Antihistaminic and antianaphylactic activity of Bresol (HK-07), a herbal formulation

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ABSTRACT
Objective: To study the antianaphylactic, antihistaminic and mast cell stabilization activity of Bresol (HK-07) in experimental animals.

Materials and Methods: Experimental studies of Bresol (HK-07) were evaluated using Wistar rats and Duncan Hartley guinea pigs. The antianaphylactic activity of Bresol (HK-07) was investigated in rats using active anaphylaxis model. The effect of Bresol (HK-07) on mast cell stabilization was performed by ex vivo challenge of antigen in sensitized rat intestinal mesenteries. Antihistaminic activity was studied in guinea pigs using histamine-induced bronchospasm where pre-convulsive dyspnea was used as an end point following exposure to histamine aerosol. Dose response studies of Bresol (HK-07) were conducted at 125, 250 and 500 mg/kg p.o. in anaphylactic shock-induced bronchospasm in rats. The optimal dose level was used for the remaining experimental models.

Results: Treatment with Bresol (HK-07) at 125, 250 and 500 mg/kg p.o. showed a significant reduction in signs and severity of symptoms (p<0.05), onset (p<0.001) and mortality rate (p<0.05) following anaphylactic shock-induced bronchospasm. It also significantly reduced the serum IgE levels (p<0.001) in animals administered with Bresol (HK-07) as compared to untreated controls. Treatment of sensitized animals with Bresol (HK-07) at a dose rate of 500 mg/kg p.o. for 2 weeks resulted in a significant reduction in the number of disrupted mast cells (p<0.001) when challenged with an antigen (horse serum). Bresol (HK-07) significantly prolonged the latent period of convulsion (p<0.008) as compared to control following exposure of guinea pigs to histamine aerosol.

Conclusion: The findings from various studies reveal that the antihistaminic and antianaphylactic activity of Bresol (HK-07) may be due to the mast cell stabilizing activity, suppression of IgE and inhibition of pathological effect induced by release of inflammatory mediators by some of the components of the herbal formulation.

KEY WORDS: Bresol (HK-07), Antianaphylactic, antihistaminic, mast cell stabilization, serum IgE

INTRODUCTION
One of the common diseases that affect mankind is allergy, in its diverse manifestations. The prevalence of allergy and asthma has risen in recent years despite the general health improvement in the population.1 Allergic diseases are responsible for significant morbidity and have severe economic impact.2 Various epidemiological studies have identified the causes for this increase in the prevalence of upper and lower respiratory tract allergic diseases. The postulated reasons are increasing environmental pollution3 and increased predisposition of individuals producing excessive IgE through a major change in the gene pool, leading to increased expression of these diseases. Other important causes include changing lifestyles and an increasing awareness of these disorders.4
Intensive research during the last several decades has highlighted the role of lymphocytes, immunoglobulins, mast cells and various autacoids in the etiopathogenesis of allergic conditions. In spite of voluminous literature on the subject, the treatment of allergic diseases continues to be far from satisfactory. The available treatment options for upper and lower respiratory tract allergic diseases have major limitations due to low efficacy, associated adverse events and compliance issues. Ayurveda, an Indian system of medicine has described several drugs from indigenous plant sources for use in the treatment of bronchial asthma and allergic disorders. Bresol (HK-07) is one such polyherbal formulation containing the extracts of Curcuma longa, Ocimum sanctum, Adhatoda vasica, Trikatu, Triphala, Embelia ribes, Cyperus rotundus, Cinnamomum zeylanicum, Elettaria cardamomum, Cinnamomum tamala and Mesua ferrea. The dry rhizome of Curcuma longa contains curcumin, the main bioactive component, demethoxycurcumin and bisdemethoxycurcumin. The traditional uses of turmeric or natural curcuminoids in folk medicine are multiple, and some of these therapeutic effects including antioxidant, anti-inflammatory and anti-allergic properties have been confirmed by various experimental studies. Curcumin is also found to be a potent blocker of nuclear transcription factor (NF)-kB, which is linked to a variety of diseases including allergy and asthma. Ocimum sanctum has been demonstrated to give protection against histamine as well as pollen-induced bronchospasm in guinea pigs and inhibited antigen-induced histamine release from sensitized mast cells and anti-inflammatory properties have also been established. Adhatoda vasica is documented for its potent anti-inflammatory, antiallergic and antitussive activities. Piper longum has been shown to reduce the passive cutaneous anaphylaxis in rats and protect guinea pigs against antigen-induced bronchospasm. Emblica officinalis was found to exhibit anti-inflammatory, antitussive and antioxidant activities. Terminalia belerica demonstrated potent antiperoxidative activity and inhibited lipid peroxide formation by scavenging hydroxyl and superoxide radicals in vitro. Zingiber officinale has been found to exert anti-inflammatory activity and is reported to be a potent inhibitor of inflammatory mediators such as prostaglandins and leukotrienes. Cyperus rotundus inhibited the nitric oxide and superoxide production in in vitro studies, which used murine macrophage cell lines.

In the present study, the effect of Bresol (HK-07), a polyherbal formulation was studied on the active anaphylaxis and mast cell stabilization in rats, and histamine-induced bronchospasm in guinea pigs.

MATERIALS AND METHODS

Animals: Inbred Wistar rats (175-200 g) and guinea pigs (400-600 g) of either sex housed in standard conditions of temperature (22 ± 2°C), relative humidity (60 ± 5%) and light (12 h light/dark cycle) were used. They were fed with standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the “National Academy of Sciences” and published by the “National Institutes of Health”.

All the chemicals and reagents were procured from Hi-Media Laboratories limited, Mumbai, except for histamine, horse serum and toluidine blue. Histamine and horse serum were procured from Sigma Chemical Co. and toluidine blue from Loba-Chemie, Mumbai. Elisa kit for IgE was supplied by Orion diagnostics, Espoc, Finland.

Active anaphylaxis: Twenty eight rats were sensitized by injecting subcutaneously 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, India). The sensitized rats were divided into 4
groups of 7 animals each. Rats of Group I received water (vehicle) and served as control. Rats of Groups II, III and IV were orally administered with Bresol (HK-07) at 125, 250 and 500 mg/kg respectively, once a day for 14 days. On day 14, after 2 h of treatment, the rats were challenged with intravenous injection of 0.25 ml horse serum. The rats were then observed for the onset of symptoms such as dyspnea and cyanosis, duration of persistence of symptoms (min) and mortality and the severity of symptoms were scored.20

Serum total IgE was quantified with an ELISA protocol according to the manufacturers instruction (Orion diagnostics, Espoc, Finland). Briefly, the plates were coated with affinity-purified rabbit anti-IgE overnight at 4°C and then blocked with 1% bovine serum albumin (BSA) in PBS for 1 h at 37°C. The serum samples and appropriate dilutions of a standard IgE preparation were placed in the wells, and the plates were incubated for 3 h at 4°C. Sample blank wells did not receive serum but were otherwise treated similarly. The bound IgE was detected with polyclonal goat anti-IgE antibodies (incubation for 1 h at 37°C), followed by HRP-conjugated rabbit anti-goat antibodies (incubation for 1 h at 37°C). The plates were developed by addition of O-phenylene diamine and read in an ELISA (Anthos HT-II, USA) plate reader at 490 nm.

Mast cell stabilizing activity: Thirty two rats were divided into four groups of eight animals in each. Rats of Group I received water (vehicle) and served as control. Rats of Group II (sensitised group, which received only water) III (Bresol (HK-07)) and IV (prednisolone) were sensitized by injecting subcutaneously 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). Rats of Group III were administered with Bresol (HK-07) at 500 mg/kg p.o., once a day for 14 days. Rats of Group IV were administered with prednisolone (reference drug) at 10 mg/kg p.o., for the same duration. On day 14, the rats were sacrificed 2 hours after the treatment and the intestinal mesentery was taken out for the study on mast cells. Mesenteries along with intestinal pieces were excised and kept in Ringer-Locke solution (NaCl 154, KCl 5.6, CaCl$_2$ 2.2, NaHCO$_3$ 6.0, Glucose 5.55 mM/l of distilled water) at 37°C. The mesenteric pieces were challenged with 5% horse serum for 10 minutes after which the mast cells were stained with 10% toluidine blue and examined microscopically for the number of intact and degranulated mast cells.20

Histamine-induced bronchospasm in guinea pigs: Bronchospasm was induced in guinea pigs by exposing them to 1% histamine aerosol under constant pressure (1 kg/cm$^2$) in an aerosol chamber. Twelve guinea pigs were divided into 2 groups of 6 animals. Group I served as control and Group II received Bresol (HK-07) at a dose rate of 500 mg/kg p.o. once a day for 5 days. Animals were exposed to 1% histamine aerosol under constant pressure (1 kg/cm$^2$) in an aerosol chamber on day 0 without any treatment. The end point, pre-convulsive dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsions.21 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. On days 1 and 5, two hours after the administration of the drug, the time for the onset of PCD was recorded as on day 0.

Statistical Analysis: The results of various studies were expressed as mean ± SEM and analyzed statistically using One Way ANOVA followed by Bonferroni’s multiple comparison post-hoc test or Chi square test or paired Student’s ‘t’ test to find out the level of significance. The minimum level of significance was fixed at 95% confidence limit. The analysis was performed using Graphpad Prism software package (Version 4.0).
RESULTS

Effect of Bresol (HK-07) on anaphylactic shock-induced bronchospasm in sensitized rats: Bresol (HK-07) protected the sensitized rats against anaphylactic shock in a dose dependent manner. In control rats, intravenous challenge dose of the antigen (horse serum) caused shock in 100% of the animals, while in treated rats (500 mg/kg), the onset of symptoms of shock was delayed \((p<0.001)\), symptoms were less severe \((p<0.05)\) and reduced the mortality rate \((p<0.05)\) (Figure 1). Bresol (HK-07) (500 mg/kg) also resulted in significant reduction of serum IgE levels \((25.80 \pm 4.85 \text{ ng/ml, } p<0.001)\) as compared to sensitized controls \((125.06 \pm 9.66 \text{ ng/ml})\). Serum IgE levels in control group was \(8.83 \pm 0.84 \text{ ng/ml (p<0.001 as compared sensitised control). Bresol (HK-07) showed optimal pharmacological effect at 500 mg /kg dose. Hence, this dose of Bresol (HK-07) was used for the remaining studies."

Mast cell stabilizing potential of Bresol (HK-07): Antigen challenge resulted in significant degranulation of the mesenteric mast cells (about 88%, \(p<0.001\)). Pretreatment of sensitized animals with Bresol (HK-07) at 500 mg/kg p.o. for 2 weeks resulted in a significant reduction in the number of disrupted mast cells \((p<0.001)\) when challenged with horse serum. The effect of Bresol (HK-07) was also comparable with the reference drug prednisolone (Table 1).

Table 1: Effect of Bresol (HK-07) on mast cell stabilization in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mast cells (%)</th>
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<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Disrupted</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>83.06 ± 3.70*</td>
<td>16.94 ± 3.70*</td>
<td></td>
</tr>
<tr>
<td>Sensitized control</td>
<td>12.33 ± 1.92</td>
<td>87.67 ± 1.92</td>
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<tr>
<td>Bresol (HK-07) (500 mg/kg)</td>
<td>64.25 ± 9.51*</td>
<td>35.75 ± 9.51*</td>
<td></td>
</tr>
<tr>
<td>Prednisolone (10 mg/kg)</td>
<td>69.19 ± 4.89*</td>
<td>30.81 ± 4.89*</td>
<td></td>
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</tbody>
</table>

\*Significantly different from sensitized control \((p<0.001)\)
Values are mean ± SE, \(n=8\) per experimental group (ANOVA followed by Bonferroni’s multiple comparison post-hoc test).

Figure 2. Effect of Bresol (HK-07) on histamine induced bronchospasm in guinea pigs. Values are expressed as Mean ± SE; \(n=6\) per experimental group. \(^{a}p<0.008\) as compared to control on Day 5 (paired Student’s ‘t’ test)

Effect on histamine-induced bronchospasm: Bresol (HK-07) significantly prolonged the latent period of PCD \((p<0.008)\) as compared to control following exposure to histamine aerosols on day 5 (Figure 2).
DISCUSSION AND CONCLUSION

Experimental animal model of asthma is characterized by allergen-induced immediate airway constriction and late airway reactivity to a pharmacological vasoconstrictor such as histamine and leukotrienes. Histamine is a central mediator in the pathogenesis of allergic and inflammatory disorders. In the present study, Bresol (HK-07) prolonged the latent period of PCD in guinea pigs following histamine aerosol. This may be suggestive of an antihistaminic activity following treatment with Bresol (HK-07). It also offered protection against anaphylactic shock-induced bronchospasm in rats.

Basophils, mast cells and their preformed and de novo synthesized mediators, play a pivotal role in the pathogenesis of allergic disorders. These molecules are potent vasoactive and bronchoconstrictor agents and they modulate local immune responses and inflammatory cell infiltration.\textsuperscript{22,23} Immunoglobulin E (IgE) mediated mast cell stimulation is an important initial event in the development of type I allergic reactions like asthma and atopic disorders. Clinical studies have found a close association between asthma and serum IgE levels as well as IgE dependent skin test reactivity to allergens.\textsuperscript{24} Antigen challenge, in sensitized animals, results in degranulation of mast cells, which is an important feature of anaphylaxis. In the present study, Bresol (HK-07) showed marked protection against the mast cell degranulation following antigen challenge in sensitized animals. Mast cell stabilizing activity of Bresol (HK-07) may be attributed to the presence of herbal extracts, which are known for their mast cell stabilizing potential against antigen-antibody reaction and/or due to the suppression of IgE antibody production, which is responsible for degranulation mast cells.\textsuperscript{9} This antianaphylactic and antihistaminic effect may be due to the stabilization of the mast cell membrane, suppression of IgE and inhibition of pathological effects induced by release of inflammatory mediators in Bresol (HK-07) treated animals.
All the above findings lend credence to the beneficial use of Bresol (HK-07) in the treatment of asthma and related conditions. However, further studies with other experimental models, especially in the role of cytokines are warranted to substantiate the antiasthmatic and antiallergic activity of Bresol (HK-07).

References
19. Seo WG, Pae HO, Oh GS, Chai KY, Kwon TO, Yun YG, et al. Inhibitory effects of methanol extract of Cyperus rotundus rhizomes on nitric oxide and superoxide


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