

Immunomodulatory Activity of Leaves of *Solanum trilobatum* in Experimental Rats

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Summary

Aqueous extract of leaves of *Solanum trilobatum* Linn (Solanaceae) was pharmacologically validated for its immunomodulatory properties in experimental animals. Oral administration of extract at dose of 100, 200 & 400mg/kg significantly increased in percentage neutrophil adhesion (P<0.001). The Delayed Type Hypersensitivity also showed a dose dependent activity (P< 0.001). Further, a dose related increase in hemagglutination antibody titer was observed with different doses as compared to control group. Carbon clearance test was conducted to establish phagocytic activity of reticuloendothelial system after treatment with aqueous extract. Phagocytic index was significantly increased after the administration of *S. trilobatum* compared to control group (P<0.001). These findings suggested that the immunostimulatory activity of *S. trilobatum* influences by potentiating humoral as well as cellular immunity.

Keywords - Immunomodulation, *Solanum trilobatum*, neutrophil adhesion, phagocytic activity.

Introduction

The immune system is involved in the etiology as well as pathophysiologic mechanisms of many diseases. Modulation of the immune responses to alleviate the diseases has been of interest for many years and the concept of 'rasayana' in Ayurveda is based on related principles[7]. Ayurveda, the Indian traditional system of medicine, lays emphasis on promotion of health a concept of strengthening host defenses against different diseases[16]. Indian medicinal plants are a rich source of substances, which are claimed to induce paraimmunity, the nonspecific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions [13].

Solanum trilobatum Linn (Family: Solanaceae) is used in the Siddha system of medicine as an expectorant and in the treatment of respiratory diseases, asthma, chronic febrile infections, and tuberculosis, cardiac and liver diseases. The leaves are used for curing cough, respiratory disorders, especially bronchial asthma [6]. It was reported that *S. trilobatum* possess anti-inflammatory[4], antioxidant activity, hepatoprotective activity[14] and protects UV induced damage and radiation induced toxicity in mice[10]. Immunosuppression is a major drawback in conventional therapy of cancer such as radiation and chemotherapy[2]. Sobatum an active fraction obtained from *S. trilobatum* found to be cytotoxic in Dalton's Lymphoma ascites (DLA), Ehrlich ascites (EA) cell lines and tissue culture cells (L929 and Vero). Sobatum significantly inhibited peritoneal tumours induced by DLA and EA tumour cells[10]. Reports showed sobatum reduced the side effects of radiation-induced toxicity and suggested that it could be used along with radiation therapy[10]. Further, *S. trilobatum* reported to enhance the nonspecific immunity in fish [3]. Hence *S. trilobatum* is known for the treatment of cancer, the present study was designed to evaluate the immunomodulatory properties in experimental animals.

Materials and methods

Plant Material and Extract Preparation - Leaves of *Solanum trilobatum* Linn (Fam: Solanaceae) were collected from wild source near Annamalai Nagar, Tamilnadu . The plant material was authenticated by Prof. A.L. Chidambaram , Department of Botany, Annamalai University and voucher specimen was lodged in the departmental herbarium. The air-dried leaves were powdered and boiled for 30 minutes with distilled water and filtered. The filtrate was subjected to freeze drying to yield 8.2%(w/w).

Animals - Wistar rats (160-180g) and Swiss albino mice (25 - 30 g) of either sex were used for the study. They were provided with a standard diet (Pranav Agro, India) and water ad libitum in animal house facility and maintained under standard laboratory conditions. The experimental protocol has been approved by institutional animal ethics committee, Annamalai University (Regd No.160/1999/CPCSEA).

Neutrophil adhesion test- Rats were administered orally with different doses of aqueous extract of *S. trilobatum* (100, 200, 400 mg/kg /day) for 14 days. Before administration of the drug on day 0 and after administration of the drug on 7th and 14th day, blood samples were collected by punching the retro orbital plexuses into heparinised vials and analysed for Total Leucocytes Count (TLC) and Differential Leucocytes Count (DLC) by fixing blood smears and staining with the field stain I & II- Leishman's Stain. After initial counts blood samples were incubated with 80 mg/ml of nylon fibres for 15 min at 37°C. The incubated blood samples were again analyzed for Total Leucocytes Count (TLC) and Differential Leucocytes count (DLC) [18]. % Neutrophil adhesion was calculated as shown below,

$$\text{Neutrophil adhesion (\%)} = \frac{N/u - N/t}{N/u}$$

Where,

N/u – Neutrophil index of untreated blood sample

N/t – Neutrophil index of treated blood sample.

Table 1. Effect of *Solanum trilobatum* on neutrophil adhesion in rats.

Treatment (mg/kg)	Percentage Increase Neutrophil Adhesion	
	7 th day	14 th day
Control	20.59 ± 1.23	20.59 ± 1.23
<i>S. trilobatum</i> 100	22.30 ± 2.86	25.75 ± 12.33
<i>S. trilobatum</i> 200	33.58 ± 7.73 *	42.13 ± 6.05*
<i>S. trilobatum</i> 400	35.96 ± 2.73 *	47.54 ± 3.45*

Values are expressed as mean ± SD. * P < 0.001 as compared to control.

Hypersensitivity (DTH) response

DTH was measured by the Mantoux test[11] was used. Sheep's erythrocytes (SRBCs) collected in elsever's solution , were washed three times with pyrogen-free sterile normal saline and adjusted to a concentration of 1×10^8 cells/ml for sensitization & challenge. After administration of various test extracts of *S. trilobatum* (100, 200, 400 mg/kg /day) On day fourteen the thicknesses of right hind footpad were measured using vernier caliper. The rats were challenged by injection of 1×10^8 Sheep's RBC in the plantar region of right hind footpad. Foot thicknesses were measured again after 24 hours of this challenge. The difference between pre and post challenge foot thickness expressed in mm was taken as a measure of DTH.

Table 2. Effect of *Solanum trilobatum* on[DTH]volume.

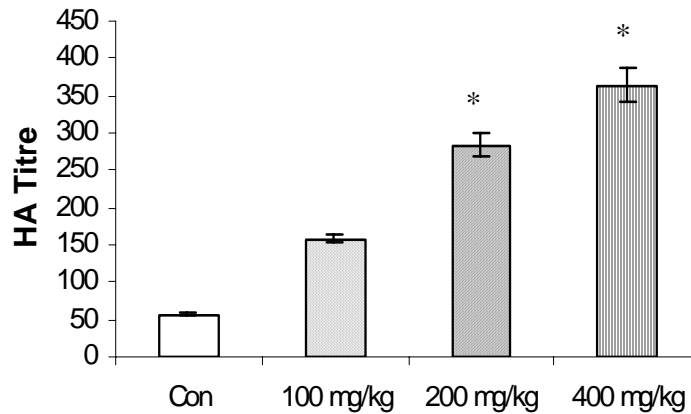
Treatment (mg/kg)	Foot Paw volume (mm)	
	Before treatment	After treatment
Control	0.87 ± 0.11	0.87 ± 0.11
<i>S. trilobatum</i> 100	0.81 ± 0.10	0.74 ± 0.04 *
<i>S. trilobatum</i> 200	0.84 ± 0.32	0.71 ± 0.02 **
<i>S. trilobatum</i> 400	0.83 ± 0.23	0.69 ± 0.01**

Values are expressed as mean ± SD *P < 0.01, ** P < 0.001 as compared to control

Haemagglutination antibody titer

Animals in Four different groups were injected i.p. 0.2 ml of 5×10^9 SRBC on day 0. Aqueous extract of *S. trilobatum* was administered orally (100, 200, 400 mg/kg /day) to animals on -4, -2, 0, 2, 4days. Control group received equal volume of PBS (pH 7.4). Blood samples were collected from retro-orbital plexus on day 7. Antibody titer was determined by the method of Nelson and Mildenhall (1967). To two-fold dilutions of serum samples made in 25 μ l volumes of normal saline containing 0.1% BSA (BSA saline) in V bottom heamagglutination plates were added 25 μ l of 0.1% suspension of SRBC in BSA saline. After thorough mixing SRBC were allowed to settle at room temperature for 90 min until control cells showed small button of cells (negative pattern). The value of the highest serum dilution causing visible haemagglutination was considered as the antibody titer [17].

Fig. 1. Effect of *Solanum trilobatum* on HA Titre

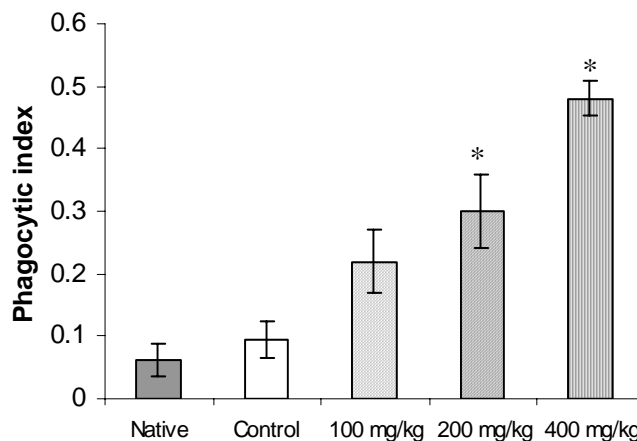


Values are expressed as mean \pm SD * P < 0.001 as compared to control

Phagocytic activity

Female albino mice (weight 25-30 g) were divided in to three groups of six animals in each. The animals were kept at room temperature and had free access to food and water. Aqueous extract of *S. trilobatum* was injected intraperitoneally in the dose of 100, 200, 400 mg/kg body wt. Respectively (group III to V), daily for 5 consecutive days. Phosphate buffer saline (pH7.4) was given to control group (II) and native group (I) without any treatment. After 48 hr. of last dose, mice of group II, III and IV were injected via the tail vein with colloidal carbon, which was diluted with PBS (pH 7.4) to eight times before use (10 μ l/g body wt.). Blood samples were drawn from the retro – orbital plexuses at intervals of 0 and 15 min. The blood (25 μ l) was dissolved in 0.1 % sodium carbonate (2 ml) and the absorbance was measured at 660 nm [5]. The phagocytic index, K, was calculated by equation:

$$K = \frac{\ln OD_1 - \ln OD_2}{t_2 - t_1}$$

Fig. 2 . Effect of *Solanum trilobatum* on phagocytic index (K)

Values are expressed as mean \pm SD *P < 0.01, ** P < 0.001 as compared to control

Statistical analysis

All the data are presented as mean \pm S.E.M. and one-way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test was applied for determining the statistical significance between different groups.

Results

Aqueous extract of *S. trilobatum* was administered at a dose of 100, 200 & 400mg/kg/oral for 14 days. Percentage of neutrophil adhesion observed were 18.30 \pm 2.86, 33.58 \pm 7.73 and 35.96 \pm 2.73 on the 7th day and after 14th day it was 25.75 \pm 12.33, 42.13 \pm 6.05 and 47.54 \pm 3.45 at a dose of 100, 200 & 400mg/kg/oral respectively. The percentage increase in neutrophil adhesion showed a dose dependent activity (P<0.001) (Table 1). The aqueous extract at a dose of 400mg/kg showed highly significant activity (P<0.001). DTH reactions in animals showed decrease in paw volume as 0.07 mm, 0.13mm and 0.24 mm at a dose of 100, 200 & 400mg/kg respectively (Table 2). The DTH showed a dose dependent activity. The aqueous extract at a dose of 400mg/kg showed significant activity (P< 0.001).

A dose related increase in hemagglutination antibody titre was observed with different doses of *S. trilobatum* compared to control group. Hemagglutination antibody titer were increased at a doses of 100, 200 & 400mg/kg from control 56.26 ± 1.16 to 158.42 ± 6.26 , 284.14 ± 16.18 , 362.36 ± 22 ($P < 0.001$) respectively (Fig. 1). Carbon clearance test was conducted to establish phagocytic activity of reticuloendothelial system after treatment with aqueous extract of *S. trilobatum* (Fig. 2). Phagocytic index was significantly increased after the administration of *S. trilobatum* compared to control group ($P < 0.001$). At dose 400 mg/kg showing phagocytic index value of 0.48 ± 0.02 compared to 0.09 ± 0.006 recorded with control.

Discussion

Intensive course of chemotherapy can have the opposing effects of tumor elimination and immunosuppression, it is incumbent upon clinicians and basic scientists to continue to explore the maximization of tumor killing balanced with the reconstitution of immunity. Control of disease by immunological means has two aspects, namely the development and improvement of protective immunity and the avoidance of undesired immunological side reactions. Modulation of the immune system by cytostatic agents is emerging as a major area in pharmacology, especially in cases where undesired immunosuppression is the result of therapy. The modulation of immune response by using medicinal plant products as a possible therapeutic measure has become a subject of active scientific investigations. The main objective of the present study was to evaluate the immunomodulatory activity of anticancer plant *Solanum trilobatum* Linn.

S. trilobatum was administered orally showed significant increase in adhesion of neutrophils to nylon fibers which correlates to the process of margination of cells in blood vessels. The neutrophil adhesion was significantly increased with the dose of 100 - 400 mg/kg/day when compared to untreated control. Neutrophils circulate in the vasculature in a passive state and become more adhesive upon stimulation at sites of inflammation. Margination to the vessel wall and subsequent transmigration and phagocytosis [15]. Delayed type hypersensitivity reaction is characterized by large influxes of non-specific inflammatory cells, in which the macrophage is a major participant. It is a type IV hypersensitivity reaction that develops when antigen activates sensitized TDTH cells. These cells generally appear to be a TH1 subpopulation although sometimes TC cells are also involved. Activation of TDTH cells by antigen presented through appropriate antigen presenting cells results in the secretion of various cytokines, macrophage migration inhibition factor and tumor necrosis factor[1]. The overall effects of these cytokines are to recruit macrophages into the area and activate them, promoting increased phagocytic activity vis-a-vis increased concentration of lytic enzymes for more effective killing. Several lines of evidence suggest that DTH reaction is important in host defense against parasites and bacteria that can live and proliferate intracellularly[17]. Treatment of aqueous extract of *S. trilobatum* enhanced DTH reaction, which is reflected from the increased footpad thickness compared to control group suggesting heightened infiltration of macrophages to the inflammatory site.

Haemagglutination antibody titre was determined to establish the humoral response against SRBC. At neutral pH, red blood cells possess negative ions cloud that makes the cells repel from one another, this repulsive force is referred to as zeta potential. Because of its size and pentameric nature, IgM can overcome the electric barrier and get cross-link red blood cells, leading to subsequent agglutination. The smaller size and bivalency of IgG, however, makes them less capable to overcome the electric barrier. This characteristic may account for, IgM being more effective than IgG in agglutinating red blood cells[8]. The augmentation of the humoral response as evidenced by an enhancement of antibody responsiveness to SRBC in rats as consequence of both pre and post-immunization drug treatment indicates the enhanced responsiveness of macrophages and B-lymphocyte subsets involved in antibody synthesis[11].

S. trilobatum treatment improved the haemagglutination antibody titre reflecting an overall elevation of humoral immune response. Phagocytosis represents an important innate defence mechanism against ingested particulates including whole pathogenic microorganisms. The specialized cells that are capable of phagocytosis include blood monocytes, neutrophils and tissue macrophages. Once particulate material is ingested into phagosomes, the phagosomes fuse with lysosomes and the ingested material is then digested [17]. Enhanced uptake of particulates with treatment of *S. trilobatum* extract is evident from carbon clearance.

In conclusion, the present study has shown the immunostimulatory activity of anticancer plant. *S. trilobatum* by potentiating humoral as well as cellular immunity. These findings also suggested that *S. trilobatum* does not suppress the immune system while treating cancer like other chemotherapeutic agents. Promising approaches to immune reconstitution using natural products regimens continue to be developed and it appears that the future of cancer chemotherapy continues to be bright. Further detailed studies are required which might establish a possible mechanism of immunomodulation effects of this plant.

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