

PHARMACOLOGICAL EVALUATION OF *PACHYRRHIZUS EROSUS* (L) SEEDS FOR CENTRAL NERVOUS SYSTEM DEPRESSANT ACTIVITY

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Abstract : The research work deals with the screening of ethanol and chloroform extracts of *Pachyrrhizus erosus* seeds for central nervous system (CNS) depressant activity. The *Pachyrrhizus erosus* seed is known to contain rotenoids, flavonoids and phenylfuranocoumarin derivatives as chemical components and is reported to have antifungal, antisecretory, insecticides, antibacterial and spasmolytic activity. Since seeds of *Pachyrrhizus erosus* is used as folk medicine in treatment of insomnia, we made an attempt to study its CNS depressant effect. The different activities studied were potentiation of pentobarbitone-induced sleep, test for locomotor activity, effect on muscle co-ordination, antiaggressive and antianxiety activities. The result of the study reflected that ethanol extract of the seeds (150 mg/kg, p.o) decreased locomotor activity, produced muscle relaxation and showed antianxiety and antiaggressive activity.

Key words : *Pachyrrhizus erosus* sedative muscle relaxant
antianxiety antiaggressive

INTRODUCTION

Advance in science and technology has contributed to an enormous improvement in the quality of life of humankind. However, modern life stress, associated trials and tribulation are responsible for the surge in incidence of variety of psychiatric disorders. Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazepam lorazepam and alprazolam etc) are the most frequently prescribed synthetic

drugs for variety of condition particularly anxiety, depression, epilepsy and insomnia. But these psychoneural drugs have very serious side effects like chronic use of benzodiazepines causes deterioration 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 (1).

In this context, a resurgence of interest

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in medicine from natural sources (mainly plant products) is seen and there is tremendous hope that drugs of plant origin will have significantly lesser side effects than that observed with synthetic drugs while having comparable efficacy.

A variety of naturally occurring drugs such as *Thymus linearis*, *Lactuca seroila*, *Papaver somniferum (opium)* and *Atropa belladonna* were tested for psychopharmacological effects and were found to be effective in the treatment of psychiatric disorders (2). The plant *Pachyrrhizus erosus* (L) is a tuberous root (*Leguminasae*), cultivated in South East Asia including India. Seeds of *Pachyrrhizus erosus* contain rotinoids, isoflavonoids and phenylcoumarine, which are reported to possess potent activity against anti-Herpes Simplex Virus type I and type II (3). Seeds of this plant were found to possess antibacterial activity against *Helicobacter pylori* (4). Tuberous root of Yam bean (*Pachyrrhizus erosus*) contains two major proteins named as YBG1 and YBG2 that were found to exhibit cysteine proteolytic activity (5). Further, seed extract of this plant is used as pesticide, anti-oxidant, anti-inflammatory, anti-fungal, anti-secretory, insecticide and spasmolytic (6).

Pachyrrhizus erosus (L) is used as folk medicine in treatment of insomnia, although it is not reported in literature. Some of the plants like *Montanoa tomentosa*, *Kunth*, *Castilleja tenuiflora* and *Penstemon barbatus* and *Byrsonima* containing chemical constituents like coumarin, flavonoids, monoterpenes, rotinoids, proanthocyanidine and glycolipids were reported to possess CNS depressant activity (6). Based on the above

information, we thought it is worthwhile to evaluate the *Pachyrrhizus erosus* (L) seeds for CNS depressant activity.

METHODS

Experimental animals

Swiss albino mice of either sex weighing between 12–35 g were used in the present study. The experimental protocol was approved by the Institutional Animal Ethics Committee. The animals were maintained under standard conditions in Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) approved Institutional Animal House.

Chemicals

Pentobarbitone sodium was obtained from Sigma-Aldrich, USA, Diazepam was procured from Cipla, Ahmedabad (India), Chloroform was purchased from Nice chemicals, Cochin (India), Chlorpromazine from Sun Pharma, Mumbai (India).

Diethyl ether from Merck Limited, Ahmedabad (India) and petroleum ether was obtained from S.D. Fine Chemicals, Mumbai (India).

Extraction of *Pachyrrhizus erosus* seeds

The *Pachyrrhizus erosus* seeds were collected from Kolkata (West Bengal, India), in winter season. They were dried, powdered and subjected to extraction by Soxhlet method using chloroform and ethyl alcohol as solvents. Extractions of dried coarse powder of *Pachyrrhizus erosus* seeds were

done in a manner, initially with petroleum ether, chloroform, and then with ethyl alcohol. Evaporation of solvents from the extract was done using rotary vacuum evaporator. A sticky mass was obtained after evaporation of solvent. The extracts were subjected to qualitative phytochemical analysis. A suspension of the extracts was prepared using 2% w/v Tween 80 in distilled water.

Acute oral toxicity study

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticides and Toxic Substance) guidelines following Up and Down procedure (7).

Selection of the extract

The chloroform and ethanol extract of *Pachyrrhizus erosus* seeds were evaluated for sedative-hypnotic activity in pentobarbitone induced sleep test. The extract, which potentiated the sedative-hypnotic activity of pentobarbitone was chosen for further study (8).

Test for locomotor activity

The spontaneous locomotor activity of each mouse was recorded individually for 10 min using actophotometer. The ethanolic extract of *Pachyrrhizus erosus* seeds (EEPES) was administered at two doses of EEPES (75 and 150 mg/kg, p.o) 60 min before the test and chlorpromazine (3 mg/kg, i.p), used as standard was given 30 min before the test. The control group was treated with 2% w/v Tween 80 orally, 60 min before test (9).

Muscle co-ordination test

This test was carried out using inclined plane and rotarod apparatus.

1. Inclined plane :

The plane consists of two rectangular plywood boards connected at one end by a hinge. One board is the base; the other is the movable inclined plane. Two plywood side panels with degrees marked on their surface are fixed on the base. A rubber mat with ridges 0.2 cm in height is fixed to the inclined plane, which was set at 65 degrees. Swiss albino mice were taken and divided into four groups, each group comprised of 6 animals. The two doses of EEPES (75 and 150 mg/kg) were administered orally, the standard group was treated with diazepam (4 mg/kg) intraperitoneally and control group received Tween 80 (2% w/v) orally. The test was carried out 30, 60 and 90 min after administration of drugs and vehicle. The animals were placed at the upper part of the inclined plane and were given 30 sec to hang on or to fall off (10).

2. Rotarod

The rotarod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6 mice. The rod is in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movements of the animals when they fall from the roller.

Swiss albino mice underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (20 rpm) for 5 min, were used for the test. The animals were treated in the same way as mentioned under inclined plane test (11).

Anti-anxiety activity

The anti-anxiety activity was evaluated using staircase test and elevated plus maize test.

1. Staircase test

Staircase consists of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along whole length of the staircase. The drugs and treatments were same as mentioned under inclined plane. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears were counted over a 3 min period. A step was considered to be climbed only if the mouse had placed all four paws on the step. In order to simplify the observation, the numbers of steps descended were not taken into account. After each step the box was cleaned in order to eliminate any olfactory cues, which might modify the behavior of the next animal (12).

2. Elevated plus maze

The apparatus consist of two open arms (5 × 10 cm) and two closed arms (5 × 10 × 15 cm) radiating from a platform (5 × 5 cm) to

form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open-arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The drugs and treatments were same as mentioned under inclined plane.

The animal was placed at the center of the maze, facing one of the closed arms.

During 5 min test period the following measures are taken :

- The number of entries into open arms
- The number of entries into closed arms
- Time spent in the open arms

Arm entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room, with observations made from an adjacent room (14).

Anti-aggressive activity

This was carried out using isolation induced aggression test. Male albino mice weighing about 12–20 gm are kept isolated in small cages for a period of 6 weeks. Prior to the administration of the drug, the aggressive behavior of the animals was tested. The male mouse being accustomed to live together with other animals was placed into the cage of an isolated mouse. The aggressive behavior of the isolated mouse was recorded. The following parameters were used to assess aggressive behavior

- Hitting the tail on the bottom of the cage
- Screaming (piercing noise) and
- Biting.

After these initial tests, the animals were subjected to different drug treatments as mentioned under inclined plane. The aggressiveness was studied again after 60 and 120 min after drug or vehicle administration (15).

Statistical analysis

Results were expressed as Mean±SEM. The differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Dunnett's test and were considered statistically significant when $P < 0.05$.

RESULTS

The preliminary phytochemical investigation of the EEPES revealed the presence of amino acids and flavones.

In acute oral toxicity study, EEPES produced death at doses of 2000 and 1000 mg/kg while with chloroform extract, there was death at doses of 2000, 1000, 750, 650, 450, 175 mg/kg, but it was safe at a dose of 75 mg/kg. EEPES was safe at a dose of 750 mg/kg orally. Hence 1/5th and 1/10th of this dose i.e. 7.5 mg/kg and 15 mg/kg of chloroform extract and 75 mg/kg and 150 mg/kg of EEPES were used for the study.

Pentobarbitone induced sleeping time

The EEPES (75 mg/kg p.o) significantly increased the pentobarbitone induced

sleeping time ($P < 0.001$). The increase in duration of action was 1075%. No significant effect was observed with the chloroform extract (Table I).

TABLE I: Effect of EEPES in pentobarbitone-induced sleep.

<i>Pentobarbitone (40 mg/kg, i.p) 30 min Post treatment of the vehicle and drugs</i>	<i>Onset of action (Min)</i>	<i>Duration of action (Min)</i>	<i>Percentage Sleeping time</i>
Control (Vehicle 6 ml/kg, p.o)	5.66±0.49	132.66±0.16	100
Chloroform extract (7.5 mg/kg, p.o)	5.83±0.16	118±14.00	89
Ethanol extract (75 mg/kg, p.o)	6.00±0.25	1420.66±0.21***	1075

All values are Mean±SEM, *** $P < 0.001$ when compared with control.

Test for locomotor activity

High dose of EEPES (150 mg/kg p.o) and diazepam (4 mg/kg i.p) decreased the locomotor activity significantly ($P < 0.01$) whereas, low dose of EEPES (75 mg/kg p.o) did not show a significant reduction in the locomotor activity (Table II).

Test for muscle coordination

1. Inclined lane

The number of animals falling from inclined plane after 60 and 90 min of treatment were significantly more in diazepam (4 mg/kg i.p) treated and EEPES (150 mg/kg p.o) treated groups when compared to control group ($P < 0.01$). Low

TABLE II: Effect of EEPES on locomotor activity in actophotometer and muscle coordination in inclined plane and rotated apparatus.

Groups	Actophotometer score in 10 min	No of animal falling down within (30 sec) from inclined plane			Time spent on revolving rod in rotarod apparatus (sec)
		30 min after administrations	60 min after administrations	90 min after administrations	
Control (Vehicle 6 ml/kg, p.o)	310.66±11.54	0±0	0±0	0±0	323.00±24.85
Ethanollic extract (75 mg/kg, p.o)	268.33±10.27	0±0	0±0	0.16±0.16	298.00±32.47
Ethanollic extract (150 mg/kg, p.o)	161.83±18.06**	0±0	0.66±0.21**	0.5±0.22**	199.5±41.59*
Standard drug	142.16±15.00**	0.66±0.21**	0.83±0.16**	0.83±0.166**	106.6±2.81**

All values are Mean±SEM, n=6, *P<0.05, **P<0.01 when compared with control.

dose of test drug was ineffective. (Table II).

2. Rotorod test

This test, EEPES (150 mg/kg) significantly reduced the time spent by the animals on revolving rod when compared to control (P<0.05). The standard drug (diazepam) also showed significant effect when compared to control (P<0.01) Low dose of drug (75 mg/kg) did not show any significant effect (Table II).

Anti-anxiety activity

1. Staircase test

The statistical summary of the rearing and number of steps climbed is presented in Table III. After 60 and 90 min of treatment, a reduction in anxiety-linked behavior was indicated by a reduction in number of rearing and sedation that was evaluated by number of steps climbed. High dose of EEPES (150 mg/kg, p.o) and standard drug (diazepam 4 mg/kg, i.p) significantly reduced the number of rearing as well as the number

of steps climbed (P<0.01). Low dose of EEPES (75 mg/kg, p.o) did not produce a significant decrease in the number of rearing or the number of steps climbed.

2. Elevated plus maze :

High dose of EEPES (150 mg/kg p.o), significantly increased the time spent in open arms (P<0.05) and number of entries into closed arms (P<0.01) but did not affect significantly the number of entries into open arms when compared with control. The standard drug (diazepam 4 mg/kg, i.p) showed a significant decrease in the number of entries into closed arms (P<0.01) and open arms (P<0.05) and also significantly increased the time spent in open arms (P<0.01). Low dose of EEPES (75 mg/kg, p.o) did not show any differences in activity compared to control (Table III).

Anti-aggressive activity

Both EEPES (150 mg/kg p.o) and diazepam (4 mg/kg i.p) produced marked inhibition of aggressiveness in isolated mice

TABLE III: Effect of EEPES and diazepam in stair case test and elevated plus-maze test.

Groups	Stair case test		Elevated plus maze test		
	No. of climbing in 3 min	No. of rearing in 3 min	No. of entry into		Time spent in open arms
			Closed arms	Open arms	
Control (Vehicle 6 ml/kg, p.o)	20.16±1.38	9.83±0.60	13.33±1.11	9.66±1.3	91.5±7.50
Ethanollic extract (75 mg/kg, p.o)	13.66±0.80	8.16±0.47	9.83±1.35	6.66±0.80	107.16±10.89
Ethanollic extract (150 mg/kg, p.o)	8.16±0.60**	6.00±0.57**	7.66±1.20**	6.83±1.66	147.33±9.97*
Diazepam (4 mg/kg, p.o)	6.00±0.68**	5.1±0.60**	7.16±0.95**	4.83±1.35*	199.51±15.84**

All values are Mean±SEM, n=6, *P<0.05, **P<0.01 when compared with control.

TABLE IV: Effect of EEPES and diazepam on isolation induced aggression.

Groups	Screaming		No. of Hitting the Tail on the bottom		Biting	
	After 60 min	After 120 min	After 60 min	After 120 min	After 60 min	After 120 min
Ethanollic extract (75 mg/kg, p.o)	1.16±0.47	0.96±0.47	7.33±1.05	7.33±1.05	3.16±0.87	2.06±1.01
Ethanollic extract (150 mg/kg, p.o)	0.50±0.22*	0.82±0.28*	1.00±0.25**	2.37±0.42*	0.66±0.21**	0.66±0.33**
Diazepam (4 mg/kg, p.o)	0.16±0.16**	0.18±2.91**	0.33±0.21**	0.16±0.03**	0.33±0.21**	0.12±0.09**

All values are Mean±SEM, n=6, *P<0.05, **P<0.01 when compared with control at the same time interval.

as indicated by a significant decrease in screaming, hitting the tail on the bottom, and biting. Low dose did not show any significant effect (Table IV).

DISCUSSION

The study showed that EEPES (150 mg/kg, p.o) possess sedative, antianxiety, muscle relaxant and antiaggressive activity. EEPES potentiated the sleep induced by

pentobarbitone suggesting that it possess some sleep inducing property. The study on the spontaneous motor activity showed that EEPES (150 mg/kg, p.o) decreased the frequency and the amplitude of movements. The reduction of the spontaneous motor activity could be attributed to the sedative effect of the extract (12).

Inclined plane method was originally developed for testing curare-like agents.

Later on, it has been used by many authors for testing compounds for muscle relaxing activity of both centrally acting and peripheral acting muscle relaxants (11). EEPES (150 mg/kg, p.o) made the animals unable to stay on inclined plane during 30 sec period. EEPES (150 mg/kg, p.o) also reduced the time spent on the revolving rod by mice in the rotarod test, a test mainly used to screen centrally acting muscle relaxants (12). This represented that EEPES may have muscle relaxant activity, which could be due to CNS depressant activity.

The mouse staircase was used for the assessment of anxiety (number of rearing) and sedation (number of steps ascended). Greater number of rear indicates anxiety like behavior and lesser number of steps ascended indicated increased sedation (13). The present investigation successfully detected the anxiolytic-like effects of EEPES and diazepam; both significantly decreased the number of rearing and number of steps ascended compared to control. This showed that EEPES has both anxiolytic and sedative properties.

The sedative, muscle relaxant and anxiolytic effects of EEPES could be due to the interaction of isoflavonoids (chemical constituent of the plant) with the GABA/benzodiazepine receptor complex in brain (16).

Elevated plus-maze test is used to evaluate psychomotor performance and emotional aspects of rodents (17). The results showed that EEPES significantly increased the time spent on the open arms and decreased the number of entries into

open and closed arms. This type of effect is observed with the drugs that act on GABA/benzodiazepine receptor complex as well with drugs that stimulate glucocorticoid production and release in the adrenal cortex (14), after administration of 5-HT_{1B} receptor antagonists and 5-HT_{1A} agonists (18). Therefore, with the present data, it is difficult to predict the precise mechanism for the anxiolytic activity of the *Pachyrrhizus erosus* seeds.

EEPES showed inhibition of aggressiveness in isolated mice. The serotonergic system is implicated in aggressive states and it has been hypothesized that decreasing serotonergic activity may encourage aggressive behavior (19). Since, both antianxiety and antiaggressive effects are seen with 5HT_{1A} antagonists, it is assumed that EEPES may also interact with the 5-HT_{1A} receptors.

To conclude, the ethanolic extract of seeds of *Pachyrrhizus erosus* possess sedative, anti-anxiety, muscle relaxant and anti-aggressive properties. The result of the present study substantiates the traditional use of seeds of *Pachyrrhizus erosus* for the treatment of insomnia.

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