Indian J Physiol Pharmacol 2001; 45 (3) : 319–328

EVALUATION OF E-721B, AN INDIGENOUS HERBAL COMBINATION IN EXPERIMENTAL MODELS OF IMMEDIATE HYPERSENSITIVITY

M. LAKSHMANA[1]*, MOHAMED RAFIQ[1] AND B. Y. SRIDHAR[2]

[1]*Department of Pharmacology, Government College of Pharmacy, P. Kalinga Rao Road, Bangalore – 560 027*

and

[2]*Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore – 560 024*

(Received on August 29, 2000)

Abstract : E-721B, an indigenous herbal combination was investigated for its usefulness in immediate hypersensitivity using different animal models. The drug inhibited the mast cell degranulation induced both by antigen and compound 48/80, the Schultz-Dale response in sensitized guinea pig ileum smooth muscle preparation and the production of precipitating antibodies in 50% of tested rats. It also inhibited the mast cell degranulation in passively sensitized rats indicating its suppressive action on production of reaginic antibody (IgE). However, the drug did not inhibit the 48 hours passive cutaneous anaphylaxis reaction in rats, indicating that a single dose of the drug does not have cromoglycate like properties. All the above results indicate the inhibitory effect of E-721B on immediate hypersensitive reactions such as asthma.

Key words : immediate hypersensitivity anti-allergic activity mast cell E-721B passive cutaneous-anaphylaxis

INTRODUCTION

The clinical manifestations in allergic and anaphylactic reactions are produced by the release of chemical mediator from tissue mast cells and circulating basophil leukocytes (1). These mediators are either preformed or stored within the cell in association with characteristic secretory granules (histamine, chemotactic factors, heparin etc..) or are generated afresh by the oxidative metabolism of membrane derived lipid components (leukotriene, prostanglandins and thromboxanes). In total, these spasmogenic vasoactive and chemotactic factors act on distinct effector cells to induce immediate and late phase reactions characteristic of asthma and other inflammatory responses. A variety of pharmacological agent are able to inhibit secretion in mast cells. Inspite of voluminous literature on the subjects,

.*Corresponding Author

treating hypersensitivity disease such as asthma continues to be far from satisfactory as treatment of acute manifestations are limited for symptomatic relief (2). Ayurveda, an Indian system of medicine cites several plants for anti-asthmatic and anti-allergic activities (3, 4). E-721B is one such formulation containing plants known for their anti-allergic actions.

METHODS

Wistar rats of body weight ranging from 180-220 gms and guinea pigs of Dunkin-Hartley strain weighing between 450-550 gms of either sex were used for the study. The animals were maintained at room temperature in a well-ventilated animal house under natural photoperiod conditions. They were provided with standard diet (Lipton India, Mumbai) and water *ad libitum*. All the animals received humane care and experimental protocol was approved from ethical committee of the institution. The drugs were administered once a day between 8 a.m. to 10 a.m. for all experiments.

The purified and concentrated horse serum was procured from Institute of Animal Health and Veterinary Biological, Bangalore. The investigative drug was obtained from The Himalaya Drug Company, Bangalore. All the other chemicals used in the study were of analytical grade. Cromolyn sodium was a generous gift from Cipla Ltd., Mumbai, India.

Preparation of E-721B

E-721B is a polyherbal formulation containing plants *Rhus succidanea* L (Anacardiaceae; galls–20%), *Solanum xanthocarpum* Schood et Wender. (Solanaceae; roots – 15%), *Tylophora indica* Bums. F. (Asclepiadeacae, leaves – 20%), *Albizzia lebbeck* Benth. (Leguminosae; bark – 25%), *Glycyrrhiza glabra* L (Papilionaceae; Roots – 5%) and *Achyranthes aspera* L. (Amaranthaceae; leaves- 5%). The plant constituents were powdered, weighed individually and then mixed in appropriate proportion.

Active anaphylaxis in rats

The rats were sensitized by injecting subcutaneously 0.5 ml horse serum along with 0.5 ml of triple antigen containing 20,000 million *Bordetella pertussis* organisms (5). The sensitized rats were divided into 4 groups of six animals each.

Group I rats received only vehicle (0.5% w/v sodium carboxy methyl cellulose in water) at a dose of 5 ml/kg orally and served as a control; group II rats received cromolyn sodium in saline (50 mg/kg per day, i.p.); group III rats received suspension of prednisolone in a vehicle (10 mg/kg per day, orally) (2). Group IV rats were treated with suspension of E-721B in a vehicle (250 mg/kg per day, orally). The drugs were administered once a day for 14 days. Dose response studies with E-721B revealed optimum effects at a dose of 250 mg/kg and hence, this dose was used in the present study (6)

On day 14, 2 hour after the assigned treatment, the rats were sacrificed by carotid bleeding under ether anesthesia and the intestinal mesentery was taken for studies on the mast cells. The mesenteries of the sacrificed rats along with pieces of intestine were kept in Ringer-locke solution

(NaCl 9.0, KCl 0.42, $NaHCO_3$ 0.15, glucose 1.0 gms/L of distilled water) at 37°C. The mesenteric pieces were challenged with 5% horse serum for 10 minutes after which the mast cells were stained with toluidine blue (2) and examined microscopically for the number of intact and degranulated mast cells in at least ten randomly selected high power fields (450 X).

Passive anaphylaxis in rats

One milliliter of the serum of the actively sensitized rats was injected intraperitoneally to 24 normal rats, which were divided into four groups of six each. Group I rats were injected with serum from control. Groups II, III and IV rats were injected with serum from cromolyn sodium, prednisolone and E-721B treated rats respectively.

Forty eight hours later, the passively sensitized rats were challenged by the intaperitoneal injection of 1 ml of horse serum. Ten minutes after the antigen challenge, the animals were sacrificed by decapitation and the intestinal mesenteries were collected in Ringer-locke solution. The intestinal mast cells was counted microscopically in at least 10 randomly selected high power fields.

Compound 48/80-induced mast cell degranulation in rats

The rats were divided into 3 groups of 6 animals each. Group I rats received vehicle (5 ml/kg of 0.5% sodium CMC orally); group II rats received cromolyn sodium (50 mg/kg per day, i.p); group III rats received suspension of E-721B (250 mg/kg per day, orally). The treatment was continued for 7 days. On day 7, 2 hours after the assigned treatment, the mast cells were collected from the peritoneal cavity (7, 8). The rats were anesthetized with ether and were injected with 10 ml of normal saline solution into peritoneal cavity and the abdomen was gently massaged for 90 seconds. The peritoneal cavity was carefully opened, and the fluid containing peritoneal mast cells was aspirated and collected in siliconised test tube containing 7 to 10 ml of RPMI-1640 Medium (pH 7.2-7.4). The mast cells were then washed thrice by centrifugation at low speed (400-500 rpm) and the pellet of mast cells was taken in the medium. The mast cells suspension approximately ($1x10^6$ cells/ml) was challenged with 0.5 µg/ml of compound 48/80 and stained with 1% toluidine blue and observed under high power microscopic field (450 X). The number of intact and degranulated cells was counted at least in ten different randomly selected fields.

Passive cutaneous anaphylaxis in rats

Cutaneous anaphylaxis (local) reaction may be specially elicited by antigen in the skin and other organs of the passively or actively sensitized animals. The effect of E-721B on passive cutaneous anaphylaxis (PCA) was studied in rats using homologous antiserum (9,10).

Homologous antiserum: Antiserum to horse serum was prepared by taking equal volumes of horse serum and complete Freunds adjuvant (Sigma Chemicals, USA) followed by thorough homogenization. Six albino rats were immunized with 0.5 ml of the above mixture subcutaneously and as well as intradermally at different sites. In addition, the rats also received 0.5 ml

of triple antigen by subcutaneous route. On day 14th the blood was collected from all the rats and serum containing heat labile homocytotropic (IgE Class) antibodies were separated and stored at –20°C.

Rat of either sex were taken for the study. The dorsal surface of the rats were shaved on both the sides, 0.1 ml of the rat homologous antiserum was injected to all the rats on shaved dorsal surface of one side and equal volume of saline was injected on the other side. The passively sensitized rat were divided into three groups of six each. Forty eight hours after sensitization, group I rats received vehicle (5 ml/kg of 0.5 sodium CMC orally): group II rats received single dose of cromolyn sodium (50 mg/kg per day, i.p) and group III rats received single does of E-721B (250 mg/kg per day, orally).

Two hours after the respective assigned treatment, the rats were lightly anesthetized with ether and mixture of 0.25 ml of 1% Evans blue dye and 0.25 ml of horse serum was injected via tail vein. After 30 minutes, the blue colored area of the skin (mm^2) was measured by taking two perpendicular longest diameter and percentage inhibition was calculated in comparison to the control group.

Schultz-dale response on sensitized guinea pig ileum preparation

The effect of formulation E-721B on sensitized guinea pigs ileum was studied (11). Fifteen guinea pigs were sensitized by two intraperitoneal injections of 0.5 ml of horse serum at 48 hours intervals. The sensitized guinea pigs were divided into 3 groups of 5 each. Group I animals received vehicle (5 ml/kg of 0.5% sodium CMC orally); group II animals were treated with cromolyn sodium (50 mg/kg per day, i.p) and group III animals were treated with E-721B (250 mg/kg per day, orally).

After sensitization the treatment was continued for 14 days. On the 15th day animals were euthanised under ether anesthesia and ileal strips from each animal were mounted in isolated organ bath (12). Tissue sensitivity was first tested with histamine. After recording the standard responses with histamine, 0.5 ml of the antigen (horse serum) was added to the bath and responses were recorded for 90 seconds using slow moving kymograph. The tissue was washed and histamine responses were again repeated. The height of contraction was recorded.

Synthesis of precipitating antibodies in rats by microagar gel immunodiffusion technique

The effect of E-721B on synthesis of antibodies was tested by the micro agar gel immunodiffusion method (13).

The agar plates were prepared by 1% agar gel in borate buffer solution (boric acid 9 gms, NaOH 2 gms, distilled water 1 liter) containing suitable bacteriostatic agent such as thiomersal or sodium azide (1 : 10000). Using 10 ml pipette the molten agar was poured on the clean glass microscopic slides to give depth of 1–2 mm of agar. The plates were solidified by keeping them at the temperature of 8–10°C and wells were made with the help of gel cutter.

Twenty four albino rats were sensitized by 0.5 ml of horse serum and 0.5ml of triple antigen containing 20,000 million *B. pertussis* organisms. The sensitized rats were divided into 4 groups six each. Group

I rats received only vehicle (5 ml/kg of 0.5% sodium CMC orally); group II rats received cromolyn sodium (50 mg/kg i.p.); group III rats received prednisolone (10 mg/kg orally) and group IV rats received E-721B (250 mg/kg orally).

The treatment was continued for 14 days. On day 14, 2 hours after the respective assigned treatment the rats were euthanised by carotid bleeding after ether anesthesia and serum was separated and used for testing the presence of precipitating antibodies by microagar gel immunodiffusion technique. The central well of the agar slide was charged with horse serum (antigen) and peripheral wells with serum samples of rats in different groups (control, cromolyn, prednisolone and E-721B treated). The charged slides were kept in a humidified atmosphere in humidity chamber at 4°C. The slides were examined for the precipitation lines between the central and peripheral wells. If no line appeared even after 5 days the result was taken as negative.

Statistical analysis

The result were expressed as Mean ± SEM and analyzed statistically using ANOVA followed by Dunnet's Student 't'-test to determine the effect of the drug.

RESULTS

Active anaphylaxis: The percentage disruption of mast cells in cromolyn sodium group, prednisolone group and E-721B treated groups were 19.0, 29.4 and 19.6 respectively as compared to 84.6 in the control group (Table I). This shows that all the drugs have significantly inhibited the mast cell disruption induced by an antigen(horse serum).

Passive peritoneal anaphylaxis: The serum of actively sensitized rats from control group was administered i.p. to fresh rats and challenged with antigen 48 hours later. The percentage disruption of peritoneal mast cells was found to be 76.3 in control group. The percentage disruption of peritoneal mast cell was significantly reduced in normal rats, which received serum from E-721B treated rats, as indicated by the marked increase in percentage of intact cells. Prednisolone, a reference drug also showed good protection on peritoneal mast cells of passively sensitized animals. In case of cromolyn sodium also statistically significant protection was observed (Table II).

TABLE I: Effect of E-721B on mast cell degranulation in activity sensitized rats.

Group	*Treatment*	*Mast cells %*		*Percent protection*
		Intact	*Disrupted*	
I	Control	15.40±1.07	84.60±1.07	–
II	Cromolyn sodium	81.00±0.67*	19.00±0.67*	77.54
III	Predinsolone	70.60±1.02*	29.40±1.02*	65.24
IV	E-721B	80.04±1.46*	19.60±1.46*	76.83

*P<0.001 as compared to Control. (Mean ± SEM of 6 animals in each group)

TABLE II: Effect of E-721B on mast cell degranulation in passively sensitized rats.

Group	Treatment+	Mast cells %		Percent protection
		Intact	Disrupted	
I	Control	23.70±2.09	76.30±2.09	–
II	Cromolyn sodium	33.10±2.50•	66.90±2.50•	12.32
III	Predinsolone	65.10±0.98*	34.90±0.92*	54.25
IV	E-721B	70.50±1.47*	29.50±1.47*	61.34

•P<0.01 and *P<0.001 as compared to Control. (Mean ± SEM of 6 animals in each group) +Serum from actively senstized rats were administered to respective groups.

Compound 48/80 induced mast cell degranulation: Compound 48/80 induced mast cell degranulation was significantly inhibited by E-721B and cromolyn sodium. E-721B and cromolyn sodium treated rat mast cells showed significant stabilizing activity when they were exposed to compound 48/80 (Table III).

Passive cutaneous anaphylaxis: The effect of E-721B on passive cutaneous anaphylaxis in rats revealed statistically no significant difference between the average diameter of the blue coloured (PCA reaction) wheal formation in E-721B treated group as compared to control Cromolyn sodium, a reference drug completely inhibited the PCA reaction in

TABLE III: Effect of E-721B on compound 48/80 induced mast cell degranulation in activity sensitized rats.

Group	Treatment	Mast cells %		Percent protection
		Intact	Disrupted	
I	Control	14.90±1.08	85.90±1.08	–
II	Cromolyn sodium	65.70±2.12*	34.30±2.12*	59.69
III	E-721B	71.10±2.12*	28.90±2.12*	66.03

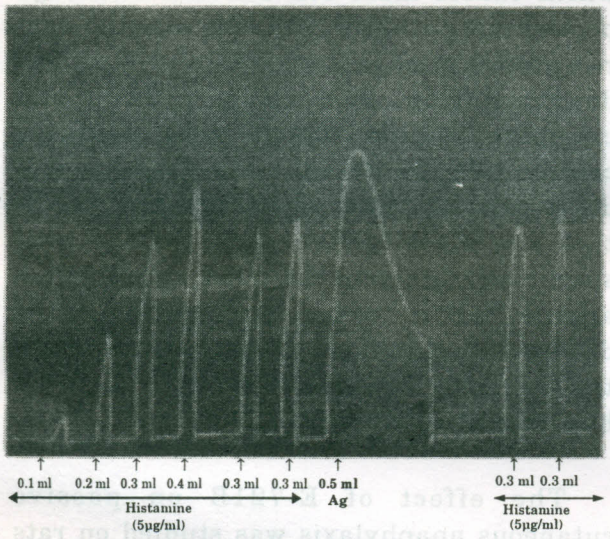
*P<0.001 as compared to Control. (Mean ± SEM of 6 animals in each group).

TABLE IV: Effect of E-721B on passive cutaneous anaphylaxis in rats.

Group	Treatment	Average area of blue colored wheal $(mm)^2$	Percent inhibition
I	Control	230.60±7.78	–
II	Cromolyn sodium	Nil	100
III	E-721B	246.20±9.38	–

rats (Table IV).

Schultz-Dale response on sensitized guinea pig ileum preparation: The *in vitro* anti-anaphylactic activity of E-721B was studied by Schultz-Dale reaction using sensitized guinea pig ileum smooth muscle preparation. It was found that the reaction was positive in all the control animals but negative in four out of five animals with E-721B for 14 days. However, the reaction was positive in all the animals treated with

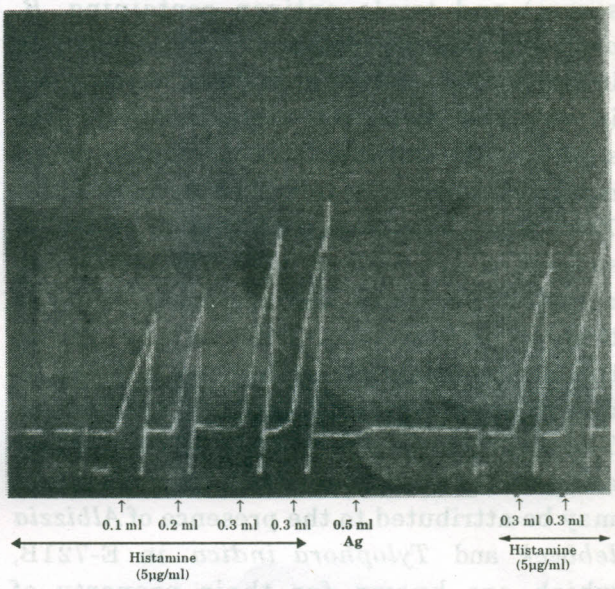


Ag : Antigen (Horse serum)

Fig. 1a : Showing the positive Schultz-Dale response in control animals.

cromolyn sodium for the same duration (Figs. 1a and 1b).

Microagar gel immunodiffusion technique: The above technique was adopted to study the effect of E-721B on production of antibodies. All the serum samples from control and cromolyn treated groups developed antigen-antibody precipitation lines against horse serum antigen, whereas five out of six prednisolone treated rats and there out of six E-721B treated rats did not show any precipitation line.



Ag : Antigen (Horse serum)

Fig. 1b : Showing the negative Schultz-Dale response in E-721B treated animals.

DISCUSSION

Immediate hypersensitivity is a state of rapidly developing immune response to antigen to which an individual is previously sensitized. Example of immediate hypersensitivity include systemic anaphylaxis, bronchial asthma, hay fever (seasonal allergic rhinitis), urticaria etc. (14). In the present study, E-721B an indigenous herbal combination was tested in different animal models of immediate hypersensitivity.

In case of active anaphylaxis, the effects of E-721B and other reference drugs on extent of degranulation of sensitized peritoneal mast cells of albino rats were studied when challenged with antigen (horse serum) and triple antigen containing *B. pertussis* as an adjuvant. The results indicate that the drug E-721B when used in selected dose and time intervals has significantly inhibited the antigen-induced mast cell degranulation. The activity of E-721B was almost equal to that of cromolyn sodium, a well known mast cell stabilizing agent. The activity was also comparable to that of prednisolone. This may be due to the direct mast cell stabilizing activity or due to suppression of antibody production. The inhibitory effect of E-721B against antigen-induced mast cell degranulation may be attributed to the presence of *Albizzia lebbeck* and *Tylophora indica* in E-721B, which are known for their property of preventing antigen induced mast cell degranulation (2,5).

In case of peritoneal passive anaphylaxis, the serum of the sensitized rats after two weeks of sensitization have been used for passive sensitization of recipient rats. This technique of sensitization of albino rats is highly successful and reproducible. The serum of the sensitized rats, which is presumed to contain the appropriate antibodies, was injected intraperitoneally to the recipient rats. It has been reported that the antibodies if present, get fixed to the peritoneal mast cells and when exposed to antigen show anaphylactic degranulation (2). The result of the passive peritoneal anaphylaxis indicate the suppressive action of E-721B on the production of antibodies of reaginic type (IgE type), which is responsible for Type 1 hypersensitivity diseases such as asthma. The suppression of antibody production by E-721B may be due to the presence of *Tylophora indica* and *Albizzia lebbeck*. *Tylophora indica* is also reported to possess immuno-suppressive and anti-inflammatory properties, which are due to the increased secretion of endogenous corticosteroids (2,15).

Compound 48/80 is a typical agent, which causes mast cell activation through peptidergic pathway (16). It is also the non-receptorial mechanism leading to mast cell degranulation. The drugs E-721B and cromolyn sodium significantly inhibited compound 48/80-induced mast cell degranulation. The above findings reveal that the investigative drug E-721B non-specifically protects the mast cells against agents, which cause degranulation by different mechanisms, thereby preventing the release of mediators of Type 1 hypersensitive diseases.

The effect of E-721B on passive cutaneous anaphylaxis was studied on rats using homologous antiserum. This test is widely used to screen compounds, which possess cromolyn sodium (DSCG) like properties (17). The drug E-721B, when used in the above mentioned dose, route and time intervals does not inhibit 48 hour PCA reaction (IgE-induced Type 1 immunologic reaction) in rats. This indicates that the single dose of drug does not have cromoglycate like properties.

The *in vitro* anti-anaphylactic activity of E-721B was studies by Schultz-Dale reaction using sensitized guinea pig ileum

preparation. Schultz-Dale response is extremely sensitive and displays a typical antigen-antibody reaction. When strips of intestinal or uterine muscle were excised from the sensitized guinea pig and suspended in a suitable physiological salt solution, the muscle contracts strongly on addition of homologous antigen to the solution (18). The negative Schultz-Dale response in animals treated with E-721B indicates that the drug had good anti-anaphylactic activity. However the response was positive in cromolyn treated group similar to the control group. This shows that cromolyn sodium had no anti-anaphylactic activity in guinea pigs and our observations are in agreement with earlier findings (19,20). The above findings reveal that while action of cromolyn sodium is species dependent, the same is not true for E-721B, which had a good anti-anaphylactic activity both in rats as well as in guinea pigs.

Agar gel immunodiffusion technique is the classic technique to demonstrate serological reaction in which the antigen and antibody diffuse from wells cut in the agar gel. When avid reactants meet, the reaction is manifested by the development of visible line of precipitate at the interface. In the present study, direct evidence of inhibition of antibody synthesis is provided, while in the sensitized control rats, antigen antibody precipitation could be demonstrated. There was no such precipitation reaction in three out of six E-721B pretreated sensitized rats, whereas five out of six prednisolone pretreated sensitized rats did not show any precipitation line. This shows the inhibitory effect of prednisolone and E-721B on production of precipitating antibodies in rats.

CONCLUSION

From the results of the various studies conducted on the drug E-721B, it appears that the drug has definite role in the treating various hypenrsensitivity such as asthma. The above results also indicate that E-721B exerts its anti-allergic activity on various stages of immediate hypersensitivity.

ACKNOWLEDGEMENT

The authors gratefully acknowledge The Himalaya Drug company, Bangalore, for their assistance and for providing the drug E-721B for the research work.

REFERENCES

1. Samuelsson B. Leukotrines: Mediators of immediate hypersensitivity reactions and inflammation. Science 1983; 220 (4597): 568–575.
2. Tripathi RM, Sen PC, Das PK. Studies on the mechanism of action of Albizzia lebbeck an Indian indigenous drug used in the treatment of atopic allergy. *J Ethnopharmacol* 1979; 1: 385–396.
3. Varier PS, editor. Indian Medicinal Plants, A compendium of 500 species, Vol. 1-5, Hyderabad, orient Longman Limited, 1996.
4. Nadkarni AK Dr. KM Nadkarni's Indian Materia Medica, Vol. 1-2, Bombay, popular prakashan, 1976.
5. Gupta SS, Tripathi RM Experimental studies on *Tylophora Indica. Aspects of Allergy and Applied Immunol* 1973; 6: 93–99.
6. Mitra SK, Gopumadhavan S, Venkataranganna MV, Anturlikar SK. Anti-asthmatic and anti-anaphylactic effect of E-721B, a herbal formulation *Indian J Pharmacol* 1999; 31: 133–137.

7. Gupta PP, Srimal RC. Anti-allergic activity of alkyl substituted pyrazolo (3.4-d) pyrimidine. *Indian J Expt Biol* 1995: 33: 38–40.

8. Lee YM, Kim SH, Kim DK, Shin TY, Kin HM. Anti-anaphylactic activity of *Poncirus trifoleata* fruit extract. *J Ethnopharmacol* 1996: 54: 77–84.

9. Adolphson RL, Finkel MP, Robichud LJ. C1-922, A novel potent anti-allergic compound-11, activity in animal models of allergy. *Intl J Immunopharmacol* 1987; 9: 51–60.

10. Agarwal MK, Shivpuri DN, Manju Gupta, Ray GN. Studies on the effect of *Tylophora indica* on the response of plain muscle to spasmogens and on the immunological reactions *in vivo* and *in vitro*. *Aspects of Allergy and Applied Immunol* 1975: 6: 107–115.

11. Tripati RM, Sen PC, Das PK . Further studies on the mechanism of anti- anaphylactic action of *Albizzia lebbeck* on Indian indigenous drug. *J Ethnopharmaco*l 1979: 1: 379–406.

12. Ghosh MN. Fundamentals of Experimental Pharmacology, Scientific Book Agency, Calcutta 1971: 27–29.

13. Crowle AJ. Applications of immunodiffusion. In: Immunodiffusion, New York, Academic press. 1961, pp 169–179.

14. Mohan H. Immunophysiology and immunopathology, In: 2nd Edition, New Delhi, Jaypee Brothers Medical Publisher Pvt. Ltd. 1994: p187.

15. Udupa AL, Udupa SL, Guruswamy MN. The possible site of anti- asthmatic action of *Tylophora asthmatica* on pituitary adrenal axis in albino rats. *Planta Medica* 1991; 57: 409–413.

16. Kassel O, Amran Y, Landry Y, Bronner C. Mast-cell activation involves plasma membrane potential and thapsigargin-sensitive intracellular calcium pools. *Fundamental Clin Pharmacol* 1995; 9: 531–539.

17. Kuriyama K, Ichikawa K. An experimental asthma model in un- anaesthetised rat and difference in effect of disodium cromoglycate between passive cutaneous anaphylaxis model and asthma model in rat. *J Pharmacodynamics* 1982: 5: 751–759.

18. Carpenter LP, Allergy, In: Immunology and serology, Philadelphia, W.B. Saunders Co., 1978; pp275–276.

19. Greaves MW. The effect of sodium cromoglycate and other ihhibitor on in vitro anaphylactic histamine realise from guinea pig basophil leucocytes. *Intl Arch Allergy and Applied Immunol* 1969; 36: 479–505.

20. Cox JS, Beach JE, Blair AM, Clarke AJ, Lee TB, loveday DE, Moss GF, Orr TS, Ritchie JT, Sheard P. Sodium cromoglycate (intal). *Adv Drug Res* 1970; 5: 115–196.