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Analgesic, anti-inflammatory and antipyretic studies of *Neolamarckia cadamba* barks

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ABSTRACT

The methanol extract of *Neolamarckia cadamba* (Family: Rubiaceae) barks showed significant analgesic, anti-inflammatory and antipyretic activity. The acute toxicity, orally evaluated in mice, was found to be higher than 3000 mg/kg. The antinociceptive response using writhing and tail immersion test in mice were examined. The antiinflammatory activity using carrageenan and antipyretic activity in yeast-induced pyrexia in rats, were also examined. The extract at the doses 400 and 600 mg/kg significantly reduced the numbers of writhings induced by intraperitoneal injection of acetic acid in mice. But the extract significantly exerted protective effects on heat-induced pain in mice at all tested doses (200, 400 and 600 mg/kg p.o.). The percentage inhibition of oedema due to injection of carrageenan was found to be in accordance with the doses tested. The extract showed significant effect on yeast-induced fever in rats at doses of 400 and 600 mg/kg and no promising results with 200 mg/kg dose level. Although these results provide a support for the traditional uses of *N. cadamba* barks, further studies are necessary to better evaluate its safety and modes of action.

Keywords: *Neolamarckia cadamba,* analgesic activity, anti-inflammatory activity, antipyretic activity.

INTRODUCTION

Neolamarckia cadamba (Roxb.) Bosser, syn. *Anthocephalus cadamba* var *A. chinensis* (Rubiaceae) commonly known as Kadam is a large tree up to 45 m high, frequently found in moist deciduous evergreen forests and widely distributed through out the greater part of India. The bark is gray, smooth in young trees, rough and longitudinally fissured in old trees[1]. The dried stem bark is used as folk medicine in the treatment of anemia, uterine complaints and for improvement of semen quality and reported to possess astringent, mucolytic, analgesic, anti-inflammatory, febrifuge and antiseptic properties[2-6]. However, only a few phytochemical and pharmacological or biological test reports have been reported on this plant in the literature. Chlorogenic acid isolated from the leaves has been reported to possess hepatoprotective activity *in vitro* and lipid peroxidation in liver microsomes *in vivo*[7]. In the present study, we investigated the anti-inflammatory activity of the methanol extract of *N. cadamba* barks in experimental animal model using carrageenin-induced paw edema in rats. The analgesic and antipyretic activities were also examined using the writhing and tail immersion tests in mice and yeast-induced pyrexia in rats respectively.

MATERIALS AND METHODS

Plant material

N. cadamba fresh barks were collected from the forests of Mayurbhanj district of Orissa during June 2008 and authenticated by the taxonomists of the Botanical Survey of India, Shibpur, Howrah. A voucher specimen [Sp. No: CNH/ I-I / (255)/2008Tech.II] has been kept in our research laboratory for further reference. The material was washed, shade dried and powdered.

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Preparation of the extract

The dried powdered plant material (500 g) was defatted with petroleum ether (40 - 60^{0}C) for 72 h and extracted with methanol for 72 h in a soxhlet extractor. Following filtration and concentration under reduce pressure, a brown sticky residue was obtained (yield: 12.46%). Aspirin (200 mg/kg, p.o.) or pentazocine (30 mg/kg, p.o.) were used as reference standards where applicable. The test samples were suspended in 1% Tween-80 in normal saline and used for the study. Preliminary phytochemical studies were performed on the extract using standard procedures.

Animals

Animals used in this study were male Swiss albino mice, weighing 20-25 g and Wistar rats with the weight ranging from 150-210 g. The animals were housed for at least one week in the laboratory animal room prior to testing in standard polypropylene cages at room temperature of 34 ± 2^{0}C and at 60-65% relative humidity Food and water were given *ad libitum* unless otherwise specified. All experimental protocols were approved by the Institutional Animal Ethics committee of Matushree V. B. Manvar College of Pharmacy, Dumiyani, Rajkot district, Gujarat. The experiments were designed in different groups containing six animals in each.

Acute toxicity study

The test was carried out as suggested by *Ganapaty et al.*[8]. The control group received only vehicle (2 ml/kg, p.o.). The other groups separately received 100, 200, 300, 600, 800, 1000, 2000 or 3000 mg/kg of the test extract respectively in a similar manner. Immediately after dosing, the animals were observed continuously for the first 4 h for any behavioral changes. They were then kept under observation up to 14 days after drug administration to find out the mortality if any.

Evaluation of analgesic activity by writhing method

The test was performed according to *Siegmund et al.*[9]. Writhing was induced in mice by single intraperitoneal injection (10 ml/kg) of 0.6% acetic acid. The number of writhings was counted over a 20

min period. Different groups of animals were treated with methanol extract (200, 400 or 600 mg/kg) through oral route just 30 min prior to injection of acetic acid. The control group received only vehicle (3 ml/kg). Aspirin (200 mg/kg) was used as reference standard for activity comparison[10]. The writhing effect indicated by stretching of abdomen with simultaneous stretching of at least one hind limb. The percentage inhibition was calculated[11].

Evaluation of analgesic activity by tail immersion method

The tail immersion test was carried out as described by *Janssen et al*.[12]. The animals were and had the last 3.5 cm of their tail immersed in hot water thermo-statistically maintained at 51°C, a procedure that caused them to rapidly withdraw their tail. Five groups of animals were held in position in a suitable restrainer with the tail extending out. The latency to withdraw the tail was recorded with a stopwatch, and a cut-off maximum latency of 10 sec was established in order to prevent tissue damage. Group I served as control, which received only vehicle (3 ml/kg, p.o.). Other groups of animals received one of the following in a similar manner: pentazocine (30 mg/kg) or methanol extracts (200, 400 or 600 mg/kg). The initial reading was taken immediately before administration of test samples and then at 15, 30, 45 and 60 min after the administration.

Evaluation of anti-inflammatory activity

The test was performed as per the method of *Winter et al*.[13]. The animals were divided into five groups. The control group was given the vehicle (2 ml/kg) through oral route. Other groups received aspirin (200 mg/kg) or the test extract at doses of 200, 400 or 600 mg/kg in a similar manner. Carrageenan (0.1 ml of 1% solution in normal saline) was administered to the rats into the planter surface of the right hind limb to induce paw oedema. Paw volume was measured with a plethysmograph after 1, 2 and 4 h of carrageenan injection and paw swellings were compared with control. Percentage inhibition of oedema was calculated[14].

Evaluation of antipyretic activity

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats [15]. Fever was induced by injecting 10 ml/kg (s. c.) of 20% aqueous suspension of Brewer's yeast in normal saline below the nape of the neck. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer. Only rats that showed an increase in temperature of at least 0.7^0C were selected for the study[16] and animals were divided into five groups. The control group received vehicle (5 ml/kg) through oral route. Other groups of animals received one of the following in a similar manner: Aspirin (300 mg/kg) or the test extracts (200, 400 or 600 mg/kg). The rectal temperature was measured at 1, 2 and 4h after treatment.

Statistical analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's-t test. A P-value < 0.05 were considered to be significant. All the values were expressed as mean ± SEM.

RESULTS

Preliminary phytochemical tests revealed presence of alkaloids, flavonoides, tannins and phenolic compounds in the methanol extract of *N. cadamba*.

Acute toxicity study

When orally administered to mice in graded doses from 100 to 3000 mg/kg, the methanol extract produced sedation and analgesia at all tested doses. However, there was no mortality in any of the above doses at the end of the 14 days of observation.

Effect of methanol extract of *N. cadamba* and aspirin on acetic acid induced writhing in mice.

The methanol extract of *N. cadamba* barks at the doses 400 and 600 mg/kg significantly reduced the numbers of writhings induced by intraperitoneal injection of acetic acid in mice. But the extract at 200 mg/kg p.o. did not elicit significant response. However, the reference drug aspirin (200 mg/kg) produced significant protective effects towards the acetic acid induced pain (Table I).

Effect of methanol extract of *N. cadamba* and pentazocine on nociceptive response induced by heat in mice.

The mean latency of nociceptive responses to thermal stimuli in the tail immersion test is summarized in Table II. The methanol extract of *N. cadamba* barks exhibited significant response at all tested dose levels in a dose dependant manner that is comparable with response of the standard drug pentazocine. The extract significantly exerted protective effects on heat-induced pain in mice.

Effect of methanol extract of *N. cadamba* and aspirin on carrageenan induced paw oedema in rats.

Oral administration of the methanol extract at the doses of 200, 400 and 600 mg/kg significantly suppressed the paw oedema at 2 and 4 hr after carrageenan injection in rats. The percentage inhibition of oedema was found to be in accordance with the doses tested. Aspirin (200 mg/kg), the standard control, also produced significant effect and reduced paw oedema in this test but the effects were observed from the 1 h of carrageenin injection in the test animals (Table III).

Effect of methanol extract of *N. cadamba* and aspirin on brewer's yeast induced pyrexia in rats.

The methanol extract of *N. cadamba* barks showed significant effect on yeast-induced fever in rats at doses of 400 and 600 mg/kg. The reference drug aspirin suppressed the fever induced by yeast in rats from 1st hour of drug administration. On the other hand, the methanol extract produced significant activity at 4th hour of test sample administration and no promising results with 200 mg/kg dose level (Table IV).

DISCUSSION

The results demonstrate that the methanol extract obtained from *N. cadamba* barks exhibited significant analgesic activity. The writhing test is generally used for screening of antinociceptive effects [17, 18]. The tail immersion test is another thermic pain model, which

Table I: Evaluation of analgesic activity of methanol extract of the barks of *N. cadamba* by acetic acid induced writhing in mice.

Group	Treatment	Dose	Avg. no. of writhing	Percentage Inhibition
I	Control	3 ml/kg	37.16±2.16	-
II	Aspirin	200 mg/kg	13.5±2.37**	63.67
III	Methanol extract	200 mg/kg	32.5±2.59	12.54
IV	Methanol extract	400 mg/kg	24.83±1.88**	33.18
V	Methanol extract	600 mg/kg	20±1.71**	46.17

*Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA; *P<0.05, **P<0.01 when compared to control.*

Table II: Evaluation of analgesic activity of methanol extract of the barks of *N. cadamba* by tail immersion method in mice.

Group	Treatment	Dose	Average tail withdrawing time (Sec)				
			0 min	15 min	30 min	45 min	60 min
I	Control	3 ml/kg	3.67±0.22	3.61±0.11	3.78±0.23	3.73±0.19	3.76±0.23
II	Pentazocine	30mg/kg	3.77±0.23	6.08±0.37**	8.15±0.07**	8.83±0.33**	8.45±0.39**
III	Methanol extract	200 mg/kg	3.58±0.25	4.04±0.21	4.65±0.14	4.99±0.1**	4.18±0.23
IV	Methanol extract	400 mg/kg	3.67±0.14	5.09±0.41*	6.32±0.38**	7.27±0.43**	6.06±0.59**
V	Methanol extract	600 mg/kg	3.60±0.11	6.27±0.38**	7.09±0.39**	8.03±0.19**	7.43±0.3**

*Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA; *P<0.05, **P<0.01 when compared to control.*

Table III: Acute antiinflammatory activity of methanol extract of the barks of *N. cadamba* on carrageenin induced rat paw oedema.

Group	Treatment	Dose	Paw Volume (ml)			
			0h	1h	2h	4h
I	Control	2 ml/kg	0.32±0.01	0.49±0.02	0.58±0.05	0.52±0.05
II	Aspirin	200 mg/kg	0.33±0.01	0.38±0.01** (22.44 %)	0.36±0.01** (37.93 %)	0.34±0.01** (34.61 %)
III	Methanol extract	200 mg/kg	0.34±0.02	0.47±0.07 (4.08%)	0.44±0.05** (24.13%)	0.43±0.03** (17.30%)
IV	Methanol extract	400 mg/kg	0.33±0.05	0.46±0.05 (6.12%)	0.42±0.06** (27.58%)	0.40±0.03** (23.07%)
V	Methanol extract	600 mg/kg	0.30±0.01	0.43±0.03 (12.24 %)	0.39±0.02** (32.75%)	0.35±0.02** (32.69%)

*Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA;*P<0.05, **P<0.01 when compared to control; Dunnet's t-test. Figures in parenthesis denote Percentage inhibition of oedema.*

Table IV: Effect of methanol extract of the barks of *N. cadamba* on yeast induced pyrexia in rats.

Group	Treatment	Dose	Average rectal temperature (°C)			
			0	1	2	4
I	Control	5 ml/kg	37.77±0.31	37.72±0.28	37.85±0.43	37.63±0.50
II	Aspirin	300 mg/kg	36.48±0.25	36.37±0.30*	36.18±0.29**	36.11±0.28*
III	Methanol extract	200 mg/kg	37.16±0.28	37.19±0.21	36.94±0.20	36.93±0.21
IV	Methanol extract	400 mg/kg	36.92±0.29	37.10±0.29	37.05±0.30	36.33±0.37*
V	Methanol extract	600 mg/kg	36.93±0.53	36.73±0.52	36.49±0.38*	36.19±0.30*

*Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA; *P<0.05, **P<0.01 when compared to control.*

assesses the way an animal responds to moderate continuous pain generated by a tissue[19]. Thermic painful stimuli are known to be selective to centrally but not peripherally acting analgesic drugs[20]. In the present study, the methanol extract significantly reduced the pain in both chemical induced stimuli and thermal stimuli indicating that the constituents present in the extract possess similar mode of action as that of pentazocine.

The methanol extract of *N. cadamba* barks suppressed the paw oedema induced by carrageenan in rats compared with aspirin, a nonsteroidal anti-inflammatory drug, which possesses analgesic, antipyretic and anti-inflammatory activities by inhibition of prostaglandin synthesis via cyclooxygenase activity[21]. Thus, the anti-inflammatory action of the extract from *N. cadamba* barks may act at some site(s) of action that are similar to those of aspirin.

The methanol extract of *N. cadamba* barks showed significant effect on yeast-induced fever in rats while the reference drug aspirin suppressed fever induced by yeast in rats by inhibiting the synthesis of prostaglandin E2 [21, 22].

Although these results provide a support for the traditional uses of *N. cadamba* barks, further studies are necessary to better evaluate its safety and modes of action.

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