ANTIHISTAMINIC AND BRONchodILATING ACTIVITY OF FRuIT BERRIES OF EMBELIA RIBES

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ABSTRACT
The present study was designed to evaluate the antihistaminic and bronchodilating activity of ethanolic extract of fruit berries of Embelia ribes (ER) by using isolated goat tracheal chain preparation and histamine-induced bronchospasm in guinea pigs. In Histamine induced contraction in isolated goat tracheal chain preparation ER inhibited dose dependent contraction of goat tracheal chain produced by histamine. In histamine-induced bronchospasm in guinea pigs, treatment with ER showed significant protection by prolonging Preconvulsion dyspnoea time (PCD). Thus, ER showed anti-histaminic and bronchodilating activity against histamine and hence possesses potential role in the treatment of asthma.

Key words: Isolated goat tracheal chain, histamine, Embelia ribes.

INTRODUCTION
Over the past decade, herbal and Ayurvedic drugs has become a subject of world importance with both medical 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 Embelia ribes fruit berries is used as appetizer, carminative, anthelmintic, laxative, curative, cures tumors, bronchitis and mental disorders. Embelia ribes Burn (Myrsinaceae), commonly known as Vishagu, is a large woody climbing shrub and is widely distributed throughout Pakistan and India. The reported activities so far are hepatoprotective, protection of pancreatic β cells in diabetes, anti-bacterial activity, wound healing activity, anticonvulsant activity, anti-fertility activity, anti-cancer activity, anti-inflammatory and analgesic activity and antioxidant activity etc. The present study was planned to evaluate the antihistaminic activity of ethanolic extract of fruit berries of Embelia ribes brum using models like Isolated Goat tracheal chain preparation and Histamine induced bronchospasm in guinea pigs.

MATERIALS AND METHODS

Animals
All experimental procedure were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee. Goat tracheal chain was obtained from slaughter house. Dunkin-Hartley Guinea pigs weighing between 350 and 400gm were used. They were housed in groups of five under standard laboratory conditions temperature (25±2°C) and 12/12 hr light/dark cycle. Animals had free access to standard pellet diet (Amrut laboratory animal feed, Sangli-Maharashtra) and water.

Plant material and preparation of extract
Fresh dried fruit berries (Myrsinaceae) was collected from Kolhapur, Maharashtra, India. The specimen was authenticated at Agharkar Research Institute, Pune. The berries were shed dried and latter powdered. This powder was defatted with petroleum ether and then subjected to Soxhle extraction using 90%ethanol. The extract obtained was then filtered and solvent was evaporated under vacuum. The yield of ethanolic extract of Embelia ribes fruit berries (ER) obtained was 3.1% (w/w).

Acute toxicity study
Acute oral toxicity study for ER was carried out according to the OECD 425 guidelines. Five albino mice weighing between 20-25 were used. ER at the dose of 2000mg/kg 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, 10th dose of above dose as 200mg/kg was taken as safe dose. (OECD 425) Dose of 100µg/ml for isolated goat tracheal chain preparation was selected by our laboratory trials. Three different doses (175, 350 and 700 mg/kg, p.o) of ER were later chosen for this study based on the acute toxicity testing. These doses were then converted in to guinea pig doses by using conversion factor.

Methods
Isolated Goat tracheal chain preparation
The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Kreb’s solution (Composition (mM): NaCl, 115; KCl, 4.7; CaCl2, 2; NaHCO3, 25; KH2PO4, 1.2; MgCl2, 1.2; glucose, 11.5). Goat trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb’s solution and maintained at 37 ± 1°C, 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 fronton 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 variant molar concentrations by maintaining 15 min time cycle. After
obtaining dose response curve of histamine (30μg/ml) on trachea, the ER (100 μg/ml) was added to reservoir and same doses of histamine were repeated. Graph of percentage of maximum contractile response on ordinate and negative log of molar concentration of histamine on abscissa was plotted to record dose response curve of histame, in absence and in presence of ER and standard drug Chlorpheniramine maleate (1 μg/ml). Histamine induced bronchconstriction in guinea pigs

Overnight fasted guinea pigs were randomly divided into five groups (n=5). Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. The Preconvulsion dyspnoea time (PCD) was noted for each animal. The Preconvulsion dyspnoea time is the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as preconvulsive dyspnoea commenced, animals were removed from the chamber and placed in fresh air to recover from dyspnoea for 24 hours. This time for preconvulsive dyspnoea was recorded as basal value. After 24 hours, animals belonging to group I served as control and were administered with phosphate buffer (1ml/kg, p.o.); Animals belonging to group II were administered with Chlorpheniramine maleate (2 mg/kg, i.p.) while group III to V were received respective doses of ER. These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hr and 24 hr and to determine Preconvulsion dyspnoea time (PCD). The protection offered by the treatment was calculated by using the following formula:

\[
\text{Percentage Protection} = \left( 1 - \frac{\text{T1}}{\text{T2}} \right) \times 100
\]

Where, \( \text{T1} \) = the mean of PCT before administration of test drugs, \( \text{T2} \) = the mean of PCT after administration of test drugs at 1hr, 4hr and 24 hr respectively.

**RESULT**

Isolated goat trachea chain preparation

In the present study, histamine (30μg/ml) produced dose dependent contraction of goat tracheal chain preparation maximum percentage of contractile response versus negative log molar concentration of histamine. The modified physiological salt solution containing Chlorpheniramine maleate (1 μg/ml) significantly inhibited (p<0.01) the contractile effect of histamine. Also the modified physiological salt solution containing ethanolic extract of ER (100 μg/ml) significantly inhibited (p<0.01) the contractile effect of histamine (Table 1).

Effect of *Embelia ribes* Extract on Histamine Induced Bronchoconstriction in Guinea Pigs

The guinea pigs when exposed to 0.2% Histamine aerosol showed signs of progressive dyspnoea leading to convulsions. Chlorpheniramine maleate (2 mg/kg, i.p) significantly prolonged (p<0.01) the preconvulsive dyspnoea in 1st, 4th and 24th hr as compared to control and the percent protection observed was 73.7, 79.6 & 61.3 respectively. ER at all doses significantly (p<0.01) prolonged the preconvulsive dyspnoea in 1st, 4th and 24th hr as compared to control. The percent protection observed for ER at the dose of 175 mg/kg was 46.8, 62.2 & 47.3 at 1st, 4th and 24th hr respectively. The percent protection observed for ER at the dose of 350 mg/kg was 70.1, 76.9 & 47.5 in 1st, 4th and 24th hr respectively. The percent protection observed for ER at the dose of 700 mg/kg was 72.3, 78.9 & 57.2 in 1st, 4th and 24th hr respectively. (Table 2 & 3)

**Statistical Analysis**

The results have been indicated in terms of mean ± SEM, (n=5). Difference between the groups was statistically determined by One way ANOVA with Dunnett’s test. The level of significance was set at **p<0.01.

<table>
<thead>
<tr>
<th>Group (n=5)</th>
<th>Percentage protection</th>
<th>1 hr</th>
<th>4 hr</th>
<th>24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>II(Std)</td>
<td>73.70</td>
<td>79.60</td>
<td>61.32</td>
<td></td>
</tr>
<tr>
<td>III(ER175)</td>
<td>46.82</td>
<td>62.20</td>
<td>47.32</td>
<td></td>
</tr>
<tr>
<td>IV (ER350)</td>
<td>70.10</td>
<td>76.90</td>
<td>47.50</td>
<td></td>
</tr>
<tr>
<td>V (ER700)</td>
<td>72.30</td>
<td>78.90</td>
<td>57.22</td>
<td></td>
</tr>
</tbody>
</table>

STD= Aerolized Histamine (0.2 μg/w) + Chlorpheniramine maleate (2 mg/kg, i.p); ER175= Aerolized Histamine (0.2 μg/w) + Ethanolic extract of fruit berries of *Embelia ribes* (175mg/kg p.o); ER350= Aerolized Histamine (0.2 μg/w) + Ethanolic extract of fruit berries of *Embelia ribes* (350mg/kg p.o); ER700= Aerolized Histamine (0.2 μg/w) + Ethanolic extract of fruit berries of *Embelia ribes* (700mg/kg p.o.)

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

<table>
<thead>
<tr>
<th>Dose of Histamine (30μg/ml)</th>
<th>-ve Log molar concentration of Histamine</th>
<th>% Maximum contraction (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0.2</td>
<td>6.38</td>
<td>17.03±0.629</td>
</tr>
<tr>
<td>0.4</td>
<td>5.91</td>
<td>29.3±0.639</td>
</tr>
<tr>
<td>0.8</td>
<td>5.34</td>
<td>48.79±2.403</td>
</tr>
<tr>
<td>1.6</td>
<td>4.89</td>
<td>68.16±1.881</td>
</tr>
<tr>
<td>3.2</td>
<td>4.56</td>
<td>86.49±1.587</td>
</tr>
</tbody>
</table>

- **p<0.01 compared to control group, Control = D.R.C. of histamine (30 μg/ml) in absence of test drug, CPM = D.R.C. of histamine (30 μg/ml) in presence Chlorpheniramine maleate (1 μg/ml); ER = D.R.C. of histamine (30 μg/ml) in presence ethanolic extract of *Embelia ribes* (100μg/ml).

Table 2: Histamine Induced Bronchoconstriction in Guinea Pigs

<table>
<thead>
<tr>
<th>Group (n=5)</th>
<th>Before Treatment</th>
<th>Preconvulsive dyspnoea (in Sec) (Mean ± SEM)</th>
<th>1 hr</th>
<th>4 hr</th>
<th>24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44.4±1.503</td>
<td>49.6±0.927</td>
<td>54.4±0.927</td>
<td>54.6±0.600</td>
<td></td>
</tr>
<tr>
<td>II (STD)</td>
<td>42.2±1.241</td>
<td>160±1.414**</td>
<td>206.8±1.020</td>
<td>108.8±1.020</td>
<td></td>
</tr>
<tr>
<td>III (ER 175)</td>
<td>43.4±1.208</td>
<td>92.6±0.871**</td>
<td>114.6±1.699</td>
<td>82.2±1.158</td>
<td></td>
</tr>
<tr>
<td>IV (ER 350)</td>
<td>42.8±1.158</td>
<td>142.8±1.497**</td>
<td>183±1.304</td>
<td>90±1.049</td>
<td></td>
</tr>
<tr>
<td>V (ER700)</td>
<td>42.8±1.158</td>
<td>154.2±0.909**</td>
<td>202.2±1.241</td>
<td>100±0.836</td>
<td></td>
</tr>
</tbody>
</table>

- **p<0.01 compared to control group, Control = Phosphate buffered saline (1ml/kg, p.o.); Aerosolized Histamine (0.2 % w/v) + Chlorpheniramine maleate (2 mg/kg, i.p); ER175= Aerolized Histamine (0.2 % w/v) + Ethanolic extract of fruit berries of *Embelia ribes* (175mg/kg p.o); ER350= Aerolized Histamine (0.2 % w/v) + Ethanolic extract of fruit berries of *Embelia ribes* (300mg/kg p.o); ER700=Aerolized Histamine (0.2 % w/v) + Ethanolic extract of fruit berries of *Embelia ribes* (700mg/kg p.o.).
DISCUSSION
Histamine is an autacoid having profound physiological effect in the body. The contraction of tracheal or bronchial smooth muscle in vitro has often been utilized for the study of contractile / dilator responses of agonists as well as antagonist. Both goat tracheal chain and strip preparations are suitable for screening the activity of a drug on respiratory smooth muscles.19 Spasmogens such as histamine, acetylcholine and barium chloride produce dose dependent contraction of goat tracheal chain preparation. The goat tracheal muscle has H1, M3 and β2 receptors. The stimulation of H1 receptor causes contraction of bronchial smooth muscle. 20 It is reported that activation of α-adrenergic and H1-histaminergic receptors causes activation of VIP (Vasoactive Intestinal Polypeptide) in cerebral cortex, which is responsible for release of histamine from sensory neurons. 22

Goat tracheal smooth muscle preparations are contracted by histamine through the H1-receptor stimulation. This leads to activation of IP3 and DAG pathway. This increased IP3 is responsible for releasing the microsomal calcium, leads to phosphorylation of actin-myosin fibers of goat trachea causing the contraction. Thus, the contraction of tracheal or bronchial smooth muscle in vitro has often been utilized for the study of contractile / dilator responses of agonists as well as antagonist.

In the isolated goat tracheal chain preparation, histamine produced dose dependent contraction of goat tracheal chain preparation while there was rightside shift of Dose Response Curve (DRC) of histamine in the presence of ER indicating antihistaminic activity. 21

Histamine when inhaled has been shown to induce bronchoconstriction by direct H1-receptor activation and also by a naturally mediated bronchoconstrictor effect via vagal reflexes. Histamine has been shown to activate action potentials in intrapulmonary vagal afferents. The guinea pigs are very much sensitive to histamine. When exposed to histamine aerosol, they showed signs of progressive dyspnoea leading to convulsions. In the present study, antihistaminic drug Chlorpheniramine maleate and ER significantly protected the guinea pigs against histamine-induced bronchospasm. ER has significantly prolonged the latent period of convulsions (PCT) as compared to control following the exposure to histamine aerosol. This indicates the utility of the ER in the treatment of asthma and bronchitis by virtue of its H1- receptor blocking or bronchodilating activity. Thus ER have anti-histaminic by blocking H1- receptor or bronchodilating activity which suggestive of its potential in prophylaxis and management of asthma.

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REFERENCES

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