EFFECT OF ARONIA MELANOCARPA FRUIT JUICE ON ALCOHOL-INDUCED DEPRESSIVE-LIKE BEHAVIOR IN RATS

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ABSTRACT

PURPOSE: The present study investigated the effects of Aronia melanocarpa fruit juice (AMFJ) on behavior and biochemical indices of liver toxicity and oxidative stress in female Wistar rats subchronically treated with ethanol.

MATERIAL AND METHODS: Ethanol was applied for 14 days at a daily dose of 8 g/kg divided in two equal oral gavages. AMFJ was applied as a pretreatment 1 hour before each ethanol administration at doses of 2,5 ml/kg, 5 ml/kg and 10 ml/kg. Effects on behavior were investigated using the open field test (OFT) for locomotor activity, elevated plus maze test (EPM) for anxiety and forced swim test (FST) for depression. The biochemical markers were: liver enzymes for liver toxicity and thiobarbituric acid reactive substances (TBARS) for oxidative stress.

RESULTS: Ethanol induced a significant reduction in locomotor activity in the OFT and EPM. The FST showed that ethanol induced a depressive-like behavior. These effects were not accompanied by liver toxicity. AMFJ did not significantly affect the reduced by ethanol central nervous excitability demonstrated in the OFT and EPM. It showed an anti-depressive effect as evidenced by the reduction of immobility time in the FST. This effect is not likely to be due to the pronounced antioxidant activity of AMFJ as ethanol did not induce oxidative stress estimated by the TBARS.

CONCLUSION: In this model of ethanol-induced changes in rat behavior, AMFJ showed an anti-depressant like effect which might be due to the polyphenolic ingredients of the juice.

Key words: Aronia melanocarpa, alcohol, behavior, anti-depressant, female rats

INTRODUCTION

Alcohol use and abuse appear to be related to neuroadaptive changes at functional, neurochemical, and structural levels leading to co-morbid expression of anxiety and depression (1). Recently, traditional herbs have provided us a prospective alternative in the treatment of depression because of better compliance and lower side effects. Well known is the anti-depressive effect of Hypericum perforatum. It is the only herbal alternative to classic synthetic antidepressants in the therapy of mild to moderate depression. Several groups of active compounds are contributing to the antidepressant efficacy of the plant. A lot of recent data indicate the significance of polyphenols for this effect (2).
Effect of *Aronia melanocarpa* fruit juice on alcohol-induced depressive-like behavior in rats

*Aronia melanocarpa* (Michx) Elliot (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.

The aim of the present study was to investigate the effects of *Aronia melanocarpa* fruit juice (AMFJ) on behavior and biochemical indices of liver toxicity and oxidative stress in female Wistar rats subchronically treated with ethanol.

**MATERIAL AND METHODS**

**Experimental substances**

Ethanol (96%) was from Geya 99, Bulgaria. The chemicals needed for the biochemical analyses were from Sigma-Aldrich Company and Merck, Germany. The assay kits for the measurement of liver enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) were from Biosystems S.A. (Barcelona, Spain) distributed by IVD Bulgaria.

**AMFJ, determination of its biologically active substances and antioxidant activity**

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

Total phenolics were determined according to the method of Singleton and Rossi (3) with Folin-Ciocalteu’s reagent and were expressed as gallic acid equivalents (GAE) per litre. Total proanthocyanidins were determined by gravimetric isolation according to the procedure described by Howell et al. (4). Phenolic acids (gallic, chlorogenic, neochlorogenic and ferulic) were determined by a high-performance liquid chromatography (HPLC) method at wavelength of λ=280 nm. Anthocyanins (cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside and cyanidin-3-xiloside) were determined by HPLC at wavelength of λ=520 nm. Agilent 1220 HPLC system (Agilent Technology, Palo Alto, Ca) was used.

Antioxidant activity of AMFJ was measured by the oxygen radical absorbance capacity (ORAC) assay and hydroxyl radical antioxidant capacity (HORAC) assay. ORAC measures the antioxidant scavenging activity against peroxyl radical induced by 2,2'-azobis (2-amidinopropane) dihydrochloride at 37°C (5). ORAC was expressed as μmol trolox equivalents per litre (μmol TE/l). HORAC measures the metal-chelating activity of antioxidants under the conditions of Fenton-like reactions employing a Co(II) complex and hence the protecting ability against formation of hydroxyl radical (6). HORAC was expressed as μmol GAE/l.

**Animals**

Female Wistar rats (200±20 g) 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 in accordance with the national laws and policies, in conformity with the international guidelines (EEC Council Directive 86/609, IL 358, 1, December 12, 1987).

**Experimental procedure**

The rats were divided in 5 groups of 12 animals each: Control, Ethanol, Eth + AMFJ₂.5, Eth + AMFJ₅ and Eth + AMFJ₁₀. The Control and Ethanol groups were pretreated twice (at 8 a.m. and 4 p.m) with distilled water (10 ml/kg) while the other three groups were respectively pretreated with AMFJ at doses of 2.5 ml/kg (diluted to a total volume of 10 ml/kg), 5 ml/kg (diluted to 10 ml/kg), and 10 ml/kg. One hour after each pretreatment the animals from Control group received distilled water (24 ml/kg), while all other groups were treated with ethanol at a dose of 4 g/kg as a 20% solution, 24 ml/kg. One hour after each pretreatment the animals from Control group received distilled water (24 ml/kg), while all other groups were treated with ethanol at a dose of 4 g/kg as a 20% solution, 24 ml/kg. Thus, the daily dose of ethanol was 8 g/kg. The experimental substances were applied orally for 14 days.

On the 15<sup>th</sup> day, 16 hours after the last ethanol exposure, the behavioral tests (open field test, elevated plus-maze test and forced swim test) were performed 1 hour one after the other. At the end of each experimental session, the floor and walls of the test apparatuses were wiped clean and dried.

**Open field test (OFT)**

This test is a common measure of exploratory behavior and general activity in rodents (7). 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 5×5 equal size 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).
Elevated plus-maze (EPM)

The EPM test is used to evaluate anxiety-like behaviors (8). The EPM apparatus consists of two opposite open arms (50×10 cm) and two opposite arms enclosed by 40 cm high walls. The arms are elevated 50 cm from the floor. Each rat was placed in the central square with the head facing the open arm and its behavior was observed for 5 min. The behaviors recorded were: entries in the open arms, entries in the closed arms and time spent in the open arms. The decreased exploration of the open arms (number of entries in the open arms and time spent there) without a decrease in the overall locomotor activity (total number of arm entries) is a measure of anxiety-like behavior.

Forced swim test (FST)

The method of Porsolt et al. (9) was used to assess depressive-like behavior. Each rat was placed in a glass cylinder pool (17 cm in diameter and 50 cm in height) for 5 min. The cylinder was filled with 30 cm water (21±1 °C). The test was performed in two sessions with a 24 hour interval. The results from the second session were recorded. Immobility is defined when no additional activity is observed other than that required to keep the rat’s head above the water. The increased immobility time is a measure of depressive-like behavior.

Serum and homogenate preparation

On the 16th day, the animals were anaesthetized with diethylether. Blood collected from the sublingual veins was centrifuged at 2000 rpm for 10 min and serum was obtained. After the decapitation of the animals liver and brain were frozen at –18 °C till the biochemical analyses. In order to prepare organ homogenates, 1 g of each organ was homogenized with ice cold Tris/HCl buffer (50 mM, pH 7.4) in 1:10 ratio for liver, and 1:5 ratio for brain.

Measurement of liver enzymes

Liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured spectrophotometrically (Aurius 2011 UV-VIS spectrophotometer, Cecil Instruments Ltd, UK) in accordance with the kits manufacturer’s instructions.

Measurement of thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation levels were estimated by the thiobarbituric acid (TBA) reaction (10). The method measures spectrophotometrically the color produced by the reaction of TBA with lipid peroxides (thiobarbituric acid reactive substances, TBARS) at 532 nm. TBARS were determined in nmol/ml serum and nmol/g tissue. Malondialdehyde, the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids, was used as a standard.

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

Biologically active substances and antioxidant activity of AMFJ

Table 1 and Table 2 show the contents of total phenolics, total proanthocyanidins, phenolic ac-
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ids and anthocyanins in AMFJ, and the ORAC and HORAC of the juice.

**Weight gain**

The mean body weight gain for the entire experimental period is presented in Table 3. Control rats increased their weight while in all other groups there was a decrease in weight.

**Open field test (OFT)**

Ethanol caused a significant reduction (p<0.05 vs. Control) in the spontaneous locomotor activity in rats as assessed by the number of crossings (horizontal activity) (Fig. 1A) and number of rearings (vertical activity) (Fig. 1B). As is evident from Fig. 1, AMFJ pretreatment caused a slight further decrease in horizontal and vertical activity (p<0.05 or p<0.01 vs. Control).

**Elevated plus-maze (EPM)**

As is obvious from Fig. 2 (A, B, C), ethanol treatment induced a decrease in open arm entries, a significant decrease in closed arm entries (p<0.01 vs. Control) and total number of arm entries (p<0.001 vs. Control) and caused a tendency to decrease the time spent in the open arms (Fig. 2D). AMFJ pretreatment resulted in an increased number of entries into the

### Table 3. Weight gain, levels of liver enzymes and TBARS in rats treated with ethanol and pretreated either with water (Ethanol), or with AMFJ (Eth + AMFJ2.5, Eth + AMFJ5, Eth + AMFJ10)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Control</th>
<th>Ethanol</th>
<th>Eth + AMFJ&lt;sub&gt;2.5&lt;/sub&gt;</th>
<th>Eth + AMFJ&lt;sub&gt;5&lt;/sub&gt;</th>
<th>Eth + AMFJ&lt;sub&gt;10&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain [g]</td>
<td></td>
<td>9,5±2.3</td>
<td>-5,9±4.4</td>
<td>-3,8±5.5</td>
<td>-5,9±4.2</td>
<td>-6,6±3.3</td>
</tr>
<tr>
<td>AST [U/l]</td>
<td></td>
<td>116,9±10,7</td>
<td>157,1±25,0</td>
<td>121,6±11,9</td>
<td>111,1±14,6</td>
<td>108,4±16,5</td>
</tr>
<tr>
<td>ALT [U/l]</td>
<td></td>
<td>29,4±2,4</td>
<td>42,0±9,2</td>
<td>42,8±5,0</td>
<td>42,3±5,2</td>
<td>41,5±6,1</td>
</tr>
<tr>
<td>AF [U/l]</td>
<td></td>
<td>214,2±16,6</td>
<td>220,6±25,3</td>
<td>210,5±24,2</td>
<td>216,4±26,9</td>
<td>215,6±29,2</td>
</tr>
<tr>
<td>TBARS Liver [nmol/g]</td>
<td></td>
<td>303,8±18,8</td>
<td>283,5±21,8</td>
<td>291,8±14,3</td>
<td>271,5±18,8</td>
<td>309,0±37,3</td>
</tr>
<tr>
<td>TBARS Brain [nmol/g]</td>
<td></td>
<td>87,8±7,1</td>
<td>93,4±4,1</td>
<td>81,4±5,6</td>
<td>87,8±3,8</td>
<td>87,8±7,5</td>
</tr>
<tr>
<td>TBARS Serum [nmol/ml]</td>
<td></td>
<td>30,8±3,0</td>
<td>36,8±3,0</td>
<td>31,2±2,3</td>
<td>31,8±2,14</td>
<td>29,1±2,3</td>
</tr>
</tbody>
</table>

![Fig. 1. Effect of AMFJ applied as a pretreatment to ethanol-treated rats on their horizontal (panel A) and vertical (panel B) locomotor activity. *p<0.05 vs. Control, **p<0.01 vs. Control](image)
open arms (Fig. 2A) accompanied by an increased number of total arm entries and a dose-dependent increase in the time spent in the open arms (Fig. 2D) but these results were not significantly different from the behavior of Ethanol group.

**Forced swim test (FST)**

In the FST, the immobility time of the animals from Ethanol group was significantly higher (p<0,01) than that of Control group (Fig. 3). The immobility time of AMFJ-pretreated groups did not differ significantly from the control time (Fig. 3). It had the lowest value for Eth + AMFJ_{2.5} group.

**Liver enzymes**

The levels of liver enzymes are presented in Table 3. Ethanol treatment caused an elevation of
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AST (135% of the control value) and ALT (145% of the control value) but the effect was not significant. AMFJ pretreatment caused a decrease in AST values to the control level and had no effect on ALT values. Neither alcohol, nor AMFJ affected AF levels.

**Thiobarbituric acid reactive substances (TBARS)**

As obvious from Table 3, neither alcohol treatment, nor AMFJ pretreatment had significant effects on TBARS contents in rat liver, brain and serum.

**DISCUSSION**

In the present experiment, ethanol caused a decrease in spontaneous locomotor activity and exploratory behavior in both OFT and EPM. This effect may be correlated with reduced level of central nervous excitability (11), elevated levels of anxiety and fear (12), loss of interest in new stimulating situations, deficit of motivation and emotionality (13). Alcohol also caused an increase in the immobility time in the FST which indicated decreased motivation and behavioural despair (9) and might be considered the behavioural expression of a depressive-like symptom in rats. All these central nervous effects of alcohol were associated with decreased body weight gain. They were not accompanied by liver toxicity as demonstrated by the liver enzyme levels and were probably not caused by oxidative stress as indicated by the levels of TBARS in the brain, liver and serum. Although, the exact mechanism by which ethanol exerts its effect is still a matter of debate, studies have shown that ethanol affecting several neurotransmitter systems, differentially modifies processes of neurotransmission in the central nervous system. Reduced cortical norepinephrine levels are considered as a major factor in alcohol-induced depression (14).

The results from the present study showed that AMFJ was extremely rich in phenolic antioxidants with pronounced ORAC and HORAC. Applied as a pretreatment, AMFJ did not counteract the alcohol-induced decrease in weight. It did not significantly affect the reduced by ethanol central nervous excitability demonstrated in the OFT and EPM. AMFJ showed an anti-depressive effect as evidenced by the reduction of immobility time in the FST. This antidepressant-like effect is not likely to be due to the high antioxidant activity of AMFJ as ethanol did not induce oxidative stress (evidenced by the levels of TBARS). The reduction of alcohol-induced depressive-like symptoms in rats by AMFJ might be due to effect on neurotransmission. Some authors suggest that polyphenols act by increasing the availability of serotonin and noradrenaline in the synaptic cleft (15,16).

**CONCLUSION**

Applied as a pretreatment in a model of ethanol-induced changes in behavior in female Wistar rats, AMFJ showed an anti-depressant like effect which might be due to the polyphenolic ingredients of the juice.

**REFERENCES**


7. Gould, T. D., T. D. Dao, C. E. Kavacsics. The open field test.- In: Mood and anxiety related pheno-


