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Evaluation of Antipyretic and Antinociceptive Activities of *Plumeria acuminata* Leaves

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The present study was designed to investigate the antipyretic and antinociceptive activity of methanol extract of *Plumeria acuminata* leaves (MEPA) (Apocynaceae) in several experimental models. A single oral administration of MEPA at different doses (100, 250 and 500 mg kg^{-1}) showed significant reduction in brewer's yeast induced hyperthermia in rats. MEPA also elicited pronounced inhibitory effect on acetic acid-induced writhing response, hot plate, tail flick and tail immersion responses in mice in the antinociceptive test. These findings suggest that the methanol extract of *Plumeria acuminata* possessed potent antipyretic and antinociceptive activity. Preliminary phytochemial screening of the extracts showed the presence of alkaloids, flavonoids, tannins, terpenes and steroids which may be responsible for antipyretic and antinociceptive activities.

Key words: *Plumeria acuminata*, apocynaceae, antipyretic, antinociceptive

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INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990). The research into plants with alleged folkloric use as pain relievers, antipyretic and antiinflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic, antipyretic and antiinflammatory drugs (Elisabetsky, 1995).

Plumeria acuminata (Apocynaceae) is commonly known as perungalli in Tamil and widely distributed throughout the Southern parts of India. The plant material is widely used as a purgative, remedy for pain, fever, diarrhea and cure for itch. The milky juice is employed for the treatment of inflammation and rheumatism. The bark has been applied as a plaster over inflammation and hard tumors. The leaves are reported to have antiinflammatory, rubefacient in rheumatism and have strong purgative effect (Nadkarni, 1976). We found no relevant literature substantiating the uses indicated. As a part of our pharmacological screening of this plant, we previously demonstrated that the methanol extract of *Plumeria acuminata* produces potent anti-inflammatory effect on experimental models (Gupta *et al*., 2006). The purpose of the present study was therefore, to evaluate the antipyretic and antinociceptive effect of the methanol extract using Brewer's yeast induced pyrexia and some acute and chronic models of pain in rats and mice.

MATERIALS AND METHODS

Plant material: The leaves of the plant *Plumeria acuminata* (Family: Apocynaceae) was collected from Erode district of Tamilnadu, India. The plant material was taxonomically identified by Botanical Survey of India, Kolkata. A voucher specimen (No. GMG 02/05) has been preserved in our laboratory for future reference. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves was defatted with petroleum ether and the marc thus obtained was then extracted with methanol in a soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained (MEPA, yield 12.4%). The dried MEPA was dissolved in normal saline and used for the present study.

Animals: Studies were conducted in Pharmacology Laboratory, Department of Pharmacology and Pharmaceutical Chemistry, Jadavpur University, Kolkata in the month of November 2006 using male Wistar albino rats weighing 180-200 g and male Swiss albino mice weighing 20-22 g. They were obtained from the animal house of Jadavpur University, Kolkata. The mice were grouped and housed in poly acrylic cages (38×23×10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25±2°C) with dark/light cycle (14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee (Ethical clearance No.: 367001//C/CPCACA).

Chemicals: Paracetamol, Aspirin (USV, Bombay) and morphine (MM Pharma, New Delhi) were used as the standard drugs. All other reagents used are of analytical grade.

Antipyretic activity: The rats were divided in to five groups containing six animals in each group and trained to remain quiet in a restraint cage. Hyperpyrexia was induced in rats by subcutaneous injection of 10 mL kg^{-1} of a 15% aqueous suspension of Brewer's yeast in the back below the nape of the rat (Al-Ghamdi, 2001). Initial rectal temperature was recorded. After 18 h animals that showed an increase of 0.3-0.5°C in rectal temperature were selected. The animals were then fasted for the duration of the experiment, water *ad libitum*. The test extract MEPA at different doses (100, 250 and 500 mg kg^{-1}; p.o.) was administered orally to groups 2, 3 and 4, respectively. Control group received normal saline (5 mL kg^{-1}; p.o.). Paracetamol (100 mg kg^{-1}; p.o.) was used as reference drug. Rectal temperature was determined by thermal probe Ellab Themistor thermometer 1-4 h after test extract and reference drug administration.

Antinociceptive activity: Evaluation of antinociceptive properties of the leaves extract of the plant was carried out by the chemical, mechanical and thermal noxious stimuli.

Acetic acid-induced writhing method: Mice were treated with 0.1 mL of 1% acetic acid by intraperitoneal administration. Mice that presented a high number of abdominal contractions in a period of 10 min were selected for the present study. They were divided into five groups of six animals in each. Writhing test was used according to the method of Turner with slight modification Turner, 1965). The MEPA at the different doses (100, 250 and 500 mg kg^{-1}; p.o.) and standard drug aspirin (100 mg kg^{-1};

p.o.) were administered orally, 1 h prior to the injection of acetic acid; vehicle control group received normal saline (0.9% NaCl, 5 mL kg^{-1}; p.o.). Writhing was induced by administering acetic acid solution (0.6%; 10 mL kg^{-1}; i.p.). The minutes after acetic acid injection, the mice were placed in a transparent box and the number of writhes was counted for a period of 10 min. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. There was a significant reduction in the number of writhes by drug treatments as compared to vehicle-treated animals. This was considered a positive analgesic response and the percentage inhibition of writhing was calculated and evaluated statistically.

Hot plate method: Five groups of six mice each were selected for the present study. Group 1 served as control and received the vehicle (normal saline, 5 mL kg^{-1}; p.o.). The extract MEPA at the concentration of 100, 250 and 500 mg kg^{-1} was administered orally to groups 2, 3 and 4, respectively and group 5 received the standard drug morphine (5 mg kg^{-1}; s.c.). The mice were placed on an aluminium hot plate kept at a temperature of 55±0.5°C for a maximum time of 30 sec (Franzotti *et al*., 2000). Reaction time was recorded when the animals licked their fore-and hind paws and jumped at before (0) and 15, 30, 45 and 60 min after administration of test drugs. The mice which reacted within 15 sec and which did not show large variation when tested on four separated occasions were selected for the studies.

Tail flick response: Mice were randomly assigned to five groups of six animals each. A control group received normal saline (0.9% NaCl, 5 mL kg^{-1}; p.o.). The methanol MEPA was given at the doses of 100, 250 and 500 mg kg^{-1}; p.o. to the second, third and fourth group, respectively. Standard drug morphine (5 mg kg^{-1}; s.c.) was given to the fifth group which served as standard. Analgesic activity was measured 30 min after the administration of test and standard drugs (D'Amour and Smith, 1941). The tail of each mouse was placed on the nichrome wire of an analgesiometer (Techno, Lucknow, India), which was fixed at 5.5±0.5 amp. The time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. The reading was taken after 30 min of administration of the test drugs. The mice which reacted within 15 sec and which did not show large variation selected for studies.

Tail immersion method: Mice were divided into five groups of six animals each. Group 1 received normal saline (0.9% NaCl, 5 mL kg^{-1}; p.o.) (control) and groups 2, 3 and 4 received 100, 250 and 500 mg kg^{-1}; p.o. of MEPA, respectively. Group 5 received the standard drug morphine (5 mg kg^{-1}; s.c.). They were held in position in a suitable restrainer with tail extending out. The tail (up to 5 cm) was then dipped into a pot of water maintained at 55±0.5°C. The time in seconds to withdraw the tail out of water was taken as the reaction time. The reading was taken after 30 min of administration of the test drugs (Palanichamy and Nagarajan, 1990). The mice which reacted within 15 sec and which did not show large variation selected for the studies.

Statistical analysis: Values are mean±SEM. Statistical significance was determined by ANOVA. Values with $p<0.01$ were considered as statistically significant.

RESULTS

Antipyretic activity: Table 1 represents the effect of MEPA on yeast induced hyperpyrexia in rats. The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 18 h of administration. The antipyretic effect of MEPA started as early as 1 h and was maintained for 4 h after administration. Treatment with MEPA at the doses of 100, 250 and 500 mg kg^{-1} decreased the rectal temperature of the rats in a dose-dependent manner. The results were comparable to that of the reference drug paracetamol (100 mg kg^{-1}; p.o.).

Antinociceptive activity

Acetic acid-induced writhing method: Table 2 shows that the methanol extract from the leaves of *Plumeria acuminata* strongly reduced the abdominal constrictions induced by the intraperitoneal administration of acetic acid solution. The effects produced by MEPA were dose-dependent and the values were found to be significant ($p<0.01$) at the doses tested, when compared to control. MEPA at the doses of 100, 250 and 500 mg kg^{-1} exhibited 33.41, 44.31 and 68.37% inhibition, respectively, where the inhibition for the standard drug aspirin was found to be 76.45%.

Hot plate method: Table 3 shows the MEPA produced significant ($p<0.01$) analgesic activity at all the doses tested. In this method MEPA considerably increased the animals' reaction time to the heat stimulus. Values were found to be significant and dose-dependent. The highest reaction time of 12.48±0.4 was observed at a dose of 500 mg kg^{-1} compared with the control group value of 5.52±0.3. The results were comparable to that of the standard drug morphine (13.34±0.18).

Table 1: Effect of methanol extract of *Plumeria acuminata* leaves on Brewer's yeast-induced hyperpyrexia in rats

Treatments	Dose (mg kg^{-1})	Rectal temperature (°C) before and after treatment					
		0 h	19 h	20 h	21 h	22 h	23 h
Control (0.9% NaCl)	5 mL	37.4±0.1	39.5±0.4	39.3±0.1	39.2±0.3	39.2±0.1	39.1±0.3
MEPA	100	37.2±0.2	39.9±0.2	39.7±0.1*	38.8±0.2*	38.5±0.1*	37.9±0.3**
MEPA	250	37.4±0.3	39.8±0.2*	39.6±0.2	38.7±0.2*	38.2±0.1	37.4±0.3
MEPA	500	37.1±0.2*	39.7±0.1	39.4±0.2*	38.3±0.2	37.7±0.1*	37.3±0.1**
Paracetamol	100	37.4±0.3	39.6±0.2*	38.3±0.2	37.9±0.1*	37.5±0.1*	37.2±0.2

Values are mean±SEM. (n = 6), *p<0.01, **p<0.001, Experimental groups compared with control group

Table 2: Effect of *Plumeria acuminata* leaves extract on acetic acid-induced writhing test in mice

Treatments	Dose (mg kg^{-1})	No. of writhes	Percentage inhibition
Control (0.9% NaCl)	5 mL	45.14±0.02	-
MEPA	100	30.06±0.01*	33.41
MEPA	250	25.14±0.02	44.31
MEPA	500	14.28±0.01*	68.37
Aspirin	100	10.63±0.01	76.45

Values are mean±SEM (n = 6), *p<0.01, Experimental groups compared with control group

Table 3: Effect of *Plumeria acuminata* leaves extract on hot plate test in mice

Treatments	Dose (mg kg^{-1})	Latency				
		0 min	15 min	30 min	45 min	60 min
Control (0.9% NaCl)	5 mL	6.38±0.2	6.44±0.6	6.01±0.4	5.96±0.1	5.52±0.3
MEPA	100	6.40±0.3	7.79±0.1*	8.47±0.1*	8.92±0.3*	9.36±0.1
MEPA	250	6.39±0.17	8.12±0.1	8.82±0.2	9.25±0.1	10.01±0.3*
MEPA	500	6.41±0.5	9.31±0.2	10.30±0.1	11.06±0.1	12.48±0.4
Morphine	5	6.40±0.2*	11.30±0.4*	13.11±0.2	13.25±0.3	13.34±0.18

Values are mean±SEM (n = 6), *p<0.01, Experimental groups compared with control group

Table 4: Effect of *Plumeria acuminata* leaves extract on tail flick response in mice

Treatments	Dose (mg kg^{-1})	Reaction time (s)	Percentage inhibition
Control (0.9% NaCl)	5 mL	4.5±0.22	-
MEPA	100	6.1±0.5*	35.56
MEPA	250	7.2±0.3	60.00
MEPA	500	8.1±0.3*	80.00
Morphine	5	8.8±0.31	95.56

Values are mean±SEM (n = 6), *p<0.01, Experimental groups compared with control group

Table 5: Effect of *Plumeria acuminata* leaves extract on tail immersion test in mice

Treatments	Dose (mg kg^{-1})	No. of writhes	Percentage inhibition
Control (0.9% NaCl)	5 mL	4.8±0.61	-
MEPA	100	7.0±0.5*	45.83
MEPA	250	7.8±0.3**	62.50
MEPA	500	8.5±0.5**	77.08
Morphine	5	9.2±0.51*	91.66

Values are mean±SEM (n = 6), *p<0.01, **p<0.001; Experimental groups compared with control group

Tail flick response: The effects of MEPA on the tail flick response of the mice were summarized in Table 4. The results shows that the administration of MEPA at different doses (100, 250 and 500 mg kg^{-1}; p.o.) and morphine (5 mg kg^{-1}; s.c.) prolonged significantly the mouse tail reaction time when the animal's tail was subjected to heat generated by the tail flick apparatus. Meanwhile, the response to heat stimuli of the control group was not altered during the period of the experiment. Administration of MEPA at the doses of 100, 250 and 500 mg kg^{-1} exhibited 35.56, 60.00 and 80.00% inhibition, respectively, while the standard drug morphine produced 95.55% inhibition.

Tail immersion method: In the tail immersion method the extract considerably increased the animals' reaction time to the heat stimulus. Values were found to be significant ($p<0.01$) and the effects were dose-dependent at the doses tested. The results were shown in Table 5. Pre-treatment with MEPA at the doses of 100, 250 and 500 mg kg^{-1} showed 45.83, 62.50 and 77.08% inhibition, respectively. And the percentage inhibition produced by the standard drug morphine was found to be 91.66%.

DISCUSSION

Preliminary screening of the methanol extract showed that the methanol extract to be more active and easier to preserve, thus it was chosen for this study. In addition the presence of methanol may help to extract some organic but polar 'active' solutes from the plant so that the extract would be more 'concentrated' for screening of pharmacological activities (Chan *et al*., 2000).

Increased body temperature and pain are known as the main symptoms of the body against an inflammatory stimulation. Hence a drug possessing anti-inflammatory activity also exhibit antipyretic and antinociceptive

properties. In our earlier studies, the methanol extract of *Plumeria acuminata* showed potent anti-inflammatory activity (Gupta *et al*., 2006). In order to determine the antipyretic effect of *Plumeria acuminata* the brewer's yeast induced hyperthermia in rat model was used. MEPA showed significant decrease in rectal temperature similar to that of paracetamol (100 mg kg^{-1}). This result suggested that the plant has some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature (Milton, 1982).

The writhing induced by chemical substances are due to sensitization of nociceptors by prostaglandins. This test is useful for evaluation of mild antinociceptive non-steroidal anti-inflammatory compounds (Ferreira and Vane, 1974; Berkenkopf and Weichman, 1988). MEPA showed significant inhibitory activity on the writhing response induced by acetic acid when compared to control. The antinociceptive effect of the extracts may be due to its anti-inflammatory action as in the case with salicylates, which are particularly effective in relieving the type of pain associated with inflammation or oedema (Passmore and Robson, 1970). Furthermore, the extract showed significant difference from that of the control with respect to hot plate, tail flick and tail immersion methods. Centrally acting antinociceptive drugs elevate pain threshold of animals towards heat and pressure. The effect of the extract on these pain models indicates that it might be centrally acting.

CONCLUSIONS

The results obtained in this study indicate that the extract possesses potent antipyretic and antinociceptive properties, which are mediated via peripheral and central inhibitory mechanisms. This could provide a rationale for the use of this plant in fever and pain conditions in folk medicine.

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