INHIBITION OF IMMEDIATE ALLERGIC REACTION BY SITOPALADI CHURNA: AN EXPERIMENTAL STUDY

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Summary

Immediate allergic reactions are potentially life-threatening immunological reactions that result from the sudden release of mast-cell-derived and basophile-derived mediators into the circulatory system. Intensive research during the last several decades has highlighted the role of lymphocytes, immunoglobulin, mast cells and several autocoids in the etiopathogenesis of allergic conditions. The available treatment option for upper and lower respiratory tract allergic diseases have major limitations owing to low efficacy, associated adverse events, and compliance issues. Ayurveda, an Indian Indigenous system of medicine, has described several drugs for the treatment of bronchial asthma and allergic disorders. One of the most commonly prescribed formulations in Ayurvedic medicine is Sitopaladi Churna in treatment of cough and various allergic conditions like bronchial asthma, chronic bronchitis. This investigation aimed at characterizing the therapeutic potential of Sitopaladi Churna in some aspects of allergy and related respiratory afflictions by studying the stabilizing effects of Sitopaladi Churna on mast cell degranulation induced by Compound 48/80 in rat as well potential of Sitopaladi Churna in controlling the milk- induced leukocytosis in rat at varying doses. Pre-treatment with Sitopaladi Churna (SC) at a dose of 36 mg, 180 mg, 360 mg /0.5 ml p.o each/ 200 g of rat produced significant ($p<0.05$) mast cell protection as 73.90%, 76.09% and 78.05% respectively. While in leukocytosis study, the group which have received average and high dose of (SC) 180, 360 mg/0.5 ml /200 g of rat shows significant ($p< 0.05$) decrease in leukocytosis as compared to control group. The effect observed with the therapeutic dose of 36 mg / 0.5 ml, p.o was not of statistical significance. These findings are clearly indicative of role of Sitopaladi Churna in immediate type of allergy as potent inhibitor of mast cell degranulation and ability to control the leukocytosis.

Key Words: Allergy, Leukocytosis, Mast cell, Sitopaladi Churna.

Introduction

Allergic reactions result from systemic release of mediators from mast cells and basophils. Again, allergic reactions are chemically and clinically indistinguishable from anaphylactic reactions except that they are not IgE mediated. Anaphylaxis occurs in an individual after re-exposure to an antigen to which that person has produced a specific IgE antibody. The antigen to which one produces an IgE antibody response that leads to an allergic reaction is called an allergen. The IgE antibodies produced may recognize various epitopes of the allergen. These IgE antibodies then bind to the high-affinity IgE receptor (FcεRI) on the surface of mast cells and basophils. Upon re-exposure to the sensitized allergen, the allergen may cross-link the mast cell or basophil surface-bound allergen-specific IgE resulting in cellular degranulation as well as de novo synthesis of mediators. Ayurveda, an Indian system of medicine, has described several drugs from indigenous plant sources for use in the treatment of bronchial asthma and allergic disorders. In Ayurved, churna was traditionally use for different therapeutic purposes. Churna/ choornam are a fine powder of a drug or drugs (1).

The term Churna may be applied to the powder prepared by a single drugs or a combination of more drugs. One of the formulations is Sitopaladi Churna which is very commonly prescribed Ayurvedic medicine in cough. According to Ashtang Hriday it consists of a mixture of powder of Sitopala (sugar), Vamasarocana (*Bambusa arundinaceae* Retz.), fruits of Pippali (*Piper longum* Linn.), Seeds of Ela (*Amomum subulatum* Roxb.) and Tvak (bark of *Cinnamomum Zeylanicum* Blume.)(2).It is recipe of traditional Ayurvedic Pharmacopoeia well known and effective in relieving coughs associated with respiratory disorder. As extract of Sitopaladi churna exhibited good anti-inflammatory effects in rats (3). In histamine induced contraction on isolated goat tracheal chain preparation, Sitopaladi churna significantly antagonize the effect of histamine-induced contraction on isolated goat tracheal chain (4). Sitopaladi churna is one of the most useful preparation in the diseases like bronchial asthma, chronic bronchitis, burning sensation of the body, hands and feet; pleurisy, distaste, anorexia and numbness of tongue (5). As expectorant useful in bronchitis, cough, tuberculosis and complaints; also of value as laxative and for relieving excessive thirst and burning sensation of extremities (6). The antibacterial potential of Sitopaladi churna and some Indian herbal preparations where investigated by preparing solvent extract using disc diffusion method against some bacterial pathogens (7). Thus validating the safety and efficacy and thus co-relating the possible mode of action of drug as a potential antiallergic herbal formulation by use of appropriate animal models.

Methods

Collection of ingredients

Raw drugs required for the preparation of Sitopaladi Churna were purchased from the reputed Ayurvedic drug store at Pune (Maharashtra), India and further subjected for the series of Quality control parameters for individual raw material to confirm its identity and purity standards as prescribed and well reported in literature. Sitopaladi Churna was prepared in laboratory according to the formula given in 'Astanga Hridaya'. The formula mentioned in 'Astanga Hridaya' was found modified in other texts.

Method of preparation

The churna was prepared in the laboratory as per the general procedure given in The Ayurvedic Pharmacopoeia of India. All the ingredient viz. *Piper longum* Linn, *Amomum subulatum* Roxb, *Cinnamomum zeylanicum* Blume, *Bambusa arundinaceae* Retz and *Saccharum officinarum* Linn were cleaned, dried, powdered individually in a pulverizer and passed through sieve number 120. Then each powdered ingredient weighed separately and mixed together in specified ratio and passed through sieve number 60 to obtain homogeneous blend. Churna thus prepared was packed in a tightly closed container to protect form light and moisture (8).

Animals

Male and female albino rats weighing150-200 gm were housed under standard laboratory light/dark cycle. Animals had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethical committee (IAEC) has approved the protocol of the study vid no. NIB/ IAEC/ 09-10/ 79 dated 15-1-2010.

Standard drug
Kitotifen fumerate

Vehicle control
1% CMC solution and 0.5 ml dose administered using intra gastric cathertization through mouth followed by 1 ml rinsing of de-ionized water.

Dose Schedule
The required dose for rat was calculated by using the standard dose calculation procedure (9) from recommended clinical dose. As human dose of Sitopaladi Churna recommended in specified ailments is 1-2 g daily in divided doses.

Chemicals and reagents
Compound 48/80 Sigma, USA; RPMI Buffer medium 1640 of Hi Media has been purchased. Toluidine blue, eosin solution was purchased from Research Lab. India. WBC diluting fluid purchased from Qualigens, India, Kitotifen fumerate purchased from Cipla, India.

***In vivo* assessment of antiallergic activity using milk-induced leukocytosis in rat** (10, 11)**:**
Rats were divided into five groups, five animals in each group. Animals belonging to Group **I** served as control and treated with vehicle 1 % CMC solution 2 ml/kg, p.o. and boiled and cooled milk in a dose of 4 ml /kg, s.c. Animals belonging to Group **II** received only vehicle (1 % CMC solution, 2 ml/kg, p.o). Animals belonging to Group **III, IV and V** received Sitopaladi churna in a dose of (36mg/0.5 ml, 180mg/0.5 ml, 360mg/0.5 ml p.o) respectively, 1 h before milk injection. Blood samples were collected from each rat from retro-orbital plexus under light ether anaesthesia. Total leukocyte count was determined in each group before drug administration and 24 h after milk administration. Blood was sucked in WBC pipette to mark 1, followed by the eosin solution to mark 11. Mixed the contents of the bulb thoroughly for 30-40 seconds and put it aside for 15-20 min for lysis and staining. The contents of the pipette were again mixed for 30 seconds. Neubauer's chamber was charged with above fluid and leukocyte count was done.

***In vivo* assessment of mast cell stabilization using Compound 48/80 induced mast cell degranulation in rat** (12).
Rats were divided into five groups of five animals in each group. Thirteen day drug treatment schedule was followed (n=5). Group **I** served as a control and received 1% CMC solution (2 ml/kg p.o) (vehicle). Animals belonging to group **II**, **III** and **IV** were administered Sitopaladi churna 36mg/0.5ml,p.o, 180mg/0.5ml,p.o, 360mg/0.5ml,p.o, respectively whereas animals belonging to group **V** received kitotifen fumerate (1 mg/kg, p.o) respectively, once a day up to 14 days. On the 14^{th} day, 2h after the assigned treatment, 10 ml of normal saline was injected into the peritoneal cavity of rats, after a gentle massage, the fluid was collected and transferred into siliconised test tube containing 7-10 ml of phosphate buffer solution (pH 7.2-7.4). Mast cells were washed thrice by centrifugation at low speed (400-500 rpm) followed by discarding the supernatant and taking the pellet of mast cells into the solution.These cells were purified and incubated with compound 48/80 (5 µg/ml) at 37^0 C for 10 min.

After incubation these cells were spun and stained with 0.1% toluidine blue and observed under microscope to count the disrupted and intact mast cells. The difference in the disrupted mast cells count of control and treated groups related to mast cells stability study and percent protection was calculated.

Statistical analysis

The data were presented as mean±SEM. The statistical significance between the groups has been tested by ANOVA followed by Dunnett's multiple test. A probability value of less than 0.05 was considered as significant.

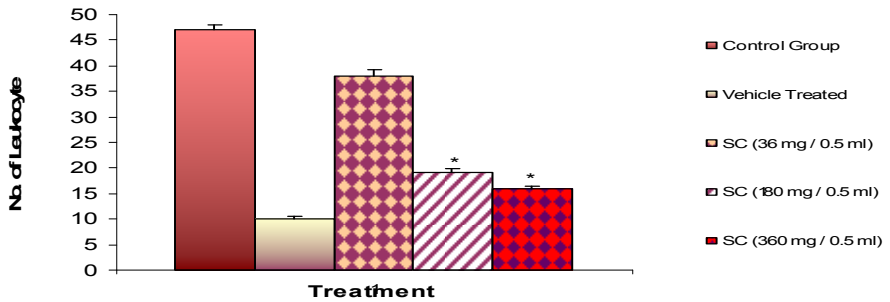
Results

Effect of Sitopaladi churna (SC) on milk-induced leukocytosis in rat

The data obtained during the study show that group II which received only vehicle 1% CMC, 2 ml/kg, p.o did not exhibit leukocytosis (10.0±0.568) while parentral administration of milk induced significant increase in total leukocyte count in milk injected control group I (47.0±0.984), while treatment group IV, V with Sitopaladi Churna (SC) at a dose of 180 mg, 360 mg /0.5 ml p.o each/ 200 g of rat in group shown significant ($p< 0.05$) decreased this milk induced leukocytosis (27.0±0.874, 16.0±0.458 respectively) when compared with control group. In the treatment group III which was given test formulation at a dose of 36 mg/0.5 ml, p.o reduced leukocytosis but not statistically significant. Results thus obtained are suggestive of relevance of Sitopaladi churna in antagonizing the milk induced blood leukocytosis by 59.57% & 65.95% respectively at a dose of 180 mg, 360 mg /0.5 ml p.o each/ 200 g of rat.

Effect of Sitopaladi Churna (SC) on Mast cells degranulation in rat

Challenge with Compound 48/80 induces mast cell degranulation in rat and Compound 48/80- induced mast cell degranulation was inhibited by standard mast cell stabilizer Kitotifen fumerate at a dose of 1mg/ kg, i.p. by (16.00±2.624) offering significant ($p<0.05$) 80.70 % protection when compared with control group. While treatment with Sitopaladi Churna (SC) as well offered significant ($p<0.05$) mast cell stabilization at a dose of 36 mg, 180 mg, 360 mg /0.5 ml p.o each/ 200 g of rat in group II, III, IV by 73.90%, 76.09% and 78.05% respectively as shown in fig.2.

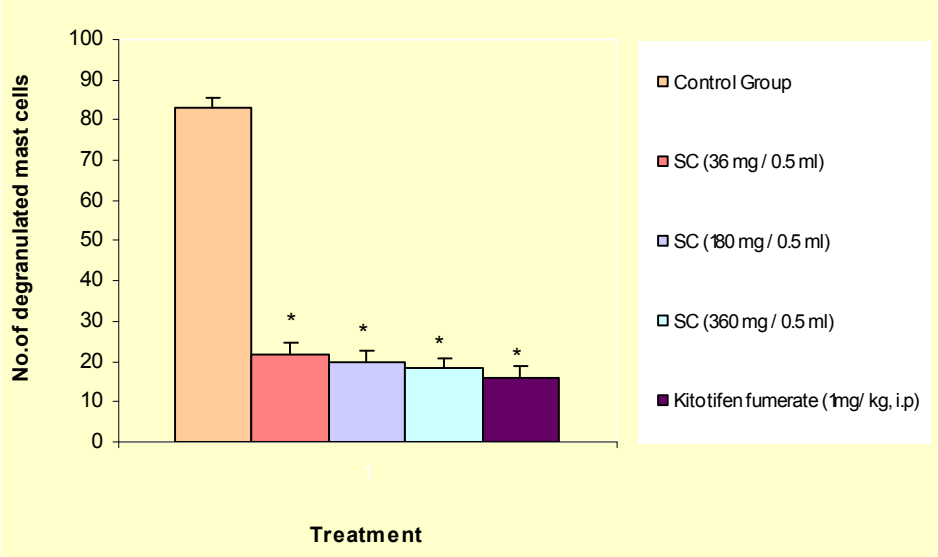


n=5, values are expressed in mean±SEM

***p< 0.05 compared with control group (ANOVA followed by Dunnett's test)**

SC- Sitopaladi churna

Figure 1: Effect of SC on Milk induced leukocytosis in rat



n=5, values are expressed in mean±SEM
Control = Vehicle (1% CMC solution, 2 ml/kg p.o)
***p< 0.05 compared with control group (ANOVA followed by Dunnett's test)**
SC– Sitopaladi churna

Figure 2: Effect of SC on Mast cells degranulation in rat

Discussion

Sitopaladi Churna has dose dependently produced significant control of milk induced leukocytosis besides exhibiting significant mast cell stabilization against substance 40/80 induced degranulation. These are some of the aspects involved in the pathological mechanism of Type-I allergic conditions which have been explained as degranulation of mast cells. Degranulation of mast cells occurs in response to immunological stimuli in which the antigen-antibody reaction predominates on the surface of mast cell resulting in release of plethora of chemical mediators and this is followed by the development of bronchospasm, together with insidious changes in the mucosal lining of the bronchi and in the mucous secretions. Release of histamine increases the permeability of the surface epithelium and allows antigen to penetrate to aggravate the reactions further. Most of the literatures do not include a diagnostic evaluation and precise practical clinical approach to leukocytosis/eosinophilia. Karnick (13) performed clinical studies and assessed the role of eosinophil in asthmatic response. It was also demonstrated that parental administration of milk produces a marked and significant increase in the leukocytes/eosinophils count after 24hr of its administration[7].The total leukocyte count reflects allergic activity and useful as a bio-marker in the management of bronchial asthma and allergic manifestations. On activation, they release inflammatory mediators such as leukotrienes and granular proteins to injure airway tissue (14).

Thus, leukocyte/eosinophils become important inflammatory cells as well as marker for allergic disorder. Bhargava (10) described that parentral administration of milk produced a marked and significant increase in the leukocytes/eosinophils count after 24 h of its administration and thus the pronounced inhibitory activity of Sitopaladi Churna in control of milk induced blood leukocytosis in rat indicates mediator's antagonist effect. Compound 48/80 was used which acts in a manner similar to immunological stimuli shows exocytosis of mast cell due to phosphorylation of cellular protein and thus further release of mediators. Dose dependent significant mast cell protection offered by Sitopaladi Churna might be due to regulation of protein phophorylation and stabilization of mast cell membrane. The results of the present investigation suggests that Sitopaladi Churna could be effective in the treatment of immediate allergic conditions because of its ability to stabilize mast cell their by leading to decrease in the mediators release and inflammation mediated by them and thus influence the course of disease. The observed effects compare well in comparison to the activity observed with standard mast cell stabilizer which was 80.70%. The bioactive component(s) responsible for the observed activities are not precisely known but it may be one or more of the phytochemical constituents established to be present in Ayurvedic mixture of powder of Sitopala (sugar), Vamasarocana (*Bambusa arundinaceae* Retz.), fruits of Pippali (*Piper longum* Linn.), Seeds of Ela (*Amomum subulatum* Roxb.) and Tvak (bark of *Cinnamomum Zeylanicum* Blume.)(15, 16). Thus the ability of Sitopaladi Churna and possible role in the treatment of immediate allergic conditions in rats needs further scientific investigation to correlate and validate its clinical utility with reliably reproducible results in humans.

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