



Evaluation of antiallergic and anti-anaphylactic activity of ethanolic extract of *Sanseveiria trifasciata* leaves (EEST) in rodents

Rohan N. Andhare^a, Mayuresh K. Raut^b, Suresh R. Naik^{b,*}

^a Department of Pharmacology, Sinhgad College of Pharmacy, Pune, India

^b Department of Pharmacology, Sinhgad Institute of Pharmaceutical Science, Lonavala, Pune, India

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ABSTRACT

Aim of the study: The leaves and rhizomes of *Sanseveiria trifasciata* are used in folk medicine for treating bronchitis, asthma, cough, snake bite and insect bite etc. The ethanolic extract elicited analgesic, anti-inflammatory and antipyretic activity. Hence, it was decided to study the antiallergic activity of ethanolic extract of *S. trifasciata* (EEST) on various animal models as well as *in vitro* conditions, and also to understand possible mechanism of action.

Materials and methods: Ethanolic extract of *S. trifasciata* leaves (EEST) were prepared by cold maceration followed by concentration and evaporation under reduced pressure on a rotary evaporator to obtain semisolid mass. The various phytoconstituents were analyzed. The acute toxicity study of EEST was carried out in mice. The antiallergic and anaphylactic activities were evaluated using animal models viz. milk induced eosinophilia and leukocytosis, compound 48/80 induced mast cell degranulation, active and passive cutaneous anaphylaxis and histamine induced pedal edema. In addition, EEST effect on Shultz–Dale reaction in sensitized guinea pig ileum *in vivo* and antioxidant activity by free radical scavenging by DPPH method (*in vitro*) were also studied.

Results: EEST treatment at 100 mg/kg and 200 mg/kg p.o inhibited (a) milk-induced increased eosinophilia, leukocytosis, monocytes and neutrophils. (b) Prevented passive cutaneous and active anaphylactoid reactions. (c) Prevented compound 48/80 induced degranulation of sensitized mesenteric mast cells. (d) Inhibited histamine induced pedal edema formation significantly. EEST pretreatment inhibited Shultz–Dale reaction in guinea pig ileum and also elicited potent antioxidant activity.

Conclusion: Experimental findings demonstrate promising antiallergic and anti-anaphylactic activity of EEST and also elicited potent antioxidant activity. The antiallergic and anti-anaphylactic activity might be due to inhibition of release of chemical mediators from mast cells largely by phytoconstituents like steroidal saponins, triterpenoids and flavonoids present in EEST.

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Introduction

The allergy is an immune dysfunction, which is a serious health problem worldwide. Substances that cause allergic reaction are called allergens including food, pollen, dust mites, cosmetics, mold spores, and animal hairs. Hypersensitivity type I, is an allergic reaction and IgE mediated immune response, largely caused by histamine secretion from mast cells and blood basophils (Tewtrakul and Itharat, 2006; Paolo et al., 2006). Blood eosinophilia and tissue eosinophilia are characteristic features of allergic inflammation and asthma (Rosenberg et al., 2007). Mast cells are one of the most important participating cells in allergic diseases (Plaut et al., 1989). When activated, mast cells release a

number of biologically active molecules viz histamine, serotonin, proteoglycans, and serine proteases through exocytosis (Yang et al., 2008). Acute allergic reactions occur due to the allergen interaction with IgE bound membrane of mast cells and basophils, and trigger the release of preformed granule-associated histamine and tryptase (Benacerraf, 1978), membrane-derived lipid mediators (leukotrienes, prostaglandins and platelet activating factor) and intracellular mediators (cytokines and chemokines) which eventually leads to physiological events such as smooth muscle contraction, vasodilatation, increased vascular permeability and mucous hyper secretion (Cavalher-Machado et al., 2008). The leaves and rhizomes of *Sanseveiria trifasciata* are used in folk medicine for treating bronchitis, asthma, food poisoning, toxemia, cough, snake bite, insect bite etc. (Seth, 2005). Furthermore, ethanolic extract of *S. trifasciata* leaves (200 mg/kg, s.c) elicited analgesic-anti-inflammatory and antipyretic activity in mice (Sunilson et al., 2009). The other documented folk medicinal uses

* Corresponding author. Tel.: +91 2114 4304322; fax: +91 2114 280205.
E-mail address: srnaik5@rediffmail.com (S.R. Naik).

of *Sansevieria* species include treatment for abdominal pains, diarrhea and hemorrhoids.

Sansevieria species also showed antioxidant and antimicrobial activity (Onah et al., 1994; Aliero et al., 2008). Considering such traditional claims and documented pharmacological activities of *S. trifasciata* leaves, it was interesting to study the effect of EEST on experimental models of allergy along with cellular mediated events in allergy and asthma, to evaluate antiallergic activity as well as its possible mechanism of action.

Experimental

Materials and chemicals

Animals

Wister rats (150–200 g), Swiss albino mice (20–25 g), Dunkon–Hartley guinea pig (350–400 g) of either sex were procured from Indian Toxicological Institute, Pune and used for experiments. All the animals were maintained in our animal house under conditions: temperature ($24 \pm 1^\circ\text{C}$), relative humidity (45–55%), light (12 h) and dark (12 h) cycle, and allowed free access to food (standard pellet diet, Amrut laboratory animal feed, Maharashtra, India) water ad libitum. Animals after one week acclimatization in our animal house were used for experiments. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) (SCOP/IAEC/Approval/2009–10/03).

Drugs and chemicals

Compound 48/80 (Sigma Aldrich, USA), histamine dihydrochloride (Analaab Fine Chemicals, Mumbai, India), egg albumin (Loba Chemei, India), horse serum (Serum Institute India Ltd., Pune, India) and the other drugs, dexamethasone, kitotifen fumarate, indomethacin, disodium chromoglycate (from local suppliers) were procured. Other chemicals and reagents used were of analytical grade.

Methods

Preparation of ethanolic extract of *S. trifasciata* leaves (EEST)

The leaves of *S. trifasciata* (agavaceae) were collected (December, 2009) from local plant nursery, Pune (India). The plant material was authenticated at Botanical Survey of India, Pune, Maharashtra and voucher specimen (ANRNSAN1) deposited on 9th Sept, 2009. After drying under oven ($38 \pm 2^\circ\text{C}$), the leaves were powdered using a ball mill. The powder was sieved through 40 mesh. The dried powder (1000 g) of the leaves was extracted by cold maceration for 72 h with ethanol (95%). The entire procedure was repeated twice to obtain maximum extraction. The ethanolic extract was filtered and concentrated *in vacuo* to yield semisolid extract (14.3 g, 0.7% w/v). The EEST was preserved in a refrigerator ($5\text{--}10^\circ\text{C}$) and used throughout the study.

Phytochemical analysis of EEST

The preliminary phytochemical analysis of EEST was carried out by the documented method (Harborne, 1984): alkaloid (Mayer's, Wagner's, Hager's and Dragendorff's test), glycosides (Borntriggers, Legal's and Keller Killiani test), saponins (Benedict's and bromine water test), steroid (Salkowskis and Libermann–Burchard test), flavonoids (ferric chloride and Shinoda test), triterpenoids and tannins (ferric chloride test, lead acetate test, potassium permanganate test). Spectral studies of the EEST were

carried out using FTIR (Perkin Elmer, USA), UV (Shimadzu, Japan) to analyze its major phytoconstituents.

HPTLC analysis

EEST 164.6 mg was dissolved (using ultra sonicator) in 16.5 ml of methanol and sample of 5, 10 & 20 μl were applied as 8 mm wide bands, using Camag Linomate V automatic sample applicator. Sample was applied with a 100 μl syringe (Hamilton, Bonaduz, Switzerland) at a constant rate of 0.1 $\mu\text{l/s}$ and the distance between adjacent bands was 15 mm. The $10 \times 10\text{ cm}$ aluminum-backed HPTLC plate coated with 250 μm layers of silica gel G 60 F254 (Merck India, India) was prewashed with methanol and activated at 110°C for 10 min and used as stationary phase. The plate was developed in an ascending manner with solvent system consisting of chloroform: methanol: water [7:4:1(v/v)]. Plate was scanned at 254, 366 and 580 nm using scanner-4 (CAMAG) operated in reflectance–absorbance, fluorescence and white light mode, and controlled by Win CATS software (Version 1.4.3). The source of radiation used was deuterium lamp (200–400 nm), mercury lamp (200–400 nm) and tungsten (400–800) emitting continuous UV and fluorescence spectra. The plates were derivatized by spraying anisaldehyde–sulphuric acid reagent (ASR) followed by heating at 110°C for 5 min, the R_f and the colors of the bands resolved were recorded.

Acute toxicity study

EEST was prepared as a fine and uniform suspension in (1% w/v) carboxy methyl cellulose (CMC) and administered at different doses viz. 500, 750, 1000, 1500 and 2000 mg/kg orally to different groups of mice for acute toxicity studies. Acute toxicity study was carried out according to the OECD Guidelines 423. The approximate lethal dose was calculated. For anti-allergic and anti-anaphylactic studies 1/20th and 1/10th of the highest dose studied was selected (OECD Guidelines for testing chemicals, 2001).

Reference drug preparation

Dexamethasone, ketotifen fumarate and indomethacin were freshly prepared as a uniform suspension using carboxy methyl cellulose (1% w/v) freshly prior to administration. The volume of administration of drugs by oral route was 1 ml/100 g body weight (B/W).

Anti-allergic activity screening:

Effect of EEST on milk-induced eosinophilia and leukocytosis in mice

Swiss albino mice weighing (20–25 g) body weight were randomly divided into 5 groups (6 mice/group). Blood samples were collected from retro orbital plexus, under light ether anesthesia. Mice from group-I were administered CMC suspension (10 ml/kg B/W, p.o), group II received boiled and cooled milk (4 ml/kg B/W, s.c) and groups III, IV received EEST (100, 200 mg/kg B/W, p.o respectively) Group V received dexamethasone (0.25 mg/kg B/W, i.p) and 1 h later all groups of mice were injected boiled and cooled milk (4 ml/kg B/W, s.c). After 24 h, blood samples were collected as described earlier. Total eosinophil and differential leukocyte count per cu. mm. were counted and recorded under microscope (Horn and Robin, 1975).

Passive cutaneous anaphylaxis in rats

Preparation of homologous rat antiserum. Male rats were injected subcutaneously (s.c) with 100 mg of egg albumin (EA) with 12 mg

of aluminum hydroxide on days 1, 3 and 5 for obtaining homologous rat antiserum. On 10th day blood was collected from the cardiac puncture under ether anesthesia and centrifuged. The homologous serum containing IgE antibody was collected after centrifugation. The rats were divided into 5 groups (6 rats/group). Rats were sensitized by injecting 0.1 ml of serum intradermally on either side of the shaved dorsal area. After 48 h, rats from Group I were administered CMC 10 ml/kg orally, Group II and Group III received EEST 100 mg/kg B/W and 200 mg/kg B/W, p.o., respectively and group dexamethasone (0.5 mg/kg B/W, i.p.). One hour later a mixture of Evans blue (0.5%) and EA (1% w/v) prepared in normal saline were injected (0.25 ml/rat) intravenously. Thirty minutes later, a blue color area of the dorsal skin (serum injected site) was accurately measured (mm^2), compared with control group and the percentage inhibition was calculated (Faulk et al., 1971).

Active anaphylaxis in rats

Twenty four rats were sensitized by injecting subcutaneously (s.c) 0.5 ml of horse serum. Sensitized rats were randomly divided into 4 groups (6 rats/group). Rats from group I served as induced control and were administered CMC suspension 10 ml/kg B/W, p.o. Rats of group II, III were administered EEST (100, 200 mg/kg B/W, p.o.), respectively and group IV with dexamethasone (0.5 mg/kg B/W, i.p) daily for 10 days. A group of rats without any treatment were kept as normal control. On 10th day, 2 h after the treatment, the rats were challenged with 0.25 ml horse serum/rat in normal saline intravenous injection (via caudal vein). Rats were observed for onset of symptoms of anaphylactic reactions such as increased respiratory rate, dyspnea, cyanosis and mortality. The symptoms of anaphylactic reactions both in induced control and treated rats following horse serum challenge were recorded using empirical respiratory severity scoring pattern. The scoring pattern with respect to symptoms were (2) increased respiratory rate, followed by dyspnea for 10 min. (4) Dyspnea and cyanosis for 10 min (8) and respiratory collapse and death (Mitra et al., 1999).

Effect of EEST on compound 48/80 (C 48/80) induced mast cell degranulation in rats

Twenty four rats were sensitized by administering C48/80 (1 mg/kg B/W, s.c.). The sensitized rats were divided into 4 groups (6 rats/group). Group II served as an induced control, and received vehicle (CMC 10 ml/kg, p.o). Rats from group II, III received EEST (100, 200 mg/kg B/W, p.o.), respectively and group V received kitotifen fumarate (1 mg/kg B/W, p.o.), daily for 14 days. A group of rats without any treatment were kept as normal control. On the 15th day, rats were injected with 10 ml of chilled phosphate buffered saline (NaCl 137 mM, NaHCO_3 12 mM, NaH_2PO_4 0.3 mM, KCl 12.7 mM, MgCl_2 1.0 mM, CaCl_2 1.8 mM, dextrose 5.6 mM) i.p. After a gentle abdominal massage, animals were killed under anesthesia, the peritoneal fluid was collected and transferred into siliconized test tube containing 7–10 ml of phosphate buffer solution (pH 7.2–7.4). Cell viability was checked by Trypan blue dye (0.4%) exclusion test. Mast cells were purified by percoll density centrifugation method. Mast cells were washed 3 times by centrifugation at low speed (400–500 rpm) and discarded the supernatant. The pellet of mast cells were resuspended in phosphate buffer solution. Mast cells from the treated as well as control and induced control were then incubated with compound 48/80 (5 $\mu\text{g}/\text{ml}$) at 37 °C for 10 min. After incubation these cells were centrifuged and stained with Toluidine blue (1%) degranulation and intact mast cells were counted under high power microscope and subsequently percent degranulation was calculated (Hachisuka et al., 1988).

Shultz–Dale inhibition test

Male guinea pigs were sensitized with EA (10 mg/kg B/W, i.p) on the 1st and 3rd day. On day 14th day guinea pigs were killed and ileal strips from each animal were suspended in isolated organ bath using Tyrodes solution (mM concentration of NaCl 136.9, Glucose 5.6, NaHCO_3 11.9, KCl 2.68, MgSO_4 1.05, CaCl_2 1.8, NaH_2PO_4 0.37) at 37 ± 0.5 °C. Tissue sensitivity was also tested with histamine hydrochloride (0.05 $\mu\text{g}/\text{ml}$). EA was added to the bath (10 $\mu\text{g}/\text{ml}$) and responses were recorded for 90 s on a Sherrington's rotating drum. Various concentrations of EEST (10–30 $\mu\text{g}/\text{ml}$) and disodium chromoglycate (5–10 $\mu\text{g}/\text{ml}$) were added to the bath prior to EA in order to see inhibitory effect on antigen-induced contraction of ileal strips (Laekeman and Herman, 1979; Baruah et al., 1998). The induced control group guinea pig ileum (sensitized but not treated with drugs) contraction with the addition of EA 10 $\mu\text{g}/\text{ml}$ was considered as 100%, and compared with treated groups ileum contraction. The percent inhibition of contraction was calculated by measuring the height of contraction of each: ileum response in different treated groups and then compared with induced control group.

Delayed type hypersensitivity (DTH) reaction

The rats were sensitized on day 0 with 0.1 ml of egg white (i.p), randomly divided into 4 groups (6 rats/group) and received the following treatment.

Group I served as egg albumin control, received 0.1 ml CMC (1 ml/100 g B/W, p.o), daily for 7 days, 1 h prior to antigen sensitization on 1st day of the experiment. Group II and III received EEST (100 and 200 mg/kg B/W, p.o), respectively and Group IV received dexamethasone (0.5 mg/kg B/W, i.p) daily for 7 days, 1 h prior to antigen sensitization. On the 7th day, rats were challenged by injecting (s.c) 0.1 ml of egg white into the sub planter region of the hind paw. The contra lateral paw received equal volume of saline and served as a control. The hind paw edema (DTH response) was measured as the difference in the paw volume between the right and the left paw at 0 and 48 h, by volume displacement method using plethysmometer. The mean values of hind paw edema formation for each group were calculated on both day (7th and 8th day) and compared with that of vehicle treated control group (Doherty, 1981).

Histamine induced paw edema

The rats were divided into 5 Groups (6 rats/group). The different groups were treated orally with EEST (100 and 200 mg/kg B/W, p.o), indomethacin (10 mg/kg B/W, p.o) and vehicle control (CMC) 1 ml/100 g, p.o. The extract and drug were administered 30 min prior to the injection of 0.1 ml of 1% freshly prepared histamine in normal saline into the sub plantar region of right hind paw of each rat (Michael et al., 1980). The paw volume was measured initially and at 1, 2 and 3 h after histamine injection using digital plethysmometer (Orchid Scientifics, Nashik, India).

Free radical scavenging activity (in vitro)

Solution of 1-diphenyl 2-picryl hydrazyl (DPPH) 0.1 mM in ethanol was prepared and 1 ml of this solution was added to 3 ml of EEST solution in ethanol at different concentration (25–200 $\mu\text{g}/\text{ml}$). Thirty minutes later, the absorbance was measured at 517 nm using spectrophotometer (Shimadzu, Japan). The inhibitory concentration for EEST in $\mu\text{g}/\text{ml}$ to scavenge 50% DPPH free radicals (IC_{50}) was calculated by applying following formula (Blois, 1958).

$$\text{DPPH free radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of DPPH; A_1 is the absorbance of DPPH solution in presence of the extract.

Statistics

The mean $\pm$ SEM values were calculated for each group. Analysis of variance (ANOVA) followed by Duncan multiple test was used for statistical analysis. The statistical significance was considered at $p < 0.05$. Data were analyzed using Primer of Biostatistics, Version 4.0.

Results

Preliminary phytochemical analysis

The preliminary phytochemical analysis of EEST showed the presence of saponins, steroids, triterpenoids and flavonoids. Physicochemical properties of EEST are outlined in Table 1.

HPTLC analysis

Standardized HPTLC chromatogram of EEST at 254 nm showed presence of total 13 components (Figs. 1 and 2). HPTLC chromatogram of EEST samples (A) exhibited ten to thirteen grey-bluish (visible) bands in the R_f range 0.1–0.9. According to the literature steroidal saponins are found in the R_f range of 0.4–0.6 and steroidal saponins and flavonoids show a strong quenching at 254 nm (B). Therefore, the chromatogram fingerprint suggests the presence of steroidal saponins and flavonoids.

Acute oral toxicity study in mice

Administration of EEST at different doses 500 mg/kg B/W–2000 mg/kg B/W, p.o did not induce mortality nor gross behavioural changes and toxic symptoms either immediately or during 96 h observation period. Hence, LD-50 was considered more than 2000 mg/kg. The 1/20th and 1/10th of highest dose used i.e., 100 mg/kg and 200 mg/kg of EEST were selected for anti-allergy and anti-anaphylaxis studies Fig. 3.

Anti allergic activity screening

Effect of EEST on milk induced eosinophilia, leukocytosis and differential leukocyte count

Administration of milk (4 ml/kg, s.c.) increased significantly ($p < 0.001$) in total eosinophils, WBC's and differential leukocyte counts. Groups of rats pretreated with EEST (100 mg/kg B/W and 200 mg/kg B/W, p.o.), showed significant ($p < 0.05$), ($P < 0.001$) reduction in milk-induced eosinophilia, leukocytosis and also in monocyte, neutrophil counts (Table 2). However, dexamethasone treatment (0.25 mg/kg) showed greater reduction in milk induced alterations in blood cells as compared to EEST treated group.

Effect of EEST on anaphylaxis (passive cutaneous and active), DTH and C 48/80 induced mast cell degranulation

In vehicle (1% CMC) treated group, egg albumin increased the local cutaneous reaction (increased blue coloration due to

increased vascular permeability) in the sensitized animals. Pre-treatment with EEST (100 mg/kg B/W and 200 mg/kg B/W, p.o) reduced ($p < 0.05$) the local blue colored area dose dependently (Table 3). Dexamethasone (0.5 mg/kg, i.p) treated rats showed greater reduction in blue coloration ($p < 0.001$).

Horse serum sensitized rats when challenged with the same antigen (horse serum) after 14 day; induced active anaphylactic reaction i.e., increased respiratory rate, dyspnea, cyanosis & mortality. The groups of rats treated with EEST (100 mg/kg B/W and 200 mg/kg B/W, p.o) and dexamethasone (0.5 mg/kg, i.p) showed a significant amelioration ($p < 0.05$) in the anaphylactic reaction, that was reflected in the decreased respiratory score (Table 3).

Rats were sensitized with C48/80 (1 mg/kg s.c) by non-immunological method. The treatment of sensitized rats with EEST 100 mg/kg and 200 mg/kg B/W or ketotifen (1 mg/kg B/W) showed a significant protection against mast cell degranulation due to the challenge of C48/80. The protective effect of EEST on degranulation of mast cells was dose dependent. Ketotifen fumarate (1 mg/kg, p.o) treatment elicited slightly a better protection than EEST (200 mg/kg) against C48/80 induced mast cell degranulation (Table 3).

Treatment with EEST (100 and 200 mg/kg B/W) and dexamethasone (0.5 mg/kg B/W) daily for 7 days inhibited induction of DTH reaction (paw edema formation) significantly ($p < 0.001$) in mice (Table 3). The treatment with EEST (200 mg/kg) elicited greater inhibition of DTH reaction as compared to dexamethasone.

Effect of EEST on histamine induced pedal edema in rats and Shultz–Dale inhibition in Guinea pig ileum (ex vivo)

The injection of 0.1 ml histamine into the sub plantar region of hind paw of rats induced a significant paw edema formation. The maximum edema formation was seen at 1 h after histamine

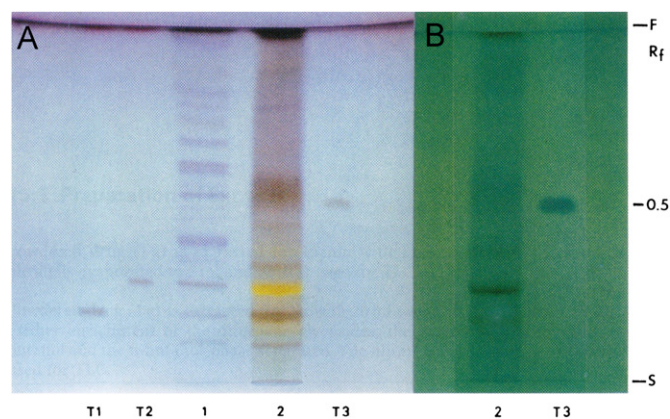


Fig. 1. HPTLC chromatogram of EEST samples (A) exhibited ten to thirteen grey-bluish (visible) bands in the R_f range 0.1–0.9. According to the literature steroidal saponins are found in the R_f range of 0.4–0.6. Steroidal saponin and flavonoids show a strong quenching at 254 nm (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Preliminary physicochemical evaluation of EEST.

Test	Observations
Nature and appearance	Semisolid, dark black brown with characteristic odour
Solubility	Soluble (water, ethanol, methanol), insoluble (toluene, acetone, benzene).
R_f value	0.75, mobile phase—chloroform: methanol:water [7:4:1(v/v)]
UV MeOH (λ_{max})	280, 360
IR spectra V_{max} at KBr	Steroidal saponin: IR cm $\sim$: 3450 (–OH stretch), 2920 (–CH stretch), 1740 (–C=O stretch), 1445, 1375, 1260, 1140, 1045, 980 (19) EEST IR values: IR cm $\sim$: 3366 (–OH Stretch), 2925 (–CH stretch), 1733 (–C=O stretch), 1053.
Phytochemicals present	Saponins (+++), steroids (+), terpenoids (+) and flavonoids (++). (+++) – high, (++) – moderate, (+) – minimum.

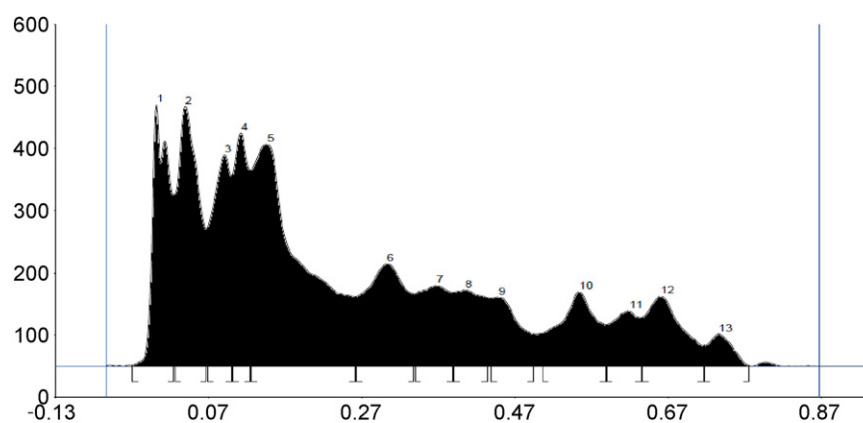


Fig. 2. Standardized HPTLC chromatogram of EEST at 254 nm showed the presence of 13 phytoconstituents including steroidal saponin and flavonoid.

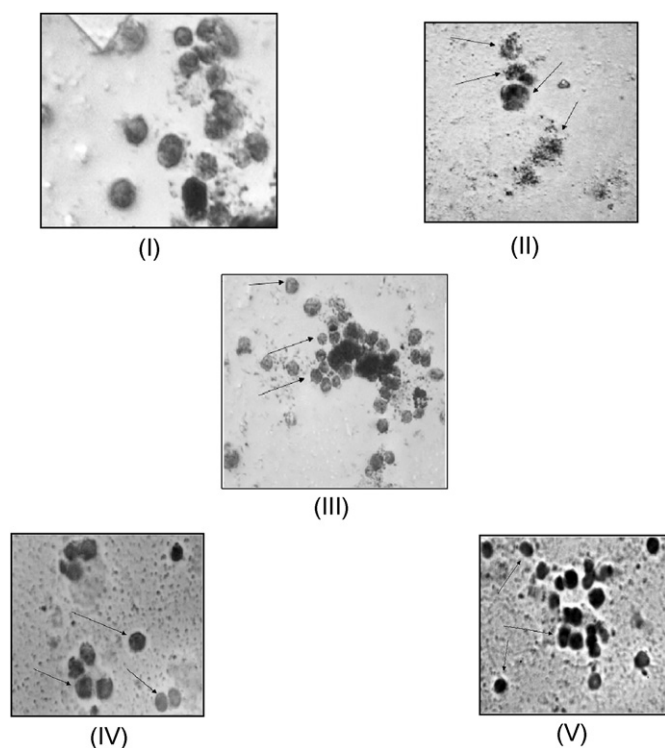


Fig. 3. Effect of EEST on mast cell in phosphate buffer (I) normal control, (II) induced control, (III) EEST 100 mg/kg B/W treated, (IV) EEST 200 mg/kg B/W treated and (V) ketotifen fumerate (1 mg/kg B/W) treated (Toluidine Blue, 400 X).

injection. The groups pretreated with EEST (100 mg/kg B/W and 200 mg/kg B/W, p.o), indomethacin (10 mg/kg B/W, p.o), showed a significant ($p < 0.05$, $p < 0.001$) inhibition in paw edema formation at different time intervals (Table 4).

EEST (10–30 $\mu\text{g/ml}$) antagonized (blocked) egg albumin (EA) induced contraction (10 $\mu\text{g/ml}$) in sensitized guinea pig ileum (*ex vivo*) dose dependently. The standard drug, disodium chromoglycate (5–20 $\mu\text{g/ml}$) also antagonized EA induced contraction dose dependently (Table 4).

Effect of EEST on DPPH free radicals scavenging activity

The DPPH free radical scavenging effects of the EEST is expressed in terms of 50% inhibition concentration (IC_{50}) values. The free radicals scavenging activity of EEST was directly proportional to its concentration. The IC_{50} value of EEST and ascorbic acid were found to be 93.15 and 50.84 $\mu\text{g/ml}$, respectively.

Discussion

Human eosinophils are distinguished phenotypically by their bilobed nuclei and large acidophilic cytoplasmic granules (Yang et al., 2008). It has been reported that IL-5 plays an important role in eosinophils differentiation, proliferation and maturation in the bone marrow. Moreover, the chemokines released by the epithelium are also responsible to eosinophil accumulation and survival (Rosenberg et al., 2007). Keeping in mind involvement of such multicellular events in immune reactions, we thought milk induced eosinophilia and leukocytosis is an ideal model to evaluate antiallergic activity of EEST, because occurrence of eosinophilia and leukocytosis is due to the participation of lymphocytes derived from bone marrow and/or thymus derived T cells (George et al., 2009).

The PCA and polybasic compound 48/80 induced allergic reactions are largely mediated through IgE effects on vascular permeability as well as histamine release by degranulation of mast cells. In local allergic skin reaction (PCA) and compound 48/80 induced systemic allergic reactions were significantly inhibited by EEST. It is well documented that during cutaneous reactions (immediate and delayed) both the inflammatory and immunological responses are induced (Pandit et al., 2008). In the present experiment EEST treatment significantly protected from IgE-mediated, local allergic skin reaction (Pepy, 1973). Hence it appears EEST has potential to regulate antiallergic activity or events associated with type I IgE-mediated anaphylactic reactions.

The protection of mast cells from degranulation in sensitized rats by EEST may presumably be due to inhibition of synthesis of reaginic antibodies (IgE) through mast cell membrane receptor interactions causing blockade of phenomenon of sensitization. Hence, EEST exerts its effect on mast cells where the decisive effects of allergen antibody reactions are determined. Alternatively, EEST may inhibit 48/80 induced degranulation of mast cells by altering the calcium fluxes into the membrane of extracellular fluid (Pepy, 1973). Furthermore, the extract mixture of *Adhatoda vasica*, *Glycyrrhiza glabra*, *Solanum xanthocarpum* bark of *Albizia lebbek* and roots of *Picrorhiza kurroa*, protects asthma patients by their mast cell stabilizing property (Mimaki et al., 1996; Iyengar et al., 1994). Considering occurrence of complex cellular events both in PCA, and compound 48/80 induced allergic reactions, the inhibitory effect of EEST on such events moot the possibility of stabilization of lipid membrane as well as G-protein activation, through intracellular Ca^{2+} release process (Han et al., 2007). The induction of active anaphylactic reaction using horse serum manifests in severe respiratory symptoms such as dyspnea, cyanosis and mortality (Mitra et al., 1999). A significant

Table 2

Effect of EEST on milk induced eosinophilia, leukocytosis, neutrophils and monocytes in mice.

Treatment (mg/kg, p.o.) n=6	Eosinophils per cu. mm ($\times 10^6/\mu\text{l}$) ^a	Differential leukocytes ($\times 10^6/\mu\text{l}$) ^b		Total leukocytes per cu. mm ^c
		Neutrophils (%)	Monocytes (%)	
Normal control	2.40 $\pm$ 0.40	3.40 $\pm$ 0.50	32.00 $\pm$ 1.14	6120 $\pm$ 280
Induced control	10.80 $\pm$ 0.58 [@]	10.40 $\pm$ 0.92 [@]	49.40 $\pm$ 1.07 [@]	9810 $\pm$ 50.96 [@]
EEST (1 0 0)	8.60 $\pm$ 0.50*	6.60 $\pm$ 0.81*	42.40 $\pm$ 1.28*	8824 $\pm$ 56.63 [#]
EEST (2 0 0)	7.80 $\pm$ 0.583 [#]	7.00 $\pm$ 0.83*	39.80 $\pm$ 1.31 [#]	8512 $\pm$ 88 [#]
Dexamethasone (0.27)	6.80 $\pm$ 0.58 [#]	6.00 $\pm$ 1.09*	33.20 $\pm$ 1.06 [#]	7900 $\pm$ 20 [#]

^a Values are expressed as mean $\pm$ SEM, n=number of mice per group, [@]p < 0.001 vs. normal control; *p < 0.05, [#]p < 0.005 vs. induced control group.^b Values are mean $\pm$ SEM, [@]p < 0.001 vs normal control; *p < 0.05, [#]p < 0.05 vs. induced control.^c Values are mean $\pm$ SEM, [@]p < 0.001 vs. normal control; [#]p < 0.05 vs. induced control.**Table 3**

Effect of EEST on anaphylaxis (passive cutaneous and active), DTH and C 48/80 induced mast cell degranulation.

Passive cutaneous anaphylaxis ^a		Active anaphylaxis ^b	DTH paw edema volume (ml) ^c		Effect on mast cell ^d	
Treatment (mg/kg, orally.) n=6	mm ^b of bluing reaction	Symptoms, respiratory score (% mortality)	0 h	48 h	Intact	Disrupted and (% protection)
Normal control	–	Normal	–	–	100	–
Induced control	74.40 $\pm$ 3.2 [@]	Respiratory collapse 7.20 $\pm$ 1.5 [@] , (80%)	0.63 $\pm$ 0.03	0.46 $\pm$ 0.06	21.20 $\pm$ 2.67	78.80 $\pm$ 2.67 [@]
EEST (1 0 0)	61.80 $\pm$ 1.4*	Dyspnea 2.80 $\pm$ 0.5*, (40%)	0.72 $\pm$ 0.05	0.44 $\pm$ 0.03 (p < 0.01)	37.20 $\pm$ 2.26	64.80 $\pm$ 1.82* (18.0%)
EEST (2 0 0)	58.80 $\pm$ 1.0*	Normal 2.40 $\pm$ 0.40*, (0%)	0.68 $\pm$ 0.06	0.30 $\pm$ 0.06 (p < 0.001)	40.00 $\pm$ 1.81	60.00 $\pm$ 1.81 [#] (24.0%)
Dexamethasone (0.5)	49.60 $\pm$ 2.8 [#]	Normal 2.00 $\pm$ 0.05 [#] , (0%)	0.65 $\pm$ 0.04	0.32 $\pm$ 0.02 (p < 0.001)	–	–
Ketotifen fumarate	–	–	–	–	46.00 $\pm$ 2.00	54.00 $\pm$ 2.00 [#] (31.5%)

^a Values are expressed as mean $\pm$ SEM, n=number of rats per group, [@]p < 0.001 vs. normal control; p < 0.05, [#]p < 0.001 vs. induce control.^b Values are expressed as mean $\pm$ SEM, [@]p < 0.001 vs. normal control; *p < 0.05, [#]p < 0 vs. induce control.^c Values are expressed as mean $\pm$ SEM, (p < 0.001) vs. vehicle treated mice.^d Values are expressed as mean $\pm$ SEM, [@]p < 0.001 vs. normal control; *p < 0.05, [#]p < 0.05 vs. induce control.**Table 4**

Effect of EEST on histamine induced pedal edema in rats and Schultz–Dale inhibition in guinea pig ileum (ex vivo).

Treatment (mg/kg, p.o.) n=6	Paw volume (ml) at different time interval			Schultz–Dale reaction inhibition (%), guinea pig ileum		
	Initial	1 h	3 h			
Normal control	0.86 $\pm$ 0.06	0.86 $\pm$ 0.06	0.86 $\pm$ 0.06	–	–	–
Induced control	0.89 $\pm$ 0.05	1.65 $\pm$ 0.038 [@]	1.62 $\pm$ 0.04 [@]	–	–	–
EEST (1 0 0)	0.85 $\pm$ 0.05	1.50 $\pm$ 0.011*	1.46 $\pm$ 0.016*	–	–	–
EEST (2 0 0)	0.87 $\pm$ 0.07	1.46 $\pm$ 0.02*	1.44 $\pm$ 0.028*	–	–	–
Indomethacin (10)	0.87 $\pm$ 0.05	1.31 $\pm$ 0.01 [#]	1.29 $\pm$ 0.08*	–	–	–
EEST (10/20/30 $\mu\text{g}/\text{ml}$)	–	–	–	55.0 $\pm$ 6.5*	62.0 $\pm$ 6.0*	70.0 $\pm$ 5.6*
Disodium chromoglycate (5/10/20 $\mu\text{g}/\text{ml}$)	–	–	–	53.0 $\pm$ 6.80*	62.0 $\pm$ 5.6*	78.0 $\pm$ 5.4*

Values are expressed as mean $\pm$ SEM, @ p < 0.001 vs. normal control; *p < 0.05, [#]p < 0.05 vs. induced control, EA 10 $\mu\text{g}/\text{ml}$ contraction was taken as 100% and compared with drug treated group.

amelioration of active anaphylactic reactions as well as protection from mortality by EEST treatment confirms its anti-allergic activity, which may be mediated through its effect on H₁ receptors (Pandit et al., 2008). Histamine is liberated in access amount during the early phase of allergic reactions, leads to plasma leakage and edema formation by binding to specific histaminergic receptors (H₁ to H₄). The inhibition of histamine-induced paw edema by EEST further establishes its antihistaminic activity (Pepy, 1973; Mimaki et al., 1996).

The anti-anaphylactic activity of EEST was further demonstrated in antigen-induced contraction of sensitized guinea pig ileum in ex vivo studies. It is presumed that inhibition of antigen-induced contraction by EEST on the sensitized guinea pig ileum might be result of Gq and Ca²⁺ mobilization (Gupta et al., 2006). These effects can also be attributed to the stabilization of the mast cell membrane of the intestine, leading to prevention of the

release of mediators of smooth muscle contraction including the inhibition of sensitization process (Blois, 1958). However, this needs further confirmation.

DTH is a part of the process of graft rejection, tumor immunity and immunity related many intracellular infectious microorganisms, especially those causing chronic diseases like tuberculosis. Furthermore, DTH requires the specific recognition of a given antigen for activation of lymphocytes, which subsequently proliferate and releases cytokines. Such events in turn increase vascular permeability, vasodilatation, macrophage accumulation, further augments increased phagocytosis and elevation of lytic enzymes for more effective repair process (Di et al., 1971). Hence, potentiation of DTH response suggest the stimulatory effect of EEST on lymphocytes and other participating immune cells essential for repair process, and also augment the cell mediated immunity (Lagrange and Mackaness, 1975).

The free radical scavenging activity of EEST was found to be concentration dependent thereby indicating its potent antioxidant property. Free radicals have been implicated in the etiology of large number of diseases and also suggested antioxidants as immunomodulators (Aher et al., 2011). Furthermore, alterations in intracellular reactive oxygen species production modulate both IL-4 gene expression and mast cell function (Wu et al., 2000). The major phytoconstituents of EEST found to be steroidal saponins, triterpenoids, flavonoids which are known potent antioxidants. Hence it is likely these phytoconstituents either alone or together might be responsible for eliciting anti-allergic activity observed in our experiments. However, it is necessary to isolate active phytoconstituents of EEST and also evaluate anti-allergic activity of constituents either alone or together with standard clinically proved anti-allergic drugs. This might be helpful to use EEST preparation or its active constituents to be used as an adjuvant along with clinically effective antiallergic drug to prove its protective role and/or potentiating effect of EEST in allergic disorders.

Conclusion

Our experimental findings provide adequate scientific basis for traditional use of *S. trifasciata* in the treatment of allergy related diseases. EEST induce potent anti-allergic activity by inhibition of chemical mediators released from mast cells through different mechanisms. EEST can be developed as an anti-allergic drug by proper standardization of its phytoconstituents and evaluation of its effects on human subjects to establish its clinical efficacy as well as an effective adjuvant in allergy-asthmatic treatment.

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