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Anti-inflammatory and analgesic activity of methanol extract of bark of *Acacia suma* (Roxb.)

Suman Acharyya[1*], H. K. Sundeep Kumar[2], Devendra Singh Rathore[3] and Sankar Narayan Bhunia[4]

1, Matushree V. B. Manvar College of Pharmacy, Dumiyani, Rajkot, (Gujarat) - India
2, Institute of Pharmacy and Technology, Salipur, Cuttack, (Orissa) - India
3, Rajasthan Pharmacy College, Bhankrota, Ajmer Road, Jaipur, (Rajasthan) - India
4, Sri Balaji College of Pharmacy, Benad Road, Jaipur, (Rajasthan) - India

Abstract

The methanol extract of *Acacia suma* (Roxb.) barks (Family-Fabaceae) showed significant anti-inflammatory and analgesic activity. The acute toxicity, orally evaluated in mice, was found to be higher than 2000 mg/kg. The anti-inflammatory activity using carrageenan was examined. The antinociceptive response using writhing and tail immersion test in mice were also examined. The percentage inhibition of oedema due to injection of carrageenan was found to be in accordance with the doses tested. The extract at the doses 200 and 400 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 (100, 200 and 400 mg/kg p.o.).The presence of flavonoids in the methanol extract may be contributory to its anti-inflammatory and analgesic activity.

Key-Words: *Acacia suma*, anti-inflammatory activity, analgesic activity, aspirin, pentazocine.

Introduction

Drugs which are in use presently for the management of pain, and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs present well known side and toxic effects. Moreover synthetic drugs are very expensive to develop since, for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs[1].

*** Corresponding Author:**
E-mail: sumanjanmenjoy@yahoo.com
Mob. +91-9712866020

Acacia suma (Roxb.) var. *Acacia polyacantha* (Family-Fabaceae) is a medium sized erect tree; trunk with fissured bark and knobby persistent prickles found in the greater part of India and costal districts of Orissa[2,3]. The bark is reported to be used as blood purifier[3] and possesses anti-cancer, insecticide and astringent properties[4-7]. The seeds are reported to have hypoglycaemic effect[4].The leaves and roots of the plant are reported to be use as insecticide, antifungal, antivenin, aphrodisiac, antimalarial, anticrustacean, stimulant and in the treatment of sores, abcesses and asthma[7-13]. Presence of proanthocyanidin[4], 5,4'-dihydroxy-7, 3'-dimethoxyflavone-3-0-D galactopyranoside[5,14], gallocatechin-5-7-digallate, quercetin and gallocatechin-7-gallate[6] in the barks have been reported earlier. An extensive literature survey does not reveal anti-inflammatory and analgesic activity of bark. So the present study was under taken to investigate the anti-inflammaory and analgesic activity of bark of *Acacia suma*.

Material and Methods

Preparation of Plant Extract

The plant material (barks) was collected from the forests of Ganjam district of Orissa during June 2007 and identified by the taxonomists of the Botanical Survey of India, Shibpur, Howrah. A voucher specimen [Sp. No: CNH/ I-I / (17)/2009/Tech.II/28] has been kept in our research laboratory for further reference. After authentication, fresh barks were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The powdered material was subjected to extract with methanol for 48 h in a soxhlet extractor. The liquid extract was concentrated under vacuum to yield dry extract. The methanol extract thus obtained was then screened for anti-inflammatory and analgesic activity.

Animals

Adult Wistar albino rats of either sex weighing between 150-200 g and Swiss albino mice of either sex weighing between 20-30 g were used for the study. The experimental protocols have been approved by the Institutional Animal Ethics Committee.

Acute toxicity study

The test was carried out as suggested by *Ganapaty et al.*[15]. Selected animals were divided into different groups of six in each. The control group received 1% Tween-80 in normal saline (2 ml/kg, p.o.). The other groups separately received 100, 200, 300, 600, 800, 1000 and 2000 mg/kg of the test extract respectively in a similar manner. Immediately after dosing, the animals were observed continuously for the first 4 hours 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 anti-inflammatory activity

The test was performed as per the method of *Winter et al* [16]. 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 100, 200 and 400 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[1].

Evaluation of analgesic activity by writhing method

The test was performed according to *Siegmund et al.*[17]. 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 (100, 200 and 400 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[18]. The writhing effect indicated by stretching of abdomen with simultaneous stretching of at least one hind limb. The percentage inhibition of writhing was calculated[19].

Evaluation of analgesic activity by tail immersion method

The tail immersion test was carried out as described by *Janssen et al.*[20]. 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 (100, 200 and 400 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.

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 was considered to be significant. All the values were expressed as mean ± SEM.

Results and Conclusion

Acute toxicity study

When orally administered to mice in graded doses from 100 to 2000 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 A. suma and aspirin on carrageenan induced paw oedema in rats.

Oral administration of the methanol extract at the doses of 100, 200 and 400 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 carrageenan injection in the test animals (Table 1).

Effect of methanol extract of A. suma and aspirin on acetic acid induced writhing in mice.

The methanol extract of *A. suma* barks at the doses 200 and 400 mg/kg significantly reduced the numbers of writhings induced by intraperitoneal injection of acetic acid in mice. But the extract at 100 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 2).

Effect of methanol extract of A. suma 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 3. The methanol extract of *A. suma* 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.

Phytochemical analysis of methanol extract of Acacia suma.

Preliminary phytochemical analysis[21,22] of the methanol extract of *Acacia suma* revealed the presence of flavonoids.

The results demonstrate that the methanol extract obtained from *A. suma* barks exhibited significant analgesic activity. The writhing test is generally used for screening of antinociceptive effects[23,24]. The tail immersion test is another thermic pain model, which assesses the way an animal responds to moderate continuous pain generated by a tissue[25]. Thermic painful stimuli are known to be selective to centrally but not peripherally acting analgesic drugs[26]. 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 *A. suma* 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[27]. Thus, the anti-inflammatory action of the extract from *A. suma* barks may act at some site(s) of action that are similar to those of aspirin. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception[28.]

The active ingredient in the extract that reduces the inflammation and pain is not known at present. There is ongoing research to isolate and characterize the bioactive compound(s) responsible for the anti-inflammatory and analgesic activity of *A. suma*.

Hence the presence of flavonoids in the methanol extract of *Acacia suma* may be contributory to its anti-inflammatory and analgesic activity.

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Table 1: Acute anti-inflammatory activity of methanol extract of the barks of *A. suma* on carrageenan induced rat paw oedema

G	Treatment	Dose ml/kg	Paw Volume (ml)			
			0h	1h	2h	4h
I	Control	2	0.43 ± 0.01	0.59± 0.05	0.73± 0.02	0.67±0 .02
II	Aspirin	200	0.42 ± 0.02	0.43± 0.01** (27.11 %)	0.47± 0.02** (35.61 %)	0.45±0 .02** (32.83 %)
III	Methanol extract	100	0.44 ± 0.03	0.58± 0.02 (1.69%)	0.53± 0.04** (27.39 %)	0.50±0 .03** (25.73 %)
IV	Methanol extract	200	0.43 ± 0.03	0.57± 0.01 (3.38%)	0.51± 0.03** (30.13 %)	0.48±0 .02** (28.35 %)
V	Methanol extract	400	0.42 ± 0.02	0.54± 0.02 (8.47 %)	0.49± 0.02** (48.97 %)	0.47±0 .02** (29.85 %)

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 edema.

Table 2: Evaluation of analgesic activity of methanol extract of the barks of *A. suma* by acetic acid induced writhing in mice

Group	Treatment	Dose ml/kg	Avg. no. of writhing	Percentage Inhibition
I	Control	3	39.05±2.82	-
II	Aspirin	200	14.15±2.2**	63.76
III	Methanol extract	100	34.75±2.57	11.01
IV	Methanol extract	200	25.67±2.21**	34.26
V	Methanol extract	400	21.6±1.46**	44.68

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.

Table 3: Evaluation of analgesic activity of methanol extract of the barks of *A. suma* by tail immersion method in mice

G	Treatment	Dose ml/kg	Average tail withdrawing time (Sec)				
			0 min	15 min	30 min	45 min	60 min
I	Control	3	4.05 ± 0.08	3.87 ± 0.13	3.9 ± 0.25	4.05 ± 0.2	4.07 ± 0.17
II	Pentazocine	30	4.12 ± 0.31	7.2 ± 0.14**	9.25 ± 0.36**	9.7 ± 0.4**	9.57 ± 0.34**
III	Methanol extract	100	4.1 ± 0.37	4.43 ± 0.22	4.82 ± 0.23*	5.26 ± 0.15**	4.21 ± 0.19**
IV	Methanol extract	200	4.25 ± 0.29	4.82 ± 0.24*	7.36 ± 0.1**	8.31 ± 0.14**	6.3 ± 0.2**
V	Methanol extract	400	4.37 ± 0.26	8.04 ± 0.23**	9.07 ± 0.14**	10.09 ± 0.11**	9.77 ± 0.15**

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.

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