ORIGINAL ARTICLE

Neuropharmacological evaluation of *Pentapetes phoenicea* Linn. extracts

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Abstract

Background: The plant *Pentapetes phoenicea* was traditionally used to treat central nervous system disorders. The present study was conducted to evaluate the neuropharmacology of the plant using various methods involving rats and mice. **Objective:** The study probes the neuropharmacological activities of *P. phoenicea* in rats and mice. **Materials and Methods:** The effect of the extracts on pentobarbitone-induced sleeping time, different models of seizures, spontaneous motor activity, exploratory behavior, and motor co-ordination were evaluated. **Results:** The extracts elicited significant prolongation of pentobarbitone-induced sleeping time, reduction in spontaneous motor activity, and exploratory behavior. The extracts prolonged the onset of seizures. Significant muscle relaxation was expressed in extract treated groups. Gamma-aminobutyric acid (GABA) levels were elevated in rats pretreated with *P. phoenicea*. Reversed-phase high-performance liquid chromatography was performed to rutin isolated from a methanolic extract of *P. phoenicea*. **Conclusion:** The results confirmed that *P. phoenicea* possesses significant anxiolytic potential with sedative action, and the mechanism underlying involves GABAnergic activity.

Key words: Anxiolytic, high-performance liquid chromatography, gamma-aminobutyric acid, neuroprotective, *Pentapetes phoenicea*, rutin, sedative

INTRODUCTION

Pentapetes phoenicea Linn. is a shrub of Sterculiaceae family, commonly known as midday flower. The capsules are mucilaginous and used for the treatment of diseases of bowels. Hypoglycemic and *in-vitro* antiradical activity was reported for the leaves of *P. phoenicea*.[1] Water of boiled leaves of the plant has been reported to be used traditionally for the treatment of inflammatory glands, cold, and cough. Plant parts are useful in fever, diarrhea, burning sensation, psychopathy, and vitiated conditions of vata and pitta.[2] Both experimental and epidemiological evidence demonstrate that bioflavonoid rutin is neuroprotective,[3] anxiolytic,[4] and sedative.[5] Flavonoids such as rutin and quercetin were proved to be cerebroprotective against ischemia-reperfusion-induced injury. As the plant was used traditionally in the treatment of psychological disorders, the study was undertaken to evaluate the benefits of *P. phoenicea* on central nervous system (CNS) with a probe on its chemical constituents.

MATERIALS AND METHODS

Plant Material

The *P. phoenicea* plants were collected from the forests of Tirupathi during August 2013. Plants were authenticated by Dr. K. Madhava Chetty and a voucher specimen was preserved at herbarium section of Department of Botany, Sri Venkateswara University.

Preparation of Extract

The plant materials were cleaned, shed-dried, and powdered. The dry powder was subjected to successive

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extraction with petroleum ether, chloroform, and methanol using soxhlet apparatus at 55°C. Methanolic extract was collected. A aqueous extract was obtained by soxhlation of the dry powder. Rotary vacuum evaporator was used to free the extract from the solvent and concentrate it. Qualitative analysis revealed the presence of alkaloids, tannins, phenolics, triterpenoids, and flavonoids in the methanolic extract. The aqueous extract was positive to tannins, phenolics, flavonoids, and glycosides.

Chemicals and Drugs

Methanol, petroleum ether, chloroform, and acetonitrile used were of the high-performance liquid chromatography (HPLC) grade. Rutin, pentobarbital, pentylenetetrazole, strychnine, diazepam, ninhydrin, trichloroacetic acid, and copper tartrate were obtained from Sigma-Aldrich.

Animals

Albino mice (20-25 g) and rats (150-200 g) of either sex were procured from National Institute of Nutrition, Hyderabad and acclimatized under standard laboratory conditions at 25 ± 2°C, relative humidity (50 ± 15%) and normal photoperiod (12 h light dark cycle) for 7 days, were used for the experiment. The animals were fed with commercial rat pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals.

HPLC Analysis

Preparation of isolated rutin sample

10 mg of isolated rutin from the methanolic extract of *P. phoenicea* was added to 25 ml of methanol (HPLC grade) and sonicated for 5 min, from that 2.5 ml solution was separated and made up to 10 ml with methanol and sonicated to make rutin solution.

Preparation of standard rutin sample

A stock solution of rutin was prepared by adding 10 mg rutin to 25 ml methanol and sonicated. From that 2.5 ml stock solution was separated and made up to 10 ml with methanol and sonicated to make standard rutin solution.

Method for Qualitative Analysis

Chromatographic analysis of *P. phoenicea* was done on reversed phase-HPLC (RP-HPLC) with Alliance waters ultraviolet 2487 Dual Lambda absorbance detector utilizing a waters stainless steel C_{18} column with dimension ×250 4.6 mm at ambient temperature. The mobile phase used was 0.5% formic acid:acetonitrile (70:30) at a flow rate 0.9 ml/min. The polyphenol identification was based on the comparison of retention time with the standard solution.

Toxicity Studies

Acute toxicity study of methanolic and aqueous extracts of *P. phoenicea* was carried out by adopting fixed dose method - OECD guideline No. 423. Observation for mortality was done up to 24 h.[6]

Behavioral screening

Mice (n = 10) after administration of methanolic and aqueous extract were screened according to Irwin's method, and observations were made for every 30 min for 2 h.

Photoactometer test

All the groups (n = 10) of mice were screened in actophotometer for 15 min both prior and after administration of vehicle/methanolic/aqueous extracts at different doses.[7]

Pentobarbitone-induced sleeping time

30 min after administration of vehicle/methanolic/aqueous extracts at various doses, pentobarbitone sodium intraperitoneally (i.p.), 40 mg/kg was administered. The time span between the loss and recovery of righting reflex was noted as sleeping time.[8]

Analgesic Activity

Acetic acid induced writhing

Different doses of methanolic and aqueous extracts of *P. phoenicea* and standard drug aspirin (200 mg/kg, i.p.) were administered to various groups of mice (n = 6), 15 min prior administration of acetic acid (1% i.p.) at a dose of 0.1 ml/10 g. A number of writhes elicited by these animals for 30 min were counted and compared with aspirin.[9]

Tail flick method

Techno analgesiometer was used to determine analgesic activity. Each animal was placed in a holder with its tail coming out through a slot in the lid. The tail was kept on the bridge of the analgesiometer with an electrically heated nichrome wire underneath. The tail received radiant heat from the wire, heated by passing current of 6 MA. The time taken for with drawl of the tail after switching on the current was taken as the latent period, tail flicking response was considered as the index of nociception. To avoid injury to skin the cut off time for determination of latent period was taken as 30 s. After recording the basal reaction time in a group of the animal at least for three consecutive trials, animals were selected for further experimentation and were administered orally with vehicle, methanolic or aqueous extracts in different doses and pentazocine 10 mg/kg was administered

i.p. as the reference standard and were tested 30 min later. All the tail flick latencies recorded are the averages of three trials.[10]

Anticonvulsant Activity

Maximum electroshock induced convulsions

Vehicle/standard drug phenytoin (25 mg/kg, i.p.)/methanolic or aqueous extracts of *P. phoenicea* at different doses were administered to a group of rats (n = 10) 30 min prior electric shock application (150 mA, 0.2 s) using electrodes. Duration of hind limb extension was noted.[11]

Pentylenetetrazole (PTZ) induced convulsions

Groups of rats (n = 6) were pretreated with PTZ (80 mg/kg, i.p.) and 30 min later, vehicle/doses of methanolic/aqueous extracts of *P. phoenicea* or phenobarbitone (20 mg/kg, i.p.) as standard drug were administered. A number of rats showing tonic convulsions, the onset of tonic convulsions and mortality was recorded in each group.[12]

Strychnine-Induced Convulsions

30 min prior administration of strychnine (4 mg/kg, i.p.) treatments with the vehicle, doses of methanolic and aqueous extracts of *P. phoenicea* or standard drug phenobarbitone (20 mg/kg, i.p.) were administered to groups of rats (n = 6). Mortality, the number of rats showing tonic convulsions were recorded.[12]

Body Temperature Variation

Electronic telethermometer was used to record the rectal temperature of mice in various groups (n = 10), prior and later administration of vehicle, doses of methanolic and aqueous extracts of *P. phoenicea.* Recordings were done at various intervals up to 4 h.

Conditioned Avoidance Response

The method according to Maffi G. 1959 was carried out. Rats were trained in a pole climbing apparatus in such a way that on hearing a buzzer sound they climb a pole suspended in the upper center of the chamber. To avoid electric shock stimulus on the grid floor termed as conditioned avoidance response. After training if the animals climb the pole even before the buzzer, it is termed as secondary conditioned response (SCR). Rats which exhibited SCR after training were selected for experimentation. Selected rats were grouped and administered with vehicle, doses of methanolic and aqueous extracts of *P. phoenicea* or standard drug (chlorpromazine 3 mg/kg, i.p.) and recordings were made 30 min, 1 h, 2 h, and 3 h later administration.[13]

Evaluation of Exploratory Behavior Pattern

Head dip test

Female mice were administered with vehicle or methanolic or aqueous extracts of *P. phoenicea* or standard drug diazepam (10 mg/kg, i.p.,). 30 min later, individual mice were placed on the wooden platform of head dip apparatus with evenly spaced holes. Number of times the head dipped into the holes in 3 min interval was recorded.[14]

Y-Maze test

30 min prior to conducting the experiment, the groups of rats (n = 6) were treated with either vehicle or methanoic or aqueous extracts of *P. phoenicea* or standard drug diazepam (10 mg/kg, i.p.). Each rat was placed individually in Y-maze runway for 5 min. A number of entries into the arms and time spent in the arm were recorded.[15]

Plus maze

30 min prior to conducting the experiment, the groups of mice (n = 6) were treated with either vehicle or methanolic or aqueous extracts of *P. phoenicea* or standard drug diazepam (10 mg/kg, i.p.). The plus maze used was elevated to 40 cm height and consisted of two closed (35 cm × 6 cm × 15 cm) and two open (35 cm × 6 cm) arms. The time spent by the mouse during the next 5 min on the open and enclosed arm was recorded.[16]

Evasion Test

Apparatus consisted of a rectangular box with an inclined plane. Mice that readily escaped from the box were selected for further experimentation. 15 min prior conducting an experiment, the groups of mice were administered vehicle or methanolic or aqueous extracts of *P. phoenicea* or standard drug diazepam (10 mg/kg, i.p.). Each group was placed in the box, and a number of mice left over after completion of 5 min was noted.[17]

Muscle Relaxant Activity

Rotarod test

Mice were placed on the rotating rod with diameter 32 mm at a speed of 5 rpm and trained on the day before the experiment was conducted. Mice remaining on the rod for not <3 min in two successive trials were elected, grouped (n = 10). Groups were administered with vehicle or methanoic or aqueous extracts of *P. phoenicea* or standard drug diazepam (10 mg/kg, i.p.) and 30 min later experiment was conducted up to 2.5 h for every 30 min. If the animal fails before 3 min, the test was given positive for the motor in co-ordination.[18]

Chimney test

The apparatus consisted of a hollow glass tube (30 cm length, 28 mm diameter) with a marking at 20 cm from the base. Mouse was placed at the end near to the mark for it to move to the other end, and the tube was made vertical and a mouse climbs upward. Mice that reached the mark in <30 s were selected, grouped (n = 10) for experimentation.

30 min later, after administration of vehicle or methanolic or aqueous extracts of *P. phoenicea* or standard drug diazepam (10 mg/kg, i.p.), the experiment was conducted and the recordings made.[19]

Traction test

A tightly pulled metal wire placed at a height with support on both the ends on the top of lab bench was used for experimentation. Mice was made to hold the wire with fore foot, and only those mice which managed to place at least one hind foot on the wire within 5 s were selected and grouped (n = 10). The test was conducted later after administration of vehicle or methanolic or aqueous extracts of *P. phoenicea* or standard drug diazepam (10 mg/kg, i.p.) and the mice that passed the test were given positive recordings.[20]

Inclined screen test

Glass plane inclined at an angle of 30° was used for experimentation. 30 min after administration of vehicle or methanolic or aqueous extracts of *P. phoenicea* or standard drug diazepam (10 mg/kg, i.p.), mouse were placed individually on the inclined screen at the top. Time taken by the mouse to slide off and reach the bottom was noted.[21]

Aggressive behavior (electroshock-induced fighting test)

The test was performed by placing pairs of mice on the grid floor of the apparatus through which a current of 3 mA was adjusted, and shock was given to mice foot for 0.2 s at five intervals.[22]

The mice which exhibited a minimum of 1 fighting episode within 3 min were selected, grouped and administered either with vehicle or methanolic or aqueous extracts of *P. phoenicea* or standard drug diazepam (10 mg/kg, i.p.).

Effect on Brain Neurotransmitter Levels

Gamma-aminobutyric acid (GABA) content of mice brain was estimated.[23] After administration of vehicle or methanolic or aqueous extracts of *P. phoenicea* at different doses, mice were sacrificed, brains isolated, blotted, homogenized in 5 ml of 10% cold trichloroacetic acid and centrifuged at 1000 rpm for 10 min at 0°C. 0.2 ml of ninhydrin (0.14 M) in 0.5 M bicarbonate buffer (pH 9.95) was added to 0.1 ml of the centrifuge, placed in a water bath for 30 min at 60°C and cooled to add 5 ml of copper tartrate reagent. After 15 min at 377/455 nm fluorescence was recorded in a spectroflourimeter.

Statistical Analysis

All the results are expressed as mean ± standard error of mean and analyzed by ANOVA followed by t-test for multiple comparisons using Graph pad prism version 6.

RESULTS

Product Identification by HPLC

Rutin isolated from methanolic extract of *P. phoenicea* was subjected to RP-HPLC under the conditions mentioned in the methodology. Rutin present in *P. phoenicea* was well resolved with good peak shape. Peak at 3.375 min was observed for standard rutin [Figure 1] and for *P. phoenicea* [Figure 2] peak at 3.339 min retention time was observed. The experimental data pertaining to retention times and area under the peaks is presented in Table 1.

Toxicity and Behavior

No deaths occurred with 2000 mg/kg of aqueous extract, and one death was observed with 2000 mg/kg of methanolic

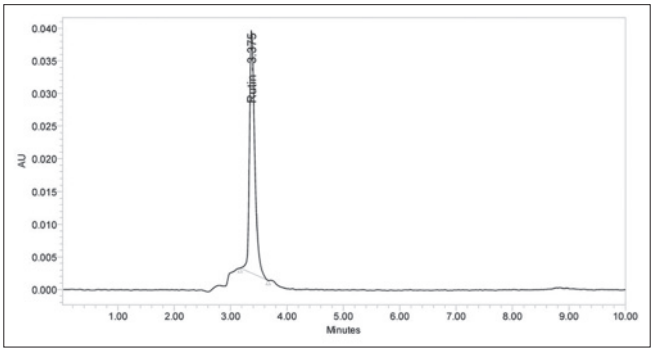

Figure 1: High-performance liquid chromatography profile of standard rutin

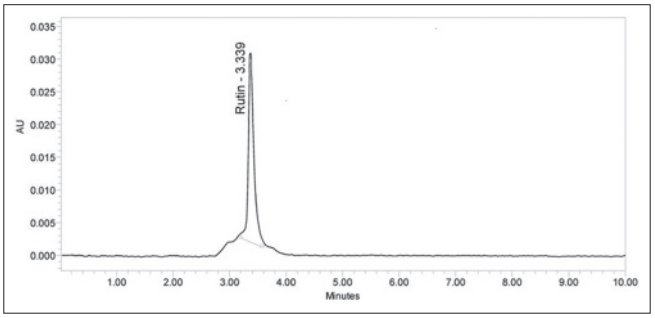

Figure 2: High-performance liquid chromatography profile of rutin isolated from methanolic extract of *Pentapetes phoenicea*

extract within 24 h. Behavioral alterations observed in these mice include a decrease in limb and abdominal tone, reduction in locomotor activity, pain response and increase in body sag. Rapid respiration was observed with various doses of methanolic and aqueous extracts of *P. phoenicea*. Staggering gait was observed only with 2000 mg/kg for 1-1.5 h.

Actophotometer Test

Significant reduction in spontaneous locomotor activity was observed in mice treated with different doses of methanolic and aqueous extracts of *P. phoenicea.* Inhibition of spontaneous activity was calculated as compared with control group [Table 2].

Pentobarbitone-Induced Sleeping Time

Administration of methanolic and aqueous extracts of *P. phoenicea* exhibited a dose-dependent increment in sleeping time [Figure 3].

Analgesic Activity

Methanolic extract at doses 100, 200, and 400 mg/kg exhibited % inhibition of 42.22%, 57.78%, and 66.67% and aqueous extract at doses 100, 200, and 400 mg/kg exhibited % inhibition of 33.33%, 46.6%, and 57.78% analgesic activity in acetic acid induced writhing test. All the doses of methanolic and aqueous extracts exhibited significant analgesic activity after 30 min and 60 min interval in tail flick method [Table 3].

Table 1: Retention time, peak area of standard rutin and rutin isolated from methanolic extract of *P. phoenicea*

Compound	Retention time	Peak area
Rutin standard	3.375	243,619
Isolated rutin from *P. phoenicea*	3.339	204,320

P. phoenicea: Pentapetes phoenicea

Table 2: Effect of *P. phoenicea* extracts on behavior of mice in actophotometer

Treatment (mg/kg)	Locomotor activity (15 min)		% Reduction in activity
	Before treatment	After treatment	
Control 10 ml/kg	523.20±7.90	510.30±8.26	-
Diazepam 4	484.20±5.09	135.30±4.98***	72.05
Methanolic 100	484.30±5.62	357.30±4.86	26.20
Methanolic 200	526.40±5.8	323.00±5.53*	38.63
Methanolic 400	502.40±6.01	159.80±6.21**	68.19
Aqueous 100	509.40±7.10	397.30±8.56	22
Aqueous 200	410.10±6.25	275.10±7.19*	32.19
Aqueous 400	530.10±5.62	222.50±5.86**	58.02

Values are expressed as mean±SEM (n=10), *P<0.01, **P<0.001, ***P<0.0001 as compared to control group.
P. phoenicea: Pentapetes phoenicea, SEM: Standard error of mean

Anticonvulsant Activity

P. phoenicea methanolic extract (400 mg/kg) and aqueous extract (200 and 400 mg/kg) exhibited a significant decrease in tonic flexion and tonic hind limb extension.

P. phoenicea extracts significantly decreased the onset and duration of strychnine and PTZ induced convulsions as expressed in Tables 4 and 5.

Body temperature

Administration of methanolic and aqueous extracts of *P. phoenicea* brought significant reduction in body temperatures of mice during first 1 h after administration [Figure 4].

Conditioned avoidance response

Unlike chlorpromazine, *P. phoenicea* did not block SCR and conditioned avoidance responding of trained rats.

Exploratory behavior pattern

Extract treated groups exhibited significantly less number of head dips compared with control group [Table 6]. In elevated

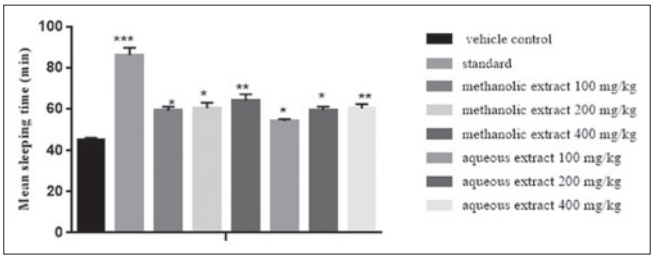

Figure 3: Effect of *Pentapetes phoenicea* extracts on pentobarbitone-induced sleeping time in mice. Results are expressed as mean ± standard error mean (n = 6), *P < 0.05, **P < 0.01, ***P < 0.001 as compared to control group using one-way ANOVA followed by t-test

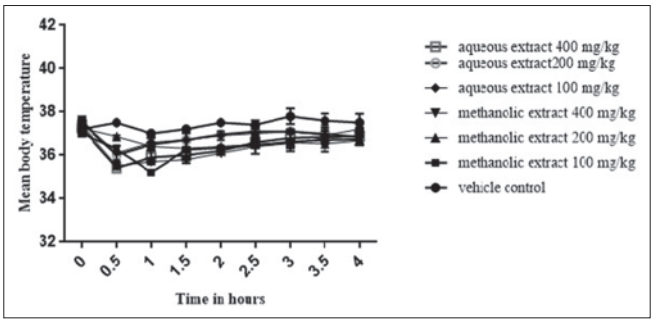

Figure 4: Effect of *Pentapetes phoenicea* extracts on body temperature of mice. Values are expressed as mean ± standard error of mean for n = 10

Table 3: Effect of *P. phoenicea* extracts on tail flick mean latent period in rats

Treatment (mg/kg)	Mean latent period of response		
	Initial	After 30 min	After 60 min
Control 10 ml/kg	9.26±0.40	9.16±0.40	9.22±0.25
Pentazocin 10	9.32±0.25	16.12±0.54***	18.33±0.47***
Methanolic extract 100	8.72±0.48	12.83±0.63**	14.33±0.40***
Methanolic extract 200	8.40±0.25	13.20±0.40**	15.33±0.47***
Methanolic extract 400	9.80±0.40	13.5±0.30***	16.16±0.25***
Aqueous extract 100	9.16±0.47	10.00±0.47	12.04±0.91*
Aqueous extract 200	8.25±0.47	12.00±0.62*	13.83±0.70**
Aqueous extract 400	8.70±0.25	13.02±0.75**	15.33±0.64***

Results are expressed as mean±SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 as compared to control group, using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

Table 4: Effect of *P. phoenicea* extracts on MES-induced convulsions in mice

Treatment (mg/kg)	Tonic flexion (time in s)	Tonic hind limb extension (time in s)	Stupor (time in s)
Control 10 ml/kg	8.46±0.26	17±0.32	54±0.17
Phenytoin 25	4.7±0.78***	1.5±0.15***	18.34±0.83***
Methanolic extract 100	6.91±0.45*	4.8±0.6	37.3±1.20
Methanolic extract 200	5.12±0.32*	3.65±0.3*	28.53±1.10*
Methanolic extract 400	4.25±0.13**	2.13±0.12**	20.78±0.76**
Aqueous extract 100	7.16±0.65	4.56±0.5	42±1.34
Aqueous extract 200	6.17±0.53*	3.83±0.5*	30.65±1.20
Aqueous extract 400	5.42±0.17**	2.67±0.3**	22.32±0.56**

Results are expressed as mean±SEM (n=10), *P<0.05, **P<0.01, ***P<0.001 as compared to control group using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

Table 5: Effect of *P. phoenicea* extracts on pentylenetetrazole and strychnine-induced convulsions in rats

Treatment (mg/kg)	Onset of tonic convulsions (s)		Duration of convulsions (s)		Percentage mortality (1 h)	
	Pentylenetetrazole	Strychnine	Pentylenetetrazole	Strychnine	Pentylenetetrazole	Strychnine
Control 10 ml/kg	193±2.7	165±3.2	93.36±1.7	73.6±0.8	98	88
Phenobarbitone 20	589.4±1.43***	463.4±1.21***	15.67±0.63***	10.67±0.3***	36	38
Methanolic extract 100	392±4.2*	369±3.5*	30.02±1.42*	32.26±1.16	79	65
Methanolic extract 200	416±3.21*	417±2.1*	23.8±1.23*	21.8±1.20**	62	60
Methanolic extract 400	467±2.54**	440±1.8**	20.34±1.02**	17.84±1.36**	56	46
Aqueous extract 100	384±4.4	327±4.24	32.12±1.04	25.2±1.0	84	74
Aqueous extract 200	491±3.4**	387±3.4**	29.4±1.21*	19.4±1.27**	75	70
Aqueous extract 400	509±2.08**	429±2.8**	24.32±1.67**	16.92±1.39**	69.2	68

Results are expressed as mean±SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 as compared to control group by using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

plus maze, *P. phoenicea* treated groups exhibited a significant change in the number of entries into open arms and rears when compared with control group [Table 7]. However, there was a significant decrease in exploratory behavior observed in Y-maze test with 400 mg/kg of methanolic (0.01) and aqueous (0.05) extracts. In the evasion test, the number of rats remained in the box after 5 min of treatment with 400 mg/kg methanolic (0.01) and aqueous (0.05) extract were 8 and 6 (n = 6). The results revealed that there was a significant reduction in curiosity with these doses of *P. phoenicea*.

Muscle Relaxant Activity

Significant muscle relaxation was expressed by rats treated with high dose of *P. phoenicea* extract in rotarod, chimney test [Tables 8 and 9], inclined plane method, and traction test [Tables 10 and 11].

Table 6: Effect of *P. phoenicea* extracts on exploratory behavior in head dip test

Treatment (mg/kg)	Mean number of head dips after 30 min	Mean number of head dips after 1 h
Control 10 ml/kg	35.33±1.54	37.3±1.6
Phenobarbitone 2	2.9±2.7***	5.2±1.58***
Methanolic extract 100	24.2±1.17*	29.00±1.3
Methanolic extract 200	26.16±1.8	25.6±1.0
Methanolic extract 400	10.33±1.2***	15.5±1.02***
Aqueous extract 100	24.02±1.8*	27.6±0.9
Aqueous extract 200	22.83±1.74**	23.00±1.00**
Aqueous extract 400	16.24±1.02***	18.6±1.78**

Results are expressed as mean±SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 as compared to control group using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

Aggressive behavior

Foot shock induced aggressive behavior was significantly inhibited with 400 mg/kg of methanolic and aqueous extracts [Table 12].

Estimation of GABA content

Results exhibited that 400 mg/kg of methanolic and aqueous extracts of *P. phoenicea* elicited a significant increase in brain GABA content in mice [Table 13].

DISCUSSION

Behavioral effects of drugs have been exploited for generations, and psychopharmacology uses smaller species and studies behavioral effects of psychoactive drugs. Synthetic anxiolytics are fraught with side effects - Addiction, severe withdrawal risk so finding a natural solution for anxiety becomes so important. In this

Table 7: Effect of *P. phoenicea* extracts on behavior of mice in elevated plus maze paradigm

Treatment (mg/kg)	Number of open arm entries	Total number of arm entries	Time spent in open arms (s)	Time spent in closed arms (s)	Number of rare in open arms
Control (10 ml/kg)	4.33±0.31	9.20±1.50	48.67±1.05	234.58±6.62	3.00±0.10
Diazepam 4	9.89±1.58**	15.20±0.97**	140.33±1.38***	179±2.29**	8.50±0.67***
Methanolic extract 100	5.17±0.48	12.6±1.29*	105.62±1.83**	209.80±2.71	4.16±0.34
Methanolic extract 200	6.18±1.33*	14.0±0.68**	126±3.24**	198±2.29*	5.17±060*
Methanolic extract 400	8.33±1.45**	14.89±1.00**	132±2.08***	174±2.25**	6.16±0.70**
Aqueous extract 100	3.17±0.60	11.6±1.29	58.62±1.83	128.80±2.71*	4.16±0.70
Aqueous extract 200	4.33±0.49	12.50±0.68*	85±1.48*	118±1.50*	4.17±0.60
Aqueous extract 400	6.02±0.33*	13.08±1.00*	119.83±4.16**	102±3.25**	5.16±0.34*

Results are expressed as mean±SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 as compared to control group using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

Table 8: Effect of *P. phoenicea* extracts on muscle in co-ordination in rotarod test

Treatment (mg/kg)	Fall of time (s)				
	15	30	45	60	75
Control (10 ml/kg)	372±13.04	348±9.29	318±7.82	287±4.92	263±3.27
Diazepam 4	258±10.69***	210±8.46***	174±9.83***	149±10.65***	98±10.45***
Methanolic extract 100	268±10.03*	225±13.97	193±11.3*	142±7.61	125±4.76
Methanolic extract 200	254±11.21*	218±12.4*	186±10.4*	129±6.92*	118±5.67
Methanolic extract 400	240±12.85**	190±10.8**	178±9.32**	118±5.82**	100±4.5**
Aqueous extract 100	289±11.30*	241±12.8*	210±10.4*	165±6.73	137±7.32
Aqueous extract 200	270±14.05**	234±12.32*	195±11.5*	128±9.32*	120±7.12
Aqueous extract 400	262±15.34**	202±11.03*	157±10.6**	114±6.93*	105±4.61*

Results are expressed as mean±SEM (n=10), *P<0.05, **P<0.01, ***P<0.001 as compared to control group using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

Table 9: Effect of *P. phoenicea* extracts on muscle tone (chimney test) in mice

Treatment (mg/kg)	Number of mice failed in the test	% failure in the test
Control 10 ml/kg	0	0
Diazepam 10	10***	100
Methanolic extract 100	0	0
Methanolic extract 200	2	20
Methanolic extract 400	6**	60
Aqueous extract 100	0	0
Aqueous extract 200	1	10
Aqueous extract 400	4*	40

Results are expressed as mean±SEM (n=10), *P<0.05, **P<0.01, ***P<0.001 as compared to control group using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

Table 10: Effect of *P. phoenicea* extracts on inclined plane test in mice

Treatment (mg/kg)	Fall off time (s)	% decrease in time
Control 10 ml/kg	238.16±9.53	0
Diazepam 10	25.01±6.28***	89.4
Methanolic extract 100	138.02±9.32*	42
Methanolic extract 200	85.0±5.38**	64.3
Methanolic extract 400	70.25±6.12**	70.5
Aqueous extract 100	156.6±8.51	34.14
Aqueous extract 200	126.06±4.82*	47.06
Aqueous extract 400	78.05±9.79**	67.22

Results are expressed as mean±SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 as compared to control group using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

Table 11: Effect of *P. phoenicea* extracts on traction test in mice

Treatment (mg/kg)	Time of holding (sec)	% Failure to put hind limb
Control 10 ml/kg	6.15±0.18	0
Diazepam 2	1.13±0.04***	98
Methanolic extract 100	4.01±0.14*	16.6
Methanolic extract 200	3.76±0.10*	50
Methanolic extract 400	2.31±0.09**	69.7
Aqueous extract 100	5.13±0.17	20.6
Aqueous extract 200	4.31±0.13*	49.0
Aqueous extract 400	3.12±0.17**	60.3

Results are expressed as mean±SEM (n=10), *P<0.05, **P<0.01, ***P<0.001 as compared to control group using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

Table 12: Effect of *P. phoenicea* extracts on aggressive behavior

Treatment (mg/kg)	Fighting (pairs)	% Fighting	% Inhibition
Control 10 ml/kg	10	100	0
Diazepam 10	0	0	100***
Methanolic extract 100	10	100	0
Methanolic extract 200	7	70	30
Methanolic extract 400	5	50	50**
Aqueous extract 100	10	100	0
Aqueous extract 200	8	80	20
Aqueous extract 400	6	60	40*

Results are expressed as mean±SEM, *P<0.05, **P<0.01, ***P<0.001 as compared to control group using one-way ANOVA followed by t-test. *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

work, we emphasized on CNS methodology for compounds with sedative and anxiolytic activity. The RP-HPLC results confirmed the presence of Rutin, an anxiolytic flavonoid in *P. phoenicea*. Thus, anxiolytic activity of *P. phoenicea* can be attributed to the flavonoid rutin. The results of the investigation like alteration in normal behavior patterns, reduction in spontaneous motility, prolongation of induced sleeping time evidenced the sedative activity of *P. phoenicea*. Locomotor activity is considered as an index of alertness, and a reduction is indicative of sedative activity.[24] The results mentioned were elicited in dose-dependent manner, and methanolic extract was comparatively more significant than aqueous extract. Moderately significant anticonvulsant activity was elicited by *P. phoenicea* against strychnine and PTZ induced convulsions. Proofs exist that anticonvulsant drugs generally increase brain GABA level,[25] here the anticonvulsant activity of *P. phoenicea* can be attributed to the GABAnergic activity of the plant extract. Hypothermia occurred in mice can be linked to alteration of various mechanisms in the brain, evidence exists that central and systemic application of GABA and GABA agonists usually causes a fall in core temperature, while the antagonists induce hyperthermia.[26] Widely used models to assess anxiety in small rodents including hole board apparatus, Y-maze, elevated plus maze, and evasion test were used to evaluate *P. phoenicea*. Significant reduction in exploratory behavior was exhibited in these tests –indicated anxiolytic activity of *P. phoenicea*. Aggressive behavior was suppressed by *P. phoenicea*. GABA is the major inhibitory neurotransmitter in the CNS and different anxiolytic, muscle relaxant, and sedative-hypnotic drugs exhibit their action via GABA.[27] Neuropharmacology of *P. phoenicea* is similar to extremely valuable natural antianxiety plant Kava.

Flavonoids have recently increased in importance because they have been identified as a new type of ligand with *in-vivo* anxiolytic properties. Flavonoids elicit anxiolytic

Table 13: Effect of *P. phoenicea* on brain GABA content in mice

Treatment (mg/kg)	GABA (µg/g)	% Increase
Control 10 ml/kg	392.5±5.9	-
Methanolic extract 100	402.4±5.1	2.52
Methanolic extract 200	448.0±4.8	14.14
Methanolic extract 400	476.0±3.4*	21.27
Aqueous extract 100	384.6±5.0	2.01
Aqueous extract 200	416.5±3.9	6.11
Aqueous extract 400	452.2±4.0*	15.21

Results are expressed as mean±SEM (n=6),*P<0.05 as compared to control group using one-way ANOVA followed by t-test.
GABA: Gamma-aminobutyric acid, *P. phoenicea: Pentapetes phoenicea*, SEM: Standard error of mean

activity through benzodiazepine receptors.[28] Rutin treatment ameliorates various impairments associated with physical fatigue. Western blot analysis of MAPK expression confirmed the antianxiety effects of rutin.[29]

Rutin shows more favorable binding to GABA-A receptor, rutin can be used as an alternative drug candidate for epilepsy treatment.[30]

Pretreatment with *P. phoenicea* significantly elevated the brain GABA levels in rats. The mechanism involved in the anxiolytic action of *P. phoenicea* may be potentiation of GABAnergic inhibitory activity possibly through action on GABA receptors and elevation of GABA secretion.

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