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Invitro and *Invivo* Anti-Inflammatory Activity of *Bauhinia X Blackeana* Linn Leaves

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Abstract

The present study is to investigate the anti-inflammatory activity of *Bauhinia X blackeana* leaves by *in vivo* on rats and *in vitro* using HRBC membrane stabilizing method. Diclofenac sodium was used as reference drug for comparision. The methanolic extract showed significant activity when compared to the standard drug.

Keywords: *Bauhinia X Blackeana;* Diclofenac Sodium; Anti-inflammatory Activity

Introduction

Inflammation is the reaction of vascularized living tissues to local injury. Inflammation comprises a series of changes in the terminal vascular bed, in blood and in connective tissues with the purpose of eliminating the offending irritant and to repair the damaged tissue [1]. Plants play an important role in maintaining human health. *Bauhinia* variety of family Caesalpiniaceae (Fabales) contains 15 species that happens in India. Some of them are bushes or trees while couples are climbers. *Bauhinia x Blackeana* (Hong Kong orchid tree) this little tree develops to around 20 feet tall with a light dark smooth bark and an umbrella-shape propensity [2,3]. It has rich rose-purple fragrant blooms with pink stamens; the brings down are bigger than on other bauhinia (5-6 creeps in distance across) and over a more drawn out period, frequently beginning in fall and reaching out to mid spring. It is additionally sterile so it doesn't set seed units. This semi-deciduous tree can be dry season deciduous in dry soils, icy deciduous in case of an ice yet even in a watered hotter areas will drop some of its dark green leaves in spring similarly as it blossoms and can in some cases be found in sprout totally without leaves so the blooms are significantly more detectable. Plant in full sun in a moderately very much depleted soil and inundate frequently too periodically. Endure of light ices and temperature down to 25 °F. An incredible tree in reasonably ice free regions with blooms that are extremely alluring to people and hummingbirds and even as they drop, make a bright groundcover [4,5]. The scientific review of the literature did not show any particulars on the anti-inflammatory studies of *Bahunia X blackenea*. In this study a trial was done to know the worth of this plant for its anti-inflammatory activity in terms of the volume of hind paw is measured prior and after inducing the inflammation by plethysmometer. This plant does not have any reported activities.

Materials and Methods

Requirement of chemicals

Diclofenac sodium was procured from Mangalam Drugs and Pharmaceuticals Ltd, Wapi, Gujarat. The solvents used was of Laboratory grade obtained from EMerck Ltd., Mumbai. All other chemicals of highest available purity were obtained from HiMedia Laboratories, Mumbai.

Plant material

For the present investigation, *Bauhinia X blackeana* leaves were collected in the month of September 2017 from timmapur village of the karimnagar district. The plant was identified and authenticated by BSI/DRC/2017-2018/TECH/779. The leaves were dried in shade and stored at 25 °c. It was powdered, passed through sieve no.40 and stored in air tight container.

Preparation of extract

Methanolic extract of *Bauhinia X blackeana* leaves were prepared by soxhlation method at suitable temperature. 50gms of powdered leaves are prepared as a thimble and placed in the condenser and in the round bottomed flask required amount of methanol (it does not have fixed amount, it should be taken 3/4th of the flask) was taken. Soxhlation process was carried out for 6 hours. The extract obtained was evaporated and dried in desiccator.

Animals

The healthy Wistar rats of either sex; about the equal age and weighing about 120-190 g used for the activity were procured from Mahaveer Enterprises, Hyderabad. Rats were feed with quality diet and liquid required. The animals were caged and maintained under quality atmospheric conditions (12 h light/12 h dark cycle; 25 ± 3 °C, 35-60% relative humidity). The rats were placed strictly according to the CPCSEA guidelines and the study was conducted after obtaining permission from Institutional Animal Ethics Committee (IAEC).

Acute Toxicity and Gross Behavioral Study

The rats were empty stomach in the night, divided into groups (n=6) and through mouth feed with increasing doses (250, 500, 750, 1000 mg/kg body weight) of methanol extract dissolved in distilled water (any extract will not be completely dissolved in water because it is a plant extract,it forms as a suspention,the extract is in dried form in order make it liquid to feed the rat it is dissolved). After feeding of the extracts, the rats were watched for first 2 h for their gross behavioral changes and once in half an hour for next 4 h and then once in 24 h for next 72 h to find out percentage mortality [6-8].

Assessment of *In-Vivo* & *In-Vitro* Anti-Inflammatory Activity

In the present study, the anti-inflammatory activity of methonolic extract of leaves of Bahunia X blakeanea were treated by (chemical) carrageenan induced rat paw edema method using Diclofenac sodium as standard and in-vitro membrane stabilization of HRBCs [7,9-12].

Anti-inflammatory assay

In-Vitro Anti-Inflammatory Activity Preparation of HRBCs (Human Red Blood Cells)

Blood was gathered from healthy human volunteer and centrifuged. The supernatant was at that point deliberately pipetted with sterile pipettes. The stuffed cells were resuspended in an equivalent volume of isosaline and centrifuged. The process was rehashed 4 times until the supernatants were clear. A 10% HRBC suspension was then arranged with typical saline and kept at 4 °C until utilize [12,13].

Effect of Plant Extracts on HRBC System

The reaction mixture (4.5 ml) consisted of 2 ml hyposaline (0.25% w/v NaCl), 1ml of isosaline buffer solution, PH7.4 (6.0 g TRIS, 5.8 gm NaCl, HCl to regulate the PH and water to make1000 ml) and varying volumes of the extract solution in isotonic buffer (concentration=100mg/ml) to make the volume to 4.0 ml. Then 0.5 ml of 10% HRBC in normal saline was added. Two controls were performed. One control with 1 ml of isosaline buffer instead of extract (control 1) and the other control with 1ml of extract solution and without red blood cells (control 2). The mixture was incubated at 56 °C for 30 mins. The tubes were cooled under running water for 20 mins. The mixture was centrifuged and the absorbance of the supernatant was read at 560 nm. The percentage of membrane stabilization was determined using the formula:

$$100 - \frac{extract\ absorbance\ value - contol\ 1\ absorbance}{control\ 2\ absorbance\ value} \times 100$$

The control 1 represents 100% HRBC lysis. The HRBC membrane stabilizing standard drug used was Diclofenac sodium [14,15].

In-Vivo Anti-Inflammatory Activity

Juvenile adult male Wistar rats weighing 190-230 g were used which are adjusted to the laboratory environment and kept on normal rat feed and water. Rats were with empty stomach for night before the activity, while allowing water throughout the experiment. Rats were divided 8 groups with each group containing 6 animals. The animals were administered control, standard or test extracts as shown in (Table 1). The control and standard samples were prepared in water. A identification was done on two hind paws just beyond the tibiotarsal junction, so that test time the paw is dipped in the mercury column up to the marked level to ensure constant paw volume. After an hour of administration of the test and standard samples, 0.1 ml of 1% carrageenan suspension (in normal saline) was injected into dorsal region of sub plantar surface of hind paw of rat subcutaneously with the help of 26 G needle. The initial paw volume of each rat was noted before drug administration. The paw volumes were measured at the end of 0.5, 1, 2, 3

and 4 h using plethysmometer. Any change in paw volume of rats was obtained by subtracting initial paw volume from the paw volumes at different time intervals. The average value of edema was calculated by taking the average of each group at different hours. Percentage inhibition of edema was calculated for each group with respect to its control group.

Group	Extract
Group I(control)	water
Group II(standard)	Diclofenac sodium (1mg/kg)
GroupIII	Methanolic extract (3 mg/kg)
Group IV	Methanolic extract (5 mg/kg)

Table 1: Division of animals for anti-inflammatory activity of Bahunia X blakenea

Percentage inhibition = (A – B) x 100/A

Where A is the mean increase paw volume in rats treated with control and B is the mean increase in paw volume in rats treated with test [16].

Results and Discussion

In acute toxicity study, all the animals were found to be surviving after 72 h. This indicates that the extracts were found to be safe up to the dose levels studied. Since, all the animals survived at a dose of 1000 mg/kg body weight, the LD50 of the extracts will be >1000 mg/kg body weight. No major l changes were observed during this period of study (Table 2).

Extracts	Group	Dose(mg/kg)	No of mice each group	After 4 hours	After 24 hours
Methanol extract	1	250	6	6	6
	11	500	6	6	6
	111	750	6	6	6
	1V	1000	6	6	6

Table 2: Acute toxicity studies

Anti-inflammatory activity of leaf extract of Bauhinia X blackeana *Invitro*

Anti-inflammatory activity for all the leaf extract was performed in-vitro by membrane stabilization of HRBCs at a dose of 3 mg/ml, 5 mg/ml. The results pertaining to this investigation were shown in the Table 3 and graph in Figure 1. The membrane stabilizing activity of methanolic extract of *Bauhinia X blackeana* at concentration 3mg/ml and 5 mg/ml was studied on heat induced lysis of human red blood cell membrane stabilization method (HRBC membrane) at 540nm. It showed at 76.05% percentage projection at a dose of 3mg/ml and 5mg/ml 89.9% indicating that the activity was dose independent. The methanolic extract showed significant activity compared to standard drug i.e., Diclofenac sodium.

Treatment	Dose mg/ml	Protection
Standard	3mg/ml	98.85%
Methanolic extract	3mg/ml	76.05%
	5mg/ml	89.9%

Table 3: *In-vitro* anti-inflammatory activity

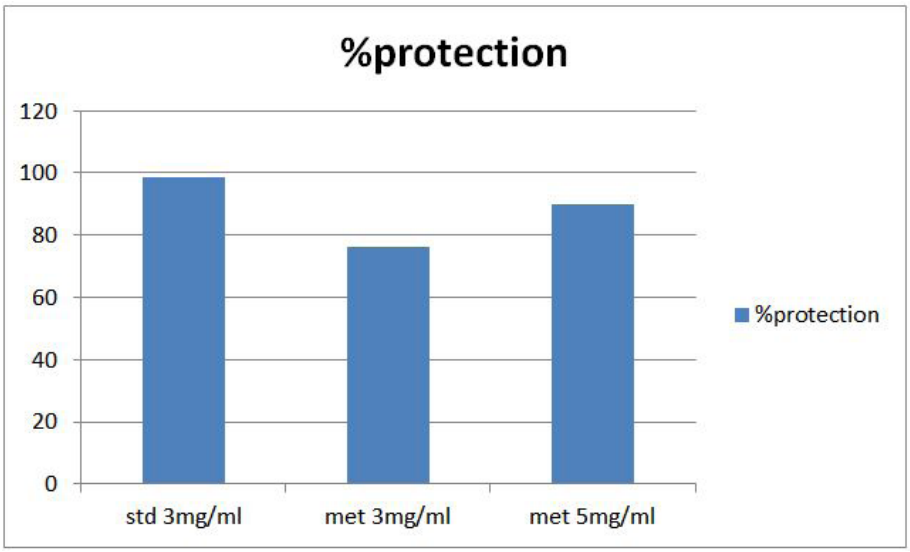

Figure 1: *invitro* Anti-inflammatory activity

Invivo

Screening for anti-inflammatory activity was performed for methanolic extract at doses of 3 and 5 mg/kg body weight in rats. 0.1 ml of 1% carrageenan suspension (in normal saline) was injected into dorsal region of sub plantar surface of hind paw of rat subcutaneously with the help of 26 G needle. This allows the evaluation of ability and potency of extract to protect from producing inflammation. The extracts were administered to the rats and after 1 hr, The paw volumes were measured after 0.5, 1, 2, 3 and 4hrs of carrageenan administration. Diclofenac sodium at a dose of 3 mg/kg was used as standard for comparison. The results pertaining to this investigation were shown in (Table 4 and 5) (Figure 2). The results acquired in this work represents that the percentage protection against edema formation with methanolic extract was significant and the extract showed not dose dependent anti-inflammatory activity. From the table it can be clear that the standard drug diclofenac sodium has protected to an extent of 34, 32.3, 50, 66 and 51% against inflammation induced by carrageenan at ½, 1, 2, 3 and 4 hrs. Methanol extract showed highest activity at dose level of 3 mg/kg at 2 hr.

Name of the drug	Dose (mg/kg)	MEAN EDEMA VOLUME(ml)				
		30min	1hr	2hr	3hr	4hr
Control	-	0.23 ± 0.02	0.34 ± 0.02	0.62 ± 0.05	0.75 ± 0.02	0.70 ± 0.03
Standard (Diclofenac sodium)	1	0.15 ± 0.03	0.23 ± 0.03	0.31 ± 0.03	0.27 ± 0.04	0.34 ± 0.03
methonal	3	0.16 ± 0.02	0.19 ± 0.03	0.21 ± 0.05	0.28 ± 0.04	0.27 ± 0.04
	5	0.18 ± 0.02	0.22 ± 0.03	0.25 ± 0.02	0.31 ± 0.03	0.29 ± 0.03

Table 4: Anti-inflammatory activity of leaf extracts of *Bahunia X blakenea*

Name of the drug	Dose (mg/kg)	% PROTECTION				
		30min	1hr	2hr	3hr	4hr
Standard	1	34	32.3	50	66	51
methanol	3	30	44.1	64.1	62.6	61.4
	5	21.7	35.2	59.6	58.6	58.5

Table 5: Percentage protection against edema formation

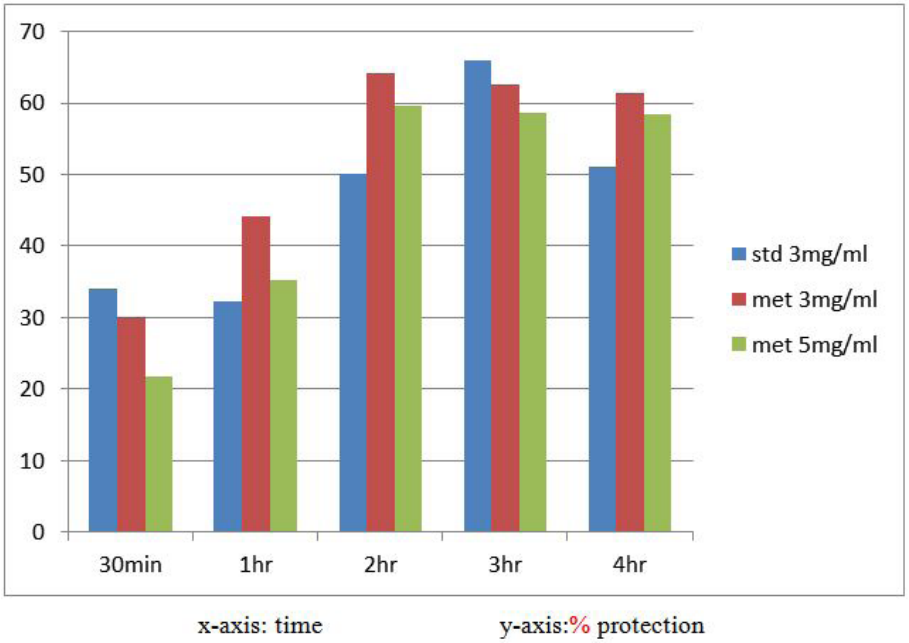

Figure 2: % protecton of ***in vivo*** Anti-inflammatory activity

References

1. Inflammation Lecture Note, 2006.

2. Gupta R, Paarakh PM (2009) Antibacterial Activity of Different Extracts of the Plant Bauhinia Variegata. Int J Chem Sci 7: 2377-83.

3. Kubdi MS, khadabadi SS, Saboo SS, Ghorpade DS, Modi AJ (2010) In-vitro Antimicrobial Activity of the Crude Extracts of Colocasia Esculenta Leaves (Araceacea). IJPSR 1.

4. Md.Reyad-UI-ferdous, Akhter S, Md.zahimel islam khan, Md.eshak khan, Md.atiqul islam, et al. (2014) Ex-vivo anti inflammatory and antimicrobial activities of the leaves of bauhinia accuminata. American J Life Sci 2: 267-70.

5. Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity. J Pharm Anal 6: 71-9.

6. Kulkarni SK (2005) Hand book of Experimental Pharmacology. 3rd edition, Vallabprakasan, Delhi.

7. Turner RA, Hebborn P (1971) Screening methods in Pharmacology 2, Academic press.

8. Amos S, Kolawole E, Akah P, Wambebe C, Gamaniel K (2001) Behavioral effects of the aqueous extract of Guiera senegalensisi in mice and rats. Phytomedicine 8: 356-61.

9. Ahmed F, Khan RA, Rasheed S (1992) Study of Analgesic and Anti-inflammatory activity from Plant extracts of Lactuca scariola and Artemisia absinthium. Med J Islamic World Acad Sci 5: 111-4.

10. Dimo T, Fotio AL, Nguelefack TB, Asongalem EA, Kamtchouing P (2006) Anti-inflammatory activity of leaf extracts of Kalanchoe crenata Andr. Indian J Pharmacol 38: 115-9.

11. Divakar MC, Jayaprakasham R (2006) Antiinflammatory and free radical activities of sea cucumber and cuttle fish glandular extracts. Indian drugs 43: 471-5.

12. Mongeli E, Desmarchelier C, Coussio J, Ciccia G (1997) Biological studies of Bolax gummifera, a plant of the falkland islands used as a treatment of wounds. J Ethnopharmacol 56: 117-21.

13. Divakar MC, Jayaprakasam R (2006) Anti- inflammatory and free radical scavenging activities of sea cucumber and cuttle fish glandular extracts. Indian Drugs 43: 471-5.

14. Puntero BF, Peinado II, Fresno AMVD (1997) Anti-inflammatory and anti-ulcer activity of Teucrium buxifolium. J Ethnopharmacol 55: 93-8.

15. Singh B, Sahu PM, Sharma MK (2002) Antiinflammatory and anti-microbial activities of triterpenoids from Strobilanthes callosus nees. Phytomedicine 9: 355-9.

16. Radhika B, Begum N, Srisailam K (2017) In-Vitro and In-Vivo Anti-Inflammatory and Analgesic Activity of Bixa orellana Linn Leaf Extracts. Int J Pharm Pharmacol 1: 108.