

# Anti-inflammatory Activity of an Ornamental Plant *Plumeria Obtusa*

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**Abstract Aims:** An experiment was conducted to evaluate the anti-inflammatory effects of ethanolic bark extract of *Plumeria obtusa*. **Material and method:** Anti-inflammatory activity was evaluated by using two different animal models namely cotton pellet method and carrageenan induce paw edema in male Wistar rats at a dose of 100,200 and 400 mg/kg respectively. **Results:** Phytochemical examination of the extract exposed the existence of alkaloids, flavonoids, tannins and terpenoids. The ethanolic extract elicited highly significant ( $p < 0.001$ ) inflammatory activity in a dose dependent manner on cotton pellet method and carrageenan induce paw edema method. This was noticed by prominently decrease of paw volume in carrageenan induce paw edema and marked increase in % inhibition of cotton pellet Granuloma pouch at the dose of 100,200 and 400 mg/kg respectively. **Conclusion:** The present investigations showed that the ethanolic extract *Plumeria obtusa* stem bark has potential anti-inflammatory activity when screened on carrageenan-induced paw edema and cotton pellet Granuloma pouch models in rats.

**Keywords** *Plumeria obtusa*, Medicinal Plant, Phytochemical Screening, Anti-inflammatory, Carrageenan, Cotton Pellet

and limiting damage[1]. Inflammation are broadly classified into two types viz; acute inflammation and chronic inflammation. With the dawn of science, traditional system of medicines begins to lose its importance and gradually synthetic drug gained more importance in the present era of allopathic system. Numerous synthetic compounds including Non-steroidal anti-inflammatory drugs (NSAIDs) were presented; but extended use of these anti-inflammatory drugs is associated with side effects. Hence, to explore for new anti-inflammatory agents that holds the therapeutic efficacy and also lacks the adverse effects associated with it [2].

*Plumeria obtusa* is a deciduous plant species belonging to the family-Apocynaceae and genus *Plumeria* usually recognized as Frangipani. *Plumeria obtusa* is innate to the northern Central America, Greater Antilles, Florida and southern Mexico. Farming is usually done in warmer parts of the world, including Southeast Asia. In traditional medicine, decoction of the bark is used as purgative or as a remedy against edemas. The ethanolic extract of stem bark of *Plumeria obtusa* exhibited antiulcer effect by ulcer induced pylorus ligation. As per our knowledge, there is scanty scientific report on the confirmation of the use of the stem bark of *Plumeria obtusa* in inflammation. Hence, the current study was commenced to discover anti-inflammatory prospective of *Plumeria obtusa* bark in rats so as to validate the traditional uses of this plant in folklore medicine.

## 1. Introduction

Primary vascular tissue has a complex biological response to harmful stimuli, such as pathogens, damaged cells, or an irritant is known as inflammation. It is a process by which the body's chemicals and white blood cells protect us from infection and foreign substances such as viruses and bacteria. Inflammation is an vital physiological reaction which happens in reaction to an extensive variety of Injurious agents (e.g. bacterial infection or physical trauma) ultimately trying to perform the dual function of promoting tissue repair

## 2. Materials and Methods

### 2.1. Collection of Plant Material

Stem bark of 2-3 years old *Plumeria obtusa*, common name frangipani, were collected at an altitude of 46ft in August 2014 from locations of Navi Mumbai, Maharashtra, India and authenticated by Dr. Rajendra D. Shinde, Botanist, Associate Professor at St. Xavier's College, Mumbai-400001.

## 2.2. Extraction & Phytochemical Evaluation

The 1 kg of dried Stem bark of *Plumeria obtusa* was cleaned by removing contaminated materials, and then were pulverized by an electric blender to give 600 gm of coarsely powder and stored in the airtight container. The coarse powder of stem bark of *Plumeria obtusa* was extracted with ethanol in Soxhlet extractor. Dark green ethanolic extract was dried under rotary vacuum evaporator, suspended in water and defatted with petroleum ether (thrice) and then successively fractionated in separating funnel with ethyl acetate (thrice) to get hydrophilic fraction (Brown color). Dried ethanolic extract was used further for the study. Further, these extracts were subjected to a battery of phytochemical tests to evaluate the constituents.

## 2.3. Animals

Male Wistar rats, weighing 150-230 g, were used. All animals were procured from Bharat serums and vaccines limited, Thane, India. The animals were kept in the animal room of measured conditions of  $24 \pm 1^\circ\text{C}$  and 12 h light – 12 h dark cycles. Food and water were given *ad libitum*. Animal Ethics Committee had approved all the animal models to be used. {Protocol number: OCP/IAEC//2014-15/05}

## 2.4. Drugs

Indomethacin (Donica® 25 mg, IPCA Limited, Mumbai) at dose of 10 mg/kg was used. Carrageenan was obtained from (S.D. Fine Chemicals, Mumbai, India).

## 2.5. Preliminary Phytochemical Screening [3].

For the confirmation of the presence of the phyto constituents such as alkaloids, glycosides, tannins, steroids, carbohydrate, proteins and amino acids, plant extract was subjected to phytochemical evaluation. Results of phytochemical screening presented in Table 1.

## 2.6. Physico-chemical Parameters [4].

The detection of several physico-chemical parameters such as total ash value, water soluble ash, acid insoluble value, sulphated ash and loss on drying were determined as per World Health Organization (WHO) guidelines for Herbal Formulation and Quality Standardized Herbs [4]. For results of physico-chemical parameters see Table 2.

## 2.7. Evaluation of *In Vivo* Anti-Inflammatory Activity of Stem Bark of *Plumeria obtusa*

The extracts were administered by oral gavage where as standard drug was injected Intra-peritoneally. Animals were randomly divided into 5 groups for each model. Each group comprised of 6 rats for the study.

Group I: Control.

Group II: Standard drug (indomethacin, 10 mg/kg)

Group III: PO (100 mg/kg)

Group IV: PO (200 mg/kg)

Group V: PO (400 mg/kg)

\*PO = Stem bark extract of *Plumeria obtusa*

### 2.7.1. Carrageenan-Induced Paw Edema in Rats [5].

The anti-inflammatory activity of the ethanolic extract of *Plumeria obtusa* was investigated on carrageenan induced inflammation in rat paw. Half an hour after the administration of the test drugs and standard, 1% carrageenan was injected to the right hind paw of each animal. The volume of paw edema was measured at 0, ½, 1, 2, 3, 4 and 6 hours using Plethysmometer after administration of carrageenan. The left hind paw served as a reference non-inflamed paw for comparison. The average percent increase in paw volume with time was calculated and compared against the control group.

**Table 1.** Results of Preliminary Phytochemical test

Sr. No.	Phytochemical test	Reagents used	Result
1	Alkaloids test	Mayer's Reagent	+
		Wagner's Reagent	+
		Hager's Reagent	+
2	Carbohydrates test	Molish's Reagent	+
		Benedict's Reagent	+
		Fehling's Reagent	+
3	Saponin test	Foam Test	+
4	Glycosides test	Modified Brontrager's	+
5	Phytosterol test	Salkowski's Test	+
6	Fats and Fixed oil test	Filter Paper press Test	-
7	Resins test	Acetone Water Test	+
8	Phenols test	Ferric Chloride Test	+
9	Tannins test	Gelatin Test	+
10	Diterpenes test	Copper Acetate Test	+
11	Flavonoids test	Alkaline Reagent Test	+
		Lead Acetate Test	+
		Shinoda Test	+
		Zinc HCl Test	+
12	Proteins and amino acids test	Xanthoproteic Test	+

+Indicates positive

-Indicates negative

### 2.7.2. Cotton-Pellet Granuloma Pouch Model [6].

The animals were administered with vehicle, standard drug and test drugs. One hour after the first dosing, the animals were anesthetized with anesthetic ether and 20 mg of the sterile cotton pellet was inserted one in each groin of rats by making small subcutaneous incision. The incisions were sutured by sterile catgut. After 10 day dosing, the animals were sacrificed by excess anesthesia on 10<sup>th</sup> day and cotton pellets were removed surgically. Pellets were separated from

extraneous tissue and dried at 60°C until weight become constant. The net dry weight that is after subtracting the initial weight of the cotton pellet was determined. The average weight of the pellet of the control group as well as of the test groups was calculated.

## 2.8. Statistical Analysis

Values are expressed as mean  $\pm$  S.D. (n = 6), one way ANOVA followed by Dunnett test; \* P < 0.05, \*\* P < 0.001 when compared with the control group.

## 3. Result and Discussion

### 3.1. Preliminary Phytochemical Analysis of *Plumeria obtusa*:

The preliminary phytochemical examination of *Plumeria obtusa* fractions revealed the presence of alkaloids, carbohydrates, flavanoids, glycosides, triterpenoids, tannins and phenolic compounds.

### 3.2. Physico-chemical Parameters

Physicochemical parameters of *Plumeria obtusa* are discovered in accordance with WHO guidelines for Quality Standardized Herbs and Herbal Formulation.

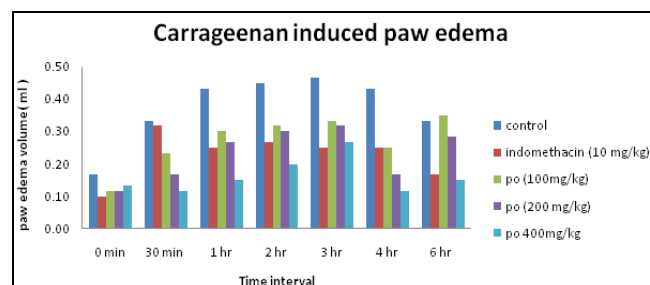
**Table 2.** Assessment of Physico-chemical parameters

Parameter	% w/w
Total ash	4.5%
Acid insoluble ash	1.53%
Water soluble ash	2.0%
Sulphated ash	5.6 %
Loss on drying	4.16%

### 3.3. Carrageenan Induced Rat Paw Edema

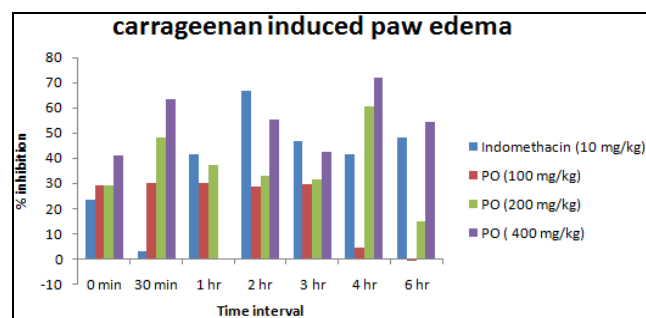
The carrageenan-induced paw edema model in rats has a direct relation to the cyclooxygenase inhibitors and has been used to investigate the effect of non-steroidal anti-inflammatory agents, which principally prevent the cyclooxygenase involved in prostaglandin synthesis [7].

There was a steady increase in edema paw volume in control group. The treatment group which was pretreated with ethanolic extract of *Plumeria obtusa* showed inhibition of edema formation in the first and second phase respectively (Fig 1) at a dose of 100mg/kg, 200mg/ kg and 400mg/kg. In 1<sup>st</sup> phase, all doses of *Plumeria obtusa* showed similar results but, there was increase in inhibition of second phase at higher dose of 400 mg/kg when compared to the lower doses of 100 mg/kg and 200 mg/kg respectively.



**Figure 1.** Effect of ethanolic extract of *Plumeria obtusa* on volume of paw edema.

Statistically significant result was obtained in terms of mean increase in paw volume at a dose of 200 mg/kg and 400 mg/kg in the given interval of time (30 min, +1 h, +2 h, +3 h, +4 h, and +6 h). After +3 h and +4 h of carrageenan administration, *Plumeria obtusa* exhibited maximum % inhibition of paw volume (P < 0.01) by 48.45%, 60.46% and 63.61%, 72.09%, at the higher two doses of plant extract 200 mg/kg and 400 mg/kg body weight respectively (Fig 2).



**Figure 2.** Effect of ethanolic extract of *Plumeria obtusa* on volume of percent inhibition of paw edema

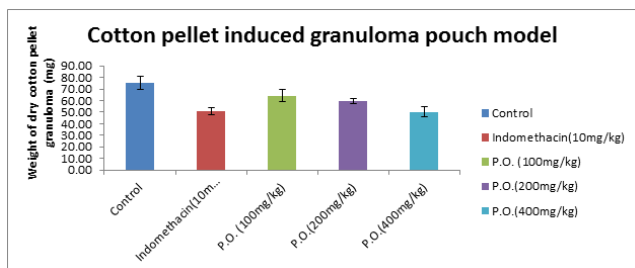
Extract given at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg significantly inhibited the 2nd phase paw edema volume induced by carrageenan, this result proposed a mechanism that there might be inhibition of cyclooxygenase synthesis by the extract and this effects are comparable to that created by nonsteroidal anti-inflammatory drugs such as indomethacin, which act in a similar manner by inhibiting cyclooxygenase enzyme.

### 3.4. Cotton Pellet Induced Granuloma Pouch Model

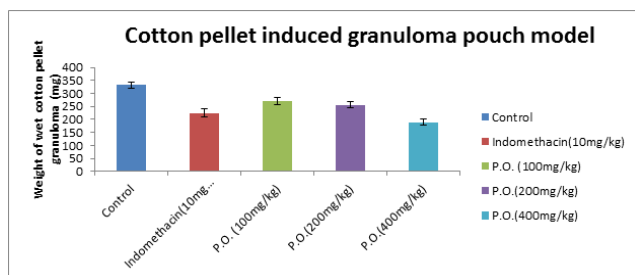
The inflammatory granuloma is associated with chronic inflammatory process [8] [9]. The cotton pellet granuloma method has been extensively used to estimate the exudative, transudative and proliferative components of chronic inflammation [10].

The ethanolic extract of *Plumeria obtusa* at a dose of 200 mg/kg and 400 mg/kg and standard drug indomethacin showed (P < 0.001) significant activity in inhibiting dry weight of granuloma. The dose administered at 200 mg/kg and 400 mg/kg showed a higher anti-granulation (20.61% and 33.24 %) effect respectively but less than indomethacin (31.91%) (Fig: 3 & 4). Hence, the reduction in the weight of granuloma specifies that the proliferative phase was efficiently repressed by ethanolic extract of *Plumeria obtusa*.

Therefore the extract of *Plumeria obtusa* was found to be effective in cotton pellet induced granuloma pouch model and it can be supposed that it is effective in all the phases of inflammation i.e., acute, sub-acute and proliferative phases. As the action also depends on the pharmacokinetic parameters, the activity of *Plumeria obtusa* extract was found to decline after 6 hrs indicating that it might be in a phase of metabolism and excretion.



**Figure 3.** Effect of ethanolic extract of *Plumeria obtusa* on weight of dry cotton pellets in Cotton pellet induced granuloma pouch model



**Figure 4.** Effect of ethanolic extract of *Plumeria obtusa* on weight of wet cotton pellets in Cotton pellet induced granuloma pouch model.

## 4. Conclusions

Ethanolic fraction of the stem-bark of *Plumeria obtusa* exhibited good anti-inflammatory activity when subjected for the screening of the anti-inflammatory activity by using in vivo animal model like carrageenan induced rat paw edema and cotton pellet induced granuloma pouch model. These activities are associated with the presence of glycosides, flavonoids, alkaloids. *Plumeria obtusa* 400 mg/kg showed highly significant result in both in vivo animal models testing, indicating that the effect of the extract was in a dose dependent manner. However, further study is required to determine the actual constituents responsible for anti-inflammatory activity and further authenticate its mechanism of action. A limitation of the study includes that only two experimental models were used. It is a well-known fact that inflammation is often associated with pain. Thus, analgesic activity of the plant *Plumeria obtusa* needs to be explored. The study holds an advantage that the species of *obtusa* was used which is commonly used as an ornamental plant whereas other studies have used different species of genus *plumeria*. This article will be helpful in shedding a

light on *Plumeria obtusa* so that it can be viewed differently; a plant, apart from being so beautiful also possesses healing properties. Future investigating studies are expected to compare the chemical constituents of this plant with available synthetic drugs. As witnessed by history, there are certain plant derived drugs like the anti-malarial drugs such as cinchona alkaloids and arte-ether are more potent and useful. Because of unwanted adverse effects coupled with synthetic drugs, researchers are more enthusiastic to unveil the hidden treasures of the flora world.

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