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Effects of Rhodiola rosea extract on passive avoidance tests in rats

Research Article

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Abstract: The purpose was to evaluate the effects of extract of Rhodiola rosea on learning and memory processes on rats. The two series of experiments were carried out - on naïve rats and on rats with scopolamine-impaired memory. The passive avoidance tests were performed - step-down and step-through. The latency of reactions in seconds was observed in both tests. Naïve rats treated with the extract in showed the prolongation of latency of reaction of both step-down and step-through passive avoidances compared to the controls. Rats with scopolamine-impaired memory showed shorter latency of reaction in both passive avoidance tests compared to the controls. Rats treated with the extract of Rh. Rosea with scopolamine-impaired memory prolonged the latency in both passive avoidance tests compared to scopolamine group. It was found that the extract of Rh. Rosea improved performance during learning session, short and long memory retrieval tests in naïve rats. Scopolamine impaired the learning and memory retrieval of rats, but Rh. Rosea pretreatment improved performance and turned off the deterioration effect of scopolamine on these brain functions probably by non-specific mechanisms on cholinergic neurons. The studied plant extract can be a candidate for treatment of dementia and other memory disturbances.

Keywords: *Extract of Rhodiola rosea • Learning and memory processes • Passive avoindance tests • Step-down • Step-through • Scopolamine-impaired memory*

1. Introduction

Rhodiola rosea L. of the family of Crassulaceae, or "golden root", is a popular plant in traditional medicine in Eastern Europe. It has a reputation for improving depression, enhancing work performance and abolishing symptoms of physical and psychological stress [1]. Because of these therapeutic properties, *Rh. rosea* is considered to be one of the most active adaptogenic drugs. *Rh. rosea* ingestion can improve cognitive function and reduce mental fatigue; it has an anti-oxidative effect and increases learning and memory [2]. In total, approximately 140 compounds were isolated from its roots and rhizome – glycosides, flavonoids, phenols, proauthocyanidins and gallic acid derivatives [3]. The main bioactive compounds of *Rh. rosea* have been reported to be rosavine, salidroside, rosin and some essential oils [4]. The extract used in the present was made from a cultivated plant; in the Bulgarian mountains Rhodope has mainly phenols and flavonoids [5].

The cholinergic neurotransmitter system in the brain plays a significant role in learning, memory and attention processes [6]. There are data, that learning and memory are closely related to cholinergic muscarinic and nicotinic receptor modulation [7].

Scopolamine is an alkaloid drug. It is among the secondary metabolites of plants from the Solanaceae (nightshade) family of plants, such as henbane, jimson weed and Angel's Trumpets. Scopolamine as a non-selective muscarinic receptor antagonist blocks cholinergic neurotransmission and impairs behavioral function, including learning and memory processes [8].

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The scopolamine model is used extensively for preclinical testing of new substances designed to treat cognitive impairment [9].

2. Materials and methods

In this study, 70 male Wistar rats with 180–220 g body weight were used, divided in 2 series, with 3 groups in the first series and 4 groups in the second (n = 10). The rats were kept under standard laboratory conditions in an 08:00–20:00 h light/dark cycle and were provided with food and water ad libitum. The extract from *Rh. Rosea* was applied per gavage.

The extract of *Rh. rosea* we studied was evaluated by the research group of Phytochemistry Division in Institute of Botanic, Bulgarian Academy of Science, leaded by Assoc. Prof. Luba Evstatieva [10,11]. The method used was HPLC on Aligent 1100 Series HPLC System with MWD UV-Vis detector. The extract was 80/20% water/alcohol, as described previously.

All experiments were carried out according to the guidelines for using laboratory animals in EU and Bulgaria. The permission for the study was obtained by the Bulgarian Food Safety Agency and the Ethic Committee of Medical University Plovdiv.

Two experimental series were studied:

1. The first experimental series: rats with normal memory.

The three groups were:

a) saline 0.1 ml/100 gr b.w. per os;

b) Rh. rosea extract at a dose of 50 mg/kg per os;

c) Rh. rosea extract at a dose of 100 mg/kg per os;

2. The second experimental series: rats with scopolamine impaired memory.

The four groups were:

a) saline 0.1 ml/100 gr per os;

b) scopolamine 1 mg/kg intraperitoneally (i.p);

c) Rh. rosea extract at a dose of 50 mg/kg per os + scopolamine 1 mg/kg (i.p);

d) Rh. rosea extract at a dose of 100 mg/kg per os + scopolamine 1 mg/kg (i.p);

Scopolamine was applied after the extract application. Both scopolamine and the extract were applied every day during the experiment.

Two passive avoidance tests were used: step-down and step-through.

The step-down passive avoidance test was performed in a set-up single-compartment cage with a plastic platform (Ugo Basile). Learning and retention sessions consisted of two trials (electrical stimulation duration of 10 sec. with intensity of 0.4 mA). The latency of reactions (the rat remaining on the platform for more than 60 sec.) was accepted as the criterion for learning and retention.

The step-through passive avoidance test was performed in an automatic set-up two-compartment cage (Ugo Basile). Learning and retention sessions consisted of three trails (door delay 7 sec., followed by electrical stimulation for 9 sec. at the intensity of 0.4 mA). The latency of reactions (the animal remaining in the light chamber for more than 180 sec.) was used as criterion for learning and retention.

In both tests, short memory retention was evaluated 24 hours after a two-day learning session. Memory retention (long memory) was evaluated on 9th day from the first day of learning for step-down test and on 11th day from the first day of learning for step-through test [12].

Statistical analysis: The values obtained were expressed as mean ± S.E.M. The comparison between groups was made by Student's t-test analysis of variance (ANOVA) in the Excel and Instat computer programs. A value of $P<0.05$ was considered as a significant difference.

3. Results

3.1. Effects of Rh. rosea extract on non-impaired memory.

In the step-down passive avoidance test, control rats significantly prolonged ($P<0.05$) the latency of reaction in short (3rd day) and long (9th day) memory tests compared with the 1st day control group (Figure 1). The animals treated with both doses *Rh. rosea* prolonged significantly ($P<0.05$) the latency of reaction on the 2nd day of learning; short and long memory retention test compared to the 1st day of the respective group and compared to the same day controls (Figure 1).

In step-through passive avoidance tests, control rats significantly prolonged ($P<0.05$) the latency of reaction in short (3rd day) and long (12th day) memory tests compared to the 1st day control group (Figure 2). Both groups of *Rh. rosea* treated rats prolonged the latency ($P<0.05$) on the 1st day of learning, compared with the control and on the 2nd day of learning, short and long memory retention test compared with the 1st day of respective group and compared with the same day controls (Figure 2).

3.2. Effects of *Rh. rosea* extract on scopolamine-impaired memory.

In the step-down passive avoidance test, rats treated with scopolamine significantly ($P<0.05$) shortened the latency of reaction during the days of learning and the

Figure 1. Effects of Rhodiola rosea extract on step-down test in naïve rats. Abscissa: 2-day learning session, short and long memory retrieval. Ordinate: the latency of reaction in seconds. ° P<0.05 compared to the first day control; * P<0.05 compared to the first day of respective group; + P<0.05 compared to the same day control.

Fig 1

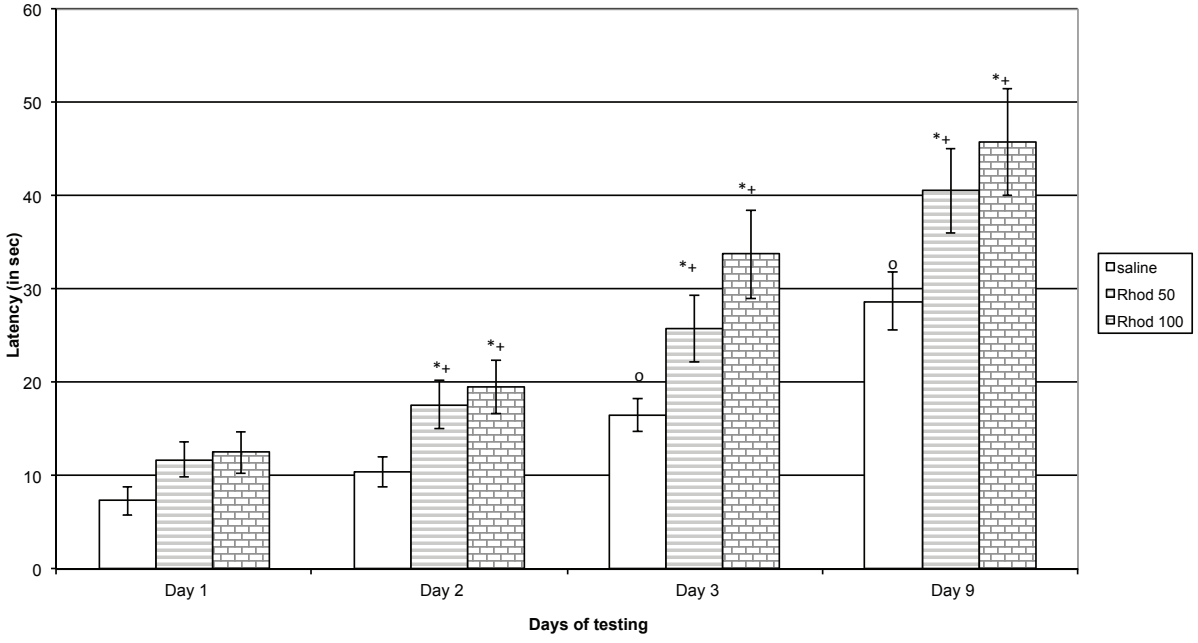


Figure 2. Effects of Rhodiola rosea extract on step-through test in naïve rats. On the Abscissa: 2-day learning session, short and long memory retrieval. Ordinate: the latency of reaction in seconds. ° P<0.05 compared with the first day control; * P<0.05 compared with the first day of respective group; + P<0.05 compared with the same day control.

Fig. 2

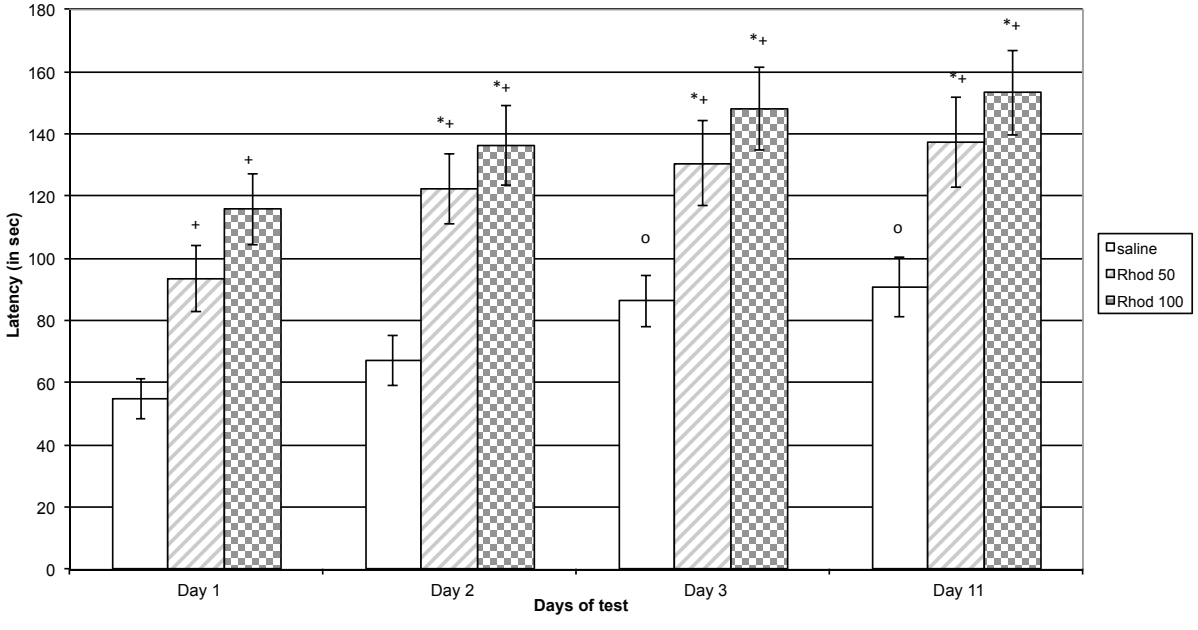


Figure 3. Effects of *Rhodiola rosea* extract on step-down test in rats with scopolamine-impaired memory. Abscissa: 2-day learning session, short and long memory retrieval. Ordinate: the latency of reaction in seconds. ° P<0.05 compared with the first day control; * P<0.05 compared with same day scopolamine group.

Fig. 3

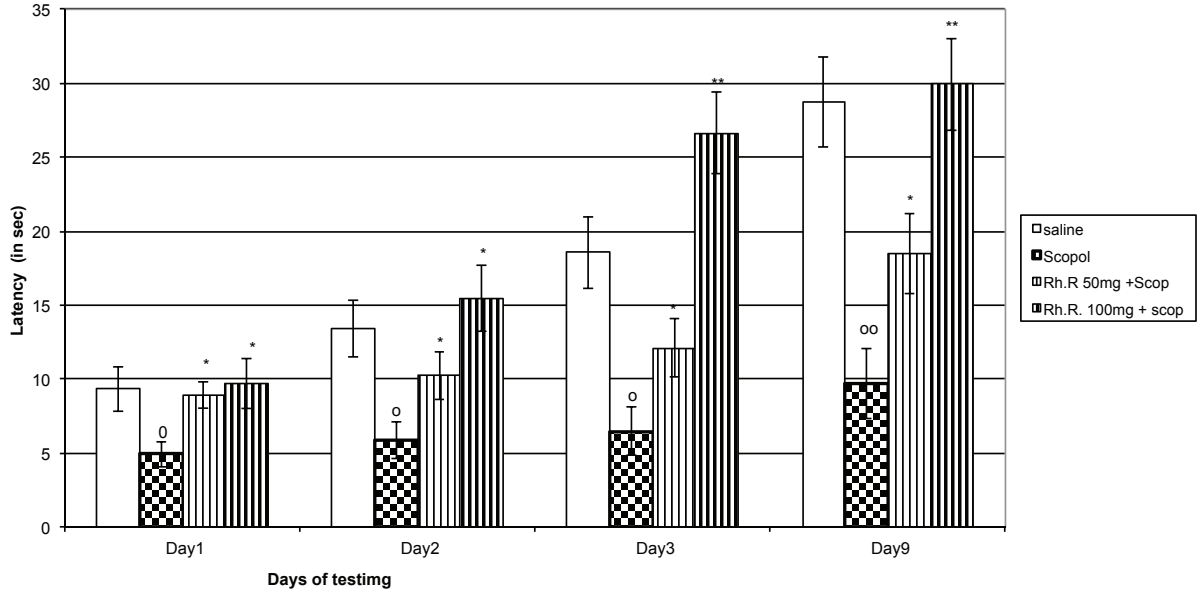
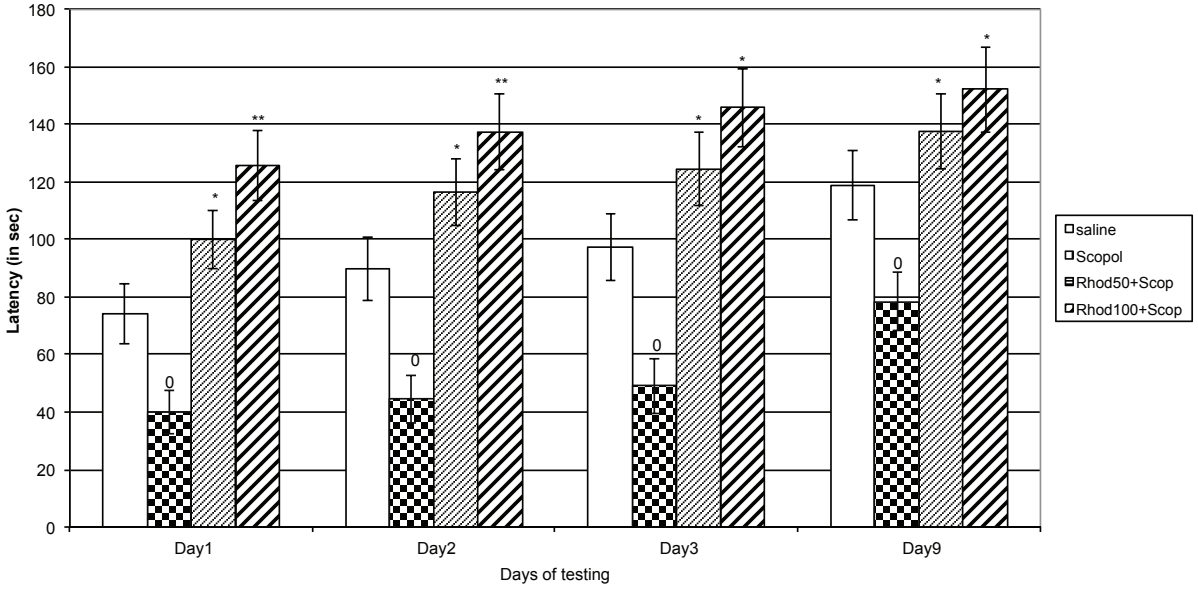


Figure 4. Effects of *Rhodiola rosea* extract on step-through test in rats with scopolamine-impaired memory. On the Abscissa: 2 days learning session, short and long memory retrieval. Ordinate: the latency of reaction in seconds. ° P<0.05 compared with the first day control; * P<0.05 compared with the same day of scopolamine group.

Fig.4



short (3rd day) and long (9th day) memory tests compared with the same-day saline group (Figure 3). The animals treated with both doses of *Rh. rosea* extract and scopolamine prolonged significantly (P<0.05) the latency of reaction in the 1st and 2nd day of learning, short and long memory retention test compared with the same day scopolamine group (Figure 3).

In the step-through passive avoidance test, scopolamine-treated rats significantly shortened (P<0.05) the latency of reaction during the learning session, and in short (3rd day) and long (9th day) memory tests compared with the same-day saline group (Figure 4). All rats received an extract of *Rh. rosea* at 50 mg/kg and 100 mg/kg and scopolamine prolonged the latency (P<0.05) during the learning sessions, short and long memory retention test compared with the same day scopolamine group (Figure 4).

4. Discussion

The extract from *Rh. rosea* L. in our experiments showed a positive effect of improvement of learning and memory processes in native rats in both passive avoidance tests. Data from another study shows that extracts from *Rh. rosea* possess effects related to memory stimulation and learning improvement, central nervous system stimulation and decreasing stress levels [13]. These authors pointed out that many health benefits are related to the *Rh. rosea* L. extract: it is known to modulate stress responses, improve exercise endurance and fatigue and elicit neuro-protective effects in vivo and in vitro. It was found that at doses up to 500 mg/kg, the extract of *Rh. rosea* increased the body weight of rats of both sexes and apparently without any changes in their organ/body weight ratio [14].

There is a tradition of study the extract from *Rh. rosea* L root on learning and memory in our country. More than 25 years previously, experiments were performed on naïve rats using a modified water maze method and staircase method, and favorable effects were found [15]. Those authors hypothesized that ingredients in the extract from *Rh. rosea* may increase learning and memory processes in rats. Present experimental practice is to perform experiments using different methods as passive avoidance tests; the extract was made from a cultivated plant that has another composition of polyphenols [11].

Our results also showed that the extract of *Rh. rosea* led to improvement of learning and memory processes in a scopolamine-impaired memory model.

Other authors have found that *Rh. rosea* extract protects rats against cognitive deficits, neuronal injury and oxidative stress [2]. There have been reports that bioactive compounds in *Rh. rosea* could reduce oxidative stress, possibly via a reactive oxygen species-scavenging capability and the enhancement of antioxidant defense mechanisms [16]. These data support our findings for improvement of learning and memory by a short *Rh. rosea* treatment, probably due to its anti-oxidative and neuroprotective effects.

Performance of a working memory task is significantly more sensitive to disruption of cholinergic mechanisms in the hippocampus than performance of reference memory task [17].

Since the scopolamine-induced amnesia was assumed to result from blockade of cholinergic neurotransmission, this substance was used to model the cognitive deficits that could be observed in aging and dementia. Scopolamine appears to be a non-selective muscarinic receptor antagonist; it has been demonstrated that scopolamine has a high selectivity for the muscarinic receptor, although it has also been reported that high doses of scopolamine are also blocking nicotinic receptors [18].

Our results have demonstrated that *Rh. rosea* extract diminishes the effects of scopolamine on cholinergic neurotransmission in the brain and improves the learning and memory. The extract lessened the memory dysfunction induced by scopolamine.

The *Rh. rosea* extract has significant free-radical scavenging activity and contains a variety of antioxidant compounds. It has been shown that P-tyrosol is absorbed in a dose-dependent manner and appears to produce a significant antioxidant. *Rh. rosea* may play a role in amelioration of cognitive deficits via its antioxidant potential and neuroprotective proteins [19].

Other authors have investigated the therapeutic effects of a 4-week treatment with *Rh. rosea* extract in subjects with life-stress symptoms: all tests showed clinically relevant improvements with regard to stress symptoms, disability, functional impairment and overall therapeutic effect. Improvements were observed even after as few as 3 days of treatment [20].

Having in mind the data above and considering our results, we may conclude the *Rh. rosea* extract in both experimental series improved learning and memory performance in rats with scopolamine-induced amnesia, probably a result of decrease of oxidative stress by antioxidant defense mechanisms. We suggest that this effect of *Rh. rosea* involved modulation of cholinergic neurotransmission, and that it may be use as a potential agent in treatment of neurodegenerative diseases such as Alzheimer's disease [2] and other type of dementia and memory deterioration.

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