

# *Solanum trilobatum* in the management of atopy: Through inhibition of mast cell degranulation and moderation of release of interleukins

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## ABSTRACT

*Solanum trilobatum* is a widely used plant in the Indian indigenous systems of medicine. It is mainly used in the treatment of respiratory diseases like bronchial asthma. In our present study, we report that the aqueous and alcoholic extracts of *S. trilobatum* exhibited inhibition of mast cell degranulation. Further, aqueous and alcoholic extracts of *S. trilobatum* significantly decreased the release of IL1 $\alpha$  and increased the release of IL8 from the cultured keratinocytes. Oral administration of the aqueous and alcoholic extracts of *S. trilobatum* stabilized mast cells in experimental rats.

**Key words:** Histamine, immunomodulation, interleukin, mast cell, medicinal plants, *Solanum trilobatum*

## INTRODUCTION

*Solanum trilobatum* belongs to the family Solanaceae, which is commonly known as *Alarka* in Sanskrit. Several reports document the diverse properties of this plant. *S. trilobatum* possesses antioxidant activity,<sup>[1]</sup> hepatoprotective activity<sup>[2]</sup> and protects against UV-induced damage and radiation-induced toxicity in mice.<sup>[3]</sup> Aqueous and solvent extracts of *S. trilobatum* were found to possess anti-inflammatory and antimicrobial properties.<sup>[4-6]</sup>

Atopic allergy implies a familial tendency to manifest conditions such as asthma, rhinitis, urticaria and eczematous dermatitis, alone or in combination. However, individuals without an atopic background may also develop hypersensitivity reactions, particularly urticaria and anaphylaxis, associated with IgE antibody. Mast cell is the key effector cell of the biologic response in allergic reaction. The use of synthetic antihistamines to control atopic allergy over a prolonged period of time could lead to potential side effects, and the relief offered by them is

mainly symptomatic and short-lived. A safe and effective management of atopy through plant resources has received much attention in recent years. This study was undertaken to evaluate the efficacy of aqueous and solvent extracts of *S. trilobatum* in regulating the mast cell degranulation event and inflammatory responses (IL1 $\alpha$  and IL8) through *in vitro* and *in vivo* studies. Further, the determination of efficacy of these extracts in preventing the disease progression in experimental model was also attempted.

## MATERIALS AND METHODS

### Extraction procedure

*S. trilobatum* plant was collected from Chennai, south India, and authenticated by the Department of Plant Biology and Biotechnology, The New College, Chennai. Nondestructive cold-process extraction was employed with different solvents (ethanol, ethyl acetate and chloroform); and in the case of aqueous extraction, heat distillation process was used. Shade-dried powdered leaves of *S. trilobatum* were soaked separately in ethanol, ethyl acetate and chloroform in a ratio of 1:5 (solute versus solvent) in a conical flask. The entire setup was kept at room temperature for 24 hours with intermittent shaking. After 24 hours, the mixture was filtered through Whatman no.1 filter paper, and the filtrate was dried to evaporate the solvent. The extract settled at the bottom was used for the experiment at varying concentrations. In

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the case of aqueous extraction, the leaves were boiled in water at 1:5 ratio at 100°C for 30 minutes.<sup>[7]</sup> After 30 minutes, the mixture was filtered and the filtrate was stored in a refrigerator until use. All animal experiments were approved by the Institutional Animal Ethics Committee.

### Collection of peritoneal mast cells

Cold normal saline (2 mL) was injected into the peritoneal cavity of the BALB/c mice under mild ether anesthesia. After a gentle massage, the peritoneal fluid was collected and transferred into siliconized test tubes containing 3 mL of RPMI-1640 medium (pH, 7.2-7.4). The cells were washed twice by low-speed centrifugation (400-500 rpm) and suspended in RPMI-1640 medium (Himedia, India).<sup>[8]</sup>

### Degranulation of mast cells

The peritoneal exudate cells containing, among other things, mast cells were incubated for 15 minutes at 37°C with antigen (0.1 mg egg albumin/mL). After incubation, the cells were stained with 1% toluidine blue<sup>[9]</sup> and observed under high-power microscope. The total number of peritoneal exudate cells and the number of mast cells (degranulated and granulated) were counted in the complete field of the microscope.

### Effect of *S. trilobatum* on rat mesenteric mast cells

Male albino Wistar rats weighing 150-200 g were sensitized with egg albumin (1 mg/rat) intramuscularly. After 10 days another dose of egg albumin was given as above. The rats were then divided into five groups (six rats in each group). The aqueous and ethanolic extracts of *S. trilobatum* in 5% Tween 80 were given orally for 3 consecutive days, following the second dose of antigen administration. The aqueous extract was administered at a dose of 5 and 10 mg/kg body weight to groups I and II, respectively. The ethanolic extract was administered at 5 and 10 mg/kg body weight to groups III and IV, respectively. Group V animals treated with cetirizine hydrochloride at 0.5 mg/kg body weight were used as control. Twenty-four hours after the last dose of drug or 5% Tween 80 treatment, rats were sacrificed by cervical dislocation and intestinal mesentery was collected.

### Collection and preparation of mesentery

This was done essentially as described by Rathinum *et al.*<sup>[10]</sup> The intestinal mesentery of overnight-fasted rats was collected, washed in normal saline, separated along with gut and suspended in RPMI-1640 medium. The mesentery was then cut into pieces of 1 cm<sup>2</sup> each and used for the experiment. Two pieces from each rat were incubated in 5 mL RPMI-1640 medium with or without 0.1 mg/mL egg albumin, for 15 minutes at 37°C. The mesentery was removed, spread on a clean glass slide, dried in air and separated from the gut using a sharp blade. The dried slide

was stained with toluidine blue and counter-stained with light green stain<sup>[9]</sup> and observed under the microscope.

### Effect of release of histamine from mast cells

Mast cells were isolated from peritoneal cavity of albino Wistar rats and maintained in RPMI-1640. Substance P (neuromediator) was used as mast cell degranulation substance. Substance P prepared at a concentration of 3.10-5 M was used for the test. Different solvent extracts of *S. trilobatum* were tested for their effect on histamine release. After treatment with the respective extracts, the cells were incubated at 37°C for 20 minutes. After incubation the reaction was stopped by changing the temperature to 4°C.<sup>[11]</sup> Later, reaction mixture was centrifuged and the supernatant was assayed for histamine by Immunotech kit (Beckman Coulter Cat. No. 2562).

### Isolation of keratinocytes

Primary cultures of human keratinocytes were established from the skin remaining of abdomen surgery from healthy donors. Skin pieces of 1 cm<sup>2</sup> were exposed to dispase 2.4 U/mL (Sigma) overnight at 4°C. The epidermis was removed from the dermal layer and incubated in 0.25% EDTA-trypsin (Sigma) for 10 minutes at 37°C. After incubation, enzymatic activity of trypsin was stopped to adding Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco) and was homogenized by repeated aspirations. Cell suspension was centrifuged at 1500 rpm for 10 minutes, and the pellet was resuspended in serum-free medium for keratinocytes (Gibco). Cells were seeded into 75-cm<sup>2</sup> culture flasks and kept at 37°C at 5% CO<sub>2</sub>. The culture medium was renewed every 3 days.<sup>[12]</sup>

### Release of IL1 $\alpha$ from keratinocytes

Keratinocytes were incubated in keratinocyte serum-free medium (KSFM) (Gibco, BRL - France) at 37°C with 5% CO<sub>2</sub>. All the extracts at all the concentrations were tested for the effect of release of IL1 $\alpha$ . Keratinocytes were irradiated with UV A and B at 0.15 J/cm<sup>2</sup>. After irradiation, the cell culture was kept at 37°C for 24 hours in KSFM; the supernatant was collected and assayed for IL1 $\alpha$  by ELISA kit (Beckman Coulter Cat. No. IM0755)

### Release of IL8 from keratinocytes

To study the release of IL8, a procedure similar to the one mentioned above was employed. Subsequently PMA (Phorbol-12- Myristate 13-Acetate) was used as IL8 stimulant. IL8 was assayed by ELISA kit (Beckman Coulter Catalog No. IM227)

### Dust inhalation assay

Twelve healthy New Zealand white rabbits (6 animals per group) orally pretreated with aqueous extract of

*S. trilobatum* at 10 mg/kg body weight for 14 days were exposed to house dust on day 15 through a nebulizer in a dust inhalation chamber for 1 hour. Four extract-pretreated animals each unexposed to house dust challenge were maintained as control. The amounts of histamine released in test and control animals were assayed using standard procedure.<sup>[13]</sup>

### Statistical analysis

The results are expressed as mean  $\pm$  SD. Significant differences between two experimental groups were analyzed by ANOVA. Values of  $P < 0.05$  were considered significant.

## RESULTS

The aqueous and alcoholic extracts of *S. trilobatum* exhibited mast cell degranulation inhibition activity at 15  $\mu\text{g/mL}$ . Increase in activity with increase in concentration with the above extracts was seen. Chloroform and ethyl acetate extracts of the above plant did not show any activity at all concentrations [Table 1].

Ten-day administration of the aqueous and alcoholic extracts of *S. trilobatum* showed mast cell stabilization in animals at 10 mg/kg body weight. The histamine release in these animals was as low as that for cetirizine hydrochloride (0.5 mg/kg body weight)-treated animals. Increase in activity with increase in concentration of the above extract was not seen [Tables 2 and 3].

Aqueous and alcoholic extracts of *S. trilobatum* at 10 and 15  $\mu\text{g/mL}$ , respectively, significantly decreased the release of IL1 $\alpha$  and increased the release of IL8 from the cultured keratinocytes [Tables 4 and 5].

## DISCUSSION

With the advent of urbanization and industrialization, allergic manifestation, especially the state of atopy, has increased significantly. As per WHO, 20% of the global population is considered to be hypersensitive/atopic in nature. Management of atopy is widely achieved either through immune-suppressive drugs such as steroids or through neutralization of the end product of allergic process — histamine with antihistamine drugs.<sup>[14]</sup> Therefore, for the management of allergy, there arises the need for either histamine inactivation or mast cell stabilization. In the current study, we have evaluated the role of *S. trilobatum* by addressing the preliminary mechanism of response in the allergic manifestation, i.e., mast cell degranulation inhibition.

The aqueous and ethanolic extracts of *S. trilobatum* showed mast cell degranulation inhibition property, whereas none of the other extracts of the same plant showed any significant activity. This was reconfirmed by quantifying the release

**Table 3: Effect of *S. trilobatum* in the release of histamine in animals exposed to dust (dust inhalation test/extract administration at 10 mg/kg body weight)**

Treatment group (Animals)	Quantity of histamine release (nM) time interval		
	6 hours	12 hours	18 hours
Untreated control	806	810	819
Negative control (cetirizine treated)	145	151	162
Aqueous extract	211	214	221
Ethanolic extract	216	219	227
Chloroform extract	800	809	815
Ethyl acetate extract	802	811	814

**Table 1: Effect of *S. trilobatum* on mast cell degranulation**

Concentrations ( $\mu\text{g/mL}$ )	Percentage of inhibition of degranulation in response to extracts				Control
	Aqueous	Ethanol	Chloroform	Ethyl acetate	
1	87 $\pm$ 1.3	87 $\pm$ 1.9	82 $\pm$ 1.3	78 $\pm$ 1.4	88 $\pm$ 1.2
5	89 $\pm$ 1.5	89 $\pm$ 1.1	85 $\pm$ 1.5	78 $\pm$ 1.9	
10	81 $\pm$ 1.2	81 $\pm$ 1.9	80 $\pm$ 1.2	81 $\pm$ 1.9	
15	64 $\pm$ 1.7	63 $\pm$ 1.6	88 $\pm$ 1.1	83 $\pm$ 1.7	
20	60 $\pm$ 1.3	61 $\pm$ 1.4	86 $\pm$ 1.2	80 $\pm$ 1.4	

**Table 2: Effect of *S. trilobatum* in the release of histamine from mast cells**

Experiment	Extracts/quantity of histamine release (nM)			
	Aqueous	Ethanol	Chloroform	Ethyl acetate
Control	312			
Mast cells treated with substance P	1543			
Mast cells treated with substance P + 1 $\mu\text{g/mL}$ extract	1242	1391	1510	1405
Mast cells treated with substance P + 5 $\mu\text{g/mL}$ extract	1132	1252	1493	1400
Mast cells treated with substance P + 10 $\mu\text{g/mL}$ extract	1100	1113	1448	1381
Mast cells treated with substance P + 15 $\mu\text{g/mL}$ extract	1064	1083	1400	1377
Mast cells treated with substance P + 20 $\mu\text{g/mL}$ extract	1018	1002	1340	1374

**Table 4: Effect of *S. trilobatum* in the release of IL1 $\alpha$  from keratinocyte culture**

Extracts	Variation of IL1 $\alpha$ by test/control				
	1 $\mu$ g/ml	5 $\mu$ g/ml	10 $\mu$ g/ml	15 $\mu$ g/ml	20 $\mu$ g/ml
Aqueous	-31 $\pm$ 5	-32 $\pm$ 6	-51 $\pm$ 8	-47 $\pm$ 6	-36 $\pm$ 6
Ethanol	-41 $\pm$ 6	-42 $\pm$ 5	-55 $\pm$ 5	-52 $\pm$ 6	-43 $\pm$ 7
Chloroform	-33 $\pm$ 5	-35 $\pm$ 7	-35 $\pm$ 6	-37 $\pm$ 6	-37 $\pm$ 5
Ethyl acetate	-32 $\pm$ 5	-34 $\pm$ 7	-35 $\pm$ 7	-37 $\pm$ 7	-37 $\pm$ 6

**Table 5: Effect of *S. trilobatum* in the release of IL8 from keratinocyte culture**

Extracts	Variation of IL8 by test/control				
	1 $\mu$ g/ml	5 $\mu$ g/ml	10 $\mu$ g/ml	15 $\mu$ g/ml	20 $\mu$ g/ml
Aqueous	-30 $\pm$ 7	-29 $\pm$ 5	-22 $\pm$ 8	-20 $\pm$ 5	-27 $\pm$ 5
Ethanol	-30 $\pm$ 5	-31 $\pm$ 6	-23 $\pm$ 6	-18 $\pm$ 7	-26 $\pm$ 6
Chloroform	-32 $\pm$ 6	-34 $\pm$ 6	-35 $\pm$ 6	-36 $\pm$ 6	-36 $\pm$ 5
Ethyl acetate	-30 $\pm$ 6	-31 $\pm$ 5	-32 $\pm$ 6	-34 $\pm$ 6	-34 $\pm$ 5

of histamines in mesenteric mast cells. The aqueous and ethanolic extracts of *S. trilobatum* showed activity for *in vitro* assay for inhibition of mast cell degranulation, whereas other extracts of the same plant did not show any activity. This plant if properly exploited can serve as an effective drug in the management of various allergic and inflammatory conditions.

The efficacy of *S. trilobatum* in the inhibition of mast cell degranulation was reconfirmed by dust inhalation study. In the *S. trilobatum*-treated animals, the release of histamine was very low in response to dust exposure. This could be due to the mast cell stabilization event, resulting in relatively low release of histamine. The *in vitro-in vivo* concordance in the result proves that *S. trilobatum* has anti-allergic properties. Studies have been carried out in the past to establish anti-inflammatory and anti-oxidant properties of *S. trilobatum*,<sup>[15-20]</sup> but its antihistaminic property, in particular, is a novel finding of this present investigation.

Keratinocytes are the chief cells which play a vital role in the skin during allergic responses. They release a variety of interleukins; IL1 $\alpha$  and IL8, in particular, are pro-inflammatory mediators. There is a dearth of studies on the effect of *S. trilobatum* extract over the cultured keratinocytes and the release of interleukins and hence this study assumes prime importance.

Biological activity of IL1 $\alpha$  is pleotropic. Many of its effects are to increase the production of other cytokines. Chemotaxis, transendothelial migration, induction of lysosomal enzyme release, respiratory burst and generation of superoxide anions are the chief effects of IL8 on neutrophils. In order to confirm the effect of *S. trilobatum* in controlling inflammatory responses, the extracts were studied for their effect on the release of IL1 $\alpha$  and IL8. The keratinocytes were treated with extracts *in vitro* and were exposed to UV radiation. The findings revealed that the

release of IL1 $\alpha$  significantly decreased while that of IL8 increased in the treated cells. Interestingly, this is the first report revealing the anti-inflammatory property *S. trilobatum* by modulating the release of interleukins by keratinocytes. *S. trilobatum* showed activity in controlling allergic responses. The aqueous and ethanolic extracts of *S. trilobatum* exhibited anti-inflammatory activities. The above activities could be attributed to the presence in these plants of specific active compounds capable of producing pharmacological effects. The analysis of the crude aqueous and ethanolic extracts showed an alkaloid content of 0.42% and the presence of tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides (data not included). The immunomodulatory and anti-inflammatory properties of *S. trilobatum* could be attributed to these constituent ingredients in the extracts. In the present study, an attempt was made to investigate the effect of whole extract as used in the traditional systems of therapy, such as Ayurveda, rather than studying the activity of specific isolated active principles. Pure phytochemicals, besides their pharmacological benefits, may also possess toxicity when used individually. However, their toxic activities may get nullified/neutralized while present together with other compounds during therapeutic use.

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