WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 13, Issue 1, 1348-1376. Research Article ISSN 2277–7105

EVALUATION OF ANTI-ANXIETY ACTIVITY OF ETHANOLIC EXTRACT OF *PHYLLANTHUS ACIDUS* LINN LEAVES IN MICE

Ragesh T.*[1], Aswathi Nambiar M. P.[2], Prof. (Dr.) Ajith Babu T. K.[3]

*[1]Lecturer, Department of Pharmacology, Malik Deenar College of Pharmacy, Kasaragod.

[2]Assistant Professor, Department of Pharmacology, Malik Deenar College of Pharmacy, Kasaragod.

Article Received on
06 November 2023,

Revised on 27 Nov. 2023,
Accepted on 17 Dec. 2023

DOI: 10.20959/wjpr20241-30775

***Corresponding Author**
Ragesh T.
Lecturer, Department of Pharmacology, Malik Deenar College of Pharmacy, Kasaragod.

ABSTRACT

Anxiety is the one of the most frequently come up disease in these times. Anxiety disorder comprises of emotional and behavioral complications. Anxiety is a situation of extreme fear along with sympathetic over action, motor stiffness and attentiveness syndrome. It alters the brainpower, recalling power, psychomotor purpose etc. An anti-anxiety drug expresses its action on central nervous system to lessen the symptoms of anxiety like apprehension, soothes the mood and progresses the sleep. Ayurveda is one of the traditional medicinal systems of India. It is known as the "Mother of all healing". World Health Organization estimated that 80% of the world's inhibitants still rely mainly on traditional medicines for their health care. The titled study is to evaluate the anti-anxiety activity of Ethanolic extract of *Phyllanthus acidus* (PAEE) leaves in experimental animal models. The anti-anxiety activity of the PAEE at doses 100 mg and 200mg/kg body weight was evaluated using the elevated plus maze, light/dark transition test, open field test and foot shock induced aggression. Diazepam (2 mg/kg) was used as the standard drug for comparison. The PAEE at doses 100 mg and 200mg/kg body weight showed significant increase in the following parameters: number of open arm entries and time spent in open arms in elevated plus maze; number of entries into the light chamber and time spent in the light chamber in Light/dark transition test; number of squares crossed, number of rearing and time spent in the central square in open field test; number of head dipping in Hole Board test. Significant anti-anxiety activity was shown by the PAEE in all fourmodels.

KEYWORDS: *Phyllanthus acidus*, Diazepam, Anti- anxiety activity.

INTRODUCTION

World Health Organization estimated that 80% of the world's inhibitants still rely mainly on traditional medicines for their health care.[1]

Ancient system of medicines like the Ayurveda, Unani, Homeopathy, Siddha, and Chinese system of medicines are the strong proof for the use of herbal medicines for maintaining a healthy lifestyle and for the treatment of various diseases**.**[2]

For majority of people anxiety is a normal and adjustive event. Some level of anxiety is a part of daily experience. High level of anxiety emotions is been experienced by some people which lead them to great distress and affect their daily normal lives things like school, job etc. and leads into impaired functioning then these anxiety emotions are called anxiety disorder**.** So, it can be said that anxiety is a very predominant mental and physiological state described by cognitive, somatic, emotional, and behavioral components.[4] The improvement of anxiety disorders shows up to result from a complex interplay of genetic, biological, developmental and different components for example, socio-economic and work environment stress. A variety of theories have been proposed to clarify how these components add to the advancement of the disorder.[5]

Peopleextremelyinfluencedbyanxietydisordersareadditionallyboundtohaveeither another sort of anxiety issue, like dysthymia, liquor or substance misuse, or an identity issue.[6] Effective treatment such as anxiolytic drug therapy or cognitive-behavioral therapy exist, but many patients experience adverse effects or do not benefit from full symptom control.

Anxiety disorders including generalized anxiety disorder (GAD), Specific and social Phobias, Post traumatic stress disorder (PSTD), Obsessive compulsive and panic disorder are typically treated with medications that target Gamma amino butyric acid (GABA) or Serotonergic system and modulate the overall effect.[7] Benzodiazepines and selective Serotonin reuptake inhibitors and β- blockers are most widely prescribed treatment for these disorders. Some forms of anxiety are relatively resistant to treatment with this agents.[8] there are many side effects such as sedation, memory impairment, potential for substance abuse and withdrawal syndrome, sexual dysfunction and weight gain. Non compliance with these pharmacological agents remains a problem leading to increased risk of relapse. Patients with

anxiety disorder also show sign of abnormal contextualconditioning.[9]

Phyllanthus acidus Skeels is a tree commonly known as star gooseberry and it belongs to the family Euphorbiaceae. Traditionally *Pacidus* fruits and leaves are used to treat ulcer, cough, scurvy, asthma and also used as an astringent and laxative. Apart from this plant also possess some other pharmacological actions such as anti-diabetic, antiinflammatory, Hepatoprotective, cytotoxic, hypotensive and anti-hyperlipidemic actions. Hence the present study is designed to evaluate the anti- anxiety activity of ethanolic extract of *Phyllanthus acidus* against experimentally induced anxiety in mice using diazepam as standarddrug.

METHODOLOGY

Drugs and Chemicals

Table 4: List of drugs and chemicals used for the study.

Sl. No.	CHEMICAL NAME
1.	*Phyllanthus acidus* leaves from Western Ghats
2.	Diazepam (Ranbaxy laboratories ltd, India)
3.	Gum Acacia (Yarrow Chem Products Mumbai, India)
4.	Distilled water

Plant material and preparation of extracts

Leaves of P. acidus was collected from forest area of Western Ghats, Dakshina Kannada (Dist.), Karnataka. Leaves was cleansed thoroughly, shade dried, pulverized mechanically and sieved (sieve no. 10/44). The extract was defatted using petroleum ether in Soxhlet apparatus. Further, hot extraction was carried out with defatted material (100 g) with ethanol (0.5L, 50°C, ≈15–17 cycles) to get ethanol extract. The extracts were dried in vacuum. Ethanolic extract of P. acidus leaves (PAEE) was stored in desiccators to avoid oxidation until further studies.

Phytochemical analysis

The dried extracts were first reconstituted in the respective solvents used for their extraction and then tested by standard phytochemical methods. Controls were maintained for each test batch.

The plant extract was subjected to preliminary phytochemical screening for the presence of several secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, saponins, sterols etc. and it was found that the extracts possessed different metabolites.

Assessment of total phenolic content in the extracts

The total phenolic content was determined by Folin–Ciocalteu method. PAEE was dissolved in methanol (1mg/ml) and then the extract solution (0.2ml) was mixed with 2ml of the Folin–Ciocalteau reagent (1:10 dilution) and 2ml of sodium carbonate(7.5%) (added 2 min after the Folin–Ciocalteau reagent). After initial mixing the reaction mixture was incubated at room temperature for 90 mins. The optical density samples were measured at 765nm and total phenolic contents were expressed as mg gallic acid equivalent.

Estimation of total flavonoids in the extracts

Flavonoid content was measured using a modified colorimetric method. Solutions of PAEE (1mg/ml) were added to test tubes and the volume was made up to 5ml. Sodium nitrite solution (5%, 0.3 ml) was added followed by Aluminium chloride (0.3ml of 10%). After 6 min, 0.5ml of 1M sodium hydroxide was added. The mixture was diluted with 2.4ml of distilled water. The absorbance of the mixture was measured at 510 nm. The data was compared to a standard curve prepared using quercetin. The flavonoid contents were expressed as mg quercetinequivalent.

Selection of Animals

Albino mice strain of either sex, 4-6 weeks, weighing 20-30g will be obtained. The animals will be grouped and housed in cages and maintained under standard laboratory conditions (temperature 25±2°) with dark and light cycle (12h/12h). The experiment will be carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and will be approved by the Institutional Animal Ethics Committee (IAEC).

Acute toxicity

Healthy Wister albino female rat weighing 180–220 gm were selected for the study. Studies carried out on three female rats under fasting condition, signs of toxicity were observed for every one hour for first 24 hours and every day for about 14 days fromthe beginning of the study. In the acute toxicity study, the rats were treated with different concentration of PAEE from the range of 5mg/kg b.wt to 2000mg/kg b.wt which did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract. In acute toxicity test the PAEE was

found to be nontoxic at the dose level of 2000mg/kg body weight known as maximum tolerated dose (MTD) as per OECD guidelines 423. Hence from the PAEE 1/20th and 1/10th of MTD was selected and the doses were fixed as 100mg/Kg and 200mg/Kg respectively.

Sub-acute toxicity

The dose selected for the sub-acute toxicity study was 100mg, 200mg/kg b.wt. All the animals were free of intoxicating signs throughout the dosing period of 28 days. No physical changes were observed throughout the dosing period. No mortality was observed during the whole experiment. No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits. There was no significant changes observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC)in all the treated groups as compared to respective control groups. The biochemical test shows the values of blood sugar, BUN, creatinine, Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP), total protein and albumin are within the normal range. The result of the lipid profile reveals that the values of Total cholesterol, Triglycerides, High density lipoprotein (HDL), Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were in normal range. Weights of organs recorded did not show any significant differences in the treatment and the control group indicating that PAEE at dosage 100mg, 200mg/kg b.wt was not toxic to kidney, liver and spleen. There is no cellular degeneration in kidney, liver and spleen both that with the 100mg and 200mg/kg b.wt. of PAEE.

Selection of dose

To evaluate the antianxiety activity of PAEE, two dose levels were selected. The doses which were administered to mice were calculated based on human dose. The human dose was converted to mice dose by using human equivalent dose method (HED). The doses of low and high dose of PAEE were selected as 100mg/kg and 200mg/kg. The antianxiety activity was evaluated using four models; Elevated plus maze, Light/dark transition test, Open field test and Hole Boardtest.

Evaluation of Antianxiety activity

Elevated Plus Maze Model[53,54]

It is a novel test used for evaluating the anxiolytic action of a drug in animals. The wooden plus maze consists of two open arms (length 16 cm × breadth 5 cm) and two closed arms of the same size (height 12 cm). The arms of the same type are placed opposite to each other, with a central square of 5 cm. The maze is in a height of 25 cm above the floor. The individual mice were placed at the center of the elevated maze with their head facing towards the open arm. Number of entries and time spent in the open and closed arms were recorded for 5 mins for each mouse. The preference of the mouse for first entry into closed arm indicates the relative safety of closed arms as compared with the relative fearfulness of open arms. Mouse being animals feels safe in dark. Hence normal animals prefer dark arm first. Anxiolytics would be expected to increase the proportion of entries and time spent in open arms.

The different groups were assigned as described below.

Table 5: Experimental design of Elevated plus maze model.

Group	Treatment
Group I	Normal control (gum acacia, 2% w/v, 10ml/kg) p. o
Group II	Standard drug (Diazepam, 2mg/kg) i. p
Group III	PAEE (low dose) p. o
Group IV	PAEE (high dose) p. o

Albino mice of either sex, weighing about 20-30g were divided into four groups of 6 animals each. Group I was served as control. Group II was treated with diazepam (2mg/kg i.p). Group III and IV were treated with two different doses of PAEE (100 mg and 200mg/kg p.o) for seven consecutive days. On the eighth day one hour after administration of the standard/test dose in respective groups, the mice were placed in a sound attenuated room, individually in the center of the maze facing one of the open arms. During a test session of five minutes, the following parameters: a) number of entries into the open arms, and time spent in the open arms were recorded. The parameterswereexpressedasapercentage (forexample, % openarmentries =100×no.of open arm entries/ Total no. of entries).

Light-dark transition test[55]

The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an

open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box. The apparatus was cleaned thoroughly between trials. All behavioral recordings were carried out with the observer unaware of the treatment the mice had received.

The different groups were assigned as described below.

Table 6: Experimental design of Light/dark transition test.

Group	Treatment
Group I	Normal control (gum acacia, 2% w/v, 10ml/kg) p. o
Group II	Standard drug (Diazepam, 2mg/kg) i. p
Group III	PAEE (low dose) p. o
Group IV	PAEE (high dose) p. o

Albino mice of either sex weighing about 20-30g were divided into four groups of 6 animals each. Group I was served as control. Group II was treated with diazepam (2mg/kg i.p). Group III and IV were treated with two different doses of PAEE (100 mg and 200mg/kg p.o) for seven consecutivedays.

On the eighth day one hour after administration of the standard/test dose in respective groups, mice were placed individually in the illuminated part of the cage and the parameters such as number of transitions between the dark and light area and during the 5 minutes time the time spent in illuminated part of the apparatus is been noted.

Open field test[56]

This method is used to evaluate exploratory activity and emotionality of animal. The apparatus consists of a wooden box (40x40x50 cm). The floor of the box was divided into sixteen squares and the center of the field was marked to differentiate from other squares. The apparatus was illuminated with a 60-W lamp suspended 100 cm above.

The different groups were assigned as described below.

Table 7: Experimental design of Open field test.

Group	Treatment
Group I	Normal control (gum acacia, 2% w/v, 10ml/kg) p. o
Group II	Standard drug (Diazepam, 2mg/kg) i. p
Group III	PAEE (low dose) p. o
Group IV	PAEE (high dose) p. o

Albino mice of either sex weighing about 20-30g were divided into four groups of 6 animals each. Group I was served as control. Group II was treated with diazepam (2mg/kg i.p). Group III and IV were treated with two different doses of PAEE (100 mg and 200mg/kg p.o) for seven consecutive days. On the eighth day one hour after administration of the standard/test dose in respective groups, the mice were placed in the center of the arena and the parameters such as total number of squares crossed, total number of rearings, and time spent in the central square were observed and recorded during a test session of 5 minutes.

Hole Board test[57]

The study was conducted using a wooden board measuring 20 cm by 40 cm with sixteen evenly spaced holes.

The different groups were assigned as described below.

Table 08: Experimental design of Hole Board test.

Group	Treatment
Group I	Normal control (gum acacia, 2% w/v, 10ml/kg) p. o
Group II	Standard drug (Diazepam, 2mg/kg) i. p
Group III	PAEE (low dose) p. o
Group IV	PAEE (high dose) p. o

Albino mice of either sex weighing about 20-30g were randomly grouped into four groups containing 6animals each. Group I served as the control group. Group II was treated with diazepam (2mg/kg i.p). Group III and IV were treated with two different doses of PAEE (100 mg and 200mg/kg p.o). Thirty minutes after treatment, the mice were placed singly on the board and the number of times the mice dipped their head into the holes at the level of their eyes during a five minute trial period was counted using a tally counter.

Statistical analysis

All the data will be expressed in mean ±SEM. The significance of differences in mean between control and treated animals for different parameters determined by one way ANOVA followed by Dunnett's multiple comparison tests. Significance for difference

between groups will be evaluated for student's t-test to come to final conclusion.

RESULTS

Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. A single plant may, for example, contain substances that stimulate digestion, anti-inflammatory compounds that reduce swellings and pain, phenolic compounds that can act as an antioxidant and venotonics, anti-bacterial and anti-fungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste products and toxins, alkaloids that enhance mood and give a sense of well-being. P. acidus is one such plant which is colloquially used to treat a number of ailments. Some of them are proved scientifically and some of them are still to be uncovered. This study is an attempt to scientifically prove some folkloric medicinal claims of this plant. The stem bark of the plant was used as the source material for conducting theexperiments.

A sincere attempt was made to study the medicinal importance of *Phyllanthus acidus* leaves. The study showed promising results pertaining to the folkloric usage of the plant, providing scientific validations to the traditional claims. The results of the study is tabulated below which gives us an insight to the pharmacological uses of the plant.

Extraction from source plant

The yield of ethanol extract was found to be 7.16%. It is evident that the extraction of leaves material with ethanol yielded the highest quantity of extract.

Table 9: Yield of P. acidus leaf extracts (% w/w).

Extract	Leaf material (g)	Solvent	Yield (%)
Ethanol	100	500	7.16%

Phytochemical analysis

Qualitative and quantitative analyses

The plant extract was tested for the probable presence of phytochemicals which actually impart the medicinal properties to the plant. The P. acidus ethanol extract (PAEE) was tested for the presence of alkaloids, flavonoids, triterpenoids, sterols, tannins and other secondary metabolites. The observations are tabulated below (Table 10).

Table 10: Qualitative phytochemical analysis of PAEE.

Sl no.	Qualitative test	Ethanol extract(PAEE)
1.	Terpenoids	+
2.	Tannins	+
3.	Saponins	+
4.	Glycosides	+
5.	Sterols	-
6.	Alkaloids	+
7.	Flavonoids	+
8.	Carbohydrates	+
9.	Proteins	-
10.	Fats and oils	-
11.	Organic acids	+
12.	Coumarins	+
13.	Quinine	+

(Phytochemical test: - negative and + positive)

The results of the tests support the presence of a number of significant secondary metabolites. The ethanol extract includes terpenoids, tannins, saponins, glycosides, alkaloids, flavonoids, organic acids, quinones and coumarins. The quantitative analyses of phenolic content in the samples were measured using a standard gallic acid curve and the flavonoids content was measured using quercitrin as the standard. Total phenolic content in extracts was expressed as equivalent to gallic acid (EGA). The total phenolic content in PAEE was found to be 152.12± 0.079 µg mg^{-1} of dry extract. Analysis of flavonoid (expressed as equivalent to quercetin) has revealed 65.82± 0.166 µg mg^{-1} in PAEE of dryextract.

Acute toxicity

Healthy Wister albino female rat weighing 180–220 gm were selected for the study. Studies carried out on three female rat under fasting condition, signs of toxicity was observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study. In the acute toxicity study, the rats were treated with different concentration of PAEE from the range of 5mg/kg b.wt to 2000mg/kg b.wt which did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract. In acute toxicity test the PAEE was found to be nontoxic at the dose level of 2000mg/kg body weight known as maximum tolerated dose (MTD) as per OECD guidelines 423. Hence from the PAEE 1/20th and 1/10th of MTD was selected and the doses were fixed as 100mg/Kg and 200mg/Kg respectively.

Table 11: Observation done in acute study.

Group	Day
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence of sign (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant colour change
Piloerection	Normal
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Urination	Normal

Table 12: Behavioural and Physiological changes noted in Acute Oral Toxicity study (Acute Toxic Class Method, OECD Guidelines 423).

Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2000	+	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-

[1. Alertness; 2.Aggressiveness; 3. Pile erection; 4. Grooming; 5. Gripping; 6.Touch Response; 7. Decreased Motor Activity; 8. Tremors; 9 Convulsions; 10. Muscle Spasm; 11.Catatonia; 12.Musclerelaxant; 13.Hypnosis; 14.Analgesia; 15.Lacrimation; 16.Exophthalmos; 17.Diarrhea; 18.Writhing;19 Respiration; 20. Mortality]

Sub-acute toxicity

The dose selected for the sub-acute toxicity study was 100mg, 200mg/kg b.wt. All the animals were free of intoxicating signs throughout the dosing period of 28 days. No physical changes were observed throughout the dosing period. No mortality was observed during the whole experiment. No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits. There was no significant changes observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) in all the treated groups as compared to respective control groups. The biochemical test shows the values of blood sugar, BUN, creatinine, Serum glutamate oxaloacetate

transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP), total protein and albumin are within the normal range. The result of the lipid profile reveals that the values of Total cholesterol, Triglycerides, High density lipoprotein (HDL), Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were in normal range. Weights of organs recorded did not show any significant differences in the treatment and the control group indicating that PAEE at dosage 100mg, 200mg/kg b.wt was not toxic to kidney, liver and spleen. There is no cellular degeneration in kidney, liver and spleen both that with the 100mg and 200mg/kg b.wt. of PAEE.

Table 13: Effect of PAEE on Hematological parameters of rats in Sub acute toxicity.

parameters	PAEE 100mg/kg	PAEE 200mg/kg
Neutrophils	14%	10%
Lymphocytes	85 %	89 %
Eosinophils	01 %	01 %
Monocytes	00 %	00 %
RBC	7.40 millions/cumm	8.98 millions/cumm
WBC	7,900 cells/cumm	8,200 cells/cumm
HB	14.8 gms%	16.9gms%
PCV	45.1 %	53.6 %
MCV	60.9 Fl	59.7Fl
MCH	20.0 pg	18.8pg
MCHC	32.8 Grams/dl	31.5 Grams/dl
PLATELET	7.11 Lakhs/cumm	7.20 Lakhs/cumm

[RBC – Red blood corpuscles, WBC- White blood corpuscles, HB- Haemoglobin, PCV- Packed cell volume, MCV- mean corpuscular volume, MCHC- Mean corpuscular haemoglobin concentration].

Table 14: Effect of PAEE on Biochemical parameters of rats in Sub acute toxicity.

parameters	PAEE 100mg/kg	PAEE 200mg/kg
Blood sugar	94 mg/dl	90 mg/dl
BUN	23.4 mg/dl	22.8 mg/dl
Creatinine	0.9 mg/dl	0.6 mg/dl
SGOT	85 U/L	72 U/L
SGPT	58 U/L	49 U/L
ALP	182 U/L	135 U/L
Total Protein	6.1 grams/dl	6.4 grams/dl
Albumin	3.0 grams/dl	3.1 grams/dl

[BUN – Blood Urea Nitrogen, SGOT – Serum glutamate oxaloacetate transaminase SGPT- Serum glutamate pyruvate transaminase, ALP- Alkaline Phosphate]

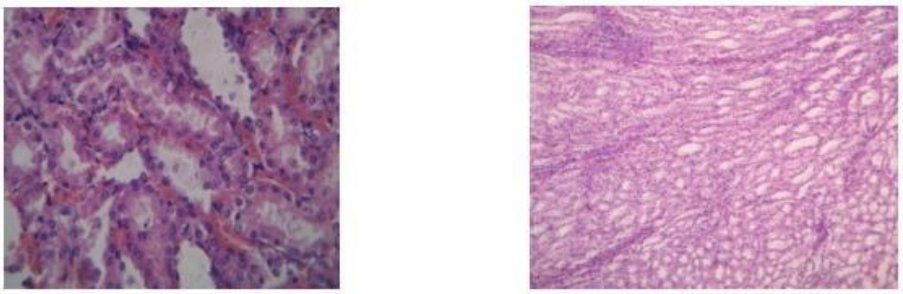
Table 15: Effect of PAEE on Lipid profile of rats in Sub acute toxicity.

parameters	PAEE 100mg/kg	PAEE 200mg/kg
Total Cholesterol	112 mg/dl	103 mg/dl
Triglycerides	65 mg/dl	69 mg/dl
HDL	23 mg/dl	26 mg/dl
LDL	67.0 mg/dl	62.2 mg/dl
VLDL	13.8 mg/dl	13.0 mg/dl
Ratio 1(T.CHO/HDL)	4.47	4.30
Ratio 2(T.CHO/HDL)	2.91	2.77

[HDL–High density lipoprotein, LDL–Low density lipoprotein, VLDL–Very low density lipoprotein]

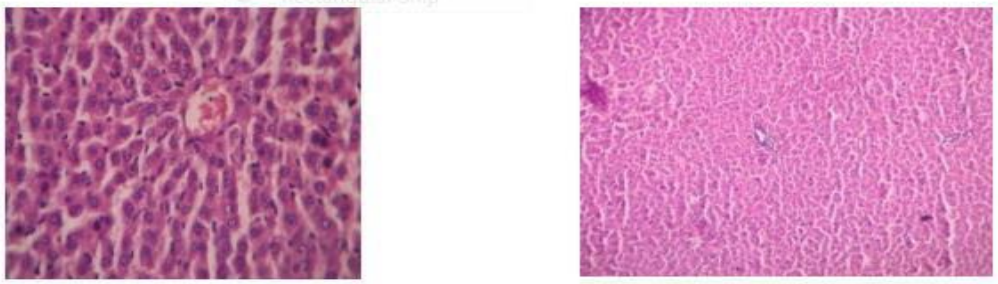
Kidney Section

PAEE100mg/kg **PAEE 200mg/kg**



Liver Section

PAEE100mg/kg **PAEE 200mg/kg**



Spleen Section

PAEE100mg/kg **PAEE 200mg/kg**

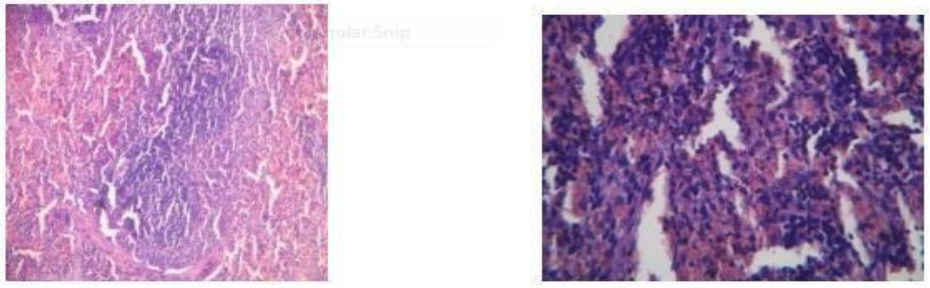


Figure 4: Histopathology of Wister rats when treated with PAEE.

Evaluation of anti-anxiety activity using *in-vivo* models Elevated Plus Maze model

a) Number of open armentries

The standard drug treated group (diazepam 2mg/kg) showed significant increase ($p<0.05$) in the number of open arm entries, when compared to the control group. The PAEE at a dose of 100mg/kg and 200mg/kg, significantly increased ($p<0.05$) the exploration activity in the open arm in a similar way to that of the standard. The results are shown in Table.

Table 16: Effect of PAEE on number of open arm entries in Elevated Plus maze.

Sl. No	Groups	Number of open arm entries Mean ± SEM
1.	Control	11.83±1.01 *
2.	Standard	35.33± 1.49 *
3.	PAEE (100mg/kg)	14.60± 0.84 *
4.	PAEE (200mg/kg)	22.80±1.70 **

Values are expressed as MEAN±SEM. n=6. *p <0.05, **p < 0.01, ***p<0.001 when compared to normal. (One way ANOVA followed by Dunnett's test).

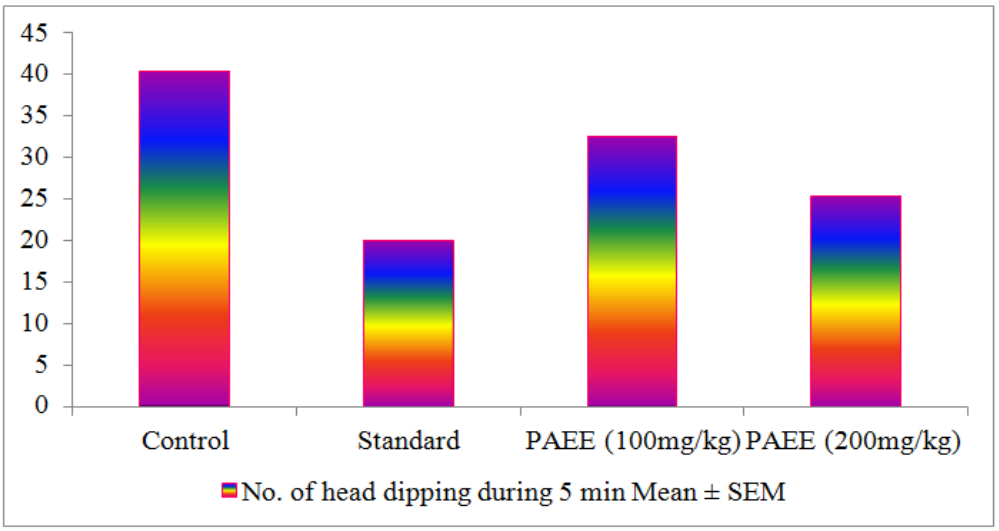


Figure 5: Effect of PAEE on number of open arm entries in Elevated plus maze.

b) Time spent in openarms

There was significant increase ($p<0.05$) in the percentage time spent in open arms by the standard drug treated group (diazepam 2mg/kg), when compared to the control group. The PAEE at a dose of 100mg/kg and 200mg/kg, significantly increased ($p<0.05$) the time spent in the open arms in a similar way to that of the standard. The results are shown in Table.

Table 17: Effect of PAEE on time spent in open arms in Elevated Plus maze.

Sl. No	Groups	Time spent in open arm Mean ± SEM
1.	Control	98.16 ± 1.01*
2.	Standard	127.83 ±0.70 *
3.	PAEE (100mg/kg)	100 ± 1.71*
4.	PAEE (200mg/kg)	110.17 ± 0.94 *

Values are expressed as MEAN±SEM. n=6. *p <0.05, **p < 0.01, ***p<0.001 when compared to normal. (One way ANOVA followed by Dunnett's test)

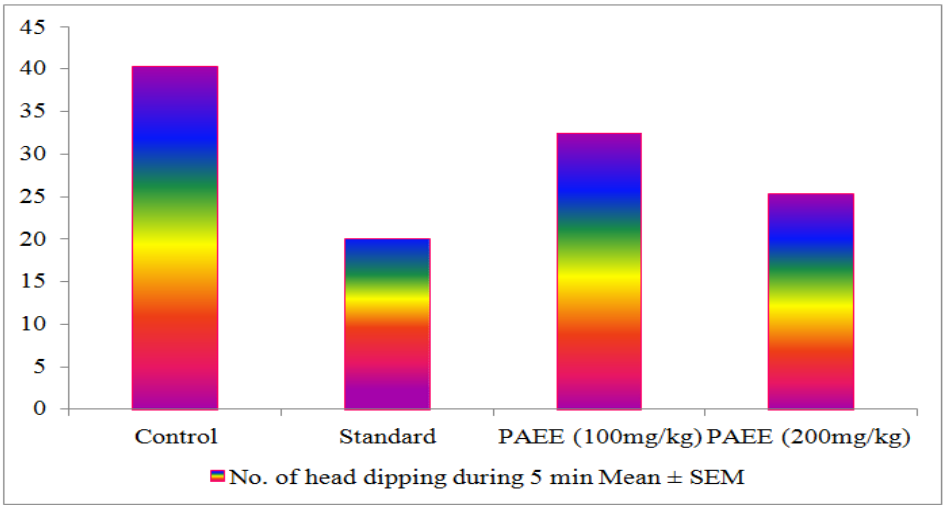


Figure 6: Effect of PAEE on time spent in open arms in Elevated plus maze.

Light/ Dark transition test

a) Number of entries into the lightchamber

The standard drug treated group (diazepam 2mg/kg) showed significant increase (p<0.05) in the number of entries into the light chamber, when compared to the control group. The PAEE at a dose of 100mg/kg and 200mg/kg, significantly increased (p<0.05) the entries into the light chamber in a similar way to that of the standard. The results are shown in Table.

Table 18: Effect of PAEE on number of entries into the light chamber in Light/dark transition test.

Sl. No	Groups	Number of entries into light chamber Mean ± SEM
1.	Control	18.66 ± 0.88 *
2.	Standard	34.83± 1.07 *
3.	PAEE (100mg/kg)	18 ± 1.54 *
4.	PAEE (200mg/kg)	27.83± 0.79 **

Values are expressed as MEAN±SEM. n=6. *p <0.05, **p < 0.01, ***p<0.001 when compared to normal. (One way ANOVA followed by Dunnett's test)

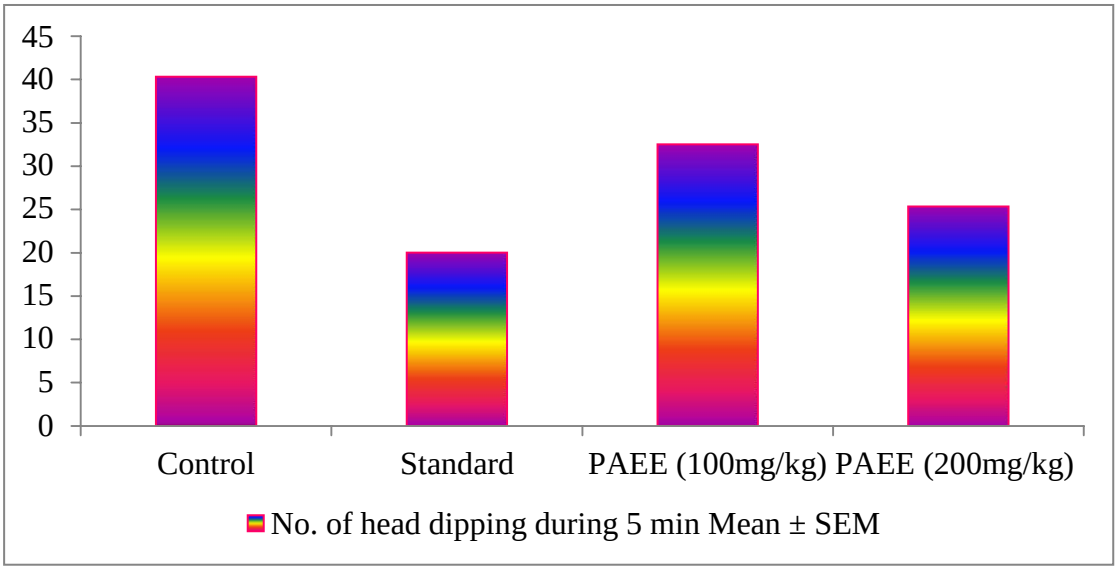


Figure 7: Effect of PAEE on number of entries into the light chamber in Light/dark transition test.

b) Time spent in the lightchamber

There was significant increase ($p<0.05$) in the duration of time spent in the light chamber by the standard drug treated group (diazepam 2mg/kg), when compared to the control group. The PAEE at a dose of 100mg/kg and 200mg/kg, significantly increased ($p<0.05$) the time spent in the light chamber in a similar way to that of the standard. The results are shown in Table.

Table 19: Effect of PAEE on time spent in the light chamber in Light/dark transition test.

Sl. No.	Groups	Time spent inthelight chamber (in sec)Mean ±SEM
1	Control	113.83 ± 0.94*
2	Standard(2mg/kg)	171.17 ± 1.01*
3	PAEE (100mg/kg)	130.17 ± 1,01**
4	PAEE (200mg/kg)	144.50 ± 1.06**

Values are expressed as MEAN±SEM. n=6. *p <0.05, **p < 0.01, ***p<0.001 when compared to normal. (One way ANOVA followed by Dunnett's test)

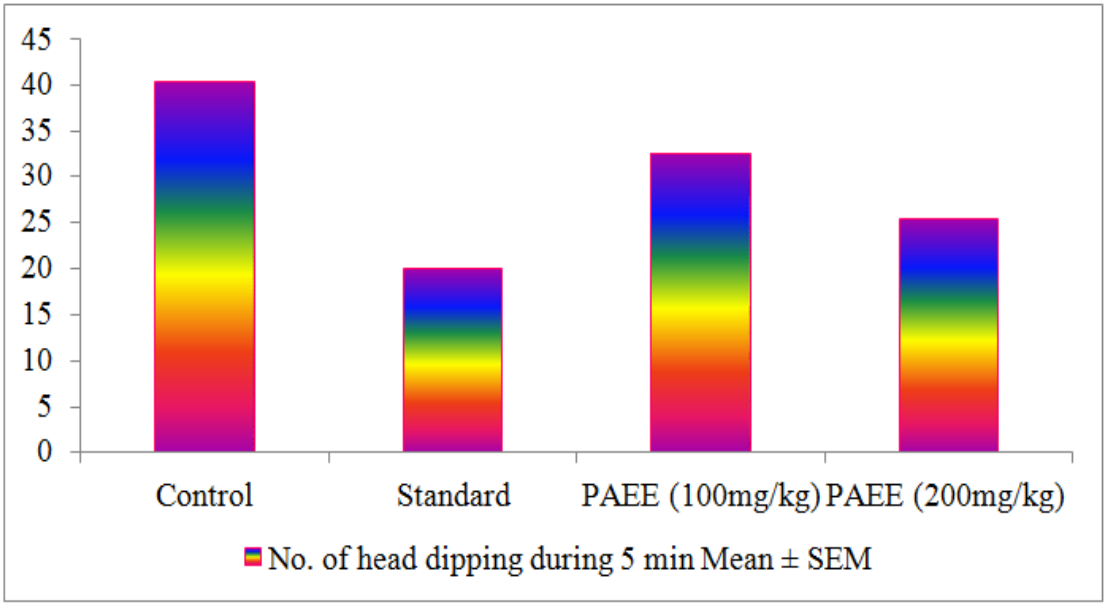


Figure 8: Effect of PAEE on time spent in the light chamber in Light/dark transition test.

Open field test

a) Number ofrearings

The standard drug treated group (diazepam 2mg/kg) showed significant increase ($p<0.05$) in the number of rearings, when compared to the control group. The PAEE at a dose of 100mg/kg and 200mg/kg, significantly increased ($p<0.05$) the number of rearings in a similar way to that of the standard. The results are shown inTable.

Table 20: Effect of PAEE on number of rearings in Open field test.

Sl. No	Groups	No of rearings in sec (Mean ± SEM)
1.	Control	6 ± 0.63*
2.	Standard(2mg/kg)	30.33± 0.84*
3.	PAEE (100mg/kg)	14.50± 0.61**
4.	PAEE (200mg/kg)	23 ± 0.96**

Values are expressed as MEAN±SEM. n=6. *p <0.05, **p < 0.01, ***p<0.001 when compared to normal. (One way ANOVA followed by Dunnett's test).

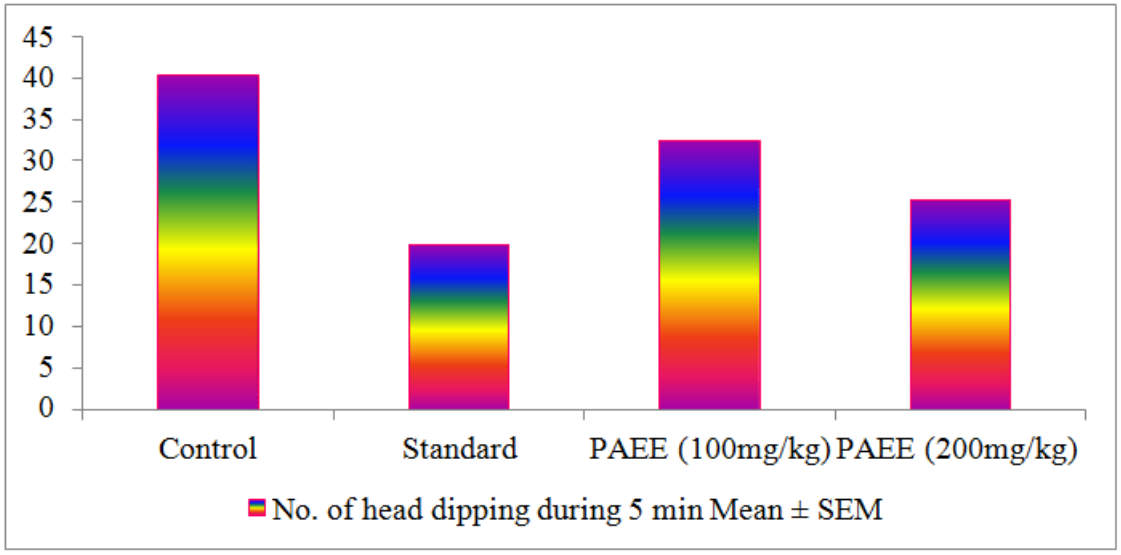


Figure 9: **Effect of PAEE on number of rearings in Open field test.**

b) Number of squarescrossed

The standard drug treated group (diazepam 2mg/kg) showed significant increase ($p<0.05$)inthenumber of squarescrossed, whencomparedtothecontrol group. The PAEE at a dose of 100mg/kg and 200mg/kg, significantly increased ($p<0.05$) the number of square crossings in a similar way to that of the standard. The results are shown in Table.

Table 21: Effect of PAEE on number of squares crossed in Open field test.

Sl. No.	Groups	Number of squares crossed Mean ± SEM
1.	Control	64.50 ± 0.76*
2.	Standard(2mg/kg)	116.33 ±0.88*
3.	PAEE (100mg/kg)	76 ± 0.81**
4.	PAEE (200mg/kg)	84.33 ± 1.05**

Values are expressed as MEAN±SEM. n=6. *p <0.05, **p < 0.01, ***p<0.001 when compared to normal. (One way ANOVA followed by Dunnett's test)

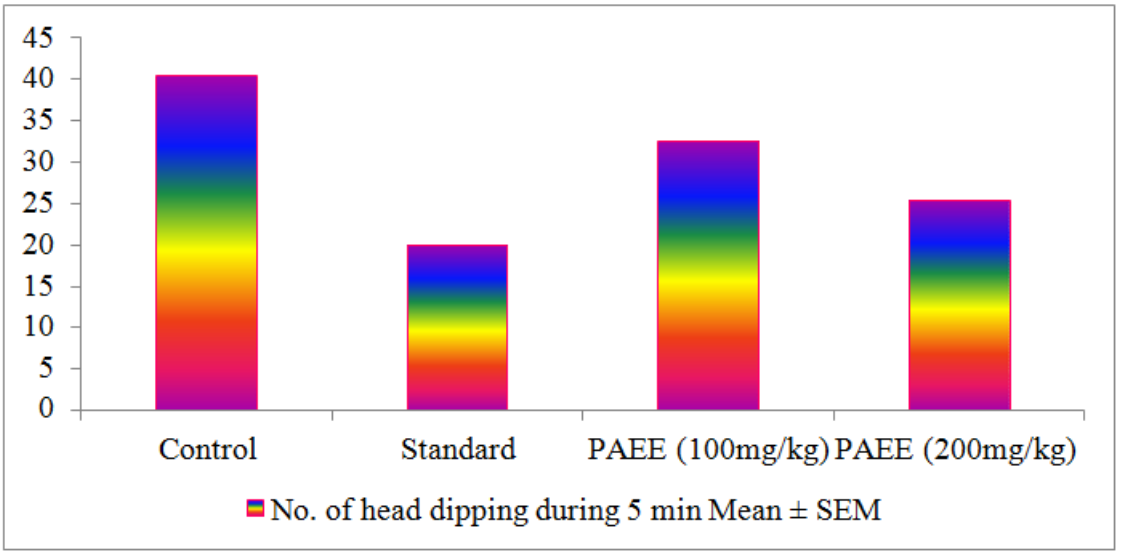


Figure 10: Effect of PAEE on number of squares crossed in Open field test.

c) Time spent in the centralsquare

The standard drug treated group (diazepam 2mg/kg) showed significant increase ($p<0.05$) in the time spent in the central square, when compared to the control group. The PAEE at a dose of 100mg/kg and 200mg/kg, significantly increased ($p<0.05$) the duration of time spent in the central square in a similar way to that of the standard. The results are shown in Table.

Table 22: Effect of PAEE on time spent in the central square in Open field test.

Sl. No	Groups	Time spent in the central square (in sec) Mean ± SEM
1.	Control	6.83 ± 0.30*
2.	Standard(2mg/kg)	35 ± 0.96*
3.	PAEE (100mg/kg)	13.50 ±0.76**
4.	PAEE (200mg/kg)	22.33 ± 0.49*

Values are expressed as MEAN±SEM. n=6. *p <0.05, **p < 0.01, ***p<0.001 when compared to normal. (One way ANOVA followed by Dunnett's test)

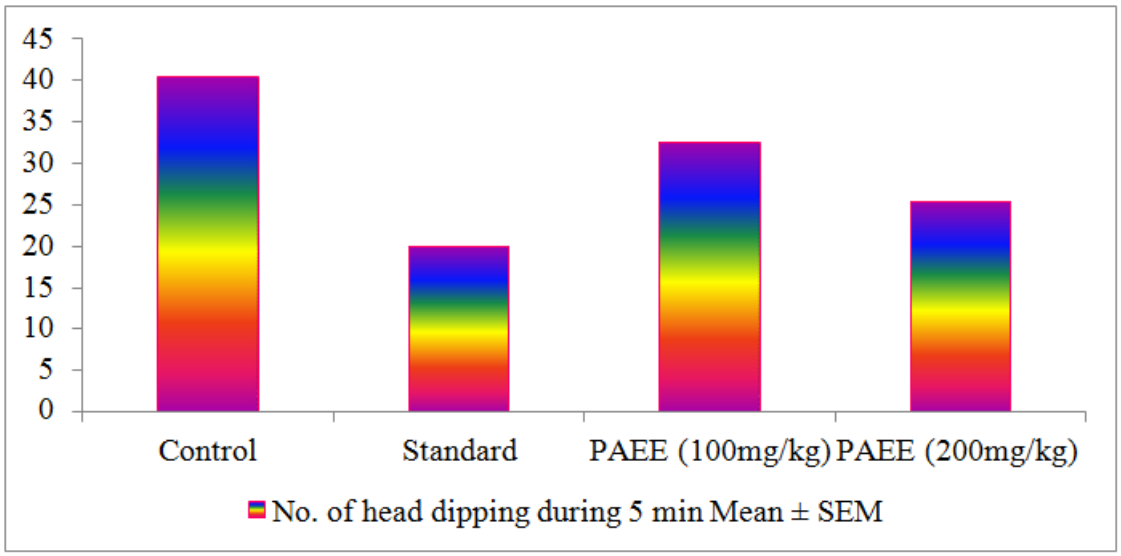


Figure11: Effect of PAEE on time spent in the central square in Open field test Hole Board test.

Standard group which received diazepam 2mg/kg showed moderately significant decrease ($p < 0.01$) in number of head dipping in 5min of time when compared with Normal group. PAEE at a dose of 100mg/kg and 200mg/kg showed significantly decrease ($p < 0.05$) in head dipping in 5min when compared with Normal group. The results are shown in Table.

Table 23: Effect of PAEE on number of head dipping in hole board test.

Sl. No	Groups	No. of head dipping during 5 min Mean ± SEM
1.	Control	40.30 ±3.22
2.	Standard(2mg/kg)	20 ± 4.12**
3.	PAEE (100mg/kg)	32.50 ±0.76*
4.	PAEE (200mg/kg)	25.33 ± 5.11*

Values are expressed as MEAN±SEM. n=6. *p <0.05, **p < 0.01, ***p<0.001 when compared to normal. (One way ANOVA followed by Dunnett's test).

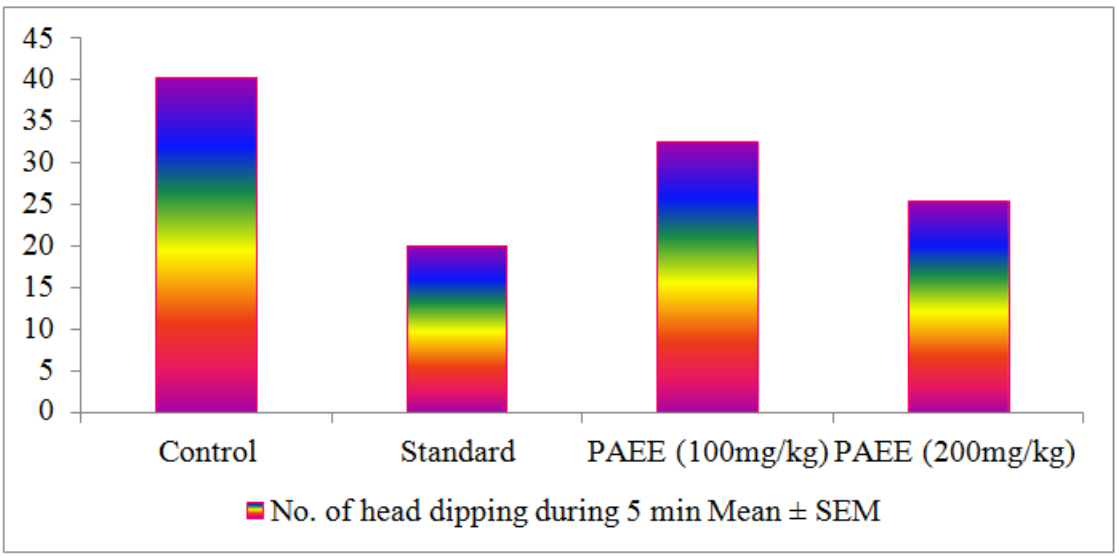


Figure 12: Effect of PAEE on Number of head dipping in hole board test.

DISCUSSION

The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. The practices continue today because of its biomedical benefits, cultural beliefs and their great contribution towards maintaining human health. The desire to capture the wisdom of traditional healing systems has led to resurgence of interest in herbal medicines.

An enormous growth in popularity of traditional healing modalities is seen, including the use of herbal remedies. Consumers have reported positive attitudes towards these products, in large part because they believe them to be of 'natural' rather than 'synthetic' origin, they believe that such products are more likely to be safe than drugs, they are considered part of a healthy lifestyle, and they can help to avoid unnecessary contact with conventional medicine. Another reason for the popularity of herbal medicine is due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures.

The extraction process of the crude extract from the plant was done using a Soxhlet apparatus. This method of extraction is convenient and widely used for extraction from plant samples because of its continuity in processing, consumption of time and limited solvent consumption.

The qualitative analysis of the phytochemicals present in the crude extract (PAEE was performed which indicated that the plant is a store for a number of phytochemicals. As

mentioned earlier the phytochemicals present in these extracts were polar in nature, they included phytochemicals belonging to the groups like Terpenoids, Tannins, Saponins, Glycosides, Alkaloids, Flavonoids, Organic acids, Coumarins and Quinine. These secondary metabolites are studied and have proved themselves to impart health benefits. There are numerous reports stating the presence of such useful secondary metabolites in a number ofplants.

The total phenolic content and the flavonoid content were measured in the extract. The total phenolic content in PAEE was found to be 152.12± 0.079 µg mg^{-1} (equivalenttogallicacid)ofdryextract. Analysisofflavonoidhasrevealed65.82±0.166 µg mg^{-1} in PAEE (equivalent to quercetin) of dry extract.

The present study was carried out to evaluate anti-anxiety activity of the PAEE using animal models based on exploratory behavior: Elevated plus maze (EPM), Light/ dark transition test, Open field test and Hole Board test. It was found that PAEE possesses significant anti-anxiety activity in a dose dependent manner.

In the 17th era, information about anxiety was designated in the anatomy of melancholy by Robert Burton. Anxiety was renowned from further negative effect and branded the anxiety as a curative ailment by Latin and Greek philosophers and general practitioner. Meanwhile at the time of 20^{th} century anxiety has become a complaint in psychiatric arrangement. The medical onset amongst daily life adaptive common anxiety and painful neurotic anxiety need management is focused to medical finding.[58]

Anxious and worry are the common symptoms in today's life. Everyone undergo such anxiety conditions. However, some persons fail to control their worries and ultimately their routine life is affected due to excessive anxiety. Lifestyle modifications like yoga, meditation and dietary alterations can be helpful in the early part of anxiety disorder.[59,60]

Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazipam, lorazepam and alprazolam) are the most frequently prescribed synthetic drugs for variety of condition particularly anxiety, tension, stress, epilepsy and insomnia. But these psychoneural drugs have very serious side effects like chronic use of benzodiazepinescausesdeterioration of cognitive function, physical dependence and tolerance. Besides addiction liabilities,

benzodiazepines adversely affect the respiratory, digestive and immune system of body and the chronic treatment with benzodiazepines often prove more harmful in the longer run.[61]

However, most of the anxiety patients are benefited by pharmacotherapy. Benzodiazepines even though established efficacy for many anxiety disorders, associated with many adverse effects.[62,63]

Oxidative stress mechanisms underlying anxiety disorder have been in existence since long time. It was claimed that nitric oxide and peroxynitrite might play a major role in setting up a vicious etiological cycle involving free radicals and inflammatory cytokines in post-traumatic stress disorder.[64,65]

Association of vitamin E depletion, increased oxidative stress markers and anxiety behaviors in phospholipid transfer protein knock-out mice has further suggested an oxidative role in the pathogenesisof anxiety.[77] Even clinical studieshave reported elevated lipid peroxidation byproducts in obsessive - compulsive disorder, panic disorder and social phobia.[66,67]

In the present study, *PAEE* was orally administered at doses 100 mg/kg and 200mg/kg for 7 days. On the 8th day, 1hr after the administration it was observed from the study that it induced an anxiolytic-like effect in mice as it increased the number of open arm entries and the time spent on open arms respectively as compared to the control group of open arm entries and time spent in open arms, in the EPM test. The Light/Dark Transition test is used to evaluate the approach-avoidance conflict in animals, as they would spent more time in the dark chamber in contrast to the light chamber due to fear of exposure to the novel environment. In this test, the number of entries into the light chamber or transitions between light and dark chamber and the time spent in the light chamber are taken as anxiety indices. Mice was treated with PAEE extract for 7 days and on the 1 hour after the administration on the 8th day, it showed significant increase in the number of entries into the light chamber and the time spent in the light chamber as compared to the controlgroup.

The open field test is used to assess the anxiety-related behavior and emotional state in animals, featured by a normal aversion to an open, illuminated space. When placed in the open field, the animal faces emotional disturbance, fear and anxiety, and tends to spend more time in the peripheral areas. In the open field test, anxiolytic-like activity is observed by an increase in the number of squares crossed, number of rearing and time spent in the central

square. PAEE was treated for the 7 days and on the 8th day of 1hr after the administration, it showed significant increase in the number of squares crossed, number of rearing and the time spent in the central square, as compared to the control group. The increase in the parameters may be linked with the anxiolytic-like effect of the PAEE.

In Hole Board tests have been effectively employed to access the neurobehavioural profile of animals under the influence of anxiogenic/anxiolytic agents. The decreased number of head dips observed in Hole Board test is reliable indices of anxiolytic effects.

In summary, the results of the present study demonstrated that the mice treated with the PAEE produced significant anxiolytic- like effects in all the animal models of anxiety, when compared to the control group.

CONCLUSION

The present study was undertaken to study the antianxiety activity of PAEE.

For the evaluation of the antianxiety activity, Elevated Plus Maze, Light Dark Transition test, Open Field Model and Hole Board test was considered. Diazepam was taken as the reference standard and the PAEE was orally administered in two different doses of 100mg/kg (lower dose), and 200mg/kg (higher dose). The antianxiety activity of the PAEE was assessed in terms of various parameters utilized in the models considered for the present study. The parameters include no. of open arm entry, time spent in open arm, no. of entries to light side, time spent in light side, no. of squares crossed, time spent in central square and no. of rearing and no. of head dips in holeboard.

The results obtained were compared with the normal group and standard group. The comparative analysis showed the significance of the PAEE in anti-anxiety ability.

Based on the results confirmed from the present study, it was concluded that the PAEE showed remarkable anti-anxiety activity in the experimental subject.

ACKNOWLEDGMENT

Behind every success there is lot of effort, but efforts are fruitful due to hands making the passage smoother. I express my deep sense of gratitude for the helping hands extended to me during my research work.

"I offer my adoration to **Almighty God** who created me, gave me the strength and courage to complete my dissertation and gave me the opportunity to thank all those people through whom his grace was delivered to me".

Finally, I would like to thank everybody who was important to the successful realization of my project, as well as expressing my apology that I could not mention personally one byone.

Place: Kasaragod

RAGESH T.

REFERENCES

1. Ashtanga Hrudaya Chikitsasthana. Sahasrayoga. Vata-pitta JwaraharaKashayam, 1: 55-58.
2. Martins Ekor. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol, 2013; 4: 177.
3. Kulkarni R, Girish KJ, Kumar A. Nootropic herbs (MedhyaRasayana) in Ayurveda: An update. Pharmacogn Rev, 2012; 6(12): 147-53.
4. Oliver Grundmann a, Jun-Ichiro Nakajima b, ShujiroSeo b, VeronikaButterweck. Anti-anxiety effects of Apocynumvenetum L. in the elevated plus maze test. Journal of Ethnopharmacology, 2007; 110: 406-411.
5. Millon T, Blaneyu PH, Davis R, ed. Oxford Text book of Psychopathology. New York: Oxford University Press, 1999.
6. Eaton WW, Kessler RC, Wittchen HU, Magee WJ. Panic and panic disorder inthe United States. Am J Psychiatry, 1994; 151: 413-420.
7. Robins LN Regier DA. Psychiatric disorder in America. The epidermologic catchment area study. New York: The free press, 1991; 211-250.
8. Sandhya S, Vinod KR, Sravan Kumar. Herbsused for braindisorders Hygeia. J Drugs Med. 2010; 2(1): 38-45.
9. KD Tripathi. Essentials of medical Pharmacology. 6th ed. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, 2008; P.386-398.
10. Yadav A V, Kawale LA, Nade V S. Effect of Morusalba L. (mulberry) leaves onanxiety in mice. Indian Journal of Pharmacology, 2008; 40: 32.
11. Desai VR, Kamat JP, Sainis KB. An Immunomodulator from *Tinosporacordifolia* with antioxidant activity in cell free systems. Proc Ind Acad Sci, 2002; 114: 713-9.

Parasuraman S, Thing GS, Dhanarajsa. Polyherbal formulation: Concept of Ayurveda. Pharmacogn Rev, 2014; 8(16): 73-80.

12. Sajeesh T and Parimelazhagan T. Analgesic, Anti-Inflammatory, and GC-MS Studies on *Castanospermumaustrale* A. Cunn. &C. Fraser ex Hook. Scientific World J, 2014; 2014: 587807.
13. Tripathi KD. Essential Medical Pharmacology. Jaypee Bros, New Delhi, 6: 388 439.
14. AshtangaHridayam, Chikitsasthana. Chapter 1, JwaraChikitsa, verse 55-58 & Sahasrayogam, Kashaya Prakarana, Vata Pitta Jwarahar Kashaya.
15. Deepashri T, Suchetakumari. Literature Review Of Draksha(*Vitisvinifera*). International Ayurvedic Medical J, 2017 Feb; 5(2).
16. K.Manjulatha, K.Saritha, O.H.Setty.Phytochemistry, Pharmacology and Therapeutics of *Hemidesmusindicus*(L.) R. Br.medicinalplants: phytochemistry, pharmacology and therapeutics, 2014; 3(1): 1-38.
17. M. Nagarajan, Gina R. Kuruvilla, K. Subrahmanya Kumar, Padma V.Pharmacology of *Ativisha*, *Musta*and their substitutes. J of Ayurveda & Integrative Medicine, 2015; 6(2).
18. Md. RubaiyatHasan, Md. Nasirul Islam, Md. RokibulIslam. Phytochemistry, pharmacological activitiesand traditional uses of *Emblicaofficinalis*: A review**.** Int Current Pharm J, 2016; 5(2): 14-21.
19. Pushpendra K. Patel, Narendra K. Prajapati, B.K. Dubey. *Madhucaindica:* A Review Of Its Medicinal Property. IntJ of Pharm Sci and Res, 2012; 3(5): 942-50.
20. Rakesh K, Nishat A, Y.C. Tripathi. Phytochemistry And Pharmacology Of*Santalum album* L.: A Review. World J of Pharm Res, 2015; 4(10): 1842-76.
21. ShashikantMP, Bhupesh RP. Phyto-Pharmacological Perspective of *Yashtimadhu* (*Glycyrrhiza Glabra*L.) - A Review. Int J of Pharm & Biological Archives, 2013; 4(5): 833-41.
22. RichaShri. Anxiety: causes and management. International journal of behavioral science, 2010; 5(1): 100-108.
23. R.S. Satoskar, Nirmala N, S.D. Bhandarkar. Pharmacology and pharmacotherapeutics. Mumbai. Popular prakashan, 2012; 22.
24. Steven D Ehrlich. Anxiety. University of maryland medical center, 2015.
25. Jean Kim, Jack M Gorman. The psychology of anxiety. Vlinical neuroscience research, 2005; 4(5-6): 335-347.
26. Kessler RC, Chiu WT, Demler O, Walters EE. Prevalence, severity, and comorbidity of twelvemonth DSM-IV disorders in the National Comorbidity Survey Replication (NCS-

R). *Archives of General Psychiatry*, 2005; 62(6): 617–627.

27. Robins LN, Regier DA, *Psychiatric Disorders in America: The EpidemiologicCatchment Area Study*. New York: The Free Press, 1991.
28. Wonderlich SA, Mitchell JE. Eating disorders and comorbidity: Empirical, conceptual, and clinical implications. *Psychopharmacology Bulletin*, 1997; 33(3): 381-390.
29. Margolin G, Gordis EB. The effects of family and community violence on children. *Annual Review of Psychology*, 2000; 51: 445–479.
30. Yehuda R. Biological factors associated with susceptibility to posttraumatic stress disorder. *Canadian Journal of Psychiatry*, 1999; 44(1): 34–39.
31. Bourdon KH, Boyd JH, Rae DS, et al. Gender differences in phobias: Results of the ECA community survey. *Journal of Anxiety Disorders*, 1998; 2: 227– 241.
32. Kendler KS, Neale MC, Kessler RC, et al. Generalized anxiety disorder in women. A population-based twin study. *Archives of General Psychiatry*, 1992; 49(4): 267–272.
33. Chantal M, Mike B. Theimportanceofnorepinephrineindepression. Neuropsychiatry Dis Treat, 2011; 7(1): 9-13.
34. StahlS, BrileyM. Understandingpainindepression. Hum Psychopharmacol, 2004; 19(1): 9-13.
35. Fricchione G, Stefano GB. Placebo neural systems: nitric oxide, morphine and the dopamine brain reward and motivation circuitries. Med SciMonit, 2005; 11(5): 54-65.
36. Karen Whalen, Richard finkel, Thomas A. Panavelil. Lippincott Illustrated Reviews Pharmacology. 6th ed. India: Wolters Kluwer, 2015; 121-122.
37. HL Sharma, KK Sharma. Principles of Pharmacology. 3rd ed. New Delhi: Paras Medical Publisher, 2017; 451-452.
38. Kessler RC et al. The use of complementary and alternative therapies to treat anxiety and depression in the united states. Am J Psychiatry, 2011; 158(2): 289-294.
39. Barbara Moquin, Marc RB, Ethel Mitty, Sandi Flores. Complementary and alternative medicine. Geriatric nursing, 2009; 0(3): 196-203.
40. VogelH.Drugdiscoveryandevaluation: methodsinclinicalpharmacology. 3rd ed. Berlin: Springer, 2011.
41. SandeepGoyal, Suresh Kumar. Anti-anxiety Activity Studies of Various Extracts of *Pulsatillanigricans*Stoerck. International Journal of Pharmaceutical Sciences and Drug Research, 2010; 2(4): 291-293.
42. Valiollah H, Mohammad R, Alireza G, Elahe D. Evaluation of antianxiety and sedative effects of essential oil of *Ducrosiaanethifolia* in mice. Clinics, 2010; 65(10).

43. Subramanian N, JothimanivannanC, Senthil R, Kameshwaran S. Evaluation of anti-anxiety activity of *Justiciagendarussa* Burm. Pharmacologia, 2013; 404- 407.
44. Saima Ahmed, SualihaLutfullah, Izhar Ahmed, Reshma Farooq, JaveidIqbal. Anxiolytic activity of *Tribulusterrestris*on elevated plus maze. Journal of applied pharmaceutical science, 2014; 4(02): 126-128.
45. Dilip Kumar Tiwari, Hemant Nagar, GauravDwivedi, Rishi Kant Tripathi, Jitendra Jena. Evaluation of anti-anxiety of *Plectranthusamboinicus*(Lour.). Asian journal of pharmaceutical and clinical research, 2012; 5.
46. Mejo CK, sJunaid RP, Sheik HS, Vivekanandhan L, Sringaravel S, Thangavel SK. Evaluation of antianxiety activity of ethanolic extract of *Sapindusemarginatus*flowers in experimental animal models. IJPCBS, 2015; 5(4): 790-795.
47. Kundan SB, Aruna D. Evaluation of anti-anxiety activityof *Melissaparviflora*(Bnth.) in rats. TJPS, 2015; 39(3): 70-75.
48. Nasreen Q, Rafeeq AK, Ghazala HR. Evaluation of antianxiety and antidepressant properties of *Carthamustinctorius*L. (safflower) petal extract. Pak. J. pharm. Sci, 2015; 28(3): 991-995.
49. Akshara, Prabha H, Amith KB, Akshaya KK, Manasa KS, Manjunatha SP. Evaluation of anti-anxiety activity of leaf of *Drypetusroxburgii*. World journal of pharmacy and pharmaceutical sciences, 2016; 5(8): 1403-1411.
50. Swetha ES, Sathisha A, Ayesha R. Evaluation of antianxiety activity of xanthine oxidase inhibitors in albino mice. Journal of pharmaceutical research and clinical practice, 2014; 4(3): 17-21.
51. New OECD 425 guidelines. OECD Guidelines for testing animals, 2001 Dec 1; 26: 1-26.
52. H. Gerhard Vogel (Ed.) Drug Discovery and Evaluation: Pharmacological assays. 2nd ed. Germany. Springer, 2008; 2; (E): 622- 626.
53. Bourin M, Petit-Demoulière B, Dhonnchadha BN, Hascöet M. Animal models of anxiety in mice. Fundam Clin Pharmacol, 2007; 21(6): 567-74.
54. Rajesh Kumar Goel, DilpreetKaur, PriyankaPahwa. Assessment of anxiolytic effect of nerolidol in mice. Indian J Pharmacol, 2016; 48(4): 450-452.
55. B.S.Thippeswamy, Brijesh Mishra, V.P Veerapur, Gourav Gupta. Anxiolytic activity of Nymphaeaalba Linn. in mice as experimental models of anxiety. Indian J Pharmacol, 2011; 43(1): 50-55a.
56. Aiyelero OM., Abdu-Aguye SN, Yaro AH, Magaji MG. Behavioural studies on the methanol leaf extract of *Securinegavirosa*(Euphorbiaceae) in mice. J. Pharmacogn.

Phytother, 2012; 4(2): 12-15.

57. Marc-Antoine C. a history of anxiety: from Hippocrates to DSM. Dialogues clinneurosci, 2015; 17(3): 319-325.
58. Longo LP, Johnson B. Addiction: partI. Benzodiazepines–sideeffects, abuseriskand alternatives. Am Fam Physician 2000; 61(7): 2121-8.
59. Lader M, Tylee A, Donoghue J. Withdrawing benzodiazepines in primary care. CNS Drugs, 2009; 23(1): 19-34.
60. Dhawan K, Dhavan S, Chhabra S. Attenuation of benzodiazepine dependence in mice by a trisubstitutedbenzoflavone moiety of *Passifloraincarnata*Linneous: A non habit forming anxiolytic. *J Pharm Pharmceu Sci*, 2003; 6(2): 215-222.
61. Taylor DP, Riblet LA, Stanton HC, Eison AS, Temple DL. Dopaine and antianxiety activity; PharmacolBiochem Behave, 1982; 17(1): 25-35.
62. Grossman E, Nadler M, Sharabi Y, Thaler M, Sanchar A, Shamiss A. Antianxiety treatment in patients with excessive hypertension. Am J Hypertense, 2005; 18(9): 1174-77.
63. Miller CS. Are we on the threshold of a new theory of disease? Toxicant- induced loss of tolerance and its relationship to addiction and abdiction. ToxicolInd Health, 1999; 15 (3-4): 284-94.
64. Pall ML, Satterlee JD. Elevated nitric oxide/peroxynitrite mechanism for the common etiology of multiple chemical sensitivity, chronic fatigue syndrome, and posttraumatic stress disorder. Ann N Y Acad Sci, 2001; 933: 323-9.
65. Ersan S, Bakir S, ErdalErsan E, Dogan O. Examination of free radical metabolism and antioxidant defence system elements in patients withobsessive-compulsive disorder. ProgNeuropsychopharmacolBiol Psychiatry, 2006; 30(6): 1039-42.
66. Atmaca M, Tezcan E, Kuloglu M, Ustundag B, Tunckol H. Antioxidant enzyme and malondialdehyde values in social phobia before and after citalopram treatment. Eur Arch Psychiatry ClinNeurosci, 2004; 254(4): 231-5.