

ANALGESIC AND ANTIINFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *ACALYPHA INDICA* LINN.

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ABSTRACT

Previous phytochemical analysis of methanolic extract of *Acalypha indica* L. has indicated the presence of steroid, flavonoid and terpenoid types of compounds. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check *A. indica* L. for possible analgesic and antiinflammatory activities. The methanolic extract of *A. indica* L. showed statistically significant ($P < 0.001$) analgesic activity in mice in a dose-dependent manner. A sustained and significant ($P < 0.001$) inhibition of carrageenan-induced inflammation of rat paw was observed with 125 mg/kg and 250 mg/kg body weight. The methanolic extract of *A. indica* L. also demonstrated antiinflammatory effect in a dose-dependent manner. Maximum inhibition by the extract was observed at 250 mg/kg body weight after three hours of ingestion, which was comparable to that of the standard drug phenylbutazone at a dose of 100 mg/kg body weight. The obtained results provide a support for the use of this plant in traditional medicine.

Keywords: *Acalypha indica*, analgesic, antiinflammatory, methanolic extract.

INTRODUCTION

Acalypha indica L. is an annual erect herb found throughout various parts of India, Bangladesh, Sri Lanka, the Philippines and tropical Africa. The plant has wide uses in the traditional medicines of various countries and reportedly possesses diuretic, purgative and anthelmintic properties, besides being also used for bronchitis, asthma, pneumonia, scabies and other cutaneous diseases (Kirtikar and Basu, 1999). A drug used for prevention and reversal of atherosclerotic disease process in the Sidha system of Indian medicine, Anna Pavala Sindhooram, contains the leaves of this plant as one of the ingredients (Shanmugasundaram *et al.*, 1983). Chemical constituents reported from this plant include acalyphamide (as acetate), aurantiamide and its acetate, succinimide calypho-lactate, 2-methyl anthraquinone, tri-*O*-methyllellagic acid, β -sitosterol and its β -D-glucoside (leaves); a cyanogenetic glucoside, acalyphine, two alkaloids, viz, acalyphine and triacetoneamine, an essential oil n-octacosanol, kaempferol, quebrachitol, β -sitosterol acetate and tannin (whole plant); stigmasterol (root) (Raj and Singh, 2000). Recently, four kaempferol glycosides, mauritianin, clitorin, nicotiflorin and biorobin have also been isolated from the flowers and leaves of this plant (Nahrstedt *et al.*, 2006).

Aqueous residues of the plant have been reported to demonstrate antibacterial activity against *Aeromonas hydrophila* and *Bacillus cereus* (Perumal Samy *et al.*, 1999). Petroleum ether and ethanol extracts of the whole plant demonstrated post-coital antifertility activity in female albino rats (Hiremath *et al.*, 1999). Ethanolic

extract of the plant showed promising wound healing activity in rats (Reddy *et al.*, 2002). Administration of ethanol leaf extract of the plant has been shown to significantly inhibit in a dose-dependent manner, the *Viper russelli* venom-induced lethality, haemorrhage, necrotizing and mast cell degranulation in rats and cardiotoxic and neurotoxic effects in isolated frog tissue (Shirwaikar *et al.*, 2004). Based on reports of the use of *A. indica* in Bangladesh traditional medicine, the present study was conducted with methanol extract of the whole plant to observe any potential analgesic and antiinflammatory actions in rats and mice.

EXPERIMENTAL SECTION

Plant material. *Acalypha indica* L. was collected at flowering stage from Khulna district, Bangladesh and identified at the Bangladesh National Herbarium, Dhaka, Bangladesh (Specimen No.DABC. No.29,635). Following collection, the whole plant was sun-dried for eight days and made into a coarse powder by grinding.

Preparation of methanol extract. The coarse powder (500 g) was extracted with methanol and concentrated in a Soxhlet apparatus to 1/3 of its initial volume, when a dark greenish mass was obtained. The concentrated mass was kept at room temperature to remove methanol by evaporation and finally warmed at 45-50°C to remove traces of methanol.

Animals and Chemicals. Swiss albino mice (20-25 g, 30 days age) and Long Evans rats (140-160 g, age 45 days) of either sex were obtained from the International Centre

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Table 1: Analgesic activity of methanolic extract of *Acalypha indica* Linn. in mice^a.

Treatment	Dose (p.o.)	Writhings ^b	t value of Writhings	Writhings Inhibition (%)
Control (Normal Saline)	10 ml/kg	39.5 ± 2.5	-----	-----
Methanolic extract	200 mg/kg	19.3 ± 1.2*	7.37	51.1
Methanolic extract	400 mg/kg	16.9 ± 1.2*	8.30	57.2
Aminopyrine	50 mg/kg	4 ± 0.4*	14.25	89.9

^aOne hour after administration of methanolic extract, mice were injected i.p. with 0.7% (v/v) acetic acid (0.1 ml/10g); five minutes after treatment, the number of writhings induced by acetic acid was counted for 10 minutes. ^bValues are mean ± SEM, (n=5), *P<0.001 vs. control; correlation coefficient (r = 0.98).

Table 2: Antiinflammatory effect of methanolic extract of *Acalypha indica* L. in carrageenan-induced rat paw inflammation^a.

Treatment	Dose (mg/kg body weight)	Increase in paw volume (ml Hg displaced X 1000) ± SEM (Percent inhibition)				
		1 h	2 h	3 h	4 h	24 h
Control (saline)	---	58.6 ± 1.5	70.9 ± 2.0	76.7 ± 2.3	89 ± 1.7	47.1 ± 1.5
Methanolic extract	125	51.1 ± 1.4 ^δ (12.8)	56.2 ± 1.5 ^φ (20.73)	60.2 ± 1.3 ^φ (21.51)	71.3 ± 2.6 ^φ (20.06)	42.1 ± 1.3 ^α (10.62)
Methanolic extract	250	47.2 ± 1.3 ^φ (19.45)	54.2 ± 1.6 ^φ (23.55)	53.2 ± 1.5 ^φ (30.64)	64.8 ± 1.8 ^φ (27.35)	41.2 ± 1.3 ^β (12.53)
Phenylbutazone	100	43.9 ± 1.5 ^φ (25.08)	46.7 ± 1.7 ^φ (34.13)	47.9 ± 1.8 ^φ (37.55)	54.4 ± 2.2 ^φ (39.01)	38.2 ± 1.3 ^δ (18.90)

^aValues are mean ± SEM (n=5); ^αP<0.1, ^βP<0.02, ^δP<0.01, ^φP<0.001 versus control, Student's t-test.

for Diarrhoeal Disease and Research, Dhaka, Bangladesh (ICDDR, B). The mice and rats were divided into four groups of five animals per group. The animals were given standard diet developed by ICDDR, B and water *ad libitum* and kept in laboratory environment for seven days. They were fasted overnight before the experiment. All experimental protocols were pre-approved by the Human and Animal Experiment Ethics Committee, University of Development Alternative (House No. 78, Road 11A, Dhanmondi, Dhaka-1205, Bangladesh) prior to the experiments. All chemicals used were of analytical grade.

Preparation of test material. The extract was suspended in saline solution containing 1% Tween 80 such that each ml of suspension contained 250 mg of the extract.

Study of analgesic activity. Analgesic activity of the methanol extract was studied in mice by acetic acid-induced writhing reflex method as described before (Vogel and Vogel, 1997). Briefly, four groups of mice, each consisting of five animals, were taken. Groups I and II were orally administered the methanol extract at doses of 200 mg and 400 mg/kg body weight, respectively. Group III (control group) was given only saline containing 1% Tween 80. Group IV was treated with the standard drug aminopyrine at a dose of 50 mg/kg body weight. One hour after administration of extract, saline or aminopyrine, mice were injected i.p. with 0.7% (v/v) acetic acid (0.1 ml/10 g body weight). Five minutes after acetic acid injection, the number of writhing induced by acetic acid was counted for 10 minutes.

Study of antiinflammatory activity. The effect of methanol extract on carrageenan (1%, w/v)-induced inflammation in rat paw was investigated following the method of Winter *et al.*, (1962) with minor modifications. Rats were randomly divided into four groups of five animals per group, of which Groups II and III were given the extract, respectively at doses of 125 and 250 mg/kg body weight. The dose of the samples was selected on the basis of folkloric use of the plant. Group I (control) was administered saline containing 1% Tween 80 (v/v) and group IV was administered the standard antiinflammatory drug phenylbutazone at a dose of 100 mg/kg body weight. Thirty minutes after oral administration of extract, saline or standard drug, 1% carrageenan solution was injected into the right hind paw of each animal. The volume of paw oedema was measured at one hour intervals for a period of 4 hours, and then a final measurement was made at 24 hour. For measurement of paw volume, the inflamed paw was immersed into mercury contained in a U-tube, which consisted of a right cylindrical glass tube (8 X 2.2 cm) connected to a narrow side-arm (10 X 0.72 cm) having a wall of uniform cross-section and open upper end. The volume of mercury displaced was recorded with a traveling microscope (ELFO Scientific Apparatus, India). Prior to immersion into mercury, each inflamed right hind paw was labeled with permanent marker pen so that the immersion would be uniform in each episode. The average percent increase in paw volume with time was calculated and compared against the control group.

Percent inhibition was calculated using the formula:

$$\% \text{ inhibition of paw oedema} = \frac{(V_c - V_t)}{V_c} \times 100$$

Where V_c and V_t represent average paw volume of control and treated animals, respectively.

RESULT

The effect of methanolic extract of *Acalypha indica* on pain perception was studied in Swiss albino mice by observing its effect on writhing reflex induced by acetic acid. The experiment showed (table 1) that the extract exhibited statistically significant inhibition of writhing reflexes by 51.1% and 57.2% at doses of 200 mg/kg and 400 mg/kg body weight, respectively within 10 minutes of administration of acetic acid. However the analgesic activity of the extract was comparatively less than that observed with aminopyrine at a dose level of 50 mg/kg body weight.

The effect of methanolic extract of *Acalypha indica* on carrageenan-induced rat paw edema at different hours of study was compared to that of control for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume. The experiment showed (table 2) that the extract exhibited statistically significant ($p < 0.001$) inhibition of paw volume in a dose-dependent manner. Significant inhibition of paw edema was observed with both doses tested till the fourth hour. However, maximum inhibition of paw edema was found to be 21.5% and 30.6% at third hour at doses of 125 mg/kg and 250 mg/kg body weight, respectively. Although the inhibition of paw edema with the extract was much less than that found with the standard drug phenylbutazone at a dose of 100 mg/kg body weight, the duration of action was found to be comparable to that of phenylbutazone till the fourth hour during investigation.

DISCUSSION

Carrageenan-induced rat paw edema has been frequently used to assess the anti-inflammatory effects of natural products (Panthong *et al.*, 2003). The course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve (Vinegar *et al.*, 1969). The first phase occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to release of histamine and serotonin (Crunkhon and Meacock, 1971). Prostaglandins (PGs) play a major role in the development of the second phase of reaction that is measured around 3-hour time (Crunkhon and Meacock, 1971; Vinegar *et al.*, 1969). The presence of PGs in the inflammatory exudates from the injected foot can be demonstrated at 3-hour and period thereafter (Vinegar *et al.*, 1969).

Non-steroidal anti-inflammatory agents are known to inhibit cyclooxygenase (COX-2) enzymes involved in prostaglandin synthesis (Robinson, 1997; Kulkarni *et al.*, 2000). Based on those reports it is possible that the inhibitory effect of the methanolic extract of *Acalypha indica* L. on carrageenan-induced inflammation in rats

could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Significant inhibition of paw edema in the first hour study could be attributed to the inhibition of histamine and/or serotonin release.

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