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Antinociceptive and sedative effects of the bark of *Cerbera odollam* Gaertn.

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SUMMARY

The crude methanolic extract of the bark of *Cerbera odollam* Gaertn. was evaluated for its possible antinociceptive and neuropharmacological activities in animal models. At the dose of 250 and 500 mg/kg body weight, the extract showed a significant antinociceptive effect in acetic acid induced writhing in mice comparable to that produced by aspirin, used as standard drug ($P < 0.001$). The extract significantly reduced the time of onset of sleep ($P < 0.01$) and potentiated the pentobarbital induced sleeping time in mice at the dose of 400 mg/kg of body weight significantly ($P < 0.001$). It also decreased the open field score in open field test significantly at the dose of 400 mg/kg of body weight ($P < 0.05$). The obtained results tend to suggest the probable antinociceptive and neuropharmacological activities of the crude extract.

Key words: *Cerbera odollam*; Antinociceptive activity; CNS depressant activity

INTRODUCTION

Cerbera odollam Gaertn. (Family: Apocynaceae) locally known as 'Dabur', 'Dhakur', is a small tree or large shrub distributed widely throughout Bangladesh, India, Malaysia, China, Australia and Philippine mostly on the sea coast. The bark and leaves of the plant are traditionally used as emetic and cathartic; kernels are used as emetic; fruit is used as a cure for hydrophobia (Kirtikar and Basu, 1987). Its bark and fruits are purgative and used for the treatment of rheumatism (Rollet, 1981). A number of research works have been performed to evaluate its biological activities as cytotoxic activity (Laphookhieo *et al.*, 2004), effect on central nervous system (Hien *et al.*, 1991), purgative and anti-rheumatic activity (Yamauchi *et al.*, 1987), cardiac stimulant activity (Chen *et al.*, 1987), neurological manifestations (Iyer *et al.*, 1975), cardiotoxic activity (Kini *et al.*, 1965), etc. The main objective of this study was to evaluate the antinociceptive and neurophamacological activities of the methanolic extract of bark of *Cerbera odollam* (*C. odollam*).

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MATERIALS AND METHODS

Plant material collection and extraction

The plants were collected in February 2003 from Sundarbans Mangrove forest of Bangladesh in July 2003 and were identified in the National Herbarium of Bangladesh (Accession no.: 29788). The bark of *C. odollam* was pulverized into fine powder. About 600 g of powered material was taken in a clean, flat-bottomed glass container and soaked in 1.8 liters of 90% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatmann

filter paper. The filtrate thus obtained was concentrated by using a rotary evaporator.

Tests for different chemical groups

The crude methanolic extract was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins (Evans, 1989). In each test 10% (w/v) solution of the extract in methanol was taken unless otherwise mentioned in individual test.

Animals

Young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B) were used for the studies. The animals were kept at animal house of Pharmacy Discipline, Khulna University, for adaptation under standard environmental condition and fed with standard diets (ICDDR, B formulated).

Drugs

Aspirin (Square Pharmaceuticals Ltd, Bangladesh), pentobarbital sodium (Sigma Chemicals, USA).

Pharmacological studies

Antinociceptive activity

The antinociceptive activity of the crude methanolic extract of *C. odollam* was studied using acetic acid induced writhing model in mice (Whitle *et al.*, 1964; Ahmed *et al.*, 2004). The animals were divided into control, positive control and test groups with five mice in each group. The animals of test groups received test substance at the dose of 250 and 500 mg/kg body weight. Positive control group was administered with aspirin (standard drug) at the dose of 25 mg/kg of body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10 ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed for specific contraction of body referred to as 'writhing' for 5 min.

Neuropharmacological activity

Pentobarbital induced hypnosis

Pentobarbital induced sleeping time test was carried out by the method of Williamson *et al.* (1996). The test animals were divided into three groups consisting of five mice in each. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the methanolic extract of *C. odollam* at the doses of 200 and 400 mg/kg body weight intra-peritoneally (i.p.), while the animals of group I (control) were administered with distilled water containing 0.1% (v/v) tween - 80 (i.p.) at the dose of 10 ml/kg of body weight. The time for onset of sleep was the time taken for the loss of righting reflex whereas total sleeping time represents the time between the loss and regain of righting reflex. The onset of sleep and total sleeping time were recorded for both control as well as for treated groups (at the dose of 200 and 400 mg/kg body weight).

Exploratory behavior

This experiment was performed by Open field test (Gupta *et al.*, 1971). The test animals were divided into three groups consisting of five mice in each. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the methanolic extract of *C. odollam* at the dose of 200 and 400 mg per kg body weight intraperitoneally (i.p.), while the animals of group I (control) were administered with 0.1% (v/v) tween - 80 (i.p.) at the dose of 10 ml per kg of body weight. The floor of an open field of half square meter was divided in to a series of squares, each alternatively colored black and white. The apparatus had a wall of 40 cm. The number of squares, traveled by the animal, was recorded for a period of 2 min.

Statistical analysis

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

RESULTS

Chemical group test

Results of different chemical tests on the methanolic extract of *C. odollam* showed the presence of steroids, flavonoids, reducing sugars, gums, saponins and tannins (Table 1).

Antinociceptive activity

Table 2 showed the effect of the methanolic extract of *C. odollam* on acetic acid induced writhing in mice. At the dose of 250 and 500 mg/kg of body weight, the extract produced 48.89% and 68.89% writhing inhibition in test animals, respectively. The results were statistically significant ($P < 0.001$) and were comparable to the standard drug aspirin, which showed 67.78% writhing inhibition at the dose of 25 mg/kg.

Neuropharmacological activity

Pentobarbital induced hypnosis

Table 3 showed the effects of methanolic extract of *C. odollam* on pentobarbital induced sleeping time. The extract decreased significantly the time of onset of sleep at the dose of 400 mg/kg body weight ($P < 0.01$); in control group the mean time of onset of sleep was 7.18 min where as in case of experimental group it was 6.72 and 6.15 min at the dose of 200 and 400 mg/kg respectively. The extract also potentiated the pentobarbital induced sleeping time significantly at the dose 400 mg/kg body weight ($P < 0.001$). The average duration of sleep was about 48 min and 74 min at the dose of 200 and 400 mg/kg respectively where as in control group it was about 43 min.

Table 1. Results of different group tests of *C. odollam*

Plant Extract	Alkaloid	Reducing Sugars	Tannins	Gums	Flavonoids	Saponin	Steroid
ME of *C. odollam*	-	+	+	+	+	+	+

ME: Methanolic extract; +: Positive result; - : Negative result

Table 2. Effect of methanolic extract of *C. odollam* on acetic acid induced writhing in mice (n = 5)

Animal Group/Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 solution 10 ml/kg, p.o.	18 ± 1.000 (100)	-
Positive control Aspirin 25 mg/kg, p.o.	5.8 ± 0.862* (32.22)	67.78
Test group-1 Me. extract 250mg/kg, p.o.	9.2 ± 0.754* (51.11)	48.89
Test group-2 Me. extract 500 mg/kg, p.o.	5.6 ± 0.509* (31.11)	68.89

Values are expressed as mean ± SEM; Me.: Methanolic * indicates $P < 0.001$ vs. control; n: Number of mice; p.o.: per oral.

Table 3. Effect of *C. odollam* on pentobarbital induced hypnosis (n = 5)

Animal group	Treatment (i.p.)	Time of onset of sleep (min)	Total sleeping time (min)
I (Control)	0.1% Tween 80 solution 10 ml/kg	7.176 ± 0.101	43.42 ± 0.873
II (Test group-I)	Me. Extract (200 mg/kg.)	6.722 ± 0.191	47.70 ± 0.935*
III (Test group-II)	Me. Extract (400 mg/kg)	6.148 ± .236**	73.66 ± 0.933***

Values are expressed as mean ± S.E.M; *** indicates $P < 0.001$, ** indicates $P < 0.01$, * indicates $P < 0.02$ vs. control; Me. = Methanol; n = number of mice; i.p.: intraperitoneal.

Table 4. Effect of *C. odollam* on exploratory behavior in mice (Open field test) (n = 5)

Group	Response at				
	0 min	30 min	60 min	120 min	240 min
I (Control)	127.13 ± 5.3	105.3 ± 6.6	71.25 ± 6.6	45.63 ± 2.1	35.87 ± 1.4
II (Me. Ext.) 200 mg/kg	130.0 ± 7.5	97.65 ± 7.5	78.65 ± 5.50	49.25 ± 4.10	42.40 ± 2.85
III (Me. Ext.) 400 mg/kg	112.64 ± 5.0	81.20 ± 4.2**	50.34 ± 3.1*	35.64 ± 2.0**	32.70 ± 1.50

Values are expressed as mean ± S.E.M.; Me.: methanolic; **indicates $P < 0.02$; *indicates $P < 0.05$vs. control; n: number of mice.

Exploratory behavior

It was observed that the extract caused a significant decrease in the open field score (Table 4) in mice at the dose of 400 mg/kg of body weight at 30 to 120 min ($P < 0.05$).

DISCUSSION

The *odollam* tree is responsible for about 50% of plant poisoning cases and 10% of all poisoning cases in Kerala. Thus, it was called "suicide plant". But the same species of Bangladesh is not too much poisonous, and even the local people use the fleshy portion of the fruit as food. Its bark is not poisonous (LD_{50}: 750 mg/kg body weight of the albino mice).

Since *C. odollam* belongs to the coastal forests, part of the plant constituents may be polar in nature. Methanol was used which has a wide range of solubility in both polar and non-polar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness.

Antinociceptive activity of the methanolic extract of *C. odollam* bark was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taesotikul *et al.*, 2003). Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt *et al.*, 1980). On the basis of the result of acetic acid induced writhing test, it can be concluded that the methanolic extract of *C. odollam* might possess an antinociceptive activity.

Central depressants elicit their effect by interfering with the functions of the cerebral cortex. A most important method of investigating the probable cortical manifestation of a drug is to check its effect on the pentobarbital narcosis as pentobarbital has multifarious effects on the cerebral cortex (Bowman and Rand, 1980). The pentobarbital sleeping time test was performed to find out whether the water extract of the plants have any effect on the cerebral cortex. Pentobarbital shorten the onset of sleep and increases sleep duration. The methanolic extract of *C. odollam* reduced the onset of sleep and potentiated the pentobarbital induced sleeping time in mice, which suggests its central depressant activity (Perez *et al.*, 1998), thus suggesting the probable tranquilizing action (Capasso *et al.*, 1996).

It has been experimentally proven that, in the absence of a special task to perform, the behavior of a given animal tend to maintain that inner activation level that is, at times, inconsistent with the actual level of activation of the animals. In order to get as accurate a picture as possible, on the effect of the drug on exploration, the open field test was performed. The extract also made mice to reduce their behavioral exploration, which further support the central sedative properties of the

extract. The overall results tend to predict the central nervous system depressant action of the extract.

In conclusion, it can be suggested that the crude extract of *Cerbera odollam* may possess anti-nociceptive and CNS depressant effects. However, further researches are necessary to find out the active principles responsible for these activities.

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