

Evaluation of Adaptogenic and Antidepressant Activity of *Rhododendron arboreum* flower

ABSTRACT

Various medications are available to treat stress and depression, but there is a growing interest in plant-based drugs due to their effectiveness and minimal side effects. As a result, researchers conducted a study to evaluate the adaptogenic and antidepressant effects of extracts from *Rhododendron arboreum* flowers using different animal models. The adaptogenic activity of the extracts was tested in mice and rats using anoxia stress and swimming endurance stress-induced models. In the anoxia stress model, animals were placed in airtight containers, and any increase in their tolerance to anoxia was considered a positive effect. In the swimming endurance model, the researchers measured various biochemical parameters such as blood glucose, cholesterol, triglycerides, blood urea nitrogen, blood cell count, and organ weights in both stressed animals and animals treated with the extract. The decrease in biochemical parameters and maintenance of blood cell counts indicated adaptogenic activity. Additionally, the extract protected against increased liver and adrenal gland weight, and decreased spleen weight under stress conditions, further confirming its adaptogenic activity. The antidepressant effect was evaluated using the tail suspension test and elevated plus maze model, where immobility was used as a behavioral parameter. Significant adaptogenic and antidepressant activity was observed after treating the animals with the ethanolic extract of *R. arboreum* flowers at doses of 200mg/kg and 400mg/kg. Interestingly, the higher dose of the extract had a more pronounced effect compared to the lower dose.

Overall, this study provides compelling evidence supporting the significant adaptogenic and antidepressant activity of the ethanolic extract derived from *R. arboreum* flowers. The results suggest that the extract has the potential to help the body adapt to stress and exhibit antidepressant effects. Furthermore, the study highlights the extract's impact on various biochemical, hematological, and organ weight parameters in rats, indicating its adaptogenic properties. The presence of flavonoids such as rutin and quercetin in the extract may contribute to its adaptogenic activity, while terpenes could be responsible for its antidepressant effects.

Keywords: *Rhododendrom arboreum*; adaptogenic activity; antidepressant activity; tail suspension test.

1. INTRODUCTION

Stress is a widespread occurrence that affects everyone, and when it reaches severe levels, it can have detrimental effects on the body and requires intervention. Stress plays a role in the development of numerous illnesses, encompassing psychiatric conditions like depression and anxiety, weakened immune function, hormonal imbalances such as diabetes and erectile dysfunction, impaired cognitive abilities, stomach ulcers, high blood pressure, and ulcerative colitis. [1].

Stress is essentially the mind and body's response to a disruption in the state of balance known as homeostasis. When stress is beneficial and encourages productivity, it is referred to as eustress. On the other hand, distress refers to harmful stress that can negatively impact well-being. When stress reaches extreme levels, the body's mechanisms for maintaining balance become impaired, posing a threat to the organism's survival. Stress has the ability to disrupt the equilibrium of various hormones, which in turn can have a significant influence on the overall immune response. The specific effect on the immune system, whether it leads to suppression or enhancement, depends on the cumulative impact of these hormonal changes. [2]

1.1 General adaptation syndrome

Physiologists describe stress as the body's response to a stressor, which can be a real or perceived stimulus that induces stress. Acute stressors have a temporary impact on an organism, while chronic stressors have a longer-lasting effect. One notable researcher in the field of stress is Selye, who extensively studied the consequences of stress.

Alarm is the initial phase in the General Adaptation Syndrome (GAS) model. It occurs when a threat or stressor is recognized by the body, triggering a state of alarm. In this stage, the body's stress response is activated, leading to the release of adrenaline to facilitate the fight-or-flight response. Additionally, the HPA axis is activated, resulting in the production of cortisol.

Resistance is the second stage of the GAS model. If the stressor persists over an extended period, the body endeavors to cope with the stress by adapting to the demands of the environment. However, the body's ability to sustain this adaptive response is limited, and its resources gradually become depleted.

"Exhaustion represents the third and final stage of the GAS model. At this point, the body's resources are completely exhausted, and it becomes unable to maintain normal functioning. Symptoms experienced during the initial alarm stage may reappear, such as increased heart rate and sweating. If stage three persists, it can lead to long-term damage as the body and immune system become depleted and impaired, resulting in decomposition. This can manifest as various physical illnesses like ulcers, cardiovascular problems, digestive issues, diabetes, as well as mental health disorders such as depression" [3].

"Depression plays a significant role in the overall burden of disease worldwide and impacts individuals in every community across the globe. Presently, approximately 350 million people are estimated to be affected by depression. According to the World Mental Health Survey conducted in 17 countries, an average of 1 in 20 individuals reported experiencing a depressive episode within the previous year. Onset of depressive disorders often occurs at a young age, resulting in reduced functioning and recurrent episodes. Consequently, depression is the leading cause of disability on a global scale, accounting for a significant number of years lost due to disability. As a result, there is an increasing demand for addressing depression and other mental health conditions worldwide". [4]

Depression is a commonly observed psychiatric disorder characterized by a wide range of symptoms and frequently co-occurs with other brain dysfunctions. The complex nature of depression has limited our understanding of the neural and genetic mechanisms involved in its development. Current antidepressant treatments often prove ineffective or have undesirable side effects in a considerable portion of patients. This has spurred efforts to search for more efficient medications that can effectively alleviate depression symptoms. [5].

Depressive episodes involve various signs such as feelings of sadness, decreased enjoyment and interest, and increased tiredness. People going through a mild depressive episode may find it difficult to perform their usual work and social activities, although they are unlikely to completely stop functioning. Engaging in extensive social or domestic tasks is highly unlikely for these individuals, except to a limited extent. Bipolar disorder often presents with alternating episodes of mania and depression, with intervals of normal mood. Manic episodes are characterized by an elevated mood, increased energy levels, leading to excessive activity, rapid speech, and reduced need for sleep [6].

1. MATERIAL AND METHOD

2.1 Drugs and chemical used

The *Withania somnifera* plant was obtained from Sapthagiri Pharma, located in Bangalore, India. Imipramine was obtained from HCG Pharma, also located in Bangalore, India. All the other chemicals and substances utilized in the experiment were of high-quality analytical grade.

2.2 Experimental animals

Albino Wistar rats weighing between 150 and 210 grams and Albino mice weighing 20 to 35 grams, regardless of their gender. The animals were randomly assigned to three groups: experimental, normal, and control. They were housed individually in sanitized polypropylene cages, and the bedding consisted of sterile paddy husk.

The experimental animals were subjected to a 12-hour light-dark cycle and were kept in an animal house with a controlled temperature range of 20 to 25 degrees Celsius and maintained humidity. The animal house adhered to standard conditions and was approved by the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3 Collection and authentication of plant material

The flowers of *R. arboretum* were collected from the mountainous region of Nepal, specifically from higher altitudes. Dr. K. Ravi Kumar, a senior botanist at FRLHT (Foundation for Revitalisation of Local Health Traditions) in JarakabandeKaval post, Attur, Yehalanka, Bangaluru (560106), verified and authenticated the plant's identification. To serve as a future reference, a specimen of the plant was preserved in the college museum's herbarium. The flowers were dried naturally in a shaded area at room temperature for a period of 15 days and then finely ground.

2.4 Extraction procedure

The flowers of *R. arboreum* were dried in a shaded area and subsequently ground into a fine powder using a mechanical grinder. The resulting coarse powder of *R. arboretum*, weighing 500 grams, was then subjected to extraction with 1000 milliliters of 70% ethanol. Prior to this extraction, defatting was carried out using petroleum ether through Soxhlet extraction for a duration of 72 hours.

During the extraction process, the extractor's body was continuously filled and emptied in an alternating manner until the solvent in the side tube of the extractor (Siphon) became discolored, indicating its depletion. Following this, the remaining residue was removed by filtration and the resulting extract was concentrated under reduced pressure. The concentrated extract was then transferred to china dishes and placed in a vacuum oven set at 45°C. The resulting extract was semisolid, exhibiting a brownish black color. To preserve its quality, the extract was stored in an airtight amber-colored bottle until it was ready for use. [7].

2.5 Preliminary Phytochemical Analysis

The ethanolic extract of *R. arboreum* flower was analyzed to identify its active components through various chemical tests. Standard procedures were followed to test for the presence of carbohydrates, glycosides, resins, tannins, alkaloids, fixed oils, flavonoids, terpenoids, proteins, saponins, anthraquinones, and amino acids. [8].

2.6 Acute toxicity and dose selection [9, 10]

The maximum lethal dose of *R. arboreum* having the same chemical constituents was found to be 2000 mg/kg body weight, hence 1/10th and 1/20th of maximum lethal dose was taken as effective dose for the ethanolic extract of *R. arboreum* for adaptogenic and antidepressant activity

2.7 Pharmacological Screening

2.7.1 Evaluation of Adaptogenic activity of *Rhododendron arboreum* by anoxia stress tolerance test [11]

Albino mice of either sexes, weighing 20-30 g, are divided into four groups of six. For this experiment, a hermetic vessel in a capacity of 500 mL of air is used. Each animal is kept in a hermetic vessel, and when the first sign of convulsion appears, it is removed from the vessel and resuscitated if necessary. The animals are subjected to anoxia stress again after one week of drug treatment. Similarly, at the end of the second and third weeks of the same treatment, the animals are observed, and the time duration for anoxia stress tolerance is recorded.

Experimental protocol

The animals were categorized into four sets, each consisting of six animals. The groupings were as follows:

Group 1: Control group (mice were given a daily dose of normal saline, 10ml/kg body weight, via oral administration) for a period of 21 days.

Group 2: Standard *Withania somnifera* group (animals were given a daily dose of *Withania somnifera*, 100 mg/kg body weight, via oral administration) for a period of 21 days.

Group 3: *R. arboreum* ethanolic extract group (animals received a daily dose of ethanolic extract of *R. arboreum*, 200 mg/kg body weight, via oral administration) for a period of 21 days.

Group 4: *R. arboreum* ethanolic extract group (animals received a daily dose of ethanolic extract of *R. arboreum*, 400 mg/kg body weight, via oral administration) for a period of 21 days.

2.7.2 Evaluation of Adaptogenic activity of *Rhododendron arboreum* by Swimming Endurance Test in Rats [11].

In the study, rats weighing between 150-200 grams were divided into four groups, each consisting of six rats. The objective was to induce stress in the rats by placing them in cylindrical vessels filled with water up to a height of 25 cm. The rats were then made to swim until they reached exhaustion, and the duration of swimming for each rat was recorded.

Over a period of 14 days, the rats in each group were given different extracts once a day. On the 15th day, the average swimming time for each group was calculated. Blood samples were taken from the rats by collecting them through the retroorbital plexus under light ether anesthesia. These blood samples were then analyzed to determine various biochemical parameters such as blood glucose, triglycerides, cholesterol, blood urea nitrogen (BUN), and blood cell count (including red blood cells, white blood cells, and differential leukocyte count).

Following the blood collection, the animals were euthanized using cervical dislocation, and the weight of organs like the liver, adrenals, and spleen was measured after rinsing them with alcohol.

The experimental protocol consisted of the following groups:

Group 1: Normal control group, where rats were given a daily dose of normal saline (10 ml/kg body weight, administered orally) for 14 days.

Group 2: Standard group, where animals were administered a single dose of *Withania somnifera* extract (100 mg/kg, administered orally) daily for 14 days.

Group 3: Rats received a single dose of ethanolic extract of *R. arboreum* (200 mg/kg, administered orally) daily for 14 days.

Group 4: Animals were given a single dose of ethanolic extract of *R. arboreum* (400 mg/kg, administered orally) daily for 14 days.

2.7.3 Evaluation of antidepressant activity of *Rhododendron arboreum* by tail suspension test (TST) [12]

Albino mice weighing approximately 20-30g were utilized in the study. The mice were transferred from their housing colony to the laboratory in their own cages and given 1-2 hours to adjust to the laboratory environment. Each mouse was individually suspended in a wooden box using a hanging clip, placing the animal 50 cm above the floor of the box. Additionally, 1 cm of the tail was clipped. To ensure isolation during the test, each animal was acoustically and visually separated from the others.

The duration of immobility for each mouse was manually recorded for a total of 6 minutes. Initially, during the first 2 minutes of the test, every animal exhibited vigorous movement. The subsequent 4 minutes were used to measure the duration of immobility. A mouse was considered immobile when it displayed no body movement, hanging passively and completely motionless. The test was conducted in a dimly lit room, and each mouse was only used once in the experiment. After 14 consecutive days of treatment with the control, standard, and extract drugs, the immobility of the mice was calculated and recorded.

The experimental groups consisted of six animals each, divided as follows:

Group 1: Normal control - Mice administered with normal saline (10 ml/kg b.w., p.o.) daily for 14 days.

Group 2: Standard Imipramine - Animals administered with a single dose of Imipramine (10 mg/kg, p.o.) daily for 14 days.

Group 3: Animals received a single dose of ethanolic extract of *R. arboreum* (200 mg/kg, p.o.) daily for 14 days.

Group 4: Animals received a single dose of ethanolic extract of *R. arboreum* (400 mg/kg, p.o.) daily for 14 days.

2.7.4 Evaluation of antidepressant activity of *Rhododendron arboreum* by elevated Plus-Maze [13]

The elevated plus-maze consists of four arms, two open and two enclosed, which extend from a central platform in the shape of a plus sign. The dimensions of the open arms are 30 cm long, 5 cm wide, and 0.25 cm high, while the enclosed arms are 30 cm long, 5 cm wide, and 15 cm high. The maze is made of black painted wood, and the open arms have a slightly raised edge (0.25 cm) to provide better grip for the animals. The entire maze is elevated to a height of 40 cm above the floor by a single central support.

The experiment is conducted during the dark phase of the light cycle, specifically from 9:00 am to 2:00 pm. Each trial begins by placing an animal on the central platform of the maze, facing one of the open arms. Over a 5-minute test period, the number of entries into each type of arm and the time spent in each arm are recorded. The percentage of entries and time spent in the open arms are used as indicators of depression. An entry into an arm is considered when all four paws of the mouse are on that arm. The maze is thoroughly cleaned between trials using damp and dry towels.

In this study, animals will be treated with a single dose of *R. arboreum* extract, and the observed effect will be compared to that of standard drugs. If the extract demonstrates a depressive effect, it will be recorded and compared to the standard drugs. However, if the extract fails to exhibit anti-depressant properties in the single-dose experiment, the dosing will be continued for 21 days, and the test will be repeated. The effects of the extract will be measured using the Elevated Plus-Maze test model.

It is important to note that all behavioral recordings will be conducted by an observer who is unaware of the treatment received by the mice.

Experimental protocol

Animals were divided into four groups of six animals each. The groups were as follows:

Group 1: Normal control (mice administered with (normal saline 10ml/kg b.w.p.o.) daily for 21 days

Group 2: Standard *Imipramine* (animals were administered with single dose of *Imipramine* (10 mg/kg, p.o.) daily for 21 days

Group 3: Animals received single dose ethanolic extract of *R. arboreum* (200 mg/kg p.o.) daily for 21 days

Group 4: Animals received single dose of ethanolic extract of *R. arboreum* (400 mg/kg p.o.) daily for 21 days

2. RESULTS AND DISCUSSION

3.1 Extraction and Phytochemical Investigation

Sequential Soxhlet extractions were carried out on *R. arboreum*, resulting in a reddish-brown, hygroscopic extract powder. The obtained extract was then analyzed using several preliminary chemical tests to identify its chemical composition. The results revealed the presence of flavonoids, steroids and triterpenoids, phenolic compounds, and tannins, as summarized in Table 1.

Table.1 Test for phytoconstituents of *R. arboreum* flower extract

Phytoconstituents	<i>R. arboreum</i> flower extract
Alkaloids	-
Flavonoids	+
Terpenoids	+
Anthraquinones	-
Tannins	+
Steroids	+
Phenolic compound	+
Glycosides	-
Reducing sugar	-

(+)- Present

(-)- Absent

3.2 Evaluation of Adaptogenic activity

Model I: Anoxia Stress Tolerance

The assessment of tolerance to oxygen deprivation was conducted by measuring convulsions as the indicator. The effectiveness of the ethanolic extract from *R. arboreum* was examined at two different doses: a low dose (referred to as EERA-I) of 200 mg/kg body weight and a high dose (referred to as EERA-II) of 400 mg/kg body weight. The results demonstrated a notable improvement in stress tolerance, as indicated by a delayed onset of convulsions, on the 7th, 14th, and 21st day compared to the control group ($p < 0.001$). These findings are summarized in Table 2 and visually depicted in Figure 1.

Table 2 Effect of ethanol extract of *R. arboreum* flower on anoxia stress tolerance test in mice

Treatment group	Dosein (mg/kg,p.o.)	Duration of Anoxia Stress Tolerance (min)			
		1st Day	7th Day	14th Day	21st Day
Control	–	20.8± 0.583	21.8±0.833	22±0.365	22.7±0.211
Standard (<i>Withania somnifera</i>)	100mg/kg	23.8±0.374**	25.8±0.749**	27.5±0.601***	32.2±0.477***
EERA-I	200 mg/kg	21.4±0.51	23.5±0.619	23.7±0.333*	25±0.365**
EERA-II	400 mg/kg	22.8±0.49*	24.5±0.428 *	24±0.365**	28.2±0.477***

expressed as mean ±SEM, data analyzed by using one way ANOVA followed by Dunnett"s. *P<0.05, **P<0.01, ***P<0.001

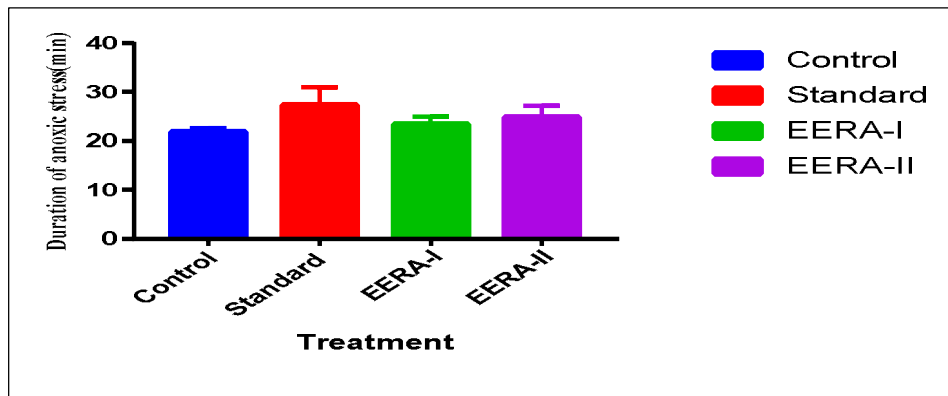


Fig.1: Effect of ethanol extract of *R.arboreum* flower on Anoxia stress test

Model II: swimming endurance test

The ethanolic extract of *R.arboreum* at low dose (i.e. EERA-I 200 mg/kg/b.w) and high dose (i.e. EERA-II 400 mg/kg/ b.w.) showed significant(p<0. 001) increase in swimming time and is supported by estimating the biochemical parameters such as blood glucose, triglyceride, cholesterol and haematological parameter such as RBCs, WBCs and DLC. The organ weight of liver, spleen and adrenal gland was reduced in groups treated with ethanolic extract of *R.arboreum* and standard *Withania somnifera* as compared to stress control. Results were depicted Table 3, 4, 5 and graphically represented in Figure 2, 3, 4.

Table 3: Effect of ethanol extract of *R.arboreum* on swimming endurance test

Treatment group	Dose in mg/kg, p.o.	Swimming survival time (Min)
Control	–	225±2.8
Standard (<i>Withania somnifera</i>)	100	368±5.88***
EERA-I	200	240±1.83*
EERA-II	400	246±2.39**

Results are expressed as mean ±SEM, data analyzed by using one way ANOVA followed by Dunnett's. *P<0.05, **P<0.01, ***P<0.001

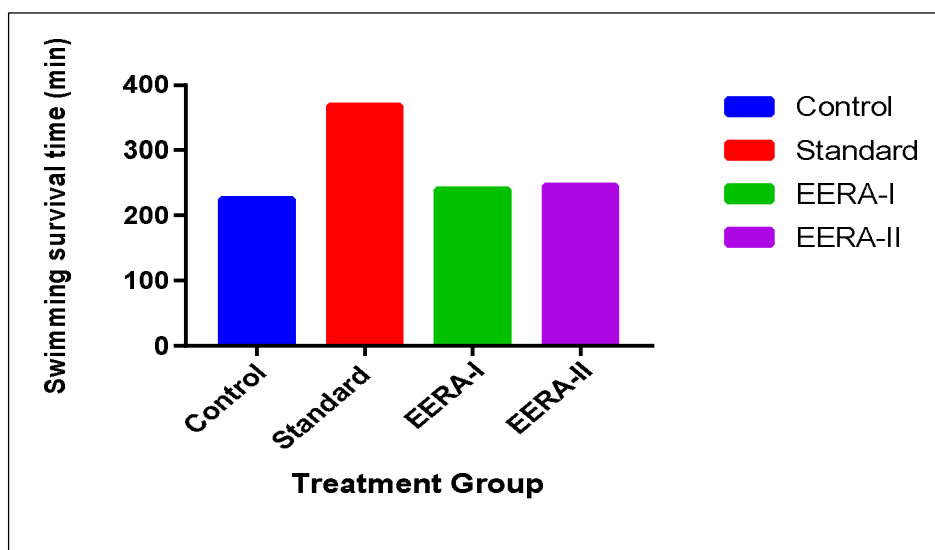


Figure 2 Effect of ethanolic extract of *R.arboreum* flower on swimming endurance test

Table 4: Effect of ethanol extract of *R.arboreum* flower on biological parameters in swimming endurance test

Groups	Glucose(mg/dl)	Cholestrol(mg/dl)	Triglycerides(mg/dl)	BUN(mg/dl)
Control	185±4.08	49.7±0.667	53.3±0.882	37.3±0.494
Standard (<i>withania somnifera</i>)	118±4.22***	41.7±1.67**	33.2±0.872***	22.8±0.703***
EERA-I	170±2.89*	44.3±1.56*	49.5±0.619*	35.3±1.67
EERA-II	163±1.3.82**	43.7±1.56*	42±0.775**	30.8±1.4**

expressed as mean ±SEM, data analyzed by using one way ANOVA followed by Dunnett's. *P<0.05, **P<0.01, ***P<0.001

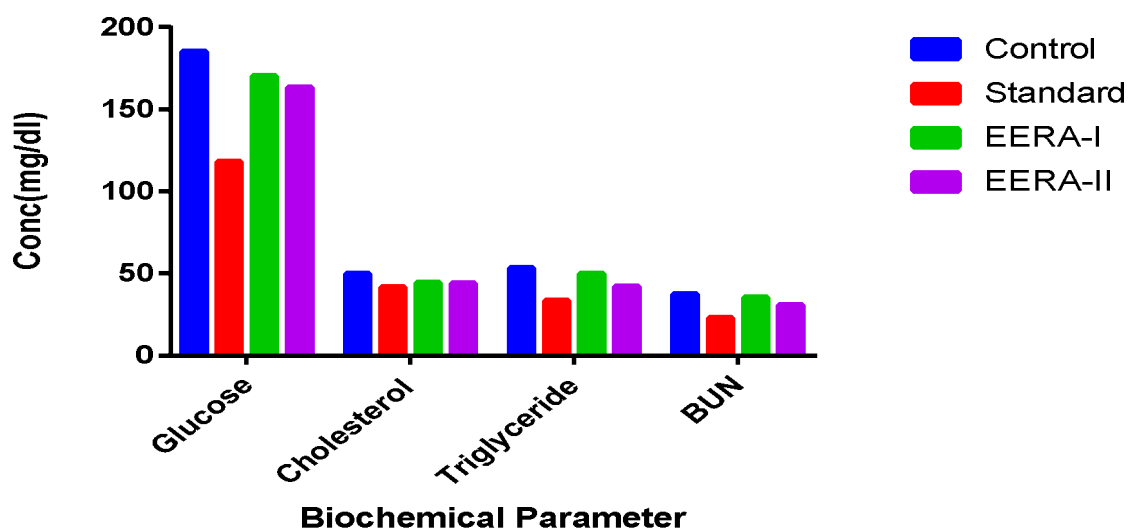


Fig.3: Effect of ethanolic extract of *R.arboreum* on biological parameters in swimming endurance test

Table 5: Effect of ethanol extract of *R.arboreum* flower on biological parameters in swimming endurance test

Groups	Glucose (mg/dl)	Cholestrol (mg/dl)	Triglycerides (mg/dl)	BUN (mg/dl)
Control	185±4.08	49.7±0.667	53.3±0.882	37.3±0.494
Standard (<i>withania somnifera</i>)	118±4.22***	41.7±1.67**	33.2±0.872***	22.8±0.703***

EERA-I	170±2.89*	44.3±1.56*	49.5±0.619*	35.3±1.67
EERA-II	163±1.3.82**	43.7±1.56*	42±0.775**	30.8±1.4**

Results are expressed as mean ±SEM, data analyzed by using one way ANOVA followed by Dunnett's. *P<0.05, **P<0.01, ***P<0.001

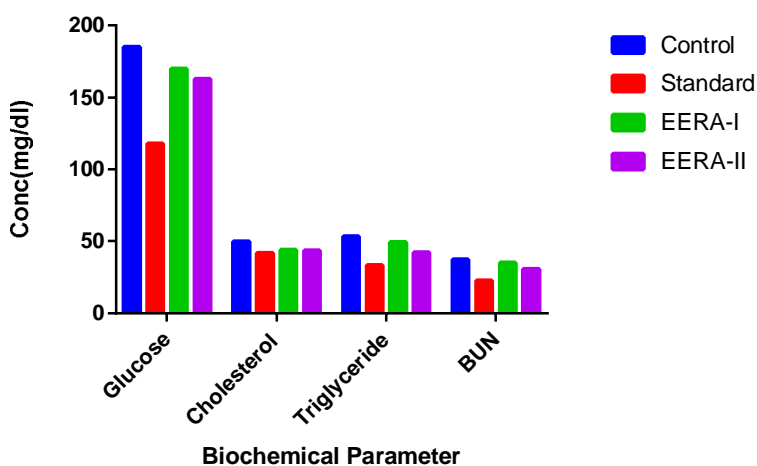


Fig.4: Effect of ethanolic extract of *R.arboreum* on biological parameters in swimming endurance test

Table 6: Effect of ethanolic extract of *R.arboreum* organ weight in swimming endurance test

Treatment group	Dose in mg/kg ,p.o.	Liver(g)	Spleen (g)	Adrenal Gland(g)
Control	-	6.6±0.13	1.09±0.0489	0.144±0.00346
Standard	100 mg/kg	3.9±0.054***	0.874±0.0246*	0.0932±0.0106**

EERA-I	200 mg/kg	5.1±0.171*	0.967±0.0989	0.112±0.00707*
EERA-II	400 mg/kg	3.88±0.215**	0.867±0.0333*	0.107±0.00516**

Results are expressed as mean ±SEM, data analyzed by using one way ANOVA followed by Dunnett"s. *P<0.05, **P<0.01, ***P<0.00

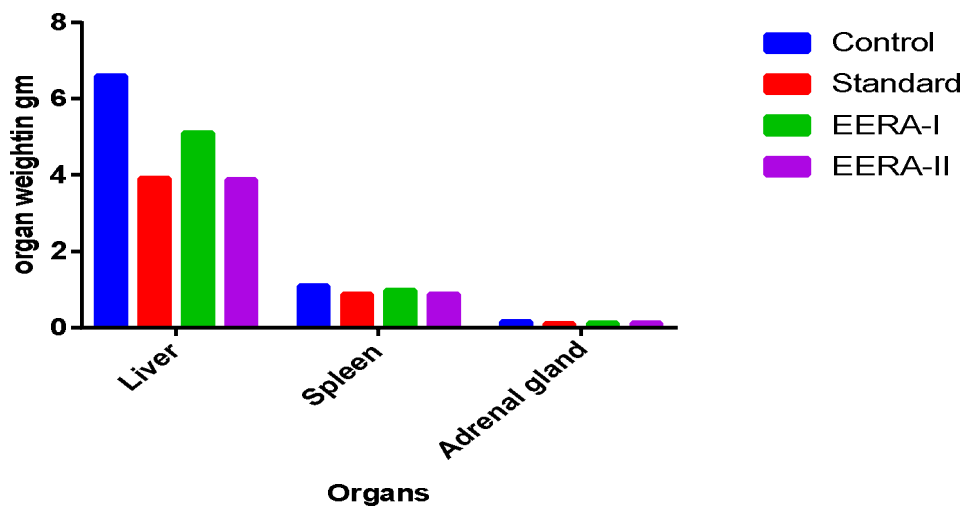


Fig. 5 Effect of ethanol extract of *R.arboreum* organ weight in swimming endurance test

Table 7: Effect of ethanol extract of *R.arboreum* on (Blood Cell Count) in swimming endurance test

Treatment group	Dose in (mg/kg .p. o.)	RBC (millions/cumm)	WBC(Cells/ cumm)	Differential Leucocytes Count (cell/cumm)		
				Lymphocytes (cell/cumm)	Neutrophils (cell/cumm)	Eosionophils(cell/cumm)

Control	–	10.7±0.441	10402±250	65.4±1.46	65.4±1,46	3.05±0.0764
Standard (<i>withania somnifera</i>)	100	8.3±0.251**	9651±143*	69.3±1.04	69.3±1.04**	2.38±0.0601**
EERA-I	200	9.69±0.396	9887±155	68.2±0.477	68.2±0.477	2.75±0.123
EERA-II	400	9.12±0.381*	9587±203*	69.2±1.28	69.2±1.28	2.72±0.0946*

Results are expressed as mean ±SEM, data analyzed by using one way ANOVA followed by Dunnett's. *P<0.05, **P<0.01, ***P<0.001

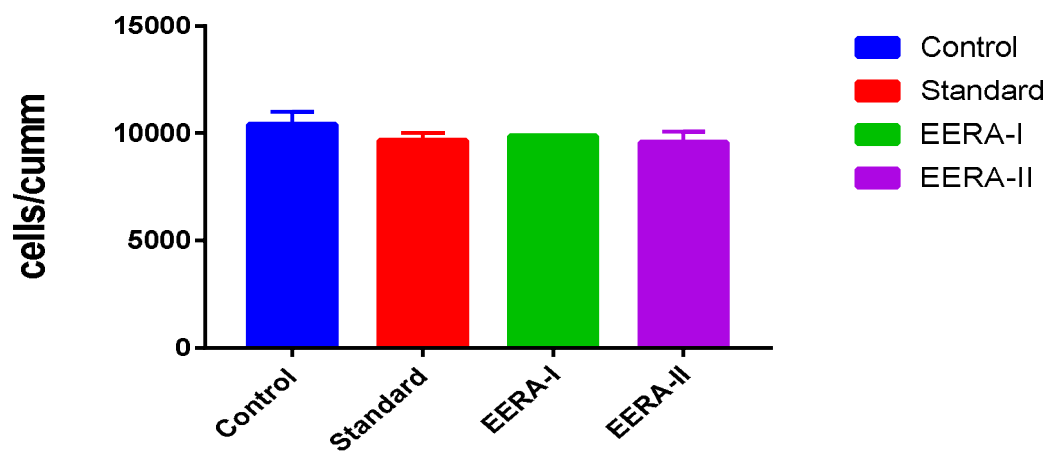


Fig.6: Effect of ethanolic extract of *R.arboreum* on WBC in swimming endurance test

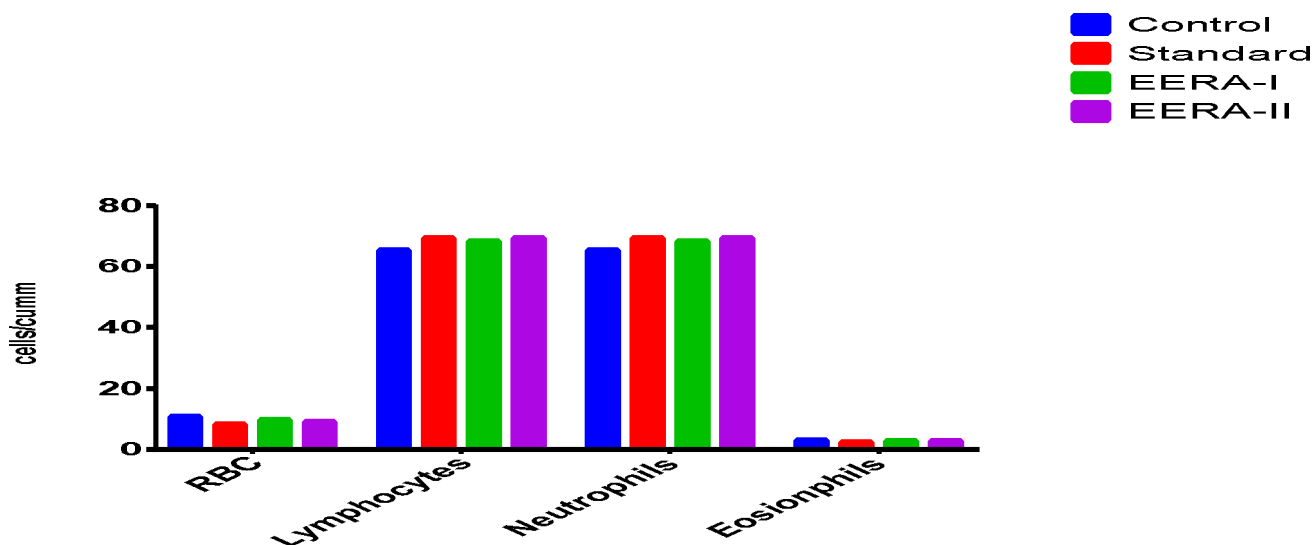


Fig.7: Effect of ethanolic extract of *R.arboreum* on (Blood Cell Count) in swimming endurance test

3.3 Tail Suspension Test

Antidepressant activity of ethanolic extract of *R.arboreum* was studied in mice by tail suspension test. The duration of immobility was reduced after treated with ethanolic extract of *R.arboreum* as compared to control. The effect of ethanolic extract of *R.arboreum* at low dose (200 mg/kg, p.o.) and high dose (400mg/kg,p.o.) was near to standard Imipramine. Study indicates that ethanolic extract at both dose (i.e.200 mg/kg, p.o and 400 mg/kg, p.o.) has considered as significant antidepressant activity. Results were depicted Table 8 and graphically represented in Figure 8

Table 8 Effect of ethanol extract of *R. arboreum* on tail suspension test

Treatment group	Dose in mg /kg (p.o.)	Duration of immobility(sec)		
		1st day	7th day	14th day
Control	-	239±1.83	236±3	231±2.39
Standard (<i>withania somnifera</i>)	100mg/kg	232±1.3*	223±2.14**	213±2.14***
EERA-I	200mg/kg	236±01.53	230±1.54	217±2.19**
EERA-II	400mg/kg	234±1.73	225±1.86**	216±2.39***

Results are expressed as mean ±SEM, data analyzed by using one way ANOVA followed by Dunnett's. *P<0.05, **P<0.01, ***P<0.001

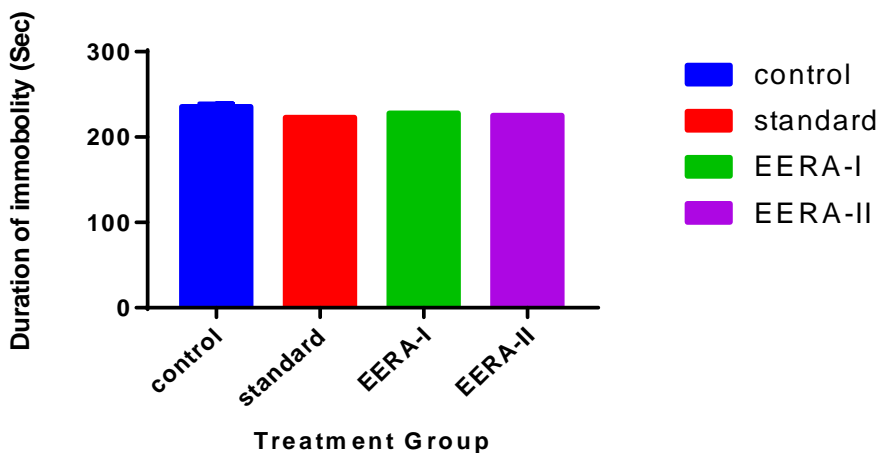


Fig.8: Effect of ethanolic extract of *R.arboreum* flower on tail suspension test

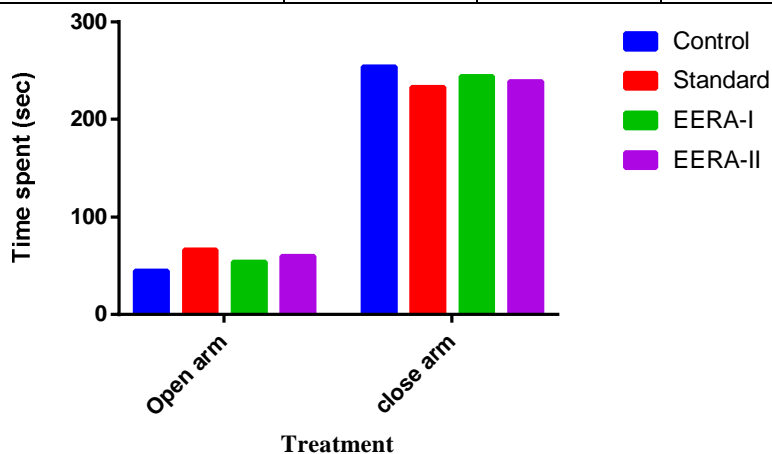
3.4 Elevated Plus Maze Test

According to the result (Table no.9) the time spend and number of entries in open arm were significantly increased in high dose and standard (figure no.9 & 10) and there was significantly decreased in time spend and number of entries in enclosed arm (figure no.9 & 10) as compared to control. The study result (table no.9) showed that the *R. arboreum* flowers extract in high dose significantly increase in the exploration time and entries in to open arm. It shows increase in the mobility of the mice in open arm which suggest the antidepressant activity.

Table 9: Effect of ethanolic extract of *R. arboreum* on Elevated plus maze test model mice

Group	Treatment	Time spend in open arm (Sec.) Mean \pm SEM	Time spend in enclosed arm (Sec.) Mean \pm SEM	No. Of entries in open arm Mean \pm SEM	No. Of entries in enclosed arm Mean \pm SEM
Control	Saline	45.2 \pm 4.91	254 \pm 5.01	5.67 \pm 0.333	10.20 \pm 0.477
Standard	Imipramine (10mg/kg)	66.5 \pm 3.91**	233 \pm 3.81 **	10 \pm 0.577**	7.83 \pm 0.601 *
Low Dose	EERA(200mg/kg)	54.2 \pm 3.52	244 \pm 3.99	7.17 \pm 0.601	8.83 \pm 0.601
High Dose	EERA(400mg/kg)	60 \pm 3.65 *	239 \pm 12.81*	8.17 \pm 0.601*	7.67 \pm 0.558 *

Results are \pm SEM(n=6), data way ANOVA *P<0.05, **P<0.01,



expressed as mean analyzed by using one followed by Dunnett's. ***P<0.

Figure 9: Effects of ethanolic extract of *R. arboreum* on Time spend in open arm & in enclosed arm.

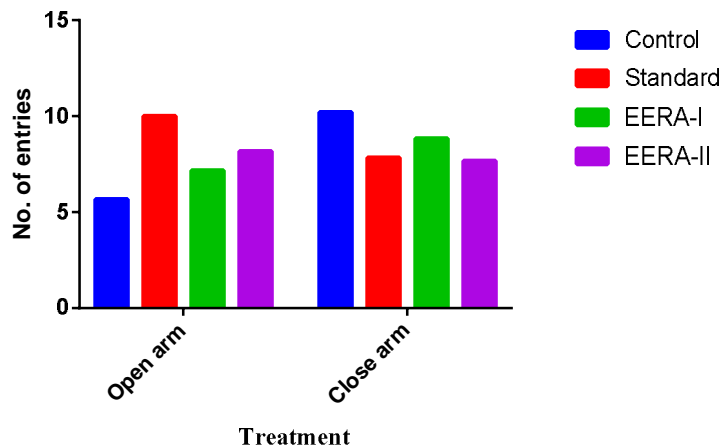


Figure 10: Effects of ethanolic extract of *R. arboreum* on number of entries in open arm & entries in enclosed arm

DISCUSSION

In The preliminary phytochemical screening of the flower extracts of *R. arboreum* Sm. Ssp. Nilagiricum (Zenker) Tagg carried out by Kiruba and co-worker showed the presence of various bioactive compounds such as phenols, saponins, steroids, tannin, xanthoprotein and coumarin. These compounds have been reported to possess several

pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. whereas on my current study it was found the presence of flavonoids, steroids and triterpenoids, phenolic compounds, and tannins in the flower extracts of *R. arboreum* Sm. ssp. *nilagiricum* (Zenker) Tagg. The presence of flavonoids and phenolic compounds is particularly noteworthy as these are well-known for their antioxidant properties and can prevent or delay the onset of chronic diseases

In another study, Mohammad Nisar and co-worker investigated the antibacterial and cytotoxic activities of various parts of the *R. arboreum* plant extract, including the flower, leaves, bark, stem, and roots. The results showed significant antibacterial activity against medically important pathogens such as *Salmonella typhi*, *E. coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. The cytotoxicity of the crude extract was also found to be effective against *A. salina* at a concentration of 1000µg/ml [14, 15].

In the various studies conducted on *Rhododendron arboreum*, Neeraj et al. found that the ethyl acetate fraction of *Rhododendron arboreum* flowers had a potent hepatoprotective effect against carbon tetrachloride-induced hepatic damage in rats. They suggested that this effect was due to glutathione-mediated detoxification and free radical scavenging activity. Sonar et al. investigated the anti-microbial and phytochemical properties of *Rhododendron arboreum* flowers. They found that the alcoholic extract of the plant was more active against bacterial strains than fungal strains. They suggested that the observed anti-microbial activity was due to the presence of Quercetin and other bioactive agents. and In another studies conducted by Roy et al. a on the leaves of *Rhododendron arboreum* found that the plant extract possessed potent anti-stress activity in test animals. They concluded that the adaptogenic activity was due to the presence of strong antioxidant activity from flavonoids and gallic acid [16-18].

Our study conducted on the ethanolic extract of *Rhododendron arboreum* to evaluate its **anoxia tolerance, swimming endurance, and antidepressant activity**. The study found that the extract exhibited significant improvements in tolerance stress time, swimming time, and exploration time in open arms in mice. Additionally, the extract showed a noteworthy reduction in the duration of immobility in the tail suspension test, suggesting its potential as an antidepressant agent.

Overall, these studies suggest that *Rhododendron arboreum* may possess various pharmacological properties, including hepatoprotective, anti-stress, anti-microbial, anoxia tolerance, and antidepressant activities. However, further studies are needed to confirm these findings and explore the underlying mechanisms of action.

The present study was carried out in healthy animals to analyze the adaptogenic and antidepressant activity. The detailed study should be conducted in the human volunteers who are suffering from depression and stress.

CONCLUSION

The present studies on *Rhododendron arboreum* have revealed significant pharmacological properties associated with its flower extracts and leaves. The flower extracts possess antioxidant properties, potentially aiding in the prevention of chronic diseases. They also exhibit antibacterial activity and cytotoxicity against certain pathogens and organisms. The ethyl acetate fraction of the flowers demonstrates hepatoprotective effects, while the alcoholic extract displays antimicrobial activity. The leaves show potent anti-stress and adaptogenic properties, attributed to strong antioxidant activity from flavonoids and gallic acid.

Moreover, research supports the adaptogenic and antidepressant activities of the ethanolic extract of *R. arboreum* flowers in animal models, with improvements observed in stress tolerance, swimming time, exploration time, and reduction in immobility duration. However, more studies are required to validate these findings and understand the underlying mechanisms of action. Human trials involving individuals with depression and stress would provide further insights. Additionally, investigating the specific bioactive compounds, such as rutin, quercetin, and terpenes, and their mechanisms of action is necessary. The studies suggest that *R. arboreum* has the potential to serve as a natural source of compounds with diverse pharmacological activities. Continued exploration of this plant may lead to the development of novel therapeutic agents for chronic diseases prevention and treatment, as well as for managing stress and depression.

CONSENT

It is not applicable

ETHICAL APPROVAL

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Office of institutional animal ethical committee (IAEC) of Mallige College of pharmacy, Bangalore. Reg. no. 1432/PO/Re/S/11/CPCSEA.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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