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Research article

Estimation of Anti-inflammatory Activity of the Ethanolic Extract of the Bark of *Albizia lebbeck* in Rat.

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ABSTRACT

The medicinal tree *Albizzia lebbeck* (Fabaceae), commonly referred to as Sirisha, is used in Indian traditional medicine to treat boils, coughs, pain, swelling, and diarrhea. Additionally, it is used in the traditional Indian Ayurvedic medical system to treat a variety of inflammatory conditions. In order to bolster the ethnopharmacological claims, the current study set out to assess any potential anti-inflammatory activity of the ethanolic extracts of A. lebbeck's barks (EEAL). Wistar rats were used in the investigation. By employing the Soxhlet extraction method, the EEAL were produced. Models of paw edema caused by carrageenan were used to investigate the anti-inflammatory properties. These investigations showed that EEAL (200, 400 mg/kg) administered orally had a markedly anti-inflammatory effect.

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INTRODUCTION

The genus Albizia, which includes trees and shrubs in the pea family (Fabaceae), is also known as the silk tree or silk plant. Although most species in the genus are native to warm parts of the old world, it is a pantropical genus [1]. *Albizia lebbeck*is a deciduous tree that typically grows to a height of 15 to 20 meters, though some exceptional examples can reach up to 30 meters. It has an open, large, spreading crown. Plantation-grown siris in India produces a premium hardwood known as "Indian walnut" or "koko" that is sold in Europe. In addition to providing valuable timber and fuel, plants are used to shade coffee and cocoa plantations. Its spreading habit provides shade, making it a popular amenity tree in the dry tropics, though its abundant litter production is sometimes considered a drawback [2-4]. For centuries, *Albizia lebbeck* has been used as a significant medication and was already recognized as a medicinal plant in Ayurveda. Numerous health benefits of *Albizia lebbeck* include its antiseptic, antibacterial, antiallergic, antidermatosis, and antidysenteric qualities, used to treat a variety of conditions, including tropical pulmonary eosinophilia, asthma, hemicranias, piles, and pneumonia. *Albizia lebbeck* is used as a tonic, an astringent, for the treatment of abdominal tumors, boils, cough, eye conditions, gingivitis, flu, and pectoral problems. Inflammation is treated medicinally with the bark [5-7]. The plant's phytochemistry is not well studied. Flavone, 3-hydroxy-4', 5-dihydroxy-4', 7-dimethoxyflavone, and a nitrogenous compound, N-benzoyl-L-phenylalaninol, friedelan-3-one, and g-sitosterol; Quercetin, unsaturated carboxylic acid methyl ester, a triterpenesaponin, albigenic, albigenin, two tri-O glycoside flavonols, namely, quercetin and kaempferol; Albizziahexoside, a hexaglycosylated saponin, and cardiac glycoside [8–12]. Antibacterial, diuretic, analgesic & anti-inflammatory, anti-tumor, in vitro antioxidant activity, antimicrobial, anti-larvae, antiulcer, antiviral, and ecbolic activities are among the biological activities linked to this species [13–26]. *Albizia lebbeck* was chosen for this study because, in traditional Indian medicine as well as other Asian countries, it is a commonly used medicinal plant in remedies to treat pain, swelling, and fever. Nevertheless, to date, no ethnopharmacological research has been done methodically to assess the plant's anti-inflammatory properties, which supports the plant's traditional uses in folk medicine. In this study, we use ethanol extract of the bark of this plant to assess the plant's overall anti-inflammatory potential in experimental animals.

MATERIALS AND METHODS

MATERIALS

Plant materials

The barks of *Albizia lebbeck* was collected from local village of Berhampur in the month of February, 2023 and were authenticated. The plant parts was washed properly, dried under shade and stored in an air tight container.

Animals

As per the OECD draft guidelines 423 received from CPCSEA, young female albino mice were used for acute toxicity study. Whereas other in vivo methods were carried out by using Sprague-Dawley (SD) rats of both sexes. All the animals for the in vivo studies, with no prior drug treatment, were procured from the animal house of Royal College of Pharmacy and Health Sciences (R.C.P.H.S.), Berhampur and housed in polypropylene cages with clean sterilized husk bedding (six mice or three rats/ cage). Bedding was changed every alternate day to maintain proper hygienic condition. Animals were maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5°C) with a 12:12 hour light: dark cycle.

The animals were fed with standard laboratory food pellets and pure drinking water ad libitum. The animals were acclimatized to laboratory hygienic conditions in the departmental laboratory for 7 days before commencing the experiment. The ethical clearance was granted for the study by Institutional animal ethics committee (IAEC) of Royal College of Pharmacy and Health Sciences (R.C.P.H.S.), Berhampur.

METHODS

Preparation of extracts

Dried and powdered plant materials (100 gm) were extracted by successive extraction process using soxhlet apparatus. Solvents were chosen depending upon their increase in polarity like Petroleum Ether (60-80° C), Chloroform, and Ethanol. The extraction was carried out for 72 hours for each solvent. All the extracts were dried using rotary vacuum evaporator and freeze dryer. Their percentage yields were determined and stored in desiccator until further use.

Phytochemical screening

Extracts obtained from the above extraction process were analyzed for presence of various phytoconstituents such as alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, fats and sugars by the method of preliminary phytochemical study (colour reactions) [15, 16, 17] .

Acute toxicity studies

The acute oral toxicity studies of extracts were carried out as per the OECD guidelines. Administration of stepwise doses of dried extracts of *Albizia lebbeck*, from the dose of 100 mg/kg up to2000

mg/kg, to young female albino mice and observed the signs of toxicity in the tested animals [18].

The albino mice were divided into different groups of six animals each. The control group received 5 ml/kg of distilled water orally. The other groups received the ethanolic extracts of *Albizia lebbeck* at dose levels of 100, 500, 1000, 1500, 2000mg/kg through oral route.

After administration of dose the animals were observed continuously for the first 4 hr and occasionally up to 24 hr and at the end of 72 hr for recording mortality, if any [19]. Additional observations like behavioral changes, somato motor activity, tremors, convulsions, tonic extension, stub tail, muscle spasm, loss of righting reflex, ataxia, sedation, hypnosis, lacrimation, diarrhoea, salivation, writhing, changes in skin, fur, eyes, mucous membranes etc were recorded [19]. One tenth of upper limit dose; and its half and double dose were selected as the levels for examination of therapeutic activity.

Carrageenan induced paw edema

Acute inflammation or edema was induced by injection of 0.1 ml of carrageenan (1 % in 0.9 % sterile saline solution) into the rat's sub plantar surface of right hind paw region. The vehicle was administered 30 min. prior to injection of carrageenan and indomethacin was orally administered 1h prior to the injection of carrageenan. The pedal volume up to the ankle joint was measured using a digital plethysmometer at 1st, 2nd, 3rd, 4th, 5th and 6th h. The percentage inhibition of edema volume between drug treated and carrageenan alone treated groups were calculated as follows.

$$\text{Percentage Inhibition} = \{(Vc-Vt)/Vc\} \times 100$$

Where, Vc-Vt and Vc represented the mean increase in paw edema volume in control and drugtreated groups.

Table 1: Animals are grouped into five categories which are as follows,

S.NO	GROUPS	TREATMENT	ROUTE
I.	CONTROL	1.0 ml (Normal saline) – [7 days]	p. o
II.	CARRAGEENAN	0.1 ml (1% in 0.9 % sterile saline solution) – [8th day]	I. p
III.	INDOMETHACIN+CARRAGEENAN	10 mg/kg – (7 days + 8th day)	p. o
IV.	EEAL 200 mg/kg +CARRAGEENAN	1.0 ml – (7 days + 8th day)	p. o
V.	EEAL 400 mg/kg +CARRAGEENAN	1.0 ml – (7 days + 8th day)	p. o|

Statistical Analysis

The mean value ± SEM calculated for each parameter. Results were subjected to statisticalanalysis using ONE-WAY ANOVA, followed by Dunnet's t-test. The values were considered significant when P< 0.001, it was calculated using Graph pad prism.

RESULTS AND DISCUSSION

Percentage of yield (w/w), colour and consistency of different extracts:

The extract of EEAL was filtered and then it was concentrated by distilling off the solvent to obtain the crude extract. The extractive values, colour is tabulated below.

Table 2: Percentage of yield (w/w) and colour of different extracts of EEAL

SL. NO.	SOLVENT	%YIELD (W/W)	COLOUR	CONSISTENCY
01	Petroleum Ether (60-80)	2.34%	Pale yellow	Greasy mass
02	Ethanol	11.75%	Reddish brown	Dry powder

Phytochemical studies:

The EEAL obtained from the above extraction process were analysed for different phytoconstituents present in it by the method of qualitative phytochemical analysis. The results are as follows.

Preliminary qualitative phytochemical screening of EEAL showed the presence of alkaloid,phenolics, tannins, saponins, triterpenoids, flavones and flavonoids. As flavones and flavonoidsare responsible for most of pharmacological activity by their antioxidant's activity, further invivo study was carried out.

Table 3: Phytochemical Study of EEAL

Sl. No.	Phytoconstituents	Presence/ Absence
1	Alkaloid	+
2	Carbohydrate	+
3	Glycoside	+
4	Tannins	+
5	Protein and Amino acid	+
6	Gum and Mucilage	-
7	Flavones and Flavonoids	+
8	Saponins	+
9	Steroids and Sterols	-
10	Triterpenoids	+

Pharmacological Study:

In carrageenan induced paw edema was measured by the displacement value of mercury in plethysmometer.Table-5 indicates that the change which occurs due to the treatment of EEAL.

Table 4: Paw volume at different time measured by mercury displacement method

Groups	Initial Paw Volume	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.	6th hr.
I.	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005	1.180 ± 0.005
II.	1.193 ± 0.007	1.908 ± 0.036****	2.253 ± 0.025***	2.368 ± 0.035****	2.437 ± 0.008****	2.577 ± 0.020****	2.692 ± 0.122****
III.	0.990 ± 0.008	2.088 ± 0.033****	1.535 ± 0.023****	1.427 ± 0.022****	1.292 ± 0.061*	1.168 ± 0.006***	1.182 ± 0.006***
IV.	1.157 ± 0.216	1.372 ± 0.026^{ns}	1.390 ± 0.157^{ns}	1.280 ± 0.051^{ns}	1.240 ± 0.040^{ns}	1.190 ± 0.026^{ns}	1.165 ± 0.007^{ns}
V.	1.000 ± 0.057	1.597 ± 0.060**	1.640 ± 0.051***	1.492 ± 0.080**	1.350 ± 0.026***	1.230 ± 0.024*	1.123 ± 0.011^{ns}

Values (ml) are expressed as mean ± SEM (n=6). Values comparison were made between Group 1 Vs Group 2,3,4,5 (****p < 0.001, ***p< 0.001, **p<0.01, ns- Non-Significant)

Table 5: Paw volume at different time measured by mercury displacement method

Group	Initial Paw Volume	6 hr. (ml)	Difference in paw	Inhibition percentage
I.	1.180 ± 0.005	1.180 ± 0.005	0.00	100
II.	1.193 ± 0.007	2.692 ± 0.122****	1.499	43.12
III.	0.990 ± 0.008	1.182 ± 0.006***	0.192	85.57
IV.	1.157 ± 0.216	1.165 ± 0.007^{ns}	0.008	84.42
V.	1.000 ± 0.057	1.123 ± 0.011^{ns}	0.123	87.24

Values (ml) are expressed as mean ± SEM (n=6). Values comparison were made between Group 1 Vs Group 2,3,4,5 (****p < 0.001, ***p< 0.001, **p<0.01, ns- Non-Significant)

CONCLUSION

Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatorydrugs, which has frequently been used to assess the anti-edematous effect of the drug.Carrageenan is a strong chemical use for the release of inflammatory and proinflammatorymediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF-α, etc.) In this model ofinflammation, *Albizia lebbeck* had very consistent anti-inflammatory activity and thus showedsignificant decrease in the paw thickness of rat. The present results suggest that *Albizia lebbeck* suppresses the first phase of carrageenan-induced paw edema, thus, confirming an NSAID-like property. The present study showed that *Albizia lebbeck* has anti-inflammatory properties.

REFERENCES

1. Balkrishna A, Sakshi, Chauhan M, Dabas A, Arya V. A Comprehensive Insight into the Phytochemical, Pharmacological Potential, and Traditional Medicinal Uses of *Albizia lebbeck* (L.) Benth. Evid Based Complement Alternat Med. 2022 Apr 21; 2022:5359669. doi: 10.1155/2022/5359669. PMID: 35497931; PMCID: PMC9050289.
2. Anonymous, The Wealth of India: A Dictionary of Indian raw materials & industrial products, Raw Materials, Vol 10 (Sp-W). New Delhi: National Institute of Science Communication and Information Resources (CSIR); 1993. p. 195-200.
3. Envis Centre on Medicinal Plants. Encyclopedia on Indian Medicinal Plants. http://envis.frlht.org/plant_details.php ?disp_id=642&parname=0. Accessed on: 05.03.18.
4. The Useful Plants of India. National Institute of Science Communication & Information Resources (CSIR), 5th ed. New Delhi, India; 2006. p. 141.
5. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Dehra Dun, India: Bishen Singh Mahendra Pal Singh; 1993. p. 549-559.
6. Gupta AK, Sharma Madhu. Reviews on Indian Medicinal Plants. Vol 7 (CI-Co). Medicinal Plant Unit. Indian Council of Medical Research. New Delhi, India; 2008. p. 561-562.
7. Asolkar LV, Kakkar KK, Charke OJ. Glossary of Indian medicinal plants with active principles, Part 1. New Delhi: National Institute of Science Communication And Information Resources (CSIR); 1992. p. 230-231.
8. El Khodary YA, Ayoub IM, El-Ahmady SH, Ibrahim N. Molecular and phytochemical variability among genus Albizia: a phylogenetic

prospect for future breeding. Mol Biol Rep. 2021 Mar;48(3):2619-2628. doi: 10.1007/s11033-021-06316-x. Epub 2021 Apr 1. PMID: 33792827.

9. Bobby N M. D., Wesely, E. G, Johnson, M. 2012; HPTLC studies on the flavonoids of *Albizia lebbeck*benth, International Journal of Advances in Pharmaceutical Research,Research Paper Vol. 3 / Issue. 3 / 830 – 836.
10. Alam P; Ali M. Aeri, Vidhu. 2012; Isolation of a new keto steroid stigmast-4, 20(21), 23-trien-3-one and a new alcohol tricontan-10a-ol from the roots of AlbizzialebbeckBenth,Journal of Natural Product & Plant Resources; Vol. 2 Issue 2, pp 234.
11. Balkrishna A, Chauhan M, Dabas A, Arya V. A Comprehensive Insight into the Phytochemical, Pharmacological Potential, and Traditional Medicinal Uses of *Albizia lebbeck* (L.) Benth. Evidence-Based Complementary and Alternative Medicine. 2022 Apr 21; 2022.
12. Kokila K, Priyadharshini SD, Sujatha V. Phytopharmacological properties of Albizia species: a review. Int J Pharm Pharm Sci. 2013 Aug 10;5(3):70-3.
13. Shirisha K, Priyanka B, Rahman H, Bardalai D, Ali F. Review on *Albizia lebbeck* (L.) Benth: a plant possessing diverse pharmacological activities. Research Journal of Pharmacognosy and Phytochemistry. 2013;5(5):263-8.
14. Kokate CK, Purohit AP, Gokhale SB. Text book of pharmacognosy. 33rd ed., Pune; NiraliPrakashan: 2001.
15. Khandelwal, KR. Practical Pharmacognosy. 14th ed., Pune; NiraliPrakashan: 2005. 146-55.
16. Evans WC. Trease and Evans Pharmacognosy. 15th ed., Edinburgh London, New York, Philadelphia, St Louis, Sydney, Toronto; W.B. Saunders: 2002.103-104, 227, 247-250, 336, 471, 519-520, 545-7.
17. MonalishaRoutaray, PoojaNayak, Subhradipta Panda, Arudeepa Dash, Satyapriya Mahapatra. Evaluation of anti-diabetic effects of ethanolic extract of *Albizia lebbeck* in rats Research Journal of Pharmacy and Life Sciences: Volume 4, Issue 2; May – August, 2023: Page 56 – 68.
18. Panigrahi, et al., Hypoglycemic and Hypolipidemic Activities of Methanolic Extract of *Glinus Oppositifolius*. Int J Pharm 2012; 2(3): 491-497.
19. Ghosh MN. Fundamentals of Experimental Pharmacology. 3rd ed., Kolkata; Hliton& Company: 2005; 190-7.