

Exploring Rhodiola Rosea L Extract: Anxiolytic Potential in Sleep-Deprived Mice

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Abstract

Aim:

This study aimed to investigate the potential anti-anxiety effects of Rhodiola Rosea L extract in sleep-deprived mice.

Background:

Anxiety disorders and sleep deprivation commonly coexist, worsening each other's symptoms and impacting overall well-being. Rhodiola Rosea L, a traditional herbal remedy, is known for its adaptogenic properties, which may offer relief from stress-related conditions.

Objective:

To assess the anti-anxiety activity of Rhodiola Rosea L extract in sleep-deprived mice using behavioral assays and to evaluate its potential as a therapeutic agent for anxiety exacerbated by sleep disturbances.

Method:

Mice were subjected to 72 hours of sleep deprivation using the grid suspended over water method. Following sleep deprivation, mice received oral administration of Rhodiola Rosea L extract at varying doses (25 mg/kg, and 50 mg/kg). Anxiety-like behaviors were assessed using

the elevated plus maze and open field tests, while locomotor activity was monitored to rule out confounding effects.

Result:

Sleep-deprived mice exhibited increased anxiety-like behaviors compared to controls, as indicated by reduced time spent in open arms and decreased entries into the center zone. However, treatment with *Rhodiola Rosea* L extract significantly attenuated these anxiety-like behaviors in a dose-dependent manner. The highest dose (50 mg/kg) showed the most pronounced effect, with mice displaying increased time spent in open arms and entries into the center zone, suggesting reduced anxiety levels.

Conclusion:

Rhodiola Rosea L extract possesses anti-anxiety properties in sleep-deprived mice, indicating its potential therapeutic utility for managing anxiety disorders exacerbated by sleep disturbances. These findings underscore the importance of further research to elucidate the underlying mechanisms and assess its efficacy in clinical settings.

Keywords: - *Rhodiola Rosea* L extract, Anti-anxiety activity, Sleep deprivation, Mice, Anxiety disorders, Behavioral assays

1. Introduction

Anxiety is a kind of psychological reaction that individuals have to situations that threaten their spiritual and financial well-being. Since anxiety disorders rank highest among mental illnesses, their prevalence is believed to be between 10 and 30 percent. Drugs that are sedative and anti-anxiety are thus often employed. Due to their calming, hypnotic, and relaxing effects on the muscles and organs, the majority of anti-anxiety medications lead to both physical and mental addiction.(1)

Diazepam is a well-known anti-anxiety medication that has been shown to have effects on the central nervous system. Because diazepam interacts with brain Gaba receptors, notably in the midbrain reticular formation, it has palliative and anti-anxiety properties.(2) Memory loss, extreme sleepiness, slurred speech, bradycardia, dyspnea, ataxia, unusual bleeding or bruises, and mouth ulcers are among the side effects of diazepam.(3)

When this medication is abruptly stopped, benzodiazepine withdrawal syndrome manifests as withdrawal symptoms. Seizures, tremors, anxiety, sleeplessness, and irritability are some of the symptoms of this condition. Herbal medications offer several benefits over chemical treatments since their active components are combined and balanced with other substances, do not build up in the body, and have no negative side effects.(4)

However, the World Health Organization (WHO) has repeatedly stressed the need for traditional medicine and herbal remedies to be used in a scientific and cost-effective manner. One of the most significant problems facing the globe in recent decades, particularly in emerging nations, is this strategy.(5) The use of medicinal plants has increased in the modern era due to the discovery of a number of their physiological characteristics, including anti-cancer, anti-sensitivity, and anti-diabetic effects, as well as their ability to lower cholesterol and prevent coronary artery blockage. There are reports on how flavonoids affect benzodiazepine receptors.(6) The active flavonoids found in medicinal plants influence the binding of benzodiazepine receptors to Gaba receptors, which has a calming and anti-anxiety effect.(7)

Rhodiola rosea L., also referred to as "roseroot," "golden root," or "arctic root," is a member of the Crassulaceae family of plants (syn. *Sedum rhodiola* DC.; *Sedum roseum* (L.) Scop). The yellow-flowered herbaceous perennial grows naturally at high elevations in the eastern coastal parts of North America, as well as in the Arctic regions of Europe and Asia (primarily Siberia), on sea cliffs and in the fissures of mountain rocks [1]. Many European and Asian nations, including Sweden, Norway, France, Germany, Iceland, Russia, and China, have included *R. rosea* L. as a beneficial therapeutic plant in their traditional and popular medicine. *R. rosea* has been traditionally used for centuries to cure weariness, depression, anemia, impotence, gastrointestinal problems, infections, and nervous system illnesses. It has also been used to promote physical endurance, lifespan, productivity at work, and resistance to high-altitude sickness [2,3,4,5].

The long-standing, well-established traditional medicinal use of *R. rosea* has sparked a great deal of contemporary scientific investigation, which has led to the identification of *R. rosea* as a "adaptogen," a material that increases an organism's resistance without specifically changing its biological parameters and having a normalizing effect on physiology [6, 7]. The Russian scientist Nikolai Lazarev is credited with coining the word "adaptogen" in 1947. He described it as an

agent that creates non-specific resistance in an organism to overcome harmful physical, chemical, or biological stresses [6]. Adaptation is necessary to deal with stress and difficult conditions. The ideal way to conceptualize adaptation would be as an organism's capacity to withstand a stressor by reacting to it by either exhibiting fewer or no typical homeostasis disruptions. Plant adaptogens have the power to direct physiological systems to initiate the process of generalized adaptation (also known as non-specific resistance) and respond more creatively to stressful environments.

The current study examined the effects of rhodiola rosea extract and diazepam on anxiety reduction in mice, taking into account the lack of previous research on the anti-anxiety properties of rhodiola rosea as well as the prevalence of anxiety in the population, the adverse effects of chemical drugs, and the superiority of herbal drugs.

2. Material and Method

Swiss male albino mice (25-35 g , 3-5 months old) were procured from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary & Animal Science (LUVAS), Hisar (Haryana). According to Zárate et al., 2017), “Female mice were excluded from the study since estrogens (female sex hormone) have been reported to possess neuroprotective effect”. The animals were housed in air-conditioned rooms (24±2°C) with 12 h light and 12 h dark cycle. The animals were housed separately in groups of 8 per cage (43×27×15 cm). The animals had free access to food and water. But food was not provided 2 h before and 2 h after drug administration, so as to rule out the effect of food on absorption of drugs. The mice were acclimatized to the laboratory conditions for 5 days before starting the experiments. The experimental protocol was approved by IAEC (Institutional Animals Ethics Committee) in its meeting held 6nd August, 2023.

2.1. Drugs and chemicals

Rhodiola rosea L (Sigma-Aldrich, USA), Diazepam injection (LORI®, NEON laboratories Ltd.; India) were employed. All other reagents used in this study were analytical grade.

2.2.Experimental design

Treatment schedule

Following eight groups of mice were constituted (n=8 each group)

Unstressed mice :

Group 1 : vehicle (0.9 % saline)administered i.p for 14 consecutive days.

Group 2 : Rhodiola rosea L (25 mg/kg) administered i.p for 14 consecutive days.

Group 3 : Rhodiola rosea L (50 mg/kg)administered i.p for 14 consecutive days.

Group 4 : diazepam (2mg/kg)administered i.p for 14 consecutive days.

Sleep deprived mice :

Group 5 : vehicle (0.9 % saline)administered i.p for 14 consecutive days.

Group 6 : Rhodiola rosea L (25 mg/kg) administered i.p for 14 consecutive days.

Group 7 : Rhodiola rosea L (50 mg/kg)administered i.p for 14 consecutive days.

Group 8 : diazepam (2mg/kg)administered i.p for 14 consecutive days.

Effect of the drugs on locomotor activity was evaluated after the administration of drugs on the last day (14st day) of the curement, for sleep deprivation mice were be placed in polypropylene cages (29x15x7cm) filled with water up to 2 cm from the bottom of cages and covered with top wire lid. The rods of top wire lid were 1 cm apart from each other. Mice will try to hold the rods and as soon as the mice will try to sleep. they will lose their muscle tone and thus grip over the rods. This will make the mice fall in water and thereby keep them awakened. Hence, sleep induced anxiety were caused.

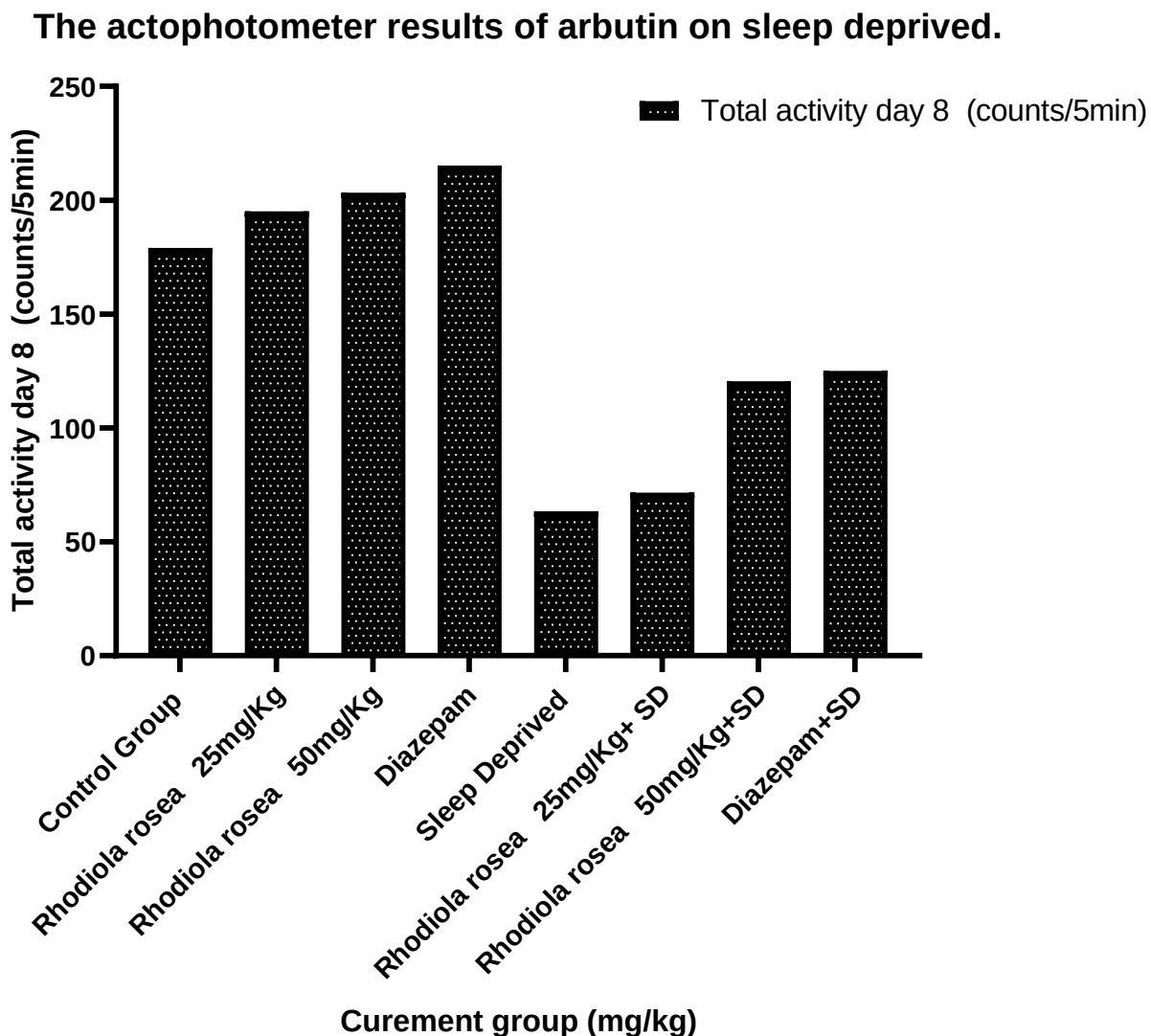
3. Result and discussion

3.1. Actophotometer

Diazepam (2mg/kg) and Rhodiola rosea (25mg/kg and 50mg/kg) groups showed CNS depressant activity in sleep deprived as compared to the control group or the group with no sleep deprived. However, when results were analyzed it was observed that diazepam and Rhodiola rosea 50mg/kg treated group showed almost equal results. As shown in table I.

Curement group (mg/kg)	Total activity day 14 (counts/5min)
Control Group	180.00±2.75
Rhodiola rosea 25mg/Kg	195.20±4.25
Rhodiola rosea 50mg/Kg	203.36±3.75
Diazepam	215.23±5.45
Sleep Deprived	63.428±5.68
Rhodiola rosea 25mg/Kg	71.66±6.36
Rhodiola rosea 50mg/Kg	119.45±5.65
Diazepam	124.04±5.35

Table I- shows the actophotometer results of Rhodiola rosea on sleep deprived.

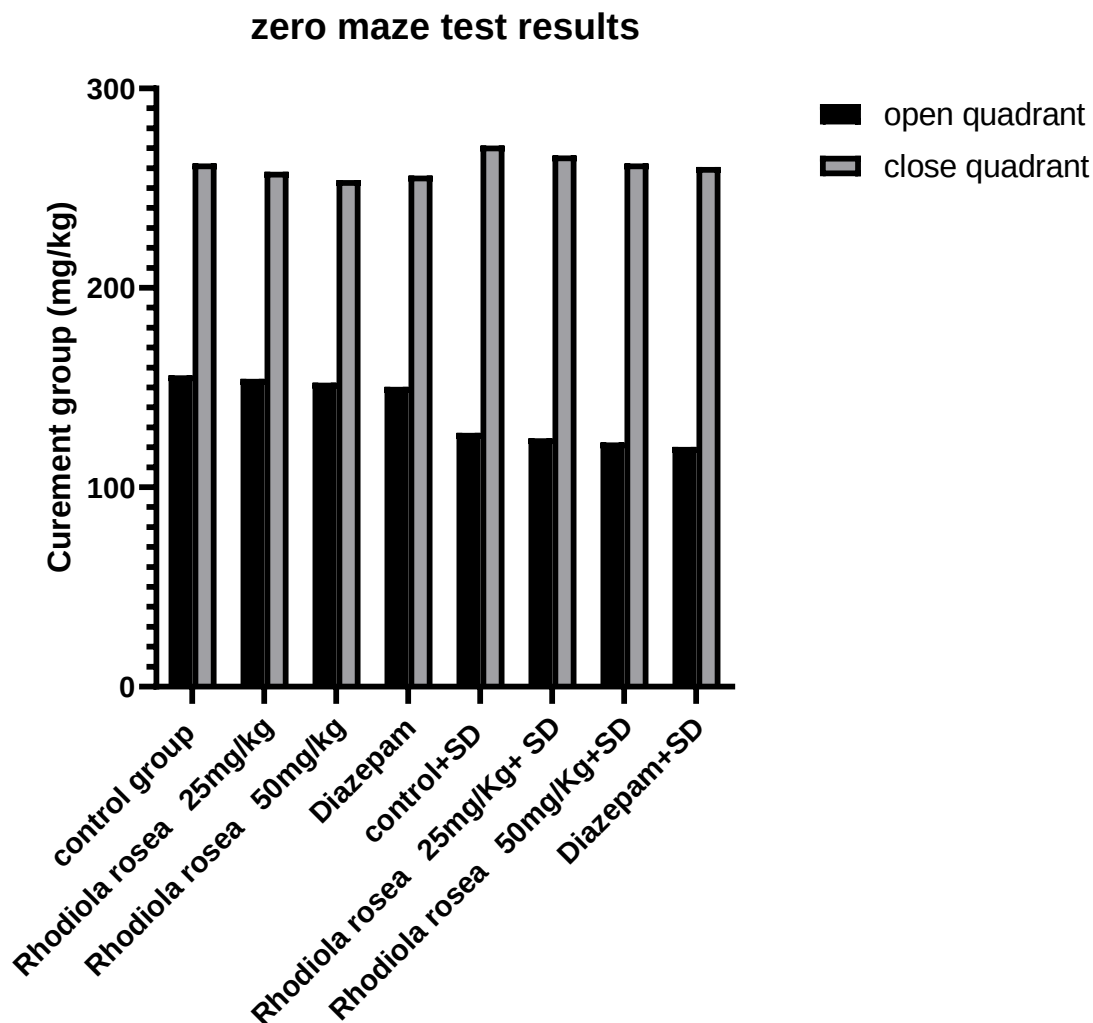


3.2. Zero maze test:

The 72-hour sleep deprivation substantially enhanced the duration in the closed quadrant, decreased the number of entries and enhanced the duration in the open quadrant compared to the control and sleep-induced anxiety group treated with *Rhodiola rosea*. Treatment with *Rhodiola rosea* (25 and 50 mg/kg) significantly enhanced the number of entries, duration in the open quadrant, and decreased the duration in the closed quadrant relative to sleep-deprived animals placed on a grid suspended over water ($P < 0.001$) (Table 1). Diazepam (2 mg/kg) substantially diminished the number of entries and duration in the open quadrant, while increasing the duration in the confined quadrant ($P < 0.005$, Table II).

Curement group (mg/kg)	open quadrant		close quadrant
	No. entries (mean±sem)	Duration (s) (mean±sem)	Duration (s) (mean±sem)
control group	5.7 ± 0.3	156.2 ± 2.4	262.5 ± 2.5
Rhodiola rosea 25mg/kg	5.2 ± 0.4	154.4± 1.6	258.2 ± 3.1
Rhodiola rosea 50mg/kg	4.8 ± 0.2	152.5 ± 2.1	254.1± 2.1
Diazepam	4.6 ± 0.2	150.4 ± 1.1	256.3± 3.5
sleep deprived	2.5 ± 0.4	127.4 ± 1.2	271.4 ± 3.8
Rhodiola rosea 25mg/kg	3.9 ± 0.2	124.6 ± 1.8	266.4± 4.3
Rhodiola rosea 50mg/kg	4.2 ± 0.2	122.5± 1.8	262.4 ± 4.5
Diazepam	4.8± 0.3	120.3± 1.8	260.7 ± 3.5

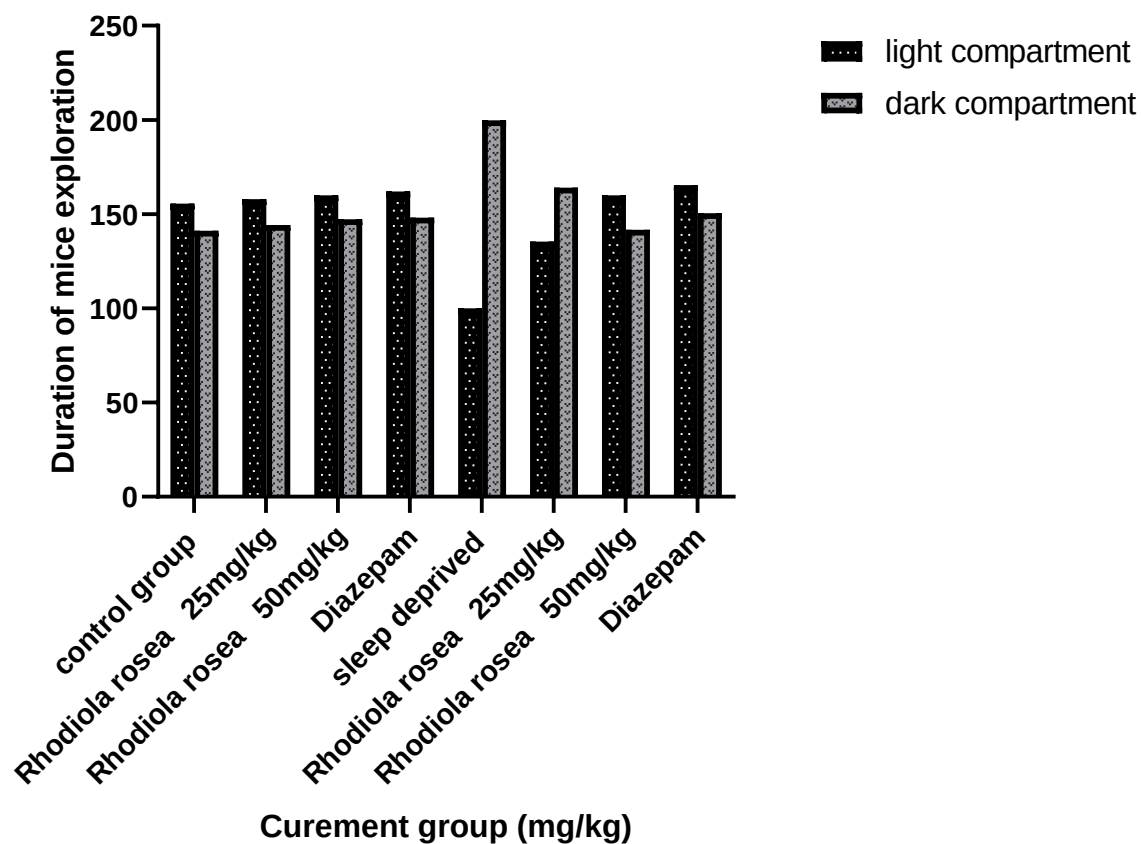
Table II:- shows the zero maze test results.



3.3.Light dark test

The light dark test results suggest that the control group with no sleep deprived spent more time in light compartment. However, the sleep deprived group spent less time in light compartment suggesting high anxiety level. Interestingly the group having sleep deprivation and were treated with *Rhodiola rosea* (25mg/kg), *Rhodiola rosea* (50mg/kg) and Diazepam (2mg/kg) showed enhance time spent in light compartment. Also the group sleep deprived group treated with *Rhodiola rosea* (50mg/kg) and diazepam (2mg/kg) showed almost equal results.

Curement group (mg/kg)	Duration of mice exploration	
	light compartment (s)	dark compartment (s)
control group	155.50±7.06	141.20±6.65
Rhodiola rosea 25mg/kg	158.00±7.65	144.20±6.25
Rhodiola rosea 50mg/kg	160.10±6.76	147.20±6.35
Diazepam	162.00±6.96	148.20±6.45
sleep deprived	100.20±5.87	199.80±5.87
Rhodiola rosea 25mg/kg	135.45±7.92	164.00±7.59
Rhodiola rosea 50mg/kg	160.00±5.67	141.70±4.31
Diazepam	165.45±6.21	150.42±4.21



Conclusion

In this study, we investigated the anti-anxiety effects of *Rhodiola Rosea* L extract at concentrations of 25 mg/kg and 50 mg/kg in sleep-deprived mice. Our findings indicate that both concentrations of *Rhodiola Rosea* L extract exhibited significant anxiolytic activity, as evidenced by behavioral assays.

Mice subjected to sleep deprivation displayed heightened anxiety-like behaviors, characterized by decreased time spent in open arms of the elevated plus maze and reduced entries into the center zone of the open field test. However, oral administration of *Rhodiola Rosea* L extract at concentrations of 25 mg/kg and 50 mg/kg effectively attenuated these anxiety-like behaviors.

Notably, the anxiolytic effects of *Rhodiola Rosea* L extract were dose-dependent, with the higher concentration (50 mg/kg) showing a more pronounced effect compared to the lower concentration (25 mg/kg). Mice treated with 50 mg/kg of *Rhodiola Rosea* L extract exhibited a significant increase in the time spent in open arms and entries into the center zone, indicative of reduced anxiety levels.

These findings suggest that *Rhodiola Rosea* L extract has potential as a natural remedy for managing anxiety disorders exacerbated by sleep disturbances. Further research is warranted to elucidate the underlying mechanisms of action and to assess its long-term efficacy and safety profiles. Additionally, investigations into optimal dosing regimens and potential synergistic effects with existing pharmacotherapies may provide valuable insights into the clinical utility of *Rhodiola Rosea* L extract in anxiety management.

Conflict of interest

The authors have no conflict of interest

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