**Rhodiola rosea L- Standardized Extract on Passive Avoidance in Rats**

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**Abstract**

**Background:** *Rhodiola rosea* L. originates from the plant family Crassulaceae and is known as an adaptogen increasing resistance to various stressors. The present study aimed to evaluate the effects of a commercially available standardized *Rhodiola rosea* L. extract administered intraperitoneally on cognitive processes in naive rats by using two passive avoidance tests.

**Methodology/Principal findings:** Male Wistar rats were pre-treated for 10 days with extract from Rhodiola in doses 50 mg/kg or 100 mg/kg. Step-through and step-down tests were used. Following two days of training memory retention tests were performed on the 3rd and 10th day. A criterion for step-through test was latency of reaction 180 s and for step-down test - latency of reaction 60 s. The statistical evaluation was done in SPSS17.0. In step-down test rats with both doses of extract decreased latency time during learning session and on long memory retention test, compared to the same day controls. In step-trough test rats with the high dose of the extract decreased significantly the latency time during two days of learning session and on short and long memory retention tests, compared to the same day controls.

**Conclusions:** Based on our results it can be speculated that the studied extract of Rhodiola influences passive avoidance condition stimuli reactions in dose-dependent manner. To clarify the exact influence of the studied extract more behavioral tests should be performed with higher doses of the studied extract. Our observations reveal some stimulating effect of Rhodiola standardized extract on the formation of memory traces.

**Keywords**
Adaptogen, Rhodiola, Conditioning, Passive avoidance, Rats.

1. **Introduction**

Adaptogenic substances are stated to have the capacity to normalize body functions and strengthen systems compromised by stress. They have a protective effect on health against a wide
variety of environmental assaults and emotional conditions (1). *Rhodiola rosea* L. belongs to the plant family Crassulaceae and is mainly known as an adaptogen increasing resistance to the harmful effects of various stressors (2). In traditional medicine in Eastern Europe and Asia it is associated with properties like stimulating the nervous system, decreasing depression, enhancing working performance, eliminating fatigue and preventing high-altitude sickness (3). It was suggested that its administration can improve cognitive functions, has antioxidative and neuroprotective effects and increase learning and memory (4-7). *Rhodiola rosea* L. is believed to have potential in treating mental disorders such as Alzheimer’s due to its mental and memory enhancing abilities (5-7). Salidroside is known as its major active ingredient and is well studied for possessing many beneficial effects itself such as antioxidant and neuroprotective properties in vivo (8-10) and protective against beta amyloid-induced cognitive impairment in vitro (11). *Rhodiola* L. appears to modulate the levels of biogenic monoamines, such as serotonin (5-HT), dopamine (DA), noradrenaline (NA), and the opioid peptides, such as the beta-endorphin (12). In most of the studies, either clinical trials or animal studies, *Rhodiola* L. extract was administered orally (2-7).

The present study aimed to evaluate the effects of a commercially available standardized *Rhodiola rosea* L. extract administered intraperitoneally on learning and memory processes in naive rats by using two passive avoidance tests.

2. Materials and Methods

2.1. Animals

Twenty-four male Wistar rats with weight 200 ±20 g were used. The rats were divided into three groups (n=8) and pre-treated daily for 10 days before the tests intraperitoneally as follows: Control group - Saline 1 ml/kg; *Rhodiola* L. extract-50 mg/kg; *Rhodiola* L. extract-100 mg/kg. Before administration the extract was suspended in saline as vehicle. The animals were kept under standard laboratory conditions in 08:00-20:00 h light/dark cycle and were provided with food and water and libitum (13). All experiments were carried out according to the guidelines for using laboratory animals in the EU-European community guidelines/EEC Directive of 1986. Permission for the study was obtained by the Bulgarian Food Safety Agency No. 98/22.05.2014 and from the Ethic Committee of the Medical University Plovdiv No. 2752/09.11.2015.

2.2. Substances

Sodium chloride solution 0.9% (saline) was purchased from B.Braun Medical EOOD (Sofia, Bulgaria). The studied extract was commercially available standardized *Rhodiola rosea* L. extract of Nature’s Way Products LLC (Green Bay, USA) purchased from a local pharmacy store in Plovdiv, Bulgaria.

2.3. Passive avoidance tests

2.3.1. The step-through test

The step-through test was performed in an automatic set-up two-compartment cage (Ugo Basile, Italy). Learning and retention sessions consisted of three trials (door delay 7 sec., followed by electrical stimulation for 9 sec. at the intensity of 0.4 mA). A criterion for learning and memory
retention was the latency of reaction – the animal remaining in the illuminated chamber for more than 180 sec.

2.3.2. The step-down test

The step-down test was performed in a set-up single-compartment cage with a plastic platform (Ugo Basile, Italy). 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.

In both test, short memory retention was evaluated on day 3 from the first day of learning session. Long memory retention was evaluated on day 10 from the first day of learning.

3. Statistical evaluation

Data management was performed using SPSS 17.0 statistical software. All observed parameters were expressed as mean ± S.E.M for each group. Comparison between groups was carried out using t-test for independent samples. Comparison within groups was done using paired t-test. A value of P<0.05 was considered representative of a significant difference.

4. Results

In step-down test controls increased significantly (p<0.05) the latency time during learning session and on memory retention tests, compared to the 1st day. Rats with both doses of extract decreased significantly (p<0.05) the latency time during learning session and on memory retention tests, compared to the same day controls. Rats with the low dose of the extract significantly increased (p<0.05) the latency time on memory retention tests, compared to the 1st day of the respective group (Figure 1).

Figure (1): Effect of Rhodiola rosea L. on step-down test

0: p<0,05 vs first day control; *: p<0,05 vs same day control; #: p<0,05 vs first day of the group

In step-through test controls increased significantly (p<0,05) the latency time on 2nd day of learning session and on short and long memory retention tests, compared to the 1st day. Rats with the low dose of the extract decreased significantly (p<0,05) the latency time during two days of learning session and on long memory retention test, compared to the same day controls. Rats
with the high dose of the extract decreased significantly \((p<0.05)\) the latency time during two days of learning session and on short and long memory retention tests, compared to the same day controls. Rats with both doses of the extract significantly increased \((p<0.05)\) the latency time on memory retention tests, compared to the 1st day of the respective group (Figure 2).

![Figure 2](image)

**Figure (2):** Effect of *Rhodiola rosea* L. on step-through test.

0: \(p<0.05\) vs first day control; *: \(p<0.05\) vs same day control; #: \(p<0.05\) vs first day of the group

5. Discussion

The rationale of passive avoidance tasks includes several criteria that have to be defined and fulfilled. Normal memory retention (as should be displayed by control animals) is indicated by an increase, between training and test sessions, of the latency to step-down from the platform or the latency of reaction (the animal remaining in the illuminated chamber) for step-trough task. This increase means that the animal has learned correctly the task. Either the difference between training and test session latencies, or the test sessions’ latency can be used as retention scores. The behavioral experiment to study memory is valid only if the control group learns adequately the task. A treatment is amnestic when, between training and test sessions, there is no significant difference at all in the latency to step-down from the platform between sessions (robust amnesia), or this latency is significantly smaller than the controls one, but still higher that its training value (partial amnesia). A treatment is facilitatory when animals learn (i.e., their latency to step-down from the platform increases between training and test sessions), and, moreover, test measurements are higher than the control-group test values. The observed differences among variable measurements, be it a decrease or an increase, must be statistically significant in order to be considered (14).

According to these definitions our results could be considered valid. In both passive avoidance tests used rats from the control group showed an increase of the latency on memory retention tests compared to the training sessions, which is showing that they have learned the task. This is crucial for the passive avoidance tests to be valid.

Our data for the studied extract of *Rhodiola* L. suggests that it influences conditioned stimuli reactions in dose-dependent manner. It can be speculated that the treatment is partially amnestic because the latency is significantly smaller than in the control group, but still higher than its training values. *Rhodiola* L. is known in the scientific literature for the stimulating effect on the CNS (2-7). One possible explanation of the tendency in our results, the experimental groups’
latencies not exceeding the controls ones, is the low doses chosen and probably the route of administration. One study with rats showed that pretreatment with higher doses of orally administered Rhodiola L. extract could significantly improve learning and memory deficits by inhibiting oxidative stress and ameliorating neuron injury in hippocampus (4). In another study was found that the extract of Rhodiola L. administered in the same doses as in the present study, but per orally, improved performance during learning session, short and long memory retrieval tests in rats on passive avoidance tests (15). The increase in the latency time in both passive avoidance conditioned stimuli tests compared to the 1st day of training suggests stimulating effects of the studied extract on the CNS influencing both short and long memory formation. Another study of ours using locomotor activity test showed that Rhodiola L. standardized extract stimulates predominant the vertical locomotor activity in rats, which also suggest stimulation of the CNS with possible involvement of the noradrenergic mechanisms (7). Moreover, healthy volunteers receiving single and repeated doses of SHR-5 extract (Rhodiola rosea L.) have demonstrated an anti-fatigue effect and improvement in cognitive functions during fatigue and in stressful conditions in one controlled clinical trial (16).

In conclusion to clarify the exact influence of the studied extract on condition stimuli reactions, more behavioral tests should be performed with higher doses of the studied extract. Our observations reveal some stimulating effect of the Rhodiola rosea L. extract used on the formation of memory traces.

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7. References
