

## Evaluation of Mast Cell Stabilizing and Anti-Anaphylactic Activity of Polyherbal Formulation

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**Abstract:** Novel polyherbal formulation (PHE) containing mainly the ethanolic extract of *Adhatoda vasica* Nees. (leaf), *Clerodendrum serratum* Linn. (root), *Curcuma longa* Linn. (rhizome), *Solanum xanthocarpum* Schrad & Wendl. (fruit) and *Piper longum* Linn. (fruit). HPTLC analysis of the herbal extracts of PHE revealed presence of 0.25%w/w vasicine, 3.4%w/w piperine, 1.56%w/w curcumin and 0.04%w/w solasodine. The mast cell stabilizing and anti-anaphylactic property of PHE was investigated against compound 48/80-induced mast cell degranulation as well as triple antigen-induced anaphylaxis in rats. PHE produced significant reduction in the mortality of rats subjected to triple antigen-induced anaphylactical shock. PHE also depicted marked protection of rat mesenteric mast cells from disruption by compound 48/80 in dose dependant manner. Our data suggest anti-anaphylactic and mast cell stabilizing property of PHE.

**Key words:** Mast cells • Anaphylaxis • Immunoglobulin E • Triple antigen • Compound 48/80

### INTRODUCTION

Allergy is one of the common diseases that affect mankind. The prevalence of allergy and asthma has risen in recent years despite the general health improvement in the population [1]. Intensive research during last several decades has highlighted the role of lymphocytes, immunoglobulin, mast cells and various autacoids in the pathogenesis of asthma and other allergic conditions but the fundamental immunologic components of immediate hypersensitivity are the mast cells and immunoglobulin E (IgE). Mast cells, the constituents of virtually all organs and tissue are important mediators of inflammatory responses, such as allergy and anaphylaxis. Anaphylaxis is mediated by histamine released in response to cross-linking of IgE bound to FCεRI on mast cells. Mast cell activation causes process of degranulation that result in releasing of mediators, such as histamine and an array of inflammatory cytokines [2, 3]. The available treatment options for upper and lower respiratory tract allergic diseases have major limitations due to low efficacy, associated adverse events and compliance issues [4]. Ayurveda, an Indian system of medicine, has described several drugs from indigenous plant sources in the treatment of bronchial asthma and

allergic disorders. PHE is one such polyherbal formulation containing mainly the extract of *Adhatoda vasica* Nees. (leaf), *Clerodendrum serratum* Linn. (root), *Curcuma longa* Linn. (rhizome), *Solanum xanthocarpum* Schrad & Wendl. (fruit) and *Piper longum* Linn. (fruit). *Adhatoda vasica* Nees. is documented for its potent anti-inflammatory [5], antioxidant [6], anti-allergic, antitussive [7], anti-asthmatic [8], bronchodilatory and smooth muscle relaxant activity [9]. *Clerodendrum serratum* Linn. traditionally used for the ailments such as asthma, bronchitis, body ache, rheumatism, inflammation, ulcers, wounds, fever, cholera, dropsy, tuberculosis, ophthalmia etc. [10]. *Solanum xanthocarpum* Schrad & Wendl. is considered to be an antispasmodic, diaphoretic, sedative, anodyne, antidotal, antiseptic, antitussive, carminative, expectorant, febrifuge, pectoral, preventative (cold) and tonic [11]. *Curcuma longa* Linn. has been used for the treatment of various diseases and disorders particularly for urticaria, skin allergy, viral hepatitis, inflammatory conditions of joints, sore throat and wounds [12]. *Piper longum* Linn. is useful in asthma, tumors, spleen disorders, inflammations, piles, tuberculosis etc. [13, 14]. Moreover, PHE is reported to have anti-inflammatory and bronchodilatory activity [15].

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In the present study, the effect of PHE, a polyherbal formulation was studied against Compound 48/80-induced mast cell degranulation as well as triple antigen-induced anaphylaxis in rats.

## MATERIAL AND METHODS

**Drugs and Chemicals:** Horse serum and triple antigen were procured from Animal Disease Investigation Office, Mehsana, India and GlaxoSmithKline, Brentford, UK, respectively. Compound 48/80 was procured from Sigma, St. Louis, MO, USA and toluidine blue from SD Fine Chemicals, Mumbai, India. Tween-80, acetone and xylene obtained from Burgoyne Burbidges & Co. Mumbai, India. Kitotifen fumarate was procured from Torrent Research Centre, Ahmedabad, India.

**Test Material:** The plant materials (leaves of *Adhatoda vasica* Nees. roots of *Clerodendrum serratum* Linn. rhizomes of *Curcuma longa* Linn. fruits of *Solanum xanthocarpum* Schrad & Wendl. and fruits of *Piper longum* Linn.) were obtained from *Government Ayurvedic Udhyan*, Gandhinagar, Gujarat, India. They were identified and authenticated by the Department of Pharmacognosy, K.B. Institute of Pharmaceutical and Education Research, Gandhinagar, India. Voucher specimens (PH/508/002, PH/508/003, PH/508/004, PH/508/005 and PH/508/006) were deposited at the Department of Pharmacognosy, K.B. Institute of Pharmaceutical and Education Research, Gandhinagar, India. The individual plants were evaluated with regard to their standard specifications according to the 'Ayurvedic Pharmacopoeia of India'. Ethanolic extracts were prepared by extracting each plant with ethanol using Soxhlet extractor and each extract was standardized to 0.25%w/w vasicine, 3.4%w/w piperine, 1.56%w/w curcumin and 0.04%w/w solasodine by HPTLC method. PHE was prepared using ethanolic extract of *Adhatoda vasica* Nees. (leaves), *Clerodendrum serratum* Linn. (roots), *Curcuma longa* Linn. (rhizomes), *Solanum xanthocarpum* Schrad & Wendl. (fruits) and *Piper longum* Linn. (fruits) in proportion of 40%, 30%, 10%, 10% and 10%, respectively [15].

**Animals:** *Wistar albino* rats (Zydus Cadila Limited, Ahmedabad, India) of either sex were selected for the study. All animals were housed at ambient temperature (22±1°C), relative humidity (55±5%) and 12h/12h light dark cycle. Animals had free access to standard pellet diet and water given *ad libitum*. The protocol of the experiment was approved by the Institutional (K.B. Institute of Pharmaceutical Education and Research)

Animal Ethical Committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

### Triple Antigen-Induced Anaphylaxis in Rats [16]:

Twelve *Wistar albino* rats of either sex weighing between 175-200 g were used for the study. All the rats were sensitized by single subcutaneous injection of 0.5 ml horse serum along with 0.5 ml of triple antigen containing 20 million *Bordetella pertusis* organisms. After sensitization, the rats were divided into two groups of 6 animals in each. Rats of group-I received vehicle and served as control. Rats of group-II received PHE (200 mg/kg, per oral route) once a day for 10 days. On day 10, two hrs after treatment, the rats were challenged with intravenous injection of 0.25 ml horse serum. The rats were observed for: onset of symptoms such as dyspnoea and cyanosis; duration of persistence of symptoms (min.); death.

The severity score with respect to symptoms were recorded using the method of Gupta *et al.* [12]: increased respiratory rate - 2, dyspnoea for 10 min. - 4, dyspnoea and cyanosis for 10 min. - 8 and collapse.

**Studies on Compound 48/80 Induced Rat Mesentric Mast Cell Degranulation [17]:** The animals were sacrificed and the pieces of mesentry were collected in petri dish containing Ringer Locke solution and then subjected to the following treatment schedules.

- Petri dish no. 1 - Ringer Locke solution (Positive control)
- Petri dish no. 2 - 0.1ml of Ketotifen fumarate (10 µg/ml)
- Petri dish no. 3 - 0.1ml of test agent in Tween-80 (PHE, 500 µg/ml)
- Petri dish no. 4 - 0.1ml of test agent in Tween-80 (PHE, 750 µg/ml)
- Petri dish no. 5 - 0.1ml of test agent in Tween-80 (PHE, 1000 µg/ml)

Each petri dish was incubated for 15 min at 37°C. Later Compound 48/80 (0.1 ml, 10 µg/ml) was added to each petri dish and again incubated for 10 min. at 37°C.

After that, all pieces were transferred to 4% formaldehyde solution containing 0.1% toluidine blue and kept a side for 20 to 25 min. After staining and fixation of mast cells, mesentry pieces were transferred through acetone and xylene two times and mounted on slides. All the pieces were examined under the high power of light microscope. Percent protection of the mast cells in the control group

and the treated groups were calculated by counting the number of degranulated mast cells from total of at least 100 mast cells counted. Percent inhibition of mast cell degranulation for each treatment was calculated by following formula:

$$\% \text{ inhibition of MCD} = \left[ \frac{1 - \text{Number of degranulated mast cell}}{\text{Total number of mast cells}} \right] \times 100$$

**Statistical Analysis:** The results were expressed as mean  $\pm$  standard error of mean. The significance was evaluated by U npaired student 't test' and One way ANOVA, followed by Tukey's multiple comparison test.  $p < 0.05$  was considered statistically significant.

## RESULTS

**Triple Antigen-Induced Anaphylaxis in Rats:** When serum containing antigen was administered into sensitized rats, it caused anaphylactic shock and death of the

animals. PHE protected the sensitized rats against anaphylactic shock. In control rats, intravenous antigen challenge (horse serum) caused shock in 84% of the animals characterised by symptoms of dyspnoea, cyanosis and collapse, while in PHE (200 mg/kg) treated rats, the onset of symptoms were delayed and symptoms were less severe ( $p < 0.001$ ) with reduced mortality (Table 1).

**Studies on Compound 48/80 Induced Rat Mesentric Mast Cell Degranulation:** Antigen challenge resulted in significant degranulation of the mast cells. Compound 48/80 (10  $\mu\text{g/ml}$ ), a known mast cell degranulating agent, produced significant rat mesentric mast cell degranulation ( $80.17 \pm 1.58$ ). Prior exposure to PHE produced significant ( $p < 0.001$ ) reduction in the Compound 48/80-induced mast cell degranulation in dose dependant manner (Figure 1). The % inhibition of MCD was found to be 54.67%, 58.83% and 66.83% with 500, 750 and 1000  $\mu\text{g/ml}$  of PHE, respectively (Table 2). Kitotifen fumarate, a known mast cell stabilizing agent, also brought significant ( $p < 0.001$ ) reduction in degranulating mast cells.

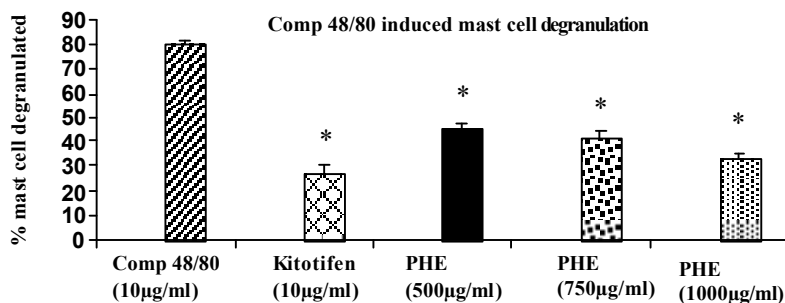


Fig. 1: Effect of PHE on Compound 48/80 induced rat mesentric mast cell degranulation  
Each bar represents mean  $\pm$  SEM (n= 6)  
\* $p < 0.001$  as compare to positive control group (One way ANOVA followed by Tukey's multiple range test)

Table 1: Effect of PHE on triple antigen induced anaphylaxis in pre-sensitized rats

Treatment groups	Dose(p.o.)	Symptoms			
		Onset (min)	Duration (min)	Severity (score)	Mortality(%)
Control	-	3.5 $\pm$ 0.5	32 $\pm$ 1.83	10.67 $\pm$ 0.85	83
PHE	200 mg/kg	4.83 $\pm$ 0.83	10 $\pm$ 1.29*	3.33 $\pm$ 0.99*	17

Results are expressed in mean  $\pm$  SEM (n=6).

\* $p < 0.001$  as compared to control group (Unpaired student 't test').

Table 2: Effect of PHE on Compound 48/80 induced rat mesentric mast cell degranulation

Treatment groups	Concentration ( $\mu\text{g/ml}$ )	% inhibition of degranulation
Positive control	-	19.83 $\pm$ 1.58
Kitotifen	10	70.67 $\pm$ 3.30*
PHE	500	54.67 $\pm$ 2.18*
PHE	750	58.83 $\pm$ 3.32*
PHE	1000	66.83 $\pm$ 2.19*

Results are expressed in mean  $\pm$  SEM (n=6).

\* $p < 0.001$  as compared to Positive control group (One way ANOVA followed by Tukey's multiple range test).

## DISCUSSION

Allergic manifestations include allergic rhinitis, anaphylaxis, urticaria and asthma - the diseases associated with inflammatory conditions. Mast cells are known to be the primary responders in allergic reactions, most of which are triggered by cross-linking of a high-affinity IgE receptor (FcεRI). Murine systemic anaphylaxis reactions are important parameters for evaluating anti-allergic property [18]. In the present study, PHE produced significant reduction in the mortality of rats subjected to anaphylactic shock induced by horse serum. After activation, mast cells exert their biological effects by releasing preformed and *de novo*-synthesized mediators, such as histamine, leukotrienes and various cytokines/chemokines [19]. IgE-mediated mast cell disruption is an important initial event in the development of type I allergic reactions such as asthma and atopic disorders. Mast cell degranulation is also elicited by synthetic Compound 48/80 and it has been used as a direct and convenient model to study the mechanism of anaphylaxis [20]. Numerous reports have established that stimulation of mast cells by Compound 48/80 initiates activation of signal transduction pathway through G-proteins, which leads to histamine release [21, 22]. PHE depicted marked protection of rat mesenteric mast cells from disruption by compound 48/80 in dose dependant manner. The anti-anaphylactic and mast cell stabilizing effect of PHE might be attributed to the presence of herbal extracts, which are known for their mast cell stabilizing potential against antigen-antibody reaction and/or due to the suppression of IgE antibody production, which is responsible for degranulation mast cells [22]. Similar results have been reported with *Clerodendrum serratum* Linn. and *Curcuma longa* Linn. [16, 23]. *Piper longum* Linn. has been shown to reduce passive cutaneous anaphylaxis in rats [24]. *Curcuma longa* Linn. and *Piper longum* Linn. showed marked inhibition of histamine release from mast cells [25, 26].

In Conclusion, PHE possesses significant anti-anaphylactic activity and this might be due to stabilization of mast cell membrane. Based on this, PHE can be used in treatment of asthma and other allergic conditions. However, further studies with other experimental models, especially the role of cytokines are warranted to substantiate the anti-asthmatic and anti-allergic activity of PHE.

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