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Research Article

STUDIES ON ANTIASTHMATIC ACTIVITY OF AQUEOUS EXTRACT OF ROOTS OF *MIMOSA PUDICA* LINN

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ABSTRACT
The present study designed to evaluate the antiasthmatic activity of aqueous extract of *Mimosa pudica* (AEMP) on *in vitro* and *in vivo* animal models. Histamine induced contraction in isolated goat tracheal chain showed that aqueous extract of *Mimosa pudica* (AEMP) inhibited the contractile effect of histamine ($P<0.05$). A dose dependent contraction of goat tracheal chain is observed. Treatment with AECP protected 74% from degranulation of mast cell as compared to control group. *AEMP* showed excellent protection in guinea pigs against the histamine-induced bronchospasm. Thus, AECP showed antihistaminic, mast cell stabilizing and protected guinea pigs against histamine induced PCD and hence possesses potential role in the treatment of asthma.

KEY WORDS: Antiasthmatic activity, Bronchorelaxant activity, membrane stabilization, anti histaminic

INTRODUCTION
Over the past decade, herbal and Ayurvedic drugs has become a subject of world importance,with both medicinal and economical implications. A regular and widespread use of herbs throughout the world has increased serious concerns over their quality, safety and efficacy. Ayurveda, an ancient system of Indian medicine, has recommended a number of drugs from indigenous plant sources for the treatment of bronchial asthma and allergic disorders. According to Ayurveda this *Mimosa pudica* is used in treatment of biliousness, leprosy,dysentry,vaginal and uterine complaints, inflammations, burning sensationmfatigue, asthma, leucoderma and blood diseases.[1,2] *Mimosa pudica* roots are used as antiasthmatic.[2,3,4.] Present study was planned to evaluate the antiasthmatic activity of aqueous extract of roots of *Mimosa pudica* using various models like Isolated Goat tracheal chain preparation, Clonidine-induced Mast cell degranulation, Histamine and Acetylcholine induced bronchospasm in guinea pigs.

MATERIALS AND METHODS
Animals
The Institutional animal ethics Committee (IAEC) approved the experimental protocol and cares of animals were taken as per guidelines of CPCSEA, Dept. of Animal Welfare, and Govt. of India. Swiss Albino mice of either sex weighing around 18-25 gms were procured from Animal house of college Sangli, India. Dunkin Hartley gunea pigs weighing between 350to 400 gram were used. They were purchased from KIMS, Karad. They were acclimatized to the standard laboratory conditions at the temperature of 25 ± 1 0C for 5 days. The animals had free access to food and water, maintained under light and dark cycles of 12 hrs each. All experiments were carried out during day time from 09.00-14.00 hrs.

Dose selection

Acute oral toxicity study of aqueous extract Up and Down procedure[5] Acute oral toxicity study was carried out according to the OECD 425 guidelines. Five albino mice and wistar rats weighing between 20-25 gm and 150-200 g respectively were used. 2000mg\kg aqueous extract was given to the animals. These are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days. Additionally observed for changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern.

As no sign of toxicity was observed, $1\backslash 10^{th}$ dose of above dose as 200mg\kg was taken as safe dose. (OECD 425) Dose of 800µg/ml for isolated goat tracheal chain preparation was selected by our laboratory trials.

MODELS

Isolated Goat tracheal chain preparation

The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in ice-cold oxygenated Kreb's solution. The spirally cut trachea was suspended in 10 ml of Kreb's solution. Goat trachea was cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Krebs-Henseleit (composition (mM): NaCl, 115; KCl, 4.7; CaCl2, 2; NaHCO3, 25; KH2PO4, 1.2; MgCl2, 1.2; glucose, 11.5) and maintained at 37 ± 1ºC, a stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S- shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min. under a load of 400 g. A dose response curve for histamine was recorded at varient molar concentrations by maintaining 15 min time cycle. After obtaining dose response curve of histamine on trachea the AEMP 800 µg/ml was added to reservoir and same doses of histamie were repeated. Graph of percentage of maximum contractile respone on ordinate and negative log of molar concentration of histamineon abscissa was plotted to record dose response curve of histame, in absence and in presence of *Mimosa pudica* root extract[6,7].

Clonidine-induced Mast cell degranulation

The histamine concentration has been calculated to be around 0.3 M in rat mast cell granules[10] Mice were divided into three groups, five animals in each group. Animals belonging to group-I received distilled water (5 ml/kg, p.o.). Animals belonging to group-II received Sodium cromoglycate (50 mg/kg, i.p.). Animals belonging to Groups-III received AEMP 200 mg/kg. Normal saline (5.0 ml) was injected into the peritoneal cavity of mice. After a gentle massage, the peritoneal fluid was collected and transferred into the test tubes containing 3-4 ml of RPMI-1640 buffer medium (pH 7.2-7.4). Mast cells were then washed by centrifugation at a low speed (400-500 rpm) followed by discarding the supernatant and taking the pellets of Mast cells into the medium. The Mast cells were treated with Clonidine (80 mcg/ml) and incubated at 37ºC in a water bath for 10 min., spread on the microscopic slide; stained with Toluidine blue containing 1% acetic acid and percentage protection against degranulation (indicated by the presence of vacuoles within the mast cells) was calculated. In the standard drug treated group, Disodium chromoglycate (200 mcg/ml) was added prior to the addition of clonidine. The mean percentages of mast cells were determined by counting 100 cells from each subcutaneous spread.

Histamine and Acetylcholine induced bronchospasm in guinea pigs [11,12,13]: Experimental bronchial asthma was induced in ten guinea pigs by exposing them to 1% Histame and 10% acetylcholine chloride under constant pressure (1 kg/cm2) in an aerosol chamber. The animals exposed to Histamine and Acetylcholine aerosol showed progressive dyspnoea. The end point preconvulsive dyspnoea (PCD) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions. As soon as PCD commenced, the animals were removed from the chamber and placed in fresh air. This PCD was taken as day 0 value. Guinea pigs were administered with AEMP 200mg/kg once a day for 7 days. On day 7, two hours after the last dose, the time for the onset of PCD was recorded as on day 0. The protection offered by the treatment was calculated by the following formula [8,9].

Percentage protection = {1- (T1/T2) }T1 x 100
where T1 is time for PCD onset on day 0 and
T2 is the time for PCD onset on day 7.

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.

RESULT

Isolated goat trachea chain preparation

It was observed that AEMP inhibits contraction produced by histamine in these tissue preparations. Histamine (10µg/ml) was taken in different dose level and DRC was plotted. Study revealed that AEMP exhibits significant ($p<0.01$) percentage decreased contraction at concentration 800 µg /ml in goat tracheal chain preparation Dose dependent response relationship was seen. As shown in Table-1 and Fig1

Effect of *Mimosa pudica* Extract on Histamine and Acetylcholine Induced Bronchoconstriction In Guinea Pigs

The guinea pigs when exposed to 0.2% Histamine aerosol showed signs of progressive dyspnoea leading to convulsions. AEMP significantly prolonged the latent period of convulsions (PCT) as compared to control following the exposure of Histamine (0.2%) aerosol ($p< 0.01$). But unable to prolong the acetylcholine induced convulsion in guinea pigs following exposure of Acetylcholine aerosol (2%). The action started within 1 hr of drug administration and there was significant effect up to 4 hr. Here aqueous extract increases latent period 89.14 % at 4 hr interval and 31.37 % at 24 hr interval As shown in Table3 and fig 2. But in case of Acetylcholine induced PCD there is no any effect of AEMP. As shown in Table 5 and fig 3. Therefore, the result of present study indicates the utility of the aqueous extract of *Mimosa pudica* root in the treatment of asthma by virtue of its H_1-receptor blocking activity.

Clonidine-Induced Mast Cell Degranulation

In the present study, the group of animals pretreated with aqueous *Mimosa pudica* extract showed significant reduction in degranulation of mast cells (52.00) when challenged with clonidine. The prevention of degranulation process by the aqueous extract ($p < 0.01$) indicates a possible stabilizing effect on the biomembrane of mast cells, indicating mast cell stabilizing activity as shown in Table 6 and fig 4.

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. In isolated goat tracheal preparation, there is preponderance of H_1 excitatory and a scanty population of H_2 inhibitory receptors[14,15]. 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 *AEMP* indicating antiashmatic action. The prevention of mast cell degranulation process by the aqueous extract ($p < 0.01$) indicates a possible stabilizing effect on the biomembrane of mast cells, indicating mast cell stabilizing activity. *AEMP* showed excellent protection in guinea pigs against the Histamine-induced bronchospasm and dose not show any protection against Acetylcholine induced broncoconstriction.

Thus the anti-asthmatic activity of *Mimosa pudica* can be attributed to bronchodilating, antihistaminic (H_1-antagonist), mast cell stabilizing, suggestive of its potential in prophylaxis and management of asthma.

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Table 1: Effect of AEMP on histamine induced contraction on isolated goat tracheal chain preparation

Sr. No.	Conc of Histamine in µg	Response in mm (Mean ± SEM)		% Maximum Response	
		Control	Test	Control	Test
1	1	7.331 ± 0.6667	5.00 ± 0.577**	15.95 ± 1.430	10.817 ± 0.8978
2	2	14.66 ± 1.453	8.666 ± 1.202*	31.96 ± 3.231	18.88 ± 2.224
3	4	23.00 ± 4.726	12.33 ± 2.603**	48.06 ± 4.267	26.04 ± 3.223
4	8	32.00 ± 5.033	14.00 ± 3.055*	69.03 ± 1.459	37.55 ± 4.463
5	16	40.66 ± 5.812	17.66 ± 2.333**	86.9 ± 4.208	38.35± 4.317
6	32	45.33 ± 6.692	23.00 ± 3.786 **	100.0 ± 0.00	49.43 ± 5.516

n=3 Values in Mean ± SEM.
Statistical analysis ANOVA followed by paired test.
*p<0.05, ** p< 0.01, Compared to control group (45.33 mm taken as 100%)
Control = D.R.C. of Histamine in absence of *Aqueous extract of Mimosa pudica.*
Test = D.R.C. of Histamine in presence of *Aqueous extract of Mimosa pudica* (800 µg/ml).

Table 2: Histamine Induced Bronchoconstriction in Guinea Pigs

Groups	Time required for Preconvulsive dysponea (In Sec)			
	Before treatment	**1 hr.**	**4 hr.**	**24 hr.**
Control	17 ±2.00	18 ± 0.5774	17± 2.00	18.5 ± 1.5
Standard	17 ± 0.5	435.0 ± 8.660**	317.5 ± 2.50**	27.5 ± 0.5
Aqueous Extract	17 ± 0.5	137.5 ± 1.44**	200.0 ± 0.00**	25.5 ± 1.5

N=2 Values in Mean ± SEM.
Statistical analysis done by ANOVA followed by Dunnett's test, ** p< 0.01 Compared to control group.

Table 3: Percentage protection against Histamine Induced Bronchoconstriction in Guinea pigs at different time intervals

Groups	Percentage protection		
	1 hr	4 hr	24hr
Standard	**95.97**	96.48	36.36
Aqueous Extract	**87.27**	89.14	31.37

Table 4: Acetylcholine Induced Bronchoconstriction in Guinea Pigs

Groups	Time required for Preconvulsive dysponea. (In Sec)			
	Before treatment	**1 hr.**	**4 hr.**	**24 hr.**
Control	14±1.00	18±1.00	17±2.00	18.5±1.500
Standard	17.00±0.00	188.5±1.5**	202.00±12.00**	75.50±6.500
Aqueous Extract	16.50±1.00	22.5±2.5	23.50±1.5	22.00±5.00

N=2 Values in Mean ± SEM.
Statistical analysis done by, ANOVA followed by Dunnet's test Compared to control group.

Table 5: Percentage protection against Acetylcholine Induced Bronchoconstriction in Guinea pigs at different time intervals

Groups	Percentage protection		
	1 hr	4 hr	24hr
Standard	90.98	91.58	77.48
Aqueous Extract	26.66	29.78	25.00

Table 6: Effect of *AEMP* on Clonidine-Induced Mast Cell Degranulation

Group	**Mast cells %**		**% protection**
	Intact	**Disrupted**	
I	25 ± 3.559	73.25 ± 2.75**	_
II	72.00± 1.780***	28.00 ± 3.55***	61.77
III	63.75 ± 3.50***	34.75 ± 3.775***	52.00

N =4 Values in Mean ± SEM.
Group II, III, IV, compared with Group I (ANOVA followed by Dunnett's test) and Group I compared with % of intact mast cells (Dunnett's - test)
**p < 0.01, ***p < 0.001
Group-I = Distilled water (5 ml/kg, p.o.) (Control)
Group-II = Sodium cromoglycate (50 mg/kg, i.p.)
Group-III = Aqueous Extract of *Mimosa pudica (200mg\kg P.O.)*

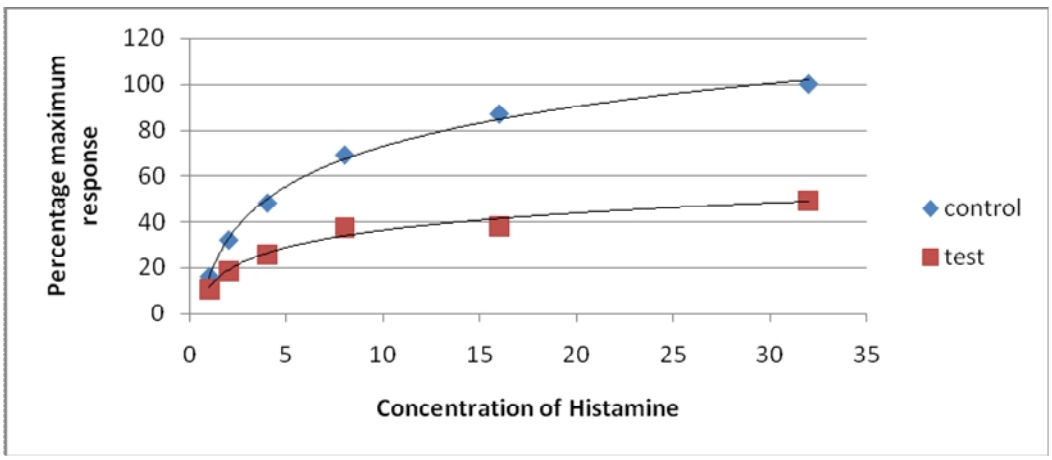

Fig 1: Effect of AEMP on histamine induced contraction on isolated goat tracheal chain preparation

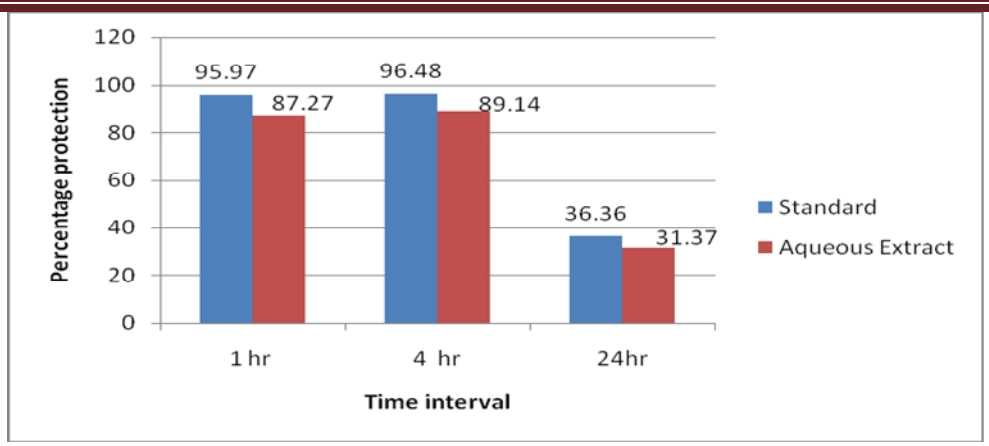

Fig 2: Percentage protection against Histamine Induced Bronchoconstriction in Guinea pigs at different time intervals

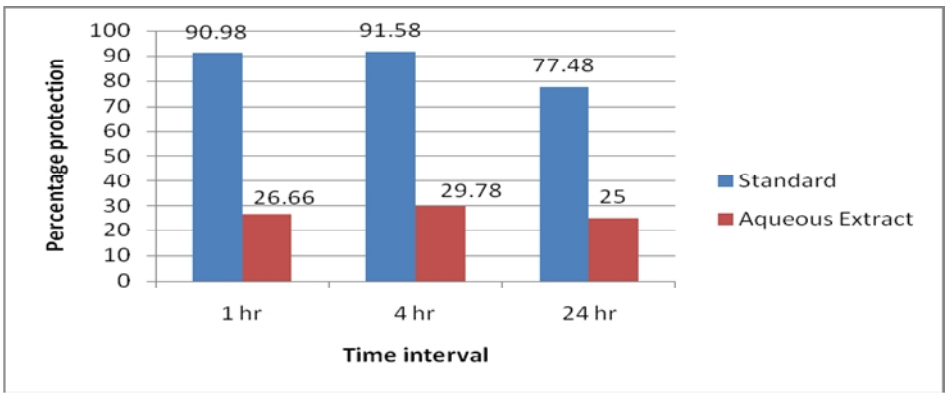

Fig 3: Percentage protection against Acetylcholine Induced Bronchoconstriction in Guinea pigs at different time intervals

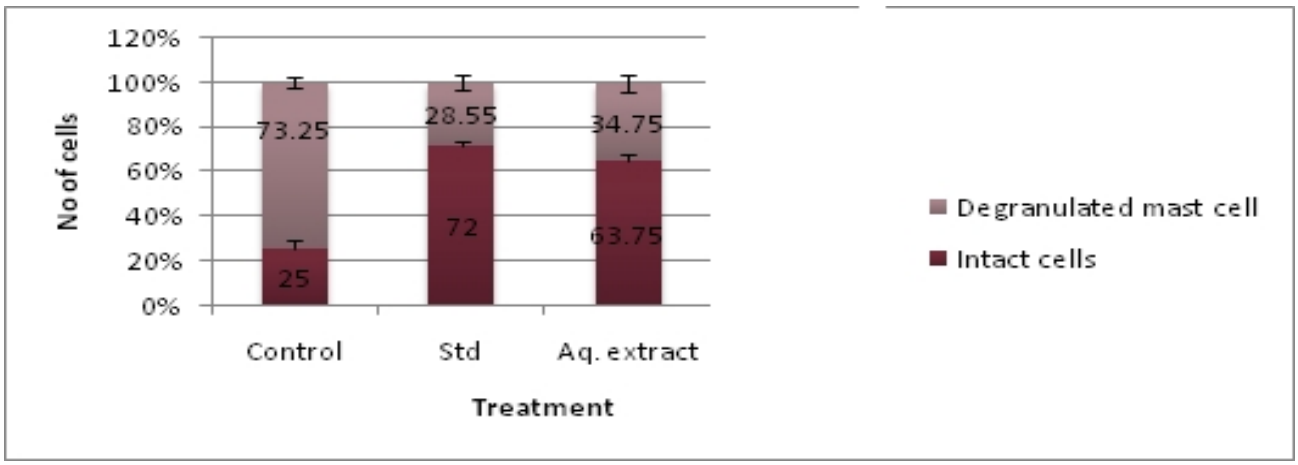

Fig 4: Effect of *AEMP* on Clonidine-Induced Mast Cell Degranulation

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