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# In Vivo Mast Cell Stabilizing Activity of Different Extracts Of Trigonella Foenum-Graecum on the Rat Mesenteric Mast Cells

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***Abstract:*** *Mast cell stabilizing activity of different extracts of Trigonella foenum-graecum was evaluated with the help of rat mesenteric mast cells. The study includes the mesenteries which are pretreated with prednisolone, petroleum ether, methanol and aqueous extract of Trigonella foenum-graecum (250mg, 500mg and 750mg) were analyzed for the degranulation of mast cell during the anaphylactic reactions. It was carried out on the mesenteries of rats, which are sensitized with sheep serum and triple antigen to induce degranulation of mast cells. Treatment with aqueous extract of Trigonella foenum-graecum (500mg) showed beneficial effect on mast degranulation of actively sensitized rats. The effect was comparable with that of standard drug, Prednisolone. Mast cell stabilizing activity of aqueous extract of Trigonella foenum-graecum on the rat mesenteric mast cells may be possibly due to the membrane stabilizing potential.*

***Keywords:*** *Mast cell stabilizing activity, Mast cell degranulation, Trigonella foenum-graecum, Membrane stabilization, Anaphylaxis.*

## I. Introduction

Anaphylaxis is one of the common diseases that affect mankind, and is responsible for significant morbidity and mortality [(1)]. Ayurveda, recommended a number of natural drugs for the treatment of various diseases like anaphylaxis, bronchial asthma and allergic disorders [(2)]. Anaphylaxis is triggered by foods (nuts, fish, wheat etc), medications (Penicillin), venom from insects, latex from natural rubber, allergy shots[(3)]. Intensive research during the last several decades has highlighted the role of lymphocytes, immunoglobulins, mast cells in the etiopathogenisis of allergic conditions[(4)]. The mast cells plays an important role in the development of anaphylaxis and allergic responses. Anaphylaxis is due to histamine release in response to antigen cross linking of immunoglobulin E (IgE) bound to Fc epsilon RI receptors on mast cells[(5)]. During anaphylaxis the mast cells are degranulated which decreases the number of intact mast cells and increases the number of degranulated mest cells[(6)].

The treatment options available for allergic diseases have major limitations owing to low efficacy, associated with different adverse events and compliance issues[(4)]. So, the current approaches are largely ameliorative rather than curative. *Trigonella foenum-graecum* has been used for allergy, in the Ayurvedic system of Indian medicine for the treatment of bronchial asthma, eczema, insect bites etc [(7)]. So, in the present study, we examined the activity of different extracts (petroleum ether, methanolic and aqueous extract) of *Trigonella foenum-graecum* on rat meenteric mast cells by the active anaphylaxis model.

## II. Materials And Methods

### Collection of plant material

The seeds of Trigonella foenum-graecum was collected from local market of Tirupathi. After taxonomic verification and were identified and authenticated in Department of Botany, S.V.University, Tirupati. The seeds of Trigonella foenum-graecum were washed, dried at room temperature for 2 to 3 days under shade and was treated with a rotary grinder for size reduction. The seeds were coarsely powdered and stored in airtight plastic containers. This powder was used for all phytochemical analysis.

### Preparation of extracts

The powder was used for preparation of extracts. The powder (100 g) was extracted with Soxhlet apparatus using 400 mL petroleum ether for about 48h. After defatting, the marc was dried in hot air oven at 50°C, packed in Soxhlet apparatus and further extracted with 400 mL 95% ethanol until it does not show the presence of any residue on evaporation. The aqueous extract was prepared by cold maceration with 3%

methanol-water for 7 days with occasional shaking. The solvents were removed from the extracts under reduced pressure by using rotary vacuum evaporator.

**Experimental animals:**

The study was conducted on Wister rats of both male and female(175 – 200 gm). They were housed in standard conditions of temperature (22 ± $2^0$C), relative humidity (60 ± 5%) and light (12h light/ dark cycle). They were fed with standard pellet diet and water. The rats were placed in wire-bottomed cages to avoid coprophagy and fighting,. All animal experiments were carried out in accordance with the guidelines of CPCSEA. Anaphylaxis is induced by sheep serum which was prepared by collecting the fresh sheep blood from the slaughter house under sterile condition.

**Active Anaphylaxis:**

72 rats are divided into 12 groups of (Group-1 is Unsensitized, Group-2 to 12 is Sensitized.) six animals each. Rats were sensitized by injecting subcutaneously 0.5 ml of sheep serum along with 0.5 ml of triple antigen containing 20,000 million Bordetella Pertusis organisms[8] ( Serum Institute of India Ltd., Pune). The six animals in Group-1 is an unsensitized group, which is a normal group and receives water (Vehicle). Rats of Group-2 received water and served as control. Group-3 rats received 10 mg/kg/day of Prednisolone (reference drug) orally for 14 days. Rats of Group-4, 5 and 6, were administered with 250, 500 and 750 mg/kg/day of petroleum ether extracts of Trigonella foenum-graecum respectively for the same duration. Rats of Group-7, 8 and 9, were administered with 250, 500 and 750 mg/kg/day of methanolic extracts of Trigonella foenum-graecum respectively for the same duration. Rats of Group-10, 11 and 12, were administered with 250, 500 and 750 mg/kg/day of aqueous extracts of Trigonella foenum-graecum respectively for the same duration. On day 14 the rats were sacrificed with intraperitoneal injection of Pentobarbitone (40 mg/kg) to avoid trauma. Intestinal mesentery was taken for the study on mast cells. Mesenteries of sacrificed rats along with intestinal pieces were kept in Ringer-Locke solution ( Nacl 9.0, Kcl 0.42, $Cacl_2$ 0.24, $NaHCo_3$ 0.15, Glucose 1.0 gm/ltr of distilled water) at $37^0$C. The Mesenteric pieces were challenged with 5% v/v Sheep serum for 10 minutes, after which the mast cells were stained and examined microscopically for the number of intact and degranulated Mast cells [9]

**Mast cell count:**

A piece of small intestine along with intact mesentery was excised and spread with out damage in a petridish, containing Ringer–Locke solution at $37^0$C. The preparation was challenged with 5% v/v Sheep serum for 10 minutes and then transferred to a wide mouthed bottle containing 10% formalin for 24 hrs. The mesenteric fans were fixed, dried and stained with thionin (0.25%) on a clean slide. The excess stain was washed with distilled water followed by dehydration in absolute alcohol. Finally the slides were cleared in Xylene and mounted in Diphenylphthalein xylene for Mast cell count [10]. The results were analysed statistically using ANOVA. The level of significance was fixed at $P<0.05$.

**Treatment Schedule Of Different Groups**

| S.No | Group | 1st Day | 1-14 days | | 14th day |
|---|---|---|---|---|---|
| 1 | Group 1 | Un sensitized | Water | | Sacrificed by intra- peritoneal injection of Pentobarbitone (40 mg/kg), The Mesenteric pieces were collected & challenged with 5% v/v Sheep serum for 10 minutes, after which the mast cells were stained and examined microscopically for the number of intact and degranulated Mast cells |
| 2 | Group 2 | Sensitized with S.C. injection of 0.5 ml sheep serum along with 0.5 ml of Triple antigen containing 20,000 million Bordetella Pertusis organisms | Water | | |
| 3 | Group 3 | | Prednisolone 10 mg | | |
| 4 | Group 4 | | Petroleum ether extract of Trigonella foenum-graecum | 250 mg | |
| 5 | Group 5 | | | 500 mg | |
| 6 | Group 6 | | | 750 mg | |
| 7 | Group 7 | | Methanol extract of Trigonella foenum-graecum | 250 mg | |
| 8 | Group 8 | | | 500 mg | |
| 9 | Group 9 | | | 750 mg | |
| 10 | Group 10 | | Aqueous extract of Trigonella foenum-graecum | 250 mg | |
| 11 | Group 11 | | | 500 mg | |
| 12 | Group 12 | | | 750 mg | |

## III. Results

After 14 days of sensitization, the antigen challenge group (Group-2) degranulated about 80% of Mast cells. Treatment of the rats (Group-3 and Group-11) with Prednisolone (10 mg), 500 mg/kg of Aqueous extract of Trigonella foenum-graecum prior to sensitization had decreased ($P<0.005$) the mast cell degranulation when compared to the petroleum ether and methanol extracts of Trigonella foenum-graecum. There was no significant difference among the Group-3 and Group-11.

**Table:** Effect of different extracts of Trigonella foenum-graecum on mast cell degranulation in actively sensitized rats

| S.No | Group | Treatment Dose(mg/kg/day p.o.) | | Intact mast cells(%) (mean ±S.E.) | Degranulated mast cells(%) (mean ±S.E.) |
|---|---|---|---|---|---|
| 1 | Group 1 | Water | | **86.42±4.53** | **13.58±4.53** |
| 2 | Group 2 | Water | | **20.31±1.65** | **79.69±1.65** |
| 3 | Group 3 | Prednisolone | 10 mg | **70.32±3.86*** | **29.68±3.86** |
| 4 | Group 4 | Petroleum ether extract of Trigonella foenum-graecum | 250 mg | **28.24±1.19** | **71.76±1.19** |
| 5 | Group 5 | | 500 mg | **44.23±2.21** | **55.77±2.21** |
| 6 | Group 6 | | 750 mg | **41.21±2.38** | **58.79±2.38** |
| 7 | Group 7 | Methanol extract of Trigonella foenum-graecum | 250 mg | 24.31±**1.47** | 75.69±**1.47** |
| 8 | Group 8 | | 500 mg | **46.34±2.52** | **53.66±2.52** |
| 9 | Group 9 | | 750 mg | **39.22±2.34** | **60.78±2.34** |
| 10 | Group 10 | Aqueous extract of Trigonella foenum-graecum | 250 mg | **33.27±2.34** | **66.73±2.34** |
| 11 | Group 11 | | 500 mg | **67.29±3.48*** | **32.71±3.48** |
| 12 | Group 12 | | 750 mg | 61.32±**2.22** | 38.68±**2.22** |

Values are mean ± S.E., n=6, *P<0.001(Students t-test).

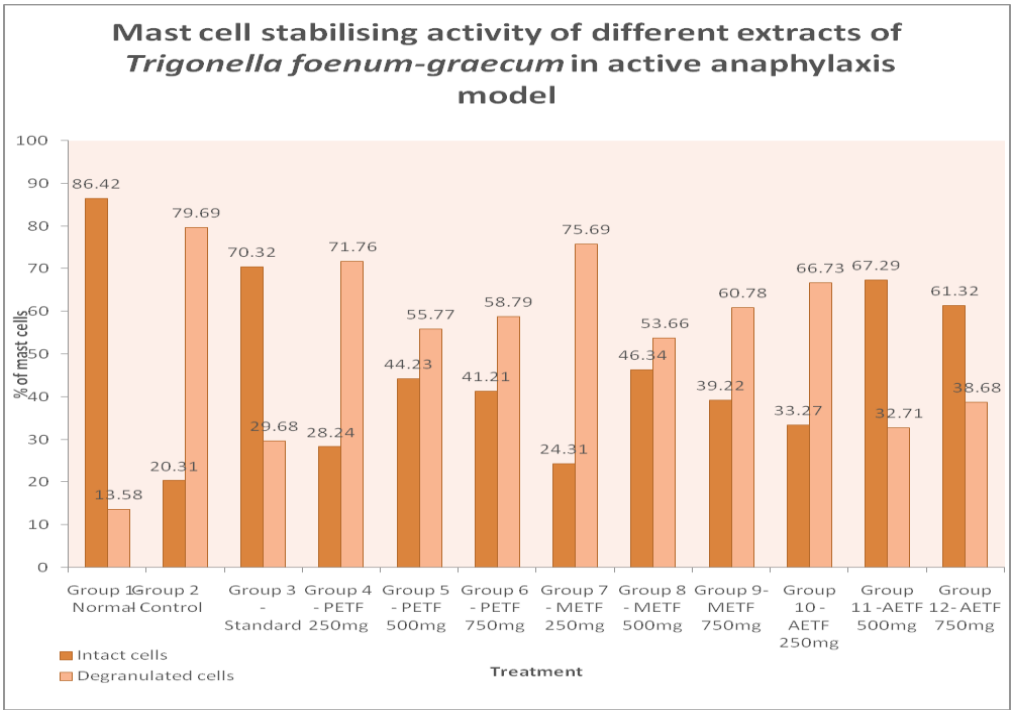
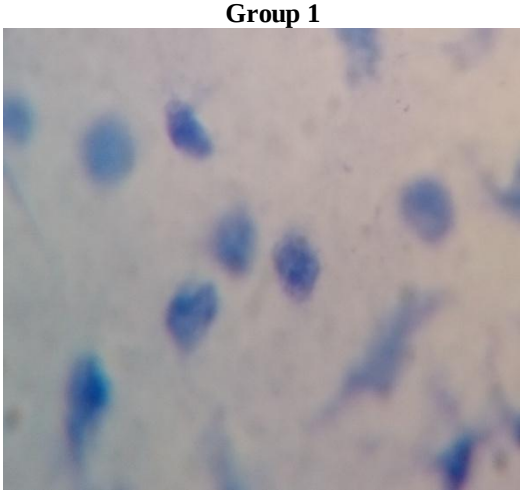



**Fig. 1:** Effect of different extracts of Trigonella foenum-graecum on mast cell degranulation in actively sensitized rats

**Group 1**



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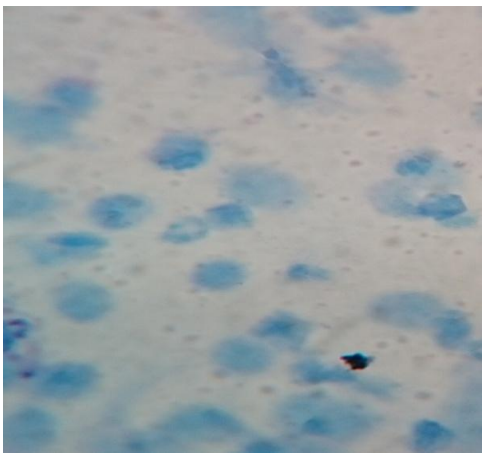

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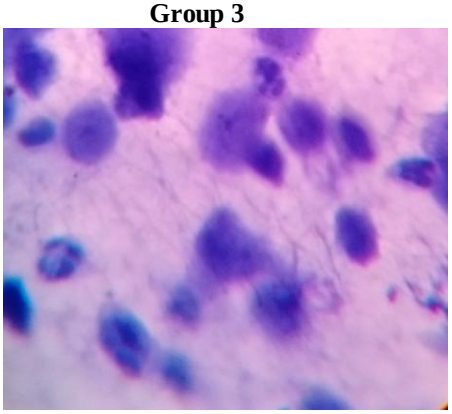

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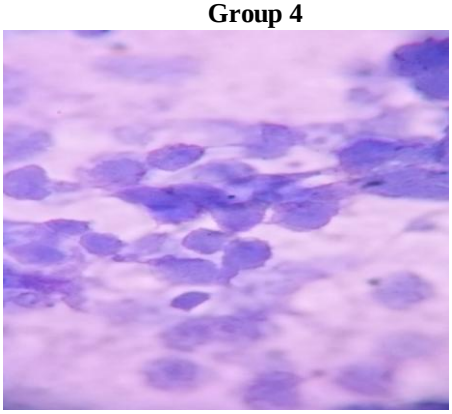

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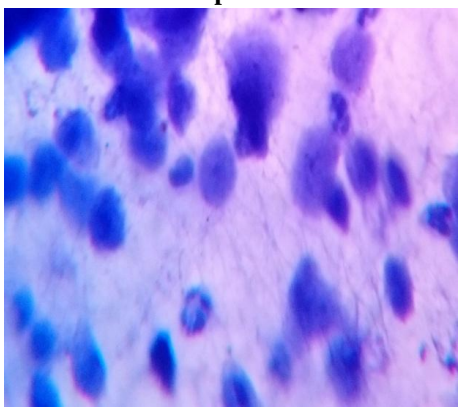
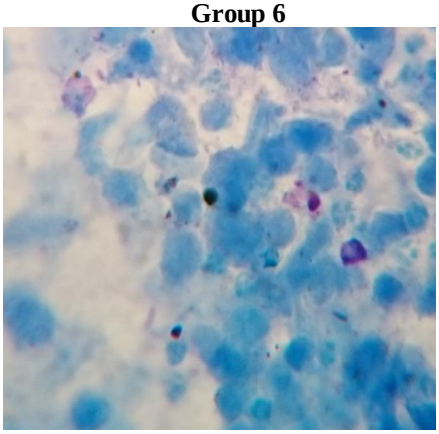


**Group 6**



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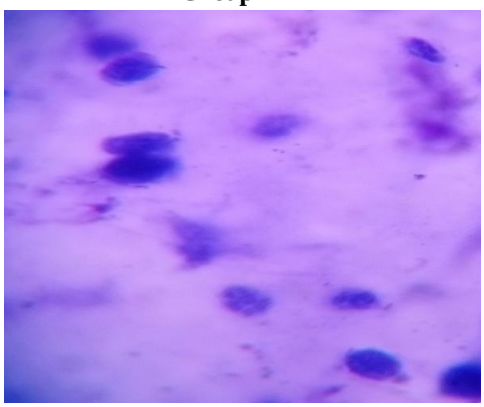
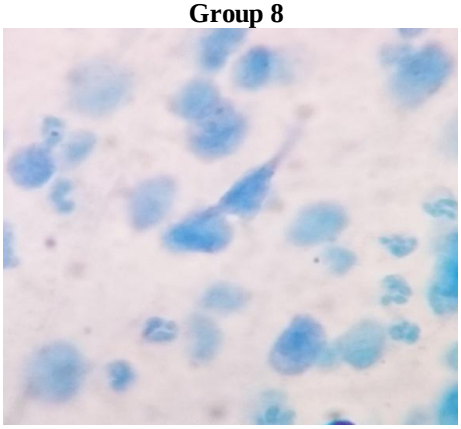
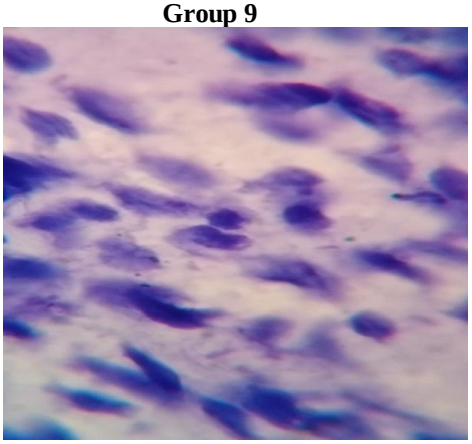


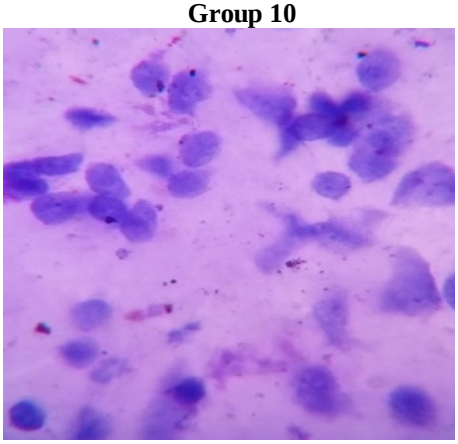
**Group 8**



**Group 9**



**Group 10**



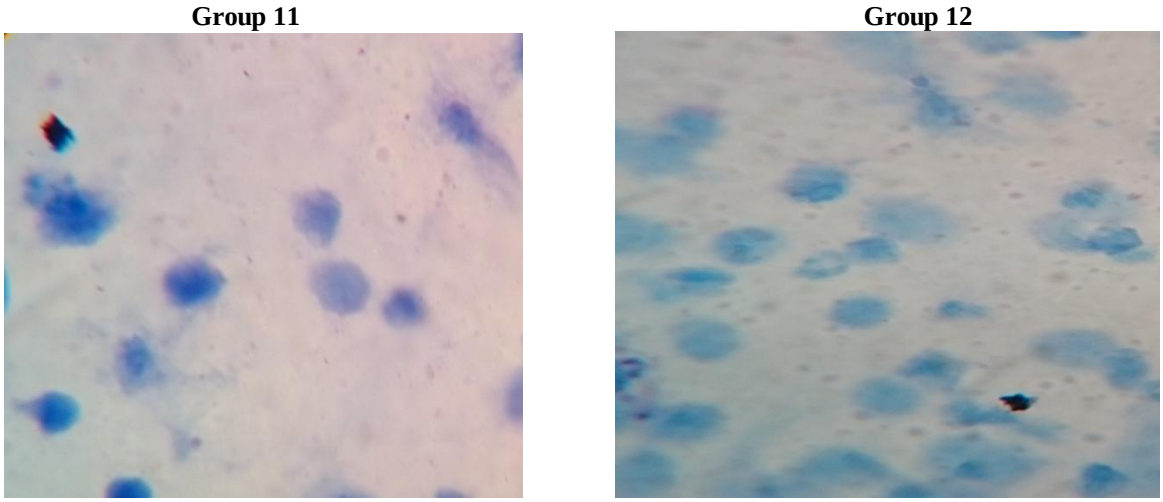



**Fig. 2:** Microscopic images of effect of different extracts of Trigonella foenum-graecum on mesenteric mast cell degranulation in actively sensitized rats.

## IV. Discussion

The activity of *Trigonella foenum-graecum* on the Mast cell stabilizing activity was studied on the rat mesenteric mast cells, following active Anaphylaxis. When compared to the petroleum ether and methanolic extracts, aqueous extract of *Trigonella foenum-graecum* has marked protection against the mast cell degranulation. The protection offered by the aqueous extract of *Trigonella foenum-graecum* may be attributed due to their mast cell stabilizing potential against antigen antibody reaction [(11)].

The stabilization of mast cell membrane, inhibition of antigen induced histamine release or non availability of antibodies on the mast cell surface is the reason for the antianaphylactic activity. It has been assumed that the process leading to histamine secretion may be mediated by calcium release from an intracellular store of mast cells [(12)]. The inhbition of degranulation of mast cells by aqueous extract of *Trigonella foenum-graecum* may be due to increase in the cyclic AMP levels by decreasing the cAMP phosphodiesterase. This inhibits the fusion of granules. It may be due to the be the flavonoids present in the plant. Further investigation may prove the exact mechanism by which aqueous extract of *Trigonella foenum-graecum* may stabilize the mast cells[(13)]. Vital organs such as liver and heart showed no significant changes. There was no significant change in the general behavior.

## V. Conclusion

All this findings reveal that, of all the three the aqueous extract of *Trigonella foenum-graecum* has the mast cell stabilizing activity. The mast cell stabilizing activity of aqueous extract of *Trigonella foenum-graecum* may be due to the mast cells stabilizing potential, suppression of antibody production and inhibition of antigen induced histamine release.

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