AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

In Vivo Antianaphylactic Activity of Different Extracts of *Glycyrrhiza Glabra* on Mesenteric Mast Cells of Rats

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ABSTRACT

Antianaphylactic activity of *Glycyrrhiza glabra* was evaluated with the help of mesenteric mast cells of rats. The study was carried out by sensitization of the rat mesenteries by using sheep serum and tripple antigen to induce degranulation of mast cells. The mesenteries which are pretreated with prednisolone (10 mg), petroleum ether, methanol and aqueous extract of *Glycyrrhiza glabra* (250mg, 500mg and 750mg) were analyzed for the mast cell degranulation during the anaphylactic reactions. Treatment with aqueous extract of *Glycyrrhiza glabra* (500mg) showed beneficial effect on degranulation of mast cells in actively sensitized rats. The activity was comparable with that of standard drug, Prednisolone. Antianaphylactic activity of aqueous extract of *Glycyrrhiza glabra* on the mesenteric mast cells of the rat may be possibly due to the membrane stabilizing potential.

Keywords: Antianaphylactic activity, Mast cell degranulation, *Glycyrrhiza glabra*, Membrane stabilization, Anaphylaxis.

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Received 09 September 2016, Accepted 17 September 2016

Please cite this article as: Reddy YN *et al*., In Vivo Antianaphylactic Activity of Different Extracts of Glycyrrhiza Glabra on Mesenteric Mast Cells of Rats. American Journal of PharmTech Research 2016.

INTRODUCTION

Ayurveda, ancient system of Indian medicine gives information about the number of medicinally useful drugs for the ailment of various diseases which includes anaphylaxis, allergy and bronchial asthma[1]. Anaphylaxis is one of the diseases with diverse manifestation that can affect mankind which may also responsible for significant morbidity and mortality [2]. Different substances which may act as the triggers for the anaphylaxis, which includes medications like Penicillin, foods like nuts, fish and wheat etc, latex from natural rubber, venom from insects, and allergy shots and the extreme temperature may also plays a role in the pathogenesis of the anaphylaxis. In the etiopathogenisis of anaphylaxis, Lymphocytes, mast cells and immunoglobulins play a crucial role [4]. Alteration in the physiology of the mast cell is responsible for the various physiological changes which are occurred during anaphylaxis. Release of the histamine from the mast cells is mainly due to the increased calcium within the cell which leads to degranualation of the mast cells[5]. Degranualation is due to the cross linking of the antigen along with the IgE antibody (immunoglobulin E antibody) which is bound to Fc epsilon RI receptors on mast cells[6].

There are number of methods of treatment was available for the anaphylaxis. Those are having limitations like adverse reactions, drug interactions and other compliance issues[4]. *Glycyrrhiza glabra* has been used in the Indian medicine for the treatment of diabetis[7], hepatotoxicity[8], inflammative arthritis[9] etc. It is also active against the diseases such as asthma and dermatitis [10]. The present research work involves the evaluation of the mast cell stabilizing activity of the different extracts of *Glycyrrhiza glabra* (petroleum ether, methanolic and aqueous extract) on the rat mesenteric mast cells by using active anaphylaxis method.

MATERIALS AND METHOD

Plant material:

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

Preparation of extracts:

The collected plant material was washed with water and it was dried at room temperature for 15-20 days. Drying was done under shade and was subjected for size reduction by using rotary grinder. The fine powder which was obtained is used for preparation of extracts. The fine powder (100 g) was extracted by using soxhlet apparatus by using 400 ml of petroleum ether for about 48 h. After

defatting, the marc was dried in hot air oven at 50°C, and it was packed in soxhlet apparatus for further extraction with 400 ml of 95% ethanol. 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. The rotary vacuum evaporator was used to remove the solvents under reduced pressure.

Experimental animals:

For this study both male and female Wister 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) were used. Rats were placed in wire-bottomed cages, to avoid Coprophagy and fighting. All animal experiments were carried out in accordance with the guidelines of CPCSEA. Sheep serum is used to induce anaphylaxis. The serum was prepared by collecting the fresh blood from sheep under sterile condition.

Active Anaphylaxis:

72 rats were taken and are divided into 12 groups of six animals each. The group-1 is unsensitized and group-2 to 12 was sensitized with sheep serum. Sensitization of rats were done by injecting subcutaneously 0.5 ml of sheep serum along with 0.5 ml of triple antigen which containing 20,000 million *Bordetella pertusis* organisms[(11)] (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 10 mg/kg/day of prednisolone (reference drug) orally for 14 days. Animals of group-4, 5 and 6, were administered with 250, 500 and 750 mg/kg/day of petroleum ether extract of *Glycyrrhiza glabra* respectively. Animals of group-7, 8 and 9, were administered with 250, 500 and 750 mg/kg/day of methanolic extracts of *Glycyrrhiza glabra* respectively. Animals of Group-10, 11 and 12, were administered with 250, 500 and 750 mg/kg/day of aqueous extracts of *Glycyrrhiza glabra* respectively for the same duration. On 14^{th} day the rats were sacrificed with intraperitoneal injection of pentobarbitone (40 mg/kg). 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 [12]. The treatment schedule of different groups was given in Table 1.

Table 1: Treatment Schedule of Different Groups

S.No	Group	1st Day	1-14 days		14th day
1	Group 1	Un sensitized	Water		Sacrificed by intra- peritoneal
2	Group 2	Sensitized with S.C.	Water		injection of Pentobarbitone (40
3	Group 3	injection of 0.5 ml	Prednisolone	10 mg	mg/kg), The Mesenteric pieces
4	Group 4	sheep serum along	Petroleum ether	250 mg	were collected & challenged
5	Group 5	with 0.5 ml of Triple	extract of	500 mg	with 5% v/v Sheep serum for
6	Group 6	antigen containing 20,000 million	*Glycyrrhiza glabra*	750 mg	10 minutes, after which the mast cells were stained and
7	Group 7	Bordetella Pertusis	Methanol	250 mg	examined microscopically for
8	Group 8	organisms	extract of	500 mg	the number of intact and
9	Group 9		*Glycyrrhiza glabra*	750 mg	degranulated Mast cells
10	Group 10		Aqueous extract	250 mg	
11	Group 11		of *Glycyrrhiza*	500 mg	
12	Group 12		*glabra*	750 mg	

Mast cell count:

For the mast cell count, a piece of intestinal mesentery was excised and spread in a petridish, containing Ringer–Locke solution at a temperature of 37^0C. The mesentery was challenged with 5% v/v sheep serum for a period of 10 minutes and it was transferred to a wide mouthed bottle containing 10% formalin, and kept aside for 24 h. After 24 h the mesenteric fans were stained with thionin (0.25%) on a clean slide and fixed and dried. Distilled water was used for washing the excess stain followed by dehydration with absolute alcohol. Finally the slides were cleared in xylene and mounted in diphenylphthalein xylene. The prepared slides were used for Mast cell count [(13)]. The results were analyzed statistically using ANOVA. The level of significance was fixed at $P<0.05$.

RESULTS AND DISCUSSION

At the end of study, the rats of group-2, antigen challenge group shows less number of intact mast cells and more number of the degranulated mast cells. About 83% of mast cells were degranulated. Animals of the group-3 and group-11, which were given with prednisolone (10 mg) and 500 mg/kg of Aqueous extract of *Glycyrrhiza glabra* prior to sensitization shows more number of intact mast cells, i.e. which has the decreased percentage of degranulation ($P<0.005$), when compared to the petroleum ether and aqueous extracts of *Glycyrrhiza glabra*. There was no significant difference among the Group-3 and Group-11. The results were depilated in Table 2, and graphically represented in Fig 1. Histopathological studies of effect of different extracts of *Glycyrrhiza glabra* on mast cell degranulation were shown in Figure 2.

Table 2: Effect of different extracts of *Glycyrrhiza glabra* 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		88.43±4.59	11.57±4.59
2	Group 2	Water		16.34±1.81	83.66±1.81
3	Group 3	Prednisolone 10		76.28±3.89*	23.72±3.89
4	Group 4	Petroleum ether extract of	250	28.31±1.26	71.69±1.26
5	Group 5	*Glycyrrhiza glabra*	500	42.29±2.21	57.71±2.21
6	Group 6		750	40.27±2.39	59.73±2.39
7	Group 7	Methanol extract of	250	27.32±1.47	72.68±1.47
8	Group 8	*Glycyrrhiza glabra*	500	51.26±2.53	48.74±2.53
9	Group 9		750	47.28±2.48	52.72±2.48
10	Group 10	Aqueous extract of	250	38.19±2.59	61.81±2.59
11	Group 11	*Glycyrrhiza glabra*	500	72.45±3.56*	27.55±3.56
12	Group 12		750	64.34±2.47	35.66±2.47

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

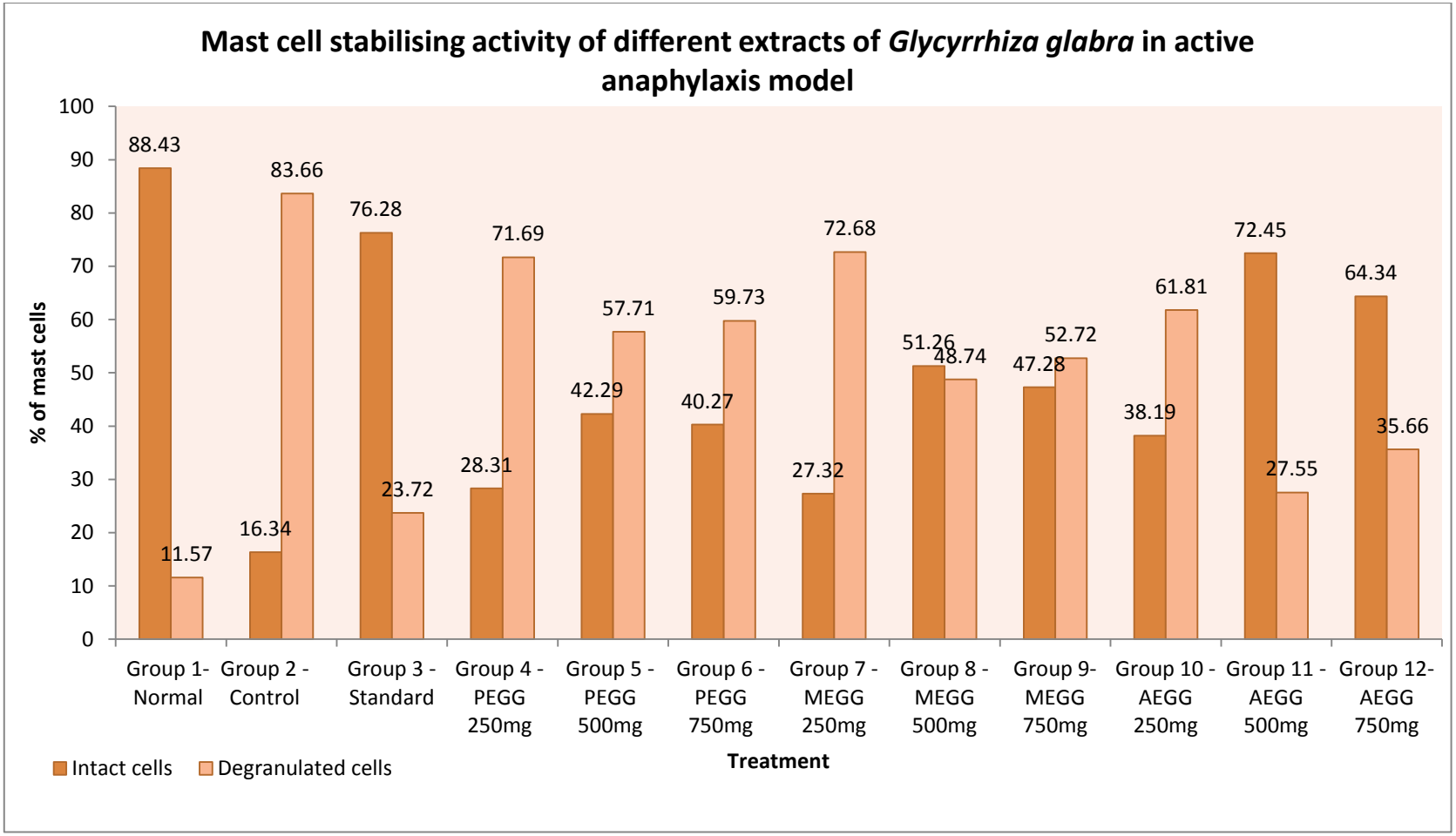
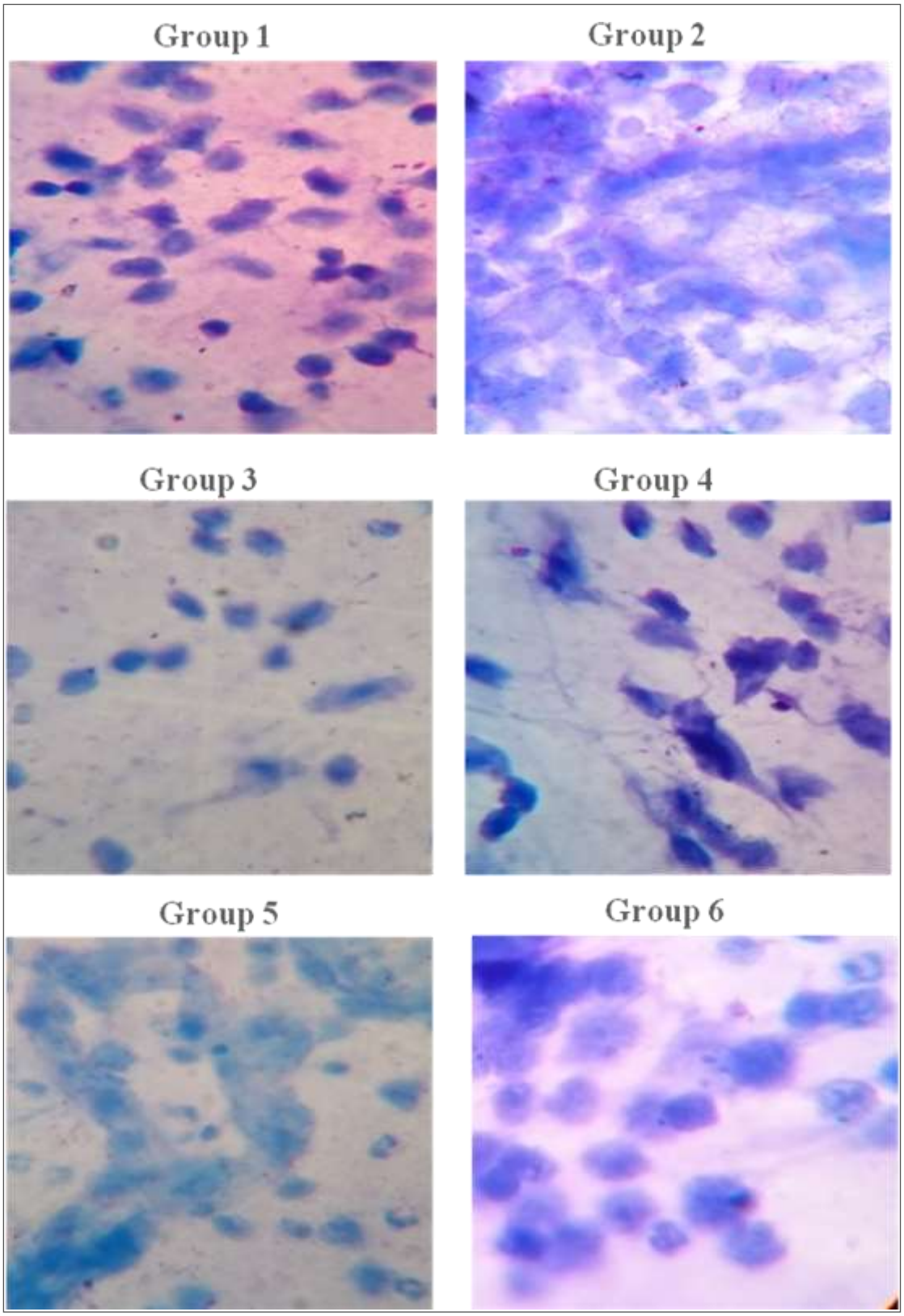


Figure 1: Effect of different extracts of *Glycyrrhiza glabra* on mast cell degranulation


Group 1
Group 2
Group 3
Group 4
Group 5
Group 6

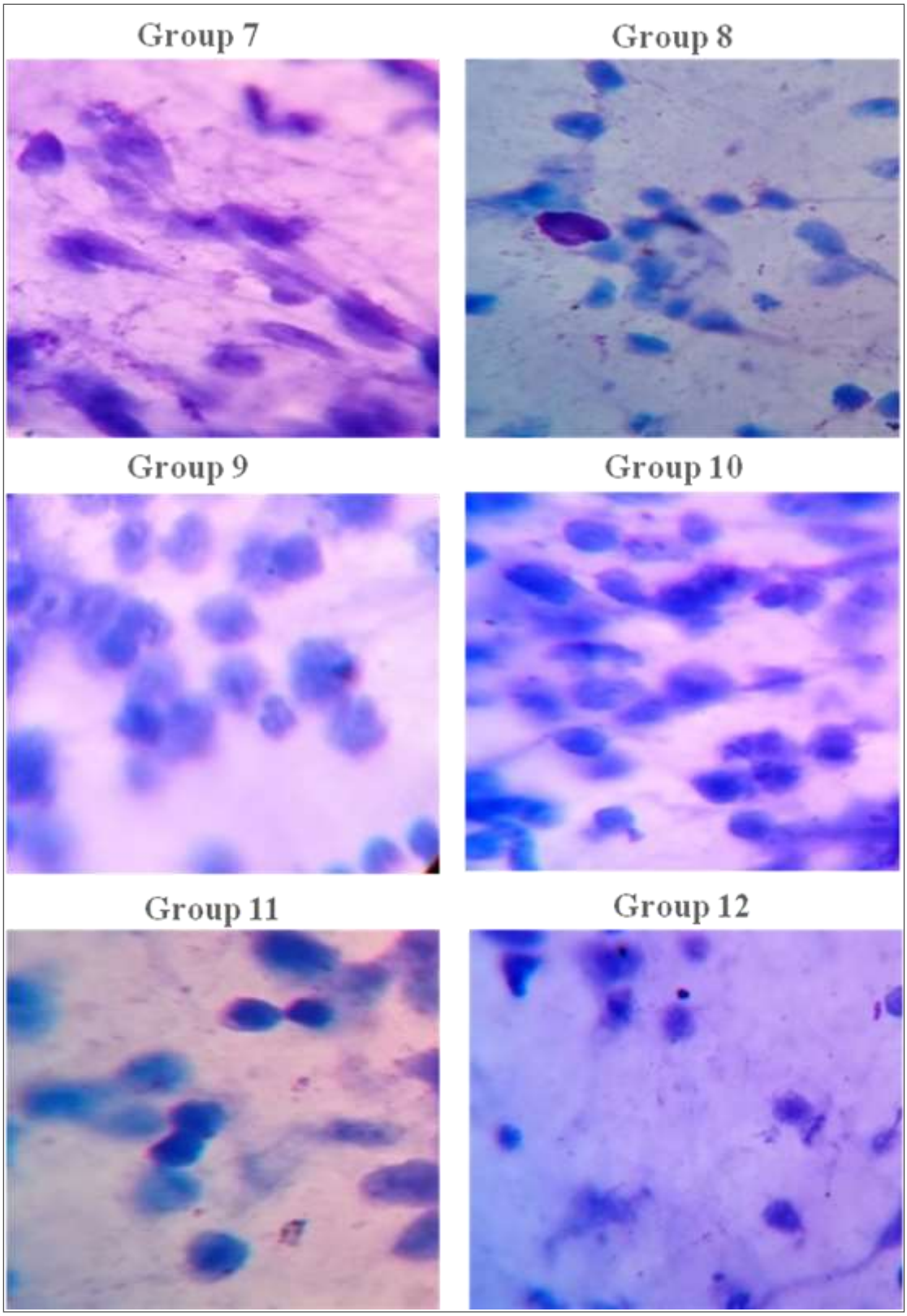


Figure 2: Histopathological studies of effect of different extracts of *Glycyrrhiza glabra* on mast cell degranulation

DISCUSSION:

The mast cell stabilizing activity of different extracts of *Glycyrrhiza glabra* was studied following active Anaphylaxis by using the rat mesenteric mast cells. Aqueous extracts of *Glycyrrhiza glabra* has marked protection against the mast cell degranulation, when compared to the petroleum ether and methanolic extract. The aqueous extract of *Glycyrrhiza glabra* shows marked protection against the degranulation which may be attributed due to their mast cell stabilizing potential against antigen antibody reaction on the mast cells [14].

Stabilization of mast cell membrane and inhibition of histamine release are the different mechanisms of the antianaphylactic activity. The histamine may be released from the mast cells are due to the calcium release from an intracellular store [15]. The aqueous extract of *Glycyrrhiza glabra* inhibits the degranulation of mast cells. It 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 *Glycyrrhiza glabra* may be responsible for this mast cell stabilizing activity. Further investigation is required to prove the exact mechanism of mast cell stabilizing activity of the aqueous extract of *Glycyrrhiza glabra*[16]. There is no significant change in the vital organs such as liver and heart and also in the general behavior.

CONCLUSION

In conclusion all the above findings reveal that, the 500 mg of aqueous extract of *Glycyrrhiza glabra* has the mast cell stabilizing activity when compared to all the three extracts (petroleum ether, methanol and aqueous extracts). The stabilizing potential of aqueous extract of *Glycyrrhiza glabra* may be due to the inhibition of antigen induced histamine release and suppression of antibody production.

REFERENCES

1. Charaka Samhita, Sri Gulabkunverba Ayurvedic Society, Jamnagar, Ayurvedic Mudranalaya, Jamnagar, 1949; 4: 1953-2032.
2. Ring J, Kramer U, Shafer T, Beherendt H. Why are allergies increasing? Curr Opinions Immunol 2001; 13: 701-8.
3. Kim et al., 2004 E.K. Kim, G.Z. Li, O.H. Chai and C.H. Song, Inhibitory effect of *Arctium lappa Linne* on compound 48/80-induced mast cell activation and vascular permeability, *Korean J. Phys. Anthropol.* 2004; 17: 55–66.
4. Salib RJ, Drake-Lee A, Howarth PH. Allergic rhinitis: past, present and the future. Clin Otolaryngol 2003; 28: 291-303.

5. G. Krishnaswamy, J. Kelley, D. Johnson, G. Youngberg, W. Stone and S.K. Huang *et al.*, The human mast cell: functions in physiology and disease, *Front Biosci*. 2001; 6: 1109–1127.
6. Metcalfe, D., Baram, D., Mekori, Y. Mast cells. Physiological Reviews. 1997; 77(4): 1033-1079.
7. Panneerselvam K, Kuppuswamy K and Kodukkur VP, Hypolipidemic activity of 18β-glycyrrhetinic acid on streptozotocin-induced diabetic rats, Eur J Pharmacology, 2009; 612(1-3): 93-97.
8. Jeong HG, You HJ, Park SJ, Moon AR, Chung YC, Kang SK and Chun HK, Hepatoprotective effects of 18β- glycyrrhetinic acid on carbon tetrachloride-induced liver injury: inhibition of cytochrome P450 2E1 expression. Pharmacological Research, 2002; 46: 221–227.
9. Chopra D, and Simon D, The Chopra Centre Herbal Handbook: Forty Natural Prescriptions for Perfect Health. Three Rivers Press, New York, 2000.
10. www.banlab.com/healingherbs/yashtimadhu.htm(12/09/2009)
11. Gupta SS, Tripathi RM. Effect of chronic treatment of the saponin of Clerodendron serratum on disruption of the mesenteric mast cells of rats. Aspects Allergy Applied Immunology 1973; 4: 177-88.
12. Norton S. Quantitative determination of mast cell fragmentation by compound 48/80. *Br J Pharmacol* 1954; 2: 484.
13. Geetha VS, Viswanathan S, Kameswaran L. Comparison of total alkaloids of *Tylophora indica* and disodium cromoglycate on mast cells. *Indian J Pharmacol* 1981; 13: 199-201.
14. Shukla R, Singh S, Bhandari CR. Preliminary clinical trials on antidiabetic actions of *A.Indica*. Medicine and surgery 1973; 134: 11-88.
15. Lee YM, Kim DK, Kim SH, Shin TY, Kim HM. Anti-anaphylactic activity of *Poncirus trifoliata fruit extract. J Ethnopharmacology* 1996; 54: 77-84.
16. Sompayrac, Lauran, How the Immune System Works. Malden, MA: Blackwell Science, Ltd. 1999; 88: 37-38.