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Research Article

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES ON LEAVES METHANOLIC EXTRACT OF *MADHUCA INDICA LINN* IN WISTER ALBINO RATS

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ABSTRACT

The Leaves extract of *Madhuca indica Linn.* obtained by cold extraction of mixture of equal proportions of petroleum ether, ethyl acetate and methanol was chosen for pharmacological screening. In rat paw edema model induced by carrageenan, the extract at the 400 mg/kg dose level showed 36.68% (p<0.001) inhibition of edema volume at the end of 4h. In the acetic acid-induced writhing test, the extract at the 200 and 400 mg/kg dose level showed 39.9 % and 52.4 % inhibition of writhing, respectively. In radiant heat tail-flick method the crude extract produced 40.74% (p<0.001) and 61.48% (p<0.001) elongation of tail flicking time 30 minutes after oral administration at the 200 and 400 mg/kg dose level, respectively.

Keywords: *Madhuca indica Linn*, analgesic activity, carrageenan, anti-inflammatory activity.

INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar *et al.*, 2004). Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa *et al*., 2002). Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary. *Madhuca indica Linn*. Family: Sapotaceae.) Madhuca fat is satisfactory for production of washing soaps. Seed Oil is used as ointment, in rheumatism and to prevent cracks in the skin in winter. It is used for edible purposes culinary, hair oil, illumination, lighting, keeps body glossy and warm. Madhuca cake can be used as cheap organic manure and possess insecticide property. Also used with shikaki for hair-wash. The flowers are used as vegetable, for making vinegar and liquor. Flower juice is used in the treatment of enlargement of axillary grand, neurotic disorder and taken with cow's milk as an aphrodisiac, in cough and bronchitis. Seed paste is applied to curve muscle fatigue and relieve pain in the muscle and joints to improve the texture and vigor of skin. Medicinal uses of madhuca indica (mahua):-this trees bark,leaves,fruits, flowers and seeds everything is useful in the making of drugs. Barks useful in bleeding gums and ulcers, and also useful in the diabetes. Moha leaves mixed with butter and applied on the burns.

Flowers are useful in the cough. This trees seeds are useful in making of soaps and good useful in skin diseases.

MATERIALS AND METHODS

Plant collection

The leaves of *Madhuca indica Linn* was collected from Badrachallam forest feb -2013. A voucher specimen (Voucher No. 2041) was kept at the Department of Botany, Kakatiya University after identification of the plant.

Extraction of the plant material and sample preparation

The dried and ground plant material (4 kg) was macerated with a mixture of solvents (12 liters)

comprising of petroleum ether, ethyl acetate and methanol, in equal proportions (1:1:1), at room temperature for 3 days. Then the extract was filtered and concentrated with a rotary evaporator and was subsequently defatted (Ahmed *et al*., 1991) to get the dried extract designated. The extract was dissolved in normal saline by using 0.1% tween-80.

Drugs and Chemicals

Aminopyrine, carrageenan and phenylbutazone were purchased from Sigma-Aldrich, Germany. Morphine was obtained from Jayson Pharmaceuticals Ltd., Dhaka, Bangladesh and acetic acid was obtained from Merck, Germany.

Experimental animal

Long-Evans rats (150-200 g) and Swiss albino mice (25- 30 g) were obtained from the Animal Research Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR,B). The animals were housed in polyvinyl cages and received feed, formulated by ICDDR, B and water *ad libitum*. To keep the hydration rate constant, food and water were stopped 12 hours before the experiments. The ethics for use of experimental animals were followed carefully.

Anti-inflammatory study

In this experiment, carrageenan-induced rat hind paw edema was used as the animal model of acute inflammation according to Winter *et al*., 1962 and described previously (Saha *et al*. 2007). Briefly, acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats 1h after the oral administration of test materials. The paw volume was measured by plethysmometer (Ugo Basile, Italy) at 1, 2, 3, and 4 h after the carrageenan injection. The extract was administered at 200 and 400 mg/kg body weight. Phenylbutazone 100 mg/kg body weight was used as standard anti-inflammatory agent.

Acetic acid induced writhing test

The peripheral analgesic activity of bark extract of AL was measured by the acetic acid induced writhing test as described earlier (Saha *et al*., 2007). Briefly, the inhibition of writhing produced by the plant extract was determined by comparing with the inhibition produced by the control group. Aminopyrine at oral dose of 50 mg/kg was used as standard analgesic agent. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhings was calculated for 10 min, 10 min after the application of acetic acid.

Radiant heat tail-flick method

The central analgesic activity of the plant material was studied by measuring drug-induced changes in the sensitivity of the pre-screened (reaction time: 2-4 sec) mice to heat stress applied to their tails by using a Medicraft Analgesiometer Mask-N (D'Amour and Smith, 1941) and described previously (Saha *et al*., 2007). Briefly, the current intensity passing through the naked nicrome wire was maintained at 5 ampere. The distance between the heat source and the tail skin was 1.5 cm and cut-off reaction time was fixed at 10 second to avoid any tissue damage. Morphine was used to compare the analgesic effect of the plant extract.

STATISTICAL ANALYSIS

Data were analyzed by one-way ANOVA followed Dunnet's test and *P* values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

In the carrageenan-induced rat paw edema test (table 1) for acute inflammation, the extract of methanol in doses of 200 mg and 400 mg/kg body weight showed 36.68% and 27.51% inhibition of edema, respectively, at the end of 4h. In the acetic acid induced writhing test the extract of methanol (200 and 400 mg/kg body weight) showed a significant (p<0.001) reduction in the number of writhes with 39.9 % and 52.4 % of inhibition, respectively (table 2). In radiant heat tail-flick test the crude extract produced 40.74% (p<0.001) and 61.48% (p<0.001) elongation of tail flicking time 30 minutes after oral doses of 200 and 400 mg/kg body weight respectively (table 3). After 60 minutes the extract showed 31.29% (p<0.001) and 41.37% (p<0.001) elongation of tail flicking time. The constriction response of abdomen produced by acetic acid is a sensitive procedure for peripheral analgesic agents. This response is believed to be mediated by the prostaglandin pathways (Ronaldo *et al*., 2000). The extract of AL produced antinociceptive activity and thus indicates the presence of analgesic components that might influence the prostaglandin pathways. In the radiant heat tail-flick test, the plant extract prolonged the stress tolerance capacity of the mice, indicating the possible involvement of a higher center (Whittle, 1964). The carrageenan-induced rat paw oedema is a biphasic process (Vinegar *et al*., 1969). The release of histamine or serotonin occurs in the first phase and the second phase is associated with the production of bradykinin, protease,

prostaglandin, and lysosome (Crunkhorn and Meacock, 1971). Therefore, the inhibition of carrageenan-induced inflammation by the extract of ME could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. The present study on leaves methanolic extract of Madhuca indica *Linn* has demonstrated that this plant has significant analgesic and anti-inflammatory properties, and it justifies the traditional use of this plant in the treatment of various types of pains, broken bones and inflammation.

Table 1: Anti-inflammatory activity of crude leaves extract of *Madhuca indica Linn* by carrageenan induced rat paw edema . % Increase in Paw Volumes (ml × 1000) ± SEM (percent inhibition)

Group	1hr	2hr	3hr	4hr
Control	70.7 ± 2.06	92.8 ± 1.19	107.2 ± 2.27	114.5 ± 3.47
methanol (200 mg/kg)	58.2 ± 1.14** (17.69)	70.3 ± 1.91** (24.24)	71.2 ± 3.44** (28.77)	83.0 ± 2.50** (27.51)
methanol (400 mg/kg)	50.3 ± 2.68** (28.77)	63.2 ± 1.74** (31.96)	69.2 ± 2.98** (35.46)	72.5 ±2.92** (36.68)
PBZ (100 mg /kg.)	47.3 ± 1.48** (33.02)	57.7 ± 2.64** (37.88)	61.3 ± 1.58** (38.72)	71.7 ± 3.04** (37.41)

*Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test):
**P<0.001 All values are means of individual data obtained from six rats (*n* = 6)

Table 2: Effects of crude extract on acetic acid induced writhing response in mice

Group	Dose (mg/kg, p.o.)	Writhingb	% Inhibition
Control		17.30 ± 1.34	-
methanol	200	10.41 ± 0.74**	39.90
	400	8.25 ± 0.63**	523.40
Aminopyrine	50	7.16 ± 0.76**	58.65
One –way ANOVA	F	25.2	-
	df	3.20	-
	p	<0.001	-

a)1hr after treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1ml/10g); 10 minutes after the injection, the number writhing was counted for 10 min.
b) Values are mean ± SEM (n = 6); One-way ANOVA; **P<0.001, compared to control.

Table 3: Effects of crude extract a on radiant heat tail-flick response in mice

Group	Dose (mg/kg)	30 min (% elongation)	60 min (% elongation)	120 min (% elongation)
Control		4.50 ± 0.15	4.63 ± 0.16	4.98± 0.20
Morphine	2b	8.37 ± 0.14** (85.93)	7.15 ± 0.19** (54.32)	6.23 ± 0.25** (25.08)
methanol	200	6.33 ± 0.15** (40.74)	6.08 ± 0.21** (31.29)	5.65 ± 0.24 (13.38)
	400	7.27 ± 0.30** (61.48)	6.55 ± 0.25** (41.37)	5.88 ± 0.22* (18.06)
One way ANOVA	F	68.5	27	5.34
	P	< 0.001	< 0.001	< 0.01
	df	7.40	7.40	7.40

a) Per oral administration of vehicle and crude extract, radiant heat intensity was 5 amp.
b) Morphine was administered sub-cutaneously.
c) Values are mean ± SEM (n = 6); One-way ANOVA; df = 7, 40; **P<0.01, *P<0.05 compared to control.

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