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Antiinflammatory activity of *Albizia lebbeck* (L.) BENTH

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ABSTRACT

Albizia lebbeck (L.) BENTH (Fabaceae), popularly known as *Sirisha*, is employed in Indian folk medicine for the treatment of boils, cough, pain, swelling and it is also used against diarrhea. The anti-inflammatory activity was assessed using acute and subacute models of inflammation in rats. These studies demonstrated that oral administration of ethyl acetate extract of *Albizia lebbeck* (EAL) (100, 200, 400 mg/kg) exhibited significant anti-inflammatory activity. In acute inflammation as produced by carrageenan 62.19% after 5h, by histamine 43.19%, by dextran 45.29%, by serotonin 68.84 while in subacute anti-inflammatory model using formaldehyde-induced hind paw edema (after 5 h) 31.5% and cotton pellets 13.48% protection from inflammation was observed at 400 mg/kg orally dose of EAL. EAL neither show ulcerogenic effect at different doses of EAL nor show any sign of toxicity and mortality up to a dose level of 5000 mg/kg, p.o. in rats & mice. These data indicate that EAL possesses significant anti- anti-inflammatory activity.

Keywords: Acute toxicity, Anti-inflammatory, *Albizia lebbeck*.

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INTRODUCTION

The genus *Albizia* also called silk tree or silk plant, genus of trees or shrubs in the pea family (Fabaceae). The genus is pantropical, though most species are native to warm regions of the old world[1]. *Albizia lebbeck* is a deciduous Tree with an open, large, spreading crown; it usually reaches a height of 15 - 20 meters, with exceptional specimens growing up to 30 meters. In India plantation-grown siris yields a high quality hardwood that is traded in Europe as 'Indian walnut' or 'koko'. Plants are used to provide shade for coffee and cocoa plantations as well as to provide a valuable timber and fuel. It is a popular amenity tree throughout the dry tropics because of its shady spreading habit, although the copious litter produced beneath it is often regarded as a disadvantage[2-4]. *Albizia lebbeck* was known as medicinal plant already in Ayurveda and it has been an important drug for centuries. *Albizia lebbeck* has many medicinal properties like Antiseptic, antibacterial, anti-allergic, antidermatosis, antidysenteric etc. Used in the treatment of Bronchitis, piles, hemicranias, cough, tropical pulmonary eosinophilia, asthma etc. *Albizia lebbeck* is used as an astringent, to treat boils, cough, to treat the eye, flu, gingivitis, lung problems, pectoral problems, to treat abdominal tumors and it is also used as a tonic. The bark is used medicinally to treat inflammation[5-7]. Phytochemically the plant is less explored. Flavone, 3', 5-dihydroxy-4', 7-dimethoxyflavone and a nitrogenous compound, N-benzoyl-L-phenylalaninol, friedelan-3-one and g-sitosterol; Hexaglycosylated saponins, quercetin, unsaturated carboxylic acid methyl ester, a triterpene saponin, albigenic, albigenin, two tri-O glycoside flavonols i.e, kaempferol, quercetin; albizziahexoside (a hexaglycosylated saponins) and cardiac glycoside[8-12]. The biological activities which are attributed to this species are antibacterial, diuretic, analgesic & anti-inflammatory, anti-tumor, *in vitro* antioxidant activity, antimicrobial, anti-larvae, antiulcer, antiviral and ecbolic activities[13-26]. In the present study, *Albizia lebbeck* was selected because it is one of the medicinal plant commonly used in remedies to treat pain, swelling and fever in Indian traditional medicine and other countries in Asia. However, upto date no ethnopharmacological data have previously been systematically conducted to evaluate the anti-inflammatory action supporting traditional uses of this plant in folklore medicine. In this work we evaluate the anti-inflammatory potential of this whole plant (including aerial part and root) in experimental animals using ethyl acetate extract.

MATERIALS AND METHODS

Chemicals

Acetic acid, formalin, carrageenan, histamine, 5-hydroxytryptamine (5-HT), prostaglandin E_2 (PGE_2), bradykinin, diclofenac and phenylbutazone (PBZ) were procured from Sigma Chemicals, St. Louis, MO, USA. Formaldehyde was purchased from Hi-Media Laboratories, Mumbai, India, and the water represents the double distilled water.

Plant material

Whole plant of *Albizia lebbeck* was collected from the Dausa, Rajasthan and after due identification with the help of floras and previous works deposited at Botanical Survey of India, Jodhpur, India herbarium vide voucher specimen number BSI/JODHPUR/AL/1.

Preparation of extract

Two hundred gram of the air dried and coarsely ground plant material was defatted with petroleum ether (60-80°c) by soxhlation for three days and then the solvent free marc was extracted with the ethyl acetate by soxhlation for 4 days and extract was concentrated *in-vacuo* in a rotary evaporator. The dried marc was extracted with distilled water by maceration in a round bottom flask for 24 h. After 24 h it was filtered though muslin cloth followed by buchner funnel. Maceration process was repeated using marc for another two times and all the three filterates were collected and combined. The solvent was removed by *in-vacuo* in rotary evaporator. The dried extracts were placed in a desiccator and used for further studies.

Animals

Thirty animals made up of equal number of male and female rats were used for this study in a complete randomized design. Animals were randomly divided into five groups with six animals per group and were orally administered as the following: Group 1, control was orally administered, once daily with 1mL of distilled water (vehicle); Groups II, III, IV, were treated with 100, 200 and 400mg/kg body weight of the ethyl acetate extract of *Albizia lebbeck*, respectively, and Group V received diclofenac (10 mg/kg body weight) reference drug, which served as a positive control. Oral administration was carried out using a metal oropharyngeal cannula. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the Institutional Ethical Committee.

Acute toxicity study

The acute toxicity test of the ethyl acetate fraction of *Albizia lebbeck* was done by up and down method [in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines, 2001]. Overnight fasted albino rats were used for study (three animals in group). The fraction was administered to all the three animals in a group at a starting single dose of 5 mg/kg. Animals were observed for a period of 2 hours, then occasionally for 4 hours for severity of any toxic signs and mortality. When no mortality was observed the same dose would be additionally administered to one more animal. If no mortality is observed at this dose, the same procedure would be repeated for dose levels of 50, 300, 2000 and 5000 mg/kg of fraction on separate newer groups. The behavioral changes closely observed for were: hyperactivity, ataxia, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The animals were kept under observation up to 14 days after drug administration to find out any delayed mortality[27].

Anti-inflammatory activity

A. Carrageenan induced rat paw edema

Carrageenan induced rat paw edema was done by the method of[28]. Inflammation was induced by injection of 0.1 ml of freshly prepared carrageenan (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats. The different groups of rats were administered with EAL (100, 200 and 400 mg/kg, p.o.) and diclofenac (10 mg/kg, p.o.). The control group received vehicle (distilled water, 10 ml/kg, p.o.). 1 h after drug treatment, paw edema was induced by the injection of carrageenan (an edematogenic agent). The paw volume was measured by a Plethysmometer. The measures were determined at 0 h (Vo: before edematogenic agent injection) and 1,2,3,4 and 5h intervals later (Vt). The difference between Vt and Vo was taken as the edema value. The percentage of inhibition was calculated according to the following formula:

$$\text{inhibition} = \frac{(Vt - Vo)\text{control} - (Vt - Vo)\text{treated}}{(Vt - Vo)\ \text{control}} \times 100$$

B. Histamine induced rat paw edema

Inflammation was induced by injection of 0.1 ml of freshly prepared histamine (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats[29]. The drug treatment and paw volume was measured in a similar manner to that of carrageenan induced paw edema model.

C. Dextran induced rat paw edema

Inflammation was induced by injection of 0.1 ml of freshly prepared dextran (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats[30]. The drug treatment and paw volume was measured in a similar manner to that of carrageenan induced paw edema model.

D. Serotonin induced rat paw edema

Inflammation was induced by injection of 0.1 ml of freshly prepared Serotonin (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats[31-32]. The drug treatment and paw volume was measured in a similar manner to that of carrageenan induced paw edema model.

E. Formaldehyde induced rat paw edema

Inflammation was induced by injection of 0.1 ml of freshly prepared Formaldehyde (3%) underneath the plantar tissue of the right hind paw of rats[33]. The test drug was administered consecutively for seven days to all the groups. On seventh day, after 1 h of drug administration, paw edema of the rat was induced by subplantar injection of formaldehyde solution. The paw volume was determined at 0 h and at 3, 24 and 48 h after formaldehyde injection as described in carrageenan model.

F. Cotton pellet induced granuloma in rats

The effect of Group III: Ethyl acetate extract of *Albizia lebbeck* on the chronic phases of inflammation was assessed in the cotton pellet induced granuloma rat model[34]. Autoclaved cotton pellets weighing 100 mg each were implanted subcutaneously. One on each side of the abdomen of the animal, through a small ventral incision of rats

anesthetized with ether. The different groups of rats were administered with EAL (100, 200 and 400 mg/kg, p.o.) and diclofenac (10 mg/kg. p.o.) once daily for 7 consecutive days from the day of cotton pellet insertion. The control group received vehicle (distilled water, 10 ml/kg, p.o.). On the eighth day the animals were sacrificed and the cotton pellets were removed, dried at 60°C for 24 h and their mass was determined. The results are expressed as mg granulation tissue formed per 100 g body weight.

G. Statistical analyses

The data were expressed as mean ± S.E.M. Results were analyzed statistically by one-way analyses of variance (ANOVA) followed by Tukey's test using the Origin version 6.0 (Microcal, Northampton, MA, USA) for Window software. *P*-value <0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

A. Carrageenan induced rat paw edema

The results of anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* on carrageenan induced paw edema is shown in Table 1 The lower dose i.e. EAL-100 showed inhibition at both early and late phase; though maximum inhibition was at late phase (67.65%, P < 0.01). The higher dose i.e. EAL-400 also showed maximum anti-inflammatory activity at late phase (39.87%) but this activity was less than that of EAL-200 (58.9%) at both early and late phase. The standard Diclofenac-10 showed maximum activity at early phase (55.98%, P<0.01). In this model, the lower dose showed more inhibition of edema formation than standard diclofenac.

Table 1: Anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* in carrageenan induced rat paw edema.

Treatment group	% Increase in paw volume									
	After 1 h		After 2 h		After 3 h		After 4 h		After 5 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	15.89 ± 2.24		24.56 ± 4.56		32.34 ± 2.38		30.13 ± 2.54		29.58 ± 2.36	
EAL-100	5.45 ± 1.42	53.54	7.09 ± 1.67	54.65	12.14 ± 1.78	48.89	11.33 ± 1.76	60.09	8.13 ± 2.65	67.65
EAL-200	7.43 ± .108*	45.65	10.06 ± 1.24**	46.09	14.46 ± 2.14**	52.43	14.87 ± 1.16**	53.76	13.40 ± 2.12	58.9
EAL-400	10.32 ± 1.46	37.87	18.03 ± 2.36	29.65	22.23 ± 2.14	28.56	22.46 ± 3.53	28.9	16.26 ± 3.16	39.87
Diclofenac-10	8.98 ± 2.08	43.45	13.45 ± 1.78*	48.78	12.76 ± 4.88**	55.98	16.76 ± 2.67*	43.23	15.01 ± 2.39*	44.43

Value are expressed as mean ± SEM, (n=6). *P<0.05; **P<0.01.

B. Histamine induced rat paw edema

The results of anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* on histamine induced paw edema is shown in Table 2. In this model, the ethyl acetate fraction of *Albizia lebbeck* at both dose levels and standard diclofenac showed anti-inflammatory activity at late phase. A clear dose dependent inhibition of paw edema was observed. The percentage inhibition of EAL-100 was 33.37% (P<0.05), EAL-200 was 38.82% (P<0.01 while that of EAL-400 was 43.19% (P<0.01). The later was nearer to standard Diclofenac-10 (55.89%, P<0.01).

Table 2: Anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* in histamine induced rat paw edema.

Treatment group	% Increase in paw volume									
	After 1 h		After 2 h		After 3 h		After 4 h		After 5 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	41.12 ± 1.82		45.67 ± 3.81		44.56 ± 2.90		42.18 ± 4.56		39.23 ± 1.79	
EAL-100	38.71 ± 2.59	5.25	38.43 ± 1.62	21.42	33.51 ± 1.32*	30.40	30.32 ± 1.30	32.40	28.47 ± 2.56**	33.37
EAL-200	40.18 ± 1.57*	7.49	35.61 ± 2.60*	25.22	29.17 ± 1.72*	33.18	27.40 ± 2.52*	35.38	24.32 ± 1.73**	38.82
EAL-400	36.25 ± 1.35	13.18	32.86 ± 1.81*	29.40	28.40 ± 2.10*	38.23	25.33 ± 3.14*	40.15	22.17 ± 1.54**	43.19
Diclofenac-10	29.28 ± 2.12*	28.10	27.12 ± 2.16**	39.89	28.60 ± 3.80**	41.16	22.15 ± 2.53**	52.12	20.18 ± 2.34**	55.89

Value are expressed as mean ± SEM, (n=6). *P<0.05; **P<0.01.

C. Dextran induced rat paw edema

The results of anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* on dextran induced paw edema is shown in Table 3. In this model, a clear dose dependent inhibition of paw edema was observed at both early and late phases. The higher dose showed distinctly more inhibition than lower dose at both phases (P<0.05). The anti-inflammatory activity of higher dose was more at later phase (50%) than early phase. The standard diclofenac showed poor anti-inflammatory activity in this model.

Table 3: Anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* in dextran induced rat paw edema.

Treatment group	% Increase in paw volume									
	After 1 h		After 2 h		After 3 h		After 4 h		After 5 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	41.33 ± 3.18		44.74 ± 4.64		39.43 ± 2.69		32.41 ± 4.43		24.37 ± 1.38	
EAL-100	30.19 ± 2.14	28.18	32.18 ± 2.52	25.46	26.32 ± 1.32	29.13	20.48 ± 2.18	33.40	17.18 ± 1.36	17.61
EAL-200	24.15 ± 1.56	22.74	26.73 ± 2.63	27.48	24.13 ± 2.44	32.27	15.43 ± 3.48	40.18	12.37 ± 3.69	32.16
EAL-400	29.14 ± 1.27*	34.37	31.47 ± 3.18*	36.18	30.38 ± 3.67*	38.47	19.79 ± 2.67**	48.37	16.82 ± 4.31*	45.29
Diclofenac-10	39.17 ± 3.17	6.84	41.04 ± 4.37	13.46	37.42 ± 1.30	12.43	27.40 ± 3.81	20.32	24.61 ± 3.62	09.33

Value are expressed as mean ± SEM, (n=6). *P<0.05; **P<0.01.

D. Serotonin induced rat paw edema

The results of anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* on serotonin induced paw edema is shown in Table 4. The maximum paw thickness was observed at 3rd h after sub-planter injection in all groups. The animals treated with lower and higher doses of EAL (100, 200 and 400 mg/kg) produced statistically significant inhibition at 1st h (P<0.05; P<0.01 & P<0.01, respectively), 2nd and 3rd h (P<0.05) and at 5th h (P < 0.01). Standard drug diclofenac-10 showed significant decrease in paw volume at 1st, 2nd h (P<0.05) and at 3rd, 4th and 5th h (P<0.01). The maximum decrease of paw volume in both the doses of EAL and diclofenac treated groups was found at 5th h (F<0.001). The percent inhibition of EAL-400 group at 5th h was same as that of standard group. The extract effectively suppressed the inflammation produced by serotonin.

Table 4: Anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* in serotonin induced rat paw edema.

Treatment group	% Increase in paw volume									
	After 1 h		After 2 h		After 3 h		After 4 h		After 5 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	15.32 ± 1.62		20.46 ± 2.15		33.46 ± 2.64		28.34 ± 1.28		23.46 ± 2.62	
EAL-100	8.14 ± 2.15	44.37	12.40 ± 3.36	38.46	16.13 ± 1.48	43.10	15.27 ± 1.54	41.07	6.43 ± 1.02	68.84
EAL-200	5.32 ± 1.62*	49.40	13.17 ± 1.02	34.31	18.14 ± 2.46*	46.32	14.32 ± 1.63**	47.40	7.38 ± 1.86**	54.32
EAL-400	6.18 ± 2.35**	57.18	15.40 ± 2.48	40.11	19.40 ± 1.37*	48.13	13.65 ± 2.65*	49.47	5.14 ± 0.89**	58.40
Diclofenac-10	7.07 ± 3.44*	61.08	10.86 ± 3.18*	64.16	13.45 ± 2.43**	67.32	8.47 ± 3.67**	72.46	5.23 ± 1.36**	75.43

Value are expressed as mean ± SEM, (n=6). *P<0.05; **P<0.01.

E. Formaldehyde induced rat paw edema

The results of anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* in formaldehyde induced paw edema is shown in Table 5. Injection of formaldehyde subcutaneously into hind paw of rats produces localized inflammation. The administration of EAL-100, EAL-200 & EAL-400 and diclofenac-10 daily for 7 days successfully significantly (F<0.001) inhibited edema induced by formaldehyde. EAL-100, EAL-200 and EAL-400 group showed maximum decrease in paw volume at 3 h (P<0.05, P<0.001 and P<0.01 respectively). Diclofenac-10 group showed decrease in paw volume at 3 h (44.37%, P<0.001) and the decreased in paw volume at 48 h was almost same (45.87%, P<0.01).

Table 5: Anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* in formaldehyde induced rat paw edema.

Treatment group	% Increase in paw volume					
	After 3 h		After 24 h		After 48 h	
	Vol. Increase	% Change	Vol. Increase	% Change	Vol. Increase	% Change
Control	52.16 ± 2.69		56.14 ± 2.46		42.18 ± 4.84	
EAL-100	38.4 ± 2.16	33.14	41.14 ± 3.78	32.47	35.67 ± 2.46	23.4
EAL-200	33.16 ± 1.45*	35.36	36.18 ± 2.14**	34.48	32.40 ± 1.49	27.6
EAL-400	31.42 ± 2.64**	40.42	35.40 ± 3.67*	37.63	29.47 ±2.69*	31.54
Diclofenac-10	28.40 ± 1.20***	44.37	32.42 ± 2.47**	41.68	21.26 ± 4.95**	45.87

Value are expressed as mean ± SEM, (n=6). *P<0.05; **P<0.01, ***P<0.001

F. Cotton pellet induced granuloma in rats

The results of anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* in cotton pellet induced granuloma is shown in Table 6. EAL-100, EAL-200 and EAL-400 groups showed 1.46%, 1.32% and 13.48% decrease in granuloma formation respectively as compared to control group, while standard diclofenac-10 group showed significant decrease in granuloma formation (33.04%, P<0.001).

Table 6: Anti-inflammatory activity of ethyl acetate fraction of *Albizia lebbeck* in cotton pellet induced granuloma in rats.

Treatment group	Pellet weight (g / 100 g b.w.)	% Change
Control	0.112 ± 0.002	
EAL-100	0.109 ± 0.005	1.46
EAL-200	0.106 ± 0.004	1.32
EAL-400	0.98 ± 0.005	13.48
Diclofenac-10	0.72 ± 0.005***	33.4

Value are expressed as mean ± SEM, (n=6). ***P<0.001

CONCLUSION

In conclusion, the present study clearly showed that ethyl acetate extract of whole plant (including aerial part and root) of *Albizia lebbeck* possessed good anti-inflammatory activity and also scientifically validated the traditional use of this plant for treating inflammatory disorders in the folk medicine. The advantages of this extract, *viz*., better and safer anti-inflammatory profile deserves further studies to identify the possible mechanism of action as well as establishing the therapeutic value in the treatment of inflammatory diseases.

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