

Anti-Allergic Activity and Mast Cell Stabilization of Areal Parts of *Solanum Torrvum* on Experimental Animals

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ABSTRACT

A *Solanum torvum* (Solanaceae) areal part of plant has been extensively in traditional medicine for the treatment of asthma and bronchitis. The present study was designed to evaluate the anti-allergic activity of hydro- alcoholic extract of *solanum torvum*. Areal parts of plant in experimental animals. The ant allergic activity of the extract was evaluated on compound 48/80 induced mast cell degranulation in rat mesentery and milk induced leukocytes and eosinophilia in mice. Treatment of HAST (300 and 600 mg/kg p.o.) showed significant ($p < 0.001$) protection against compound 48/80 (1 mg/kg s.c.)-induced mast cell degranulation mesenteric pans. The hydro alcoholic extract of *S. torvum* Areal parts of plant inhibited milk induced model Pre-treatment of HAST (200 and 400 mg/kg, p.o.) to group of mice exhibited significantly ($p < 0.001$) reduced the milk (4 ml/kg s.c.)-induced elevated levels of blood total leukocyte and eosinophils counts. The observed beneficial effect of title plant may be attributed to reported bioactive compounds and their synergistic outcome.

Keywords: *Solanum torvum*, Anti-allergic, Leukocytes, Eosinophils, Mast cells.

1. INTRODUCTION

Allergies represent a significant global health challenge, characterized by an abnormal immune response to ordinarily harmless environmental substances, known as allergens. This chronic condition, though seemingly innocuous at times, carries the hypothetical risk of being life-threatening and can manifest with rapid onset, affecting individuals across all age groups [1]. The spectrum of allergic responses is vast, directed against various environmental proteins, and clinically presents as a range of conditions including allergic rhinitis, allergic asthma, food allergy, urticaria, allergic conjunctivitis, and severe anaphylaxis [2].

The underlying mechanism of allergic diseases involves a complex interplay of immune responses. These conditions typically unfold in two phases. The initial phase involves the development and sensitization of T and B cell responses, leading to the production of Immunoglobulin E (IgE) antibodies. The subsequent phase is marked by the IgE-dependent activation of mast cells and the infiltration of inflammatory cells, including eosinophils and innate lymphoid cells. These cellular events are orchestrated by a surge in activated CD4+ T helper type 2 (Th2) lymphocytes, which play a critical role in driving allergic inflammation, ultimately leading to severe allergic disorders and potential tissue injury [3].

The escalating prevalence of allergic diseases is a growing concern globally. Several factors contribute to this surge, including environmental health troubles, rising dust mite populations, evolving dietary factors, and an increasingly sedentary lifestyle [4]. The economic implications of allergic diseases are substantial, playing an increasingly important role in healthcare decisions. The World Allergy Organization has underscored this burden, estimating that approximately 300 million people worldwide suffer from asthma, while 200 to 250 million contend with food allergies. Furthermore, around

one-tenth of the global population is affected by drug allergies, and a staggering 400 million individuals suffer from rhinitis [5].

Regional statistics further illuminate the alarming rates of allergic disease. The European Academy of Allergy and Clinical Immunology (EAACI) reported in 2014 that over 150 million people in Europe were grappling with chronic allergic disorders. Projections indicate a grim future, with estimates suggesting that by 2025, over 50% of the population in the European region could be affected by allergic conditions [6]. Beyond Europe, the global prevalence ranges between 10-30%, with India reporting similar figures, where 20-30% of the population suffers from these types of diseases. Studies specifically in India highlight that allergic rhinitis and asthmatic disorders among children range between 20-30% [7, 8].

The economic impact extends to healthcare costs. In the United States of America, the cost of asthma care alone was estimated to be US\$ 11.3 billion in 1998, nearly doubling from US\$ 6.2 billion in 1990. Current estimates suggest the average total annual cost of asthma per person in the USA is approximately \$4,912, with direct costs (medications, hospitalizations) accounting for \$3,180 (65%) and indirect costs (lost productivity) making up \$1,732 (35%) [9]. These rising figures underscore the urgent need for more effective and sustainable treatment strategies. Current conventional anti-allergic drugs encompass a range of pharmacological agents, including antihistamines, mast cell stabilizers, immunosuppressants, and corticosteroids. While these medications offer symptomatic relief and work by targeting various cytokines involved in the inflammatory cascade, they are not without significant limitations and adverse effects [10, 11].

First-generation H1 receptor antagonists, for instance, are notoriously associated with side effects such as sedation, cognitive dysfunction, and undesirable anticholinergic effects. While newer generations of antihistamines have improved safety profiles, they may still not provide complete relief for all individuals. Corticosteroids, despite their potent anti-inflammatory properties, are linked to a spectrum of adverse reactions with prolonged use. These can include dermatological changes, an increased risk of cardiovascular diseases, and potential gastrointestinal adverse effects [10, 11]. Such limitations in current treatments highlight a critical unmet need for new therapeutic strategies that can offer better efficacy, fewer side effects, and potentially prevent, retard, or even reverse allergic reactions. This imperative drives current research efforts towards discovering novel compounds and approaches for managing allergic disorders.

For millennia, medicinal plants have served as the cornerstone of healthcare systems across diverse cultures. In India, they are particularly favored for treating various common ailments, owing to their deep-rooted traditional values, perceived fewer side effects, ease of accessibility, and affordability [12]. The global herbal repertoire represents an extensive collection of authentic early medicines that continue to prevent and cure diseases even today. This enduring legacy has bestowed upon herbal medicines a valuable status, making them readily available options for primary healthcare. The World Health Organization (WHO) has further endorsed their safe and effective use, acknowledging their crucial role in global health [13].

The Indian Materia Medica alone lists approximately 2000 drugs of natural origin, with a significant majority—around 1600—derived from plants, while the remainder are of mineral and animal origin. This vast botanical resource continues to inspire modern pharmacological research [13]. Over the past few decades, there has been a burgeoning global interest in studying medicinal plants and their traditional applications. Documenting this indigenous knowledge through ethnobotanical studies is vital, not only for the conservation of biological resources but also for identifying potential new drug candidates [17].

Plants possess an almost limitless capacity to synthesize a wide array of aromatic substances, primarily secondary metabolites. It's estimated that over 12,000 such compounds have been isolated to date, though this number is believed to represent less than 10% of the total existing in nature. These substances often serve as the plant's defense mechanisms against predation by microorganisms, insects, and herbivores. Beyond their ecological roles, many of these phytochemicals possess significant medicinal properties, contributing to plant odour (terpenoids), pigmentation (tannins and quinones), and flavour (capsaicin) [18]. Numerous important medicinal plants are utilized in traditional systems like Ayurveda, Unani, Siddha, and various folk medicine practices for treating a multitude of ailments, including microbial infections, diarrhoea, and diabetes [18].

Among the vast array of medicinal plants *S. torvum* Sw. (Solanaceae), commonly known as "turkey berry" or "pea eggplant," stands out due to its wide distribution across tropical and subtropical regions globally, including India. This plant has a long and documented history of medicinal use, particularly highlighted in Ayurveda and Chinese pharmacopoeia. Beyond its therapeutic applications, *S. torvum* is also widely consumed as a food source in many traditional communities worldwide [14].

Traditional medicinal applications of *S. torvum* are extensive and varied. It is frequently employed to address conditions such as asthma, diabetes, hypertension, cold and coughs, and to help reduce body heat. Globally, it is intensively used in traditional medicine as a poison antidote and for the treatment of fever, wounds, tooth decay, various reproductive problems, and gastrointestinal diseases [14]. Furthermore, its uses extend to liver problems, sore throats, seizures, epilepsy, skin diseases, painful periods, jaundice, general pain, stomach upset, or as a sedative, diuretic, and haemostatic [19]. The fruits are specifically used for hypertension, cough, enlarged spleen and liver, and anemia, while the leaf juice and unripe fruits are

utilized to strengthen immunity, act as haemostatic and haemopoietic agents, or to treat wounds and female infertility [19]. The diverse therapeutic claims associated with *S. torvum* are supported by its rich phytochemical profile. Scientific investigations have identified the presence of various important bioactive compounds, including alkaloids, flavonoids, saponins, glycosides, tannins, fixed oils, steroids, solanolid, rutin, iso-quercetin, kaempferol, and quercetin [15]. These compounds are largely responsible for the plant's observed medicinal properties.

Based on this extensive history of traditional claims and its well-documented phytochemical composition, researchers have embarked on investigations to scientifically validate the anti-allergic and mast cell stabilizing effects of *S. torvum* in experimental animal models [15, 19]. The primary goal of current research is to systematically prove its potential for mast cell stabilizing activity and its impact on airway hyper-responsiveness under different allergic and allergic inflammatory conditions in animal models [15, 19]. This line of inquiry aims to explore *S. torvum* as a viable herbal alternative or adjunct therapy, offering new hope in the ongoing global fight against the escalating burden of allergic diseases

2. MATERIALS AND METHODS

Chemicals: Compound 48/80, Dexamethasone, Alcohol (Ehtanol), Ketotifen fumarate, RPMI 1640 medium (at150), Toluidine blue.

Animals: Male wistar albino rats weighing around 200-250 g and Swiss albino mice of either sex weighing around 25-30 g with no sign of allergic conditions were selected for the present study. Experimental animals were obtained from animal house Sree Siddaganga College of Pharmacy. Experimental animals were housed in an appropriate polypropylene cages with free access to food and water with a sterile paddy husk as a bed and maintained in a standard condition with temperature $22 \pm 2^\circ\text{C}$, relative humidity of 45–60%, and a 12h light: 12 h dark normal cycle (lights on at 7 am) in a quarantine room. The animals were randomized according to body weight and grouped into experimental and control groups for further studies. So that the mean body weight difference would not be statistically different from each other. Animals were adapted to laboratory conditions 48 h prior to initiation of experimental studies to minimize any non-specific stress. All the animal experiments were initiated after obtaining prior permission by Institutional ethical committee of Sree Siddaganga College of Pharmacy, Tumakuru, and Karnataka, Approval No. SSCP/IAEC clear/212/20-21, according to prescribed guidelines of committee for the Purpose of Control and Supervision of experiments on Animals (CPCSEA), government of India

Plant material and extraction

Collection of plant material.

The Areal parts of the plant *solanum torvum* were collected from local region of Tumakuru, Karnataka. The collected samples identified and authenticated by Prof. Chidananda Dept. of Botany, Sree Siddaganga college of arts, science and commerce, Tumakuru. and specimen voucher (Herbarium) is preserved in the department.

Preparation of Hydro-alcoholic extract of *solanum torvum*.

The Areal parts of the plant *solanum torvum* were shade dried until the rinds became brittle. Later the dried fruit rinds were grounded into course powder and subjected to Soxhlet extraction process using Ethanol and water as solvents in the ratio of 70:30. The hot extraction was carried out using Soxhlet apparatus. After the process the solvent obtained was dried using water bath and a semi solid residue of Hydro-alcoholic extract of *solanum torvum* was obtained.

Preparation of extract and test drugs

Hydroalcoholic extract of *Solanum torvum*: The hydroalcoholic extract of *solanum torvum* (HAST) was dissolved in 0.3%w/v CMC (Carboxymethyl cellulose) solution and administered orally to experimental animals.

Preparation of Compound 48/80

Compound 48/80 was prepared using normal saline and administered through subcutaneous injection to experimental animals.

Pharmacological screening models.

Compound 48/80 induced mast cell degranulation's on rat mesentery²⁰

Mast cells are the major source of mediators of allergy. Mast cells are located mainly in the connective tissue throughout the body, particularly near small blood vessels they are found in abundance in the mesentery of rats. The activation of mast cells is induced by compound 48/80 substance called secretagogues of mast cells in the intestinal mesenteric pieces of sensitized albino rats.

Procedure:

Swiss albino rats of either sex (180-200 g) were divided into six groups, selected and each group containing six animals. Animals belonging to Group-I received normal saline (1ml/kg, p.o) while Group-II was treated with HAST alone (600 mg/kg,

p.o). On 1st day the rats of Group-III and Group-VI were sensitized with compound 48/80 (1 mg/kg, s.c). Group-III served as inducer control. Group-IV and Group-V served as extract group and administered HAST (300-600 mg/kg, p.o) whereas Group-VI received disodium chromoglycate (10 mg/kg, i.p) as reference standard drug. On 7th day 2h after assigned treatment, rats were sacrificed and intestinal mesentery were taken for study of mast cells. Mesenteries of sacrificed rats along with intestinal pieces were spread on Petri dish containing Ringer Locke's solution at 37°C which was transferred on a slide and stretched with the help of needles. The intestinal tissues pieces were cut and removed. The pieces of mesentery were challenged with 5µg/ml of compound 48/80 solution *in-vitro* for 10 mins and then stained with 0.1% toluidine blue in 4% aqueous formalin solution. The stained cells are immersed in xylene for 5-10 mins and finally rinsed 2 or 3 times with acetone then observed under microscope (45x). Total 100 mast cells were counted from different visual areas. The numbers of intact and degranulated cells were counted and percentage protection was calculated.

Experimental Protocol for Mast Cell Stabilization Study in Swiss Albino Rats

Male and female Swiss albino rats (180-200 g) were randomly assigned to six experimental groups (n=6 per group):

Groups	Treatment
1	Vehicle (1 ml/kg, p.o)
2	HAST (600 mg/kg)
3	Compound 48/80 (1 mg/kg s.c)
4	Compound 48/80 (1 mg/kg s.c) + HAST (300 mg/kg, p.o)
5	Compound 48/80 (1 mg/kg s.c) + HAST (600 mg/kg, p.o)
6	Compound 48/80 (1 mg/kg s.c) + Disodium chromoglycate (10mg/kg, i.p)

Experimental Design for Leukocyte and Eosinophil Analysis in Swiss Albino Mice

The study utilized Swiss albino mice (20-25 g body weight) randomly allocated into five experimental groups, with six animals per group. Prior to treatment administration, baseline blood samples were collected from each mouse via retro-orbital plexus puncture under light inhalation anesthesia, with initial total leukocyte and eosinophil counts recorded for all groups.

The treatment protocol was as follows:

- **Group I (Control):** Received vehicle only (1 ml/kg, oral administration)
- **Group II (Milk Challenge):** Administered freshly boiled and cooled milk (4 ml/kg, subcutaneous injection)
- **Groups III & IV (Treatment Groups):** Pretreated with hydroalcoholic extract of *S. torvum* at doses of 100 mg/kg and 200 mg/kg respectively (oral administration), followed by boiled/cooled milk challenge (4 ml/kg, subcutaneous injection) 5 minutes later
- **Group V (Standard):** Milk (4 ml/kg, s.c) + Dexamethasone (50 mg/kg, i.p)

Twenty-four hours post-milk administration, blood samples were again collected from all animals via retro-orbital puncture under light anaesthesia. Total leukocyte counts and eosinophil differentials were determined for each treatment group to evaluate the inflammatory response and potential modulatory effects of the *S. torvum* extract.

Inhibiting Compound 48/80-Induced Mast Cell Degranulation

To assess mast cell stabilization, researchers used the Compound 48/80-induced mast cell degranulation model in rat mesentery. A subcutaneous injection of Compound 48/80 (1 mg/kg) caused a significant increase in mast cell degranulation, with 86.05% of mast cells degranulating in the control group that received only Compound 48/80 (p<0.001 compared to the normal control group).

However, treatment with HAST at different doses (300 mg/kg and 600 mg/kg), as well as the standard mast cell stabilizer Disodium Cromoglycate (10 mg/kg), significantly reduced this degranulation (p<0.001 for all). Specifically, mast cell degranulation was reduced to 48.61% with HAST 300 mg/kg, 30.25% with HAST 600 mg/kg, and 20.64% with Disodium Cromoglycate. These reductions correspond to impressive percentage protections of 63.01%, 74.46%, and 70.49%

respectively. Interestingly, the higher dose of HAST (600 mg/kg) alone did show a significantly increased degranulation of mast cells when compared to the normal group ($p < 0.05$), indicating a complex dose-response or potential for different effects at higher concentrations without the degranulating agent.

Mitigating Milk-Induced Leukocytosis and Eosinophilia in Mice

The study also explored HAST's impact on inflammation-related leukocyte and eosinophil counts using a **milk-induced model in mice**. Subcutaneous administration of milk (4 ml/kg) led to a significant increase in both leukocyte and eosinophil counts after 24 hours, compared to the normal control group ($p < 0.001$). This indicates a clear inflammatory response.

In contrast, mice pre-treated with **HAST at doses of 200 mg/kg and 400 mg/kg** exhibited a significant decrease in both leukocyte and eosinophil levels ($p < 0.001$). This protective effect was comparable to the reference standard, **Dexamethasone (50 mg/kg)**, which also showed a significant reduction in leukocyte ($p < 0.01$) and eosinophil ($p < 0.001$) counts. These findings suggest that HAST possesses anti-inflammatory properties capable of reducing the influx of inflammatory cells characteristic of allergic and inflammatory conditions.

3. DISCUSSION

Allergic reactions represent a significant health challenge worldwide, stemming from an immune dysfunction that triggers hypersensitivity, particularly Type I or IgE-mediated allergic responses. These reactions are primarily driven by the systemic release of inflammatory mediators, especially histamine, predominantly secreted from activated mast cells and blood basophils [21]. Anaphylaxis, a life-threatening form of allergic reaction, exemplifies the severe consequences of this sudden, systemic release of inflammatory mediators and pro-inflammatory cytokines, often triggered by various stimuli, including substances like Compound 48/80 and anti-IgE antibodies [21]. The focus of this study was to evaluate how HAST (an experimental compound) impacts key aspects of allergy, such as mast cell degranulation, and leukocyte and eosinophil infiltration, using diverse experimental models [21].

In allergic asthma and other inflammatory allergic conditions, numerous inflammatory cells are involved in the pathogenesis, including eosinophils, mast cells, macrophages, and neutrophils. Among these, mast cells are recognized as one of the most crucial participants in allergic diseases [22]. When activated, mast cells release a complex array of biologically active molecules that lead to various physiological events characteristic of allergic responses.

The contents of mast cell granules are veritable arsenals of pre-formed and newly synthesized mediators. These include critical substances like histamine (a key mediator of immediate hypersensitivity), serotonin, proteoglycans, and serine proteases. Beyond these pre-formed mediators, activated mast cells also synthesize and release membrane-derived lipid mediators such as leukotrienes, prostaglandins, and platelet-activating factor. Furthermore, they produce and secrete intracellular mediators including a wide variety of cytokines and chemokines. The combined action of these diverse mediators ultimately leads to physiological changes observed in allergic reactions, such as smooth muscle contraction (e.g., in the airways during asthma), vasodilation, increased vascular permeability, and mucous hypersecretion [22]. The continuous release of these mediators promotes the ongoing inflammatory process.

The inflammatory process in allergic conditions, particularly allergic asthma, is further exacerbated by the infiltration of leukocytes. These white blood cells play a crucial role in potentiating inflammation. During asthmatic inflammation, leukocytes release a range of inflammatory mediators, including various cytokines, histamine, and major basic protein. These substances actively promote the continuation of the inflammatory cycle [22]

A significant consequence of this leukocyte infiltration is the release of reactive oxygen species (ROS) into the surrounding tissue. This release leads to an increase in oxidative stress, a state of imbalance between the production of free radicals and the body's ability to counteract their harmful effects. This heightened oxidative stress is strongly associated with many pathogenic features of asthma and other allergic diseases, contributing to tissue damage and perpetuating the inflammatory response [22]. The oral administration of milk, for instance, has been shown to produce a distinct increase in both leukocyte and eosinophil counts within 24 hours in experimental models, serving as a reliable method to induce such an inflammatory response [22].

To systematically study the mechanisms of anaphylaxis and mast cell degranulation, synthetic compounds are often employed as direct activators. Compound 48/80 is a notable example, being one of the most potent secretagogues of mast cells. This polymer is formed through the condensation of N-methyl-P-methoxy phenethylamine with formaldehyde [23].

The mechanism by which Compound 48/80 triggers mast cell degranulation is well-established. Compound 48/80, along with other polybasic compounds, directly activates G-proteins, which are crucial components of cell signaling pathways. This activation results in increased permeability of the lipid bilayer membrane, causing a significant perturbation in the membrane's integrity. Crucially, the intracellular calcium pathways are fundamental to the degranulation process of mast cells; the membrane perturbation induced by Compound 48/80 leads to an influx of calcium ions, which then triggers the release of the granular contents [23].

Mast cells, widely distributed throughout the connective tissue, are preferentially localized adjacent to small blood vessels [23]. This strategic positioning allows them to rapidly respond to stimuli. Numerous reports have firmly established that stimulation with Compound 48/80 initiates the activation of mast cells, leading to the process of degranulation. This degranulation results in the swift release of a wide array of mediators, including histamine, as well as other critical inflammatory mediators such as leukotrienes, prostaglandins, proteases, and various pro-inflammatory and chemotactic cytokines via complex signal transduction pathways [23].

4. CONCLUSION

The present study confirms the anti-allergic activity of the hydroalcoholic extract of *Solanum torvum* (HAST), attributing its effects to the inhibition of mediator release from mast cells. HAST demonstrated significant anti-allergic potential in both Compound 48/80 and milk-induced allergic models in mice and rats, suggesting prominent mast cell stabilizing and anti-inflammatory actions. This validates the ethnopharmacological claims for *S. torvum*'s use in allergic conditions. Further research is necessary to fully uncover the exact mechanisms behind these beneficial anti-allergic properties.

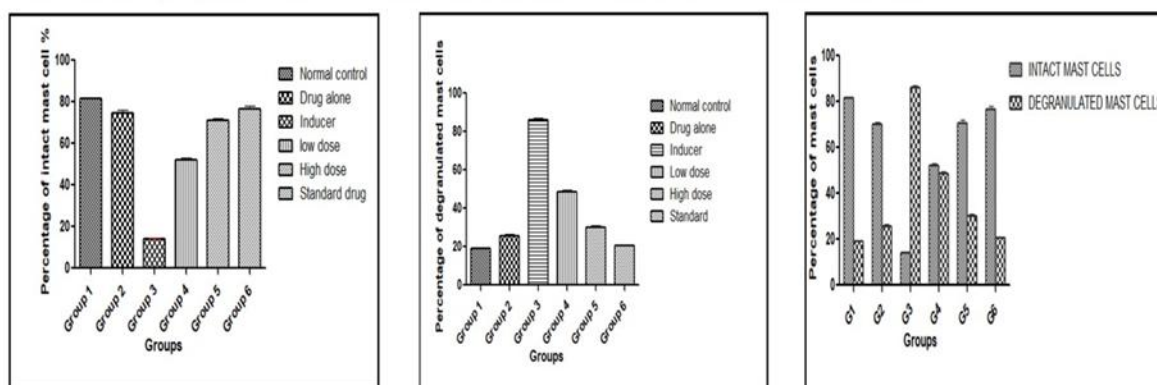
Table 1. Effect of Hydroalcoholic extract of *Solanum torvum* (HAST) in compound 48/80 – induced mast cell degranulation in rat mesentery

Groups	Treatment	%Protection intact cells	For %Protection degranulated cells	for Percentage protection
I	Normal control Vehicle (1ml/kg, p.o)	81.45±0.65	19.18±0.49	78.0%
II	HAST alone (600 mg/kg p.o)	74.78±1.06	25.46±1.12	70.5%
III	Inducer control (C-48/80+1 mg/kg s.c)	14.26±0.50###	86.05±.21###	-----
IV	C-48/80 + HAPN (300 mg/kg p. o)	52.07±1.21***	48.61±1.19***	43.6%
V	C-48/80 + HAPN (400 mg/kg p.o)	71.07±1.22***	30.25±0.67***	64.9%
VI	Disodium chromoglycate (10 mg/kg i.p)	76.77±1.38***	20.64±0.68***	76.1%

Each Value represent the Mean ± S.E.M (n = 6),### $P < 0.001$ compared to Normal control; *** P

<0.001 compared to Compound 48/80 group. Statistical evaluation was done by One-way ANOVA followed by Tukey's posthoc test

Fig.1 Graph showing Mast cell stabilizing effect of different doses of Hydroalcoholic extract of *Solanum torvum* (HAST) in compound 48/80 induced mast cell degranulation in rat mesentery.



Each Value represent the Mean \pm S.E.M (n = 6),### $P < 0.001$ compared to Normal control; *** $P < 0.001$ compared to Compound 48/80 group. Statistical evaluation was done by One-way ANOVA followed by Tukey's posthoc test.

Table 2. Effect of hydroalcoholic extract of *Solanum torvum* (HAST) in milk- induced leukocytosis and eosinophilia in mice

Groups	Treatment	Difference in no. of Leucocytes(per mm ³)	Difference in no. of Eosinophils (%)
I	Normal control	725.0 \pm 25.00	0.553 \pm 0.19
II	Inducer control (Milk 4ml / kg)	7042 \pm 684.4###	15.13 \pm 0.68###
III	HAST (200 mg/kg p.o.)	3463 \pm 232.1***	2.253 \pm 0.56***
IV	HAST (400 mg/kg p.o.)	4083 \pm 285.1***	1.251 \pm 0.12***
V	DEXO (50mg/kg)	5460 \pm 119.2***	1.654 \pm 0.42***

Values are given as Mean \pm S.E.M. for group of six animals each. The intergroup variation was measured by One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ when compared with Milk alone group at significance level $P < 0.001$ confidence interval.

Fig. 3 Effect of Hydroalcoholic extract of *Solanum torvum* (HAST) in milk-induced eosinophilia in mice.

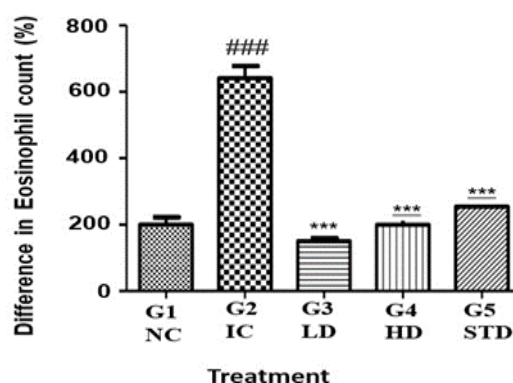
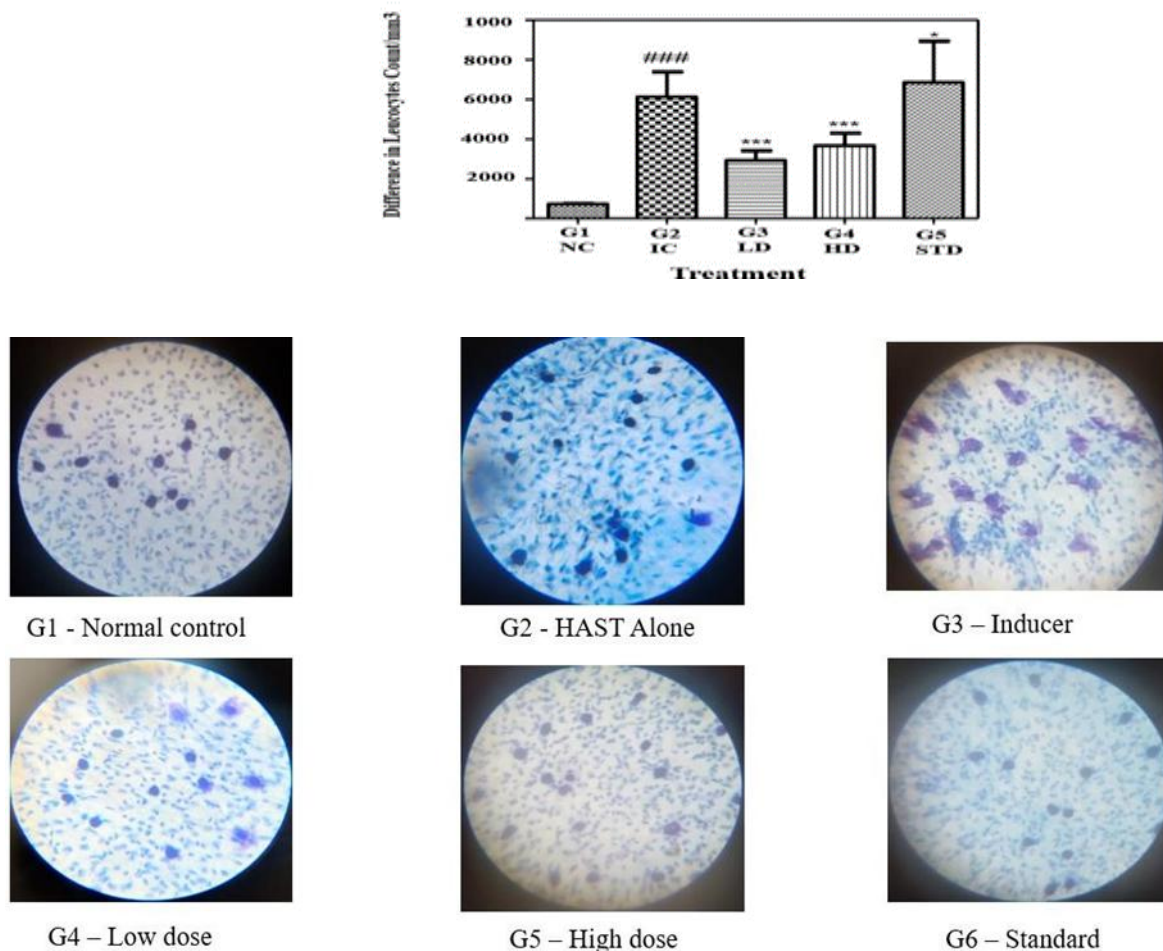


Fig.2. Effect of Hydroalcoholic extract of *Solanum torvum* (HAST) in milk induced leukocytosis



Protective effect of C-48/80 induced Mast cell degranulation activity in rat mesentery

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