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Central nervous system depressant activity of *Leucas aspera* root

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SUMMARY

The ethanolic extract of *Leucas aspera* root was studied for its effect on the central nervous system (CNS) using pentobarbitone induced sleeping time test, the open field test and the hole cross test in Swiss albino mice. The present investigation revealed that the extract at the doses of 250 and 500 mg/kg, significantly prolonged the pentobarbitone induced sleeping time in mice though the onset of sleep was delayed as compared to control. In open field test the depressing effect was prominent from the second observation period (30 min) and persisted throughout the entire experimental period (240 min). In the hole cross test, the depressing effect was observed significant from the third observation period (60 min) and persisted up to the seventh observation period (240 min) except at fourth observation (90 min) for 250 mg dose group and depressing effect was significant from second observation (30 min) up to seventh observation period (240 min) for 500 mg dose group. These results support the finding that *Leucas aspera* root may contain biologically active constituent(s) having CNS activity.

Key words: *Leucas aspera*; Central nervous system; Pentobarbitone induced sleeping time; Open field test; Hole cross test

INTRODUCTION

Leucas aspera Link (Family: Labiatae) (Darkolos or Dandokolos in Bangladesh) is a common aromatic herb of waste places grows in Dhaka, comilla, chittagong and chittagong hill tracts (Gani, 2003). Traditionally, the decoction of the whole plant is taken orally for analgesic-antipyretic, antirheumatic, anti-inflammatory and antibacterial treatment etc., and its paste is applied topically to inflammed areas (Gani, 1998). Leaves contain glucosides, tannins, saponins and sterols.oleic, linoleic, palmitic, stearic, oleanolic and ursolic acids have been isolated from this plant (Chaterjee and Majumder, 1969; Chowdhury and ghosh, 1969; Khaleque *et al.*, 1970; Badami and Palil, 1975). Chloroform and ether extracts possess antifungal activity (Thakur *et al.*, 1987). Eight lignans and four flavonoids, LA-1 - LA-12 have been isolated from whole aerial part and among which PG inhibition was observed in LA-1, LA-2 and LA-5 and antioxidant activity in LA-1 - LA-3 and LA-8 - LA-12 (Sadhu SK *et al.*, 2003). Particularly the root part is used traditionally for different ailments of human being in different parts of Bangladesh. There has been no scientific report on biological activity of this part of this plant. The present study was aimed to evaluate the effect of *Leucas aspera* root extract on the central nervous system on some neuro-pharmacological experimental models.

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MATERIALS AND METHODS

Plant material and extraction

Leucas aspera Link (Labiateae) were collected from Pabna district of Bangladesh during the month of February, 2005 and was identified by Botanist Professor Abdul Matin, Forestry Discipline, Khulna University, Khulna. The specimen sample was preserved in the Phytochemistry Laboratory (No. PL -77) of Khulna University. The root parts are carefully cut with the help of a scissor and separated from other parts. About 400 g of the root was dried for 15 days without the direct contact of sunrays. The dried root was finally ground and extracted by maceration over 20 days with 1,000 ml of 80% ethanol. The solution was filtered and solvent was evaporated under normal environment by an electric fan to get the dried extract (approx. yield 9.5%). Phytochemical investigation indicated the presence of alkaloid, reducing sugar, flavonoid and tannin. After that the extract was used for pharmacological screening.

Animals

Male and female mice (*Swiss-webstar* strain, 20 - 25 g body weight) bred in the animal house of the Department of Pharmacy, Khulna University, were collected from animal resources branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDRB) and used for the experiments. The animals were housed under standard laboratory conditions (relative humidity 55 - 65%, room temperature 23.0 ± 2.0°C and 12 h light: dark cycle).The animals were provided with standard laboratory food and tap water *ad libitum*. The animals were divided in-groups of 6, with each group balanced for sex and body weight. All the experiments were conducted on every evening in an isolated and noiseless condition.

Acute toxicity test

Test animals were divided into different groups containing six animals in each. The groups received *Leucas aspera* root extract orally at the doses of 62.5, 125, 250, 500, 1,000, 2,000 and 4,000 mg/kg body weight whereas the control group received distilled water. General signs and symptoms of toxicity and mortality were recorded for 24 h (Lork, 1983).

Hypnotic action of pentobarbital

Pentobarbital sleeping time test was carried out by the method of (Williamson *et al*., 1996). The animals were randomly divided into four groups containing five mice each. The test group received *Leucas aspera* root extract at the doses of 250 and 500 mg/kg body weight while positive control was treated with diazepam (1 mg/kg i.p.) and control with vehicle (1% Tween 80 in water). Thirty minutes later, pentobarbitone (50 mg/kg i.p.) was administered to each mouse to induce sleep. The animals were observed for latent period (time between pentobarbitone administration to onset of sleep) and duration of sleep (time between the loss of rithing reflex to recovery of rithing reflex).The test drugs were administered per oral 30 min before the administration of pentobarbital. The animals were observed for the onset and the duration of sleep, as evidenced by the observation of the loss of writhing reflex.

Open field test

This experiment was carried out as described by (Gupta *et al*., 1971).The animals were divided into control and test groups containing five mice each. The test group received *Leucas aspera* root extract at the doses of 250 and 500 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water).The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, 120, 180, and 240 min after oral administration of the test drugs.

Hole cross test

The method was adopted as described by (Takagi *et al*., 1971). A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90, 120, 180 and 240 min after oral administration of the test drugs.

Statistical analysis

All analyses were done using the SPSS 7.5 for windows. Experimental values were expressed as Mean ± S.E.M. (Standard Error of Mean). Independent samples *t*-test was done for statistical comparison. Statistical significance was considered to be indicated by a *P* value of less than 0.05 in all cases.

RESULTS

Intact animals are considered to be the best method for investigating the action of drugs on CNS. In the acute toxicity test the animal exhibited decreased mobility but no convulsions or loss of rithing reflex and at the highest dose tested, 4,000 mg/kg, no mortality was observed in the test animals.

In the pentobarbitone induced sleeping time test, *L. aspera* root extract, at doses of 250 and 500 mg / kg, was found to induce sleep at a delayed stage as compared to control but increased the duration of sleep. This effect was observed to follow a dose dependant manner and the results were statistically significant (Table 1).

In the open field test, from the second observation period (30 min) the result showed a prominent decrease in locomotion in the test animals of both dose groups (250 and 500 mg/kg) and it was continued till the seventh observation (240 min). With the passage of time, the depressing effect was more intense and persisted throughout the entire observation period with little variation. The effect was dose dependent and results were statistically significant (Table 2).

In the hole cross test, a depressing effect was observed in the test animals beginning from the third observation period (60 min) for dose group 250 mg/kg and from second observation (30 min) for dose group 500 mg/kg, which increase with the passage of time. Maximum depression occurred during the third (60 min), fifth (120 min) and seventh (240 min) observation periods for dose group 500 mg/kg but for dose group 250 mg/kg these are third (60 min) and fifth (120 min) periods of observation. The results were statistically

Table 1. Results of different group tests of *Leucas aspera* root

Root Extract	Alkaloids	Reducing Sugars	Taninns	Gums	Flavonoids	Saponins	Steroids
Ethanolic extract of *Leucas aspera* root	+	+	+	-	+	-	-

+ : Positive; - : Negative

Table 2. Effect of *Leucas aspera* root on the hypnotic action of pentobarbital

Treatment	Dose (mg/kg)	Route of dministration	Onset of sleep (min)	Duration of sleep(min)
Control (1% tween 80 in water)	10 ml/kg	p.o.	5.82 ± 0.43	70.0 ± 2.89
Diazepam	1	i.p.	4.15 ± 0.38[c]	89.14 ± 2.14[a]
L. aspera	250	p.o.	6.84 ± 0.28	81.54 ± 1.52[b]
L. aspera	500	p.o.	7.01 ± 0.38	105.50 ± 2.84[a]

Values are mean ± S.E.M. (n = 6), n = number of mice. [a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$ vs control, Students *t*-test

Table 3. Effect of *Leucas aspera* root on open field test

Treatment	Dose (mg/kg, p.o.)	Number of movement						
		0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control (vehicle, 10ml/kg)		105 ± 3.54	91 ± 2.04	94 ± 2.55	90 ± 2.82	84.75 ± 2.39	70 ± 3.70	65 ± 3.32
L. aspera	250	104 ± 4.02	78.12 ± 2.31[b]	55.19 ± 2.17[a]	45.19 ± 2.73[a]	42.17 ± 2.65[a]	30.55 ± 3.17[a]	28.33 ± 3.30[a]
L. aspera	500	96.04 ± 3.12	70.13 ± 2.86[a]	42.05 ± 2.10[a]	30.16 ± 2.05[a]	32.14 ± 2.30[a]	26.10 ± 3.09[a]	25.16 ± 3.39[a]

Values are mean ± S.E.M. (n = 6), n = number of mice. [a]P< 0.001, [b]P< 0.01 vs control, Students *t*-test

Table 4. Effect of *Leucas aspera* root on Hole cross test

Treatment	Dose (mg/kg, p.o.)	Number of movement						
		0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control (vehicle, 10ml/kg)		8.50 ± 0.62	8.25 ± 0.75	10.50 ± 0.29	8.50 ± 0.65	7.21 ± 0.61	6.75 ± 0.48	6.01 ± 0.33
L. aspera	250	9.60 ± 1.21	8.13 ± 0.70	6.14 ± 0.45[a]	6.03 ± 0.73	3.10 ± 0.19[a]	4.12 ± 0.52[b]	3.01 ± 0.12[a]
L. aspera	500	8.25 ± 0.78	6.05 ± 0.83[c]	4.50 ± 0.65[a]	4.05 ± 0.59[a]	2.25 ± 0.63[a]	2.15 ± 0.24[a]	1.25 ± 0.48[a]

Values are mean ± S.E.M. (n = 6), n = number of mice. [a]P< 0.001, [b]P< 0.01, [c]P< 0.05 vs control, Students *t*-test.

significant up to seventh observation period (240 min) for both dose groups (Table 3).

DISCUSSION

A most important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. inhibitory effects on spontaneous motor activity of the ethanol extract indicated depressant activity.

A number of methods have been developed to evaluate the effect of substances on the CNS. In the present study *L. aspera* root extract was investigated for its possible effect on the CNS via a number of methods, including pentobarbitone induced sleeping time test, the open field test and the hole cross test. In the pentobarbitone induced sleeping time test the extract exhibited significant potentiation of sleeping time, indicating a depressing effect on the CNS. Pentobarbitone is a barbiturate type of hypnotic agent. When given at appropriate dose, it induces sedation or hypnosis in animal by potentiating the GABA mediated post synaptic inhibition through an allosteric modification of GABA receptors (Goodman and Gilman, 2001). Typically, substances that have CNS depressant activity either decrease the time to onset of sleep or prolong the duration of sleep or both. Diazepam in this test used as a positive control, belongs to the benzodiazepine group of anxiolytic and hypnotic agents. In the present study it both decreased the latent period for onset of sleep as well as increased the duration of pentobarbitone induced sleeping time. While the *Leucas aspera* root extract significantly increased the duration of sleeping time in test animals it paradoxically increased the latent period for onset of sleep. Increasing the total sleeping time indicates that the extract may have a depressing effect on the CNS. But the reason is not clear why the extract increased the time for onset of sleep as compared to the control. The extract was also studied to verify its effect on the CNS by the open field test and the hole cross test that demonstrate psychological effects of the test substances on the CNS. The extract demonstrated a decrease in locomotor activity in test animals in both the open field test and the hole cross test. The mechanism of this depression is not clearly understood, the drug may exert central depressant effect by interfering with the functions of the cortex.

Further Studies to determining underlying mechanism of action and to isolate the active principle(s) responsible for such activity are also needed.

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