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Action of Areal Parts of *Solanum Torvum* on Allergic Action and Mast Cell Stabilization on Laboratory Animals

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ABSTRACT

Solanum torvum (Solanaceae) Areal parts of plant has been extensively in Indian traditional medicine for the treatment of asthma and bronchitis. The present study was designed to evaluate the anti-allergic activity of hydro- alchoholic 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 Solanum 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 elevatedlevels 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, Compound48/80.

INTRODUCTION

An allergy is a long-term medical disorder characterized by an aberrant response to an allergen, which is a normally innocuous chemical. It is hypothetically life threatening and rapid- onset disease reported in all age of groups[1]. The allergic response is directed against various environmental proteins (allergen) and manifests clinically as allergic rhinitis, allergic asthma, food allergy, urticarial, allergic conjunctivitis and anaphylaxis[2]. The allergic diseases are of two phases which includes development and sensitization of T and B cell responses and IgE dependent activation of mast cells and infiltration of eosinophil, innate lymphoid cells that are arranged by numbers of activated CD4+ T helper type 2 (Th2) lymphocytes. These play a critical role in allergic inflammation leading to severe allergic disorders, which causes tissue injury[3]. Environmental health troubles, rising dust mite populations, dietary factors, and deskbound lifestyle are causing a surge in allergic diseases. Formal economic evaluation is playing an increasingly important role in health care decisions and hence allergic diseases are on the rise at alarming rates[4]. World Allergy Organization summarized the burden of allergic diseases and it is estimated that around 300 million people suffer from asthma and about 200 to 250 million people suffer from food allergies. Drug allergies affect 10% of people, and 400 million people have rhinitis5. As per the European Academy of Allergy and Clinical Immunology (EAACI), in 2014, more than 150 million people in Europe are suffering

from chronic allergic disorders andit has been estimated that by 2025, over 50% of the population in the region will be affected with allergy diseases[6]. Globally 10-30% of people was suffering from these types of diseases and in India this was around 20-30% population, studies in India also reports that allergic rhinitis and asthmatic disorders. among children ranges between 20-30%[7,8].

In 1998, the cost of asthma care was estimated to be US$ 11.3 billion in United State of America; nearly a double increase from US $ 6.2 billion was estimated in 1990. A research estimates that the average yearly cost per person in the USA for asthma is $4,912, of which $3,180 (65%) comes from direct and indirect expenditures. additionally, $ 1,732 (35 %), Respectively[9]. Currently antiallergic drugs used in treatment include antihistamines, mast cell stabilizers,immune suppressors and corticosteroids which works also as anti- inflammatory agents bytargeting on several cytokines. Despite of their advantages, these medications have severaladverse effects. Where, first H1 receptor antagonist exhibited some sort of sedation, cognitive dysfunction and unwanted anti-cholinergic effects. Corticosteroids usage increases dermatological changes, CVS diseases and increases risk of GI adverse Effects[10,11]. These treatments are less satisfactory leading towards need of new strategies to treat disorders. Hence, the goal of the current research is to develop treatments that canprevent, retard or reverse allergic reactions. Since the beginning of time, medicinal plants have been utilized in healthcare. Hence, they are considered as the preferred treatment option for various common ailments in almost all parts of India because of their traditional values, lesser known side effects, easy accessibility, affordability and so on[12]. The global herbal cornucopia represents an eclectic collection of the most authentic early medicines that even today continued to prevent andcure diseases. Thus, herbal medicines have been given a valuable status and readily available products for primary health care, and WHO has endorsed their safe and effective use[13]. Indian Materia-Medica includes about 2000 drugs of natural origin of which approximately 400 are of mineral and animal origin while the rest are of vegetable origin.*Solanum torvum* (Solanaceae), which is widely spread throughout the tropical and subtropical countries of the world including India. Traditional medicinal use of solanum torvum have been highlighted in the Ayurveda and Chinese pharmacopoeia and it is widelyused like food and in folk medicine around the world. *Solanum torvum* is often used in the traditional system of medicine for a variety of ailmentsincluding asthma, diabetes, hypertension, cold and coughs, to reduce body heat. and it is intensively used worldwide in the traditional medicine as poison antidote and for the treatment of fever, wounds, tooth decay, reproductive problems, gastrointestinal diseases[14]. *Solanum torvum* is having medicinal importance which including alkaloids, flavonoids, saponins, glycosides, tannins, fixed oil, steroids, solanolide, rutin, iso-quercetin, kaempferol and quercetin. Based on the above traditional claims we have planned to investigate the anti-allergic and mast cell stabilizing effect of *Solanum torvum* in experimental animals[15].based on these above facts and ethno medical claim, we thought it worthwhile to prove its potential a mast cell stabilizing activity Using different allergy and allergic inflammatory condition in animal model.

Solanum torvum plants have been used in traditional medicine for several thousand years[16].Based on several medical systems like Ayurveda, Unani, and Siddha, the knowledge of medicinal plants has been amassed over many years. It is said that 2500 plant species are used by traditional healers in India, and 100 plant species are regularly used as sources of medication. In recent years, there has been a growing interest in researching medicinal plants and their traditional uses throughout many regions of the world. Documenting theindigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources[17].Plants have an almost limitless ability to synthesize aromatic substances mainly secondarymetabolites of which at least 12,000 have been isolated a number estimated

to be less than10% of the total. In many cases these substances serve as the molecules of plant Defense against predation by microorganisms, insects and herbivores. However, there are medicinal properties to some of these molecules. For the treatment of a number of illnesses, including bacterial infections, diarrhoea and diabetes, some important medicinal plants are used in Ayurveda, Unani, Siddha and folk medicine. *Solanum torvum* Sw. used in treatment of liver problems, cough, sore throat and stomach, seizures, epilepsy, diarrhoea, skin diseases, diabetes, toothache (tooth decay), sores, painful periods, jaundice, colds, pain, fever, stomach upset or as a sedative, diuretic, haemostatic or poison antidote. The fruits are used in the treatment of hypertension, cough, enlarged spleen and liver, anaemia, or as an analgesic, the leaf juice and unripe fruits are used to reduce body, to strengthen the immunity of the body, haemostatic, haemopoietic or to treat wounds and female infertility. based on these above facts and ethno medical claim, we thought worthwhile to prove its mast cell stabilizing activity and airway hyper-responsiveness Using different allergy and allergic inflammatory condition in animal model[19].

Materials and methods:

Chemicals: Compound 48/80, Dexamethasone, Alcohol (Ehtanol), Ketotifen fumerate, 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 presentstudy. 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 ± 2°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.

Approval No. from the institutional ethics council of Sree Siddaganga College of Pharmacy, Tumakuru, Karnataka, was obtained before any animal studies were conducted. SSCP/IAEC clear/212/20-21, according to prescribed guidelines of committee for thePurpose 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 obtainedwas 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 throughsubcutaneous injection to experimental animals.

Pharmacological screening models.

Compound 48/80 induced mast cell degranulation's on rat mesentery[20]

The primary source of allergy mediators is mast cells. 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 mesentericpieces of sensitized albino rats.

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)

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 wastreated 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. Rats were slaughtered and their intestinal mesentery was removed for mast cell research on the second day of the seventh treatment cycle. 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 invitro for 10 mines 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.

% protection =[1-(T/C)] ×100

where T is the number of test degranulated cells.

C=no. Of degranulated cell of inducer control

Milk induced leucocytosis and eosinophilia in mice[21]:

One of the most prevalent allergens is milk. The symptoms of a milk allergy can range from mild to severe

and include vomiting, hives, asthma, and digestive issues.

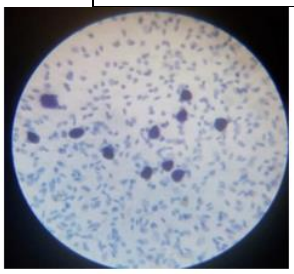
Milk allergy can also cause anaphylaxis and severe, life-threatening reaction. The symptoms of a milk allergy can range from mild to severe and include vomiting, hives, asthma, and digestive issues. Milk allergy can alsocause anaphylaxis, severe life-threatening reactions.

Procedure:

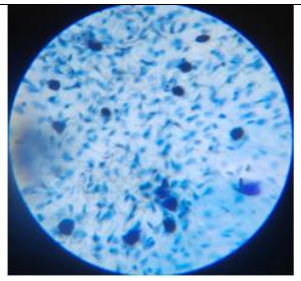
Swiss albino mice (20-25 g) were randomly divided into five groups and each group consists of six animals. Blood samples were collected from each mice from the retro orbital plexus, under light inhalation anaesthesia before treatment, Total leucocytes and eosinophil count was recorded in each group. Following single dose treatments were given Animals belonging to Group- I received Vehicle (1 ml/kg, p.o). Animals belonging to Group- II received freshly boiled and cooled milk (4 ml/kg, s.c). Animals belonging to Group- III and IV were pre-treated with hydro alcoholic extract of *solanum torvum* (100 and 200 mg/kg p.o respectively) and 4tract 5minutes later boiled and Cooled milk (4 ml/kg, s.c) was administered to the same animal. Blood samples were collected after 24 h after milk administration from the retro orbital plexus, under light inhalation anaesthesia. Total leukocyte and eosinophils count were recorded in each group aftertreatment.

Milk-induced leukocytosis and eosinophilia in mice.

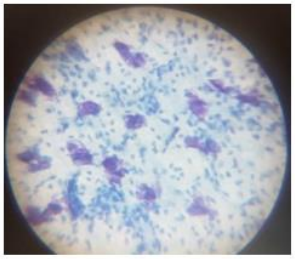
Groups	Treatment
I	Vehicle (1 ml/kg, p.o)
II	Milk (4 ml/kg, s.c)
III	Milk (4 ml/kg, s.c) + HAST (200mg/kg, p.o)
IV	Milk (4ml/kg, s.c) + HAST (400mg/kg, p.o)
V	Milk (4 ml/kg, s.c) + Dexamethasone (50 mg/kg, i.p)



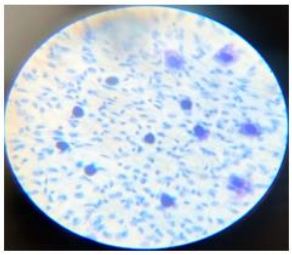
G1 - Normal control



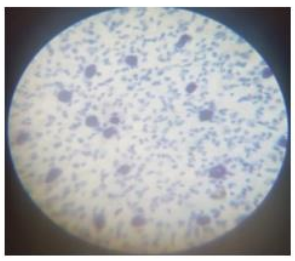
G2 - HAST Alone



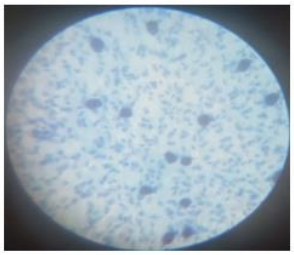
G3 – Inducer



G4 – Low dose



G5 – High dose



G6 – Standard

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

Compound 48/80 induced mast cell degranulation in rat mesentery

Subcutaneousinjection of Compound 48/80 (1mg/kg) significantly increases ($p<0.001$) the degranulation of mast cells in C-48/80 alone group (86.05±.21) Compared to normal controlgroup. In the treatment

groups with different doses of HAST (300, 600 mg/kg) and Disodium chromoglycate (10 mg/kg) Showed 48.61±1.19, 30.25±0.67, 20.64±0.68 degranulation of mast cellswith a significant reduction ($p< 0.001$) and percentage protection was found 63.01, 74.46 & 70.49 % respectively. HAST (600 mg/kg) alone showed significantly increased ($p< 0.05$) in the degranulation of mast cells when compared to normal group.

Groups	Treatment	%Protection For intact cells	%Protection for degranulated cells	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 ANOVAfollowed by Tukey's posthoc test.

Milk induced leukocytosis and eosinophilia in mice

Subcutaneous administration of milk (4 ml/kg) showed significant increase ($p< 0.001$)in the leucocytes and eosinophils count after 24 h compared to normal control group. Whereas, group of mice pre-treated with HAST (200 mg/kg and 400 mg/kg) exhibited significant decrease ($p<0.001$) in leucocytes and eosinophils levels. Reference standard of Dexamethasone (50 mg/kg) showed significant reduction ($p<0.01$; $p< 0.001$) in leucocytes and eosinophils counts respectively.

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

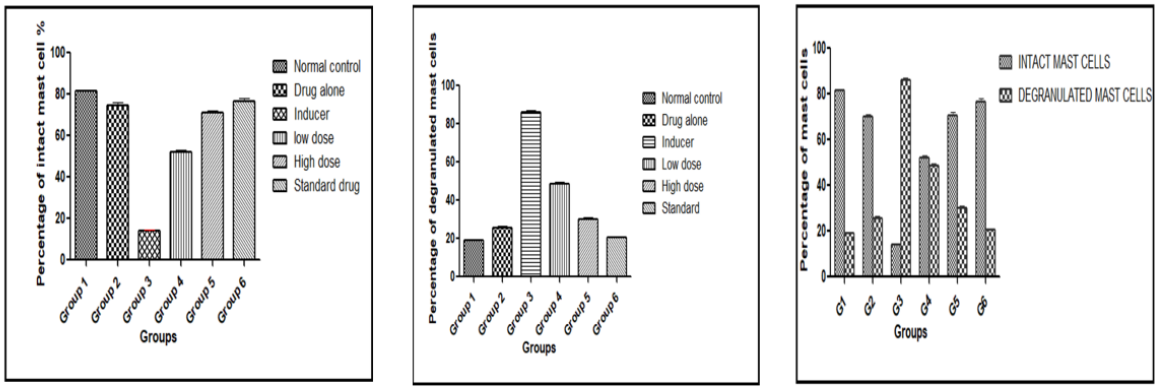
Groups	Treatment	Difference in no. of Leucocytes(permm^3)	Difference in no. of Eosinophils (%)
I	Normal control	725.0±25.00	0.553±0.19
II	Inducer control (Milk 4ml / kg)	7042±684.4###	15.13±0.68###
III	HAST (200 mg/kg p.o.)	3463±232.1***	2.253±0.56***

IV	HAST (400 mg/kg p.o.)	4083±285.1***	1.251±0.12***
V	DEXO (50mg/kg)	5460±119.2***	1.654±0.42***

Values are given as Mean ± 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 Milkalone group at significance level P<0.001 confidence interval.

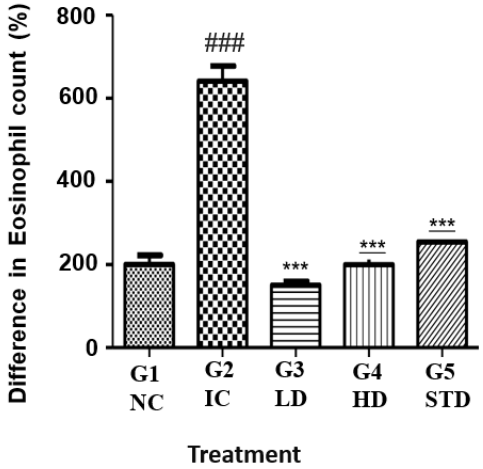
Fig.5.4. Effect of Hydroalcoholic extract of *Solanum torvum (HAST)* in milk-induced leukocytosis.

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



Conclusion

The results obtained in this work showed that the hydroalcoholic extracts of areal parts of *Solanum torvum* have the antiallergic activities, and *Solanum torvum* is having medicinal importance which including alkaloids, flavonoids, saponins, glycosides, tannins, fixed oil, steroids, solanolide, rutin, iso-quercetin, kaempferol and quercetin.

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