

**STUDIES ON ANTI-ASTHMATIC ACTIVITY OF  
AQUEOUS EXTRACT OF *CLERODENDRON PHLOMIDIS***

**Gautam P. Vadnere<sup>\*a</sup>, Rahul S. Somani<sup>b</sup>, Abhay K. Singhai<sup>c</sup>**

**a. Smt.S.S.Patil College of Pharmacy, Chopda-425 127(MS), India.**

**b. Sinhgad College of Pharmacy, Pune-411 041(MS), India.**

**c. Dept. of Pharm. Sci., Dr. HS Gour University, Sagar-470003(MP), India.**

**Summary**

The present study designed to evaluate the antiasthmatic activity of aqueous extract of *Clerodendron phlomidis* (AECF) on *in vitro* and *in vivo* animal models. Histamine-induced contraction in isolated goat tracheal chain showed that aqueous extract of *Clerodendron phlomidis* inhibited the contractile effect of histamine ( $P < 0.05$ ). A dose dependent contraction of goat tracheal chain is observed. Treatment with AECF ( $100 \text{ mg kg}^{-1}$ , *i.p.*) in mice ( $n=5$ ) decreased blood eosinophilia by 68% while mast cells were protected 74% from degranulation as compared to control group. Also, AECF decreased capillary permeability by 63% in mice was evident from its effect on optical density of the dye. Thus, AECF showed antihistaminic, mast cell stabilizing and decreased capillary permeability effect and hence possesses potential role in the treatment of asthma.

**Keywords:** *Clerodendron phlomidis*; antiasthmatic activity; eosinophilia; capillary permeability

\*Corresponding author: E-mail: [gautamvadnere31@rediffmail.com](mailto:gautamvadnere31@rediffmail.com)

## Introduction

Among several respiratory diseases affecting man, bronchial asthma is the most common disabling syndrome. Nearly 7–10% of the world population suffers from bronchial asthma. Despite the availability of a wide range of drugs, the relief offered by them is mainly symptomatic and short lived. Moreover the side effects of these drugs are also quite disturbing. Hence a continuous search is on going to identify effective and safe remedies to treat bronchial asthma. *Clerodendron phlomidis* (L.F.) family-Verbenaceae is deciduous and evergreen shrubs grow wild in tropical region of North Maharashtra in India. More than hundred species of *Clerodendron* have been discovered, but very few are in cultivation. In Indian system of medicine as leaf juice believed to have function in management of asthma. The family of *Clerodendron* has yielded several secondary metabolites; most notably terpenoides, steroids and flavonoids [1]. Several species have been evaluated for their claimed medicinal properties in traditional medicine. The aqueous extract of *Clerodendron phlomidis* (AECF) shows significant anti ulcer effect in rats [2]. Whereas the methanolic extract inhibit castor oil induced diarrhea [3]. *Clerodendron indicum* methanolic extract is a potent inhibitor of lipid peroxidation [4]. Leaf juice of the plant widely used by practitioners of the Indian system of medicine to treat respiratory diseases [5]. The present study was undertaken as no such effort have made till time to evaluate the claimed therapeutic effect *i.e.* asthma relieving or antispasmodic property of *Clerodendron phlomidis*, *in vitro* on isolated goat tracheal chain and *in vivo* studies on milk- induced eosinophilia, mast cell degranulation and capillary permeability in mice.

## Methods

### Collection of plant material

The fresh leaves of *Clerodendron phlomidis* were collected in the month of June 2005 near Chardi village Chopda Dist. Jalgaon, MS, India. Dr.A.K.Singhai, Dept. of Pharmaceutical Sciences Dr.H.S. Gour University, Sagar authenticated the plant material. A voucher specimen (No. 28575) was deposited in the Botanical Survey of India, Pune, India.

### Extraction of plant material

*Clerodendron phlomidis* leaves were shed dried and reduced to coarse powder using pastel and mortar. The powdered material (1000 g) was macerated overnight with purified water at room temperature. The maceration was repeated twice. The filtered extracts were combined and evaporated under reduced pressure (yield 7.9 %w/w). The phytochemical test of the crude extract showed the presence of flavonoids, terpenoids and steroids.

### Animals

Male albino mice (Swiss strain) weighing 22- 25 g were housed under standard laboratory condition in a group of five each. Animals had free access to food and water. The Institutional Animal Ethical committee (IAEC) has approved the protocol of the study.

### **Chemicals**

Histamine Sigma USA, Clonidine Unichem, India, Disodium cromoglycate Cipla, RPMI Buffer medium 1640 of Hi Media was purchased. Reagents like Eosin solution WBC diluting fluid was purchased from Qualigens India.

### **Goat tracheal chain preparation**

Goat trachea brought from slaughterhouse was cut into individual ring and tied together in series to form a chain [6]. It was suspended in a bath containing Kreb's solution (Concentration in mMoles/ L as NaCl, 118; KCl, 4.7; CaCl<sub>2</sub>, 2.5; MgSO<sub>4</sub>, 1.2; NaHCO<sub>3</sub>, 25.0; KH<sub>2</sub>PO<sub>4</sub>, 1.2; Glucose, 11.1) maintained at 37 ± 1<sup>0</sup>C a stream of CO<sub>2</sub> in O<sub>2</sub> was bubbled through the organ tube. One end was tied to aerator tube and other attached to isotonic frontal writing lever to Kymograph paper on Sherrington rotating drum. Tissue was allowed to equilibrate for 45 min. under to load of 400 mg [6]. A dose response curve for histamine was taken in variant molar concentration. After obtaining a dose response curve of histamine on goat trachea aqueous solution of extract (n= 4) was added to reservoir and same dose of histamine were repeated.

### **Milk- induced eosinophilia**

A blood eosinophilia is hallmark of both allergic and non allergic asthma. Mice were divided into five groups, five animals each. Blood samples were collected from retro-orbital plexus under light ether anesthesia, the eosinophil count was done in each group before drug administration and 24 h after the milk injection (boiled and cooled, 4 ml/kg, s.c.). Difference in the eosinophil count before and 24 h after milk administration was noted using modified method described by Brekhman [7].

### **Mast cell degranulation**

Mice divided in five groups, five animals each. The three days drug treatment schedule was followed. Group I received vehicle (10 ml/kg, i.p.). Group- II treated with standard drug disodium cromoglycate (DSCG, 200µg/ml, i.p.). Group-III, IV and V were treated with extract 25, 50 and 100 mg/kg, i.p. respectively. On day fourth each animal were injected with 4 ml/kg, 0.9% NaCl solution into peritoneal cavity. By gentle massage, peritoneal fluid was collected after 5 min. and transferred into siliconised test tube containing 7-10 RPMI- 1640 buffer medium (pH 7.2- 7.4). This solution was then centrifuged at 400- 500 rpm. Pellets of mast cell were washed with same buffer medium twice by centrifugation, discarding supernatant. The cells were challenged with clonidine (50 µg) incubated at 37 <sup>0</sup>C in a water bath for 10 min. Followed by staining with 1 % toluidine blue and observed under microscope (45 X). Total 100 cells were counted from different visual area. Percent protection against degranulation was calculated using method described by Lakdawala [8].

### **Capillary permeability**

Mice were divided into four groups of five animals each. The mice in the control group were sensitized with bovine albumin and Freund's adjuvant 0.05 ml given i.p. Three weeks later the animals were challenged with the same dose of bovine albumin. At the same time Evan's blue injected i.v. in a dose of 200mg/kg. The mice were sacrificed. 30 min. later 5 ml of saline was injected i.p. and the abdominal wall was gently massaged for a minute. The abdomen was then incised and peritoneal fluid was collected and filtered after passage through glass wool. It was centrifuged at 3000 rpm for 15 min. The group II, III and IV received AECF in a dose of 25, 50 and 100 mg/kg, i.p. 24 h and 2 h before the challenge. The transmittance of the dye depends on capillary permeability was

determined by measuring the optical density on a spectrophotometer by modification of the method used by Tagoki and Fukao, [9].

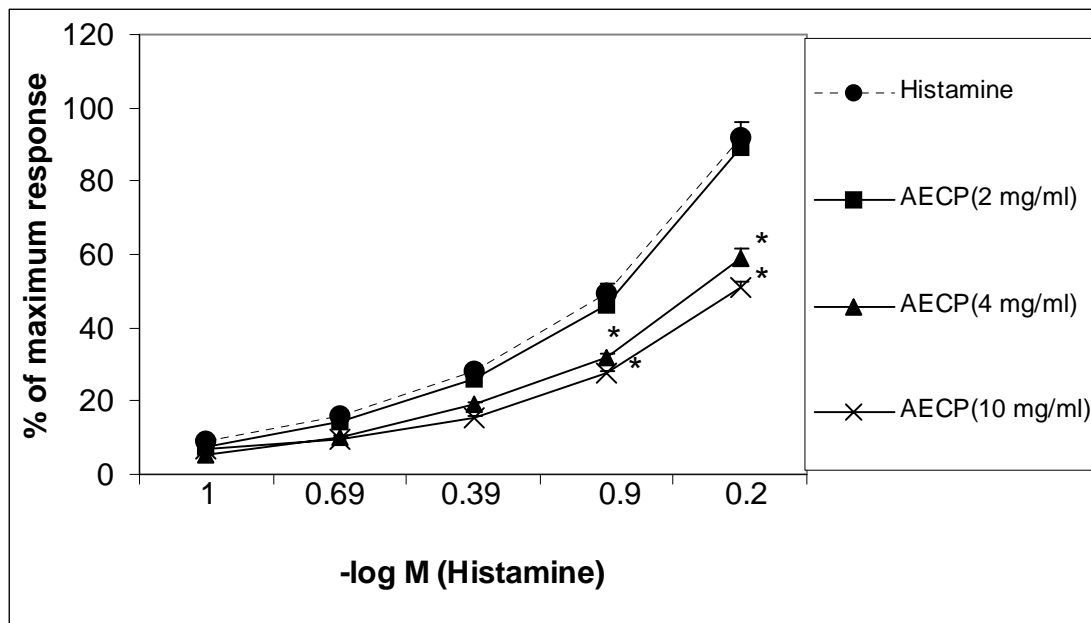
#### Statistical analysis

The data is presented as mean $\pm$ SEM. The statistical significance between the groups was tested by ANOVA followed by Dunnett's test. A probability values less than 0.05 were considered as significant.

### Results

#### Effect of AECP on isolated goat tracheal chain

Histamine at a dose of 1.6  $\mu$ g/ml was able to produce notable contraction on isolated goat tracheal chain (82 mm taken as 100 %). Goat tracheal chain is known as a sensitive tissue for studying the effect of histamine. We studied the response produced by histamine in this tissue in presence of the plant extract. AECP exerted antagonistic effect on histamine-induced contraction ( $p < 0.05$ ). Significance seen at a dose of 4 mg/ml and 10 mg/ml ( $59.14 \pm 2.70$  and  $50.91 \pm 1.81$  respectively) Figure 1 is indicative of this activity might be due to relaxant effect exerted by AECP i.e. depression of histamine H<sub>1</sub> receptor.



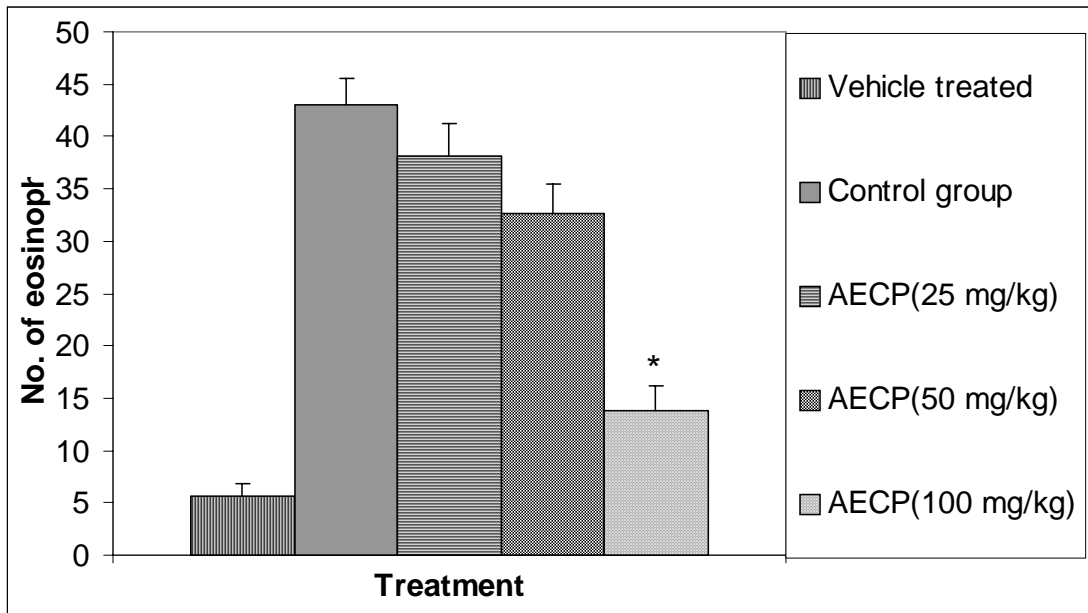
**Figure 1: Effect of AECP on isolated goat tracheal chain**

n= 4, values are expressed in mean $\pm$ SEM.

\* $p < 0.05$  compared with histamine induced contraction (82 mm taken as 100 %).

#### Effect of AECP on milk induced eosinophilia

Mice treated with of milk (boiled and cooled, 4 ml/kg, s.c.) showed a significant increase in total eosinophil count. AECP (100 mg/kg, i.p.) treatment significantly ( $p < 0.05$ ) reduced milk induced eosinophilia ( $13.8 \pm 2.4$ ) as compared control group ( $43.1 \pm 1.25$ ). Figure 2 is suggestive of relevance of AECP in antagonizing the milk induced blood eosinophilia.



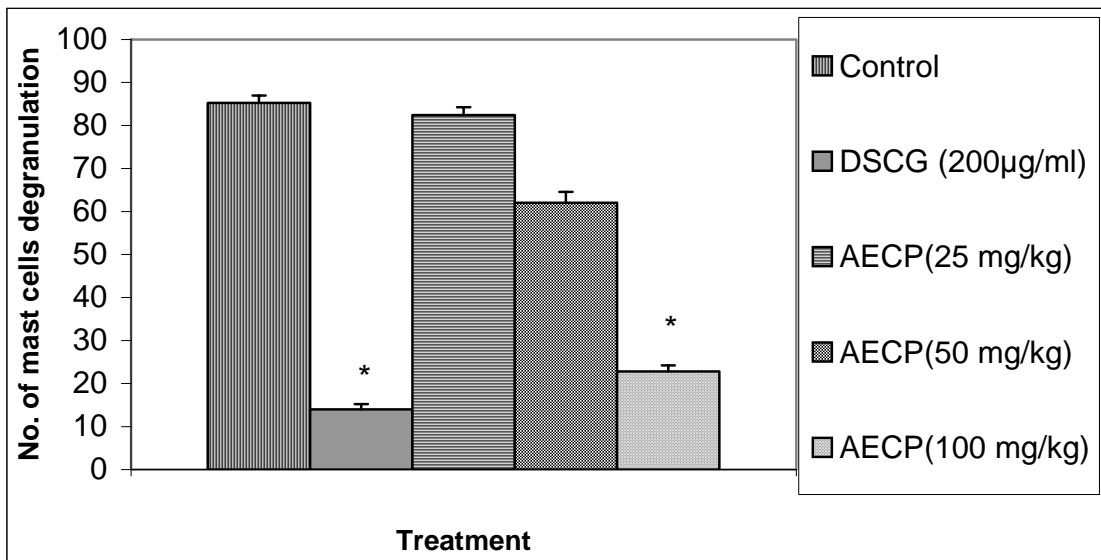
**Figure 2: Effect of AECP on milk induced- eosinophilia in mice**

n= 5, values are expressed in mean±SEM.

\*p< 0.05 compared with control group (ANOVA followed by Dunnett’s test).

**Effect of AECP on mast cell degranulation**

Clonidine challenge resulted in significant degranulation of mast cell. Pretreatment of sensitized animal with standard drug DSCG showed protection 83.57% and AECP at a dose of (100 mg/kg, i.p) showed percentage protection of 73.25% as in figure 3.



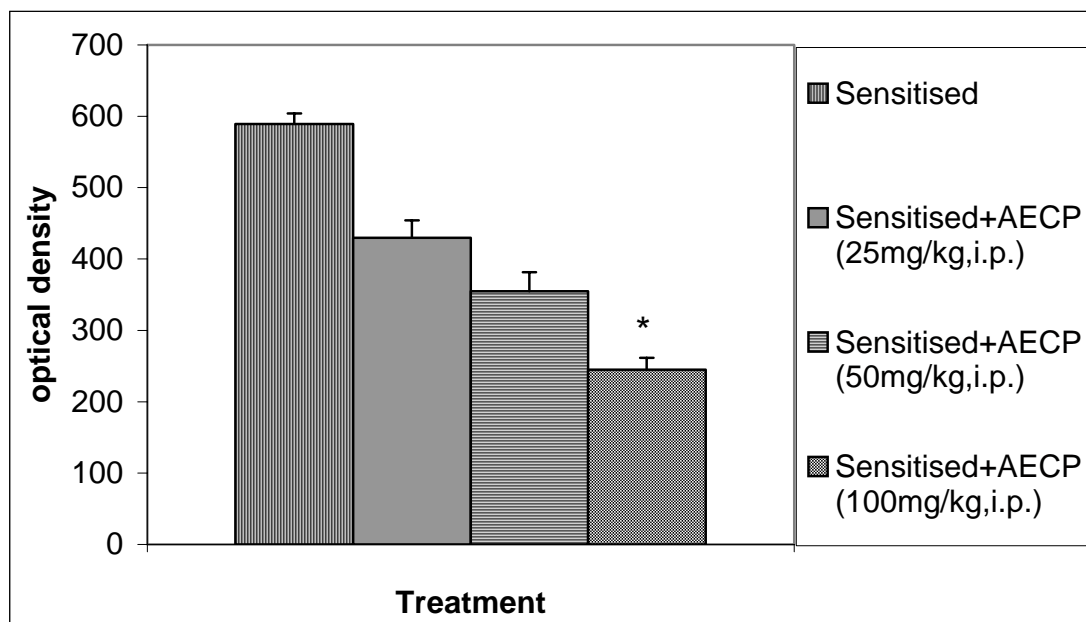
**Figure 3: Effect of AECP on mast cell degranulation in mice**

n= 5, values are expressed in mean±SEM.

\*p< 0.05 compared with control group (ANOVA followed by Dunnett’s test).

**Effect of AECP on capillary permeability**

The data in controlled group showed higher percent transmittance of the dye. The treatment of AECP (100 mg/kg, i.p) significantly decreased the percentage transmittance as is evident from its effect on optical density of the dye (Figure 4).



**Figure 4: Effect of AECP on capillary permeability in mice**

n= 5, values are expressed in mean±SEM.

\*p< 0.05 compared with sensitised group (ANOVA followed by Dunnett's test).

### Discussion

Histamine contracts the tracheobronchial muscle of dog, horse, guinea pig and man [10]. Sensitivity of goat trachea to various drugs / agents has been studied by [06]. The dose dependent contraction by spasmogens such as histamine (0.1-102.4 mg), acetylcholine (0.1-12.8 µg), and barium chloride (0.1-51.2 mcg) using goat tracheal chain preparation demonstrated by Kulshreshtha [11]. They have reported antihistaminic effect (H<sub>1</sub>-antagonism) of mepyramine maleate by using goat tracheal chain preparation. Histamine antagonism modulated by the relaxing factors involved and may be due to the suppression of histamine H<sub>1</sub>-receptor [12]. The present study was carried out to investigate the potential of aqueous extract of leaves of *Clerodendron phlomidis*.in asthma and showed significant (p< 0.05) antihistaminic activity in histamine induced contraction in goat tracheal chain preparation. Dose of AECP 2 mg/ml showed no such significant relaxation.

A blood eosinophilia is hallmark of both allergic and non-allergic asthma. Activated eosinophil cause desquamation and damage to respiratory epithelial cells. The eosinophil count increases in body fluids and tissues, emphasis is placed on the number of eosinophils in blood. Eosinophilia is associated with respiratory disorder, often allergic in nature together with pulmonary infiltrates that are detectable on chest films [13].

A recurrent milk aspiration produces changes in airway mechanics; lung eosinophilia in animal model. The observations of the present study indicate that AECP significantly reduced milk-induced eosinophilia.

Uvnas [14] studied the mast cell degranulation and its correlation with the release of histamine after administration of compound 48/ 80, the mast cell degranulating agent. Both clonidine and compound 48/80 act through the dynamic expulsion of granules without causing any damage to the cell wall [15]. Lakadawala [08] have shown that clonidine releases histamine from mast cells in a similar manner to a selective liberator like compound 48/80. It is known that disodium cromoglycate a standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate [16]. It has been known that all pharmacological agents that increase intracellular levels of AMP relax airway smooth muscle and inhibit the release of autocoids from the tissue and basophils. Present study shows statistically significant stabilization of mast cell by AECP.

Histamine and 5 HT are known to be principle mediators of allergy [17]. The transmittance of the dye (Evans blue) depends on capillary permeability was determined by measuring the optical density on a spectrophotometer by modification of the method used by Tagoki and Fukao [9]. The treatment group of AECP (100 mg/kg, i.p) significantly decreased the percentage transmittance as is evident from its effect on optical density of the dye.

All the above findings lend credence to the beneficial use of aqueous extract of *Clerodendron phlomidis* (AECP) in the treatment of asthma and related conditions. However, further studies with other experimental models, especially to explore the role of cytokines are warranted to substantiate the antiasthmatic and antiallergic activity of *Clerodendron phlomidis*.

### References

- [1] Glasby JS. Dictionary of Plants containing secondary metabolites. Taylor and Francis Ltd., London, 1991, 59.
- [2] Tan PV, Nijimi CK, Ayafor JF. Screening of some African medicinal plants for antiulcerogenic activity. *Phytotherapy Research* 1997, 11 (1):45-47.
- [3] Rani S, Ahmed N, Rajaram S, Saluja R, Thenmozhi S, Murugesan T. Anti-diarrhoeal evaluation of *Clerodendron phlomidis* leaf extract in rats. *J Ethnopharmacol* 1999, 68 (1-3):315-319.
- [4] Kumar S, Mullar K. Medicinal plants from Nepal II. Evaluation of inhibitor of lipid peroxidation in biological membranes. *J Ethnopharmacology* 1999, 64 (2):135-139.
- [5] Nadkarni, Indian Materia Medica Vol. I&II, Popular Book Depot. Mumbai. 1954, 87.
- [6] Nag Chaudhari AK, Lahiri SC. Use of goat trachea for an isolated tracheal chain preparation. *Indian J Pharmacol* 1974, 6:149-151.
- [7] Brekhman II, Dardymov IV. New substances of plant origin which increases nonspecific resistances. *Annu Rev Pharmacol* 1969, 9:419-430.
- [8] Lakadawala AD, Dadkar MK, Dohadwala AN. Action of clonidine on mast cells of rat. *J Pharm Pharmacol* 1980, 32:790-791.

- [9] Tagoki K, Fukao T. Effect of some drugs on capillary permeability in anaphylaxis of the mouse. *Japanese J Pharmac* 1971, 2 :455-465.
- [10] Ehrright T, Chua S, Lim DJ. Pulmonary eosinophilia syndromes. *Ann J Allergy* 1989, 62:277-83.
- [11] Kulshreshtha S, Misra SS, Sharma AL, Sharma P, Singhal D. Response of the goat trachea to some autonomic drugs. *Indian J Pharmacol* 1983, 15:107-110.
- [12] Shi CC, Liao JF, Chen CF. Spasmolytic effect of three harmala alkaloids on guinea pig isolated trachea. *Pharmacol Toxicol* 2001, 89 (5):259-264.
- [13] Ehrright T, Chua S, Lim DJ. Pulmonary eosinophilia syndromes. *Ann J Allergy* 1989, (62):277-283.
- [14] Uvnas B. Mast cell and Histamine release. *Indian J Pharmacol* 1969, 1: 23-24.
- [15] Stanworth DR, Immediate Hypersensitivity, In: *The molecular basis of allergic response*, edited by Neuberger A, Tatum EL, North Holland Publishing Company, Amsterdam, 1973, 73.
- [16] Geetha VS, Viswanathan S, Kameswaran L, Comparison of total alkaloids of *Tylophora indica* and effect of disodium cromoglycate on mast cell stabilization. *Indian J Pharmacol* 1981, 13:199-201.
- [17] Nayampalli SS, Desai NK, Ainapure SS. Antiallergic properties of *Tinospora cordifolia* in animal models. *Indian J Pharmacol* 1986, 18:250-252.