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INVESTIGATION OF ANXIOLYTIC POTENTIAL OF ETHANOLIC EXTRACT OF *PUTRANJIVA ROXBURGHII* WALL LEAVES IN RAT MODELS

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ABSTRACT

Anxiety disorders, marked by persistent fear and excessive worry, impair daily functioning and quality of life. While conventional anxiolytics are effective, their long-term use often leads to side effects, prompting interest in safer, plant-based alternatives. *Putranjiva roxburghii* Wall, a traditional medicinal plant, has been used to treat various ailments. This study aimed to investigate the anxiolytic potential of the ethanolic extract of *Putranjiva roxburghii* wall leaves in rat models through behavioral and biochemical assessments. **Methodology:** EEPR was prepared using Soxhlet extraction from shade-dried leaves, yielding 20.6%. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, tannins, steroids, saponins, triterpenes, resins, amino acids, and carbohydrates. The study was conducted with four groups of rats: control, standard (diazepam), low dose (250 mg/kg), and high dose (500 mg/kg) EEPR. Behavioral tests were performed weekly, including: EPM: Assessed open arm and center activity. OFT: Evaluated exploratory behavior and locomotor activity. HBT: Measured exploratory behavior through head-dipping. Rotarod test: Monitored motor coordination and spontaneous activity. On the 29th day, biochemical parameters such as monoamine oxidase (MAO) activity and lipid peroxidation were assessed to measure neurochemical effects and oxidative stress. **Result:** The ethanolic extract of *Putranjiva roxburghii* leaves (20.6% yield) contained alkaloids, glycosides, flavonoids, tannins, steroids, saponins, and other bioactive compounds. Behavioral tests demonstrated that the high dose (500 mg/kg) significantly improved open-arm exploration in the (EPM) and enhanced head-dipping behavior in the (HBT), comparable

to diazepam. Both doses increased time spent in the central zone of the (OFT) and reduced muscle rigidity in the rotarod test. Biochemical analysis revealed dose-dependent reductions in MAO activity and lipid peroxidation, highlighting its anxiolytic and antioxidative potential. **Conclusion:** The EEPR showed dose-dependent anxiolytic effects, supported by behavioral and biochemical improvements. Phytochemicals like alkaloids, glycosides, and flavonoids may underlie its activity, highlighting its potential for further clinical exploration.

KEYWORDS: *Putranjiva roxburghii* wall, Ethanolic extract (EEPR), Alkaloids, Flavonoids, Diazepam, Elevated plus maze (EPM), Open field test (OFT), Hole board test (HBT), Rotarod test, Monoamine oxidase (MAO), Lipid peroxidation, Dose-dependent effect.

INTRODUCTION

Anxiety is a medical state related to our psychological as well as physiological behaviour having numerous characters like cognitive, emotional, behavioral and somatic. The term anxiety actually came from a Latin word “Ango” which means “to vex or torment” may be in the absence or presence of any psychological stress.[1]

Anxiety disorders are highly prevalent psychiatric disorders, affecting an estimated 25% of the adult population at some point during their lifetime. A large survey found that less than 14% of people with psychiatric disorders receive treatment. Anxiety disorders are highly comorbid, occurring in about 58% of patients with major depressive disorder and 93% of patients with bipolar disorder.[2]

Anxiety usually refers to the experience of fear, apprehensiveness, nervousness, panic, restlessness, tension, and agitation. Manifest symptoms include trembling, fainting, headaches, and sweating, possibly elevated blood pressure, and changes in other psychophysiological indices such as heart rate, muscle tone, and skin conductance.[3]

Benzodiazepines, Tricyclic antidepressants (TCA’s), Monoamine oxidase inhibitors, SSRIs, and azapirones are commonly used conventional medicines fortreating anxiety disorders; however, β –blockers, Hydroxyzine, and antipsychotic drugs are sometimes useful in treating anxiety and other medical disorders. These conventional drugs produce significant adverse effects in their long-term use as anticholinergic effects, hypotension, weight gain, sleepiness and fatigue.[4]

Hence, Medicinal plants and herbs are often used to cure or prevent diseases. According to

WHO (World Health Organization), medicinal herbs account for 80% of raw materials used in herbal medicine. About 80% of the global population relies on plant-based medicine for their primary health care.[5]

Putranjiva rouxburghii Wall. (*P.roxburghii*) belongs to the family euphorbiaceae that cultivated in mostly Asian tropical regions *P.roxburghii* is known for its therapeutic properties, and grown all over India, abundantly in khammam forests of Andhra Pradesh It is used by tribes in treating a number of health problems like fever, cold, illness, phlegm, skin ailment, acidity and sterility.[6]

P. roxburghii Wall is a large spreading, evergreen tree with numerous pendant branches, and height ranging from 15-20 m The trunk is light brown to greyish in colour, bearing horizontal lenticels. The leaves are elliptical to oblong in shape, with a slightly pointed apex, dark green in colour and glabrous, arranged alternately on the branches. The flowers are small, unisexual, greenish yellow in colour. Male flowers are found in axillary clusters, whereas female flowers are usually solitary. Fruits are rounded to elliptical drupes, with a single hard, stony seed.[7]

There is a tire need for mechanistically novel, more effective treatments for anxiety. Therefore, more recent drugs trails have focused on screening for natural source that have multiple targets to control anxiety with minimal side effects. Hence the present study focused on to evaluate the ethanolic extract of *Putranjiva roxburghii* wall. and screen for anxiolytic potential in rats. Literature study reveals that, no significant data on anxiolytic activity of *Putranjiva roxburghii* wall.

MATERIALS AND METHODS

Chemicals

Ethanol (Extraction solvent), Diazepam, Phosphate buffer (pH 7.8), 650µl of Hydrogen Peroxide solution. (7.5 mm), Thiobarbituric acid (TBA), 0.2 N Hydrochloric acid (HCL), L tyramine plus /L pargyline, 4-aminoantipyrine, Horseradish peroxidase, 15% trichloroacetic acid. Normal saline, were purchased from Yarrow chem products, Mumbai, India. The remaining reagents were of analytical grade.

Collection and Authentication of plant

Leaves of *Putranjiva roxburghii* wall. were collected from local areas of Chitradurga, Karnataka. They were washed, and then the leaves were dried in fresh circulating air under shade. The leaves material was identified and authenticated by Mrs. Niveditha B T, Assistant Professor, Dept of Studies in Botany, Jyanagangothri PG Center, GR Halli, S Davanagere University, Chitradurga, Karnataka.

Preparation of Plant Extract[8]

The leaves of *Putranjiva roxburghii* Wall. were collected from a local garden, shade-dried, and ground into a coarse powder using a grinder. The powdered leaves were packed in a Soxhlet apparatus and extracted with 70% ethanol (1:10 w/v) at 40°C for 48 hours. The extraction process was repeated as needed, and the hot extract was filtered. Excess solvent was removed using a rotary flash evaporator, and the crude extract was stored in an air-tight container at below 10°C for further studies

Preliminary phytochemical screening[9,10]

Preliminary phytochemical investigations were carried out on the ethanolic extract of *Putranjiva roxburghii* wall. leaves for the detection of various phytochemicals by using standard methods prescribed in practical pharmacognosy by C K Kokate and R K Khandelwal.

Experimental Animals

Animal ethical clearance was obtained from Institution Animal Ethics Committee (IAEC) for experimental purpose **(Ref No:03C/SJMCP/IAEC/SEPT2023/2022-23)**. Healthy Adult Wistar Albino rats weighing about 150-200g of either sex was used for this study. The animals were obtained from Biogen Laboratory Animal Facility, Bangalore – 562107. Before the initiation of the experiment, the animals were acclimatized for 10 days and randomized under standard environmental conditions such as temperature (26±2^{0}C), relative humidity (45-55%), and 12hrs light/dark cycle maintained as per Committee for Control and Supervision of Experiments on Animal (CCSEA) guidelines. All the animals were allowed free access to standard laboratory pellets and drinking water ad libitum under strict hygiene conditions.

Selection of Screening Dose[8]

The selection of doses for the ethanolic extract of *Putranjiva roxburghii* Wall. in this study was guided by the findings of an acute oral toxicity study conducted by *Lakshmi Rajahamsa* et al. (2013). The study, following OECD guidelines 423, reported that the median lethal dose (LD_{50}) of the extract was 5000 mg/kg (5 g/kg). Based on these findings, $1/10^{th}$ & $1/20^{th}$ of the LD_{50} is considered as high dose and low dose respectively.

Low dose: - 250mg/kg.

High dose: - 500mg/kg.

Experimental design[11]

The present study aimed to investigate the potential anxiolytic effects of *Putranjiva roxburghii*. Wall in Albino Wistar rats. The experimental design included four groups: Group 1 served as the negative control, Group 2 received the standard drug, while Groups 3 and 4 were administered EEPR at low dose (250 mg/kg) and high dose (500 mg/kg) doses, respectively. Treatments were given once daily for 4 weeks. Behavioral tests were performed on Days 1, 7, 14, 21, and 28, 1 hour after administration of the treatment, distilled water, or the standard drug. Biochemical assays were conducted at the end of the 4-week treatment period.

Table No 3: Experimental design.[11]

The animals were divided into five groups with six rats each: (n=6).

Sl. no	Groups	Treatment
1	Group 1 (Control)	Standard diet and water *ad libitum.*
2	Group 2 (Standard)	Diazepam 1mg/kg p.o for 28days.
3	Group 3 (Test group I)	Low dose of EEPR 250mg/kg p.o for 28 days.
4	Group 4 (Test group II)	High dose of EEPR 500 mg/kg p.o for 28 days.

Behavioral parameters assessment

1. Elevated plus maze[12]

The elevated plus maze consisted of two open arms (50×10 cm) and two enclosed arms (50×10×40 cm) with an open roof, elevated 50 cm above the ground. Animals in the control, standard, and treatment groups received a single oral dose of normal saline, standard drug, or *Putranjiva roxburghii* extract (250 mg/kg and 500 mg/kg) one hour before testing. Each rat was placed at the junction of the four arms, facing an open arm, and underwent a 5-minute

session. The number of entries and time spent in each arm were recorded, with increased open arm activity indicating anti-anxiety behavior.

2. Open field test[13]

The apparatus used was a wooden box measuring 60 × 60 × 30 cm, with the floor divided into 16 squares (15 × 15 cm) and illuminated by a 40-W lamp suspended 100 cm above. Animals were administered saline, extract, or the standard drug diazepam (1 mg/kg, p.o) one hour before testing. After 30 minutes, each animal was placed individually in one of the corner squares, and their behavior was observed for 5 minutes. The number of rearing, assisted rearing (forepaws touching the walls), and squares crossed were recorded, with an increase in square crossings indicating enhanced locomotor activity.

3. Hole Board Test[14]

The hole board apparatus was a wooden box (60 × 60 × 30 cm) with four equidistant holes, each 2 cm in diameter, on the floor. Animals were administered saline, extract, or the standard drug, and 10 minutes later, they were allowed to explore the hole board. The number of head dips and the time spent head-dipping were recorded over a 10-minute period. Head dipping was defined as the animal lowering its head into a hole at least to eye level. To prevent interference from odour cues, the board was cleaned with 10% alcohol after each trial. An increase in the number and duration of head dips indicated enhanced exploratory activity, suggesting anxiolytic-like behavior, while a decrease in these parameters reflected sedative effects.

4. Rotarod test[12]

The effect on motor coordination was evaluated using a Rota-rod apparatus, which consisted of a base platform and a 3 cm diameter, 30 cm long iron rod with a non-slippery surface, divided into four sections by three disks. Animals underwent a training session 24 hours before the test, where those able to remain on the rotating rod (20-25 rpm) for 5 minutes were selected. During the test, four rats walked simultaneously on the rod at the same speed (20-25 rpm). Each group received saline, extract, or the standard drug diazepam (1 mg/kg, p.o) one hour prior to testing. The time interval between mounting the rod and falling off was recorded automatically as the performance time, with observations made over a 5-minute period (300 seconds).

Biochemical assessment of brain homogenate includes the following

1. Lipid peroxidation[11]

Brain tissue was homogenized in ice-cold Tris–HCl buffer (50 mM, pH 7.4) for 2 minutes at 5,000 rpm, followed by centrifugation at 5,000g for 60 minutes. The sample was then mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate proteins. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) tertiary butyl alcohol (TBA) in a boiling water bath for 10 minutes. After cooling, the absorbance was measured at 532 nm using a spectrophotometer.

Calculation

$$Conc.of\ MDA = \frac{Abs\ 532 \times 100 \times VT}{(1.56 \times 105) \times WT \times VU}$$

Where,

Abs_{532} is absorbance

V_T is total volume of mixture (4ml)

1.56×10^5 is molar extinction co-efficient

W_T is weight of dissected brain

V_U is aliquot volume (1 ml)

2. Monoamine oxidase assay[11]

After completing the behavioral tests, animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight). The brain regions (cortex and striatum) were dissected and homogenized in ice-cold 100 mmol/L potassium phosphate buffer (pH 7.4) using a glass Potter-Elvehjem homogenizer. The homogenates were incubated with 500 mmol/L tyramine and 500 nmol/L pargyline to inhibit MAO-B activity. A chromogenic solution, prepared with 1 mmol/L vanillic acid, 500 mmol/L 4-aminoantipyrine, and 4 IU/mL horseradish peroxidase in 0.2 mol/L potassium phosphate buffer (pH 7.6), was used in the assay. Absorbance was measured spectrophotometrically at 490 nm, and the MAO-A activity was expressed as mmol/min per gram of tissue.

STATISTICAL ANALYSIS

The data obtained from the above findings was subjected to statistical analysis using one-way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the result.

RESULTS

Percentage yield of ethanolic extract: **20.06%.**

Colour and consistency: Dark green and Thick Semi-solid.

Preliminary phytochemical screening

The preliminary phytochemical investigation of EEPR leaves confirms alkaloids, glycosides, flavonoids, tannins, steroids, saponins, triterpenes, resins, and carbohydrates. (The Results are shown in Table No.5)

Table No 5: Preliminary Phytochemical Investigation of EEPD leaves.

Sl. No	Phytoconstituents	Ethanolic Extract
1.	Tannins	+
2.	Saponins	-
3.	Triterpenoids	-
4.	Flavonoids	+
5.	Resins	+
6.	Glycosides	+
7.	Alkaloids	+
8.	Steroids	+
9.	Carbohydrates	+
10.	Phenols	+

(+): Present, (-): Absent

Anti-anxiety activity

A test sample of ***Putranjiva roxburghii* wall** was screened for anxiolytic potential by employing the Elevated Plus Maze Model, Rota Rod Test, Hole Board Test, and Open field Test and Biochemical parameters like Monoamine-oxidase assay and lipid peroxidation in Wistar albino rats of either sex weighing 150-200g.

Behavioral Results

Elevated plus maze model

The ethanolic extract of *Putranjiva roxburghii* Wall. leaves was evaluated for anxiolytic activity using the elevated plus maze (EPM) model in Wistar albino rats (150–200 g) of both sexes. The parameters measured included the number of entries and the time spent in the open and closed arms, leveraging the rats' natural aversion to heights and open spaces to assess anxiety-related behavior. The experiment spanned 28 days, with weekly assessments on the 7th day of each week, comparing a control group to three treatment groups: a standard group receiving Diazepam (1 mg/kg), a low dose of ethanolic extract (250 mg/kg), and a high dose (500 mg/kg). Results showed that the diazepam group exhibited a highly significant

increase (****P < 0.0001) in both entries and time spent in the open arms, reflecting strong anxiolytic effects. The high dose of the extract (500 mg/kg) also produced significant improvements (***P < 0.001) compared to the low dose, which achieved significance by the 3rd week (*P < 0.05) and further increased by the 4th week (**P < 0.01). A progressive enhancement in anxiolytic-like behavior was observed across all groups over time, demonstrating a dose-dependent effect throughout the study.

Table No. 6: Effect of EEPR on the number of entries in open arms.

Group	Treatment	Number of entries in Open-arm (Counts/5min)			
		1st Week	2nd Week	3rd Week	4th Week
Group I	Control (Normal saline)	3.00 ± 0.36	2.83 ± 0.30	2.66 ± 0.49	3.00 ± 0.57
Group II	Standard (Diazepam)	7.00 ± 0.73***	9.83 ± 1.10****	11.00 ± 1.03****	12.50 ± 1.17***
Group III	Low Dose of EEPR (250mg/kg)	3.66 ± 0.42^{ns}	4.33 ± 0.49^{ns}	5.50 ± 0.76*	10.66 ± 1.52**
Group IV	High Dose of EEPR (500mg/kg)	5.66 ± 0.66**	7.33 ± 0.49***	7.83 ± 0.47***	11.16 ± 1.60***

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

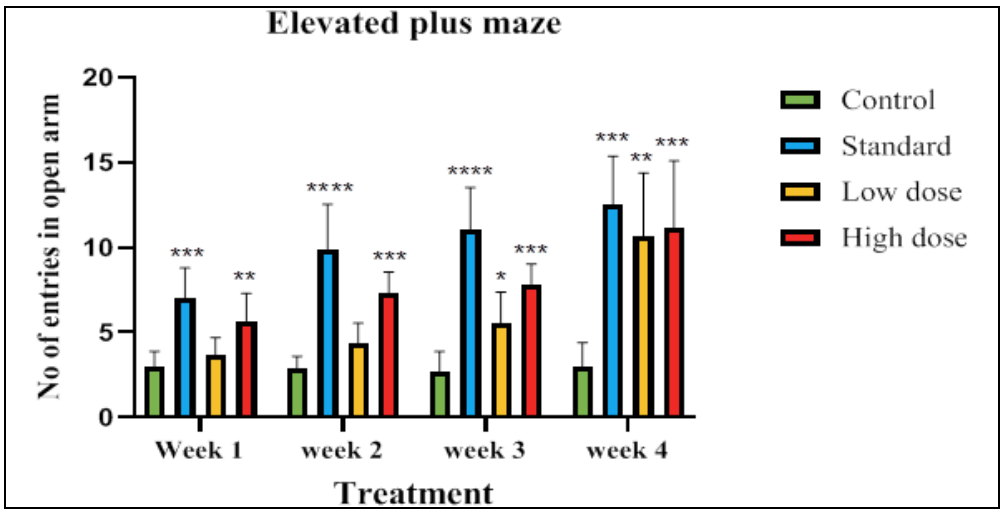

Fig No. 10: Effect of EEPR on the number of entries in open arm.

Table No. 7: Effect of EEPR on the time

Group	Treatment	Time spent in Open arm (Counts/5min)			
Group I	**Control (Normal saline)**	17.83 ± 2.08	44.33 ± 3.49	41.16 ± 4.82	48.66 ± 4.08
Group II	**Standard (Diazepam)**	117.33 ± 10.97****	122.17 ± 8.83****	156.83 ± 7.79****	164.67 ± 6.55****
Group III	**Low Dose of EEPR (250mg/kg)**	21.83 ± 4.11^{ns}	69.33 ± 4.23*	71.83 ± 6.46*	87.00 ± 8.83**
Group IV	**High Dose of EEPR (500mg/kg)**	53.16 ± 3.15**	80.50 ± 3.38***	143.00 ± 7.55****	125.50 ± 6.91****

spent in open arms.

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

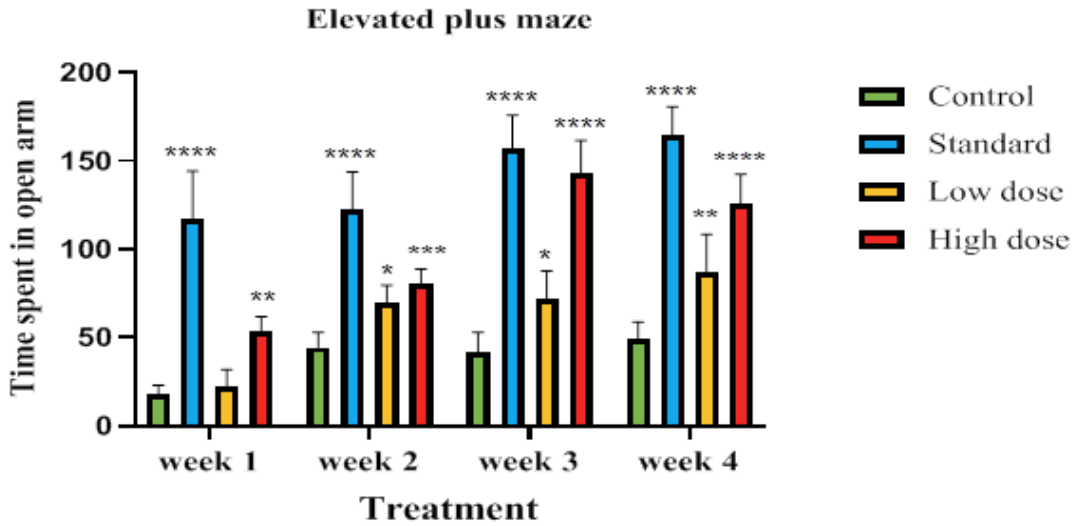

Fig No. 11: Effect of EEPR on the time spent in open arms.

Table No. 8: Effect of EEPR on the number of entries in closed arms.

Group	Treatment	Number of entries in Closed-arm (Counts/5min)			
		1st Week	**2nd Week**	**3rd Week**	**4th Week**
Group I	**Control (Normal saline)**	11.00 ± 1.31	10.33 ± 2.04	9.16 ± 1.85	9.16 ± 1.66
Group II	**Standard (Diazepam)**	5.16 ± 0.90***	2.83 ± 0.60***	2.66 ± 0.49**	2.66 ± 0.33***

Group III	**Low Dose of EEPR (250mg/kg)**	2.66 ± 0.49****	6.33 ± 0.76 ns	6.00 ± 1.34 ns	5.16 ± 0.70*
Group IV	**High Dose of EEPR (500mg/kg)**	2.66 ± 0.34****	5.33 ± 0.61*	4.50 ± 0.56*	4.50 ± 0.76**

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

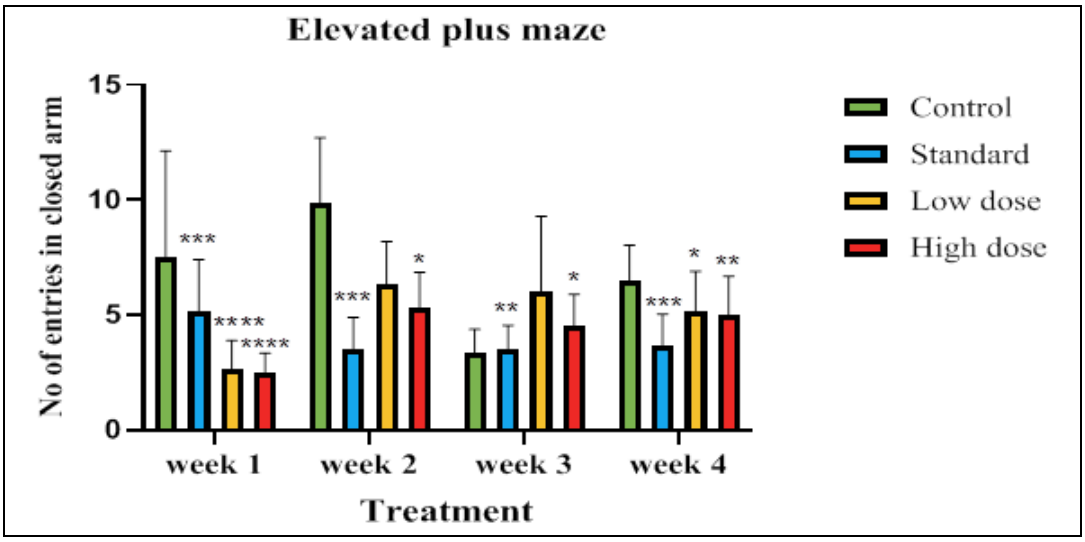

Fig No. 12: Effect of EEPR on number of entries in closed arm.

Table No. 9: Effect of EEPR on time spent in closed arms.

Group	Treatment	Time spent in Closed arm (Counts/5min)			
		1st Week	2nd Week	3rd Week	4th Week
Group I	**Control (Normal saline)**	200.83 ± 14.83	196.2 ± 18.27	204.17 ± 14.41	205.33 ± 13.363
Group II	**Standard (Diazepam)**	113 ± 8.14****	77.16 ± 6.45****	73.33 ± 7.05 ns	101.17 ± 8.25****
Group III	**Low Dose of EEPR (250mg/kg)**	172.17 ± 8.36	146.50 ± 8.91*	140.83 ± 4.90 ns	158.00 ± 6.99**
Group IV	**High Dose of EEPR (500mg/kg)**	128.33 ± 8.10***	96.00 ± 7.28****	110.67 ± 5.23 ns	100.00 ± 5.07****

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

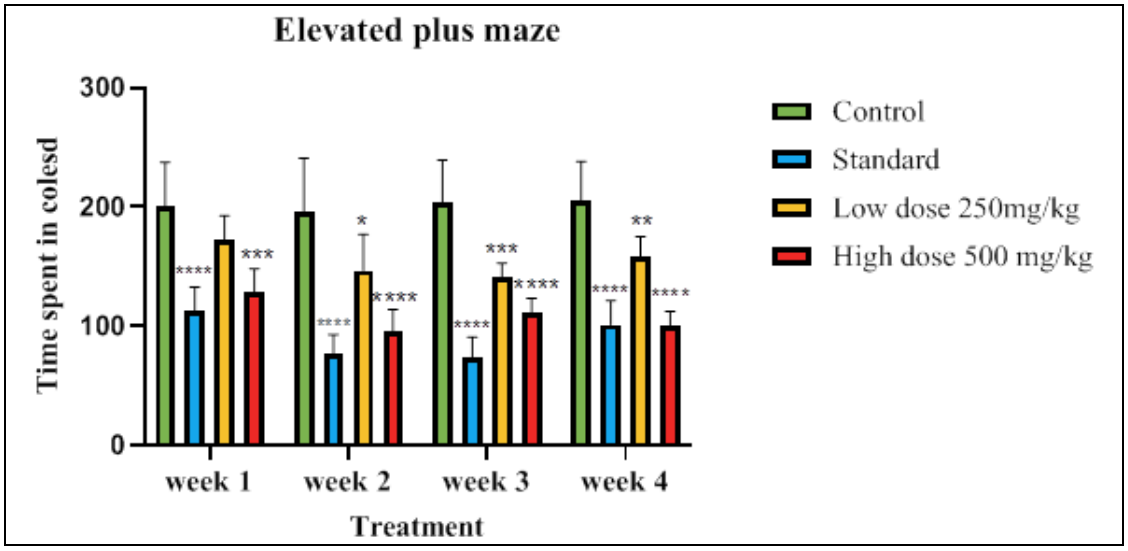

Fig No. 13: Effect of EEPR on the time spent in closed arms.

2. Investigation of anxiolytic potential of *Putranjiva roxburghii* wall leaves in Open field model

In our study, locomotor and exploratory behavior were assessed using the open-field test to evaluate anxiety levels, measuring parameters such as rearing, line crossings (locomotion), and crossings into the center square (exploration). The experiment spanned 28 days, with evaluations conducted weekly on the 7th day of each week, comparing a control group to three treatment groups: a standard group receiving Diazepam (1 mg/kg), a low dose of EEPR (250 mg/kg), and a high dose of EEPR (500 mg/kg). The diazepam-treated group showed a highly significant increase in both locomotor and exploratory activities (****P < 0.0001), indicating strong anxiolytic effects. The low dose of EEPR (250 mg/kg) produced a modest increase, achieving significance in the 3rd week (*P < 0.05) and the 4th week (**P < 0.01), though these effects were less pronounced than those of diazepam and diminished over time. In contrast, the high dose of EEPR (500 mg/kg) resulted in a moderately significant increase in activity (***P < 0.001) compared to the control group. A progressive rise in significance across weeks highlighted the dose-dependent nature of the responses and the gradual development of anxiolytic effects throughout the study.

Table No. 10: Effect of EEPR leaves in Open field model 1st Week.

Open Field Model 1st Week				
Group	**Treatment**	**Rearing**	**Assisted rearing**	**Number of squares traversed in 5 mins**
Group I	**Control (Normal saline)**	4.5 ± 0.76	3.66 ± 0.88	81.83 ± 3.21
Group II	**Standard group (Diazepam 1 mg/kg)**	12.66 ± 1.02****	12.5 ± 1.76 ****	110.00 ± 1.46****
Group III	**Low dose of EEPR (250 mg/kg)**	5.33 ± 0.49 ns	4.5 ± 0.42 ns	80.66 ± 3.43 ns
Group IV	**High dose of EEPR (500 mg/kg)**	8.33 ± 0.95*	10.50 ± 1.17**	102.00 ± 4.40***

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

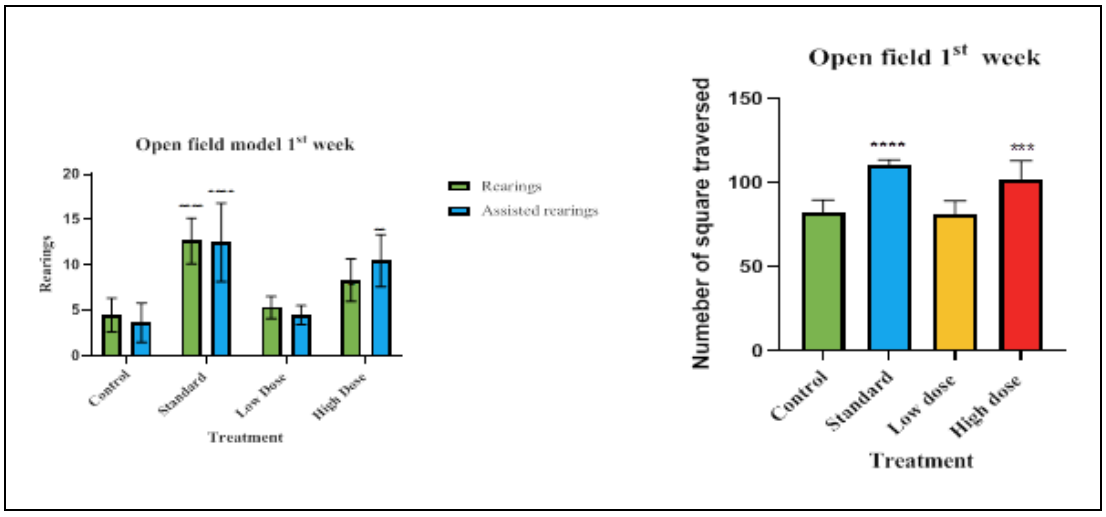

Fig No. 14: Effect of EEPR leaves in Open field model 1st Week.

Table No. 11: Effect of EEPR leaves in Open field model 2nd Week.

Open Field Model 2nd Week				
Group	**Treatment**	**Rearing**	**Assisted rearing**	**Number of squares traversed in 5 mins**
Group I	**Control (Normal saline)**	4.83 ± 0.60	4.66 ± 0.55	81.83 ± 83
Group II	**Standard group**	10.5	11.66	110

	(Diazepam 1 mg/kg)	± 0.99***	± 1.38****	± 1.46****
Group III	**Low dose of EEPR (250 mg/kg)**	6.16 ± 0.70 ns	3.16 ± 0.47 ns	88.50 ± 4.66 ns
Group IV	**High dose of EEMZ (500 mg/kg)**	9.00 ± 0.93**	9.50 ± 0.99**	97.00 ± 3.55*

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

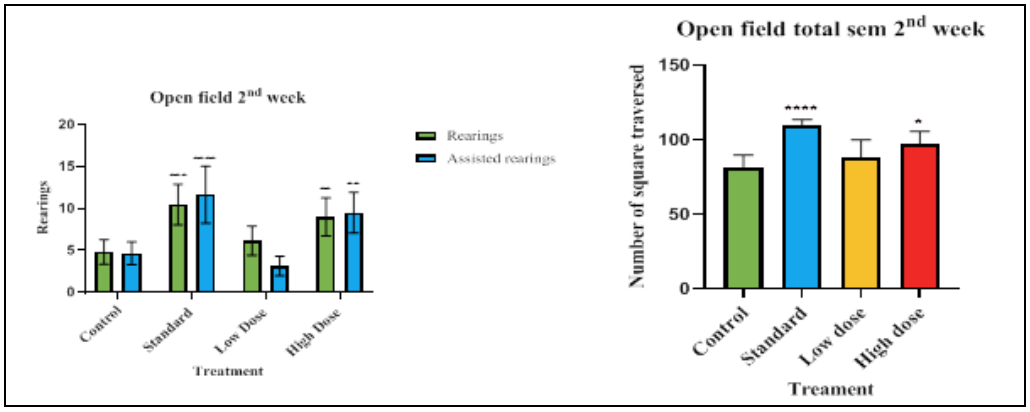

Fig No. 15: Effect of EEPR leaves in Open field model 2nd Week.

Table No. 12: Effect of EEPR leaves in Open field model 3rd Week.

Open Field Model 3rd Week				
Group	**Treatment**	**Rearing**	**Assisted rearing**	**Number of squares traversed in 5 mins**
Group I	**Control**	5.33 ± 0.49	4.66 ± 0.66	83.33 ± 3.53
Group II	**Standard group (Diazepam 1 mg/kg)**	11.83 ± 1.24**	12.33 ± 1.66***	120 ± 3.29****
Group III	**Low dose of EEPR (250 mg/kg)**	6.66 ± 1.33 ns	5.16 ± 0.60 ns	96.66 ± 5.00
Group IV	**High dose of EEPR (500mg/kg)**	10.66 ± 1.60*	10.00 ± 0.96**	100.00 ± 2.85*

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

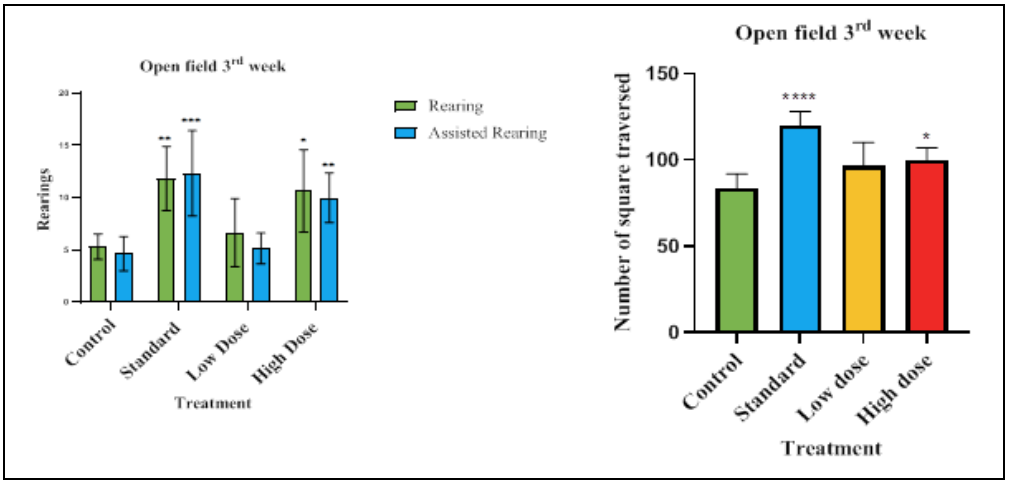

Fig No. 16: Effect of EEPR leaves in Open field model 3rd Week.

Table No. 13: Effect of EEPR leaves in Open field model 4th Week.

Open Field Model 4th Week				
Group	**Treatment**	**Rearing**	**Assisted rearing**	**Number of squares traversed in 5 mins**
Group I	**Control(Normal saline)**	5.16 ± 0.79	2.50 ± 1.38	76.16 ± 2.21
Group II	**Standard group (Diazepam 1 mg/kg)**	13.00 ± 1.06**	15.50 ± 1.38****	126.67 ± 3.44****
Group III	**Low dose of EEPR (250 mg/kg)**	8.50 ± 0.88 ns	7.66 ± 0.66*	96.50 ± 2.65**
Group IV	**High dose of EEPR (500 mg/kg)**	10.50 ± 0.88*	10.50 ± 1.60***	100.00 ± 5.37***

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

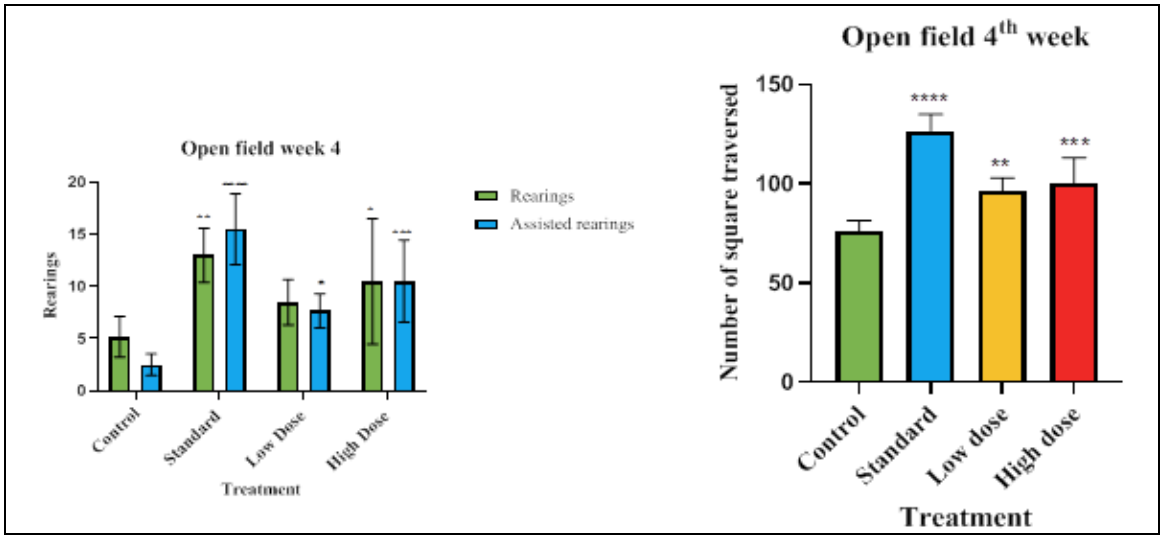

Fig No. 17: Effect of EEPR leaves in Open field model 4th Week

3. Investigation of anxiolytic potential of *putranjiva roxburghii* wall leaves in Hole board apparatus

The ethanolic extract of *Putranjiva roxburghii* Wall. leaves (EEPR) was evaluated for anxiolytic activity using the Hole Board model in Wistar albino rats of either sex, weighing 150–200 g. The 28-day experiment included weekly behavioral assessments on the 7th day, with exploratory behavior measured through head-dipping activity. The rats were divided into four groups: a control group, a standard group receiving Diazepam (1 mg/kg), a low-dose EEPR group (250 mg/kg), and a high-dose EEPR group (500 mg/kg). Diazepam significantly increased the number of head dips (****P < 0.0001), indicating strong anxiolytic effects compared to the other groups. The low-dose EEPR group (250 mg/kg) exhibited a mild but statistically significant increase in head dips (*P < 0.05) relative to the control group, while the high-dose EEPR group (500 mg/kg) demonstrated a more substantial increase (***P < 0.001). Notably, the anxiolytic effect in the high-dose group improved progressively throughout the study, with significance levels increasing each week, culminating in a pronounced effect by the final assessment, highlighting a time-dependent enhancement of efficacy.

Table No. 14: Effect of EEPR leaves in hole board model.

Group	Treatment	Number of head dipping in 5 mins			
		1st Week	2nd Week	3rd Week	4th Week
Group I	Control (Normal saline)	4.16 ± 0.65	3.83 ± 0.70	4.16 ± 1.04	2.66 ± 0.42
Group II	Standard	10.16	12.83	14.66	18.16

	(Diazepam 1mg/kg)	± 0.70**	± 1.55***	± 1.66***	± 3.04****
Group III	**Low Dose of EEPR (250mg/kg)**	5.16 ± 1.70 ns	7.66 ± 2.09 ns	6.66 ± 1.62 ns	6.66 ± 1.62 ns
Group IV	**High Dose of EEPR (500mg/kg)**	10.16 ± 6.44**	11.00 ± 1.06**	11.83 ± 1.27**	13.00 ± 1.21**

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

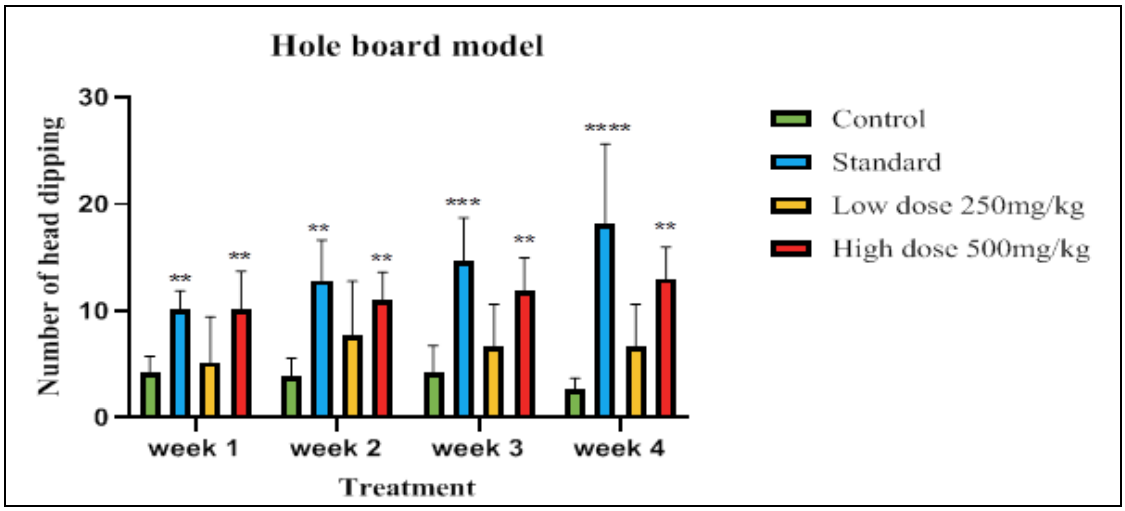

Fig No. 18: Effect of EEPR leaves in hole board mode.

4. Investigation of anxiolytic potential of *Putranjiva roxburghii* wall leaves in Rota-rod apparatus

The ethanolic extract of *Putranjiva roxburghii* Wall. leaves (EEPR) was evaluated for anxiolytic activity using the rotarod model in Wistar albino rats (150-200 g) over 28 days, with behavioral assessments conducted weekly. The study focused on spontaneous activity, muscle coordination, and balance, as stress-induced rigidity can impair neuromuscular function. Diazepam (1 mg/kg) served as the standard, showing a highly significant reduction in locomotor activity and motor coordination (****P < 0.0001) compared to all other groups, indicating a strong anxiolytic effect. The low-dose EEPR group (250 mg/kg) showed a significant reduction (**P < 0.01), while the high-dose group (500 mg/kg) exhibited a moderately significant reduction (***P < 0.001) in motor coordination, both compared to the control group. The high-dose group demonstrated a progressive, time-dependent

enhancement in anxiolytic effects, with results improving over the 28-day period, suggesting the extract's potential in improving motor coordination with sustained use.

Table No. 15: Effect of EEPR muscle grip strength by Rota-rod model.

Group	Treatment	Fall off time in 5 mins (Sec)			
		1st Week	2nd Week	3rd Week	4th Week
Group I	Control (Normal saline)	140 ± 8.26	138.83 ± 3.44	136.16 ± 5.06	126.33 ± 2.48
Group II	Standard (Diazepam 1mg/kg)	93 ± 3.51****	83.83 ± 4.12****	77.00 ± 5.79****	75 ± 2.95****
Group III	Low Dose of EEPR (250mg/kg)	104.50 ± 5.82**	103.67 ± 2.95***	111.67 ± 3.43**	102.67 ± 5.03**
Group IV	High Dose of EEPR (500mg/kg)	101.50 ± 5.77***	102.67 ± 4.46****	99.83 ± 4.65****	101.33 ± 4.84***

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

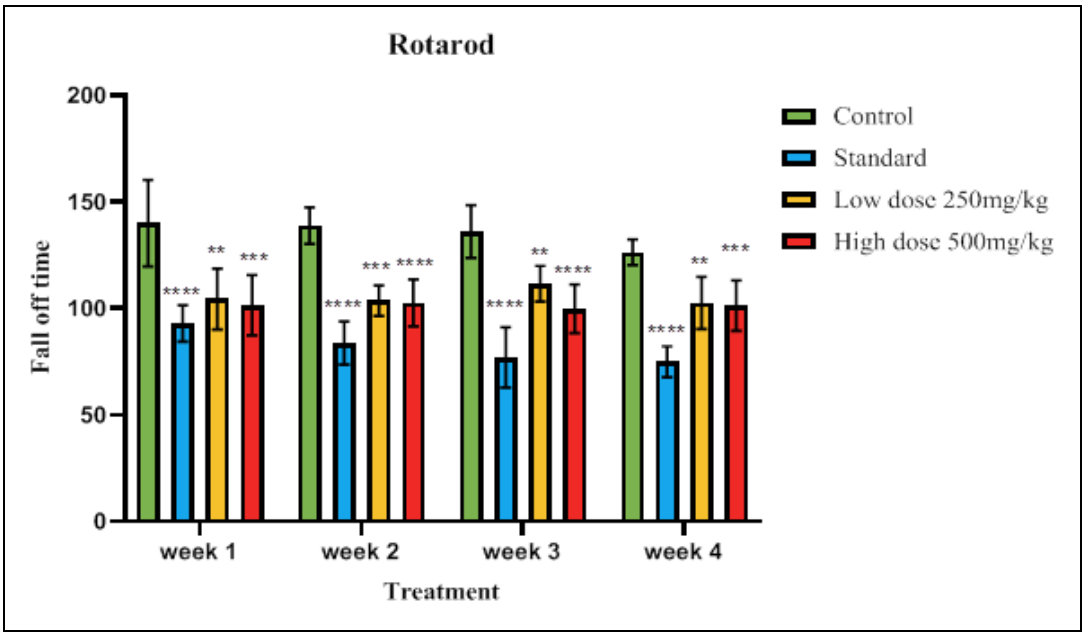

Fig No. 19: Effect of EEPR on muscle grip strength by Rota-rod model.

5. Lipid peroxidation assay of *Putranjiva roxburghii* wall leaves

In the investigation of the anxiolytic potential of the ethanolic extract of *Putranjiva roxburghii* leaves, rats were divided into four groups—control, standard (Diazepam 1 mg/kg),

low-dose (250 mg/kg), and high-dose (500 mg/kg)—and treated over 28 days, with lipid peroxidation assessed on the 29th day as a key biochemical parameter. Malondialdehyde (MDA), a marker of lipid peroxidation, increases with oxidative stress caused by free radicals. Scopolamine-induced cognitive impairment can elevate reactive oxygen species (ROS), damaging lipids and raising MDA levels. The lipid peroxidation assay revealed a dose-dependent reduction in oxidative stress, with the standard group showing the greatest decrease (**P < 0.01), followed by the high-dose group (**P < 0.01) and the low-dose group (*P < 0.05) compared to the control. These results suggest that the extract not only exhibits anxiolytic properties but also mitigates oxidative stress, with higher doses offering greater protection against lipid peroxidation, thereby contributing to its therapeutic potential.

Table No. 16: Effect of EEPR leaves on Lipid peroxidation assay.

Group	Treatment	MDA (nmol /mg of protein)
Group I	**Control (Normal saline)**	6.58±0.92
Group II	**Standard Diazepam (1mg/Kg)**	3.35±0.12**
Group III	**Low dose of EEPR (250mg/Kg)**	4.064±0.56*
Group IV	**High dose of EEPR (500mg/Kg)**	3.92±0.31**

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

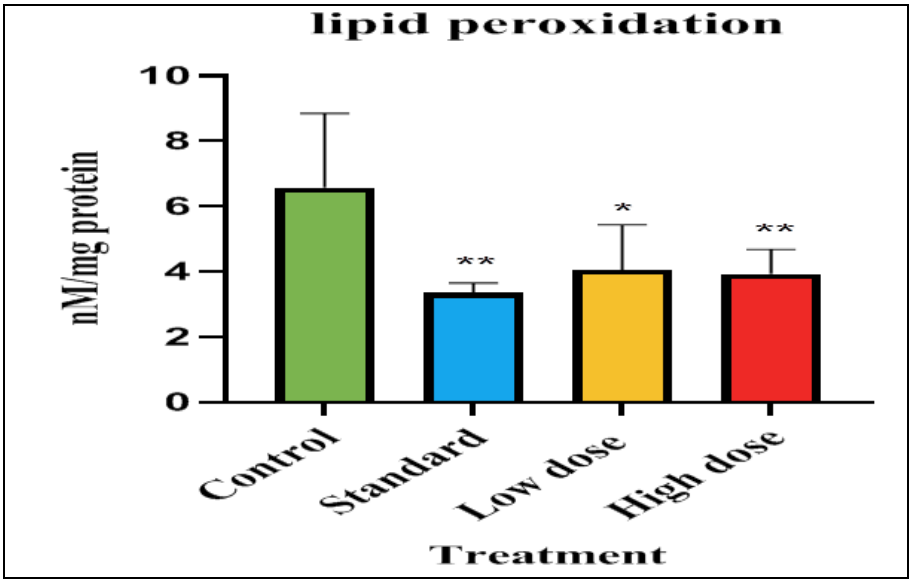

Fig No. 20: Effect of EEPR leaves on Lipid peroxidation assay.

6. Monoamine oxidase assay of *Putranjiva roxburghii* wall leaves

The anxiolytic potential of the ethanolic extract of *Putranjiva roxburghii* Wall. leaves was evaluated over 28 days, with animals divided into four groups: control, standard (Diazepam 1 mg/kg), low-dose (250 mg/kg), and high-dose (500 mg/kg). On the 29th day, a monoamine oxidase (MAO) assay was conducted to assess the extract's biochemical effects. MAO breaks down neurotransmitters like serotonin, dopamine, and norepinephrine, which regulate mood and anxiety, and elevated MAO activity is linked to anxiety and depression. Inhibiting MAO increases neurotransmitter levels, promoting anxiolytic effects. The results showed a dose-dependent reduction in MAO activity, with the standard group exhibiting the most significant reduction (****P < 0.0001), followed by the high-dose group (****P < 0.0001) and the low-dose group (*P < 0.05). These findings indicate that the extract exhibits anxiolytic properties by inhibiting MAO activity, with higher doses providing greater therapeutic benefits.

Estimation of EEPR on monoamine oxidase (MAO)-A assay.

Group	Treatment	(µmol /min/g tissue)
Group I	**Control (Normal saline)**	2.22±0.04
Group II	**Standard Diazepam (1mg/Kg)**	1.21±0.08****
Group III	**Low dose of EEPR (250mg/Kg)**	1.73±0.09***
Group IV	**High dose of EEPR (500mg/Kg)**	1.37±0.42****

Values were expressed as Mean ± SEM (n=6); Significance values are: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05 and ns P > 0.05. Control group vs all groups. (By one-way ANOVA followed by Tukey-Kramer Multiple comparison tests).

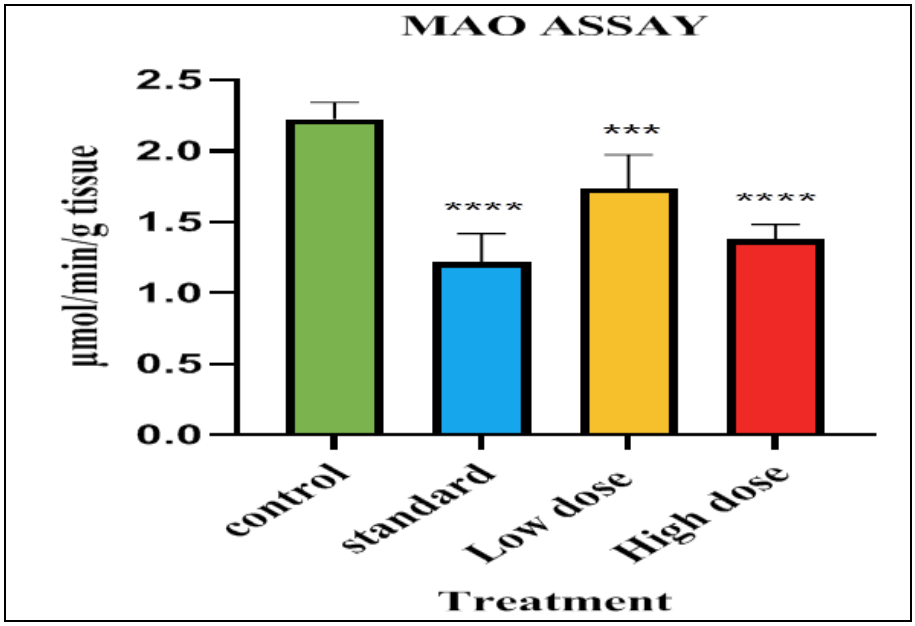

Fig No. 21: Estimation of EEPR on monoamine oxidase assay.

DISCUSSION

In response to the growing reliance on plant-based therapies for safer alternatives to synthetic drugs, this study investigated the anxiolytic potential of the ethanolic extract of *Putranjiva roxburghii* Wall. leaves over a 28-day period using rat models. Behavioral assessments included the elevated plus maze (EPM), open field (OFT), hole board (HBT), and rotarod tests. The extract, prepared by Soxhlet extraction and yielding 20.6%, was tested at low (250 mg/kg) and high doses (500 mg/kg), alongside a control and standard group treated with Diazepam (1 mg/kg). Biochemical parameters—monoamine oxidase (MAO) activity and lipid peroxidation—were evaluated on the 29th day.

Behavioral tests indicated that the high-dose extract enhanced exploration and reduced anxiety, with significant increases in time spent in the open arms (EPM), central area exploration (OFT), and head-dipping behavior (HBT). Importantly, motor coordination was not impaired in the rotarod test, suggesting the extract provides anxiolytic benefits without affecting motor function, unlike conventional anxiolytics.

Biochemical assays showed dose-dependent reductions in MAO activity and lipid peroxidation, with the high-dose group demonstrating the most significant effects. Reduced MAO activity suggests increased neurotransmitter levels (serotonin, dopamine, norepinephrine), contributing to mood regulation. Decreased lipid peroxidation reflects antioxidant properties, potentially mitigating oxidative stress associated with anxiety.

Phytochemical analysis identified alkaloids, flavonoids, tannins, and saponins, known for their neuroprotective, antioxidant, and CNS-modulating effects. The dose- and time-dependent improvements observed suggest that the extract's therapeutic potential increases with prolonged use. In conclusion, the ethanolic extract of *Putranjiva roxburghii* Wall. exhibits promising anxiolytic effects by modulating neurotransmitter systems and reducing oxidative stress, offering a natural alternative for anxiety management. Further studies are warranted to explore its clinical applications and mechanisms of action.

CONCLUSION

The present investigation on the anxiolytic potential of the ethanolic extract of *Putranjiva roxburghii* Wall. in rat models yielded promising results. Authenticated by Niveditha BT, the extract underwent phytochemical analysis, revealing bioactive compounds like alkaloids, flavonoids, tannins, and saponins, known for their therapeutic effects. Over 28 days,

behavioral assessments—including the elevated plus maze, open field, hole board, and rotarod tests—showed dose-dependent anxiolytic effects, with higher doses (500 mg/kg) producing more significant improvements than lower doses (250 mg/kg). On the 29th day, biochemical evaluations through MAO and lipid peroxidation assays further confirmed these effects, demonstrating reduced oxidative stress and enhanced neurotransmitter availability. The findings suggest that the extract's anxiolytic properties increase with time and higher dosages, indicating its potential as a natural therapeutic agent for anxiety management. Future studies should explore its underlying mechanisms and clinical applications

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