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Antianaphylactic Activity Polyherbal Formulation on Mast Cells of Rats against Active Anaphylaxis

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ABSTRACT

Anaphylaxis is an acute systemic reaction, which is produced by the mast cells due to release of different chemical mediators. Anaphylaxis is the result of alteration in physiology of mast cell and is responsible for the various physiological changes. Objective of the present study was to evaluate the antianaphylactic activity of polyherbal formulation against the active anaphylaxis with the help of mesenteric mast cells of rats. The study was carried out by sheep serum induced active anaphylaxis model, triple antigen induced symptomatic active anaphylaxis model and compound 48/80 induced rat paw edema model. The rats which are pretreated with standard drug prednisolone (10 mg) and polyherbal formulation (250 mg) shows beneficial effect against active anaphylaxis. Antianaphylactic activity of polyherbal formulation against active anaphylaxis may be possibly due to the membrane stabilizing potential.

Keywords: Antianaphylactic activity, Mast cell degranulation, Membrane stabilization, Anaphylaxis, Polyherbal formulation

INTRODUCTION

The Indian traditional ayurvedic system of medicine is deep rooted and manifested the existing life to lead healthy and blissful. It gives information about the number of medicinally useful drugs for the treatment of different types of diseases which includes anaphylaxis, allergy and bronchial asthma [(1)]. Anaphylaxis is one of the common diseases which affect mankind and also responsible for significant morbidity and mortality [(2)]. Anaphylaxis is an acute Type I hypersensitivity reactions, which is produced by the release of different types of chemical mediators especially from the mast cells and also from the basophil cells. The triggers for the anaphylaxis are drugs like Penicillin, foods like nuts, , latex from natural rubber, fish and wheat etc, venom from insects, and allergy shots and sometimes extreme temperature may also involved in the pathogenesis of the anaphylaxis[(3)]. Mast cells, Lymphocytes and immunoglobulins play an important role in etiopathogenesis of anaphylaxis [(4)]. The symptoms of anaphylaxis are mainly due to the release of the histamine from the mast cells. The histamine release is initiated by the increased intracellular calcium concentration and which is responsible for degranualation of the mast cells [(5)]. Degranualation is due to the cross linking of the antigen along with the IgE (immunoglobulin E) antibody which is bound to Fc epsilon RI receptors on surface of mast cells [(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 *Leptadenia reticulata, Nigella sativa, Withania*

somnifera and aqueous extract of *Trigonella foenum graecum, Glycyrrhiza glabra* in equal ratio. *Leptadenia reticulata* has been used in the treatment of bronchial asthma, eczema and insect bites [7]. *Nigella sativa* also has antiallergic activity, and also used to treat asthma [8]. *Withania somnifera* has hypoglycemic activity [9], antiplasmodic activity [10], hypolipidemic activity [11], anthelmintic activity and antiinflammatory activity [12] etc. *Trigonella foenum-graecum* is also has analgesic and antiinflammatory activity [13]. *Glycyrrhiza glabra* has been used for the treatment of diabetis [14], hepatotoxicity [15], inflammative arthritis [16] etc. The present research work involves the evaluation of the antianaphylactic activity of polyherbal formulation against active anaphylaxis and the study was carried out by sheep serum induced active anaphylaxis model, triple antigen induced symptomatic active anaphylaxis model and compound 48/80 induced rat paw edema model.

MATERIALS AND METHODS

Plant material: The plant materials were collected from the local market of Tirupati. The plant materials were identified and 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 is 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 (175 – 200 gm) were used. The animals were housed in standard conditions of temperature (22 ± 2^0C), relative humidity (60 ± 5%) and light (12h light/ dark cycle). To avoid Coprophagy and fighting rats were placed in wire-bottomed cages. All animal experiments were carried out in accordance with the guidelines of CPCSEA.

Sheep serum induced active anaphylaxis model: 42 rats were taken and are divided into 7 groups of six animals each. The group-1 is unsensitized and group-2 to 7 was sensitized with sheep serum. Sensitization was done by administration of 0.5 ml of sheep serum along with 0.5 ml of triple antigen subcutaneously [17] (Serum Institute of India Ltd., Pune). The animals of group-1is an unsensitized group is a normal group which receives water (Vehicle). Animals of group-2 received water and served as control. Rats of group-3 served as standard group, received prednisolone (10 mg/kg/day) (standard drug) orally for 14 days. Animals of group-4, 5, 6 and 7, were administered with 100, 250, 500 and 750 mg/kg/day of polyherbal formulation respectively. On 14th day all the rats were sacrificed with intraperitoneal injection of 40 mg/kg of pentobarbitone. After sacrification the intestinal mesentery along with intestinal pieces was taken for the study on mast cells. Mesenteries were kept in Ringer-Locke solution (NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.15, Glucose 1.0 gm/ltr of distilled water) at a temperature of 37^0C. The collected mesenteries were challenged with 5% v/v sheep serum for a period of 10 minutes. After challenging they were stained with thionine and examined the number of intact and degranulated mast cells by using the microscope [18].

Triple antigen induced symptomatic active anaphylaxis model: 30 wistar rats were taken and are divided into 3 groups of ten animals each. All the rats were sensitized by administration of 0.5 ml of sheep serum and 0.5 ml of triple antigen containing 20 million *Bordetella pertussis* organisms by subcutaneous route. The rats of group-1control group which receives water (Vehicle). Rats of group-2 receive 10 mg/kg/day of prednisolone (standard drug). Rats of group-3 received 250 mg/kg of polyherbal formulation for 10 days. On 10th day two hours after the treatment, all the rats were challenged by intravenous administration with sheep serum. The rats are observed for the onset of symptoms like increased rate of respiration, cyanosis, duration of persistence of symptoms, and death if any. The severity score of the symptoms was noted using the method of Gupta [17].

Score 2 - Increased rate of respiration.
Score 4 - Dyspnoea for 10 mins.
Score 8 - Cyanosis and dyspnoea for 10mins.
Score 12 - Death or collapse.

Compound 48/80 induced rat paw edema model.

24 wistar rats were taken and are divided into 4 groups of six animals each. The rats of group-1serves as normal group which receives water (Vehicle). Rats of group-2 received water and served as control. Rats of group-3 served as standard group, received prednisolone (10 mg/kg) (standard drug) Rats of group-4 received 250 mg/kg of polyherbal formulation. One hour after the treatment, rats of all groups except group-1, administer 0.1 ml of the

compound 48/80 was injected into the plantar region of right hind paw by subcutaneous route, which produces edema. The paw volume was measured immediately after administration of the compound 48/80, and at 1, 2 and 3 h of administration by using Plethysmometer [19]. Mean increase in paw volume and percent inhibition of inflammation was calculated by using the following formula.

$$\% \text{ inhibition} = 100 (1-Vt/Vc)$$

Where

'Vt' represents edema volume in test group.

'Vc' represents edema volume in control group.

RESULTS:

Sheep serum induced active anaphylaxis model:

The results after screening of polyherbal formulation at doses of 100, 250, 500 and 750 mg shows that PHF at a dose of 250 mg/kg showed highest percentage of intact mast cells (71.21), as that of the standard prednisolone treated group (73.34), which was compared with the control group (21.34). So treatment with the 250 mg of PHF and 10 mg of prednisolone prior to sensitization had decreased the degranulation of mast cells. There was no significant difference among group-3 and group-5 with P value less than 0.05. The results were depilated in Table 1, and graphically represented in Figure 1. Histopathological studies of effect of polyherbal formulation on mast cell degranulation were shown in Figure 2.

Triple antigen induced symptomatic active anaphylaxis model:

The results after the screening, states that the PHF at a dose of 250 mg/kg showed low score of the severity of symptoms (3.24), as that of the standard prednisolone treated group (2.32), which was compared with the control group (11.23). So treatment with the 250 mg of PHF and 10 mg of prednisolone prior to sensitization has decreased the severity of symptoms. 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.

Compound 48/80 induced rat paw edema model:

The results after the screening states that the PHF at a dose of 250 mg/kg showed decrease in the mean paw volume (0.23), as that of the standard prednisolone treated group (0.18), which was compared with the control group (0.51). There was no significant difference among group-3 and group-4 with P value less than 0.05. The results were shown in Table.3 and graphically shown in Figure 4.

DISCUSSION:

The antianaphylactic activity of polyherbal formulation against active anaphylaxis was evaluated by using sheep serum induced active anaphylaxis model, triple antigen induced symptomatic active anaphylaxis model and compound 48/80 induced rat paw edema model. 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 [20]. Antianaphylactic activity of polyherbal formulation may be due to decreased cAMP phosphodiesterase enzyme which leads to increase in the cyclic AMP levels which is responsible for the fusion of granules. The flavonoids present in the different extracts of polyherbal formulation may be responsible for this antianaphylactic activity [21]. Further investigation is required to prove the exact mechanism of antianaphylactic activity of polyherbal formulation.

CONCLUSION:

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

Table 1: Effect PHF on mast cell degranulation in actively 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		85.41±4.43	14.59±4.43
2	Group 2	Water		21.34±1.57	78.66±1.57
3	Group 3	Prednisolone 10		**73.34±3.74***	26.66±3.74
4	Group 4	PHF	100	32.21±1.21	67.79±1.21
5	Group 5		250	**71.21±2.24***	28.79±2.24
6	Group 6		500	62.24±2.39	37.76±2.39
7	Group 7		750	58.23±2.41	41.77±2.41

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

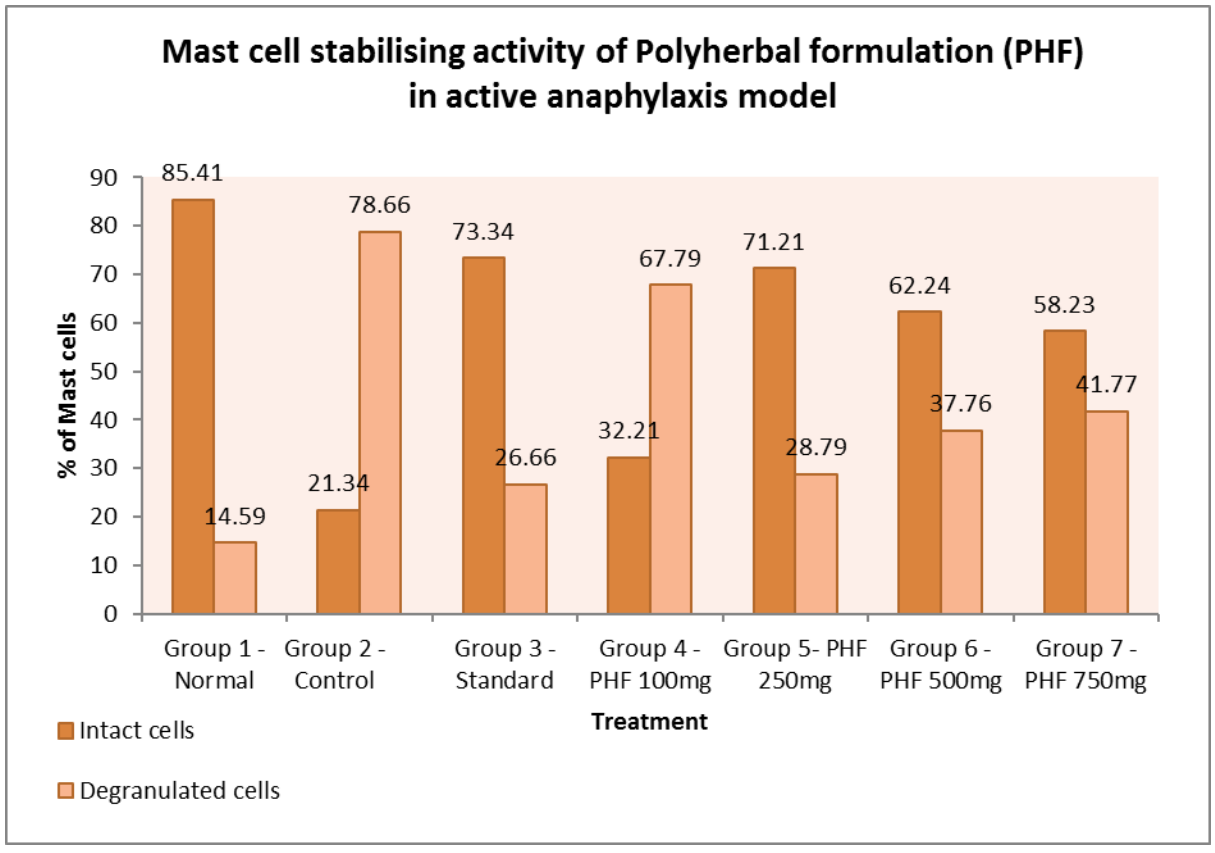

Figure 1: Effect of PHF on mast cell degranulation in actively sensitized rats

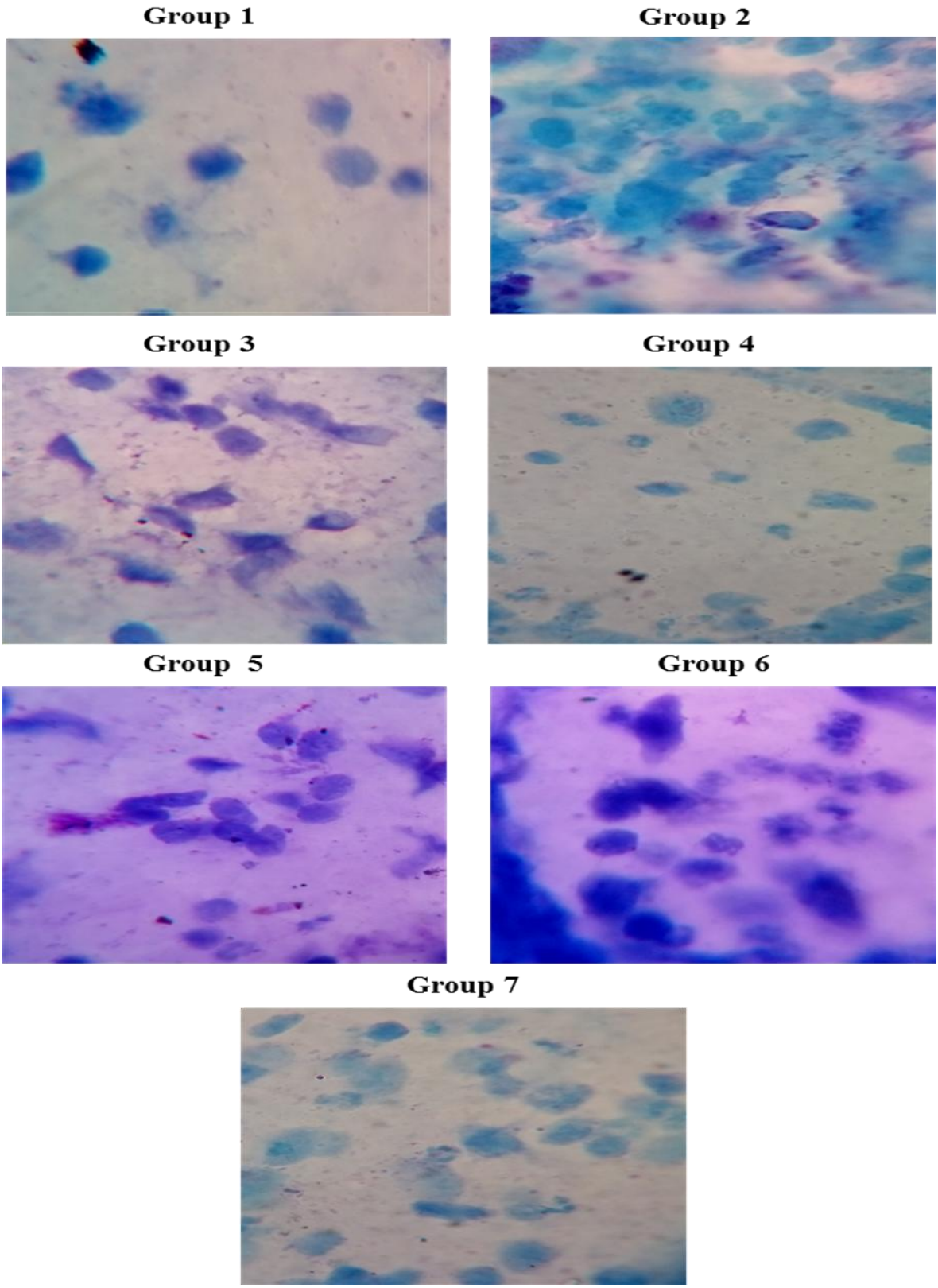

Figure 2: Histopathological studies of effect of PHF on mast cell degranulation in actively sensitized rats

Table 2: Effect of PHF on Triple antigen induced symptomatic active anaphylaxis model

S.No.	Groups	Treatment	Symptoms			% of Mortality
			Onset (min)	Duration (min)	Severity (Score)	
1	Group 1	Water	1.26±0.43	31.53±5.32	11.23±0.73	90
2	Group 2	Prednisolone (10mg/kg)	2.54±0.32	5.36±0.34	**2.32±0.12***	0
3	Group 3	PHF (250mg/kg)	2.37±0.45	6.12±0.42	**3.24±0.25***	10

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

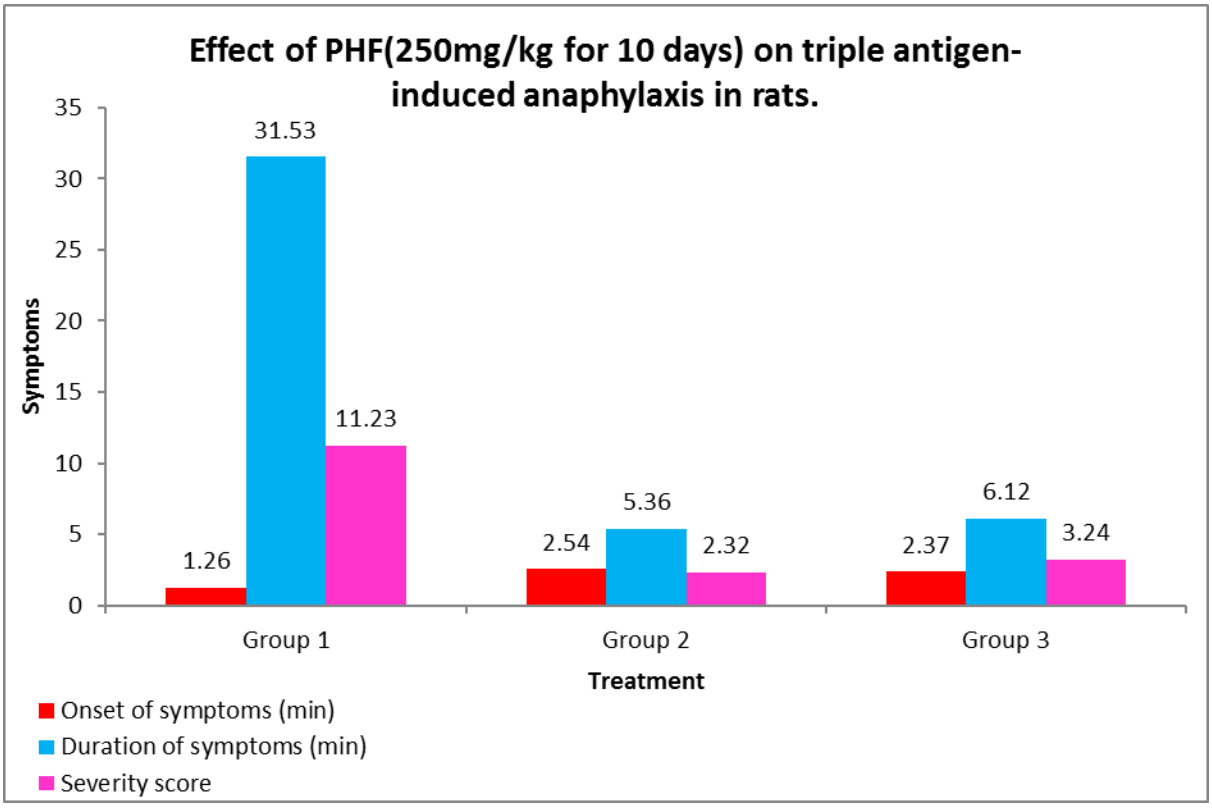

Figure 3: Effect of PHF on triple antigen induced symptomatic active anaphylaxis model

Table. 3: Effect of PHF on Compound 48/80 induced rat paw edema

Groups	Treatment	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	Water	0.83 ± 0.04	0.83 ± 0.03	0.84 ± 0.03	0.83 ± 0.04	0.01 ± 0.01	98.04
Group 2	Water	0.85 ± 0.02	1.57 ± 0.05	1.43 ± 0.04	1.36 ± 0.04	0.51 ± 0.01	---
Group 3	Prednisolone (10 mg/kg)	0.84 ± 0.04	1.23 ± 0.02	1.12 ± 0.03	1.02 ± 0.02	0.18 ± 0.01	**64.70***
Group 4	PHF (250 mg/kg)	0.86 ± 0.05	1.29 ± 0.03	1.18 ± 0.02	1.09 ± 0.04	0.23 ± 0.01	**54.90***

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

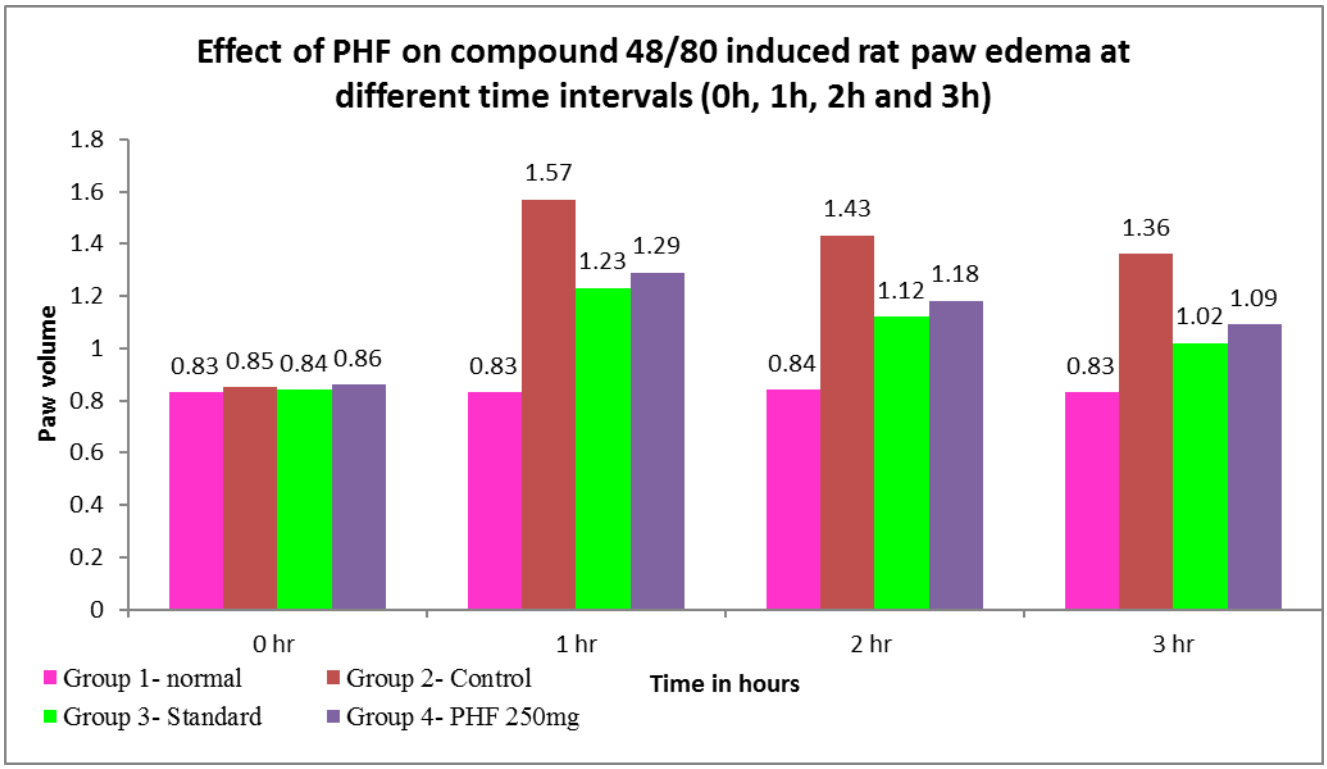

Figure 4: Effect of PHF on Compound 48/80 induced rat paw edema

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