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EFFECTS OF *ARONIA MELANOCARPA* FRUIT JUICE ON EXPLORATORY BEHAVIOUR AND LOCOMOTOR ACTIVITY IN RATS

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The main bioactive substances in *Aronia melanocarpa* fruit juice (AMFJ) are polyphenols (flavonoids, procyanidins, and phenolic acids). A great number of polyphenols are able to traverse the blood-brain barrier. In recent years more attention is drawn to the ability of these substances to influence central nervous system functions. The aim of the present study was to investigate the effects of AMFJ on exploratory behaviour and locomotor activity in male Wistar rats. AMFJ was administered orally for 7, 14, 21, and 30 days at three increasing doses (2.5, 5, and 10 ml kg^{-1}). The changes in exploratory behaviour and locomotor activity were recorded in an Opto Varimex apparatus. It was found that the low doses of AMFJ (2.5 and 5 ml kg^{-1}) for all treatment periods did not significantly affect exploratory behaviour and locomotor activity of rats compared to the saline-treated controls. AMFJ at the highest dose of 10 ml kg^{-1} had no significant effect on exploration and locomotion for the treatment periods of 7 and 14 days, while for the periods of 21 and 30 days it significantly decreased the number of horizontal and vertical movements, which might be the result of a sedative effect. At all the doses and testing periods, AMFJ did not disturb the progressive decrease in motor behaviour, suggesting habituation.

Keywords: exploratory behaviour, locomotor activity, *Aronia melanocarpa*, rats

Aronia melanocarpa (Michx.) Elliot, also known as black chokeberry, is a shrub, member of the Rosaceae family. Native to North America, it is now extensively cultivated in Europe. *Aronia melanocarpa* fruit juice (AMFJ) is one of the richest sources of natural polyphenols. These polyphenols are flavonoids (mainly from the subclass of anthocyanins), procyanidins, and phenolic acids (Denev et al., 2012).

Plant polyphenols are able to access the brain via the blood brain barrier and represent novel therapeutic agents in diseases of the central nervous system. In aged rats, anthocyanins from blueberry have been found in the cerebellum, cortex, hippocampus, or striatum in their unmetabolized forms (Andres-Lacueva et al., 2005). These findings were the first to suggest the ability of polyphenolic compounds to cross the blood brain barrier and localize in various brain regions. Williams and co-workers (2008) reported that flavanol levels were higher than anthocyanin levels in brain tissue of aged rats supplemented with blueberries. Rangel-Ordonez and co-workers (2010) detected relatively high concentrations of quercetin in the hippocampus, striatum, and cerebellum. ^{14}C-labelled plant polyphenols found in the brain

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tissue and brain microdialysate indicated that these phytochemicals or their metabolites are able to cross the blood-brain barrier (Janle et al., 2010).

Given to rats as a single dose of 5 or 10 ml kg^{-1}, AMFJ did not significantly affect locomotor activity in the open field test (Valcheva-Kuzmanova & Zhelyazkova-Savova, 2009). However, there are findings that flavonoids and polyphenols from berries do accumulate in the brain following long-term consumption (Willis et al., 2009).

The aim of the present study was to investigate whether AMFJ applied subchronically to male Wistar rats could have an effect on exploratory behaviour and locomotor activity.

1. Materials and methods

1.1. AMFJ preparation

AMFJ was produced from *Aronia melanocarpa* (Michx.) Elliot fruit grown in the Balkan Mountains, Bulgaria. 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. 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 (Singleton & Rossi, 1965); total flavonoids, 189.4±8.6 mg as catechin equivalents, measured by a colorimetric assay developed by Zhishen and co-workers (1999); total anthocyanins, 106.8±6.2 mg as cyanidin-3-glucoside equivalents, determined by a pH-differential spectrophotometry at pH 1.0 and pH 4.5 (Giusti et al., 1999); quercetin, 11.8±0.8 mg, measured by a high-performance liquid chromatography method (Hertog et al., 1992). The values were the mean of duplicate determinations of three samples.

1.2. Animals and treatment

Male Wistar rats (180–200 g; n=160) were housed in polypropylene boxes with free access to food and 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 (EEC, 1986).

The rats were divided into 16 groups of 10 animals each. They were treated intragastrically through an orogastric cannula in the course of 7 days (one week), 14 days (2 weeks), 21 days (three weeks), or 30 days (one month). Rats from AMFJ groups were treated with AMFJ at doses of 2.5 ml kg^{-1}, 5 ml kg^{-1}, or 10 ml kg^{-1}. The control groups were treated with saline. There were four groups of rats for each treatment period: Control, $AMFJ_{2.5}$, $AMFJ_{5}$, and $AMFJ_{10}$ (the index indicates AMFJ dose).

1.3. Exploratory behaviour and locomotor activity

Exploratory behaviour and locomotor activity were recorded in an Opto Varimex apparatus (Columbus Instruments, USA) according to the method of Köhler & Lorens (1978). The experimental chamber was 50 cm×50 cm×25 cm. This apparatus records the number of photobeam interruptions during the animal movements. It provides selective counting of the number of horizontal movements (ambulation) and vertical movements (rearings) in arbitrary units (AU). The information obtained was automatically recorded every minute for the first 5 min of the test and for the next 5 min thereafter. The number of horizontal and vertical movements recorded every minute for the first 5 min served as a measure of exploratory

activity and habituation to the new environment. The total number of movements during the first 5 min and during the whole 10-min period of observation was used as a measure of locomotor activity. The experiments were carried out at the same time (between 9:00 a.m. and 1:00 p.m.). The rats were placed in the central quadrant of the activity monitor. The different groups were tested on the 7th, 14th, 21st, and 30th day 60 min after the last AMFJ application. Before each test, the apparatus was wiped clean and dried.

1.4. Statistical analysis

Behavioural data were analysed by analysis of variance (ANOVA). Separate two-way repeated measures ANOVA was used to process the data obtained for horizontal and vertical movements between subject factors: drug (four levels: saline, AMFJ 2.5 ml kg^{-1}, AMFJ 5 ml kg^{-1}, and AMFJ 10 ml kg^{-1}) and time (five levels: 1st, 2nd, 3rd, 4th, and 5th min). Separate one-way repeated measures ANOVA was used to process the data obtained for the total number of horizontal and vertical movements during the whole 5-min and 10-min periods of observation. ANOVA data were further analysed by post hoc *t*-test. A level of $P<0.05$ was considered significant. GraphPad Prism statistical software was used.

2. Results and discussion

2.1. Effect of AMFJ on exploratory behaviour

2.1.1. Effect of AMFJ on the horizontal movements during the first 5 min. Repeated two-way ANOVA of the number of horizontal movements recorded every minute for the first 5 min did not demonstrate a significant effect of the factor dose on the 7th ($F_{3.199}=0.87$, $P\leq0.46$) and 14th day ($F_{3.199}=0.72$, $P\leq0.54$) and demonstrated a significant effect of the factor time on the 7th ($F_{4.199}=54.18$, $P\leq0.001$) and 14th day ($F_{4.199}=61.34$, $P\leq0.001$). Repeated two-way ANOVA on the 21st and 30th day revealed significant effects of both factors: dose ($F_{3.199}=10.75$, $P\leq0.001$; $F_{3.199}=4.93$, $P\leq0.002$, respectively) and time ($F_{4.199}=20.03$, $P\leq0.001$; $F_{4.199}=112.21$, $P\leq0.0001$, respectively).

Separate post-hoc *t*-test comparisons for each minute demonstrated that AMFJ applied at doses of 2.5 ml kg^{-1} and 5 ml kg^{-1} for periods of 7, 14, 21, and 30 days had no significant effect on the number of horizontal movements (Figs 1A, 2A, 3A, 4A). Post-hoc *t*-test comparisons for each minute showed that AMFJ at the dose of 10 ml kg^{-1} applied for 7 and 14 days did not significantly reduce the number of horizontal movements (Figs 1A, 2A). The dose of 10 ml kg^{-1} significantly decreased the horizontal movements on the 21st day on the 1st ($P<0.05$), 2nd ($P<0.05$), 3rd ($P<0.01$), 4th ($P<0.05$), and 5th min ($P<0.05$) as well as on the 30th day on the 1st ($P<0.05$), 2nd ($P<0.01$), 3rd ($P<0.01$), 4th ($P<0.05$), and 5th min ($P<0.05$) compared to the respective saline-treated controls (Figs 3A, 4A).

ANOVA of the total number of horizontal movements throughout the initial 5-min observation period showed that the factor dose was not significant on the 7th ($F_{3.39}=0.55$, $P\leq0.65$) and 14th day ($F_{3.39}=0.91$, $P\leq0.45$), while on the 21st ($F_{3.39}=7.41$, $P\leq0.001$) and 30th day ($F_{3.39=}4.744$, $P\leq0.006$) it was significant. The post-hoc *t*-test demonstrated that AMFJ at the dose of 10 ml kg^{-1} significantly reduced the total number of horizontal movements for the first 5 min on the 21st ($P<0.001$) and 30th day ($P<0.001$) as compared with the respective saline-treated controls (Table 1). The greatest effect was observed on the 30th day.

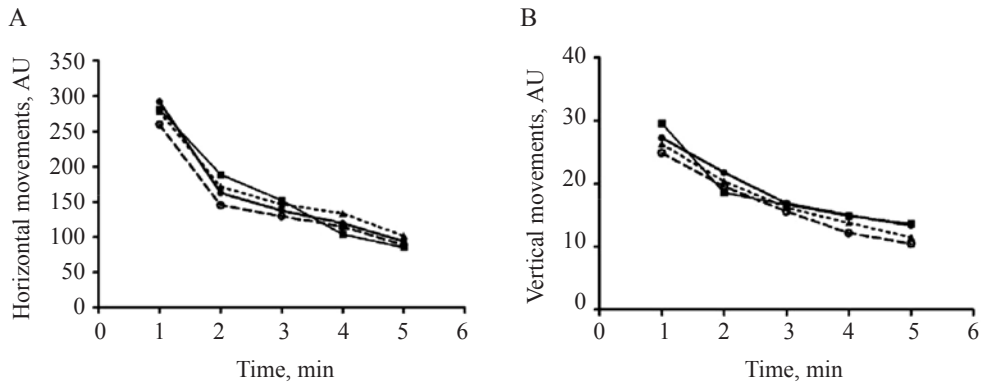

Fig. 1. Effect of AMFJ at doses of 2.5 ml kg^{-1} (AMFJ$_{2.5}$), 5 ml kg^{-1} (AMFJ$_5$), and 10 ml kg^{-1} (AMFJ$_{10}$) applied orally to rats for 7 days on the number of horizontal movements (panel A) and vertical movements (panel B) recorded every minute for a 5-min observation period. AU: arbitrary units; n=10;
—●—: Control; —■—: AMFJ$_{2.5}$; - -▲- -: AMFJ$_5$; - -○- -: AMFJ$_{10}$

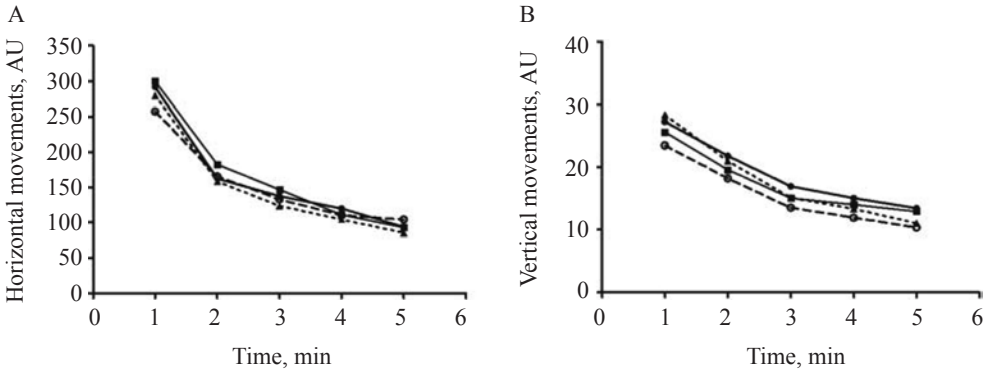

Fig. 2. Effect of AMFJ at doses of 2.5 ml kg^{-1} (AMFJ$_{2.5}$), 5 ml kg^{-1} (AMFJ$_5$), and 10 ml kg^{-1} (AMFJ$_{10}$) applied orally to rats for 14 days on the number of horizontal movements (panel A) and vertical movements (panel B) recorded every minute for a 5-min observation period. AU: arbitrary units; n=10;
—●—: Control; —■—: AMFJ$_{2.5}$; - -▲- -: AMFJ$_5$; - -○- -: AMFJ$_{10}$

2.1.2. Effect of AMFJ on the vertical movements during the first 5 min. Repeated two-way ANOVA of the number of vertical movements recorded every minute for the first 5 min did not demonstrate a significant effect of the factor dose on the 7th ($F_{3.199}$=0.88, P≤0.45) and 14th day ($F_{3.199}$=1.695, P≤0.169) and demonstrated a significant effect of the factor time on the 7th ($F_{4.199}$=21.12, P≤0.001) and 14th day ($F_{4.199}$=22.91, P≤0.001). Repeated two-way ANOVA revealed significant effects of the factors dose and time on the 21st day ($F_{3.199}$=10.750, P≤0.001; $F_{4.199=}$20.030, P≤0.001, respectively) and 30th day ($F_{3.199}$=16.805, P≤0.001; $F_{4.199=}$28,437, P≤0.0001, respectively).

Separate post-hoc *t*-test comparisons for each minute demonstrated that AMFJ applied at doses of 2.5 ml kg^{-1} and 5 ml kg^{-1} for periods of 7, 14, 21, and 30 days had no significant effect on the number of vertical movements (Figs 1B, 2B, 3B, 4B). Post-hoc *t*-test comparisons for each minute showed that AMFJ at the dose of 10 ml kg^{-1} applied for 7 and 14 days did not significantly reduce the number of vertical movements (Figs 1B, 2B) and significantly

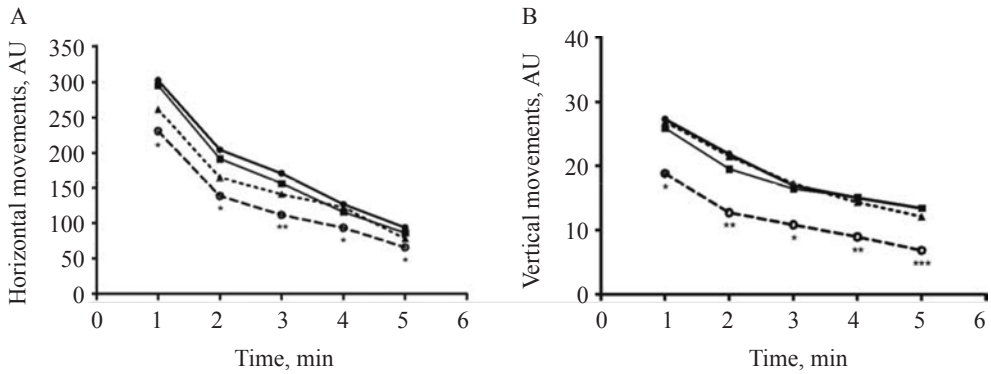

Fig. 3. Effect of AMFJ at doses of 2.5 ml kg^{-1} (AMFJ$_{2.5}$), 5 ml kg^{-1} (AMFJ$_5$), and 10 ml kg^{-1} (AMFJ$_{10}$) applied orally to rats for 21 days on the number of horizontal movements (panel A) and vertical movements (panel B) recorded every minute for a 5-min observation period. AU: arbitrary units; n=10; —●—: Control; —■—: AMFJ$_{2.5}$; - -▲- -: AMFJ$_5$; - -○- -: AMFJ$_{10}$; *P<0.05; **P<0.01; ***P<0.001 vs. the respective saline-treated control groups

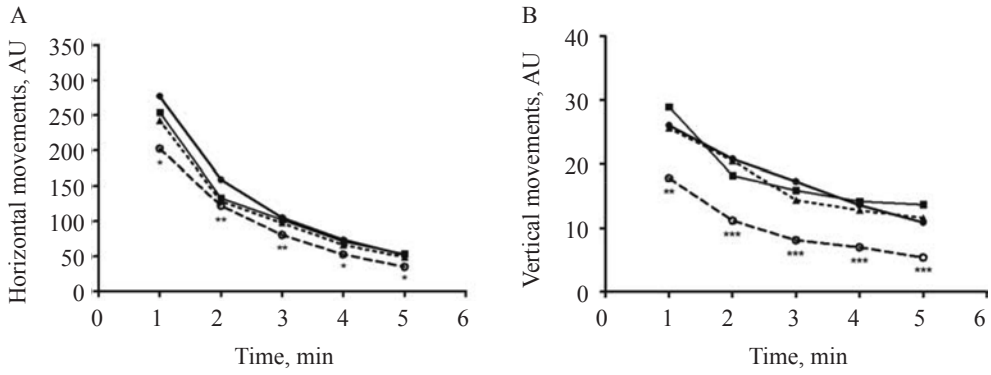

Fig. 4. Effect of AMFJ at doses of 2.5 ml kg^{-1} (AMFJ$_{2.5}$), 5 ml kg^{-1} kg (AMFJ$_5$), and 10 ml kg^{-1} (AMFJ$_{10}$) applied orally to rats for 30 days on the number of horizontal movements (panel A) and vertical movements (panel B) recorded every minute for a 5-min observation period. AU: arbitrary units; n=10; —●—: Control; —■—: AMFJ$_{2.5}$; - -▲- -: AMFJ$_5$; - -○- -: AMFJ$_{10}$; *P<0.05; **P<0.01; ***P<0.001 vs. the respective saline-treated control groups

decreased the number of these movements on the 21st day on the 1st (P<0.05), 2nd (P<0.01), 3rd (P<0.05), 4th (P<0.01), and 5th min (P<0.001) as well as on the 30th day on the 1st (P<0.01), 2nd (P<0.001) , 3rd (P<0.001), 4th (P<0.001), and 5th min (P<0.001) compared to the respective saline-treated controls (Figs 3B, 4B).

ANOVA for the vertical movements during the whole period of 5 min did not show a significant effect of the factor dose on the 7th ($F_{3.39}$=0.77, P≤0.52) and 14th day ($F_{3.39}$=0.86, P≤0.47), while on the 21st ($F_{3.39}$=4.72, P≤0.007) and 30th day ($F_{3.39}$=7,680, P≤0.0004) the factor dose was significant. The post-hoc *t*-test comparisons demonstrated that AMFJ at the dose of 10 ml kg^{-1} significantly reduced the total number of vertical movements for the period of 5 min on the 21st (P<0.001) and 30th day (P<0.001) as compared with the respective saline-treated controls (Table 1).

Table 1. Effect of AMFJ at doses of 2.5 ml kg^{-1} (AMFJ$_{2.5}$), 5 ml kg^{-1} (AMFJ$_5$) and 10 ml kg^{-1} (AMFJ$_{10}$) applied orally to rats for 7, 14, 21, and 30 days on the total number of horizontal and vertical movements, measured in arbitrary units (AU) in Opto Varimex for the whole 5-min and 10-min periods of observation. Results are presented as mean±S.E.M.; n=10; ***P<0.001, ****P<0.0001 vs. the respective saline-treated control groups

Treatment period	Groups	Horizontal movements		Vertical movements	
		5 min	10 min	5 min	10 min
7 days	Control	805.2±38.33	282.8±29.98	94.4±7.05	45.0±4.86
	AMFJ$_{2.5}$	811.8±72.67	333.1±76.86	93.5±10.77	48.0±8.88
	AMFJ$_5$	831.4±51.83	341.0±38.76	88.2±5.67	37.1±4.65
	AMFJ$_{10}$	737.9±50.68	260.5±33.42	82.8±6.62	40.1±3.64
14 days	Control	805.2±38.33	282.8±29.98	94.4±7.05	45.0±4.86
	AMFJ$_{2.5}$	832.6±39.17	318.2±41.57	87.1±6.61	44.2±5.80
	AMFJ$_5$	751.4±58.57	243.6±36.37	88.8±8.27	39.8±7.28
	AMFJ$_{10}$	769.8±77.55	297.5±37.00	77.4±8.39	38.8±4.61
21 days	Control	896.8±60.60	285.9±33.13	94.4±7.05	45.0±4.86
	AMFJ$_{2.5}$	844.4±28.32	278.1±33.47	90.3±9.87	44.7±5.40
	AMFJ$_5$	766.8±52.71	276.6±43.02	92.0±8.59	40.5±7.56
	AMFJ$_{10}$	639.4±37.30***	209.1±28.21****	58.0±5.46****	21.4±2.39****
30 days	Control	666.6±41.11	328.2±31.54	88.6±5.25	48.1±4.61
	AMFJ$_{2.5}$	613.4±34.98	311.2±53.03	90.9±8.12	46.4±4.27
	AMFJ$_5$	583.0±31.27	286.4±47.64	84.9±8.01	36.8±5.57
	AMFJ$_{10}$	492.0±23.40***	241.2±35.19****	49.5±6.31***	18.9±2.66****

These results for the horizontal and vertical movements showed that AMFJ applied orally to rats decreased the exploratory behaviour only at the highest dose of 10 ml kg^{-1} applied for 21 and 30 days.

2.2. Effect of AMFJ on locomotor activity

2.2.1. Effect of AMFJ on the horizontal movements the period of 10 min. ANOVA of the total number of horizontal movements for the period of 10 min did not demonstrate a significant effect of the factor dose on the 7th ($F_{3.39}$=0.75, P≤0.53) and 14th day ($F_{3.39}$=0.91, P≤0.45), while on the 21st ($F_{3.39}$=5.14, P≤0.004) and 30th day ($F_{3.39}$=4.74, P≤0.006) the factor dose was significant. The post-hoc *t*-test revealed that AMFJ applied to rats at doses of 2.5 and 5 ml kg^{-1} for all periods (7, 14, 21, and 30 days) had no significant effect on the horizontal activity recorded during the 10-min observation period (Table 1). The post-hoc *t*-test demonstrated that AMFJ at the dose of 10 ml kg^{-1} caused a decrease in the horizontal movements on the 21st (P<0.0001) and 30th day (P<0.0001) in comparison with the saline-treated controls (Table 1).

2.2.2.Effect of AMFJ on the vertical movements during the period of 10 min. ANOVA of the total number of vertical movements for the period of 10 min did not demonstrate a significant effect of the factor dose on the 7th ($F_{3.39}$=0.77, P≤0.52) and 14th day ($F_{3.39}$=0.86, P≤0.47), while on the 21st ($F_{3.39}$=7.41, P≤0.0005) and 30th day ($F_{3.39}$=16.80, P≤0.0001) the factor dose was significant. Post-hoc *t*-test comparisons demonstrated that AMFJ applied to rats at doses of 2.5 and 5 ml kg^{-1} for all periods (7, 14, 21, and 30 days) did not induce significant changes in the vertical movements (Table 1). Post-hoc *t*-test comparisons showed that AMFJ at the dose of 10 ml kg^{-1} significantly reduced the number of vertical movements on the 21st (P<0.0001) and 30th day (P<0.0001) (Table 1).

In the present study, exploratory behaviour and locomotor activity of rats were tested when the animals were placed in an environment that was unfamiliar to them – the chamber of the Opto Varimex apparatus. This test is a common measure of exploratory behaviour and general activity in rodents (Gould et al., 2009). The expected pattern of behaviour is that animals will tend to highly explore the novel arena initially, and eventually habituate to the environment (Daenen et al., 2001). Exploration has been defined as active investigation (e.g., locomotion) that might lead an animal to gain information about its environment (Lynn & Brown, 2009). The short length of time emphasizes exploratory behaviour (Gould et al., 2009).

The present study showed that AMFJ applied at doses of 2.5 and 5 ml kg^{-1} for 7, 14, 21, and 30 days did not significantly affect the exploratory behaviour and locomotor activity of rats. At the highest dose of 10 ml kg^{-1} applied for 21 and 30 days, AMFJ inhibited the exploratory behaviour and reduced the horizontal and vertical locomotor activity. That effect of AMFJ is probably not due to toxic effects as the weight gain of rats was similar across treatments (data not shown). There are also no literature data for toxic effects of *Aronia melanocarpa* fruit and juice. The observed effects of AMFJ on locomotor activity are probably due to the activity of its ingredients – flavonoids, mainly from the subclass of anthocyanins, and other polyphenols. Decrease in spontaneous motor activity, such as ambulation and rearing might result from reduced excitability of the central nervous system and sedation (Prut & Belzung, 2003). The brain GABAergic system is responsible for sedation and depressive behaviours. There are data that flavonoid compounds may interact with the $GABA_A$ receptors, (Fernandez et al., 2009), thus producing sedation, anxiolytic or anticonvulsive effects **(**Jäger & Saaby, 2011**)**. The sedative and partly the anticonvulsant actions are attributed to the activation of α_1-containing receptors, while suppression of anxiety – to the α_2/α_3 subtypes (Rudolph & Möhler, 2006). Sedative effects have been demonstrated for flavonoids (Martínez et al., 2009; Vissiennona et al., 2011) and plant extracts containing procyanidins, flavonoids, and other polyphenols (Jiang et al., 2007). There are also data that flavonoids may have anxiolytic and sedative effects that could be mediated by activation of GABAergic nonbenzodiazepine binding sites (de Carvalho et al., 2011).

Flavonoids are important components of AMFJ. We could suppose that the possible sedative effect of the highest AMFJ dose in the present study might be due to the activation of α_1-containing $GABA_A$ receptors occurring with the accumulation of flavonoids and polyphenols in the brain following long-term consumption (Willis et al., 2009) or could be mediated by GABAergic nonbenzodiazepine binding sites.

In the present study, the progressive decrease in motor behaviour, suggestive of habituation, was similar across groups. Habituation to a novel environment is believed to be one of the most elementary forms of learning, in which the decreased exploration is taken as an index of memory (Thiel et al., 1999).

3. Conclusions

The results showed that AMFJ applied orally to rats for 7, 14, 21, and 30 days at doses of 2.5 and 5 ml kg^{-1} had no effect on exploratory behaviour and locomotor activity. After 21 and 30 days of treatment, AMFJ at the highest dose (10 ml kg^{-1}) decreased the number of horizontal and vertical movements, which might be a result of a sedative effect. At all the doses and

testing periods, AMFJ did not disturb the progressive decrease in motor behaviour, suggesting habituation.

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