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Investigation of Anti-inflammatory activity of *Plumeria pudica* Linn.

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Abstract

Plumeria pudica Linn. is an indigenous medicinal of India and have been used scientifically for its beneficial effects. The present investigation was carried to study the anti-inflammatory activity of the plant and to evaluate the crude extracts of leaves for pharmacological potential to confirm and provide scientific basis for its use in traditional medicine. For the above mentioned purpose, the crude extracts derived were screened pharmacologically.

Keywords: Inflammation, *Plumeria pudica*, Leaf

Introduction

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen.[1-2]

Plumeria pudica Linn commonly known as Nag champa is a fast-growing, medium size tree, that is botanically belongs to family Apocynaceae. The plant can reach a height up to 5-8 feet with many branches on the upper part. Small trees or herbs with obanceolate leaves. Leaves are alternate, bounded at twig tips, strongly recovered margin, flowers are white, fragrant, in corymbose clusters. The white flowers bearing five petals and have fragrance. The plant is used for the cure of rheumatism, diarrhoea, blennorhea, venereal disease, leprosy, psychosis and diuresis etc. *Plumeria* species have also been investigated for isolation of irridoids and triterpenoids, which exhibited algicidal, antibacterial and cytotoxic activities.[3] Despite, the usefulness and importance of the selected species of *Plumeria* accurate information on anti-inflammatory approaches were not being carried out so far with proper validation and documentation. Therefore, the present work was conceived.

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Fig. 1:*Plumeria pudica* Linn.:A twig

Material and Methods

Selection, Collection and authentication of Plant / plant Material

The plant *Plumeria pudica* Linn. is widely found to occur through Central India. The parts (leaves) was collected in the month of Jan-Feb.' 2017 from the Medical Malwa region of Madhya Pradesh and identified and authenticated by Dr. S.N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, A.P.S. university, Rewa, M.P. and was deposited in our Laboratory Voucher specimen No. PCog/PI/110.

Extraction of Plant Material

The shade dried coarsely powdered leaves of *Plumeria pudica* Linn. (250 g) were loaded in Soxhlet apparatus and was extracted with ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator.[4]

Pharmacological Screening

Acute Toxicity Studies of Extracts

Organization for Economic co-operation and Development (OECD) regulates guideline for oral acute toxicity study. It is an international organization which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing.[5]

Procurement of experimental animals

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee, after scrutinization.

***In-vivo* Anti – inflammatory activity**

Carrangeenan induced paw edema

Animals

Adult Albino rats of both sex (200-250 gm) were procured from Veterinary college, Mhow, Indore, (M.P.) maintained under ideal feeding and management practices in the laboratory. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment.

Study Design

The animals were divided into 6 groups each containing six animals. Group I served as untreated control and received 0.9 normal saline, group II served as positive control and received Indomethacin (10 mg/kg, i.p.) and others group were treated with different doses of *P. pudica* aqueous and ethanolic extracts.[6]

Anti-inflammatory Screening

The aqueous and ethanolic extract of *Plumeria pudica* and standard drug Indomethacin were administered in prescribed doses. Control received 0.1 ml of 1% carrageenan in normal saline. The administration of extract and drug was 30 min prior to injection of 0.1 ml of 1% carrageenan[6] in the right hind paw sub platar of each rat. The paw volume was measured plesthysmometrically (model 7140, Ugo Basil, Italy). Prior to injection of carrageenan, the average volume of the right hind paw of each rat was calculated. At 1, 2, 3, 4 and 5 hr after injection paw volume was measured. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.

***In-vitro* Anti-inflammatory activity**

HRBC Methods

The HRBC membrane stabilizing activity assay was carried using 10% (v/v) Human erythrocyte suspension while Indomethacin was used as standard drugs. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 10% (v/v) human erythrocyte suspension, 1.0 ml of drugs (standard and extracts) and final reaction mixtures were made up to 4.5 ml with isosaline. To determine the anti-inflammatory activity by HRBC membrane stabilization method, the following solutions were used.[7-8]

Test solution (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v),1ml of phosphate buffer (pH7.4), 1ml of test extract (1mg/ml – 5 mg/ml) in normal saline and 0.5ml of 10% w/v human red blood cells in isotonic saline.

Test control (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of isotonic saline and 0.5ml of 10%w/v human red blood cells in isotonic saline.

Standard solution (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of Indomethacin (2.5mg/ml) and 0.5ml 10%w/v human red blood cells in isotonic saline. Drug was omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was measuredspectrophotometrically at 560 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated using the following equation[9-10]

% Inhibition of haemolysis = 100 x (A1-A2/A1)

Where: A1 = Absorption of hypotonic buffered saline solution alone A2 = Absorption of test sample in hypotonic solution.

Statistical analysis

Results were tabulated and the data was expressed as mean ± SEM. The difference between experimental group were determined using one way analysis of variance (ANOVA) followed by Dunnet test. $P \leq 0.01$ was considered significant.

Results and Discussion

The aqueous and ethanolic extracts of leaves of plant of *Plumeria pudica*Linn. were screened for acute toxicity study by OECD guideline no. 423 for determination of LD50. The results showed that the aqueous and ethanolic extracts i.e., EE and AE were belonging to category 5 (unclassified). Hence, LD50 was 5000 mg/kg, therefore, ED50 was 500 mg/kg. Therefore, two doses of 250 and 500 mg were selected for present investigation.

The aqueous and ethanolic extract of *Plumeria pudica* leaves evaluated for anti-inflammatory activity in animal models and results are summarized in table 1 and 2. The results obtained pudicates that the extract found to have significant ($P<0.01$) anti inflammatory activity in rats. The AE at the test doses 250 and 500 mg/kg b.w. reduced the edema induced by carrageenan by 38.78% & 32.72% respectively at 5hr, whereas the EE at the test doses 250 and 500 mg/kg b.w. reduced the edema induced by carrageenan by 33.93% & 33.33% as compared to standard drug which showed 55.75% of inhibition as compared to the control group (graph 1). The aqueous and ethanolic extract of the leaves of *Plumeria pudica* was studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Five different concentration of aqueous and ethanolic extract were used among which concentration at 5 mg/ml showed 56.38 % &protection of HRBC in hypotonic solution. All the results (table 3) were compared with standard indomethacin, which showed 89.50% protection. The activity may be due to the presence of one or more phytochemical constituents present in the extract. The result obtained have been supported by Photomicrographical pictures of the HRBC (Fig 2,3,4). The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. The erythrocyte membraneis analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is importantin limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release.

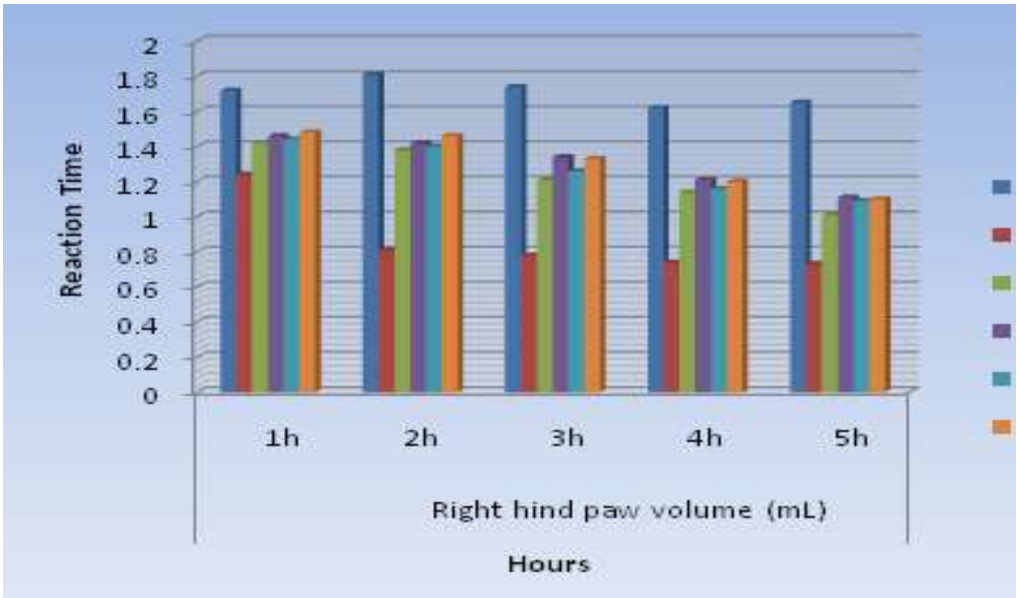
Table 1: Effect of *Plumeria pudica* (L.f.) Linn. Extracts on carrageenan induced Edema

Treatment	Dose (mg/kg)	Right hind paw volume (mL)				
		1h	2h	3h	4h	5h
C	-	1.72±0.0058	1.81±0.008	1.74±0.007	1.62±0.0083	1.65±0.0039
SD	10	1.24±0.0037	0.81±0.0037*	0.78±0.0031	0.74±0.0024	0.73±0.0019*
AE	250	1.42±0.0037*	1.38±0.0037	1.21±0.0031	1.14±0.0024*	1.01±0.0024*
AE	500	1.46±0.0031	1.42±0.0037*	1.34±0.0037	1.21±0.0024	1.11±0.0024*
EE	250	1.44±0.0024	1.40±0.0037*	1.26±0.0037*	1.16±0.0019	1.09±0.0019*
EE	500	1.48±0.0037*	1.46±0.0024	1.33±0.0024	1.20±0.0024*	1.10±0.0019*

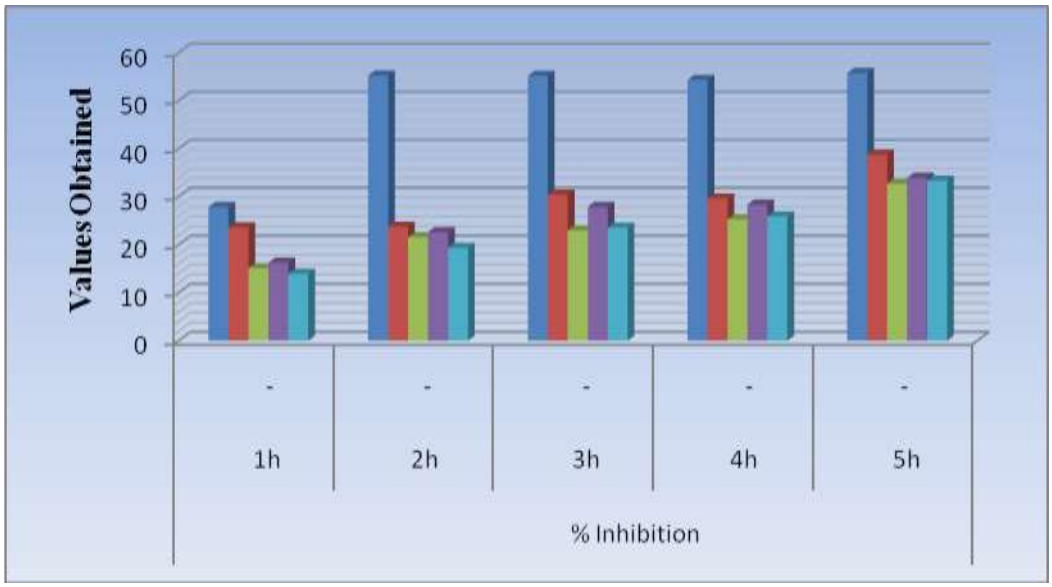
Note: All values are mean ± SEM, n=6, * $P < 0.01$ found to be significant: Abbr.: SD: Standard Drug, AE=Aqueous extract; EE: Ethanolic extract

Table 2: % Inhibition of *Plumeria pudica* (L.f.) Linn. Extracts on carrageenan induced Edema

Treatment	Dose (mg/kg)	% Inhibition				
		1h	2h	3h	4h	5h
C	-	-	-	-	-	-
SD	10	27.90	55.24	55.17	54.32	55.75
AE	250	23.62	23.75	30.45	29.62	38.78
AE	500	15.11	21.54	22.98	25.30	32.72
EE	250	16.27	22.65	27.90	28.39	33.93
EE	500	13.95	19.33	23.56	25.92	33.33



Graph 1: Effect of *Plumeria pudica* (L.f.) Linn. Extracts on carrageenan induced Edema

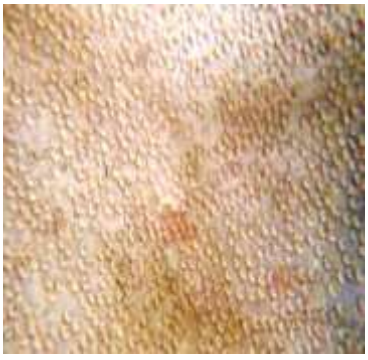


Graph 2: % Inhibition of *Plumeria pudica* (L.f.) Linn. Extracts on carrageenan induced Edema

Table 3: HRBC membrane stabilization method for extract of the leaves of *Plumeria pudica* Linn.

Treatment	Conc. mg/ml	Absorbance	%Inhibition
Control	-	0.6670±2.99	-
AEPIL	1	0.3370±2.44[a]	49.47
	2	0.3210±2.44 [c]	51.87
	3	0.3009±2.44 [b]	54.88
	4	0.2993±2.44 [a]	55.12
	5	0.2909±0.03[c]	56.38
EEPIL	1	0.7308±2.00 [c]	9.5
	2	0.7680±3.16 [b]	15.14
	3	0.7827±2.44 [a]	16.68
	4	0.8113±2.44 [c]	21.63
	5	0.8890±2.00[b]	33.28
Standard drug Indomethacin	2.5	0.0700	89.50

Values are expressed as X (Mean) +SEM, n=3. (One way ANOVA followed by Student t-test). Statistically significance of aP< 0.05, bP<0.01, cP<0.001 and dNS in comparison to respective control.



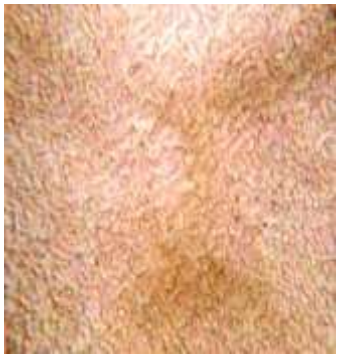


Fig. 2: HRBC in Isotonic Solution
Fig. 3: HRBC in Hypertonic Solution -Control (Lysis of Hypertonic induced HRBC membrane)
Fig. 4: RBC in Hypertonic solution with Plant extract (AE) (5mg/ml) (Protection of Hypertonic induced HRBC membrane lysis)

Conclusion

The present investigation reveals that the ethanolic extract of *P. pudica* exhibit its maximum anti-inflammatory activity of 33.33%, by Carrangeenan induced paw edema method at the given dose of 500mg/kg, followed by 33.93% at the dose 250 mg/kg, and it was significant when compared with control and standard group. The aqueous extract showed a moderate anti-inflammatory activity of 32.72 % at the dose 500 mg/kg and 38.78% at the dose 250 mg/kg when compared with control and standard group. The present investigation reveals that the ethanolic extract of *P. pudica Linn.* exhibit its maximum anti-inflammatory activity of by HRBC Method at the given dose of 500mg/kg, and it was significant when compared with control and standard group. The aqueous extract showed a moderate anti-inflammatory activity when compared with control and standard group. Hence, from the present work it was concluded that the selected medicinal plants *P. pudica Linn*. of Indian origin possess optimum anti-inflammatory activity which will claims their folk-lore uses as mentioned in traditional system of medicine.

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