Anti-Asthmatic and Anti-Anaphylactic Activity of Helicanthus Elastica Desr

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Summary

Asthma is a complex and multifactorial airway disease. Due to side effects and resistance like limitations, development of new treatments represents a major goal for the researchers. So the purpose of the present study was to evaluate ethyl acetate fraction of Helicanthus elastica (H. elastic) for anti-asthmatic and anti-anaphylactic activity by various animal models. The antiasthmatic activity of H. elastica was studied on the bronchial hyperactivity models like histamine induced bronchospasm in guinea pigs. While for anti-anaphylactic activity was studied by active and passive anaphylaxis in rats. Treatment with H. elastica showed significant protection against histamine aerosol-induced bronchospasm in guinea pigs. H. elastica treatment for 14 days resulted significant mast cells stabilization of actively and passively sensitized rats, also reduction in eosinophil cell count, IgE count and give relief from nasal acoustic symptom. Anti-asthmatic and anti-anaphylactic activity of H. elastica may be due to anti-histaminic property and mast cell membrane stabilizing potential, suppression of IgE antibody production, suppression of eosinophil cell activation respectively.

Keywords: H. elastica, anti-asthmatic, anti-anaphylactic, mast cell stabilization, anti-eosinophilic.
Introduction

Respiratory diseases like asthma, tuberculosis etc are major cause of death and disability in all over the world as per global health situation. Asthma is a chronic inflammatory disorder of the airways responsible for morbidity and mortality worldwide (1, 2, 3). Corticosteroid treatment remains the first preference of treatment in asthma; however these therapies are not always completely effective (4). Intensive research during the last several decades has highlighted the role of responsible factors in the etiopathogenesis of asthmatic conditions and airway hyperresponsiveness. Insipe of voluminous literature on the subject, the treatment of allergic diseases is still far from satisfactory. The available treatment options for upper and lower respiratory tract allergic diseases have major limitations due to lack of efficacy, associated adverse events, and compliance issues on prolong use (4). Therefore, there is a need to explore for new anti-asthmatic agents.

Plants containing flavonoids have been reported to possess antiasthmatic, antiallergic, and mast cell stabilization properties (5-9). Hemiparasitic plants contain higher amounts of polyphenolic compounds than their host plant (10). *Striga orobanchioids* a hemiparasitic plant have been reported to possess antihistaminic and mast cell stabilizing activity (11). Presence of common flavonoids, quercetin and some cytotoxic proteins was conformed in hemi parasites like *Helicanthus elastic* (12). Therefore present study was undertaken to study the anti-asthmatic and anti-anaphylactic activity of extract using different animal models.
METHODS

Collection and authentication of plant material
The mistletoe, *Helianthus elastica* Desr. parasitic on *Syzygium cumini* Linn. (Myrtaceae) was collected from Western Ghats (Latitude 17° 55' 0N and Longitude 73° 40' 0E and at altitude 1352 m) in November 2007. The plant specimen (Voucher no. LOT-1) was authenticated by Dr. P.S. N. Rao (Botanical Survey of India, Pune).

Extraction
About 700gm of powdered material was extracted with methanol (1.5 L×3) by cold maceration method for 24 h. The extract was collected, combined and concentrated using rotary vacuum evaporator which yields (9.48%w/w) of extract. The aqueous extract was partitioned between ethyl acetate and n-butanol successively. The ethyl acetate fraction (4.08%w/w) enriched with polyphenolic compounds was used in animal experimentation.

Animals
The adult guinea pigs (Dunkey-Hartley, 300-400 g) of either sex were obtained from Haffkin Institute, Mumbai. The rats (Albino Wistar, 170-200 g) of 8-9 weeks age were obtained from commercial breeder. The animals were maintained under standard conditions of temperature (22 ± 2°C), relative humidity (60 ± 5%) and light (12 hrs light/dark cycles) with free accesses to feed and water. The study has got approval from Institutional Animal Ethics Committee (IAEC) of R.C.P., Shirpur, India and was in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on Animals (CPCSEA). Registration number 651/02/C/CPCSEA.
Histamine induced bronchospasm in guinea pigs

Histamine induced bronchospasm in guinea pigs was carried out by reported method (13, 14). Briefly, experimental bronchial asthma was induced in guinea pigs by exposing them to 0.5 % histamine diphosphate (Sigma chemicals) with constant flow rate 5 mL/min in an aerosol chamber to induced experimental bronchial asthma. The animals exposed to the histamine 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. The onset time of PCD before and after extract treatment was designated as T1 and T2 respectively. The animals divided into five groups, each group containing four. Animals in a control group received water, at a dose of 10mL/Kg p.o. Chlorpheniramine maleate (2 mg/Kg, p.o) and H. elastica extract in graded dose (25, 50, 100 mg/Kg p.o) were administered. After two and half hours, the time for the onset of PCD was recorded (T2). The protection offered by the extract treatment was calculated by the following formula.

\[
\text{Percentage protection} = 1 - \frac{T_1}{T_2} \times 100
\]

Where: T1 is time for PCD before treatment and T2 is the time for PCD after drug treatment.

Active anaphylaxis in rat

Active anaphylaxis in rat was performed according to the reported method (15, 16). Briefly, the rats were divided into six groups, each with six animals. Except normal control group, all five groups were sensitized by subcutaneous injection of 0.5 mL of horse serum along with 0.5 ml of triple antigen containing 20,000 million Bordetella pertussis.
organisms (Serum Institute of India Ltd., Pune). Group I receiving water 10mL/Kg p.o served as normal control. Group II served as sensitized control receives water10mL/Kg p.o. Group III, IV, V treated with the *H. elastica* extract in graded dose (25, 50, 100 mg/Kg p.o.) respectively and Group VI treated with reference standard, Prednisolone (10mg/Kg, p.o.) once a day for 14 days. Blood samples were collected from tail vein and eosinophil cell count was taken on 1st, 3rd, 5th, 7th, 9th, 11th, 13th and 14th days of treatment. The frequency of sneezes and other nasal symptoms such as the nasal acoustic phenomenon was evaluated every day using a scoring system (17). Symptom scores were graded on a four-point scale. Each grade was assigned a numerical score (0-4), and data were analyzed both as separate symptoms and as a total symptom score. Nasal acoustic symptom scores were graded in points as follows: 0 – none; 1 – impaired inspiration; 2 – nasal crackles; 3 – intensive nasal crackles and severe breathing impairment; 4 – death. Gross morphology was observed every day. On day 14, two hours after drug administration, the rats were anesthetized by anesthetic ether and blood was collected from cardiac puncture. The serum was separated and IgE analyzed using Alpha light fully automated chemiluminescence immunoassay system (15). The intestinal mesentery was isolated from sacrificed animals and was kept in Ringer-Locke solution at 37°C until used. The mesenteric pieces were challenged with 5% horse serum for 10 min and the mast cells were stained with toluidine blue and examined microscopically for the number of intact and degranulated mast cells (18).
Passive anaphylaxis in rat

The passive anaphylaxis in rats was studied according to the reported method (15, 16). In brief, following active anaphylaxis, the rats was sacrificed and the blood was collected by decapitation and the serum separated. One mL of serum of the actively sensitized rats were injected intraperitoneally to 30 normal rats comprising of 6 each in group II, III, IV and V,VI. Rats of Group I received water 10mL/Kg (vehicle) and served as control. Rats of Group II received 1mL anaphylactic rat serum and water (vehicle) 10mL/Kg, Group III, IV, V were administered with *H. elastica* extract 25, 50, 100mg/Kg p.o. respectively, once a day for 14 days .Group VI rats received 10mg/Kg of Prednisolone (reference drug) orally for the same duration. Forty eight hours later, passively sensitized rats were challenged by the intraperitoneal injection of horse serum (1 mL). Ten minutes after the antigen challenge, the rats were sacrificed and the intestinal mesenteries were collected in Ringer-Locke solution. The mesenteric mast cells were stained with toludine blue as stated above and examined microscopically. The number of intact and disrupted mast cells was counted in at least 10 randomly selected fields for each tissue.

Statistical analysis

The results are expressed as mean ± SEM and analyzed statistically using ANOVA with Tukey- Kramer multiple comparison, Bonferroni’s multiple comparison post-hoc post test. The minimum level of significance was fixed at p < 0.05.
Results

Histamine induced bronchospasm in guinea pigs
The dyspnoea was evoked within 26.83 seconds in control group animals on exposure of histamine aerosol, while after the treatment with *H. elastica* extract 25, 50, 100 mg/Kg p.o, of extract prolonged the PCT up to 35, 95.6, 106.3 seconds and also standard chlorpheniramine maleate prolonged the PCT upto 201.3 seconds under same conditions (Figure1).

![Figure 1 Effect of *H. elastica* extract on histamine aerosol induced bronchospasm](image)

Values are mean ± SEM, n=3 in each group. One-way ANOVA (P<0.05) followed by Tukey- Kramer multiple comparison post-hoc test * P<0.05, ***P<0.001 significantly different from control, ns- not significant.

The results indicate that extract at 50 and 100 mg/kg and chlorpheniramine maleate (2 mg/Kg), significantly prolonged the latent period of convulsion (PCT) as compared to control following exposure to histamine aerosol. Extract 25, 50, 100
mg/Kg demonstrated 24.86%, 72.49% and 75.26% protection respectively while reference standard chlorpheniramine maleate, showed 86.94% protection against histamine induced bronchospasm (Figure 2). The antihistaminic drug Chlorpheniramine maleate used in the study produced a significant increase the PCT.

Figure 2 Effect of H. elastica extract on % protection against histamine induced bronchospasm

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std CPM</td>
<td>86.94%</td>
</tr>
<tr>
<td>HE 25 mg/kg</td>
<td>72.49%</td>
</tr>
<tr>
<td>HE 50 mg/kg</td>
<td>75.26%</td>
</tr>
<tr>
<td>HE 100 mg/kg</td>
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</tbody>
</table>

Active anaphylaxis in rat
Two weeks after sensitization, the antigen challenge degranulated about 80% of the mast cells in sensitized control group. The sensitized group treated with 25, 50,100 mg/Kg p.o for 2 weeks and then challenged with an antigen there was shown significant reduction in the number of disrupted mast cells (Table 1). H. elastica also control the elevating eosinophil cell count at early stage of anaphylaxis as compare to Prednisolone.
Table 1 Effect of *H. elastica* extract on mast cell stabilization in actively sensitized rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose p.o</th>
<th>Mast cells %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td>Control</td>
<td>Water 10mL/Kg</td>
<td>85 ± 0.96***</td>
</tr>
<tr>
<td>Sensitized control</td>
<td>Water 10mL/Kg</td>
<td>24.33 ± 0.98</td>
</tr>
<tr>
<td><em>H. elastica</em></td>
<td>25 mg/Kg</td>
<td>29.33 ± 1.7 ns</td>
</tr>
<tr>
<td><em>H. elastica</em></td>
<td>50 mg/Kg</td>
<td>59.88 ± 1.30***</td>
</tr>
<tr>
<td><em>H. elastica</em></td>
<td>100 mg/Kg</td>
<td>75.5 ± 1.05***</td>
</tr>
<tr>
<td>Std drug (Prednisolone)</td>
<td>10 mg/Kg</td>
<td>77.5 ± 1.52***</td>
</tr>
</tbody>
</table>

Values given as mean ± SEM, n=6. One-way ANOVA (P<0.05) followed by Bonferroni’s multiple comparison post-hoc test (ns= non significant, ***P<0.001).

Significantly reduction was shown in eosinophil cell count by 50 and 100 mg/Kg *H. elastica* as compared to sensitized control (Figure 3).
Blood IgE level was increased by sensitization by horse serum. In sensitized group rats showed raised IgE level up to 10.48 IU/mL (International Unite/milliliter). *H. elastica* 100 mg/Kg and Prednisolone (std. drug) treated sensitized rats showed significant reduction in serum IgE levels as compared to sensitized control (Table 2).
Table 2 Effect of *H. elastica* extract on IgE level in actively sensitized rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose p.o</th>
<th>IgE (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Water 10 mL/Kg</td>
<td>3.16±0.43***</td>
</tr>
<tr>
<td>Sensitized control</td>
<td>Water 10 mL/Kg</td>
<td>10.48±1.09</td>
</tr>
<tr>
<td><em>H. elastica</em> 50 mg/kg</td>
<td></td>
<td>7.46±0.33*</td>
</tr>
<tr>
<td><em>H. elastica</em> 100 mg/kg</td>
<td></td>
<td>3.64±3.51***</td>
</tr>
<tr>
<td>Std drug (Prednisolone)</td>
<td>10 mg/Kg</td>
<td>3.28±1.04***</td>
</tr>
</tbody>
</table>

Values given as mean ± SEM, n=6. One-way ANOVA (P<0.05) followed by Bonferroni’s multiple comparison post-hoc test (ns- non significant, ***P<0.001).

*H. elastica* extract gives relief from frequency of sneezes and nasal acoustic symptoms as compared to sensitized control. Significant differences in symptom score between extract treated and sensitized control groups occurred from the 3rd day of sensitization and persisted till the 9th and 10th days of provocation (Figure 4).

**Figure 4** Effect of *H. elastica* extract on nasal acoustic symptoms

![Graph showing respiratory score over days for different groups: sensitized control, std drug, extract 50mg/kg, extract 100mg/kg, and control.](image)
*H. elastica* treated rats showed no inflammation anywhere on the body as compared to sensitized control rats. (Data not shown)

**Passive anaphylaxis in rat**

When the serum of the actively sensitized rats from control group were administered intraperitoneal to the fresh rats and challenged with antigen 48 hr later, the percentage degranulation of the peritoneal mast cells was about 84.5 %. The percentage degranulation was significantly reduced in case of extract treated group. Prednisolone, the reference drug, also showed significant mast cell stabilization activity in passively sensitized rats (Table 3).

**Table 3 Effect of *H. elastica* extract on mast cell stabilizing activity in passively sensitized rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drug p.o</th>
<th>Mast cells %</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact</td>
<td>Disrupt</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Water 10 mL/Kg</td>
<td>84.50 ± 1.9***</td>
<td>15.5 ± 1.9***</td>
<td></td>
</tr>
<tr>
<td>Sensitized control</td>
<td>Water 10 mL/Kg</td>
<td>20.17 ± 1.1</td>
<td>79.83 ± 1.1</td>
<td></td>
</tr>
<tr>
<td><em>H. elastica</em></td>
<td>25 mg/kg</td>
<td>26.16 ± 1.2 ns</td>
<td>73.84 ± 1.2 ns</td>
<td></td>
</tr>
<tr>
<td><em>H. elastica</em></td>
<td>50 mg/kg</td>
<td>51.67 ± 2.1***</td>
<td>48.33 ± 2.1***</td>
<td></td>
</tr>
<tr>
<td><em>H. elastica</em></td>
<td>100 mg/kg</td>
<td>71.83 ± 2.6***</td>
<td>28.17 ± 2.6***</td>
<td></td>
</tr>
<tr>
<td>Standard drug (Prednisolone)</td>
<td>10 mg/Kg</td>
<td>74.50 ± 2.8***</td>
<td>25.5 ± 2.8***</td>
<td></td>
</tr>
</tbody>
</table>

Values given as mean ± SEM, n=6. One-way ANOVA (P<0.05) followed by Bonferroni’s multiple comparison post-hoc test (ns- non significant, ***P<0.001).
Discussion

In the present study, histamine aerosol was used for induction of broncho-constriction, dyspnoea, asphyctic convulsions. The pre-convulsive time (PCT) was counted. The antihistaminic drug Chlorpheniramine maleate (H\textsubscript{1}: H\textsubscript{2} 15000:1 antagonist) used in the study produced a significant increase the PCT. Therefore, the result of present study indicates the utility of the *H. elastica* in the treatment of asthma and bronchitis by virtue of its may be anti-histaminic broncho-dilating activity (16, 19).

The protection offered by *H. elastica* on the mast cells stabilizing potential against antigen-antibody reaction due to membrane stabilization. Allergic asthma is a chronic inflammatory process occurring due to exposure of allergen resulting in the activation of T-lymphocytes with subsequent release of inflammatory mediators. Mast cell activation initiated by horse serum (allergen) via cross linking of IgE antibodies on the cell. Horse serum antigen sensitizes the animal and stimulates production of IgE antibody. These IgE binds to FcεRI receptors on mast cells. During challenge, re-introduction of antigen leads to cross-linking of mast cell bound IgE which leads to mast cell activation and degranulation and release of mediators. The disruption of mast cells which causes histamine release is an important feature of anaphylaxis. It has been assumed that the process leading to histamine secretion may be mediated by calcium release from an intracellular store of mast cells (19).

Significantly reduction in eosinophil cell count means that *H. elastica* 50 and 100 mg/Kg may inhibit clustering of eosinophil cell around the nerves and inhibit release of major basic protein (MBP) or inhibit eosinophil cell recruitment by
inhibition of (interleukins) IL-4, IL-5 and IL-13 which play important role in eosinophil cell recruitment (19). Activated eosinophils release eosinophil major basic protein (MBP), which is an endogenous antagonist for M_2 muscarinic receptors. The M_2 receptors on the parasympathetic nerves in the lungs normally inhibit release of acetylcholine. This means that the eosinophils increase release of acetylcholine from the parasympathetic nerves. When M_2 receptors are blocked by MBP, acetylcholine release is increased, resulting in hyper responsiveness (20).

IgE mediated mast cell stimulation is an important initial event in development of type I allergic reaction such as asthma and atopic disorders. Clinical studies have found a close association between asthma and serum IgE levels. Clinical studies have found a close association between asthma and serum IgE levels, as well as IgE- dependent skin test reactivity to allergens (21). In active anaphylaxis model sensitized control rats were showed significantly increased IgE level as compared to normal rats IgE level. Significant suppression of IgE indicates that *H. elastica* inhibit activation of B cell, CD^4^ T helper cell, plasma cell after sensitization with antigen (horse serum). Antigen challenge, in sensitized animals, results in degranulation of mast cell (16, 19). The anti-anaphylactic action of *H. elastica* was due to the suppression of elevating IgE antibody production which was studied directly in rat serum.

Due to sensitization by horse serum anaphylactic hypersensitivity reaction was occurred in sensitized control group of rats. The redness, difficulty in breathing, allergic inflammation at all over the body was observed on 14^{th} d of sensitization. This may be due to anaphylactic and allergic shock. *H. elastica* was found to be active in immediate phase
of asthma. Consistent with these observations, the extract treated rats showed less score in nasal acoustic symptom as compared to sensitized control (17). Inhibition of inflammation may be due to mast cell stabilization, suppression of IgE and inhibition of inflammatory mediators (COX$_1$, COX$_2$ related PGD$_2$) releases (19).

The anti-anaphylactic action of *H. elastica* was may be due to the suppression of elevating IgE antibody production and eosinophil cell and moderate mast cell stabilization which was studied directly in rat serum. *H. elastica* have moderate mast cell stabilizing activity in passively sensitized animals. In passive anaphylaxis, the serum of the actively sensitized rats which contain the appropriate IgE antibodies was injected intra-peritoneally into the recipient rats. The present antibodies become fixed to the peritoneal mast cells of fresh recipient rat and when exposed to antigen will show anaphylactic degranulation. Cross-linking between mast cell bound IgE and antigen leads to mast cell activation and release of primary and secondary mediators like histamine, prostaglandins, leukotrienes (16, 19). *H. elastica* showed moderate mast cell stabilization in passive anaphylactic rats due to low serum IgE level of active anaphylactic rat.

All this findings revealed that the anti-asthmatic and anti-anaphylactic activity of *H. elastica* extract may be due to anti-histaminic spasmolytic activity, suppression of IgE antibody production, mast cell stabilizing potential and inhibition of antigen- immediate hypersensitivity reaction. Anti-asthmatic and anti-anaphylactic activities of *H. elastica* may be due to presence of polyphenolic compounds present in it.
References

1. Asthma facts, United States Indoor Environments Division Environmental Protection Agency Office of Air and Radiation (6609J) EPA-402-F-04-019 (6609J), 2009.


