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EVALUATION OF PSYCHOPHARMACOLOGICAL EFFECTS OF PETROLEUM ETHER EXTRACT OF *CUSCUTA REFLEXA* ROXB. STEM IN MICE.#

DILIPKUMAR PAL[1]*, CHINMAY PANDA, SAMIR SINHABABU, ARNAB DUTTA and SHILADITYA BHATTACHARYA[2]

[1] Division of Pharmaceutical Chemistry, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj–757 086, Orissa, India.
[2] Department of Pharmaceutical Technology, Jadavpur University, Kolkata–700 032, West Bengal, India

Abstract: The petroleum ether extract of *Cuscuta reflexa* Roxb. stem (PECR) was evaluated for its psychopharmacological activities in several experimental models using Swiss albino mice. The PECR was found to cause significant reduction in spontaneous activity and exploratory behavioral profiles. It also showed reduction in muscle relaxant activity by rotarod, 30° inclined screen tests and showed significant analgesic properties as well as potentiated remarkably the pentobarbitone sodium, diazepam and meprobamate – induced sleeping time. All these results were compared with respective controls for the evaluation of significance. The presence of steroids in the PECR might be responsible for psychopharmacological activities.

Keywords: *Cuscuta reflexa*, psychopharmacology, PECR, sleeping time, general behaviour, analgesic, experimental animals

Cuscuta reflexa Roxb. (Family: Convolvulaceae, *swarnalata* in bengali, *amarvel* in hindi) is a very long, twining, branched, golden yellow parasite. The plant is found throughout the greater part of India, sometimes completely covering bushes and trees. The plant is bitter, astringent, antihelmintic, purgative, useful in jaundice, pains in the muscles and the joints. The stems are specially useful in bilious disorders (1,2). *Cuscuta reflexa* stem on preliminary analysis is found to contain a large quantity of flavonoids (3,4). The methanolic extract of the plant has been found to possess antisteroidogenic activities in mice (5). It has been found that the various parts of the plant were used by tribes for the diseases like fits, melancholy and insanity. The present study was undertaken to evaluate various psychopharmacological activities of the petroleum ether extract of *Cuscuta reflexa* Roxb. stem (PECR) to substantiate the folklore claim.

EXPERIMENTAL

The stems of *C. reflexa* Roxb. were collected from the Panua, Bankura district region of West Bengal, India, and the taxonomic identification was made by the division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Calcutta. The voucher specimen has been preserved in our research laboratory (Ref No.: DPCR 1) for further reference. Shade–dried, powdered and sieved (40 mesh size) plant materials were Soxhlet extracted with petroleum ether (40–60°) and the excess solvent was removed completely by vaccum pressure and a yellowish green residue was obtained (yield 14.9% w/w with respect to the dry starting material) which was stored in a desiccator. On preliminary phytochemical analysis, the extract showed positive tests for the presence of steroids, which was confirmed by thin layer chromatography (TLC). For pharmacological studies, the petroleum ether extract of *C. reflexa* (PECR) was dissolved in arachis oil.

Animals and treatment

Adult albino mice of either sex (22 ± 2 gm; Swiss strain) were acclimatized to normal laboratory conditions for one week and given pellet diet (Hindustan Lever Ltd., India) and water *ad libitum*. The experiment was performed under the guidance of the Ethical Committee.

Toxicity Study

An acute toxicity study relating to the determination of the LD_{50} value was performed with

* Author for correspondence:
Telephone: +91–3244–243265; E–mail: drdilip2003@yahoo.co.in; Fax: + 91–6791–222238

different doses of the extract in albino mice as per the method described by Ghosh and Kulkarni (6,7).

Effect on sleeping time

The mice were divided into 4 groups, each group containing 10 mice. The animals of group I received a vehicle (arachis oil, 5 ml/kg); groups II, III, IV received PECR at low, medium and high dose (100 mg/kg, 130 mg/kg and 60 mg/kg, respectively). Vehicle and the extracts were injected intraperitoneally 30 min prior to the adminstration of pentobarbitone sodium (40 mg/kg, *i.p.*), diazepam (3 mg/kg, *i.p.*) and meprobamate (100 mg/kg, *i.p.*). The sleeping time was noted by recording the interval between the loss and regaining of righting reflex (8).

General behavioural tests (9,10).

Swiss albino mice were divided into 5 groups (10 in each group). The activities were recorded at a 30 min interval for the next 4 h, for the following parameters.This method gives the effect of the drugs on the instinctive hyperactivity seen in animals placed in new surroundings, explorative or oriental hyperactivity (11).

- Spontaneous motor activity. This was evaluated by placing a mouse into a photoactometer. The activities of the mice were recorded from the count. Locomotor activity was measured of all groups of normal mice before treatment with the vehicle and the processed extract. The number of counts on interruption of the light beam were recorded after 30, 60, 120, 150 and 180 min, respectively from the time of injection for 10 min (12).
- Awareness and alertness. This was done by placing a mouse in a bell jar. It usually shows a moderate degree or inquisitive behaviour.
- Touch response (13). It was noted that when the animal was touched with a forcep (or) pencil at various parts (i.e., on the side of the neck, on the abdomen and on the groin) (13).
- Pain response (13). This response was graded when a small artery clamp was attached to the base of tail (13).
- Righting reflex. A mice was placed gently on its back on an undulated surface made of white iron at 30°C. If the animal remains on its back for 30 sec, loss of the righting reflex are said to occur.
- Pinna reflex. It was tested by touching the center of the pinna with a hair of fine instruments. The withdrawal of pinna from the irritating hair was considered to be that response.
- Corneal reflex. A stiff hair touched to the cornea, causes the animals to close the eyelids.

Muscle relaxant activity

30° inclined screen test. Swiss albino mice (male) 15 min after injection of either arachis oil (5 ml/kg), diazepam (10 mg/kg) or PECR (100, 175 and 250 mg/kg) were left on the screen for at least 4 h to observe whether the paralysing effect was severe enough to cause the mice to slide off the screen (14). Groups of 10 mice were used for each group of control and experimental batches.

Rotarod test. Untreated fresh mice were placed on a horizontal wooden rod (32 mm in diameter), rotating at a speed of 5 rpm. The animals on the rod for 3 min or more in two successive trials were selected for the test and were divided into 5 groups of 10 animals each. The first three groups were injected (*i.p.*) with PECR at different doses (100, 175 and 250 mg/kg) while the forth and fifth groups of animals received arachis oil (5 ml/kg) and diazepam (10 mg/kg) respectively. The animals were then placed on the rod at intervals of 30, 60, 90, 120 and 150 min. If the animals failed more than once to remain on the rotating rod for 3 min, then it was considered to be positive (15).

Exploratory behaviour

Head dip test. The animals (adult female swiss mice) were divided into 5 groups of 10 for the test. Thirty min after *i.p.* injection of arachis oil (5 ml/kg), diazepam (10 mg/kg) or the PECR (100, 175 and 250 mg/kg), the mice were placed on a wooden board with 16 evenly spaced holes. The number of time they dipped their heads into the holes during each 3 min trial was counted (16).

Analgesic properties

Writhing test. This method involved *i.p.* injection of freshly prepared 1.2 % acetic acid. The number of abdominal constrictions in the following 10 min was noted. For this test, PECR was tested at 100, 175 and 250 mg/kg. Acetylsalicylic acid, paracetamol and morphine sulphate were used as reference standard at 68 mg/kg, 68 mg/kg and 1.15 mg/kg, respectively (17, 18, 19).

Statistical analysis. Results were expressed as the mean ± SEM. Significance was evaluated using Student's t test in all the experiments. The Chi–squared test was used to assess muscle relaxant activity (20). $p<0.05$ was considered significant.

RESULTS

On the basis of the toxicity studies, it was observed that the LD_{50} dose of PECR is 700 mg/kg, *i.p.*

The extract (PECR) at low, medium and high dose level lengthened significantly the sleeping time induced by standard sedatives in the order: sodium pentobarbitone 104.7 %, 223.8 %, 342.2 %; diazepam 115.3 %, 189.8 %, 279.6 %; meprobamate 117 %, 207.8 %, 285.8 % compared with vehicle control mice (Table 1).

The results obtained from general behavioral profiles are exhibited in Table 2 and Figure 1. It was noted that PECR depressed spontaneous motor activity studied in the photoactometer; affected pain and touch responses; altered righting, pinna and corneal reflexes at doses of 175 mg/kg and above; produced moderate depression in patterns concerned with alertness and awareness when compared to control (arachis oil, 5 ml/kg.). However, chlorpromazine hydrochloride (standard) produced a profound depression of these responses in comparison with PECR.

From the results of the rotarod test and 30° inclined screen test, it was noted that the PECR produced significant loss of motor coordination in animals. The PECR also produced a significant loss of muscle tone as evident from the 30° inclined screen test (Table 3).

Table 1. Effect of petroleum ether extract of *C. reflexa* stem (PECR) on sleeping time induced by standard sedatives in mice

Treatment	Sleeping time (min) induced by		
	Pentobarbitone (40 mg/kg, *i.p.*)	Meprobamate (100 mg/kg, *i.p.*)	Diazepam (3 mg/kg, *i.p.*)
Normal saline (5 ml/kg, *i.p.*)	36.0 ± 1.25	56.7 ± 2.10	68.2 ± 1.75
Vehicle (arachis oil) (5 ml/kg, *i.p.*)	40.3 ± 1.55	60.7 ± 1.94	74.0 ± 1.87
PECR (100 mg/kg, *i.p.*)	82.5 ± 2.19*	130.7 ± 2.85*	160.6 ± 3.10*
PECR (130 mg/kg, *i.p.*)	130.5 ± 2.98*	175.9 ±3.65*	227.8 ± 4.75*
PECR (160 mg/kg, *i.p.*)	178.2 ± 3.72*	230.4 ± 3.88*	285.5 ± 4.98*

Values are mean ± SEM, *p< 0.05 as compared with vehicle control (Student's 't' test), *i.p.*–intraperitoneal, n = 10.

Table 2. Effect of petroleum ether extract of *C. reflexa* stem (PECR) on behavioral profile in mice

Treatment	Awareness	Touch response	Pain response	Righting reflex	Pinna reflex	Corneal reflex
Normal saline (5 ml/kg, *i.p.*)	0	0	0	0	0	0
Vehicle (arachis oil) (5 ml/kg, *i.p.*)	0	0	0	0	+	0
CPZ (5 mg/kg, *i.p.*)	4+	4+	4+	4+	4+	4+
PECR (100 mg/kg, *i.p.*)	2+	2+	3+	2+	3+	2+
PECR (130 mg/kg, *i.p.*)	3+	3+	4+	3+	4+	3+
PECR (160 mg/kg, *i.p.*)	4+	4+	4+	4+	4+	4+

Key for scoring: 0= no effect (normal); + = slight depression; 2+ = moderate depression; 3+ = strong depression; 4+ = very strong depression; *p< 0.05 as compared with vehicle control (Student's 't' test), *i.p.*–intraperitoneal, n = 10.

On the basis of the results obtained from the head dip test, mice treated with low, moderate and high doses of PECR showed a significant decrease (26.2%, 44.2% and 47.7%, respectively) in head dip responses as compared to control (Table 3).

As can be seen in Figure 2, PECR significantly reduced the number of writhes and stretches induced in mice by acetic acid 1.2% solution, with a dose of 100 mg/kg, *i.p.*, the percentage of protection being

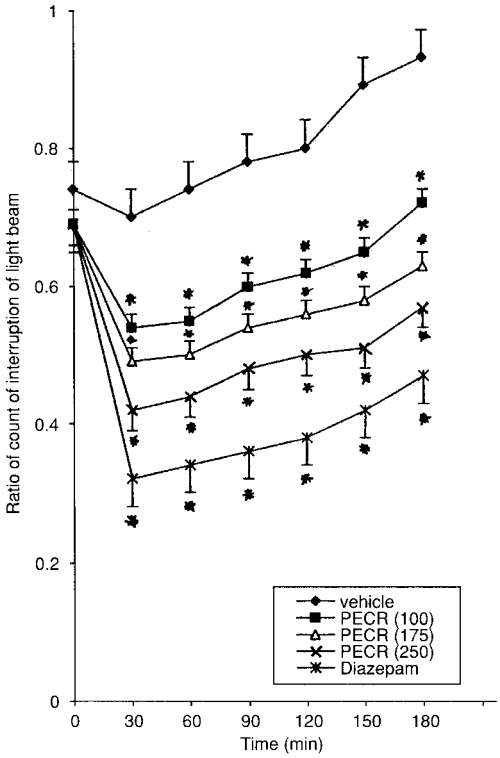

Figure 1. Effect of petroleum ether extract of *C. reflexa* (PECR) on spontaneous motor activity (photoactometer study) in mice. Values are mean ± SEM. *p<0.05 *vs* vehicle, n=10.

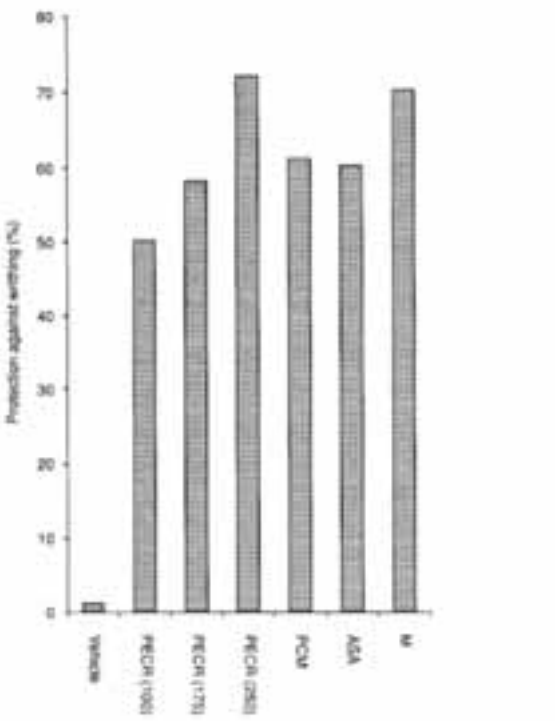

Figure 2. Influence of PECR (100, 175, 250 mg/kg), paracetamol (68 g/kg), acetylsalicylic acid (68 mg/kg) and morphine sulphate 1.15 mg/kg) on the writhing and stretching induced in mice by acetic acid 1.2% solution (writhing test). *p<0.05.

Table 3. Effect of petroleum ether extract of *C. reflexa* stem (PECR) on exploratory behaviour (head dip test) and muscle relaxant activity (30° inclined screen test and rotarod test) in mice

Treatment	Head dips •	30° inclined screen test φ	Rotarod test φ
Normal saline (5 ml/kg, *i.p.*)	27 ± 0.85	0	0
Vehicle (5 ml/kg, *i.p.*)	26 ± 1.92	0	0
Diazepam (10 mg/kg, *i.p.*)	8.7 ±1.10*	100*	100*
PECR (100 mg/kg, *i.p.*)	19.2 ±1.27*	43*	51.4*
PECR (130 mg/kg, *i.p.*)	14.5 ±1.57*	62*	66.0*
PECR (160 mg/kg, *i.p.*)	13.6 ± 1.62*	71*	74.5*

• Values are the number of head dips in 3 min. (mean ± SEM).
φ Values are the percentage animals showing a negative test. Statistical significance test for comparison of test with control was done using the „Chi–square test",
* p<0.05, n= 10.

50%. This dose dependent effect reached 72% with a dose of 250 mg/kg, *i.p.* Analgesic compound acetyl salicylic acid tested at 68 mg/kg, exerted a significant protective effect, inducing a protection of 60%. Morphine sulphate and paracetamol gave 70% and 61% protection, respectively.

DISCUSSION AND CONCLUSIONS

The prolongation of sleeping time by PECR may be due to dopamine receptor blockage or due to enhancement of GABAergic transmission. PECR was found to prolong diazepam induced sedation. Benzodiazepins are believed to act at specific binding sites which are closely linked to gamma–aminobutyric acid (GABA) receptors, the binding of benzodiazepines enhances GABA ergic transmission (21). Although the actual cause of prolongation of diazepam induced sleeping time is not known, the enhancement of GABAergic transmission might be related to its prolongation of sleeping time (22). PECR prolonged the pentobarbitone induced sleeping time, probably by tranquilizing action as well as CNS depressant action (9, 23). PECR also enhanced the sleeping time induced by meprobamate. Although the exact mechanism responsible for the enhancement is not clear, it may be due to CNS depressant action or due to enhancement of GABAergic transmission (23).

The experimental results indicate that the PECR influences general behavioral profiles, as evidenced in the spontaneous motor activity, awarness, alertness, touch and pain responses, righting reflex, pinna, corneal reflex. A dose graded reduction in the motor activity of the mice indicates sedation or CNS depressant action (24, 25). It has been found that there was an increase in the epinephrine, norepinephrine and dopamine in the extract treated mice (unpublished data). Therefore, the reduced locomotor activity might be due to the increase concentration of these catecholamines in mice brain (26). The reduction of pinna reflex may be due to stimulation of some part of sensory nerve or the spinal synapses or the efferent pathway (27). The possible CNS activity of the PECR was further tested against other common psychopharmacological tests (i.e., the rotarod test, 30° inclined test). The reduction in exploratory behaviour in animals treated with the petroleum ether extract is in keeping with the action of other CNS depressant agents (28).

The profound analgesia produced by PECR is probably mediated by inhibition of a post synaptic specific sensitive mechanisms either by depleting endogenous levels *via* dopamine –β–hydroxylase inhibition or by blocking its effects at the receptor level (21). It is found that PECR increases the brain serotonin level in mice (unpublished data). Therefore, the analgesia produced by PECR may be related to the increased brain serotonin level in mice. Phytochemical tests indicate the presence of steroids in PECR. Since various steroids have been reported to possess analgesic (29, 30, 31) and anxiolytic activities (32, 33), the psychopharmacological effects of PECR in mice might be due to the presence of such compounds.

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