ANTIANAPHYLACTIC, ANTIHISTAMINIC AND MAST CELL STABILIZATION ACTIVITY OF HN-08, A POLYHERBAL FORMULATION

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Summary

The present paper reports the antianaphylactic, antihistaminic and mast cell stabilization activity of HN-08, a polyherbal formulation in various experimental models. HN-08 is an herbal formulation containing extracts of various plant constituents. The compound HN-08 was evaluated using Wistar rats and Duncan Hartley guinea pigs. The antianaphylactic activity was investigated in rats using the active anaphylaxis model. The effect 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 preconvulsive dyspnea was used as an end point following exposure to histamine aerosol. Dose response studies on test formulation were conducted using different levels of post oral doses (125, 250, and 500 mg/kg, p.o) in anaphylactic shock-induced bronchospasm in rats. Based on the activity profile optimum dose was selected for further studies.

Keywords: Hypersensitivity, IgE, mast cell degranulation

Introduction

Allergy is one of the common diseases that affect mankind with diverse manifestations. The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population (1). Allergic diseases are responsible for significant morbidity and have severe economic impact (2). Various epidemiological studies have identified the causes for an increase in the prevalence of upper and lower respiratory tract allergic diseases. Some of the postulated reasons are increasing environmental pollution (3) and increased predisposition of individuals producing excessive IgE through a major change in the gene pool, changing lifestyles, and an increasing awareness of the 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. Inspite of the 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 owing to low efficacy, associated adverse events, and compliance issues (5).

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. HN-08 is one such polyherbal formulation containing mainly the extracts of Curcuma *longa,* Ocimum *sanctum,* Piper *longum,* Heliotropium *indicum and* Naravelia *zeylanica* . The dry rhizome of *Curcuma longa* contains curcumin, demethoxycurcumin, and bisdemethoxycurcumin as main bioactive component. The traditional uses of turmeric or natural curcuminoids in folk medicine are multiple, and some are based on their antioxidant, antiinflammatory and antiallergic properties which have been confirmed by various research studies (6). Curcumin is also found to be a potent blocker of nuclear transcription factor (NF)-kB, (7) which is linked to a variety of diseases including allergy and asthma (8). *Ocimum sanctum* has been demonstrated to protect against histamine, as well as pollen-induced bronchospasm in guinea pigs and inhibited antigen-induced histamine release from sensitized mast cells(9) apart from its established anti-inflammatory(10) and antioxidant(11) properties. Piper *longum* has been shown to reduce the passive cutaneous anaphylaxis in rats and protect guinea pigs against antigen-induced bronchospasm(12). In ayurveda the Naravelia *zeylanica* plant has been extensively used by native peoples as an astringent, bitter, antipruritic and anti-inflammatory. It is also useful in pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, cephalalgia, colic inflammation, wound healing & ulcer protection. The root and stem have a strong penetrating smell and is used to relieve malarial fever and headache. Root and stem paste is applied externally for psoriasis, itches and skin allergies. Naravelia zeylanica is used as a source of drug for intestinal worms, skin disease and toothache (13). The juice of Heliotropium *indicum* leaves applied on boils, pimples, ulcers, sores and wounds to cure. In Belize, the plant used for diarrhea, malaise or vomiting in infants. The leaves are used for the treatment of ophthalmic disorders, erysipelas, pharyngodynia, and anti-inflammatory, anti-tumour. The roots are used as astringent, expectorant and febrifuge. The aqueous extract of leaves was proved to be active against Schwart's leukemia (14).

In the present study, a polyherbal formulation HN-08 was prepared combining all the mentioned herbs. The prepared formulation was evaluated for it's antianaphylactic, antihistaminic and mast cell stabilization activity on various experimental models. The therapeutic activity of the test formulation was studied on the active anaphylaxis, mast cell stabilization in rats, and histamine-induced bronchospasm in guinea pigs.

Materials and Methods

Plant material

Plant materials were collected from local markets. The plants materials were authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (H.S.198) was deposited in the herbarium of our institute. Test formulation was prepared (Combination of all the powders in equal proportion), same formulation which was used in the study was stored in the amber colored air tight glass container and deposited in the laboratory specimen cupboard.

Animals

Wistar rats (175-200 g) and guinea pigs (400-600 g) of either sex are procured from Indian Institute of Sciences. They are maintained under standard conditions (temperature 22 ± 2^{o}C, relative humidity 60±5% and 12 h light/dark cycle)0.The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to 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 Institute of Health".

Chemicals

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

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 each. Group I served as control and received water (vehicle). Groups II, III and IV were administered HN-08 at 125, 250, and 500 mg/kg respectively, orally, once a day for 14 days. On day 14, after 2 h of treatment, the rats were challenged with intravenous injection (tail vein) of 0.25 ml horse serum in normal saline. They were then observed for the onset of symptoms such as dyspnea and cyanosis, duration of the persistence of symptoms (min), and mortality. The severity of symptoms was scored (21). The optimal pharmacological effective dose of HN-08, which is derived from this dose response study, was used for the remaining studies.

Serum total IgE was quantified with an ELISA protocol according to the manufacturer's instructions. 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 phosphate buffered saline (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 were treated similarly but without serum. 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 the 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 group. Group I served as control and received vehicle (water). Group II (sensitized control group, received only water), Groups III (HN-08) and IV (prednisolone) were sensitized by injecting 0.5 ml of horse serum subcutaneously along with 0.5 ml of triple antigen containing 20,000 million *Bordetella pertussis* organisms (Serum Institute of India Ltd., Pune). Group III were administered HN-08 500 mg/kg, p.o., once a day for 14 days. Group IV were administered prednisolone (reference drug) 10 mg/kg, p.o., for the same duration. On day 14, the rats were sacrificed 2 h after the treatment and the intestinal mesentry was taken out for the study on mast cells. Mesentries 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 37oC. The mesenteric pieces were challenged with 5% horse serum for 10 min after which the mast cells were stained with 1.0% toluidine blue and examined microscopically for the number of intact and degranulated mast cells (15).

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 (24 × 14 × 24 cm) made of perplex glass. Of the two groups of six animals each, Group I served as control and Group II received HN-08 500 mg/kg, p.o., once a day for 5 days. The 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, preconvulsive dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsions (16). As soon as PCD commenced, the animals were removed from the chamber and exposed to fresh air. This PCD was taken as day 0 value. On days 1 and 5, 2 h 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 unpaired Student's ' *t* ' test to find out the level of significance. $P<0.05$ was considered statistically significant. The analysis was performed using Graphpad Prism software package (Version 4.0).

Results

Effect of HN-08 on anaphylactic shock-induced bronchospasm in sensitized rats

HN-08 protected the sensitized rats against anaphylactic shock in a dose-dependent manner. In control rats, intravenous antigen challenge (horse serum) caused shock in 100% of the animals, while in treated rats (500 mg/kg of HN-08), the onset of symptoms of shock was delayed and symptoms were less severe with reduced mortality as shown in Table-1. HN-08 (500 mg/kg) also resulted in significant reduction of serum IgE levels (25.80 ± 4.85 ng/ml) as compared to sensitized controls (125.06 ± 9.66 ng/ml). Serum IgE levels in control group was 8.83 ± 0.84 ng/ml. HK07 showed optimal pharmacological effect at 500 mg/kg dose. Hence, this dose of HN-08 was used for the remaining studies.

Table 1: Effect of HN-08 test formulation on anaphylactic shock- induced bronchospasm in rats

Groups	Scores/min/percentage		
	Total score x 10	**Onset of symptoms (min)**	**Mortality**
Control	120	70	105
HN-08 (125mg/Kg)	116	70	90
HN-08 (250mg/Kg)	79	80	57
HN-08 (500mg/Kg)	68*	115$^{\#}$	41*

Values are mean ± SEM except for mortality, which is expressed as percentage, n=7 in each group; Total score: F=50508, df =27, P=0.0050;

Onset of symptoms: F=20.51, df = 27, P=0.0001. *P<0.05, $^{\#}$P<0.001 as compared to control. (ANOVA followed by Bonferroni's multiple comparison post hoc tests for total score and onset of symptoms. Chi-square test for mortality).

Mast cell stabilizing potential of HN-08

Antigen challenge resulted in significant degranulation of the mesentric mast cells (approximately 88%). Pretreatment of sensitized animals with HN-08 at 500 mg/kg, p.o., for 2 weeks resulted in a significant reduction in the number of disrupted mast cells when challenged with horse serum. The effect of HN-08 was also comparable with the reference drug prednisolone as shown in table-2.

Table 2: Effect of HN-08 on mast cell stabilization in sensitized rats

Groups	Mast cells (%)	
	Intact	Disrupted
Control	83.06 ± 3.70*	16.94 ±3.70*
Sensitized control	12.31 ± 1.92	87.69 ±1.92
HN-08 (500 mg/Kg)	64.25 ± 9.51*	35.75 ± 9.51*
Prednisolone (10mg/Kg)	69.19 ± 4.89*	30.81 ± 4.89*
One-way F	129.7	129.7
ANOVA df	31	31

Values are mean ± SEM, n=8 in each group. * Significantly different from sensitized control (P<0.0001).

Effect on histamine-induced bronchospasm

HN-08 at 500 mg/kg, p.o., significantly prolonged the latent period of PCD as compared to control, following exposure to histamine aerosols on day 5 as shown in table-3.

Table 3: Effect of HN-08 on histamine induced bronchospasm in guinea pigs

Groups	Pre-convulsion dyspnea (sec)		
	Day 0	Day 1	Day 5
Control	188	192	190
HN-08 (500mg/Kg)	186	198	440*
P	0.008	0.008	0.008

Values are mean ± SEM, n=6 in each group. * (P<0.008) as compared to control on day 5 (unpaired Student's't' test)

Discussion

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, HN-08 prolonged the latent period of PCD in guinea pigs following histamine aerosol. This may be suggestive of an antihistaminic activity following treatment with HN-08. It also offered protection against anaphylactic shock-induced bronchospasm in rats.

Basophils, mast cells, and their preformed *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 (17-18).Immunoglobulin E (IgE)-mediated mast-cell stimulation is an important initial event in the development of type I allergic reactions such as 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 (19). Antigen challenge, in sensitized animals, results in the degranulation of mast cells, which is an important feature of anaphylaxis. In the present study, HN-08 showed marked protection against the mast cell degranulation following antigen challenge in sensitized animals. Mast cell stabilizing activity of HN-08 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 (9). This antianaphylactic and antihistaminic effect may be caused by the stabilization of the mast cell membrane, suppression of IgE, and inhibition of pathological effects induced by the release of inflammatory mediators in HN-08 treated animals.
Experimental results indicated the potent benefits of HN-08 (polyherbal formulation) in the treatment of asthma and related conditions. However, further studies with other experimental models, especially to explore the role of cytokines are warranted to substantiate the antiasthmatic and antiallergic activity of HN-08 before putting it out as commercial formulation.

Conclusion

The findings from various studies reveal that the antihistaminic and antianaphylactic activity of HN-08 is mainly due to its mast cell stabilizing potential, suppression of IgE, and inhibition of release of inflammatory mediators. Thus prepared formulation has the strong rationale behind the mentioned therapeutic activities.

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