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Anti-inflammatory Activity of Ethanolic Extract of Plumeria Acuminata

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ABSTRACT: Plumeriaacuminata belonging to the family Apocynaceae. In traditional medicinal system different parts of the plant have been mentioned to be useful in a variety of diseases. The plant material is widely used as a purgative, remedy for diarrhoea and cure for itch. The milky juice is employed for the treatment of inflammation and rheumatism. The objective of the present study was to evaluate theantiinflammatory activity of ethanol extract of leaves of Plumeriaacuminata on carrageenaninduced inflammation in rat hind paw oedema models. Wistar albino rats of either sex weighing 180–200 g were used. The results indicate the effectiveness and relative safety of drug extract for the treatment of conditions associated with inflammation. Carrageenan-induced oedema has been commonly used for acute inflammation. The rats were divided into three groups (n = 3). The different groups were treated orally with Ethanolic extract of plumeraacuminata (250 and 500 mgkg-1 b.w), Diclofenac (10 mgkg-1 b.w), and vehicle. control (0.9% NaCl, 5 mlkg-1 b.w). The administration of extract and drugs was 30 min prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw subplantar of each rat. The paw volume was measured initially and then at 1, 2, 3 and 4 h after the carrageenan injection by using plethysmometer. The inhibitory activity shown by the extract of Plumeriaacuminata leaves (250 mgkg-1 b.w) over a period of 4 h in carrageenan induced paw inflammation shown good results and did comparisionwith group treated with diclofenac and control group.Results checked for 0min,15min,30min,60min,120mins.Based on the results of percentage inhibition of paw volume at 120 mins shows 40.69%,38.37%. based on results be concluded that the ethanolic extract of leaves of Plumeriaacuminatapossess an antiinflammatory property in acute phase of inflammation.

Keywords: Anti-inflammatory, diclofenac, paw volume, carrageenan, plethysmometer

I. INTRODUCTION

Inflammation is an evolutionarily conserved process of protection and a critical survival mechanism. It is composed of complex sequential changes in the tissue to eliminate the initial cause of the cell injury, which may have been caused by infectious agents or substances from their metabolism (microorganisms and toxins), as well as by physical agents (radiation, burn, and trauma), or chemicals (caustic substances). The signs of inflammation are local redness, swelling, pain, heat, and loss of function[1]. Medicinal plants with Secondary metabolites like terpenoids, flavonoids, phenolic compounds, and alkaloids, steroids, shows antinflammatory activity are
Fragariachiloensis, Ugnimolinae, Buddlejaglobosa, Aristoteliachilensis, Berberismicrophylla,
and Quillajasaponaria are some native species in the coastal and Andes Mountain ranges in the south-central region of Chile[2]. Aeglemarmelos,Bryophyllumpinnatum,Albizialebbeck,C,assia fistula Sidacordifolia Linn. Moringaoliefera, Hibiscus rosa- sinensis, Cynodon dactylon[3]can interfere in the pathophysiological process of inflammation, to minimize tissue damage.
Types of Inflammation [4]

Acute Inflammation

Tissue damage due to trauma, microbial invasion, or toxic compounds can induce acute inflammation. It starts rapidly, becomes severe in a short time and symptoms may last for a few days for example cellulitis or acute pneumonia.

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Subacute inflammation is the time period between acute and chronic inflammation and may last 2 to 6 weeks.

Chronic Inflammation

Chronic inflammation is also referred to as slow, long-term inflammation lasting for prolonged periods of several months to years. Generally, the extent and effects of chronic inflammation vary with the cause of the injury and the ability of the body to repair and overcome the damage.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a drug class FDA-approved for use as anti-inflammatory agents.These effects make NSAIDs useful for treating muscle pain, dysmenorrhea, arthritic conditions, pyrexia, gout, migraines, and used as opioid-sparing agents in certain acute trauma cases.

NSAIDs are typically divided into groups based on their chemical structure and selectivity: acetylated salicylates (aspirin), non-acetylated salicylates (diflunisal, salsalate), propionic acids (naproxen, ibuprofen, acetic acids (diclofenac, indomethacin), enolic acids (meloxicam, piroxicam) anthranilic acids (meclofenamate, mefenamic acid), naphthylalanine (nabumetone), and selective COX-2 inhibitors (celecoxib, etoricoxib)[5].

Topical NSAIDs (diclofenac gel) are also available for use in acute tenosynovitis, ankle sprains, and soft tissue injuries.

Some of the FDA-approved NSAIDs are

s.no	**Non-selective NSAIDs**	**COX-2 Selective NSAIDs**
1.	Diclofenac	Celecoxib
2.	Diflunisal	Rofecoxib
3.	Etodolac	Valdecoxib

- The main mechanism of action of NSAIDs is the inhibition of the enzyme cyclooxygenase (COX).it convert arachidonic acid into thromboxanes, prostaglandins, and prostacyclins. The therapeutic effects of NSAIDs are attributed to the lack of these eicosanoids. Specifically, thromboxanes play a role in platelet adhesion, prostaglandins cause vasodilation, increase the temperature set-point in the hypothalamus, and play a role in anti-nociception.
- There are two cyclooxygenase isoenzymes, COX-1 and COX-2. COX-1 gets constitutively expressed in the body, and it plays a role in maintaining gastrointestinal mucosa lining, kidney function, and platelet aggregation. COX-2 is not constitutively expressed in the body; and instead, it inducibly expresses during an inflammatory response. Most of the NSAIDs are nonselective and inhibit both COX-1 and COX-2[6].

Plumeriaacuminata is used as medicinal plant, eight species are known in India and of which Plumeriaacuminata and P.rubra are commonly grown. They are commonly known as "Temple tree" or "Champa" in India. The plant material is widely used as purgative, remedy for pain, fever, diarrhoea and cure for itch. The milky juice is employed for the treatment of inflammation. The excessive doses of the latex derived from Plumeriaacuminata are poisonous and the root is a violent cathartic. The essential oil from the flowers possesses antifungal activity. Leaves-The leaves crowed at the terminal end of the branch, commonly oblong in shape, reaching a length of 40cm and a width of 7cm.It is simple, opposite, rarely whorled or alternate, stipules absent or rarely present family Apocynaceae[7].

Phytochemical constituents of Plumeriaacuminata are Plumieride as the main irridoidglucoside from the stem bark of P.rubra and P.lancifolia. The stigmast-7-enol, lupeol carboxylic acid, lupeol acetate and ursolic acid had been isolated from leaves. The researchers have successfully isolated Fulvoplummierin, Plumericin along with three new compoundsisoplumericin, β-dihydroplumericin and βdihydroplumericinic acid from roots of Plumeriaacuminata The steam distillate of Plumeriaacuminata yields an essential oil (0.04-0.07 %) which mainly consist of primary alcohols, geraniol, citronellol, farnesol and phenylethylalchol with little amount of aldehyde and ketones (6.8 %)[7].

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Fig1: Plumeriaacuminata

II. MATERIALS AND METHODS:

Plant material collection and preparation :

The leaves of the plant Plumeriaacuminata (Family: Apocynaceae) was collected from Raghuvanahalli, bangalore, karnataka India. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves was defatted with petroleum ether and the marc thus obtained was then extracted with ethanol in a soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained and yield obtained is 12% .

Drugs and chemicals

Drugs Carrageenan (vasa scientific lab, Bangalore), Diclofenac (vasa scientific lab, Bangalore) was used as the standard drug.

Animals

Animals Studies were carried out using Wistar albino rats of either sex weighing 180–200 g They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than three animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light circle (14/10 h). They were allowed free access to standard dry pellet diet and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were reviewed and approved by the university animal ethical committee.REG.NO.1959/PO/Re/517/CPCSEA.

Preliminary phytochemical screening of the powdered leaf ethanol shows the presence of Alkaloids, Cynogenic glycosides, Phenolic compounds, Flavonoids, Terpenoids, Tannins and Saponins.

Acute toxicity test : The animals were divided into three groups containing three animals in each group. Drug extract was suspended in normal saline and administered orally as a single dose to groups of rats at different concentrations (500, 750, 1000, 1250, 1500 and 2000 mgkg-1 b.w). These animals were observed for a 72 h period. The number of deaths was expressed as a percentile and the LD50 was determined by probit a test using the death percentage versus the log dose .

Antiinflammatory activity: Carrageenan-induced rat paw oedema:The rats were divided into three groups (n = 3). The different groups were treated orally with Ethanolic extract of plumeraacuminata (250 and 500 mgkg-1 b.w), diclofenac (50 mgkg-1 b.w), and vehicle. control (0.9% NaCl, 5 mlkg-1 b.w). The administration of extract and drugs was 30 min prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw subplantar of each rat. The paw volume was measured initially and then at 1, 2, 3 and 4 h after

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the carrageenan injection by using plethysmometer[8].

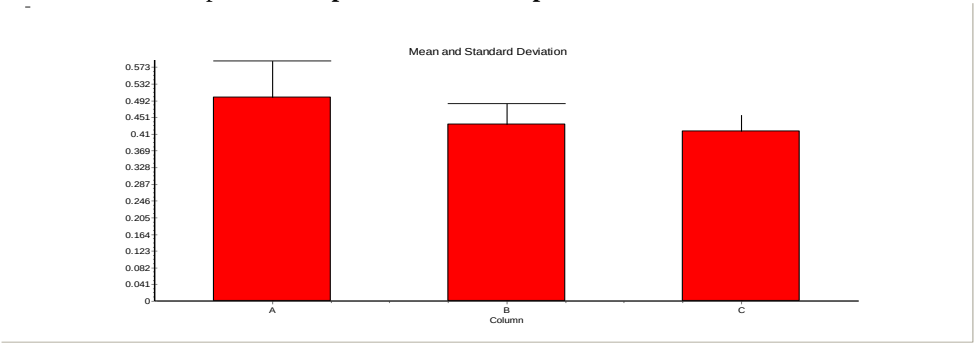
Fig no 2: Plethysmometer

III. RESULTS :

Group	Test material (dose mg/kg)	Mean Increase In The Paw Volume ± SEM(Ml)					% inhibition in Paw volume at 120 mins
		0min	15min	30 min	60 min	120 min	
1.	Control	0.33±0.021	0.5±0.036	0.63±0.021	0.76±0.016	0.8±0.021	--
2.	Diclofenac (50 mg/kg)	0.35±0.022	0.41±0.042	0.45±0.021**	0.5±0.025**	0.51±0.034**	40.69%
3.	Ethanolic Extract (250mg/kg)	0.4±0.025	0.43±0.021	0.53±0.021*	0.5±0.022**	0.53±0.033**	38.37%

Values represent mean ± SEM, n=6,
One way ANOVA followed by Dunnett's multiple comparison test * And ** indicates significant anti-inflammatory activity at P<0.05 and P < 0.01 respectively compared with the control group

Graphs 1: **Comparision of Mean paw volumes at 15 mins**



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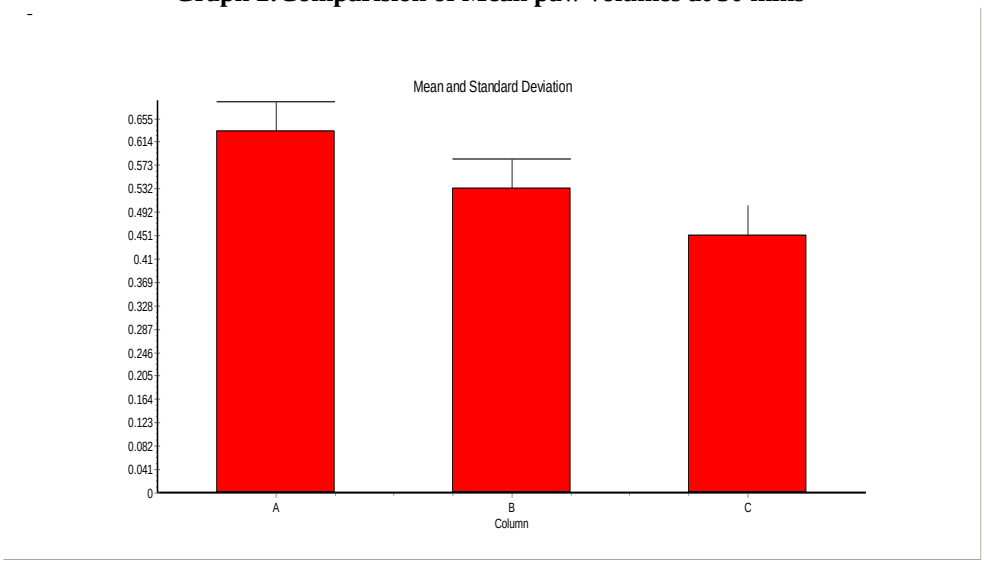
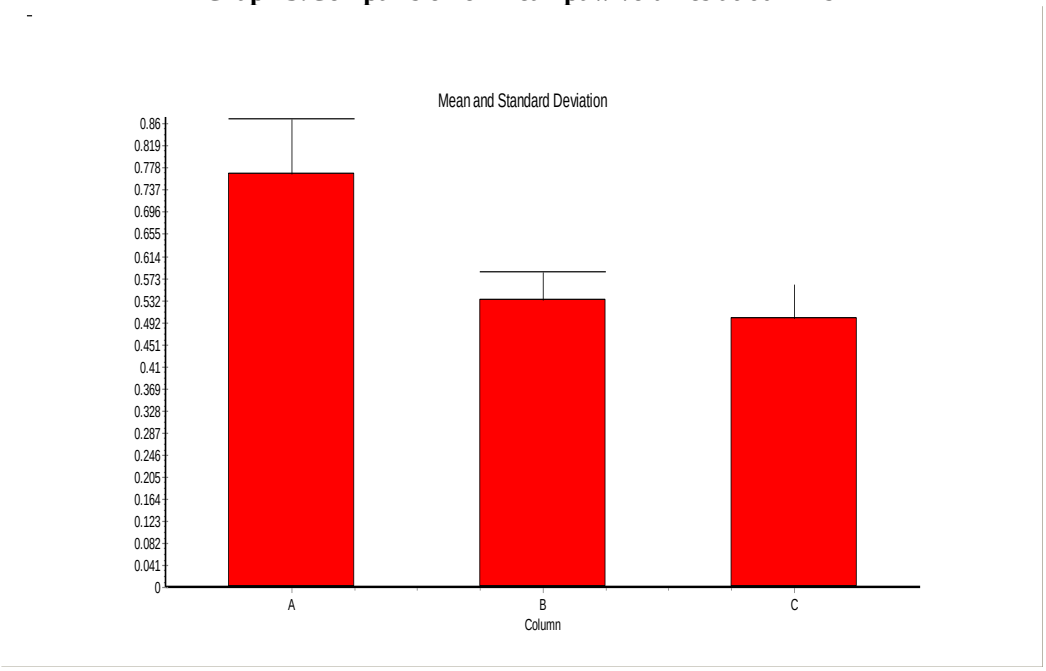
Graph 2:Comparision of Mean paw volumes at 30 mins



Graph 3:Comparision of Mean paw volumes at 60 mins



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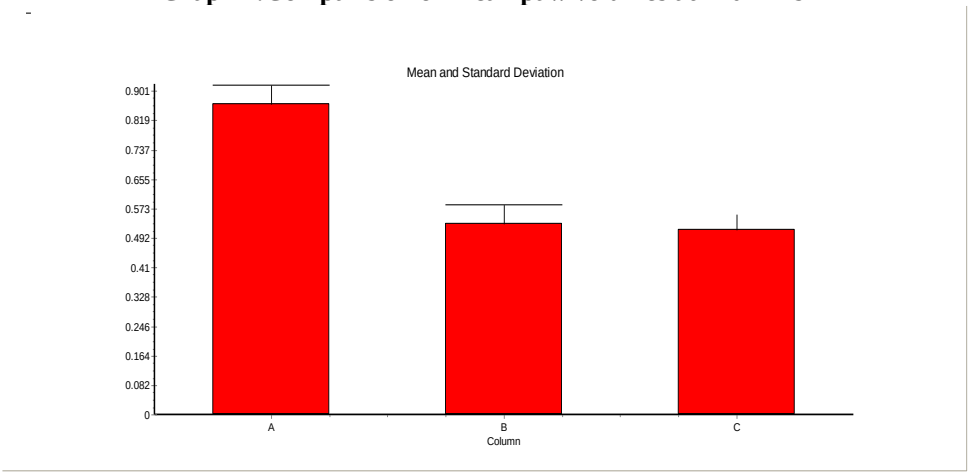
Graph 4:Comparision of Mean paw volumes at 120 mins



IV. DISCUSSION:

The present study establishes the antiinflammatory activity of the ethanol extract of the leaves of Plumeriaacuminata by animal studies. Using acute toxicity assay, the median lethal dose, LD50 was determined is higher than 2.0 gkg-1 b.w. In this assay, either deaths or symptoms associated with toxicity such as convulsion, ataxy, diarrhoea or increased diuresis occurred during the 72 h observation period. These results indicate the effectiveness and relative safety of drug extract for the treatment of conditions associated with inflammation. Carrageenan-induced oedema has been commonly used for acute inflammation. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandinsproduced by tissue macrophages[9]. The inhibitory activity shown by the extract of Plumeriaacuminata leaves (250 mgkg-1 b.w) over a period of 4 h in carrageenan induced paw inflammation shown good results and did comparisionwith group treated with diclofenac and control group.Results checked for 0min,15min,30min,60min,120mins.

V. CONCLUSION

Based on the results of percentage inhibition of paw volume at 120 mins shows 40.69%,38.37%. It can be concluded that the ethanolic extract of leaves of Plumeriaacuminatapossess an antiinflammatory property in acute phase of inflammation.

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