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PHARMACOLOGICAL SCREENING OF ETHANOLIC LEAF EXTRACT OF *MYXOPYRUM SERRATULUM*

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ABSTRACT

Antiasthmatic activity of ethanolic leaf extract of *Myxopyrum serratulum* (EEMS) by ***in-vitro*** and in vivo methods were carried out. EEMS exhibited anti-histaminic and anti-cholinergic activity in isolated goat tracheal muscle. It also protected egg albumin induced degranulation of mast cells in dose dependant manner. For in vivo studies EEMS significantly inhibited the duration of catalepsy and showed a significant decrease in leukocytes and differential count in dose dependent manner. Thus the present study suggests that EEMS exhibited significant antihistaminic, mast cell stabilizing and anti-allergic activity in dose dependent manner. So, it can be utilized for the treatment of asthma and further studies are required to elucidate its exact mechanism of action to support this traditional claim.

KEYWORDS: Antiasthmatic, *In vitro.* In vivo.

INTRODUCTION

Anti- asthmatic drugs currently used gives only symptomatic relief but no cure. To add on to this is the undesirable adverse effects they cause such as tachycardia, tolerance etc. Herb has been part of mankind life since times immemorial. One such herb namely, *Myxopyrum serratulum* has been used as a traditional folklore medicine for treating asthma. Antioxidant,[1] anti-inflammatory,[2] antimicrobial,[3] and wound healing[4] properties of the plant have been reported earlier. Also phytochemical investigations on *Myxopyrum serratulum* revealed the presence of active constituents such as flavonoids and saponins which may account for its anti-asthmatic activity.[3] Hence the aim of the present study was to investigate the antiasthmatic activity of *Myxopyrum serratulum by* ***in-vitro*** and in vivo

methods. In vitro anti-asthmatic activity was determined by isolated goat tracheal chain method and mast cell stabilizing activity on rat mesentery. To evaluate ***in-vivo*** activity clonidine induced catalepsy and milk induced leukocytosis and eosinophilia in mice were carried out.

MATERIALS AND METHODS

Animals

Adult albino Wistar rat of either sex weighing between 150 – 180g were used for evaluating mast cell stabilising activity. Swiss albino mice of either sex weighing between 25- 30 g were used for evaluating in vivo anti-asthmatic activity. They were housed at standard conditions of temperature (22 ± 2ºC), humidity (55 ± 5%) and 12h/12h light-dark cycle. Animals received standard pellet diet and drinking water *ad libitum*. The protocol for the experiment was approved by Institutional Animal Ethics Committee as per the CPCSEA guidelines.

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). All statistical comparisons between the groups were made by means of One Way Analysis of Variance followed by Dunnett's multiple comparision test using Graph pad Prism 7 software.

Collection and Authentication of leaves of Myxopyrum serratulum

Leaves of *Myxopyrum serratulum* were collected from the Thiruvananthapuram, Kerala, in the month of March, 2017. The plant was identified and authenticated at Institut Franceais de Pondichery, Pondicherry.

Extraction of Plant leaves

Leaves were dried in shade at room temperature and ground in electric grinder. 50g of the coarse powdered Myxopyrum *serratulum* leaves were packed in a Soxhlet and extracted with 500 ml of ethanol for 72 hours. The solvent were removed from the extract by distillation method. The solvent free extract is packed in an airtight container and was used for further studies. The extract was subjected to preliminary phytochemical screening. Previous studies using aqueous, petroleum ether, chloroform and ethanolic extract of *Myxopyrum serratulum* showed that phytochemical constituent were present more in ethanolic extract when compared to others.[3] Hence the current study was designed using ethanolic extract of *Myxopyrum serratulum.*

Isolated goat tracheal chain preparation method

Adult goat trachea was obtained immediately after slaughter of the animal. Trachea was cut into small individual rings and tied together in series to form a chain. Trachea was suspended in organ bath with Krebs solution and maintained at 37^0c. Dose response curve of histamine, acetylcholine were recorded in presence and absence of EEMS extract and their respective antagonist, chlorpheneramine maleate (CPM) and atropine.[5]

Mast cell stabilizing activity on rat mesentry

Adult albino Wistar rat was sacrificed and pieces of rat mesentery with connecting lobes of fat and blood vessels were rapidly dissected out and placed in Ringers Locke solution. All the petri dishes were incubated for 30 min as per following schedule of treatment.

Petri dish no.1: Normal control - distilled water
Petri dish no.2: Asthmatic control - 0.1ml of 1% w/v egg albumin
Petri dish no.3: Standard drug - 0.1 ml of 1% w/v egg albumin + ketotifen (20µg/ml)
Petri dish no.4: Test drug 1 - 0.1 ml of 1% w/v egg albumin + *Myxopyrum serratulum* ethanolic leaf extract (100 µg/ml)
Petri dish no.5: Test drug 2 - 0.1 ml of 1% w/v egg albumin + *Myxopyrum serratulum ethanolic* leaf extract (200 µg/ml)

Then mesenteries were transferred to petri dishes containing 0.1ml of 1% w/v egg albumin for 20 min separately. Then all these mesenteries were transferred in 4% formaldehyde containing 0.1% toluidine blue dye and kept for 20 min. After staining and fixation of mast cells, mesenteric pieces were transferred through acetone and xylene two times and mounted on slides. Four pieces of mesentery were used for each concentration of drug and observed under microscope.[6]

Percentage protection of mast cell= (1-T/C)×100
T= Percentage of disrupted cells in test drug.
C= Percentage of disrupted cells in egg albumin control.

Clonidine induced catalepsy in mice

Overnight fasted mice were divided into 4 groups (n = 5). Treatment were given according to the groups and Ih later all the groups received clonidine(1mg/kg s.c). Group 1 served as control and group 2 received standard drug treatment with chlorpheniramine maleate (3.25

mg/kg po). Group 3 and 4 received 200mg/kg and 400 mg/kg oral administration of EEMS respectively. The forepaws of mice were placed on horizontal bar and duration of catalepsy catalepsy (time required to remove the paws from bar was noted for each animal) were measured at 0, 15,30,60,90,120,150 and 180 minutes.[5]

Milk induced leukocytosis and eosinophilia in mice

Albino mice were divided into four groups (n=5). Animals received respective treatment and all the groups were injected boiled and cooled milk (4 ml/kg, sc) 30 min after treatments. Group 1 served as control, group 2 received dexamethasone (1 mg/kg po) and boiled and cooled milk (4ml/kg s.c). Group 3 and 4 received boiled and cooled milk (4ml/kg s.c) and 200 mg/kg and 400 mg/kg oral administration of EEMS respectively. After 24 hours blood samples were collected from retro-orbital plexus from all animals and total leukocytes count in mice blood was noted.[7]

RESULTS

Preliminary phytochemical screening of EEMS revealed the presence of alkaloids, flavonoids, saponins glycosides, tannins and phytosterols.

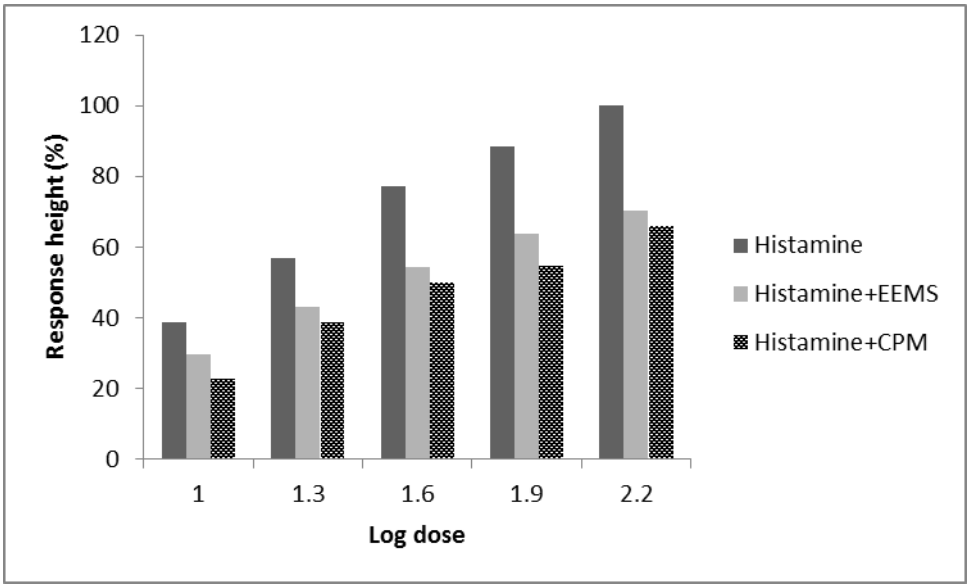

Figure 1: Effect of EEMS on histamine action in isolated goat tracheal chain.

7.9 µg of histamine produced the contraction of 50% response where as in presence of Chlorpheniramine maleate (CPM) and EEMS it was increased to 19.9 µg and 14.2 µg respectively. This was evidenced by the right hand shift in log dose response curve of histamine in presence of EEMS and CPM.

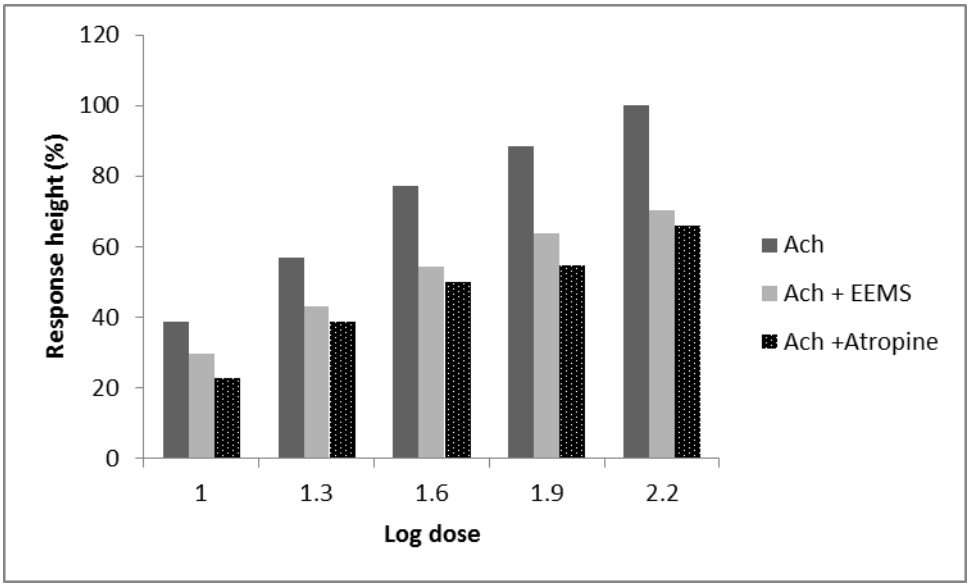

Figure 2: Effect of EEMS on Ach action in isolated goat tracheal chain.

14.12 µg of Acetylcholine produced the contraction of 50% where as in presence of atropine and EEMS it was increased to 35.4 µg and 22.6µg respectively. This was evidenced by the right hand shift in log dose response curve of acetylcholine in presence of EEMS and atropine.

Table 1: Effect of EEMS on mast cell stabilization in rat mesentery.

Groups	Mast cell		Percentage protection
	Intact cells (%)	Disrupted cells (%)	
Normal	95.750 ± 1.79	4.250±1.79	-
Control	43.500 ± 2.17	56.500 ± 2.17	-
ketotifen (20µg/ml)	80.500 ± 2.06*	19.500 ± 2.06*	65.5%
EEMS (100µg/ml)	60.500 ± 2.10*	39.500 ± 2.10*	30.1%
EEMS (200µg/ml)	68.750 ± 1.97*	30.750 ± 2.28*	45.7%

Values are expressed in mean ± SEM, n=4. Statistical analysis done by ANOVA followed by Dunnett's multiple comparison test. *P<0.01, compared to control group. n = 4.

Ketotifen significantly (*P<0.01) inhibited egg albumin induced mast cell degranulation and percentage protection was found to be 65.5%. In groups pre-treated with EEMS (100 and 200 µg/ml) there was significant protection of mast cells and the percentage protection (*P<0.01) was found to be 30.1% and 45.7% respectively. The percentage protection increases with the concentration of test drug and revealed that EEMS shows dose dependent activity, but it was less potent when compared to the standard ketotifen.

Clonidine (1 mg/kg, sc) produced catalepsy in mice, which remained for 3 hr. The vehicle treated group showed maximum duration of catalepsy (28 ± 23.02 sec) at 90 minute after the administration of clonidine. There was significant inhibition (*P<0.05) of clonidine-induced catalepsy in the animals pretreated with EEMS (200 and 400 mg/kg, p.o) in dose dependent manner and the duration of catalepsy was found to be 22 ± 11.10 and 12 ± 4.04 seconds respectively at 90 minute after the administration of clonidine. Chlorpheniramine maleate (10 mg/kg, i.p.) significantly inhibited (9.8 ± 2.57 sec) the clonidine-induced catalepsy in mice at 90 minute after the administration of clonidine.

Table 2: Effect of EEMS on clonidine induced catalepsy in mice.

No	Groups	Duration of catalepsy (sec)							
		0 min	**15 mins**	**30 mins**	**60 mins**	**90 mins**	**120 mins**	**150 mins**	**180 mins**
1	Control	1± 0.00	9.4 ± 2.06	17 ± 10.84	18 ± 10.51	28 ± 23.02	22.4 ± 17	14 ± 9.6	11.382 ± 7.62
2	CPM 3.25 mg/kg po	2.8 ± 0.06	11.2 ± .31	13.4 ± 2.04*	11.4± 1.69*	9.8 ± 2.57*	8.4 ± 3.20*	5.800 ± 1.71*	4.4 ± 1.568*
3	EEMS 200 mg/kg po	1.4 ± 0.24	7.8 ± 2.24	15 ± 5.39*	16 ± 11.11*	22 ± 11.09	15.4 ± 9.6*	12.6 ± 7.82	9.4 ± 5.98
4	EEMS 400 mg/kg po	2.80 ± .80	13.2 ± .73	15.4 ± 3.66*	16.8 ± 3.66*	12 ± 4.03*	11 ± 2.84*	9.2 ± 2.47*	7 ± 2.02*

Values are expressed in mean ± SEM, n=5. Statistical analysis done by ANOVA followed by Dunnett's multiple comparison test. * P<0.05 when compared to control group.

The maximum increase in total leukocytes count (5566.6±233.33) was observed in control group 24 hrs after administration of milk (4ml/kg, s.c). In the groups of mice pretreated with EEMS at doses of 200 and 400 mg/kg p.o., there was significant inhibition of milk-induced leukocytosis * P<0.05 and **P<0.01 (4266.6 ± 272.85 and 3433.3 ± 260.34) in dose dependent manner. Dexamethasone (1mg/kg po) also showed more significant activity **P<0.01 (3733.3 ± 352.77) on milk induced leukocytosis.

Table 3: Effect of EEMS on milk induced leukocytosis in mice.

S.no	Group treatment	Total leukocyte count (cells/cu.mm)
1	Normal	2533.3±371.18
2	Control	5566.6±233.33
3	Dexamethasone (1mg/kg po)	3733.3±352.77**
4	EEMS (200mg/kg po)	4266.6±272.85*
5	EEMS (400 mg/kg po)	3433.3±260.34**

Values are expressed in mean ± SEM. (n=5). Statistical analysis done by ANOVA followed by Dunnett's multiple comparision test. * P<0.05, **P<0.01, compared to control group.

DISCUSSION

The goat tracheal muscle has H1, M3 and B2 receptors. Spasmogens cause dose dependent contraction of goat tracheal chain preparation and are suitable for screening the activity of a drug on respiratory smooth muscles. In the present study the histamine and acetylcholine induced contraction of isolated goat tracheal muscle were effectively blocked in presence of EEMS which is comparable to that of standard drug, chlorpheniramine maleate and atropine. The stimulation of H1 receptors cause contraction of bronchial smooth muscle.[8] Further the log dose response curve of histamine and acetylcholine was shifted towards right hand side. This confirms the antagonistic activity of EEMS against histamine and acetylcholine. These above findings are also clearly shows that the extract had possessed significant anti-histamine activity through histamine receptor antagonist activity comparable to that of chlorpheniramine.

Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Degranulated mast cells liberate mediators of inflammation such as histamine, leukotrienes, platelet activating factors and chemotactic factors.[6] Ketotifen, a standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate which relaxes airway smooth muscle and inhibit the release of autocoids. In our study EEMS at doses of 200 and 400 mg/kg significantly protected egg albumin induced degranulation of mast cells in dose dependant manner and was comparable to that of standard drug, ketotifen. This indicates that EEMS is effective in stabilizing mast cell.

Catalepsy is a condition in which the animal maintains imposed posture for long time before regaining normal posture. Catalepsy is a sign of extrapyramidal side effects of drugs that inhibit dopaminergic transmission or increase histamine release in brain. It has been suggested that the cataleptic effect of clonidine in the mouse be mediated by histamine, which is released from the brain mast cells in response to stimulation of α2 adrenoreceptors by clonidine.[9] The observation of this study indicated that EEMS significantly inhibited the duration of catalepsy in dose dependant manner which shows that it reduced the release of histamine. Finally the result reveals that EEMS possess anti-asthmatic activity.

Parental administration of milk produced a marked increase in the leukocytes and eosinophils count after 24 h of its administration. An increase in the number of leukocytes i.e., leukocytosis and an increase in the number of eosinophils i.e., eosinophilia in the blood is

often an indicator of allergies such as asthma, hay fever and hives and also parasitic infections.[10] Leukocytes release variety of inflammatory mediators like cytokines, histamine, and major basic protein. The infiltration of leukocytes potentiates the inflammatory process by the release of reactive oxygen species into the surrounding tissue, resulting in increased oxidative stress and associated with many pathogenic features. In the late phase, especially in the development of allergic asthma, eosinophil plays a role as an inflammatory cell and EEMS significantly decreased the total leucocyte count.

The phytochemical screening of EEMS revealed presence of alkaloids, flavonoids, saponins, glycosides, phenols and tannins. Previous report revealed that saponins possess mast cell stabilizing activity. Flavonoids possess smooth muscle relaxant activity, bronchodilator activity and in-vivo antiallergic activity. These flavonoids also inhibited allergen induced histamine release and neutrophil beta glucuronidase release.[11] Hence all this shows that antiasthmatic activity of EEMS may be due to presence of these Phytoconstituents. In this study we observed that mice pretreated with EEMS at a dose of 200 and 400mg/kg po showed a significant decrease in leukocytes and differential count in dose dependent manner. Result suggests that EEMS decreases milk induced leukocytes count by normalizing oxidative stress and it shows EEMS possess the anti-asthmatic activity.

CONCLUSION

The present study suggests that ethanolic leaf extract of *Myxopyrum serratulum* exhibited significant antihistamine, mast cell stabilizing and anti-allergic activity in dose dependent manner. So, it can be utilized for the treatment of asthma and further studies are required to elucidate its exact mechanism of action to support its traditional claim.

REFERENCES

1. Sheelarani T, Gopal V, Seethalakshmi S, Chithra K. Vitro anti-oxidant activity of Myxopyrum serratulum A.W.Hill. International Journal of Pharmacy and Pharamaceutical sciences, 2013; 5(4): 545-6.
2. Rajalakshmi K, Mohan VR. Anti inflammatory activity of stem and leaf of Myxopyrum serratulum A.W.Hill (Oleaceae). International Journal of Current Pharmaceutical Review and Research, 2016; 7(4): 190-2.
3. Gopalakrishnan S, Rajameena R, Vadivel E. Antimicrobial activity of the leaves of Myxopyrum serratulum A.W.Hill. International Journal of Pharmaceutical Sciences and Drug Research, 2014; 4(1): 31-4.

4. Gopalakrishnan S, Rajangam RR. Wound healing activity of the ethanol extract of the leaves of Myxopyrum serratulum A.W.Hill in rats. International Journal of Pharmaceutical Review and Research, 2013; 22(1): 143-7.
5. Pravin S. Derle D, Tyothi V. Preclinical Evaluation and Antiasthmatic Activity of *Euphorbia hirta* Linn. *Int. J. Pure App. Biosci.*, 2014; 2(2): 262-6.
6. Limbasiya KK, Modi VR, Tirgar PR, Desai TR, Bhalodia PN. Evaluation of antiasthmatic activity of dried whole plant extract of *Leucas aspera using* various experimental animal models. International Journal of Phytopharmacology, 2012; 3(3): 291-8.
7. Dnyaneshwar JT, Ravindra YP. Effect of *Abrus precatorius* leaves on milk induced leukocytosis and eosinophilia in the management of asthma. Asian Pacific Journal of Tropical Biomedicine, 2012; S40-S42.
8. Tayade PM, Borde SN, Jagtap SA, Patil VP, Vaishnav G, Girbane YR et al. Effect of *Tamarindus indica* linn. against isolated goat tracheal and guinea pig ileum preparation. International Journal of Comprehensive Pharmacy, 2010; 2(6): 1-3.
9. Ghaisas MM, Ninave PB, Ganu GP, Zope VS. Effect of *Randia dumetorum* lam on clonidine and haloperidol induced catalepsy in mice. Pharmacologyonline, 2008; 2: 42-50.
10. Ehright T, Chua S, Lim DJ. Pulmonary eosinophilic syndromes. Ann. Allergy, 1989; 62: 277-83.
11. Taur DJ, Patil RY. Evaluation of antiasthmatic activity of *Clitoria ternatea* L. roots. Journal of Ethnopharmacology, 2011; 136(2): 374-6.