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IN-VITRO AND IN-VIVO ANTI ASTHMATIC ACTIVITY OF *CLERODENDRUM PHLOMIDIS LINN.* IN GUINEA PIGS

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ABSTRACT

Objective: To study the In-vitro and In-vivo Anti asthmatic Activity of *Clerodendrum phlomidis Linn.* . (Family: Verbaneceae) in Guinea Pigs

Methods: The ethanolic extract of leaves of *Clerodendrum phlomidis was* administered orally at the dosage levels of 100, 200, mg/kg/day and 400 mg/kg/day body weight in Guinea Pigs. The assessment of Anti asthmatic Activity activity were studied by isolated guinea pig ileum preparation, histamine induced bronchoconstriction and Histamine induced bronchoconstriction in Guinea pig has performed.. The significantly prolonged the latent period of convulsions followed by exposure to histamine aerosol at the dose of 400mg/kg, (per oral)

Results: The results of the present study Isolated guinea pig ileum preparation ethanol extract of *Clerodendrum phlomidis* exhibited significant (***p< 0.01, <0.001) percent decreased contraction at 100mg/ml in isolated guinea pig ileum preparation. (Table: 1, Figure: 1) Histamine induced bronchoconstriction in Guinea pig. The ethanol extract of leaves of *Clerodendrum phlomidis* significantly prolonged the latent period of convulsions followed by exposure to histamine aerosol at the dose of 400mg/kg, (per oral) and Showed maximum protection of 59.04 % at 4th hour as compared to Chlorpheniramine maleate (Standard) 1mg/kg, (per oral) (p.o). This offered maximum protection of 65.04 % at 4th hour. (Table 2 & 3, Figure: 2&3).

Conclusion: In the present investigation, it can be concluded from the result obtained that *Clerodendrum phlomidis* possesses significant dose-dependent anti asthmatic activity at 100,200 and 400mg/kg (per oral).

Key Words: *Clerodendrum phlomidis* Linn, Histamine, Chlorpheniramine maleate, Histamine Chamber.

INTRODUCTION

Clerodendrum phlomidis (Verbaneceae) known as Arni Hindi. It is distributed More or less throughout India, Ceylon, and Malay, Peninsula. The sandals rub the plant over their bodies in dropsy and also give it to their cattle to cure them of diarrhea and worms, or when the stomach swells (1). The decoction of roots is used as a demulcent in gonorrhea. The juice of leaves is used as bitter tonic (2) and also given in neglected syphilitic complaints (3). The plant has been found to possess hypoglycemic activity (4). The ethanol extract of *Clerodendrum phlomidis* Linn. A leaf shows most of the pharmacological activities characteristic of minor tranquilizers (5). It is used in Amrita Nectar tablets (Amrita nectar tablets containing 38 herbs) the effects of aqueous & alcoholic extract of Amrita nectar tablet on rat liver microtonal lipid per oxidation are good(6). Ethanol extract of leaves of *Clerodendrum phlomidis* Linn (MECP) showed significant inhibitory activity against castor oil induced diarrhea and PGE2 induced enter pooling in rats. The extract also showed a significant reduction in gastrointestinal motility in charcoal meal test in rats (7). The ethyl acetate and hexane extracts of leaves of C. phlomidis showed antifungal activity against plant and human pathogens but it is more effective in plants. It was tested by poison plate Technique (8).

MATERIALS AND METHODS

PLANT MATERIAL:

The leaves of the *Clerodendrum phlomidis* were collected from the foothill of Annavasal, Pudukkottai (DT), and Tamil nadu in the month of June2010. The collected plant was identified and Authenticated by a botanist Dr. P.Jayaraman, Director, Plant Anatomy Research Centre, and Chennai. A voucher specimen (PARC/2010/574.).

PREPARATION OF EXTRACTS

The leaves 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 40 C, yielding a dark brown Colored viscous mass 40g (08.0%).

Animals

Guinea pigs (300-400g) of either sex were procured from IRTT Perundurai Medical College, Perundurai, Tamil Nadu, and India. Protocols were in accordance with and approved by the institutional animal ethical committee (JKKMMRFCP/IAEC/2010/012). These animals were maintained under controlled conditions of temperature 26 ± 2°C, relative humidity 44-56%, and photo-schedule (12 h light and 12 h dark).Animals were provided with standard diet The animals were fed with rat pellet feed supplied by Hindustan Lever Ltd., Bangalore, India and water ad libidum.

Acute toxicity studies

Acute toxicity studies were performed according to OECD-423 guidelines (acute toxic class method). Albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4hrs with free access to water only. The plant extract of *Clerodendrum phlomidis* was administered orally with an initial dose of 1000 mg.kg-1 body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose such as 50,300 and 2000 mg.kg-1(9).

Evaluation of anti asthmatic activity isolated guinea pig ileum preparation (invitro)

Experimental design:

Experimental rats were divided into 3 groups of five animals each and treated for 10 days as follows.

Group-I : Control Histamine (0.2% aerosol)

Group-II : Chlorpheniramine maleate (2 mg/kg, i.p.)

Group-III : Ethanol extract of *Clerodendrum phlomidis* 100mg/kg/p.o

PROCEDURE

Overnight fasted guinea pig was sacrificed and ileum was mounted in an organ bath containing Tyrode solution. The Tyrode solution was continuously aerated and maintained at 37 ± 0.5ºC. The tissue was allowed to equilibrate for 30 min. under a load of 500 mg, contact time of 30 sec. and the response of Histamine was recorded by 5 min time cycle. After obtaining a dose response curve of histamine (10µg/ml) on ileum, ethanol extract of leaves of *Clerodendrum phlomidis* (100µg/ml) was added to the reservoir and same doses of histamine was repeated in presence of plant extract. Same procedure was repeated for standard drug (CPM 10µg/ml) as ethanol extract. Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on Abscissa was plotted to record dose response curve of histamine, in absence and presence of plant extracts (10, 11).

Histamine induced Bronchoconstriction in Guinea pigs (In-vivo)

Experimental design

Experimental rats were divided into four groups of five animals each and treated for 10 days as follows.

Group-I : Chlorpheniramine maleate (2 mg/kg, i.p.)

Group-II : Ethanol extract of *Clerodendrum phlomidis* 100mg/kg/p.o

Group-III : Ethanol extract of *Clerodendrum phlomidis* 200mg/kg/p.o

Group-IV : Ethanol extract of *Clerodendrum phlomidis* 400mg/kg/p.o

PROCEDURE:

Overnight fasted guinea pigs were divided into four groups, (n=5). Group-I received Chlorpheniramine maleate (2 mg/kg, i.p), Group-II, III and IV received ethanol extract of *Clerodendrum phlomidis* 100,200 and 400 mg/kg, (p.o) respectively. Bronchospasm was induced in guinea pigs by exposing them to histamine aerosol (0.2%) produced by an ultra-sound Nebulizer in an aerosol chamber (24×14×24 cm) made of Perspex glass. The time required for appearance of pre-convulsive dyspnoea caused by the histamine was recorded for each animal. Prior drug treatment, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The pre convulsion time (PCT) i.e. the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion, was noted. As soon as the preconvulsion dyspnoea (PCD) was noted, the animals were removed from the chamber and placed in fresh air to recover. This time for preconvulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 24 hrs. After 24 hrs. The animals of group I received Chlorpheniramine maleate (2mg/kg i.p), group II, III and IV received ethanol extract of *Clerodendrum phlomidis*. (100,200,400 mg/kg p.o). These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hrs

and 24hrs to determine preconvulsion time (PCT). The protection offered by the treatment was calculated by using the following formula (12, 13).

$$\text{Percentage Protection} = (1 - T_1/T_2) \times 100$$

T_1 = the mean of PCT before administration of test drugs.

T_2 = the mean of PCT after administration of test drugs at 1 hr, 4 hr and 24 hrs.

Statistical analysis

All the values of *in vitro* and *in vivo* anti asthmatic activity were expressed as mean ± Standard error of mean (S.E.M) and was analyzed for significance by ANOVA and groups were compared by Dunnett's multiple comparisons of groups with control. P Value were considered moderate significant at P<0.01, <0.001 level.

RESULTS AND DISCUSSION

Isolated guinea pig ileum preparation

Ethanol extract of *Clerodendrum phlomidis* exhibited significant (***p< 0.001) percent decreased contraction at 100mg/ml in isolated guinea pig ileum preparation. (Table: 1)

Histamine induced Bronchoconstriction in Guinea pig

The ethanol extract of leaves of *Clerodendrum phlomidis* significantly prolonged the latent period of convulsions followed by exposure to histamine aerosol at the dose of 400mg/kg, (p.o) and Showed maximum protection of 76.40 % at 4th hour as compared to Chlorpheniramine maleate (Standard) 1mg/kg, (p.o). This offered maximum protection of 62.92 % at 4th hour. (Table 2 & 3)

Asthma, the atopic disease with the greatest clinical and economic effect is an allergic and inflammatory outward sign of respiratory disorders. It is essentially characterized by the restriction of tracheal muscle obstruction (14). The syndrome of bronchial asthma is characterized by wide spread narrowing of the bronchial tree due to contraction of the smooth muscle in response to multiple stimuli resulting in the release of chemical mediators such as Histamine(15).Guinea pig ileum is used for screening of antihistaminic activity. The stimulation of H1 receptors produces graded dose related contraction of isolated guinea pig ileum (16, 17). In the present study, *Clerodendrum phlomidis* (100 μg/ml) significantly inhibited the histamine induced Contraction of isolated guinea-pig ileum preparation indicating its H_1 receptor antagonistic activity and supports the anti asthmatic properties of the plant.

Histamine induced bronchoconstriction is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to convulsion in guinea pigs and causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in cardiovascular system. A prominent effect caused by histamine leads to severe bronchoconstriction in the guinea pigs that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms (18).

The results of the study thus confirmed the bronchodilator properties of the plant, justifying its traditional claim in the treatment of asthma.Drugs effective in the asthma are mostly steroidal in nature. Phytochemical profile of the plant reveals the presence of steroidal nucleus in form of triterpenoids and various sapogenins and saponin glycosides. The antiasthmatic activity showed by the plant may be because of these chemical moieties (19).However this claim demands for further research and the studies are infact underway to isolate & characterize the active principles responsible for the anti-asthmatic activity.

	Control (10µg/ml)	C.phlomidis(100µg/ml)	CPM(10µg/ml)
0.1	42.12±2.17	17.26±0.97**	19.29±1.34**
0.2	62.23±3.01	28.07±1.65**	33.67±1.73**
0.4	65.74±2.29	30.17±1.14**	38.72±2.69**
0.8	76.4±2.53	35.22±1.31**	41.22±2.33**
1.6	80.4±1.52	44.63±0.71**	50.20±1.89**
3.2	100±1.67	46.26±0.93**	61.00±2.21**

Table – 1 Effect of ethanol extract of leaves of Clerodendrum phlomidis Linn. (100µg/ml) on histamine induced contraction on isolated guinea pig ileum.

n=5, Values are in Mean ± SEM. ** p< 0.01, *** p< 0.001
Control = DRC of Histamine in absence of ethanol extract.
Test = DRC of Histamine in presence of ethanol extract (100mg/ml).

Groups	Latent period of convulsion (in sec.)(Mean ± SEM)			
	Before	**1 hr**	**4hr**	**24hr**
STD	14.44±0.87	50.6±2.61**	61.2±2.11**	26.7 ±1.02**
Ret 100	15.7±1.02	32.2±2.17**	42.3±2.07**	24.2±0.83**
Ret 200	15.2±0.83	42.3±1.67**	41±2.01**	24.8±1.40**
Ret 400	16.4±0.73	38.6±1.53**	40.9±1.95**	26.2±1.27**

Table – 2 Effect of ethanol extract of leaves of Clerodendrum phlomidis Linn against Histamine induced bronchoconstriction in guinea pigs.

Groups	% Protection		
	1 hr	**4hr**	**24hr**
STD	71.46	76.40	45.91
Ret 100	51.24	62.88	35.12
Ret 200	64.06	62.92	38.70
Ret 400	57.51	59.90	37.40

Table – 3 % Protection of leaves of Clerodendrum phlomidis against histamine induced Bronchoconstriction in guinea pigs

N =5

Control = Distilled water (10 ml/kg, p.o.)
STD. = Chlorpheniramine maleate (2 mg/kg, i.p.)

Ret 100 = Ethanol extract of leaves of *Clerodendrum phlomidis* Linn. (100 mg/kg, p.o.)
Ret 200 = Ethanol extract of leaves of *Clerodendrum phlomidis* Linn. (200 mg/kg, p.o.)
Ret 400 = Ethanol extract of leaves of *Clerodendrum phlomidis* Linn. (400 mg/kg, p.o.)

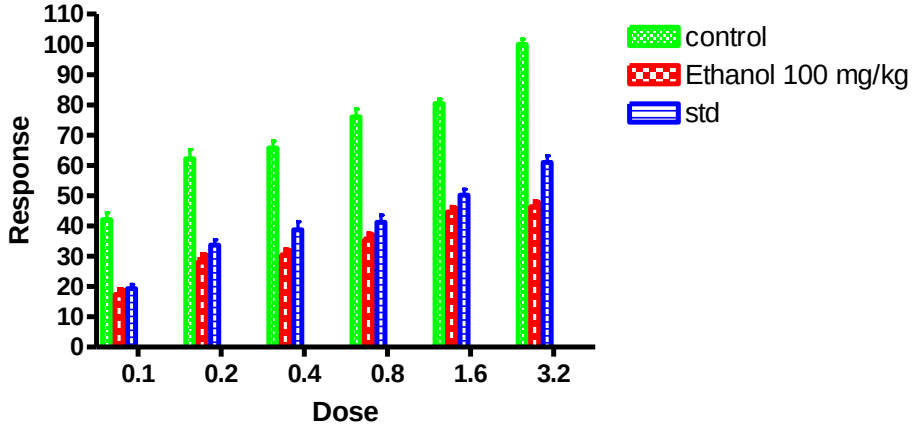

Figure - 1

Effect of ethanol extract of leaves of Clerodendrum phlomidis Linn. (100µg/ml) on histamine induced contraction on isolated guinea pig ileum.

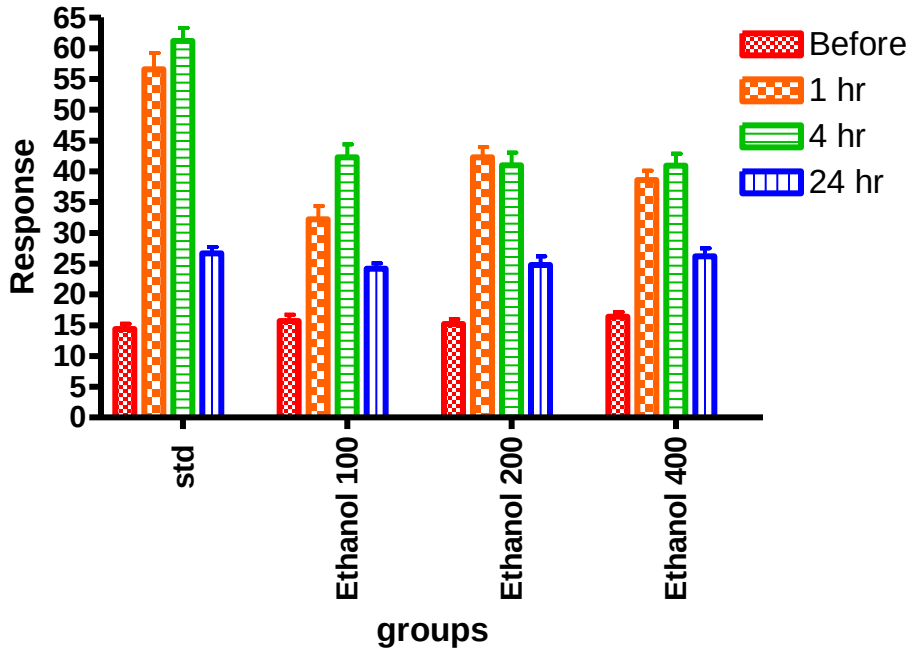

Figure - 2

Effect of ethanol extract of leaves of Clerodendrum phlomidis Linn against Histamine induced bronchoconstriction in guinea pigs.

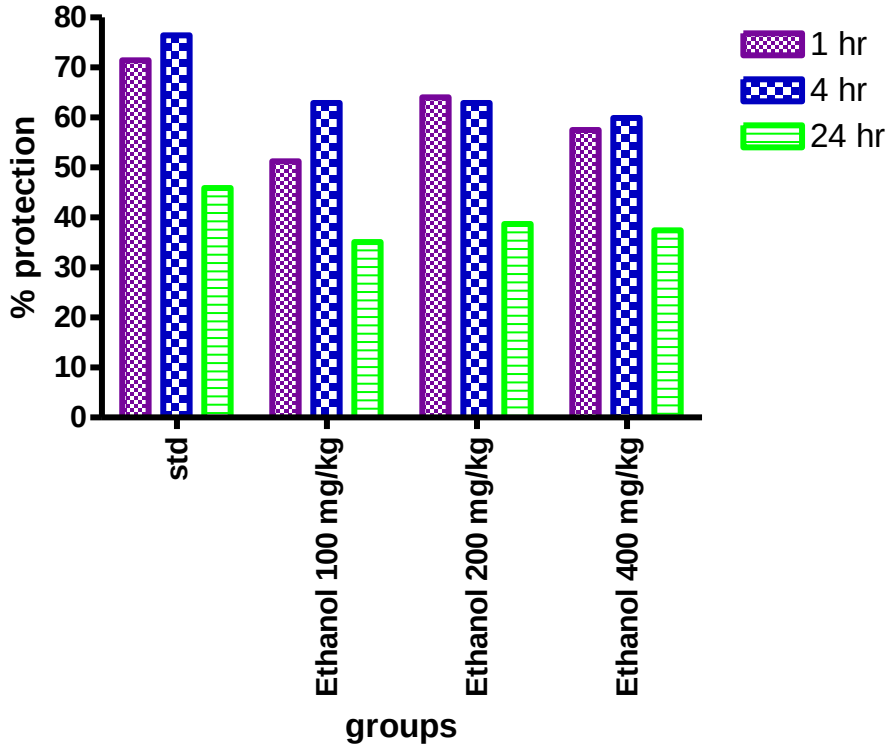

Figure - 3

% Protection of leaves of Clerodendrum phlomidis against histamine induced Bronchoconstriction in guinea pigs

CONCLUSION

In the present investigation, it can be concluded from the result obtained that *Clerodendrum phlomidis* possesses significant dose-dependent anti asthmatic activity at 100,200 and 400mg/kg (p.o

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