

**IN VIVO AND IN VITRO ANTI-ASTHMATIC STUDIES OF
Clerodendrum serratum Linn**

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Summary

Ethanollic extract of roots of *Clerodendrum serratum* Linn(Verbenaceae) was evaluated for antiasthmatic activity by employing isolated goat tracheal chain preparation (in-vitro), Clonidine induced catalepsy, Milk induced leucocytosis and eosinophilia studies (in-vivo) in mice. The results of the study revealed that the ethanolic extract produced significant dose-dependent antiasthmatic activity at 50, 100 and 200mg/kg p.o

Keywords: Antiasthmatic activity, Catalepsy, *Clerodendrum serratum*, Total Leukocyte count.

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Introduction

Clerodendrum Serratum Linn. (Verbenaceae), known as bharangi in ayurveda and Sirutekku in siddha system of medicine, is traditionally claimed to be useful in treating pain, inflammation, rheumatism, respiratory diseases, and malarial fever¹. Owing to its importance in traditional medicine the plant was investigated for its anti-inflammatory, analgesic, antipyretic² and hepatoprotective properties³. The plant is reported to contain β -sitosterol, 24(S)-ethyl cholesta-5,22,25-trien-3 β -ol, 5-hydroxy-7,4'-dimethoxy flavone, luteolin, apigenin, scutellarien, ursolic acid and two iridoid glucosides namely 7 β -coumaroyloxyugandoside and 7 β -cinnamoyloxyugandoside.⁴The vast ethnomedical uses of the plant inspired us to investigate the anti asthmatic potentials of the plant

Materials and Methods

Plant Material and Extraction:

The plant was collected from foothills of Sinhagad Pune(India), and was authenticated from Botanical Survey of India, Pune with voucher specimen no. SSBC1. The roots were dried under shade and coarsely powdered and passed through 40 mesh sieve. The powdered material (500g) was extracted with ethanol using Soxhlet apparatus. The extract obtained was dried in rotary vacuum evaporator at 400 C, yielding a dark brown colored viscous mass 50g (10.0%).

Animals

Isolated adult goat tracheal tissue and albino mice (Wistar Strain) of either sex weighing 20-25 g were used for studies. The tissue was obtained immediately after slaughter of the animal. Pieces of the trachea were collected in the ice cold oxygenated Krebs solution. The albino mice were obtained from animal house of National Chemical Laboratory, Pune (India) and were housed for 2 weeks prior to the experiment for acclimatization in the animal house of Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune 411018, India. Animals were maintained under controlled conditions of temperature $26 \pm 2^\circ\text{C}$, relative humidity 44-56%, and photo-schedule (12 h light and 12 h dark). Animals were provided with standard diet (Amrut feeds, Mumbai, India) and water ad libitum. The food was withdrawn 18 h, before the start of the experiment. Institutional Animal Ethics Committee approved the experimental protocol (198/99/CPCSEA). The pharmacological work was carried out as per norms of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Acute toxicity studies:

Mice were selected for this study. They were divided into eight groups each containing six animals. Alcoholic extract of *C.serratum* was administered orally in varying doses (0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.50g/kg) to these animals. They were continuously observed for 2h to detect changes in the autonomic or behavioral responses like alertness, spontaneous activity, irritability, urination, etc. Any mortality during experimentation and the following 7 days was also recorded. A group of animals treated with vehicle (distilled water) was served as control. Based on the results of preliminary toxicity testing the doses of 50, 100 and 200mg/kg p.o were chosen for further experiments.

Antiasthmatic Activity:**1) Isolated goat trachea chain preparation**

Isolated adult goat tracheal tissue was obtained immediately after slaughterhouse of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Krebs solution and was continuously aerated at $37 \pm 0.5^\circ\text{C}$. Dose response curve (DRC) of histamine in plane Krebs solution and in 80 $\mu\text{g/ml}$ *Clerodendrum serratum* extract in Krebs solution was taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of drug extract^{5,6}.

2) Clonidine induced catalepsy in mice

Albino mice were divided into five groups ($n = 5$). Control group received saline (10ml/kg) and other groups received single dose of extract (50,100,200 mg/kg p.o. Body weight) respectively. Chlorpheniramine maleate (10 mg/kg, i.p.) was used as standard. All the groups received clonidine (1 mg/kg s.c.) one hour after the drug administration and the duration of catalepsy was measured at 15, 30, 60, 90, 120, 150 and 180 min^{7,8}.

3) Milk- induced eosinophilia and leucocytosis:

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 retroorbital plexus under light ether anesthesia, the eosinophil and total leucocyte count is 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 and total leucocyte count before and 24 h after milk administration was noted using modified method which is previously described^{8,9,10}.

Statistical Analysis

The statistical analysis was performed by using one-way analysis-of-variance (ANOVA) Followed by Dunnett's test for individual comparison of groups with control.

Results**1) Isolated goat trachea chain preparation**

Clerodendrum serratum extract inhibited contraction produced by histamine in the tissue preparations. Histamine (50 $\mu\text{g/ml}$) was taken in different dose level and DRC was plotted. Study revealed that *Clerodendrum serratum* extract exhibits significant ($p < 0.01$) percentage decreased contraction at concentration 80 $\mu\text{g/ml}$ in goat tracheal chain preparation Dose dependent response relationship was seen. (Table-1)

Table 1) Effect of *Clerodendrum serratum* extract on histamine induced contraction on isolated goat tracheal chain preparation.

	Dose of Histamine (50 µg/ml) (ml)	Control group % max. Contraction (Mean ± SEM)	Test group % max. Contraction (Mean ± SEM)
1.	0.1	25±0.8944	9.72±0.4944**
2.	0.2	45.13±1.108	21.5±0.6009**
3.	0.4	70.48±1.925	31.25±1.118**
4.	0.8	77.08±1.204	46.5±1.249**
5.	1.6	99.29±1.905	53.45±0.9458***
6.	3.2	100±2.017	54.83±1.138***

n = 6

Values are in Mean ± SEM.

Control = D.R.C. of Histamine in absence of *Clerodendrum serratum* extract.

Test = D.R.C. of Histamine in presence of *Clerodendrum serratum* extract.(80µg/ml)

Statistical analysis done by using Student's 't'-test. **p<0.01, significantly different from control.

2) Clonidine induced catalepsy in mice:

Clonidine (1mg/kg, s.c.) produced catalepsy in mice, which remained for 3 hours. The vehicle treated group has shown maximum duration of catalepsy (78.8 ± 14.548) at 180 minute after the administration of clonidine. There was significant inhibition (**p<0.01) of Clonidine induced catalepsy in the animal pretreated with *Clerodendrum serratum* extract (50,100,200 mg/kg, p.o.) and the duration of catalepsy was found to be 44 ± 2.966 , 32.8 ± 2.728 and 27 ± 3.108 seconds respectively. Chlorpheniramine maleate, (10 mg/kg, i.p.) treated group significantly reversed (p<0.01) the Clonidine induced catalepsy in mice.

Table 2: Effect of *Clerodendrum serratum* on clonidine induced catalepsy in mice.

Groups	15min	30 min.	60min	90min	120min	150min	180min
CONTROL	41.8 ± 1.655	59.4 ±6.425	66.6 ±5.972	74.4 ±3.487	83.8 ±2.596	100.2 ±5.962	78.8 ±14.548
STD	16.8 ± 1.068	15.8 ±1.02**	20 ±2.345**	26.4 ±4.434**	26.6 ±4.366**	23 ±2.775**	16.2 ±2.709**
RET 50	24 ±7.127	39.6 ±3.385*	35.8 ±2.478**	51.4 ±9.244	38.5 ±6.478**	46 ±2.793**	44 ±2.966**
RET 100	26.8 ±6.135	26.6 ±3.059**	28.6 ±2.358**	39.6 ±6.99**	35.8 ±4.598**	36.6 ±2.926**	32.8 ±2.728**
RET 200	26.6 ±4.925	32.2 ±2.672**	33.6 ±1.99**	38 ±1.378**	34 ±1.612**	32 ±3.277**	27 ±3.108**

Where, n=6,

Control = Distilled Water (10 ml/kg, p.o.)

STD= Chlorpheniramine maleate (10 mg/kg, i.p.)

RET 50 = Ethanolic extract of *Clerodendrum serratum* root (50 mg/kg, p.o.)

RET 100 = Ethanolic extract of *Clerodendrum serratum* root (100 mg/kg, p.o.)

RET 200 = Ethanolic extract of *Clerodendrum serratum* root (200 mg/kg, p.o.)

Statistical analysis done by ANOVA followed by Dunnett's test.

*p<0.05, **p<0.01, compared to control group.

3) Milk- induced eosinophilia and leucocytosis:

Mice treated with of milk (boiled and cooled, 4 ml/kg, s.c.) showed a significant increase in total eosinophil and count. Ethanol extract *Clerodendrum serratum* at dose of 50, 100 and 200 mg/kg, p.o., mg/kg, i.p.) significantly (**p< 0.01) reduced milk induced eosinophil and leucocyte count as compared control group which receives only vehicle and milk (Table 3).

Table 3: Effect of ethanol extract of *Clerodendrum serratum* on milk induced leucocytosis and eosinophilia in mice.

Groups	Difference in no. of eosinophils (per cu mm) (Mean ± SEM))	Difference in no. of Leucocytes per cu mm (Mean ± SEM))
Control	18±2.51	78±8
Intoxicated	146.6±4.686##	4100±222.36 [#]
RET 50	88.6±4.622**	3160±160**
RET 100	64.6±4.493**	2300±130.38**
RET 200	44.6±4.686**	1460±116.62**

Where, n=6,

Control = Distilled Water (10 ml/kg, p.o.)

Intoxicated = Distilled water (10 ml/kg, p.o.) + Milk (4 ml/kg, s.c.)

RET 50 = Ethanolic extract of *Clerodendrum serratum* root (50 mg/kg, p.o.) + Milk (4 ml/kg, s.c.)

RET 100 = Ethanolic extract of *Clerodendrum serratum* root (100 mg/kg, p.o.) + Milk (4 ml/kg, s.c.)

RET 200 = Ethanolic extract of *Clerodendrum serratum* root (200 mg/kg, p.o.) + Milk (4 ml/kg, s.c.)

Statistical analysis was performed by using Student's t-test (Intoxicated Group were compared with Control Group) and ANOVA followed by Dunnett's test.

Discussion

Histamine contracts the tracheo-bronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is easier to handle and to prepare; it is also much more sensitive than guinea pig tracheal chain. Therefore, the dose relative contractile responses of different agonists like ACh, histamine, 5-hydroxytryptamine and bradykinin can be observed in isolated goat trachea⁴. In the present study the isolated goat tracheal chain preparation; there is right side shift of Dose Response Curve (DRC) of histamine in the presence of *Clerodendrum serratum* ethanolic extract indicating antiasthmatic action¹¹.

Clonidine, a α_2 -adrenoreceptor agonist induces dose dependent catalepsy in mice, which is inhibited by histamine (H_1) receptor antagonists but not by H_2 receptor antagonist. It is known that Clonidine releases histamine from mast cells. Brain histamine does play a definite role in the production of the extra pyramidal motor it has been suggested that the cataleptic effect of Clonidine in the mouse be mediated by histamine (via H_1 receptors) which is released from brain mast cells in response to stimulation of α_2 adrenoreceptors by Clonidine¹². The extract also significantly inhibited the clonidine induced catalepsy. The inhibition of clonidine induced catalepsy by *Clerodendrum serratum* may be due to the potential to antagonize H_1 receptor or inhibition of mast cell degranulation induced by clonidine.

Eosinophil have special propensity to collect in tissues in which allergic reactions occur, this is caused at least partly by the fact that many mast cells and basophils participate in allergic reactions. The mast cells and basophils release an eosinophil chemotactic factor that causes eosinophils to migrate toward the inflamed allergic tissue. The eosinophils are believed to detoxify some of the inflammation-inducing substances released by the mast cells and basophils and probably also to phagocytize and destroy allergen-antibody complexes, thus preventing excess spread of the local inflammatory process¹³.

After parenteral administration of milk there is increase in total leucocyte count and this stressful condition can be normalized by administration of an antistress or adaptogenic drug. It was demonstrated that there is increase in leucocyte count after parenteral administration of milk (4 ml/kg, s.c.)¹⁴.

In the present study the vehicle treated group of mice, after parenteral administration of milk (4 ml/kg, s.c.) showed significant increase in leukocyte and eosinophil count after 24 hour, where as the groups in which fractions of methanol extract of *Clerodendrum serratum* was administered, have shown normalization of leukocyte and eosinophil count. This indicates the antiallergic activity of fractions of the Methanol extract of *Clerodendrum serratum*.

In conclusion the present study confirmed that the ethanolic extract of *Clerodendrum serratum* roots exhibits significant dose dependent anti - asthmatic activity in various in-vitro and in-vivo animal models and supports the traditional claim of plant in the treatment of asthma. The toxicity studies of the plant suggest that it has reasonable safety margin justifying its wide application in various communities and lack of any reported side effects with traditional use of this plant. Further studies are infact underway to isolate and characterize the active principle responsible for the anti - asthmatic activity.

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