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Anti-inflammatory, antinociceptive and diuretic activities of *Amoora cucullata* Roxb.

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SUMMARY

The crude methanolic extract of the leaves of *Amoora cucullata* Roxb. was investigated for its possible anti-inflammatory activity using carrageenin induced rat paw edema model and cotton pellet implantation method in rat. The extract was also studied for its antinociceptive activity using acetic acid induced writhing model in mice. At the doses of 200 and 400 mg/kg body weight, the extract showed significant anti-inflammatory activity in both models. At the same doses, the extract also significantly reduced the number of acetic acid-induced abdominal constriction (writhing) in mice. The crude extract also showed significant diuretic activity in albino mice.

Key words: Amoora cucullata; Anti-inflammatory activity; Anti-nociceptive activity; Diuretic activity

INTRODUCTION

Amoora cucullata Roxb., syn.: *Aglaia cucullata*, (Roxb.) Pellegr., (Meliaceae) is a long tree mostly grown in coastal forests of Bengal, Burma, Malay peninsula, Andamans and Borneo. In Bangladesh, it grows in the Sundarbans mangrove forest located in the southern region of the district of Khulna. Locally it is known as 'Amur', 'Latmi' and 'Natmi'. In the local traditional medicinal practice, the leaves are used in the treatment of inflammation (Shahid, 2003). The juice of the leaves is antibacterial and extensively used for the treatment of dysentery, skin diseases and in cardiac diseases (Kirtikar and Basu, 1999). In view of this and evidence from the existing information show that this plant may possess some important biological activities. The present study was carried out to evaluate the anti-inflammatory, antinociceptive and diuretic activities of the methanolic extract of the leaves of *Amoora cucullata* (*A. cucullata*).

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MATERIALS AND METHODS

Plant material and extraction

The leaves of *A. cucullata* were collected in January 2003 from the Sundarbans Mangrove Forest, Khulna, Bangladesh and were identified at Forestry Discipline, Khulna University. The dried leaves of *A. cucullata* were pulverized into a fine powder. The extracts of approximately 400 g of powdered material was obtained by soxhlet apparatus with 90% aqueous methanol at 55°C. The extract was filtered and evaporated (approximate yield 14%) using vacuum rotary evaporator.

Animals

Swiss-albino mice of either sex, weighing 22-25 g, bred in the animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh were used for antinociceptive and diuretic activity test. Wistar rats of either sex, weighing 180-200 g, were purchased from the Animal Resources Branch of the International Center for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) and were used for anti-inflammatory activity tests. All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2.0°C and 12 hours light: dark cycle). The animals were fed with standard diet (ICDDR, B formulated) and had free access to tap water.

Drugs

Carrageenin (Sigma Chemicals, USA), Aspirin (Square Pharmaceuticals Ltd, Bangladesh), Furosemide (Square Pharmaceuticals Ltd, Bangladesh).

Pharmacology

Anti-inflammatory activity

Carrageenin-induced hind paw edema in rats

Anti-inflammatory activity of *A. cucullata* was tested using the carrageenin-induced rat paw edema model as described by Winter *et al.* (1962). Experimental animals (Wistar rats) were randomly divided into four groups with six animals in each group. Control group received vehicle (1% Tween 80 in water) at the dose of 10 ml/kg body weight. Positive control group received aspirin (standard drug) at the dose of 150 mg/kg and the test groups were treated with *A. cucullata* extract at the doses of 200 and 400 mg/kg. The drugs were administered orally 1h prior to the injection of 0.1 ml of 1% freshly prepared suspension of carrageenin into the left hind paw of each rat. The paw volume was measured by using a plethysmometer (Ugo Basile 7140, Italy) every hour for 5 hours after the carrageenin injection.

Cotton pellet implantation

Wistar rats were anesthetized and 10 mg of the sterile cotton pellets were inserted in each axilla of rats. *A. cucullata* extracts (200 and 400 mg/kg), aspirin (150 mg/kg), and control vehicle (1% Tween 80 in water, 10 ml/kg) were administered orally for seven consecutive days starting from the day of cotton pellet implantation. The animals were anesthetized again on the 8th day and cotton pellets were removed surgically, freed from extraneous tissue. These pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight (D'Arcy *et al.*, 1960).

Antinociceptive activity

The antinociceptive activity was studied using acetic acid induced writhing model in mice (Koster *et al.*, 1959). The animals were divided into control, positive control and test groups with ten mice in each group. The animals of test groups received test substance at the doses of 200 and 400 mg/kg body weight. Positive control group was administered aspirin (standard drug) at the dose of 100 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 orally 45 min before intraperitoneal administration of 0.7% acetic acid. After an interval of five minutes, the mice were observed for specific contraction of body referred as 'writhing' for 15 min.

Diuretic activity

Diuretic activity of the extract was investigated using the method as described by Lipschitz *et al.* (1943). The test animals were randomly chosen and divided into five groups having ten mice in each. Twenty-four hours prior to the experiment, the test animals were placed in to metabolic cages with the withdrawal of food and water. Group-1 or the control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. Group-2 was provided with urea solution at a dose of 500 mg/kg. Group-3 was provided with standard

diuretic drug furosemide at a dose of 0.5 mg/kg. Group-4 and group-5, the test groups were treated with the methanol extract of *A. cucullata* leaves at the doses of 200 and 400 mg/kg respectively. From the graduated urine chamber of metabolic cage, the urinary output of each group was recorded 5 h after the above treatments. Collected urine was centrifuged and then estimated for sodium and potassium by using digital flame photometer (Elico Pvt. Ltd., model CL 22D). Chloride was estimated by the Schales and Schales method reproduced by Godkar (1994).

Statistical analysis

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

RESULTS

Anti-inflammatory activity

Carrageenin-induced rat paw edema

In the carrageenin induced rat paw edema model of anti-inflammatory activity, the methanolic extract of leaves of *A. cucullata* showed a significant inhibitory effect on the edema formation from the first hour to fifth hour. The highest inhibitory effect was found during the third hour where the inhibition was 24.59% ($P<0.001$) and 40.98% ($P<0.001$) at the doses of 200 and 400 mg/kg respectively. These findings were comparable to standard drug aspirin where the inhibition was 51.23% (Table 1).

Cotton pellet implantation

In the cotton pellet implantation model for anti-inflammatory activity, the extract showed a marked reduction in the weight of the cotton pellet in test animal compared to control (Table 2). At the doses of 200 and 400 mg/kg, the extract exhibited 23.31% and 34.41% reduction of the weight of the cotton pellets respectively which was comparable to that of the standard drug aspirin where the reduction was 41.79%. These results were statistically significant ($P<0.001$).

Antinociceptive activity

Table 3 shows the effect of the methanolic extract of *A. cucullata* on acetic acid induced writhing in mice. At the doses of 200 and 400 mg/kg, the extract produced 32.00 and 51.34% writhing inhibition in test animals, respectively. The results were statistically significant ($P<0.001$) and were comparable to the standard drug aspirin, which showed 63.64% writhing inhibition at the dose of 100 mg/kg.

Diuretic activity

The effect of the methanolic extract of *A. cucullata* leaves on the urination of mice was observed for

Table 1. Effect of methanolic extract of *A. cucullata* on carrageenin-induced rat paw edema

Animal group/ Treatment	Time after carrageenin injection				
	1 h	2 h	3 h	4 h	5 h
	Edema volume × 1000 (ml) (Percent inhibition)				
Control 1% Tween 80 10 ml/kg; p.o.	15.0 ± 0.47	164.5 ± 1.61	244.5 ± 1.82	272.0 ± 1.92	231.0 ± 1.23
Positive control Aspirin 150 mg/kg; p.o.	10.54 ± 1.23** (29.71)	100.5 ± 2.34* (38.91)	119.25 ± 1.92* (51.23)	172.8 ± 1.72* (36.47)	155.4 ± 2.11* (32.72)
Test group-1 AC extract, 200 mg/kg; p.o.	12.86 ± 0.52* (14.26)	133.8 ± 0.98* (18.68)	184.38 ± 1.43* (24.59)	224.4 ± 1.66* (17.51)	194.7 ± 1.76* (15.71)
Test group-2 AC extract 400 mg/kg; p.o.	11.43 ± 1.04* (23.80)	113.3 ± 1.10* (31.13)	144.30 ± 2.21* (40.98)	192.6 ± 1.72* (29.18)	170.5 ± 1.27* (26.18)

Values are expressed as mean ± SEM (Number of animals, n=6); *indicates $P<0.001$, **indicates $P<0.05$ vs. control; AC: *A. cucullata;* p.o.: per oral

Table 2. Effect of methanolic extract of *A. cucullata* on cotton pellet-induced granuloma pouch in albino rat

Animal Group/ Treatment	Mean weight of granuloma pouch (mg)	Inhibition (%)
Control 1% tween-80 solution in water; p.o.	19.84 ± 0.75	-
Positive control Aspirin 150 mg/kg; p.o.	11.55 ± 0.24*	41.79
Test group-1 AC extract 200 mg/kg; p.o.	15.22 ± 0.31*	23.31
Test group-2 AC extract 400 mg/kg; p.o.	13.01 ± 0.26*	34.41

Values are expressed as mean ± SEM (Number of animals, n=6);* indicates $P<0.001$ vs. control; p.o.: per oral; AC: *A. cucullata*

Table 3. Effect of methanolic extract of *A. cucullata* on acetic acid induced writhing in mice

Animal Group/Treatment	Number of writhes	Inhibition (%)
Control 1% tween-80 solution in water; p.o.	18.7 ± 0.52	-
Positive control Aspirin 100 mg/kg; p.o.	6.80 ± 0.69*	63.64
Test group-1 AC extract 200 mg/kg; p.o.	12.70 ± 0.70*	32.00
Test group-2 AC extract 200 mg/kg; p.o.	9.10 ± 1.00*	51.34

Values are expressed as mean ± SEM (Number of animals, n=10);* indicates $P<0.001$ vs. control; p.o.: per oral; AC: *A. cucullata*

Table 4. Effect of methanolic extract of *A. cucullata* on urine excretion parameters in mice

Treatment	Dose (mg/kg; p.o.)	Volume of urine (ml)[b]	Concentrations of ions (m.eq.l^{-1})			
			Na^+	K^+	Cl^-	Na^+/K^+
Group-1(Control)	-	2.75 ± 0.08	75.67 ± 1.25	49.75 ± 1.18	77.56 ± 1.24	1.52
Group-2(Urea)	500	3.81 ± 0.09	113.67 ± 1.36**	76.56 ± 1.27**	86.75 ± 1.38*	1.48
Group-3(Furosemide)	0.5	4.75 ± 0.13	125.86 ± 1.75**	85.46 ± 1.67**	94.39 ± 1.49*	1.47
Group-4(ME)	200	4.36 ± 0.07	117.50 ± 1.18**	79.34 ± 1.87**	91.76 ± 1.68*	1.48
Group-5(ME)	400	4.88 ± 0.05	132.75 ± 1.56**	91.23 ± 1.79**	97.59 ± 1.87*	1.45

ME: Methanolic extract of *A. cucullata*; Values are expressed as mean ± SEM (Number of animals, n = 10); *indicates $P<0.01$, **indicates $P<0.001$ vs. control; [b]Collected for 5 hours after treatment.

5 h which revealed that the extract has a marked diuretic effect in the test animals. This was comparable to that of standard drug furosemide and diuretic agent urea (Table 4). Electrolyte loss showed similar ratio (Na^+/K^+ excretion ratio was 1.48 and 1.45 at the doses of 200 and 400 mg/kg respectively) as that of the loop diuretic furosemide (1.47).

DISCUSSION

Since *A. cucullata* 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.

Carrageenin induced rat paw edema model is one of the most widely used primary test for the screening of new anti-inflammatory agents (Winter *et al.*, 1962). The edema formation is a biphasic event. The initial phase, observed during the first hour, is attributed to the release of histamine and serotonin (Vinegar *et al.*, 1969) and the delayed edema is due to the release of bradykinin and prostaglandins (Di Rosa *et al.*, 1971; Flower *et al.*, 1985). These results tend to suggest that the inhibitory activity of the extracts observed in the first phase of carrageenin induced inflammation

may be due to inhibition of early mediators, such as histamine and serotonin. The action on the second phase be due to the inhibition of bradykinin and prostaglandins.

The results of the cotton pellet implantation model for anti-inflammatory activity further support the anti-inflammatory activity of the crude extract.

Inhibition of prostaglandin synthesis could give rise to analgesic activity. So the extract was further investigated for its possible anti-nociceptive activity. Antinociceptive activity of the methanolic extract of *A. cucullata* leaves 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 *A. cucullata* possesses an antinociceptive activity.

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, cirrhosis of liver. Furosemide, used as the standard drug in this experiment belongs to the loop or high-ceiling diuretics, which act by inhibiting $Na^+/K^+/Cl^-$ co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na^+ and Cl^- from the body. The extract was able to increase the volume of urine with statistical significance along with a considerable Na^+ and Cl^- load which was comparable to that of furosemide. The diuretic action of the extract may be due to its action on the kidney. The extract may also contain a high proportion of osmotically active compounds or their metabolites that lead to an increased urine volume. Further studies may be carried out to identify whether these actions are associated with the same agent or a number of agents that are responsible for such activities.

In conclusion, it can be suggested that the crude extract of *Amoora cucullata* may possess anti-inflammatory, anti-nociceptive and diuretic effects, which correlate well with the traditional use of the plant. Therefore, further researches are essential to find out the active principles responsible for these activities.

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