

**TO INVESTIGATE THE MAST CELL STABILIZATION  
AND ANTIANAPHYLACTIC ACTIVITY OF ETHANOLIC  
EXTRACT OF *THESPESIA POPULNEA* BARK.**

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**Summary**

To Investigate the mast cell stabilization and antianaphylactic activity of ethanolic extract of *Thespesia populnea* bark.

*Thespesia populnea* is (Family Malvaceae) is a small or large tree; more commonly found in tropical and sub tropical area. *Thespesia populnea* is widely used traditionally in various inflammatory conditions as well as the anti inflammatory activity has been also proven scientifically. Histamine is key parameter for evaluating any target for its anti-allergic potential. Mast cell stabilization activity was investigated by Compound 48/80 induced mast cell degranulation in rat peritoneal mast cell and Antianaphylactic activity was performed by determining the mortality rate of rat upon exposure to compound 48/80. Significant reduction of % mast cell degranulation was observed at 60 mg/ml dose of TPEE (Ethanolic extract of *Thespesia populnea* bark) and Antianaphylactic activity of TPEE at dose of 400mg/kg which was comparable with Ketotifen. This finding provides evidence that TPEE inhibits mast cell-derived immediate-type allergic reactions and mast cell degranulation.

**Key Words:** TPEE, Mast cell, Histamine, Compound 48/80.

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## Introduction

Mast cells are constituents of virtually all organs and tissues and are important mediators of inflammatory responses such as anaphylaxis and allergy<sup>[1]</sup> in which histamine is most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity upon release<sup>[2]</sup>. Mast cell degranulation can also be evoked by the compound 48/80, which is a mast cell degranulator and has been used as a direct and convenient reagent to study the mechanism of anaphylaxis<sup>[3]</sup>. The influence of natural products derived from plants is broadly recognized for their great structural diversity as well as their wide range of pharmaceutical activities<sup>[4]</sup>. *Thespesia populnea* is (Family: Malvaceae) is a small or large tree, up to 3 mt height and more commonly found in India up to an altitude of 1,200 mt with reddish brown bark, cordate leaves, and yellowish flowers with crimson centre<sup>[5]</sup>. *Thespesia populnea* is widely used traditionally in various inflammatory conditions. The therapeutic attributes of plants have been inquired in the light of recent scientific developments throughout the world, due to their pharmacological activities like anti-inflammatory activity<sup>[6]</sup>. With this background, the study was designed to establish the mast cell stabilization and antianaphylactic activities of ethanolic extract from *Thespesia populnea*.

## Materials and Methods

### Chemicals:

Compound 48/80 (Sigma), Toluidine blue dye (Hi-media), Ketotifen fumarate (Gift sample from Torrent).

### Experimental animals:

All animals were housed at ambient temperature (22±1°C), relative humidity (55 ±5° %) and 12:12 hrs light/dark cycle. Animal had access to standard pellet diet (Pranav Agro Industries Ltd., Sangali) and water *ad libitum*. The Protocol approved by the institutional animal ethics committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on animals (CPCSEA), Ministry of social Justice and Empowerment, Government of India.

**Collection of plant material:**

The Bark (2 kg) of *Thespesia populnea* was collected from surrounding field of Mehsana district of Gujarat between February-March, 2008. The Bark was authenticated by the Dr. Ritesh Vaidya, Assoc. Professor, Bioscience department, Ganpat University, Kherva, Mehsana. The shade-dried bark was ground. It was stored in an airtight, hard polyethylene container with silica pouch up to 10-12 days.

**Preparation of plant extract:**

The bark powder was packed in a soxhlet apparatus and extracted with 95% ethanol for 18 hrs. Appearance of colorless solvent in the siphon tube was taken as the termination of extraction. After each extraction, the solvent was recovered using distillation assembly, and the extract was concentrated under reduced pressure. Then, the extract was transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50°C to get ethanolic extract. The marc was finally air dried thoroughly to remove all traces of the solvent.

**Studies on compound 48/80 induced mast cell degranulation<sup>[7]</sup>:**

**Preparation of peritoneal mast cell suspension:**

Normal saline containing 5 units /ml of heparin was injected in the peritoneal cavity of male rats lightly anaesthetized with ether. After a gentle abdominal massage, the peritoneal fluid containing mast cells was collected in centrifuge tubes placed over ice. Peritoneal fluid of 4-5 rats was collected and centrifuged at 2000 rpm for 5 min. Supernatant solution was discarded and the cells were washed twice with saline and resuspended in 1 ml of saline.

**Procedure:**

Take 7 test tubes and transfer respective solution as follows. Test tube no 1: 0.1 ml peritoneal fluid, Test tube no.2: 0.1 ml peritoneal fluid + 0.1 ml Compound 48/80 , Test tube no.3: 0.1 ml peritoneal fluid + 0.1 ml Compound 48/80 + 0.1ml of 10µg/ml of Ketotifen fumarate, Test tube no.4: 0.1 ml peritoneal fluid + 0.1 ml Compound 48/80 +0.1ml of TPEE 10 mg/ml , Test tube no.5: 0.1 ml peritoneal fluid + 0.1 ml Compound 48/80 +0.1ml of TPEE 20 mg/ml, Test tube no.6: 0.1 ml peritoneal fluid + 0.1 ml Compound 48/80 +0.1ml of TPEE 40 mg/ml , Test tube no.7: 0.1 ml peritoneal fluid + 0.1 ml Compound 48/80 +0.1ml of TPEE 60 mg/ml.

Each test tube was incubated for 15 min at 37°C with peritoneal fluid and respective drug treatment and then Compound 48/80 (0.1 ml, 10µg/ml) was added to each test tube except test tube no. 1. After further incubation for 10 min. at 37°C, the cells were stained with 0.1% toluidine blue solution made in distilled water and examined under the high power of light microscope. Same procedure was repeated in triplicate manner. Percent degranulation of the mast cells in the different test tubes treated with different doses of TPEE was calculated by counting the number of degranulated mast cells from total of at least 100 mast cells counted under electron microscope with 45X magnification and from it % protection against degranulation was calculated.

**Systemic anaphylaxis model using rat** <sup>[8,9]</sup>:**Procedure:**

Albino wistar rats of either sex weighing range of 200-250g were used and divided into four groups, six animals in each group. Group I: Disease Control (vehicle), Group II TPEE: 100 mg/kg, Group III: TPEE 200 mg/kg, Group-IV: TPEE 400 mg/kg. Mast cell degranulator, Compound 48/80 (8 mg/kg) and TPEE were dissolved in saline. The various doses of TPEE i.e. 100, 200, 400 mg/kg were administered orally except disease control group. Compound 48/80 was administered i.p. in all groups after 1 hr respective treatments. Mortality was monitored for 1 hr after induction of anaphylactic shock. This model was performing in triplicate manner.

**% Mortality:** No. of dead rat X 100/ Total no of experiment rats at respective dose.

**Stastical Analysis:**

Results were expressed as Mean ± S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA (One way analysis of variance) followed by Dunnett's Multiple Comparison test using computer based software program (Graph pad prism, version 5.0). Comparison was carried out in manner of disease control group versus all treated groups.

### Results and Discussion

The results from earlier studies showed significant anti-inflammatory activity in several experimental studies and also used traditionally in inflammatory conditions. So, we chose this plant to give scientific background for mast cell stabilization and anti-anaphylactic activity.

#### **Effect of Ethanolic Extract of *Thespesia populnea* on Compound 48/80 induced mast cell degranulation.**

Degranulation of mast cells causes release of pre-formed and newly formed mediators leading to acute and late phase allergic reactions depending on the allergic disease<sup>[10]</sup>. Compound 48/80 is one of the most potent mast cell degranulators, which causes liberation of inflammatory mediators such as histamine, leukotriene, platelet activating factors, chemotactic factors for eosinophils and neutrophils etc. from mast cell<sup>[11, 12]</sup>. They play a significant role in airway inflammatory response such as airway eosinophilia, late asthmatic response, and airway hyperresponsiveness as well as immediate hypersensitivity reaction like bronchial contraction. Degranulation of mast cell has been taken as the positive criteria for anaphylaxis.

Compound 48/80 challenged resulted in significant degranulation of rat peritoneal mast cells ( $79.67 \pm 1.358$ ). Preincubation of rat peritoneal mast cells with Test drug at 10 mg/ml, 20 mg/ml, 40 mg/ml, and 60 mg/ml for 15 minutes resulted in significant reduction of % mast cell degranulation in dose dependant manner  $40 \pm 1.483$ ,  $24.67 \pm 1.453$ , and  $17 \pm 1.432$ , and  $5 \pm 0.792$  respectively. The effect of Test drug (TPEE) was also comparable with Standard drug (Ketotifen Fumarate) ( $18.67 \pm 1.764$ ) were shown in Figure: 1. A significant protection showed against compound 48/80 induced rat peritoneal mast cells disruption by Ethanolic Extract of *Thespesia populnea* indicates its ability to interfere the release and/or synthesis of mediators of inflammation, exhibiting its mast cell stabilizing activity.

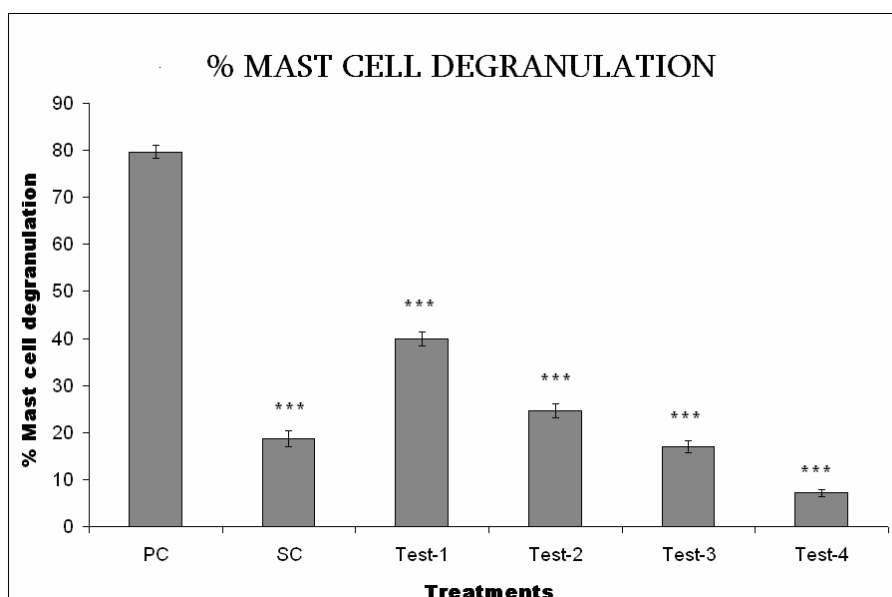


Figure 1: % mast cell degranulation

PC: Positive control, SC: Standard control, Test-1: TPEE (10 mg/ml); Test-2: TPEE (20 mg/ml); Test-3: TPEE (40 mg/ml), Test-4: TPEE (60 mg/ml). All values represented as Mean  $\pm$  S.E.M. of six animals. Significance at the level of \*\*\*  $p \leq 0.001$ .

#### Effect of ethanolic extract *Thespesia populnea* on compound 48/80 induced systemic anaphylaxis in rats.

Stimulation of mast cells with compound 48/80 is believed to initiate the activation of a signal transduction pathway, which leads to histamine release. There have been some reports that compound 48/80 is able to activate G proteins [13, 14]. Chadi et al. announced that compound 48/80 activates mast cell phospholipase D (PLD) via heterotrimeric GTP-binding proteins [15]. They identified recombinant  $G\beta_2\gamma_2$  subunit markedly synergized PLD activation by compound 48/80 in permeabilized RBL-2H3 cells. Murine mast cells are a good experimental model for the study on compound 48/80-induced histamine release [16]. The report that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane [17] indicates that the membrane permeability increase may be an essential trigger for the release of mediators from mast cells.

To assess the contribution of TPEE in anaphylactic reactions, systemic anaphylaxis model was used. In disease control group, compound 48/80 showed 100 % mortality. Where as TPEE (100, 200, 400 mg/kg) showed protection against mortality in dose-dependant manner ( $66.66 \pm 0.00$ ,  $33.33 \pm 0.00$ ,  $11.11 \pm 11.11$  respectively). Were shown in figure: 2. Our results showed that TPEE pretreatment profoundly affected compound 48/80-induced systemic anaphylaxis. Thus, TPEE might act on the lipid bilayer membrane affecting the prevention of the perturbation induced by compound 48/80.

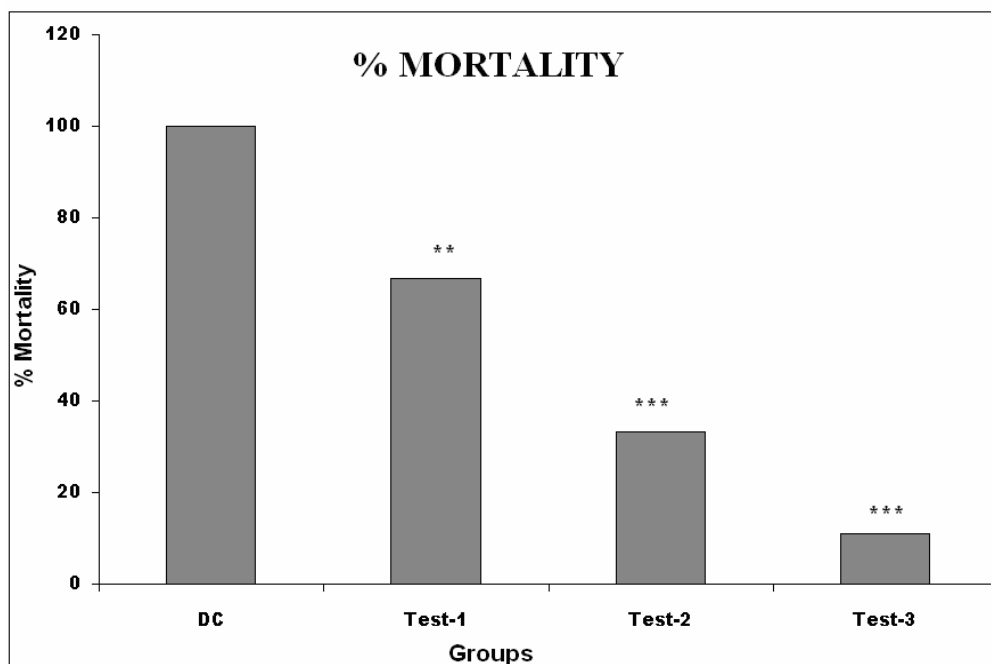


Figure: 2 % Mortality

DC: Disease control, Test-1: 100 mg/kg TPEE, Test-2: 200 mg/kg TPEE, Test-3: 400 mg/kg TPEE

\*\*\* $P < 0.001$ ; significantly different from the control value.

### Conclusion

The results revealed that mast cell stabilization and antianaphylactic activities, which are closely mimicked to the standard, Ketotifen and it is worth isolating the phytochemical from this source for benefit of being potential anti-allergic drug, which is under progress in our laboratory.

**Future action plan:**

However, further investigation should be carried out to isolate active chemical constituents that are responsible for Anti Asthmatic activity and includes analysis of active principles by UV, IR, MS and NMR spectroscopy along with separation techniques by HPTLC.

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