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Analgesic, anti-inflammatory and antipyretic properties of *Acacia suma* stem bark

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Abstract

Acacia suma (Fabaceae) is a medium sized erect tree found in the greater part of India. Present study was carried out for evaluation of ethanolic extract of stem bark of *Acacia suma* (EEAS) at 200 and 400 mg/kg, p.o. for analgesic, anti-inflammatory and antipyretic activity. EEAS was screened for analgesic activity by writhing, tail flick, tail immersion and hot plate method in mice. The anti-inflammatory activity by acute carrageenan induced paw oedema and chronic Freund's adjuvant arthritis models in rats. The antipyretic activity was evaluated using Brewer's yeast induced pyrexia in rabbits. Acute toxicity in mice was found to be higher than 2000 mg/kg., p.o. Analgesic activity revealed that test dose of 400 mg/kg, p.o., had significant activity in various teste models.

Anti-inflammatory studies at 200 and 400 mg/kg., p.o., of extract showed significant activity ($P<0.01$). The extract showed significant ($P<0.01$) effect on yeast-induced fever in rabbits in dose dependant manner. Preliminary phytochemical tests revealed presence of carbohydrates, tannins, alkaloids, saponins and phenolic compounds in the ethanol extract of *A. suma* bark. The present study therefore provides scientific base for its use in the folklore remedies as an analgesic, anti-inflammatory and antipyretic properties of natural origin.

Keywords: Acacia suma stem bark, Acute toxicity study, Tail flick method, Freund's adjuvant arthritis, Brewer's yeast induced pyrexia.

Introduction

The use of herbal heritage has become a part of general health care by the tribes since time immemorial. The use of modern medicines of synthetic origin imparting dramatic results in a short span in the therapeutic field laid several side effects upon long term use. Traditional medicaments, chiefly obtained from plants have played a vital role in sustaining disease free human existence on this planet. It is rather difficult to date back the origin of these medicaments as a means of therapy. In spite of overwhelming influence of modern medicine and tremendous advances made on the production of synthetic drugs, traditional medicaments designated now a days as herbal drugs in different places in literature, have retained their place in therapy. Their effectiveness, low cost and comparative freedom from serious toxic effects makes these medicaments not only popular but also an acceptable mode of treating diseases even in modern times [1,2].

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 [3,4]. The dried stem bark is used as folklore medicine in the treatment of anemia, uterine complaints and reported to possess astringent, analgesic, anti-inflammatory and antiseptic properties [5]. The seeds are reported to have hypoglycaemic effect and bark is reported to be used as blood purifier, possesses anti-cancer and astringent properties [6], similarly the various extracts of stem bark is also reported for hypoglycaemic activity in normoglycaemic and alloxan induced hyperglycaemic rats [7] and according to Mondal [8] reported the diuretic and laxative effects of aqueous extract of *A. suma* barks at 200 and 400 mg/kg, p.o. Acacia polyphenol also inhibited the lipase and glucosidase activities [9]. Indole alkaloid namely tryptamine, N-N- dimethyl is isolated from leaves [10]. Presence of proanthocyanidin, quercetin [11] and 5, 4'-dihydroxy-7, 3'-dimemethoxyflavone-3-0-D galactopyranoside in the stem bark have been reported earlier Ayub *et a* [12]. Keeping this in view, the present study was undertaken to investigate analgesic, anti-inflammatory and antipyretic properties of ethanolic extract of *Acacia suma* (EEAS) stem bark in experimental animal model to provide a scientific support to the folklore claims.

Materials and Methods

Plant material

The stem bark were collected from young matured plant from Ganjam district of Odisha and authenticated by the taxonomists of 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. The material was washed, shade dried and powdered.

Preparation of the extract

The powdered plant materials were defatted with petroleum ether (60^0-80^0C) in a soxhlet extractor. The marc was then air-dried and extracted with ethanol (90%) and then the ethanolic extract was concentrated in rotary evaporator (*Evator*, Media Instrument Mfg. Co., Mumbai, India) at reduced pressure to obtain a dark greenish-brown residue (12.75%). Preliminary phytochemical studies were performed on the extract using standard procedures [13].

Animals

Animals used in this study were male Swiss albino mice (20-25 g), Wistar rats of both sex (150-210 g) and New Zealand white rabbits of both sex (1.5-2.0 kg). 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 ± 2^0C 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 GITAM Institute of Pharmacy, Visakhapatnam, Andhra Pradesh, India (Regd. No.1287/ac/09/CPCSEA). 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 [14]. The control group received only vehicle (2 ml/kg, p.o.). The other groups separately received 100, 200, 400, 600, 800, 1000 or 2000 mg/kg, p.o. 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 Bose *et al* [15]. 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. Group I serve as control received only vehicle (3 ml/kg, p.o.), the second group received aspirin (200 mg/kg, p.o.), which was used as reference standard for activity comparison; group III and IV received ethanol extract (200 and 400 mg/kg, p.o). The writhing effect indicated by stretching of abdomen with simultaneous stretching of at least one hind limb. The percentage inhibition was calculated by Mondal et al method [16].

Evaluation of analgesic activity by tail flick method

Before the study [17], Swiss albino mice were screened for sensitivity test by placing the tip of the tail on the radiant heat source. Any animals that held to withdraw its tail in 5 second were rejected from the study. The selected animals were divided into four groups of six mice each the first group received vehicle (3 ml/kg, p.o.); the second group received pentazocine (30 mg/kg p.o.); other groups received doses of ethanol extract *A. suma* bark (200 and 400 mg/kg, p.o.) respectively. Analgesia was assessed with tail flick apparatus (Analgesiometer). The basal reaction time was measured initially and another set of four measures were taken as 15, 30, 45 and 60 minutes interval and the reaction of the animals considered as the post drug reaction time. A cut-off period of 10 sec. was observed to prevent tissue damage of the tail of the animals.

Evaluation of analgesic activity by tail immersion method

The tail immersion test was carried out as described by Bose *et al* [15]. In this method, Swiss albino mice weighing between 20-25 g, deprived of food and water for 18 hours prior to the experiment, were divided in four groups of six mice in each. 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, p.o.) or ethanol extracts (200 or 400 mg/kg, p.o.). The distal part of the tails of the animals was immersed in hot water maintained at 55.0±1.0^0C. The time taken to withdraw the tail was noted as reaction time. A cut off time 10 sec was maintained at 55.0^0C to prevent tissue damage. The reaction time measured at 0, 15, 30, 45 and 60 min after treatment.

Evaluation of analgesic activity by hot-plate test

Mice (20-25g) of both sexes were fasted overnight before the study. Hot-pate was used to measure response latencies according to the methods of Reanmongkol, *et al* [18]. In this study, the hot-plate was maintained at 55 ± 1^0C and the animals were individually placed on the heated surface. The time in seconds between placement and shaking, paw licking and jumping off the plate was recorded as response latency. Four groups of six animals each the first group received vehicle (3 ml/kg, p.o.); the second group received morphine sulphate (10 mg/kg, p.o.); other groups received doses of ethanol extract A. suma bark (200 and 400 mg/kg, p.o.) respectively. Measurements were taken at zero, 30, 60 and 120 minutes after the treatment of animals.

Evaluation of anti-inflammatory activity

Carrageenan-induce rat paw oedema

The test was performed as per the method of Mondal *et al* [16]. The animals were divided into four groups. The control group was given the vehicle (2 ml/kg) through oral route. Other groups received diclofenac (12.5 mg/kg, p.o.); ethanol extract of *A. suma* stem bark (200 and 400 mg/kg, p.o.) 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.

Adjuvant-induce polyarthritis

The arthritic syndrome was induced in rats by an injection of 0.1 ml of Freund's complete adjuvant into the subplantar region of the right hind paw [15]. Animals were treated orally either vehicle (2 ml/kg, p.o.), diclofenac (12.5 mg/kg, p.o.), ethanol extract (200 and 400 mg/kg, p.o.) for 30 days. Plethysmographic determination of paw volume was performed on both injected and contra-lateral foot. Paw volume after 18 h of adjuvant injection was taken as subacute phase of inflammation and that of the 30th day was observed as an index of chronic inflammation.

Evaluation of antipyretic activity

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rabbits [15]. Fever was induced by injecting 3 ml/kg (s.c.) of 10% aqueous suspension of Brewer's yeast in normal saline below the nape of the neck. After 5 hr, animals showing at least an increase of 1^{0}C of rectal temperature were selected for the experiment. The test animals were administered with vehicles (3 ml /kg, p.o.), Paracetamol 100 mg/kg, p.o., or EEAS (200 and 400 mg/kg, p.o.) orally. The rectal temperature was measured at 1, 3 and 5 h 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. P value < 0.05 was considered to be significant. All the values were expressed as mean ± SEM.

Results and Discussion

Preliminary phytochemical tests revealed the presence of saponins, carbohydrates, alkaloids, flavonoids, tannins and phenolic compounds in ethanolic extract of *Acacia suma* (EEAS) stem bark. In acute toxicity study, EEAS when administered orally to mice in graded doses 100 to 2000 mg/kg, p.o., EEAS did not produce any significant changes in general behavior, cutaneous effects, breathing, sensory nervous system responses and GI effects during the study. However, there was no mortality in tested doses at the end of 14 days of observation.

Oral administrations of EEAS significantly ($P < 0.01$) reduce the writhings induced by acetic acid in mice; the activity was compared with that of aspirin (Table 1). Analgesic studies against thermal noxious stimuli the extract shows dose dependent analgesic effect (Table 2 and 3). In tail flick method, EEAS at 200 mg/kg, p.o., showed significant activity (3.70 ± 0.24) ($P < 0.05$) after 30 minutes whereas at a dose of 400 mg/kg, p.o., showed significant analgesic activity (3.78 ± 0.33) ($P < 0.05$) at 15 minutes but in tail immersion method, EEAS showed significant activity after 30 minutes interval of experiment at all tested dose levels. Pentazocine (30 mg/kg p.o.) used as standard, which showed significant activity throughout the

Table 1: Evaluation of analgesic activity of ethanol extract of the stem bark of *A. suma* by acetic acid induced writhing in mice

Treatment	Dose	Avg. no. of writhing	Percentage Inhibition
Control	3 ml/kg	37.16±2.16	-
Aspirin	200 mg/kg	13.5±2.37**	63.67
EEAS	200 mg/kg	24.83±1.88**	33.18
	400 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 2: Evaluation of analgesic activity of ethanol extract of *A. suma* stem bark by tail flick method in mice

Treatment	Dose	Average tail withdrawing time (Sec)				
		0 min	15 min	30 min	45 min	60 min
Control	3 ml/kg	2.33±0.41	2.38±0.21	2.14±0.33	2.30±0.15	2.10±0.28
Pentazocine	30mg/kg	2.60±0.28	4.14±0.41*	5.50±0.31**	6.93±0.72**	8.26±0.59**
EEAS	200 mg/kg	2.02±0.14	2.48±0.19	3.70±0.24*	5.68±0.54**	4.60±0.49*
	400 mg/kg	2.62±0.36	3.78±0.33*	5.00±0.43**	6.31±0.22**	7.28±0.56**

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 3: Evaluation of analgesic activity of ethanol extract of *A. suma* stem bark by tail immersion method in mice

Treatment	Dose	Average tail withdrawing time (Sec)				
		0 min	15 min	30 min	45 min	60 min
Control	3 ml/kg	2.00±0.22	2.40±0.19	2.54±0.13	2.60±0.19	2.16±0.15
Pentazocine	30mg/kg	2.43±0.11	4.10±0.41*	5.60±0.31**	7.00±0.23**	8.50±0.21**
EEAS	200 mg/kg	2.18±0.15	3.58±0.19*	4.60±0.21*	6.10±0.23**	7.20±0.57*
	400 mg/kg	2.62±0.36	3.38±0.28*	4.90±0.43**	6.60±0.42**	7.80±0.45**

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.

course of study. The result of hot plate method (Table 4) indicated that the reaction time for mice was significantly increased in a dose dependent manner with percentage analgesic activity of 16.81% at 200mg/kg, p.o. and 54.47% at 400mg/kg, p.o., whereas (standard drug) morphine sulphate showed 89.86% at 10 mg/kg, p.o., at second hour of oral administration. In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects [19]. EEAS protected against both thermal and chemical induced stimuli, which were evidence from tail flick, tail immersion, hot-pate and acetic acid induced writhing test. 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. EEAS also produced antinociceptive activity and thus indicates the presence of analgesic components that might influence the prostaglandin pathways [20]. In the radiant heat tail immersion, hot-pate and tail-flick test the plant extract prolonged the stress tolerance capacity of the mice, indicating the possible involvement of a higher center [21]. EEAS at doses of 200 and 400 mg/kg was shown significant decrease in yeast-induced fever. This result seems to support the view that the extract has some influence prostaglandin biosynthesis because prostaglandin is believed to be regulator of body temperature [15].

Table 4: Effect of ethanol extract of *A. suma* stem bark on pain induced by hot plate method

Treatment	Dose	Latency period			
		0 min	30 min	60 min	120 min
Control	3 ml/kg	4.74±0.35	4.6±0.17	3.60±0.17	3.86±0.22
Morphine sulphate	10 mg/kg	4.34±0.11	6.14 ±0.17**(41.47)	7.30±0.66**(68.20)	8.24±0.35**(89.86)
EEAS	200 mg/kg	4.40±0.09	4.46±0.04(1.36)	4.72±0.08*(7.27)	5.14±0.25**(16.81)
	400 mg/kg	3.76±0.11	5.13±0.08*(36.43)	5.14±0.25**(36.70)	5.82±0.06**(54.47)

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.

The inhibitory activity on carrageenan-induced acute inflammation model is represented in (Table 5). Diclofenac, a cylcooxygenase inhibitor, at dose of 12.5 mg/kg, p.o., exhibited significant paw edema inhibition, whereas EEAS at dose of 200 and 400 mg/kg, p.o., body weight possessed significant inhibitory effect on paw edema. Similarly oral administration of EEAS at 200 and 400 mg/kg p.o., in adjuvant-induce polyarthritis significantly inhibited paw volume edema in a dose dependent manner ($P < 0.01$) after 30 days course of study (Table 6). The effect of yeast-induced pyrexia in rabbits is depicted in Table 7. It was found that EEAS also showed significant lowering of body temperature in a dose dependant manner. Subcutaneous (s.c) injection of yeast suspension markedly elevated the rectal temperature after 5 h of administration. EEAS treatment with tested dose reduced the rectal temperature of rabbits in dose dependent manner. Both the EEAS and Paracetamol 100 mg/kg, p.o., standard drug significantly reduced the yeast elevated rectal temperature compared to control group. Carrageenan-induced rat paw edema is a biphasic process [22]. The release of histamine or serotonin occurs in the first phase and the second phase is associated with the production of lysosome, bradykinin, protease and prostaglandin [23]. Therefore, the inhibition of carrageenan-induced inflammation by EEAS could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. In Freund's adjuvant induces arthritis with synovitis and erosions histologically resembling rheumatoid arthritis. Both T cell and B cell activation is important in collagen induced arthritis. Cytokines of both Th1 and Th2 cells are produced, and at disease onset a Th1 profile predominates. It may be thus hypothesised that EEAS inhibits these inflammatory cytokins, as well. The results obtained are comparable with Bose *et al* [15]. Presence of phytoconstituents like saponins, alkaloids, tannins, flavonoids, and phenolic compounds

Table 5: Effect of ethanol extract of *A. suma* stem bark on carrageenan induced rat paw oedema

Treatment	Dose (ml/kg)	Time after 1% carrageenan injection					
		1 h		2 h		4 h	
		EV	% EI	EV	% EI	EV	% EI
Control	2	0.26±0.04	-	0.79±0.04	-	0.70±0.03	-
Diclofenac	12.5	0.06±0.01**	76.92	0.36±0.03**	54.43	0.30±0.02**	57.14
EEAS	200	0.12±0.01*	53.84	0.52±0.07*	34.17	0.39±0.06**	44.28
	400	0.08±0.01**	69.23	0.42±0.01**	46.83	0.31±0.03**	55.71

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. EV = oedema volume (ml) at time; %EI = percent oedema inhibition of test substance at time.

Table 6: Effect of the ethanol extract of *A. suma* stem bark on adjuvant induced paw arthritis in rats

Treatment	Dose	Paw volume (ml)		
		0 h	18 h	30th day
Control	2 ml/kg	0.33±0.01	0.72±0.02	0.64±0.02
Diclofenac	12.5 mg/kg	0.31±0.02	0.48±0.01**(33.33)	0.28±0.02**(56.25)
EEAS	200 mg/kg	0.34±0.01	0.63±0.02*(12.5)	0.38±0.02**(40.62)
	400 mg/kg	0.32±0.01	0.59±0.02*(18.05)	0.32±0.01**(50.00)

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 7: Effect of EEAS on yeast induced pyrexia in rabbit

Treatment	Dose	Rectal temperature (^{0}C)		Rectal temperature after administration of drugs (^{0}C)		
		Normal	5 h after yeast administration(0 h)	1 h	3 h	5 h
Control	3 ml/kg	38.5±0.1	40.0±0.1	40.0±0.2	40.0±0.2	39.6±0.2
Paracetamol	100 mg/kg	38.7±0.3	40.7±0.2	39.1±0.2**	38.3±0.2**	38.2±0.2**
EEAS	200 mg/kg	38.5±0.3	40.0±0.2	39.6±0.1	39.4±0.2*	38.6±0.1*
	400 mg/kg	38.8±0.4	39.9±0.2	39.4±0.2*	38.4±0.2**	38.3±0.2**

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.

has been previously found to be responsible for analgesic, anti-inflammatory and antipyretic activities in plants [15]. The presence of the above said phytoconstituents in EEAS may be probably responsible for the observed activities.

Conclusion

In conclusion, the present study demonstrated that the ethanol extract obtained from stem bark of *Acacia suma* exhibited analgesic, antipyretic, acute and chronic anti-inflammatory properties in the different tested experimental animal models. The acute toxicity studies revealed no mortality was recorded. The present study therefore provides scientific base for its use in the folklore remedies as an analgesic, anti-inflammatory and antipyretic drug of natural origin. Hence there is a need of further study to identify the chemical entity and characterization of the active constituents from the plant extract.

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