

Central nervous system depressant activity of *Diospyros peregrina* bark

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SUMMARY

The methanol extract of *Diospyros peregrina* bark was studied for its effect on the central nervous system (CNS) using the 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 the 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 from the second observation period (30 min) and persisted up to fifth observation period (120 min) for 250 mg dose group and up to sixth observation period (180 min) for 500 mg dose group. These results support the finding that *D. peregrina* bark extract at the above doses has CNS depressing effects and indicate that *D. peregrina* bark may contain biologically active constituent(s) having CNS depressant activity.

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

INTRODUCTION

Diospyros peregrina Gruke. (Ebenaceae), a middle sized evergreen tree grows throughout Bangladesh. The bark and seeds of the plant are used to treat dysentery, diarrhoea and intermittent fever (Yusuf *et al.*, 1994 Kiritikar and Basu, 1999). A paste made from the bark is applied to boils and tumors. Flowers and fruits are given for hiccups in children. The calyx and peduncle of the fruit are used in the treatment of coughs and dyspnea. Infusion of the fruit is used as gargle in aphthae and sore throat. Fruit juice is used as an application for wounds and ulcers (Kiritikar and

Basu, 1999; Joshi, 2000).

During the pharmacological screening of *D. peregrina* bark extract, the test animals exhibited a marked depression. So, the present study was conducted to evaluate the effect of *D. peregrina* bark extract on the central nervous system.

MATERIALS AND METHODS

Plant material and extraction

The bark of *D. peregrina* was collected from southern Khalishpur, Khulna, Bangladesh in July 2003 and was identified by the experts of Bangladesh National Herbarium where a voucher specimen has been submitted for future reference (Voucher specimen no. DACB: 30323). The dried bark was ground into coarse powder by a hammer mill. The dried powdered plant part was subjected to maceration by methanol at room temperature

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overnight. The solution was filtered and the solvent was evaporated using a rotary evaporator (approx. yield 16%). Phytochemical investigation indicated the presence of sterols, flavonoids, tannins and saponins (Harborne, 1984).

Animals

Swiss albino mice (22-25 g) of either sex obtained from the Animal Resources Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature $23.0 \pm 2.0^{\circ}\text{C}$ and 12 h light: dark cycle). The animals were fed with standard diet and water *ad libitum*.

Acute toxicity test

Test animals were divided into different groups containing six animals in each. The groups received *D. peregrina* bark 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 24hr (Lorke, 1983).

Pentobarbitone induced sleeping time test

The animals were randomly divided into four groups containing five mice each. The test groups received *D. peregrina* bark 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 the latent period (time between pentobarbitone administration to onset of sleep) and duration of sleep (time between the loss of righting reflex to recovery of righting reflex) (Williamson *et al.*, 1996).

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 groups received *D. peregrina* bark 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 3min at 0, 30, 60, 90, 120, 180 and 240 min during the study period.

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 passages 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 during the study period.

RESULTS

In the acute toxicity test, the animals exhibited decreased mobility but no convulsions or loss of righting 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, *D. peregrina* bark extract, at the 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 2nd observation period (30 min) the results showed a noticeable

decrease in locomotion in the test animals of both dose groups (250 and 500 mg/kg). 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 dependant and the results were statistically significant (Table 2).

In the hole cross test, a depressing effect was

observed in the test animals beginning in the 2nd observation period (30min), which increased with the passage of time. Maximal depression occurred during the 3rd (60 min) and 4th (90 min) observation periods. The results were statistically significant up to 5th observation period (120 min) for 250 mg dose group and the 6th observation period (180 min) for 500 mg dose group (Table 3).

Table 1. Effect of *D. peregrina* bark extract on pentobarbitone induced sleeping time in mice

Treatment	Dose (mg/kg)	Route of administration	Onset of sleep (min)	Duration of sleep (min)
Control (1% tween 80 in water)	10 ml/kg	p.o.	5.52 ± 0.53	65.14 ± 2.63
Diazepam	1	i.p.	4.08 ± 0.38 ^c	83.14 ± 2.14 ^a
<i>D. peregrina</i>	250	p.o.	6.92 ± 0.33 ^c	77.4 ± 1.61 ^a
	500	p.o.	7.82 ± 0.35 ^b	86.52 ± 2.95 ^a

Values are mean ± SEM (n = 5).

^aP < 0.001, ^bP < 0.01, ^cP < 0.05 vs control, students *t*-test.

Table 2. Effect of *D. peregrina* bark extract in open field test

Treatment	Dose (mg/kg, p.o.)	Number of movements						
		0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control (vehicle, 10ml/kg)		113.6 ± 7.28	58.8 ± 4.27	45.6 ± 3.04	40.8 ± 2.82	49.6 ± 3.84	39.2 ± 4.63	43 ± 3.32
<i>D. peregrina</i>	250	116.8 ± 7.07	34.6 ± 2.66 ^b	23.4 ± 3.14 ^a	19.2 ± 2.24 ^a	24.2 ± 2.50 ^a	21.8 ± 2.89 ^c	27.8 ± 3.64 ^c
	500	108 ± 8.72	36.2 ± 2.91 ^b	17.2 ± 2.01 ^a	15.8 ± 2.27 ^a	17.4 ± 2.11 ^a	22.2 ± 2.75 ^c	19.6 ± 2.27 ^b

Values are mean ± SEM (n = 5).

^aP < 0.001, ^bP < 0.01, ^cP < 0.05 vs control, students *t*-test.

Table 3. Effect of *D. peregrina* bark extract in hole cross test

Treatment	Dose (mg/kg, p.o.)	Number of movements						
		0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control (vehicle, 10ml/kg)		10.2 ± 0.80	7.6 ± 1.03	7 ± 0.71	6.4 ± 0.68	5 ± 0.71	5.8 ± 1.11	5 ± 0.71
<i>D. peregrina</i>	250	10.6 ± 1.08	5 ± 0.71 ^c	2.4 ± 0.93 ^a	2.2 ± 0.86 ^a	2.8 ± 0.58 ^c	4 ± 0.45	3.8 ± 0.58
	500	9.6 ± 1.21	4.6 ± 0.51 ^c	1.6 ± 0.51 ^b	1.8 ± 0.58 ^a	2.6 ± 0.75 ^c	2.6 ± 0.81 ^c	3.2 ± 0.86

Values are mean ± SEM (n=5).

^aP < 0.001, ^bP < 0.01, ^cP < 0.05 vs control, students *t*-test.

DISCUSSION

Drugs that act upon the central nervous system (CNS) can produce specific physiological and psychological effects. Agents affecting the function of the CNS can be useful in the treatment of disorders related to the CNS. Therefore a number of methods have been developed to evaluate the effects of substances on the CNS. In the present study *D. peregrina* bark extract was investigated for its possible effect on the CNS via a number of methods, including the 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 animals 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, used as the positive control in this test, 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 *D. peregrina* bark 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, tests that demonstrate psychological effects of 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. All of these results support the finding that *D. peregrina* bark extract possesses CNS depressing activity.

Further studies are needed to determine if the extract produces CNS depression alone or whether the CNS depression is associated with any other psychoactive property. Studies to determine underlying mechanism of action and to isolate the active principle(s) responsible for such activity are also needed.

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