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CNS activity of methanol extract of *Parthenium hysterophorus L.* in experimental animals

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ABSTRACT

The plant Parthenium hysterophorus l.was found causing toxicity in grazing animals. But the exact cause and nature of toxicity was vague till date in absence of any scientific investigation. The present study was carried out to evaluate the effect of methanol extract of parthenium hysterophorus l (PH) on psychological behavior of animals. The PH was administered orally in Swiss albino mice and the CNS effects were evaluated by general behavior, exploratory behavior, and phenobarbitone sodium induced sleeping time using standard procedures in experimental animal models.The results revealed that PH at 2.5 and 5 mg/kg caused a significant reduction in the spontaneous activity (general behavioural profile), exploratory behavioural pattern (Y maze and head dip test and significantly potentiated phenobarbitone sodium–induced sleeping time.The results conclude that the extract exhibit CNS depressant activity in tested animal models.

Keywords: parthenium hysterophorus l; muscle relaxant; phenobarbitone induced sleeping time; CNS depressant activity.

INTRODUCTION

Parthenium hysterophorus L. an aggressive and exotic weed of family Asteracea, at present has occupied almost all parts of India [1]. It is native to subtropics of North and South America [2] and was accidentally introduced in subcontinent in 1955 through imported food grains [3,4,5]. It is also known as congress weed, carrot weed, star weed, white top, chatak chandani, bitter weed, ramphool and gajar grass. Direct contact with plant and plant parts results in dermatities in mankind [6,7]. Presence of pollen in air causes diseases like hay fever, eczema, asthma and rhinitis in human [8,9,10,11,12,13]. In cattle the main problem due to parthenium intoxication are fever, rashes, ulcerations, necrosis in different parts of body etc. The impact of parthenium weed on livestock production is diverse affecting grazing land, animal health, milk

and meat quality and marketing of pasture seed and grains [14, 15, 16, 17]. The chemical analysis has indicated that all the plant parts including trichomes and pollens contain toxins called sesquiterpene lactones. The major components of toxin being 'Parthenin' and other phenolic acids such as caffeic acid, vanillic acid, anisic acid, chlorogenic acid, parahydroxy benzoic acid and p-anisic acid are lethal to human beings and animals [18, 19, 20]. In addition to health hazards a lot of available data also highlights its impact on agriculture as well as natural ecosystem [14,21]. Sesquiterpene lactones (SQLS) exhibit a wide spectrum of biological activities like cytotoxicity, antitumour, allergic, antimicrobial, antifeedant, phytotoxic, anticancers, hypoglycemic and other pharmacological activities [22].

MATERIALS AND METHODS

Plant material and extraction

The plant was collected from agriculture farms of Pune District, India in September 2010 and identified by botanical survey of India, Pune. The voucher specimen was deposited in herbarium of Pharmacognosy department JSPM'S Charak College of Pharmacy & Research, Wagholi, Pune, India. One kg of coarsely powdered plant material was successively extracted with three volumes of 60% methanol for 72 h at room temperature. The whole extract was collected in a 5 litre conical flask, filtered, and the solvent was evaporated to dryness under reduced pressure in rotary evaporator at 40-45ºC. The w/w yield of the prepared extract was 9.1% with respect to the dry powder. The preliminary phytochemical group tests of the plant extract were done by standard methods[23,24,25] for the presence of alkaloids, terpenoids, steroids, amino acids, flavonoids, gums, reducing sugars, tannins and saponins.

Animals

Swiss albino mice (20-25g) of either sex were used. They were obtained from the animal house, JSPM'S Charak College of Pharmacy & Research, Wagholi, Pune, India. The animals were housed in groups of 4 per cage (standard polypropylene cage) prior to pharmacological studies. The animals were provided with free access to standard diet and water ad libitum for at least 2 weeks on a 12/12 h light/dark cycle. All animals were fasted overnight before test while providing tap water ad libitum. The ambient temperature was 22±1ºC, except phenobarbitone sodium induced sleeping time experiments, which were carried out at 30±1ºC.All procedures described were reviewed and approved by the Institutional Animal EthicsCommittee (IAEC).

Chemicals

Chlorpromazine hydrochloride (Indus Pharmaceuticals Limited, India), diazepam (Lupin Laboratories Limited, India), phenobarbitone sodium (Rhone-Poulenc India Limited, India), pethidine (Ranbaxy Laboratories Limited, India), Diclofenac sodium. were procured and used in the study. All other chemicals of highest available purity were obtained from Merck, Mumbai, India.

Awareness, alertness and spontaneous activity

The awareness and alertness were recorded by visual measure of the animal's response when placed in different positions and its ability to orient itself without bumps or falls [26]. The normal behavior at resting position was scored as 0. Similarly little activity (+), moderate flexibility (++), strong response (+++) and abnormal restlessness (++++) were recorded. The

spontaneous activity of mice was recorded by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behavior. Less or moderate activity was scored as ++ and strong activity as +++. If there is slight or little motion, the score was + while the animal sleeps, the score was -. Excessive or very strong inquisitive activity like constant walking or running was scored as ++++.A similar test was performed with the same scoring, when the animal are removed from the jar and placed on a table [26,27].

Touch, pain and sound responses:
The touch response was recorded by touching the mice with a pencil or forceps at a various parts of the body (i.e. on the side of the neck, abdomen and groin).The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted. Albino mice normally utter no sound, so that vocalization may indicate noxious stimulus.

Analgesic activity:
Analgesic activity was studied by using tail immersion and tail-flick methods.

Tail immersion test:
Swiss albino mice of either sex were divided into four groups of 10 animals each. Saline solution (5ml/kg), extract at the doses of 2.5 and 5 mg/kg and pethidine (5mg/kg) were administered intraperitonially. The tail (up to 5cm) was then dipped into a pool of water maintained at 55±0.5ºC. The time in seconds to withdraw the tail out of water was taken as the reaction time. The reading was taken after 30 minutes of the administration of the test drug [28].

Tail flick test
Wistar rats of either sex weighing 150 - 180g were divided into 4 groups of 6 animals each. The tail of the rat was placed on the nichrome wire of an analgesiometer (Techno, Lucknow, India) and the time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. Extract at the doses of 2.5 and 5 mg/kg and pethidine (5mg/kg) were administered intraperitonially. Saline solution (5ml/kg) was served as a control. Analgesic activity was measured after 30 min of administration of test and standard drugs [28].

Sleeping time:
Mice were divided into 4 groups of 6 animals each. Animals received 40 mg/kg i.p. phenobarbitone sodium 30 min after the injection of Saline solution (5 ml/kg) and the extract at the doses of 2.5 and 5 mg/kg. The sleeping time was recorded, and measured as the time interval between the loss and remaining of the light reflux [28, 29].

Exploratory behavior
Exploratory behavior of the animals was evaluated using Y-maze and head dip tests.

Y-maze test
The test was performed in 4 groups of 6 albino wistar rats (weighing 150-180gm) at 30, 60, 90 and 120 min after injection of either Saline solution (5ml/kg), extract (2.5 and 5 mg/kg) and diazepam(10 mg/kg) respectively. The rats were placed invidually in a symmetrical Y-shaped runway (33 × 38 × 13cm) for 3 min and the number of times a rat entered in the arm of the maze with all 4ft (an ‘entry’) were counted [29,30].

Head dip test
Five groups of female albino mice (n=6) were placed on the top of a wooden box with 16evenly spaced holes, 30 min after injection of the extract (2.5 and 5 mg/kg, vehicle (5ml/kg, Saline solution) and diazepam (10mg/kg) respectively. The number of times that each animal dipped the head into the hole was counted for a period of 3 min [31].

Statistical analysis
The results were expressed as mean ± S.E.M. Statistical analysis of difference between groups was evaluated by ANOVA followed by Dunnett'sposthoc test. A p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of PH was performed by standard methods and the results indicated the presence of tannins, terpenoids, flavonoids, steroids and reducing sugars.

Effect on behavioral profiles
The results obtained from the experiments are presented in Table 1. The extract affected spontaneous activity, sound and touch responses at a dose of above 5 mg/kg and produced moderate or slight depression relating to awareness and alertness. However, the standard drug chlorpromazine hydrochloride caused significant depression of all these responses compared with methanol extract. The results indicate that the extract influences general behavioural profiles, as evidence in the spontaneous activity, touch, sound and pain responses.

Analgesic activity
The results of the analgesic activity by tail immersion and tail flick methods were presented in Table 2. In both the tests the reaction time was significantly increased in treated animals when compared to control. This indicates that the extract have shown significant analgesic activity compared with the control in a dose dependent manner. The activity may be due to its action on central nervous system similar to that of pethidine.

Exploratory behavior potentials
In the Y-maze test, the animals treated with the extract in tested doses have shown a marked decrease in exploratory behavior compared with controls (Table 3). In head dip test, there was a significant reduction in the head tip responses occurred in mice treated with the extract, compared with the control (Table 4).

Effect on phenobarbitone sodium-induced sleeping time
The extract significantly potentiated the phenobarbitone sodium-induced sleeping time at the doses studied, with respect to the control (Table 5). The potentiation of phenobarbitone sodium-induced sleeping time is possibly through a CNS depressant action [32] or a tranquilizing action [30].

Table 1 Effect of PH on general behavioural profiles in mice and rats (n=6)

Behaviour	PH (mg/kg)		Chlorpromazine (5 mg/kg)	Saline solution (5 ml/kg)
	2.5	5		
Spontaneous activity	+	++	++++	-
Alertness	+	+++	+++	-
Awareness	+	+++	+++	-
Sound response	+	++	++++	-
Touch response	++	+++	++++	-
Pain response	+	+	++++	-

Depression levels: -, no effect; +, slight; ++, moderate; +++, strong; ++++, very strong

Table 2 : Analgesic effect of PH on tail flick and tail immersion tests in mice and rats

Treatment	Dose	Tail flick test (Reaction time in sec)	Tail immersion test (Reaction time in sec)
Saline solution	5 ml/kg	2.26±0.14	2.22±0.17
Pethidine	5mg/kg	4.28±0.18	4.45±0.11
PH	2.5 mg/kg	2.68±0.17	2.68±0.04
PH	5 mg/kg	2.88±0.06	3.01±0.03

Values are mean ±S.E., n=6. All the data are signi ficant at P<0.001 vs. control, Students t-test.

Table 3: Effect of PH on exploratory behaviour (Y-maze test) in rats

Experiment	Dose	Number of entries after treatment (min)			
		30	60	90	120
Saline Solution	5ml/kg	9.2 ± 0.2	9.3 ± 0.2	9.3 ± 0.2	9.4 ± 0.2
Diazepam	10 mg/kg	3.0 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.3 ± 0.1
PH	2.5 mg/kg	6.4 ± 0.05	6.4 ± 0.05	6.6 ± 0.05	6.7 ±0.05
PH	5mg/kg	5.0 ± 0.2	5.0 ± 0.2	5.1 ± 0.2	5.1 ± 0.2

Values are mean ±S.E., n=6. P<0.001 compared with control

Table 4: Effect of PH on exploratory behavior (head dip test) in mice

Experimental group	Dose	No. of head dips
Saline solution	5 ml/kg	98 ± 1.0
Diazepam	10 mg/kg	35 ± 2.0***
PH	2.5mg/kg	76 ± 3.0*
PH	5 mg/kg	62 ± 2.0**

*Values are number of head dips in 3 min (mean ±S.E., n=6). *P < 0.05, **P<0.01 and ***P<0.001 compared with control*

Table 5: Effect of PH on phenobarbitone sodium-induced sleeping time

Experimental group	Dose	Sleeping time (min)
Saline	5 ml/kg	63 ± 0
PH	2.5 mg/kg	72 ± 2.0*
PH	5 mg/kg	85 ± 3.0*

Values are mean ±S.E., n=6. P<0.001 compared with control

CONCLUSION

The possible CNS activity of methanolic extract of parthenium hysterophorus l was investigated by common psychopharmacological tests. The reduction in exploratory behavior in animals is

similar with the action of other CNS depressant agents. The results altogether indicates that the extract shows CNS depressant activity.

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