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

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ABSTRACT

PURPOSE: The main biologically active constituents of Aronia melanocarpa fruit juice (AMFJ) are polyphenolics, amongst them proanthocyanidins, flavonoids and phenolic acids. The aim of the present study was to investigate the effects of Aronia melanocarpa fruit juice (AMFJ) on anxiety in subchronically treated (21 and 30 days) male Wistar rats utilizing the social interaction test.

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 social interaction test was carried out 1 hour after the treatment on the 21st and 30th day. The time of social interaction between the test partners was used as a measure of anxiety. The longer time for social contacts showed lower degree of anxiety.

RESULTS: In rats treated with AMFJ for 21 days, the time of social contacts between the test partners increased dose-dependently and at the dose of 10 ml/kg it was significantly higher (p < 0.05) than the control time. Applied for 30 days, AMFJ did not increase the time of social interaction between the rats which might be attributed to the fact that at such duration of treatment AMFJ could decrease the general locomotor activity of the animals.

CONCLUSION: The findings from the present study suggest an anxiolytic-like effect of AMFJ in rats which could be due to its polyphenolic ingredients.

Key words: Aronia melanocarpa, social interaction test, behavior, anxiolytic, male rats

INTRODUCTION

Millions of people around the world suffer from mental disorders. Two of the most common mental disorders are anxiety and depression. Anxiety can be a normal emotional response to threat, but when it is inappropriate, extreme and persistent, it is classified as a pathological condition. It is estimated that one-eighth of the total population worldwide suffers from inappropriate anxiety (3). The most commonly prescribed drugs for the treatment of anxiety disorders are benzodiazepins. They have many side-effects, such as sedation, myorelaxation, ataxia, amnesia and dependence. Recently, research has been conducted to investigate natural anxiolytic agents with fewer harmful effects (2). In recent years, drug screening from traditional medicinal herbs has attracted much attention in the hope to identify novel therapeutics for the treatment of various diseases. The discovery of chrysin, one of the first flavonoids shown to possess in vivo activity through interaction with the benzodiazepine receptors (11), has marked the search for natural anxiolytics. Different plant species have been submitted to neuropharmacological evaluation. A number of flavonoids have been found to possess partial allosteric modulatory action at the GABAA receptor complex (17,23), and therefore constitute a
promising class of naturally occurring compounds for the treatment of anxiety.

*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 – mainly proanthocyanidins, phenolic acids and flavonoids from the subclass of anthocyanins.

The aim of the present study was to investigate the effect of *Aronia melanocarpa* fruit juice (AMFJ) on anxiety in subchronically treated male Wistar rats utilizing the social interaction 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. (6); phenolic acids (gallic – 6.9 mg/l, chlorogenic – 691 mg/l, neochlorogenic – 840 mg/l and ferulic – 19.9 mg/l) were 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-xiloside – 0.6 mg/l) were 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±20g 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 in 8 groups of 6 pairs of animals each. Four of the groups designated as Control, AMFJ1, AMFJ2, and AMFJ3 were treated for 21 days. The other 4 groups also designated as Control, AMFJ4, AMFJ5 and AMFJ6 were treated for 30 days. The two control groups were treated orally with distilled water (10 ml/kg) once daily for 21 and 30 days, respectively. AMFJ was applied orally through an orogastric cannula once daily at doses of 2.5 ml/kg (to the two AMFJ2 groups), 5 ml/kg (to the two AMFJ4 groups) and 10 ml/kg (to the two AMFJ6 groups) for periods of 21 and 30 days, respectively. The social interaction test was performed on the 21st and 30th days one hour after last treatment.

**Social interaction test**

Rats were tested according to the method developed by Sandra and Hyde (5) 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.

**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**

The results from the experiment showed that applied for 21 days, AMFJ increased dose-dependently the time of social contacts between the test partners. The social interaction time of the Control group was 45.0±5.3 sec. It was increased to 57.0±7.7...
sec in AMFJ2.5 group (126.7% of the control time), to 56.9±12.0 sec in AMFJ group (126.4% of the control time) and to 65.8±4.9 sec in AMFJ10 group (146.3% of the control time). The increase of the social interaction time was statistically significant (p<0.05 vs. Control) for AMFJ10 group treated 21 days (Fig. 1).

**DISCUSSION**

AMFJ used in the experiment is rich in polyphenolic substances – proanthocyanidins, phenolic acids and flavonoids from the subclass of anthocyanins. *Aronia melanocarpa* anthocyanins are cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside and cyanidin-3-xyloside (12). These anthocyanins cross the blood brain barrier and can act centrally (1). 14C-labeled plant polyphenols found in the brain tissue and brain microdialysate indicated that these phytochemicals or their metabolites are able to cross the blood-brain barrier (8). Williams et al. (21) reported that flavanol levels were higher than anthocyanin levels in brain tissue of aged rats supplemented with blueberries. Rangel-Ordonez et al. (14) detected relatively high concentrations of quercetin in the hippocampus, striatum and cerebellum. There are literature data that polyphenols from berries do accumulate in the brain following long-term consumption (22).

The brain GABAergic system is responsible for sedation and depressive behaviors. There are data that flavonoid compounds may interact with the GABA<sub>α</sub> receptors (4), thus producing sedation, anxiolytic or anticonvulsive effects (7). The sedative and partly the anticonvulsant actions are attributed to the activation of α<sub>1</sub>-containing GABA<sub>α</sub> receptors, while suppression of anxiety – to the α<sub>2</sub>/α<sub>3</sub> subtypes (15). Sedative effects have been demonstrated for flavonoids (10,18) and plant extracts containing procyanidins, flavonoids and other polyphenols (9).

Two possible explanations have been proposed for the mechanisms underlying the separation of anxiolytic-like effect of flavonoids from the sedation, myorelaxation, cognitive impairment, motor incoordination, constituting undesirable effects of the benzodiazepines. The first concept assumes that flavonoids may act as partial agonists at the benzodiazepine site thus retaining anxiolytic activity with limited side effects (19,20), unlike classical benzodiazepines, which are full agonists at GABA<sub>α</sub> receptors and frequently give side effects. According to the second hypothesis flavonoids exert a selective anxiolytic-like effect through α<sub>2</sub>- and α<sub>3</sub>-containing receptor subtypes (20). Therefore, the α<sub>2</sub>- and α<sub>3</sub>-containing GABA<sub>α</sub> receptors are shown to be useful drug
targets for naturally occurring flavonoid anxiolytics, promising alternatives to classical benzodiazepines.

The results from the present study showed that applied for 21 days, AMFJ dose-dependently increased the time of social interaction between the test partners, the effect being significant at the highest dose. This result is consistent with an anxiolytic-like effect of AMFJ. Applied for 30 days, AMFJ did not affect the state of anxiety measured in the social interaction test. One possible explanation for that observation could be the decreased locomotor activity of the animals. The locomotor activity interferes with the results from the social interaction test. Probably at that longer period of administration, the polyphenolic ingredients of AMFJ could accumulate in the brain (22) leading to manifestation and prevalence of the sedative effect as a result of their agonistic activity at all subtypes of GABA_A receptors. The decrease of locomotor activity is consistent with reduced excitability of the central nervous system and sedation (13) and could explain the lack of increase of the social interaction time after 30 days of AMFJ application in the present experiment.

**CONCLUSION**

In conclusion, the results of the present study demonstrate that AMFJ, applied to rats for 21 days, exerts an anxiolytic-like effect in the test of rat social interaction. This effect might be due to the polyphenolic ingredients of the juice.

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