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MEMORY EFFECTS OF *ARONIA MELANOCARPA* FRUIT JUICE IN A PASSIVE AVOIDANCE TEST IN RATS

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ЭФФЕКТ ФРУКТОВОГО СОКА *ARONIA MELANOCARPA* НА ПАМЯТЬ КРЫС В ТЕСТЕ ПАССИВНОГО ИЗБЕЖАНИЯ

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ABSTRACT

Aim: To study the effect of *Aronia melanocarpa* fruit juice on memory in male Wistar rats. **Materials and methods:** The juice was administered orally for 7, 14, 21 and 30 days at doses of 2.5 ml/kg, 5 ml/kg and 10 ml/kg. Memory was assessed in the one-way passive avoidance task (step through) which consisted of one training session and two retention tests (3 hours and 24 hours after training). The variables measured were the latency time to step into the dark compartment of the apparatus and the learning criterion (remaining in the illuminated compartment for at least 180 sec). **Results:** Oral administration of *Aronia melanocarpa* fruit juice for 7 and 14 days resulted in a dose-dependent tendency to increase the latency time and the learning criterion compared to saline-treated controls but the effect failed to reach statistical significance. After 21 days of treatment, the juice dose-dependently prolonged the latency time at the retention tests, the effect being significant at doses of 5 ml/kg and 10 ml/kg. Applied for 30 days, the juice in all the tested doses increased significantly the latency time at the retention tests and the dose of 10 ml/kg significantly increased the percentage of rats reaching the learning criterion. **Conclusion:** These findings suggest that *Aronia melanocarpa* fruit juice could improve memory in rats. The effect is probably due to the polyphenolic ingredients of the juice which have been shown to be involved in learning and memory processes.

Key words: *Aronia melanocarpa, memory, passive avoidance, rats*

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РЕЗЮМЕ

Цель: Настоящая работа ставит себе целью исследовать эффект сока *Aronia melanocarpa* на память мужских крыс породы Wistar. **Материалы и методы:** Плодовый сок *Aronia melanocarpa* применен перорально в течение 7, 14, 21 и 30 дней в дозах 2.5 мл/кг, 5 мл/кг и 10 мл/кг. Память оценивали с помощью метода обучения однонаправленному пассивному избежанию - step through, состоящему из одной тренировочной сессии и двух тестов, предназначенных для определения памяти (3 часа и 24 часа после тренировки). Прослежены латентное время перехода в темное отделение аппарата и критерий обученности (оставание в освещенное отделение не менее 180 секунд). **Результаты:** Применен в течение 7 и 14 дней сок *Aronia melanocarpa* вызывает доза-зависимую тенденцию к повышению латентного времени и критерия обученности по сравнению с контрольной группой крыс, получившей физиологический раствор, но эффект оказался статистически незначительным. После применения сока в течение 21 дня доза-зависимо удлиняет латентное время в тестах для определения памяти, при чем эффект статистически значим при дозах 5 мл/кг и 10 мл/кг. После 30-идневного применения все исследованные дозы сока значимо удлиняют латентное время в тестах для определения памяти, а доза 10 мл/кг значимо увеличивает и процент крыс, достигших критерия обученности. **Заключение:** Полученные авторами результаты показывают, что сок *Aronia melanocarpa* может улучшить память крыс. Этот эффект вероятно можно объяснить полифенольными составляющими сока, о которых доказано, что участвуют в процессах обучения и памяти.

Ключевые слова: *Aronia melanocarpa, память, пассивное избежание, крысы*

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INTRODUCTION

Aronia melanocarpa [Michx.] Elliot (black chokeberry) originates from the eastern parts of North America and East Canada. Around 1900 the plant migrated to Europe and Russia. *Aronia* fruits are commonly used for production of juice, syrup, jellies, tea and wine. Chokeberry fruits are extremely rich in polyphenols.[1] These polyphenols are flavonoids (mainly from the subclass of anthocyanins), procyanidins and phenolic acids.

Several studies have shown that flavonoids and other fruit- and vegetable-derived phytochemicals have beneficial effects on learning and memory. Berry fruit supplementation has continued to demonstrate efficacy in reversing age-related cognitive decline in animal studies.[2]

In aged rats, anthocyanins from blueberry have been found in the cerebellum, cortex, hippocampus or striatum in their unmetabolized forms.[3] These findings are the first to suggest that polyphenolic compounds are able to cross the blood brain barrier and localize in various brain regions important for learning and memory. Interestingly, Williams et al.[4] reported that flavanol levels were higher than anthocyanin levels in brain tissue of aged rats supplemented with blueberries.

A number of animal studies have demonstrated that berries[2,5,6] as well as pure flavonoids[7], are capable of affecting several aspects of memory and learning.

Most studies have been carried out on aged animals and in experimental models of memory impairment. To date, there have been only a limited number of studies investigating the effects of flavonoid-rich foods on cognition in young/healthy animals.[6] So far, *Aronia melanocarpa* (chokeberry) fruits have not been investigated for their effect on memory.

AIM

The aim of the present study was to investigate the effect of *Aronia melanocarpa* fruit juice (AMFJ) on memory in male young/healthy rats using the one-way passive avoidance task (step through).

MATERIALS AND METHODS

Aronia melanocarpa fruit juice (AMFJ) preparation

AMFJ was produced from *Aronia melanocarpa* (Michx.) Elliot fruits 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[8]; total flavonoids, 189.4 ± 8.6 mg as catechin equivalents, measured by a colorimetric assay developed by Zhisten et al.[9]; 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[10]; quercetin, 11.8 ± 0.8 mg, measured by a high-performance liquid chromatography method[11]. The values were the mean of duplicate determinations of three samples.

Animals and treatment

Male Wistar rats (200-240 g at baseline) 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 of 1986 (86/609/EEC).

The experiments were performed on 224 rats divided into 16 groups of 14 animals each. The animals were treated orally through an orogastric cannula for 7 days (one week), 14 days (two weeks), 21 days (three weeks) or 30 days (one month). Rats from the AMFJ groups were treated with AMFJ at doses of 2.5 ml/kg, 5 ml/kg and 10 ml/kg. The doses of 2.5 ml/kg and 5 ml/kg were diluted with distilled water to a total volume of 10 ml/kg. The control groups were treated with saline (10 ml/kg). There were four groups of rats for each treatment period: Control, $AMFJ_{2.5}$, $AMFJ_5$, and $AMFJ_{10}$ (the index indicates AMFJ dose).

One-way passive avoidance task – step through

In the passive avoidance test in order to avoid a mild foot shock, the rat must learn to remain in a brightly lit compartment and not enter the preferred dark compartment. The passive avoidance task was carried out using a step through apparatus. The apparatus had two chambers: a dark one (30 x 30 x 30 cm) with metal grid floor and a brightly illuminated (100 W) one (8 x 7 x 30 cm). The two chambers were separated by a guillotine-type door. One training trial and two retention tests were conducted according to the method of Gozzani and Izquierdo.[12] The training trial started by placing the rat in the brightly lit compartment. Once the rat entered the dark compartment, the guillotine door was closed and an electric shock (0.30-0.35 mA for 3 sec) was delivered to the animal through the

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grid floor. Each rat underwent one trial. Retention tests (no shocks) were performed 3 hrs and 24 hrs after the acquisition trial. At that time, the animal was returned to the illuminated compartment, and the step-through latency was estimated by measuring the time (latency time) for the rat to move to the dark compartment. A latency of at least 180 sec was used as a criterion for learning. The last AMFJ application was 60 min before the training trial. The juice was not given to the rats before the two retention tests. Before each test, the apparatus was wiped clean and dried. Experiments were performed between 9.00 h and 13.00 h.

Statistical analysis

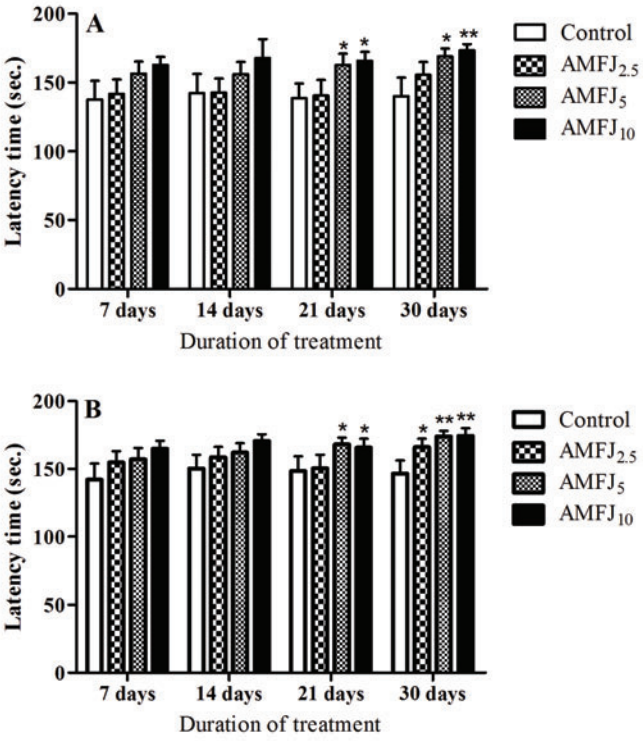
Results are presented as mean ± S.E.M. One-way ANOVA was used for the latency time in the retention tests. Findings from the ANOVA were post-hoc analyzed using the Student-Newman-Keuls test. A level of $p < 0.05$ was considered significant. Analysis of the data for the learning criterion was performed using χ^2 test. GraphPad Prism statistical software was used.

RESULTS

AMFJ oral administration for 7 and 14 days, at all the tested doses (2.5 ml/kg, 5 ml/kg and 10 ml/kg) resulted in a dose-dependent tendency to increase the latency time at the retention tests at 3 and 24 hrs but the effect was not statistically significant (Fig. 1). The effect of the juice on the learning criterion at the retention tests for these treatment periods was also not significant (Table 1).

After 21 days of application, AMFJ at a dose of 2.5 ml/kg did not significantly affect the latency time and the learning criterion at the retention tests (Fig. 1, Table 1). Applied for 21 days, the dose of 5 ml/kg significantly increased the latency time at the retention tests at 3 hrs ($t = 1.786$, $p \leq 0.04$) and 24 hrs ($t = 1.637$, $p \leq 0.05$) (Fig. 1) but did not have a significant effect on the percentage of rats that reached the learning criterion (Table 1). The dose of 10 ml/kg received for 21 days significantly increased the latency time at the retention tests at 3 hrs ($t = 2.182$, $p \leq 0.02$) and 24 hrs ($t = 1.707$, $p \leq 0.05$) (Fig. 1) without a significant effect on the percentage of rats reaching the learning criterion (Table 1).

Administered for 30 days at a dose of 2.5 ml/kg, AMFJ significantly increased the latency time



Results are presented as mean ± S.E.M.; n = 14; *p < 0.05 vs. control; **p < 0.01 vs. control

Figure 1. Effect of AMFJ on the latency time during the retention tests at 3 hours (A) and 24 hours (B) in the passive avoidance task (step through).

Table 1. Effect of AMFJ administered for 7, 14, 21 and 30 days on the learning criterion of rats during the retention tests 3 hrs and 24 hrs after training (n = 14; *p < 0.05 vs. control)

Groups	Learning criterion (% of rats)							
	7 days		14 days		21 days		30 days	
	3 hrs	24 hrs	3 hrs	24 hrs	3 hrs	24 hrs	3 hrs	24 hrs
Control	50	50	57	57	43	50	50	50
$AMFJ_{2.5}$	50	50	50	57	50	57	57	64
$AMFJ_5$	50	57	64	64	57	64	79	86*
$AMFJ_{10}$	57	64	71	71	64	71	86*	93*

only at the retention test at 24 hrs ($t = 1.679$, $p \leq 0.05$) (Fig. 1) and did not significantly affect the learning criterion (Table 1). At a dose of 5 ml/kg for that treatment period, AMFJ significantly increased the latency time at the retention tests at 3 ($t = 1.954$, $p \leq 0.03$) and 24 hrs ($t = 2.349$, $p \leq 0.01$) (Fig. 1) as well as the percentage of rats that reached the learning criterion at 24 hrs ($\chi^2 = 4.094$, $p \leq 0.05$) (Table 1). After a treatment period of 30 days at a dose of 10 ml/kg, AMFJ significantly increased the latency time at the retention tests at 3 hrs ($t = 2.349$, $p \leq 0.01$) and 24 hrs ($t = 2.487$, $p \leq 0.01$) (Fig. 1) and also significantly increased the number of rats reaching the learning criterion at 3 hrs ($\chi^2 = 4.094$, $p \leq 0.05$) and 24 hrs ($\chi^2 = 6.301$, $p \leq 0.02$) (Table 1).

DISCUSSION

The passive avoidance task is a one trial fear-motivated avoidance task in which the rat learns to refrain from stepping through a door to an apparently safer but previously punished dark compartment. The latency to refrain from crossing into the punished compartment serves as an index of the ability to avoid, and allows memory to be assessed.

Latency time, the time required to re-enter the dark room again, is influenced essentially by the basic movement of animals and their learning ability. At the doses and treatment durations used in the present study, AMFJ had no significant effect on the locomotor activity (unpublished results). Thus, the latency time determined in this study reflected predominantly the memory functions of the rats.

The biological actions of AMFJ are probably due to its polyphenolic ingredients. In this study, the memory of rats tested by the passive avoidance task was improved by AMFJ, especially for the treatment periods of 21 and 30 days. These data are in accordance with previous findings that flavonoids and other polyphenols from berries do accumulate in the brain following long-term consumption.[2]

There have been studies demonstrating that some of the biological effects of polyphenols on the brain are due to their antioxidant actions, through their ability to scavenge reactive species, to induce antioxidant enzymes and to reduce oxidative damage to cellular components.[5,13] Indeed, AMFJ has been shown to possess pronounced antioxidant and radical scavenging activities[1,14] which might contribute to its beneficial effects on memory.

However, this classical antioxidant activity probably does not account for all biological actions of flavonoids in vivo, particularly in the brain, where they are found at only very low concentrations.[15] Emerging findings suggest a variety of potential mechanisms of action of flavonoids and their bioavailable metabolites in cytoprotection against oxidative stress, which may be independent of conventional antioxidant activities.[15] Flavonoids induce beneficial effects on the vascular system leading to changes in cerebrovascular blood flow capable of causing angiogenesis, neurogenesis and changes in neuronal morphology.[7] Perez-Vizcaino et al.[16] have suggested that the improvement of cerebrovascular blood flow by quercetin might be due to its ability to cross brain endothelium where it is likely to exert an endothelium independent vasodilatory effect. This effect may result from inhibition of protein kinases such as myosin light chain kinase and, possibly, other kinases involved in Ca^{2+}-sensitizing mechanisms.[16] This vasodilatory effect may lead to enhanced cerebrovascular blood flow and improved memory functions.

In vitro work has indicated that flavonoids and their physiological metabolites are capable of activating signaling pathways, critical in controlling synaptic plasticity,[17] but only at low nanomolar concentrations similar to those reported in the brain. Such signaling pathways are the extracellular receptor kinase (ERK) and protein kinase B/Akt pathways.[18] These pathways are known to be critical in controlling the morphological mechanisms behind memory storage in the hippocampus and cortex of the brain. Flavonoids have the potential to enhance memory and learning by activating kinases within these pathways. One way they act is by regulating proteins such as the cAMP response element-binding protein (CREB), which is involved in the expression of important genes linked to memory. For example, CREB is crucial for the production of neurotrophins such as the brain-derived neurotrophic factor (BDNF), which are known to be required during memory acquisition and consolidation.[19] Rendeiro et al.[6] suggest that consumption of flavonoid-rich blueberries has a positive impact on spatial learning performance in young healthy animals, and these improvements are linked to the activation of ERK-CREB-BDNF pathway in the hippocampus.

The central cholinergic system is essential for the regulation of cognitive functions. There are data that polyphenols are able to inhibit acetylcholinesterase activity[5,13] and to restore acetylcholine brain contents in cognitively impaired rats.[20]

From the review of literature, it is clear that

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plant polyphenolic substances which are components of *Aronia melanocarpa* fruits, might improve memory by several mechanisms: antioxidant activity, vascular effects, activation of signaling pathways and inhibition of acetylcholinesterase activity. The exact mechanism of AMFJ-induced improvement of memory in rats remains to be elucidated.

CONCLUSIONS

The present results showed that AMFJ, administered for 21 and 30 days, improved memory in young/healthy male rats. This effect was more pronounced after 30 days of treatment at doses of 5 ml/kg and 10 ml/kg, i.e. the effect was time- and dose-dependent. However, further research is required to find out the exact mechanisms of this effect.

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