juice administered subchronically to male Wistar rats utilizing the forced swim test.

MATERIAL AND METHODS

AMFJ preparation and determination of its biologically active 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, 6652 mg as gallic acid equivalents per litre, determined spectrophotometrically according to the Folin-Ciocalteu procedure (16); total proanthocyanidins, 3926,2 mg/l, determined by gravimetric isolation according to the procedure described by Howell et al. (5); phenolic acids (gallic – 6,9 mg/l, chlorogenic – 691 mg/l, neochlorogenic – 840 mg/l and ferulic – 19,9 mg/l) determined by a high-performance liquid chromatography (HPLC) method at wavelength of λ=280 nm; anthocyanins (cyanidin-3-galactoside – 20,0 mg/l, cyanidin-3-glucoside – 4,4 mg/l, cyanidin-3-arabinoside – 8,2 mg/l and cyanidin-3-xyloside – 0,6 mg/l) determined by HPLC at wavelength of λ=520 nm. Agilent 1220 HPLC system (Agilent Technology, Palo Alto, Ca) was used.

Animals

Male Wistar rats with a mean weight of 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 conformity with the National and International laws and policies (EEC Council Directive 86/609, IL 358, 1, December 12, 1987).

Experimental procedure

The experiments were performed on 96 rats. They were divided in 8 groups of 12 animals each and were treated orally through an orogastric cannula for 21 (four groups) or for 30 days (the other four groups). The groups were designated as Control, $AMFJ_{2.5}$, $AMFJ_{5}$ and $AMFJ_{10}$. Control groups were treated with distilled water 10 ml/kg once daily for 21 and 30 days, respectively. AMFJ was applied once daily at doses of 2,5 ml/kg diluted to a total volume of 10 ml/kg (to $AMFJ_{2.5}$ groups), 5 ml/kg diluted to a total volume of 10 ml/kg (to $AMFJ_{5}$ groups) and 10 ml/kg (to $AMFJ_{10}$ groups) for periods of 21 and 30 days to the respective experimental groups. The dose and the treatment periods were chosen on the basis of previous investigations (unpublished data), which have shown that AMFJ at that dose and treatment durations had significant effects on central nervous functions in rats. The forced swim test was performed on the 21st and 30th days one hour after last treatment.

Forced swim test

The method of Porsolt et al. (13) was used to asses the immobility of the rats as a measure of their depressive-like behavior. Each rat was placed in a

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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. Inactivity (immobility) and swimming were distinguished as mutually exclusive behavioral states. Swimming behavior was defined as movement throughout the cylinder. Immobility was measured when no additional activity was observed other than that required to keep the rat's head above the water. The increased immobility time is a measure of the depressive-like behavior.

Statistical analysis

Results are presented as mean±S.E.M. The data were tested by Student's *t*-test. All analyses were performed using GraphPad Prism statistical software. A level of $p<0,05$ was considered significant.

RESULTS

On the 22nd day, the immobility time of the control group was 51,6±5,5 sec. It was 30,9±5,1 sec in $AMFJ_{2.5}$ group (59,9% of the control time), 55,9±8,3 sec in $AMFJ_5$ group (108,3% of the control time) and 29,6±4,3 sec in $AMFJ_{10}$ group (57,4% of the control time). Thus, applied for 21 days, AMFJ decreased significantly ($p<0,05$ vs. Control) the immobility time in the groups treated with the doses of 2,5 ml/kg and 10 ml/kg, and caused a tendency to increase the immobility time in the group treated with the dose of 5 ml/kg (Fig. 1).

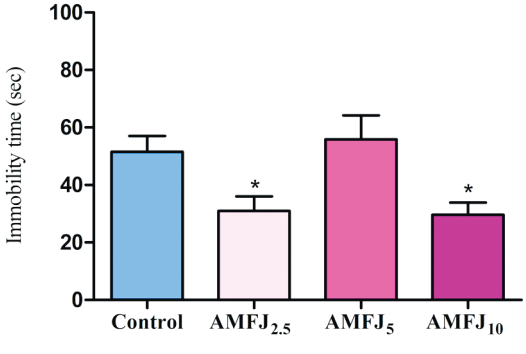

*Fig. 1. Effect of AMFJ applied for 21 days on the immobility time in the FST. Results are presented as mean±S.E.M.; n=12; *p<0,05 vs. Control*

On the 31st day, the immobility time of the control group was 77,3±14,7 sec. It was 68,2±11,2 sec in $AMFJ_{2.5}$ group (88,2% of the control time), 53,4±9,6 sec in $AMFJ_5$ group (69,1% of the control time) and 38,5±6,2 sec in $AMFJ_{10}$ group (49,8% of the control time). Thus, applied for 30 days, AMFJ dose-dependently decreased the immobility time of the treated rat groups. The decrease of the immobility time was statistically significant ($p<0,05$ vs. Control) for $AMFJ_{10}$ group (Fig. 2).

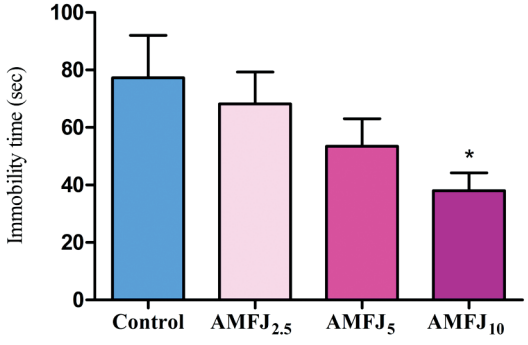

*Fig. 2. Effect of AMFJ applied for 30 days on the immobility time in the FST. Results are presented as mean±S.E.M.; n=12; *p<0,05 vs. Control*

DISCUSSION

The main bioactive substances in AMFJ are polyphenols – proanthocyanidins, phenolic acids and flavonoids from the subclass of anthocyanins. *Aronia melanocarpa* anthocyanins are cyanidin-3-galactoside, cyanidin-3-glucoside, and cyanidin-3-arabinoside (12). There are literature data that polyphenols from berries are able to traverse the blood-brain barrier (1,6,14) and do accumulate in the brain following long-term consumption (19).

A dysregulation of the CNS involving the neurotransmitters norepinephrine, serotonin and dopamine has been suggested to play a role in the pathogenesis of depression. A decrease mainly in serotonergic and noradrenergic activity is associated with depressive symptoms.

Literature data show that plant extracts rich in polyphenols as well as individual phenolic substances possess antidepressant activity. There are many investigations trying to elucidate the mechanisms underlying of the antidepressant effects of herbal

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preparations. *Hipericum perforatum* extract has complex actions that include: weak inhibiton of monoamine oxidase (MAO) A and B; inhibition of synaptosomal uptake of serotonin, dopamine and norepinephrine; affinity for adenosine, $GABA_A$, $GABA_B$ and glutamate receptors; down-regulation of beta-adrenergic receptors and an up-regulation of 5-HT_2 receptors; regulation of genes that control hypothalamic-pituitary-adrenal axis function (3). The rich in catechins, procyanidins and flavonoids *Cecropia glazioui* Sneth extract and six of its purified constituents inhibit the uptake of [3H]-serotonin, [3H]-dopamine and [3H]-noradrenaline by synaptosomes of different brain regions. The antidepressant-like effect of the extract is most likely due to the blockade of the monoamines uptake in the CNS (15). *Mentha aquatica* contains naringenin and is used in traditional medicine for depression-like conditions. It possesses MAO-inhibitory activity (11).

Using a mouse model, Takeda et al. (17) have demonstrated that rosmarinic acid and its major metabolite the phenolic caffeic acid significantly decreased immobility and increased the anti-resignation behavior of mice in the FST. Machado et al. (8) used the tail suspension test (another behavioral test for depression) and revealed that rutin significantly decreased the duration of immobility behavior of mice. The authors suggest that the antidepressant-like effects of this polyphenols is due to the increase of the availability of serotonin and noradrenaline in the synaptic cleft (8,17).

The results from present study showed that AMFJ after 21 days of application to rats significantly decreased the immobility time in the FST at the doses of 2,5 ml/kg and 10 ml/kg but surprisingly increased the immobility time at the dose of 5 ml/kg. Applied for 30 days, AMJF dose-dependently decreased the immobility time, the effect being significant at the highest dose. On the basis of these results, we could suppose that AMFJ showed an antidepressant-like effect in rats. The antioxidant effect is the most pronounced and well-studied effect of polyphenolic substances. AMFJ has been shown to possess a powerful antioxidant activity. However, in a previous investigation (18), AMFJ did not reduce the brain lipid peroxidation products in an alcohol-induced depression-like model in rats. Thus, it is not probable the antidepressant effect of AMFJ in young/healthy rats in this study to be due to its antioxidant actions. These previous results together with the other author's data allow us to suppose that AMFJ-induced reduction of the depressive-like behavior in rats might be due to effect on neurotransmission.

CONCLUSION

In conclusion, the results of the present study demonstrate that AMFJ, applied subchronically to rats, exerts an antidepressant-like effect in the FST. This effect might be due to the polyphenolic ingredients of the juice.

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