



Research Article

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Therapeutic Activity of Polyherbal Formulation against Passive Anaphylaxis by Using Rat Mesenteric Mast Cells

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ABSTRACT

Anaphylaxis is the result of alteration in mast cell physiology and is responsible for the various physiological changes which are observed during anaphylaxis. The symptoms of anaphylaxis are due to the release of the histamine from the mast cells and it results mainly due to the degranulation of the mast cells. Antianaphylactic activity of polyherbal formulation against passive anaphylaxis was evaluated with the help of sheep serum induced passive anaphylaxis model and passive paw anaphylaxis model. Sheep serum induced passive anaphylaxis model was carried out by wistar rats. The selected rats were administered intraperitoneally with serum of active anaphylactic rats. After 48 h all the rats are administered intraperitoneally with 1ml of sheep serum. The rats which are pre-treated with prednisolone (10 mg), polyherbal formulation (250 mg) were analyzed for the mast cell degranulation. Passive paw anaphylaxis model was carried out by subcutaneous administration of 0.1 ml serum of active anaphylactic rats into the plantar region of left hind paw of rats. After 24 h the left hind paw of rats was administered subcutaneously with 0.1 ml of egg albumin. The rats which are pre-treated with prednisolone (10 mg), polyherbal formulation (250 mg) were analyzed for the increase in paw volume. Antianaphylactic activity of polyherbal formulation on the mesenteric mast cells of the rat may be possibly due to the membrane stabilizing potential.

Keywords: Mast cell degranulation, Membrane stabilization, Passive anaphylaxis, Polyherbal formulation.

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INTRODUCTION

Ayurveda, the Indian traditional system of medicine is deep rooted and manifested the life to lead healthy and blissful. It gives information about the number of medicinally useful drugs for the treatment of various diseases which includes anaphylaxis, allergy and

bronchial asthma. [1] Anaphylaxis is one of the diseases which affect mankind and is also responsible for significant mortality and morbidity. [2] Anaphylaxis is an acute Type I hypersensitivity reactions which is produced by the release of different types of chemical mediators from the mast cells and also from the basophil cells. The triggers for the anaphylaxis are foods like nuts, latex from natural rubber, drugs like Penicillin, venom from insects, fish and wheat etc, and allergy shots. Sometimes extreme temperature may also play an important role in the pathogenesis of the anaphylaxis. [3] Mast cells, lymphocytes and

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immunoglobulins play a vital role in etiopathogenesis of anaphylaxis. [4] Histamine release from the mast cells is responsible for the symptoms of anaphylaxis. Release of histamine is triggered by the increased intracellular calcium concentration and which is responsible for degranulation of the mast cells. [5] Cross linking of the antigen with the IgE (immunoglobulin E) antibody and is bound to Fc epsilon RI receptors on surface of mast cells is responsible for mast cell degranulation. [6] The available treatments for the anaphylaxis have major limitations like adverse reactions, drug interactions and other compliance issues.

Polyherbal formulation is prepared by mixing the methanolic extract of three plants (*Leptadenia reticulata*, *Nigella sativa*, *Withania somnifera*) and aqueous extract of two plants (*Trigonella foenum graecum*, *Glycyrrhiza glabra*) in equal ratio. *Leptadenia reticulata* has been used in the treatment of eczema, bronchial asthma and insect bites. [7] *Nigella sativa* also has antiallergic activity, and also used in the treatment of asthma. [8] *Withania somnifera* has hypoglycemic activity [9], antiplasmodic activity [10], hypolipidemic activity [11], anthelmintic activity [12] and also used in the treatment of inflammation [13] etc. *Trigonella foenum-graecum* is also has anantidiabetic [14], anthelmintic [15], analgesic and also used in the treatment of inflammation. [16] *Glycyrrhiza glabra* has been used for the treatment of diabetes [17], hepatotoxicity [18] and inflammatory arthritis [19] etc. The objective of the present study involves the evaluation of the antianaphylactic activity of polyherbal formulation against against the passive anaphylaxis

MATERIALS AND METHODS

Plant material

The plant materials were collected from the local market of Tirupati. The plant materials were identified and they are authenticated in Department of Botany, S. V. University, Tirupathi. The plant materials were coarsely powdered by using the rotary grinder and stored in airtight plastic containers and the prepared powder was used for extraction.

Preparation of extracts

The fine powder was used for preparation of extracts. The fine powder (100 g) was extracted by using soxhlet apparatus by using 400 ml of 95% methanol. Extraction was continued, until it does not show the presence of any residue on evaporation. The aqueous extract was prepared by cold maceration with 3% methanol-water for 7 days with frequent shaking. Rotary vacuum evaporator was used to remove the solvents under reduced pressure.

Experimental animals

Both male and female Wistar rats of weight 175-200 g were used. The animals were housed in standard conditions of temperature ($22 \pm 2^\circ\text{C}$), relative humidity ($60 \pm 5\%$) and light (12 h light/ dark cycle). Rats were placed in wire-bottomed cages to avoid Coprophagy and fighting of animals. All animal experiments were

carried out in accordance with the guidelines of CPCSEA.

Sheep serum induced passive anaphylaxis model

Wistar rats of both sex were used for this study. 18 rats were taken and are divided into 3 groups of six animals each. Blood was collected from the active anaphylactic groups of rats and serum was separated. Administration of the collected serum by intraperitoneal route to all the three groups at a dose of 1ml induces mast cell degranulation. The group-1 serves as control group which receives water only. The group-2 serves as standard group which receives standard drug, Prednisolone at a dose of 10 mg/kg. Animals of group 3 were administered with 250 mg/kg of polyherbal formulation. After 48 hours all the 3 groups were administered with 1 ml of sheep serum through intraperitoneal route. After 10 mins all the rats are sacrificed by pentobarbitone and mesenteries were collected in petridish and transferred to formalin solution for 24 h. The mesenteries were stained with toluidine blue and fixed by using Xylene and Diphenylphthalein xylene. Mesenteries were examined under microscope for mast cell count. [20]

Passive paw anaphylaxis model

Wistar rats were used for this study. Rats were administered with *Bordetella pertussis* vaccine and egg albumin through intraperitoneal route. The blood was collected from the above rats by orbital plexus under ether anesthesia and the serum was separated by centrifugation at 1500 rpm. The serum was stored at cool place of temperature 20°C .

18 Wistar rats of either sex were taken and are divided into 3 groups of six animals each. The group-1 serves as control group which receives water only. The group-2 serves as standard group which receives standard drug, Prednisolone at a dose of 10 mg/kg. Animals of group 3 were administered with 250 mg/kg of polyherbal formulation. Treatment was given for seven days. On the seventh day two hours after the treatment, the rats were passively sensitized by administration of 0.1 ml of undiluted serum by subcutaneous route into the plantar region of left hind paw. After 24 h of the passively sensitization, the left hind paw was injected with 10 mg of egg albumin in saline at a dose of 0.1 ml by subcutaneous route. This leads to the development of edema in the paw. The hind paw volume was measured by using volume displacement method using Plethysmometer. [21] Percent inhibition of inflammation and Mean increase in paw volume was calculated by using the following formula.

$$\% \text{ inhibition} = 100(1 - V_t/V_c)$$

Where, 'Vt' represents edema volume in test group, 'Vc' represents edema volume in control group.

RESULTS

Sheep serum induced passive anaphylaxis model

The results states that the polyherbal formulation at a dose of 250 mg/kg showed, highest percentage of intact mast cells (73.52), as that of the standard

prednisolone treated group (76.46), which was compared with the control group (19.18). So, treatment with the 250 mg of PHF and 10 mg of prednisolone prior to sensitization had decreased the mast cell degranulation. There was no significant difference

among group-2 and group-3 with *P* value less than 0.05. The results were shown in Table 1 graphically shown in Figure. 1 and histopathological changes were shown in Figure. 2.

Table 1: Effect of PHF on mast cell degranulation in passively sensitized rats

S. No.	Groups	Treatment Dose (mg/kg)	Intact mast cells (%) [Mean ± S.E.M]	Degranulated mast cells (%) [Mean ± S.E.M]
1	Group 1	Water	19.18 ± 1.47	80.82 ± 1.47
2	Group 2	Prednisolone (10)	76.46 ± 3.64*	23.54 ± 3.64
3	Group 3	PHF (250)	73.52 ± 2.32*	26.48 ± 2.32

Values are mean ± S.E.M., n=6, **P*<0.05 as compared with the control group

Table 2: Effect of PHF on passive paw anaphylaxis

Groups	Paw edema (ml) at different time interval				Mean increase in paw volume (ml)	% decrease in paw volume
	Initial	1 h	2 h	3 h		
Group 1	0.83 ± 0.04	1.55 ± 0.03	1.47 ± 0.02	1.32 ± 0.04	0.49 ± 0.01	---
Group 2	0.85 ± 0.05	1.21 ± 0.04	1.09 ± 0.03	1.01 ± 0.03	0.16 ± 0.01	67.35*
Group 3	0.83 ± 0.03	1.26 ± 0.03	1.15 ± 0.02	1.04 ± 0.05	0.21 ± 0.01	57.15*

Values are mean ± S.E.M., n=6, **P*<0.05 as compared with the control group

Passive paw anaphylaxis model

The results obtained states that the polyherbal formulation at a dose of 250 mg/kg showed decrease in the mean paw volume (0.21), as that of the standard prednisolone treated group (0.16), which was compared with the control group (0.49). There was no significant difference among group-2 and group-3 with *P* value less than 0.05. The results were shown in Table. 2 and graphically shown in Figure 3.

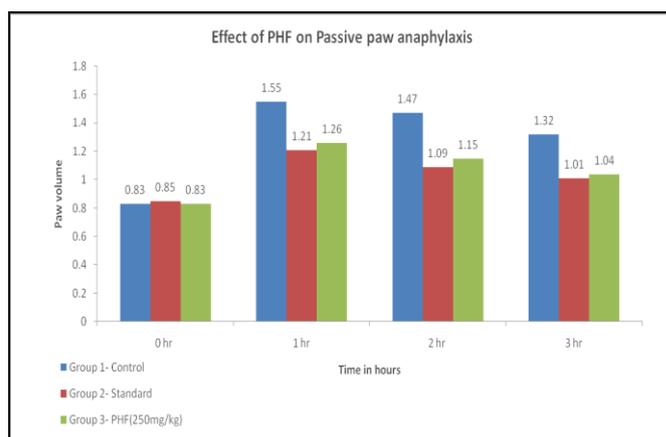


Fig. 3: Effect of PHF on passive paw anaphylaxis

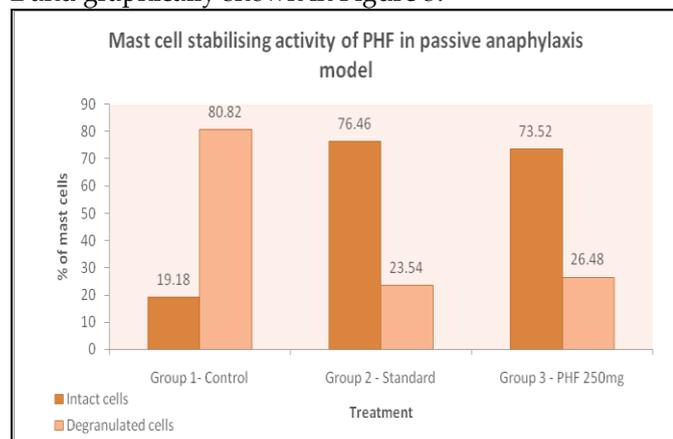


Fig. 1: Effect of PHF on mast cell degranulation in passively sensitized rats

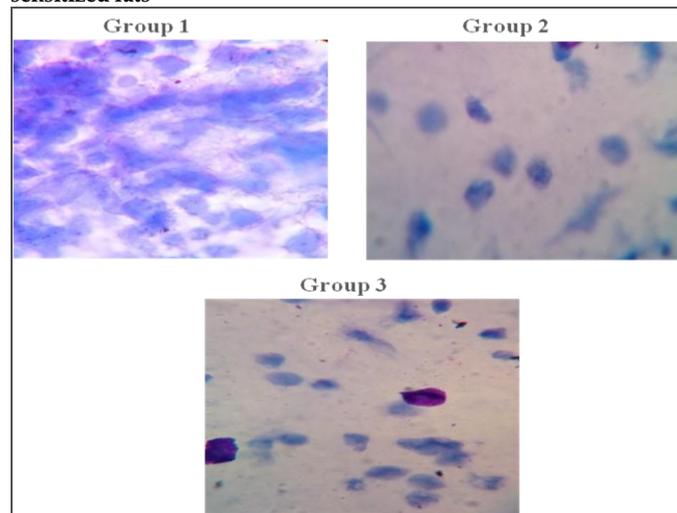


Fig. 2: Histopathological studies of Effect of PHF on mast cell degranulation in passively sensitized rats

DISCUSSION

The antianaphylactic activity of polyherbal formulation against passive anaphylaxis was evaluated by sheep serum induced passive anaphylaxis model and passive paw anaphylaxis model. The serum of active anaphylactic rats induces in other group of the rats due to the second exposure of the rats to the sheep serum. This induces degranulation of the mast cells present in the intestinal mesentery. The polyherbal formulation shows marked protection against the degranulation of mast cell which may be due to their mast cell stabilizing potential against antigen antibody reaction on the mast cells. [22] Administration of serum separated from the rats treated with the egg albumin and *Bordetella pertussis* vaccine, to the wistar rats bu subcutaneous route induce edema of the hind paw. The polyherbal formulation also shows protection against edema of paw. Antianaphylactic activity of polyherbal formulation may be due to decreased cAMP phosphodiesterase enzyme which leads to increase in the cyclic AMP levels and which is responsible for the fusion of granules. The flavonoids present in the various extracts of polyherbal formulation may be responsible for this antianaphylactic activity against passive anaphylaxis. [23] Further investigation is required to prove the exact mechanism of

antianaphylactic activity of polyherbal formulation against passive anaphylaxis.

In conclusion all the above findings reveal that, the 250 mg of polyherbal formulation has the antianaphylactic activity against passive anaphylaxis. The stabilizing potential of polyherbal formulation against passive anaphylaxis may be due to suppression of antibody production and the inhibition of antigen induced histamine release.

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