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ANTIANAPHYLACTIC ACTIVITY OF *NIGELLA SATIVA* SEEDS ON THE RAT MESENTERIC MAST CELLS

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ABSTRACT

Anti-anaphylactic activity of three extracts (petroleum ether, methanol and aqueous extract) of *Nigella sativa* seeds was evaluated on the rat mesenteric mast cells. Mast cell degranulation is induced by sensitizing with sheep serum and triple antigen. Mesenteries pretreated with prednisolone, petroleum ether, methanol and aqueous extract of *Nigella sativa* (250mg, 500mg and 750mg) were analyzed for the mast cell degranulation during the anaphylactic reactions. Methanolic extract of *Nigella sativa* (500mg) shows effect treatment on degranulation of actively sensitized rats which are compared with that of Prednisolone. The possible mechanism of anti anaphylactic activity of methanolic extract of *Nigella sativa* on the rat mesenteric mast cells may be the membrane stabilizing potential, and inhibition of antigen induced histamine release.

Keywords: Antianaphylactic activity, mast cell degranulation, *Nigella sativa*, membrane stabilization, degranulated mast cells.

INTRODUCTION:

Various diseases, like anaphylaxis, bronchial asthma and allergic disorders are treated in ayurveda, by a number of drugs[1]. One of the common diseases that affect mankind with diverse manifestations and is responsible for significant morbidity and mortality is allergy[2]. Anaphylaxis is triggered by different substances like foods (nuts, fish and wheat etc.,), medications (Penicillin), venom from insects, latex from natural rubber; allergy shots and extreme temperature also act as stimuli for anaphylaxis.[3]Lymphocytes, immunoglobulins, mast cells play an important role in the etiopathogenisis of allergic conditions[4] and in the development of many physiological changes during anaphylaxis and allergic responses. Anaphylaxis is due to antigen cross linking of immunoglobulin E (IgE) bound to Fc epsilon RI receptors on mast cells which induces degranulation and releases histamine[5]. During anaphylaxis the mast cells are degranulated which decreases the number of intact mast cells[6].

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The available treatment for anaphylaxis has major limitations which are associated with adverse events and compliance issues[4]. So, the current approaches are largely ameliorative rather than curative. *Nigella sativa* has been used in the ayurvedic system of Indian medicine for the treatment of bronchial asthma, eczema etc[7]. So, the present study involves the evaluation of the effect of different extracts (petroleum ether, methanolic and aqueous extract) of *Nigella sativa* on the active anaphylaxis in rats by using rat mesenteric mast cells.

MATERIALS AND METHODS:

Collection of plant material

The seeds of *Nigella sativa* were 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 *Nigella sativa 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 preparation of different extracts.

Preparation of extracts

The powder was used for preparation of extracts. The powder (100gm) was extracted with soxhlet apparatus using 400mL 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 400mL of 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 male and female wistar rats (175–200gm) housed in standard conditions of temperature (22±2^{0}C) relative humidity (60±5%) and light (12 h light/dark cycles) were used. They were fed with standard pellet diet and water. To avoid coprophagy and fighting, the rats were placed in wire-bottomed cages. All animal experiments were carried out in accordance with the guidelines of CPCSEA. Sheep serum to induce anaphylaxis 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 0.5ml of sheep serum along with 0.5ml 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 10mg/kg/day of prednisolone (reference drug) orally for 14 days. Rats of group-4, 5 and 6, were administered with 250, 500 and 750mg/kg/day of petroleum ether extracts of *Nigella sativa* respectively for the same duration. Rats of group-7, 8 and 9, were administered with 250, 500 and 750mg/kg/day of methanolic extracts of *Nigella sativa* respectively for the same duration. Rats of group-10, 11 and 12, were administered with 250, 500 and 750mg/kg/day of aqueous extracts of *Nigella sativa* respectively for the same duration. The treatment schedule is given in table-1. On day 14 the rats were sacrificed with intraperitoneal injection of pentobarbitone (40mg/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 and Glucose 1.0gm/ltr of distilled water) at 37^{0}C. The mesenteric pieces were challenged with 5%v/v sheep serum for 10min, 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 10min and then transferred to a wide mouthed bottle containing 10% formalin for 24h. The mesenteric fans were fixed, dried and stained with toluidine blue (0.1%) 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 $P<0.05$.

Table-1: 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 (40mg/kg), The Mesenteric pieces were collected & challenged with 5% v/v Sheep serum for 10min, 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 0.5ml sheep serum along with 0.5ml of Triple antigen containing 20,000 million Bordetella Pertusis organisms	water		
3	Group 3		Prednisolone	10 mg	
4	Group 4		Petroleum ether extract of *Nigella sativa*	250 mg	
5	Group 5			500 mg	
6	Group 6			750 mg	
7	Group 7		Methanol extract of *Nigella sativa*	250 mg	
8	Group 8			500 mg	
9	Group 9			750 mg	
10	Group 10		Aqueous extract of *Nigella sativa*	250 mg	
11	Group 11			500 mg	
12	Group 12			750 mg	

RESULTS:

After 14 days of sensitization, the effect of different extracts of *Nigella sativa* on intact and degranulated mast cells in actively sensitized rats was shown in Table-2. Fig-1 and Fig-2 explains the graphical representation of the effect of different extracts of *Nigella sativa* on intact mast cells and degranulated mast cells in actively sensitized rats. Microscopic images of different groups of rat mesenteric mast cells as per the treatment schedule (table-1) are shown in Fig -3. The antigen challenge group (group-2) degranulated about 77% of mast cells. Treatment of the rats (group-3 and group-8) with prednisolone (10mg) and 500mg/kg of methanolic extract of *Nigella sativa* prior to sensitization had decreased ($P<0.05$) the mast cell degranulation when compared to the petroleum ether and aqueous extracts of *Nigella sativa*. There was no significant difference among the group-3 and group-8.

Table-2: Effect of different extracts of *Nigella sativa* 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		88.39.±4.59	11.52±1.78
2	Group 2	water		22.32±1.78	77.87±4.71
3	Group 3	Prednisolone	10 mg	**71.23±3.87***	28.21±1.29
4	Group 4	Petroleum ether extract of *Nigella sativa.*	250 mg	27.27±1.198	72.28±2.49
5	Group 5		500 mg	42.29±2.14	57.37±2.27
6	Group 6		750 mg	37.32±2.37	62.22±2.19
7	Group 7	Methanol extract of *Nigella sativa.*	250 mg	34.31±2.59	65.27±1.39
8	Group 8		500 mg	**68.37±3.46***	31.57±1.26
9	Group 9		750 mg	63.28±2.32	36.29±2.23
10	Group 10	Aqueous extract of *Nigella sativa.*	250 mg	24.36±1.42	75.53±3.47
11	Group 11		500 mg	47.21±2.59	52.28±2.42
12	Group 12		750 mg	43.28±2.39	56.37±2.26

Values are mean ± S.E, n=6, *P<0.05

Fig -1: Effect of different extracts of *Nigella sativa* on intact mast cells

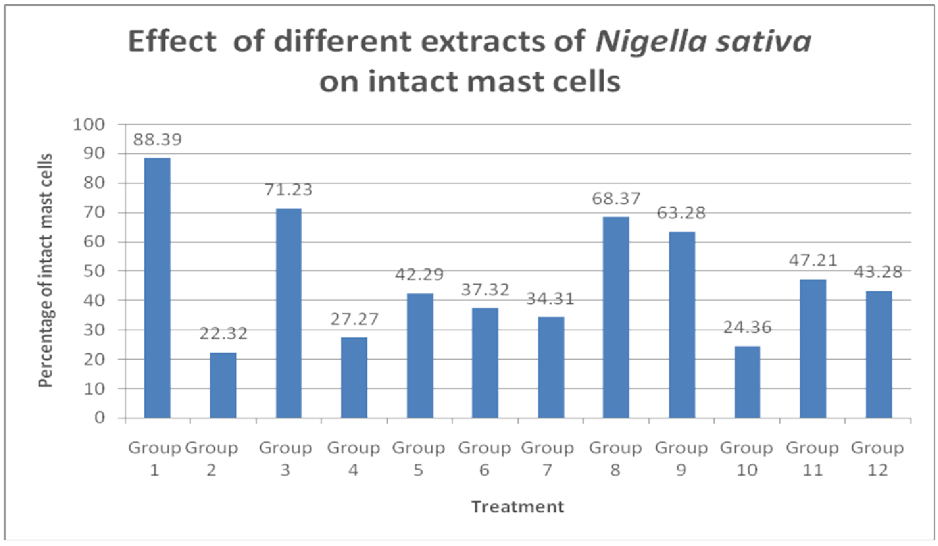


Fig -2: Effect of different extracts of *Nigella sativa* on degranulated mast cells

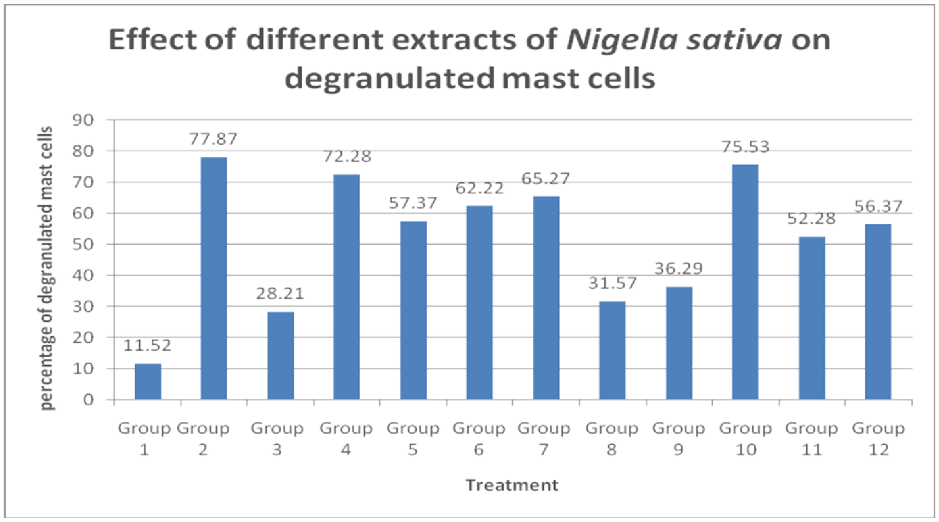
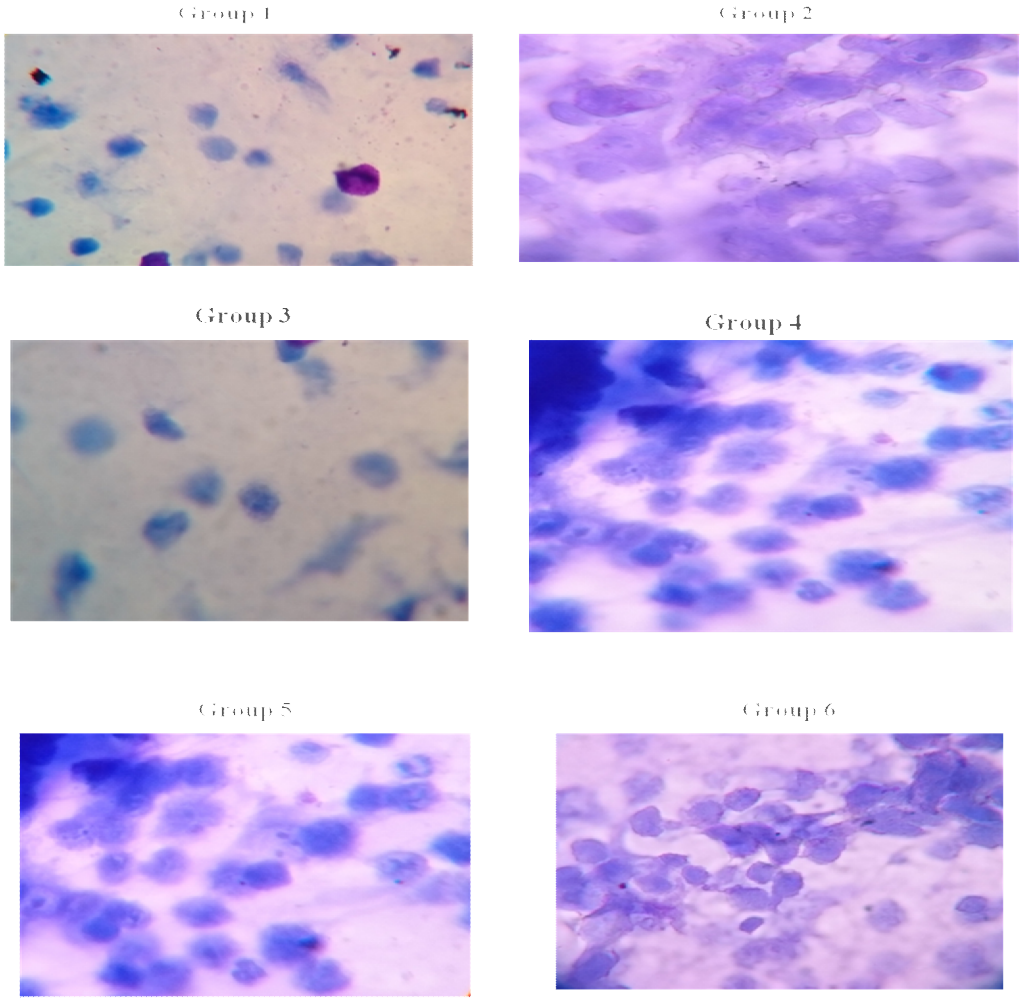
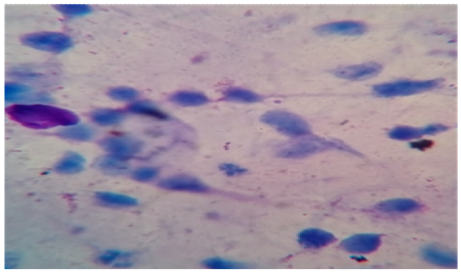


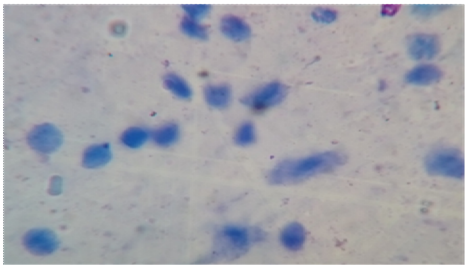
Fig -3: Microscopic images of different groups of rat mesenteric mast cells as per the treatment schedule (Table-1).



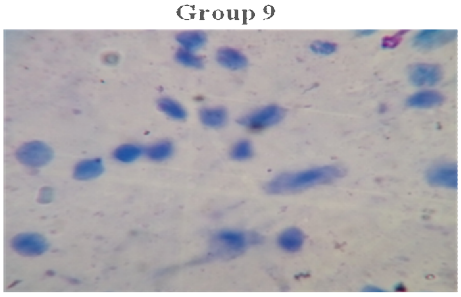
Group 7



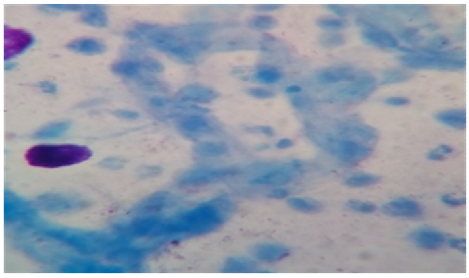
Group 8



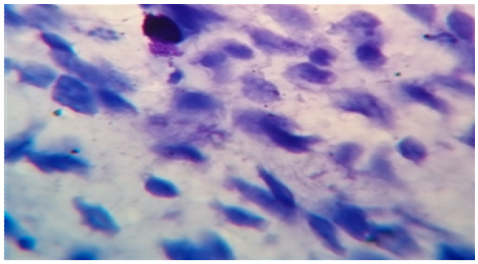
Group 9



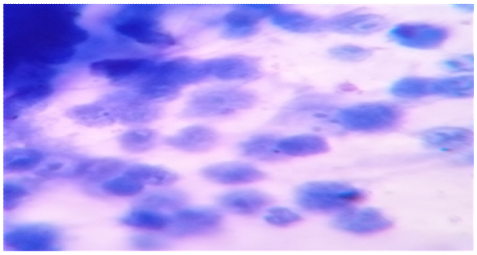
Group 10



Group 11



Group 12



DISCUSSION:

Methanolic extract of *Nigella sativa* has marked protection when compared to the petroleum ether and aqueous extracts of *Nigella sativa* against the mast cell degranulation following antigen challenge in sensitized animals. The protection offered by the methanolic extract of *Nigella sativa* may be due to their mast cell stabilizing potential against antigen antibody reaction [(11)]. The antianaphylactic effect may be due to stabilization of mast cell membrane which inhibits histamine release mediated by calcium release from an intracellular store of mast cells[12]. The methanolic extract of *Nigella sativa* inhibits the degranulation of mast cells may be by decreasing the cAMP phosphodiesterase which may increase the cAMP levels which inhibits the fusion of granules. May be the flavonoids present in the plant could be responsible for this activity. Further investigation may prove the exact mechanism by which methanolic extract of *Nigella sativa* may stabilize the mast cells[13]. There was no significant change in the general behavior.

CONCLUSION:

In conclusion all this findings reveal that, of all the three extracts (petroleum ether, methanol and aqueous extracts) the methanolic extract of *Nigella sativa* has the mast cell stabilizing activity. The antianaphylactic activity of methanolic extract of *Nigella sativa* may be due to the mast cells stabilizing potential, suppression of antibody production and inhibition of antigen induced histamine release.

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