THE ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF THE EXTRACT OF *ALBIZIA LEBBECK* IN ANIMAL MODEL

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ABSTRACT

The extract of the bark of *Albizia lebbeck* Benth. 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: *Albizia lebbeck*, analgesic activity, writhing response, 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.

Albizia lebbeck Benth. (Bengali name: Shirish, Koroi; Family: Leguminosae) is a deciduous tree with compound leaves, flat oblong fruits, round cream colored seeds, grows wild and planted in almost all districts of Bangladesh (Ghani, 2003). The plant is found throughout India, Bangladesh, tropical and subtropical Asia and Africa (Kirtikar and Basu, 1980). Barks are used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhea. Ethanolic extract of pods possesses antiprotozoal, hypoglycemic and anticancer properties. The methanolic extract of the pod was investigated for antifertility activity (Gupta *et al*., 2004; 2005). The plant extract also evaluated in allergic rhinitis (Pratibha *et al*., 2004) and memory and learning of mice (Chintawar *et al*., 2002). Previous phytochemical investigations showed that the pod of the *A. lebbeck* contains 3',5 Dihydroxy 4', 7 dimethoxy flavone and N-Benzoyl L phenyl alaninol (Rashid *et al*., 2003). The beans of the plant contain albigenic acid-a new triterpenoid sapogenin (Barua and Raman, 1959). The plant also contains saponins (Pal *et al*., 1995; Ueda *et al*., 2003), macrocyclic alkaloids (Misra *et al*., 1995; Dixit and Misra, 1997), phenolic glycosides (Maa *et al*., 1997) and flavonols (El-Mousallamy, 1998). Although *A. lebbeck* has traditionally been used in the treatment of many types of pain and inflammatory conditions in Bangladesh, no scientific report is available to date to validate these folkloric uses. As a part of our continuing studies on the medicinal plants of Bangladesh (Ahmed *et al.*, 2001; Saha *et al*., 2007; Shrotriya *et al*., 2007), we now report on the anti-inflammatory and analgesic activities of extract of *A. lebbeck.*

MATERIALS AND METHODS

Plant collection

The bark of *A. lebbeck* was collected from Sherpur in October 2004. A voucher specimen (Voucher No. 1616) was kept at the Department of Botany, University of Dhaka 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 as AL. The extract was dissolved in normal saline by using 0.1% tween-80.

Drugs and Chemicals

Aminopyrine, carrageenan and phenylbutazone were

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Table 1: Anti-inflammatory activity of crude extract of *A. lebbeck* by carrageenan induced rat paw edema

Group	% Increase in Paw Volumes (ml × 1000) ± SEM (percent inhibition)			
	1hr	2hr	3hr	4hr
Control	70.7 ± 2.06	92.8 ± 1.19	107.2 ± 2.27	114.5 ± 3.47
AL (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)
AL (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[a] on acetic acid induced writhing response in mice.

Group	Dose (mg/kg, p.o.)	Writhing[b]	% Inhibition
Control	-	17.30 ± 1.34	-
AL	200	10.41 ± 0.74**	39.90
	400	8.25 ± 0.63**	52.40
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)	Reaction time (sec)[c]		
		30 min (% elongation)	60 min (% elongation)	120 min (% elongation)
Control		4.50 ± 0.15	4.63 ± 0.16	4.98± 0.20
Morphine	2[b]	8.37 ± 0.14** (85.93)	7.15 ± 0.19** (54.32)	6.23 ± 0.25** (25.08)
AL	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.

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 by 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 AL 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 AL (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 edema 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 AL could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis.

The present study on extract of *A. lebbeck* 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 and inflammation.

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