Antianaphylactic and mast cell stabilization activity of *Strychnos potatorum* Linn. seed

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ABSTRACT

Aim: The antianaphylactic activity of *Strychnos potatorum* Linn seed extract was evaluated by using compound 48/80 induced anaphylaxis and mast cell stabilization was studied by using peritoneal mast cells of rats. The possible antianaphylactic and mast cell stabilization mechanism was evaluated by using compound 48/80 induced mast cell activation and level of nitric oxide in rat peritoneal mast cells. **Materials and Methods:** Anaphylactic shock in mice was induced by the intraperitoneal administration of 8 mg/kg compound 48/80, prior to induction of anaphylaxis the animals were treated with *S. potatorum* Linn. seed extract administered orally 1 h before administration of compound 48/80, the rate mortality was observed in each group of animals. Mast cell stabilization was seen by preincubation of mast cells with the compound 48/80 and the extracts. **Results:** This study indicates that the chloroform, petroleum ether, and methanolic extracts were shown potent and has significant ($P < 0.01$ and $P < 0.001$) inhibitory effects on compound 48/80 induced anaphylactic reaction and mast cell activation. This compound also inhibited significantly compound 48/80 induced increased level of nitric oxide in rat peritoneal mast cells. **Conclusion:** We conclude from this study that the different extracts of *S. potatorum* seed have potent antianaphylactic activity through mast cell stabilization and inhibition of nitric oxide synthesis. The inhibitory effect of *S. potatorum* Linn. on release of histamine and nitric oxide protects from compound 48/80 induced anaphylactic reaction may be through blocking vasodilatation, decrease vascular resistance, hypotension and tachycardia induced by immunogenic agent used in this study.

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INTRODUCTION

Depending on the amount of allergen entered into the body, it will induce different kind of changes including running nose, sneezing, cutaneous wheel and flare reaction, and wheezing occurs within few minutes.[1] This type of reaction is called as an immediate type of hypersensitivity reactions.[1] Recently, it has been reported that in an immediate type of allergic reaction the allergen triggers B-cells to produce IgE and IgG antibodies, which react with these allergens, and bind to high affinity receptors for IgE (FcεRI) and circulating basophils and tissue mast cells.[2] Mast cells are well known as critically important components in various biologic processes of allergic diseases. These are found relatively large numbers in the mucosa of respiratory, gastrointestinal, urinary tract, skin and near blood or lymphatic vessels, these cells are supposed to express surface membrane receptors with high affinity and specificity for IgE.[3] Activation of these cells known to release proinflammatory cytokines, proteases, histamine, prostaglandins and leukotrienes which are known to be involved in chemotaxis and phagocytosis of macrophages.[3] It has also been reported that activation of mast cells and subsequent release of cytokines are involved in stimulation of inducible nitric oxide synthase (iNOS) leading to generation of nitric oxide at relatively and sustained level. Now, it is well established that many cell types involved directly or indirectly in immunity and inflammation synthesize nitric oxide.[4]

The modern medicines available for stabilizing the mast cells include sodium cromoglycate, cyclosporine, and glucocorticoids, but these drugs are associated with

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unwanted effects including local irritation and transient bronchospasm. These drugs are also no mean for use in pregnancy and prolonged use.[5] However, there are several plant-derived preparations in the ancient text of Ayurveda and Siddha for the treatment of allergic conditions, including asthma. It is required for the plants or their preparations to prove scientifically with their clinical applicability. In this view this study has been conceived to evaluate and investigate the Ayurveda and Siddha importance of *Strychnos potatorum*[6,7] for its antiallergic property.

MATERIALS AND METHODS

Materials

Compound 48/80 (C2313) was purchased from Sigma Aldrich (St. Louis USA), toluidine blue (02912ED-016) was purchased from SA (St. Louis USA), other chemicals including formaldehyde, methanol, DMSO, and organic solvents used were of AR grade. The Griess reagent was purchased from (G4410-10G) SA (St. Louis, USA).

Plant collection and preparation of extract

Strychnos potatorum Linn. (Loganiaceae) seeds were purchased from Dorle Ayurved, Kolhapur after authenticated by Prof. V. V. Sidlingappanavar, Head, Department of Botany, B.V.V.S Science College, Bagalkot, by the studies include macroscopic and microscopic observations. Then, the voucher specimen was deposited at Science College and number is BSC /BOT/07/04. Soon after authentication, all seeds were dried at room temperature, until they were free from the moisture. Finally, 5 kg of seeds was powdered to fine texture and passed through seive no. 40 to obtain uniform texture and extracted successively using the Soxhlet extractor, with petroleum ether, chloroform, and methanol for 24 h single cycle. The extract obtained was concentrated using a rotary evaporator at 50°C. The extract was dried in the freeze dryer after evaporating the solvent, preserved in an amber-colored well-stoppered bottle at room temperature; before the experiment the percent yield of petroleum ether, chloroform, and methanol was calculated and found to be 0.22%, 0.154%, and 0.506%, respectively. The dried extract was reconstituted in (50% DMSO diluted by methanol) DMSO for further study.

Animals

Albino Swiss mice of either sex weighing to 18–35 g were used for acute toxicity, anaphylactic reaction, and release of nitric oxide from mast cells study. *Male Sprague Dawley rats* weighing 150–250 g were used for mast cell stabilizing activity. These animals were maintained under standard conditions of temperature at 25°C and humidity 50–60% in animal house of H. S. K. College of Pharmacy, B.V.V.S. campus, Bagalkot, Karnataka. The animals were provided with standard diet *ad libitum* tap water. All the experiments using animals were carried out as per guidelines of institutional animal ethics committee (REG. NO: 821/01/a/CPCSEA) of college, after the approval (HSK/IAEC.Clear/2004–2005) dated 27/12/2004.

Methods

Acute toxicity study

The systemic acute toxicity (LD_{50}) profile of the extract was evaluated in *Swiss Albino* mice according to OECD No. 407 guidelines, with some modification. Briefly this method was carried out in two steps, the initial investigation in which 10 animals were used, three animals per treatment group with widely differing dose ranges 50, 500, and 5000 mg/kg, respectively, of the extract as per body weight and they were observed for 24 h.[8] However, in this study no mortality was observed with any of the above doses.

Antiallergy

Compound 48/80-induced systemic anaphylactic reaction was examined as previously described.[9] Mice were given an intraperitoneal injection of 8 mg/kg of the mast cell degranulator compound 48/80. Each extract of *S. potatorum* Linn in a dose of 200, 400, and 800 mg/kg were given orally at 1 h before compound 48/80 injection. Mortality was monitored for 1 h after induction of anaphylactic reaction (n = 6/group). Along with mortality, behavioral changes were observed and time of onset of tremors and time of death were recorded.

Mast cell stabilization

Isolation and purification of mast cells: Rats were anesthetized by ether, and injected with 20 ml of Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM NaH[CO_3], 2.7 mM KCI, 0.3 mM Na[H_2][PO_4], and 0.1% gelatin), into the peritoneal cavity. The abdomen was gently massaged for about 90 s. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells were aspirated using a Pasteur pipette. Thereafter, the peritoneal cells were sedimented at 150 × g for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells, i.e., macrophages and small lymphocytes. These peritoneal cells suspended in 1 ml Tyrode buffer B and centrifuged at room temperature for 15 min at 400 × g. The cells remaining at the buffer metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCI, 1.4 mM Ca[Cl_2], 1 mM Mg[Cl_2], 5.6 mM glucose and 0.1% BSA) containing calcium. Mast cell preparations were about 95% pure as assessed by toluidine blue staining. Mast cell suspension was incubated with each extract of a dose of 1, 5, 10, and 25 mg/ml for 30 min. After that 0.1 ml of

2 µg/ml mast cell activator compound 48/80 was added and incubated for 15 min, two to three drops of suspension were fixed on glass slide by solution of formaldehyde and methanol (1:3), stained by using toluidine blue (0.1%). Slides were observed under a research microscope at 40× and total 100 activated and nonactivated cells were counted.[9-11]

Release of nitric oxide from mice peritoneal mast cells

Suspension of mice peritoneal mast cells were obtained as per above-described procedure in mast cell stabilization. To 2 ml suspension, each extract was added in a concentration of 1, 5, 10, and 25 mg/ml and incubated for 24 h, after that suspension incubated for 1 h with 0.1 ml of compound 48/80, normal with 50% DMSO only and control with 0.1 ml compound 48/80 incubated for 1 h. Centrifuged at 400 × *g* and 1 ml of supernatant were collected. By adding 1 ml of the Griess Reagent to each, absorbance was measured in a spectrophotometer at 546 nm.[12]

Generation of the standard curve using sodium nitrate as the standard

The 1000 µM stock solution was prepared by dissolving 6.9 mg of $NaNO_2$ in 10 ml of distilled water. From this stock solution, 100 µl added to 9.9 ml of distilled water to obtain 100 µM solution of $NaNO_2$.[12]

From the above 100 µM solution by dissolving sufficient amount in distilled water to obtain solutions of the 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µM concentration. One milliliter solution from each concentration was added to 1 ml Griess reagent, placed in a dark room for 10 min. Lastly, absorbance was measured in a photometer at 546 nm. A standard curve was obtained by plotting the concentration of $NaNO_2$ on the *X*-axis and absorbance at 546 nm on the *Y*-axis.

Statistical analysis

The experimental results obtained from this study are represented as mean ± SEM (standard error mean). The data were evaluated by using unpaired Student '*t*' test and $P < 0.05$ was accepted as significant.

RESULTS

Antianaphylactic activity

Intraperitoneal administration of compound 48/80 induces anaphylactic shocks in mice, when *S. potatorum* administered orally 1 h before administration showed significant antianaphylactic effects as compared to control on mortality. The petroleum ether extract in doses of 200, 400, and 800 mg/kg inhibited 16.66%, 66.67%, and 66.67% mortality in mice, respectively, while the chloroform extract of *S. potatorum* Linn. in doses of 200%, 400%, and 800% mg/kg inhibited 100% mortality in mice, and the methanolic extract in doses of 200, 400, and 800 mg/kg inhibited 50%, 66.67%, 66.67% mortality in mice, respectively. Behavioral observation shows that it delayed or totally inhibited tremors induced by compound 48/80 [Table 1].

Mast cell stabilization

After incubation of peritoneal mast cell obtained from *Sprague-Dawely* rats with 50% DMSO activated 11.66 ± 3.55% of mast cells in the normal group. Incubation of mast cells with compound 48/80 and 50% DMSO, 0.1 ml of concentration 2 µg/ml activated 91 ± 2.22 activated in the control group. Preincubation of rat peritoneal mast cells with the petroleum ether extract of *S. potatorum* Linn. of a strength of 1 mg/ml significantly reduced total number of activated mast cells and the percent protection was 38.59. Preincubation of rat peritoneal mast cells with the petroleum ether extract of *S. potatorum* Linn. of strength 5, 10, and 25 mg/ml significantly reduced total number of activated mast cells and percent protection was 49.45, 61.36, and 65.93, respectively. More significant effects on compound 48/80 activated peritoneal mast cells were observed with the chloroform extract. The dose of *S. potatorum* of strengths 1, 5, 10, and 25 mg/ml prevented mast cell granulation. Preincubation with all strengths of the methanolic extract has shown dose dependently inhibitory effects on compound 48/80 induced mast cell activation. Methanolic extracts of concentrations 1, 5, 10, and 25 mg/ml have protected 34.25%, 54.21%, 56.96%, and 70.87%, respectively [Table 2].

Release of nitric oxide from peritoneal mast cell

Release of nitric oxide from mast cells after incubation with DMSO was evident of the control group sample preparation and was measured to be 19 ± 0.34 of compound 48/80 an allergen known to produce,

Table 1: Effect of *Strychnos potatorum* Linn. on compound 48/80 induced anaphylactic shock in mice

Treatment	Mortality (%)	Protection (%)
Control	100	0
200 mg/kg (p.o) petroleum ether extract	83.34	16.66
400 mg/kg (p.o) petroleum ether extract	33.33	66.67
800 mg/kg (p.o) petroleum ether extract	33.33	66.67
200 mg/kg (p.o) chloroform extract	0	100
400 mg/kg (p.o) chloroform extract	0	100
800 mg/kg (p.o) chloroform extract	0	100
200 mg/kg (p.o) methanolic extract	50	50
400 mg/kg (p.o) methanolic extract	33.33	66.67
800 mg/kg (p.o) methanolic extract	33.33	66.67

The percent mortality and percent protection were calculated by comparing with the control. The more is the percent protection that was more significant.

Table 2: Effect of *Strychnos potatorum* on peritoneal mast cells

Treatment	% Mast cells activated (mean ± SEM)	Protection (%)
DMSO (50%)	11.66 ± 3.55	
2 µg/ml of compound 48/80	91 ± 2.22	
1 mg/ml petroleum ether extract	65.66 ± 2.53***	38.59
5 mg/ml petroleum ether extract	46 ± 2.38***	49.45
10 mg/ml petroleum ether extract	35.16 ± 2.55***	61.36
25 mg/ml petroleum ether	31 ± 1.23***	65.93
1 mg/ml chloroform extract	50.66 ± 1.14***	34.25
5 mg/ml chloroform extract	28 ± 1.86***	54.21
10 mg/ml chloroform extract	20.16 ± 1.87***	56.96
25 mg/ml chloroform extract	19.5 ± 1.93***	70.87
1 mg/ml methanolic extract	59.83 ± 1.58***	34.25
5 mg/ml methanolic extract	41.66 ± 1.86***	54.21
10 mg/ml methanolic extract	39.16 ± 2.40***	56.96
25 mg/ml methanolic extract	26.5 ± 2.04***	70.87

****P* value is highly significant ($P < 0.001$)

Table 3: Effect of *Strychnos potatorum* Linn. on release of nitric oxide from peritoneal mast cells

Treatment	Amount of nitric oxide released (µM/ml) (mean ± SEM)	Protection (%)
Normal	19 ± 0.340	–
Control	30.9 ± 3.22	–
1 mg/ml petroleum ether extract	35.5 ± 1.72	19.94
5 mg/ml petroleum ether extract	28.4 ± 1.07	10.83
10 mg/ml petroleum ether extract	24.4 ± 1.91*	28.17
25 mg/ml petroleum ether extract	24 ± 1.88*	29.91
1 mg/ml chloroform extract	30.9 ± 2.24	0
5 mg/ml chloroform extract	23.9 ± 0.88*	30.34
10 mg/ml chloroform extract	20.2 ± 0.68**	46.38
25 mg/ml chloroform extract	20.6 ± 1.35**	44.65
1 mg/ml methanolic extract	25.7 ± 2.008*	22.54
5 mg/ml methanolic extract	29.6 ± 1.43	5.63
10 mg/ml methanolic extract	30.1 ± 8.77	3.46
25 mg/ml methanolic extract	29.2 ± 1.90	7.36

**P* value is significant ($P < 0.05$), ***P* value is medium significant ($P < 0.01$)

anaphylactic reaction significantly increased the level of nitric oxide release from mast cells. It was from 19 ± 0.34 to 30.9 ± 3.22. Preincubation of mice peritoneal mast cells with the petroleum ether extract of *S. potatorum* Linn. of strength 1 mg/ml has shown an increase in production of nitric oxide and percent elevation was -19.42. Preincubation of mice peritoneal mast cells with the petroleum ether extract of *S. potatorum* Linn. of strength 5 mg/ml not shown significant effects on nitric oxide release, but percent inhibition was 10.88. Preincubation of mice peritoneal mast cells with the petroleum ether extract of *S. potatorum* Linn. of a strength of 10 and 25 mg/ml has significantly reduced nitric oxide production and percent protection was 28.17 and 29.91, respectively. Preincubation of mice peritoneal mast cells with the chloroform extract of *S. potatorum* Linn. of a strength 1 mg/ml have not shown any significant effect on nitric oxide production. Preincubation of mice peritoneal mast cells with the chloroform extract of *S. potatorum* Linn. of a strength 5, 10, and 25 mg/ml significantly reduces nitric oxide production and percent inhibition was 30.34, 46.38, 44.65 [Table 3].

DISCUSSION

Anaphylaxis is a life-threatening syndrome induced by the sudden systemic release of inflammatory mediators, such as histamine, heparin from mast cells, and production of nitric oxide in response to IFN-γ particularly in combination with tumor necrosis factor-α from immunocompetent cells.[10,13] In the anaphylactic shock release of histamine and cytokines were causes profound vasodilation, decreased systemic vascular resistance, hypotension, trachycardia, flushing, an increase in bronchial and gastrointestinal smooth muscle contraction and glandular secretion leading to death.

In earlier studies, it has been proved that application of stimulant such as compound 48/80 triggers degranulation of mast cell by the aggregation of high affinity receptor for the fc region of IgE (fcεRI) caused by cross linking of IgE by polyvalent antigens, leading to activation of PL_C.[14] When PL_C in activated in mast cells, phosphatidyl inositol 4,5-biphosphate (PIP_2) is hydrolyzed to produce IP_3 and DAG. IP_3 binds to its receptors on the intracellular $[Ca^{++}]i$ storage site to release Ca^{++}, while DAG activates PK_C. Then increase [Ca] and activation of PK_C lead to degranulation, which are required to release histamine.[13] During degranulation, storage granules are transformed into secreting granules; the initial ultrastructural sign of this transformation is the granule matrix disorganization. Mast cell tryptase released after degranulation has a longer half-life in the circulation and reaches the maximum level in 60 min after the onset of symptoms.[14] In this study, similar to earlier studies compound 48/80 induced 100% mortality when treated intraperitoneally at 8 mg/kg dose in mice. In relation to longer half-life of tryptase, the period of observation was carried out for 1 h; the behavioral changes and alteration were similar to anaphylactic shock confirming similarity in the present model to clinical conditions. In this study, the chloroform extract of *S. potatorum* significantly ($P < 0.001$) inhibited compound 48/80 induced mortality and 100% protection was observed with all doses. The petroleum ether extract also was shown significant inhibition ($P < 0.001$) and protection, and the same kind of action was also shown by the methanolic extract. An active constituent of *S. potatorum* extracted by chloroform including the nitrogenous component or alkaloids may be a potent inhibitor of any one above said mechanisms thereby inhibiting compound 48/80 induced anaphylactic reactions in mice. Compound 48/80 is a condensation product of *N*-methoxy phenyl amine with formaldehyde.[11,15] This

compound is employed as classic mast cell secretagogue that release histamine,[16] a high condensation compound 48/80 induce about 90% release of histamine from mast cells and perturbation of membrane with increased permeability of membrane.[17] Thus an appropriate amount of compound 48/80 (8 mg/kg i.p.) was used as a direct and convenient agent to study anaphylactic reactions 2 μg/ml strength for an *in vitro* study. Potent inhibitory activity of the chloroform extract followed by the petroleum and alcoholic extract on compound 48/80 induced activation and degranulation of mast cell indicates its membrane stabilization and decreased membrane permeability, thereby inhibiting release of the histamine cytokines and tryptase and protecting the experimental animals from anaphylactic death.[9-11,18]

Mast cells are known as the main effector cells in Ig-E-mediated allergic responses, but they also play an important role in immune responses by releasing free radicals and cytokines released from mast cells including IFN-γ and TNF-α produce an increased level of nitric oxide which converts cGTP to cGMP and leads to allergic responses.[19]

The chloroform extract also shows a significant inhibitory effect on release of nitric oxide from mast cell; however, the same effect was not shown by the petroleum ether and methanolic extract possibly due to difference in active constituent present in the petroleum ether and methanolic extract *S. potatorum*. Inhibition in release of nitric oxide from peritoneal mast cells by preincubation with *S. potatorum* Linn. extracts may be through its inhibitory effect on release of TNF-α, IFN-γ, and cytokines as evidenced in an earlier study.[12]

CONCLUSION

The results obtained from this study substantiated the use of *S. potatorum* Linn. according to Ayurveda and Siddha in inflammation and asthma. The extract of *S. potatorum* Linn. shows a significant antiallergic property by stabilizing peritoneal mast cell membranes and inhibiting the release of nitric oxide from mast cells.[20]

On the basis of earlier studies, we propose the probable mechanism of antiallergic activity of *S. potatorum* as inhibition of perturbation induced by compound 48/80 by stabilizing and strengthening mast cell membrane and pregranular membrane, thereby inhibiting expression of Fc expression on mast cells. Inhibition of Fc receptor expression on mast cell membranes protects immunostimulant-induced IgE cross linking, successively activation of phospholipase C, followed by degranulation and release of histamine from mast cells. The inhibitory effect of *S. potatorum* on release of histamine and nitric oxide protects from compound 48/80 induced anaphylactic reaction may be through blocking vasodilatation, decrease vascular resistance, hypotension and tachycardia induced by the immunogenic agent used in this study.

Hence, this study indicates its possible applicability and use in local, as well as systemic allergic reactions; however, detailed studies are required to obtain lead molecules from the crude extract and elaboration of a molecular mechanism of antiallergic action.

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