Original Article

Herbex-kid Inhibits Immediate Hypersensitivity Reactions in Mice and Rats

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Herbex-kid (HK), a polyherbal formulation was evaluated in various experimental allergic models of Type I hypersensitivity reactions. Compound 48/80 (C 48/80) has been shown to induce rat mesentery mast cell degranulation and HK (1.07, 10.75 and 107.5 mg ml^{-1}) inhibited the mast cell degranulation in a dose dependent manner. HK (1.07, 10.75 and 107.5 mg kg^{-1}; p.o.) showed dose-dependent protection against C 48/80 induced systemic anaphylaxis in male Balb/C mice. In active anaphylaxis model, male Wistar rats orally administered with 10.75 and 107.5 mg kg^{-1} of HK showed significant ($P < 0.01$) protection against mast cell degranulation, while in passive anaphylaxis model, only at 107.5 mg kg^{-1} showed significant ($P < 0.01$) reduction in mast cell degranulation. HK at all dose levels was able to significantly decrease the time spent in nasal rubbing in Wistar rats sensitized to ovalbumin, while only at 107.5 mg kg^{-1} it showed significant ($P < 0.01$) reduction in number of sneezes. In C 48/80-induced skin itch model, all dose levels of HK significantly ($P < 0.001$) decreased the time spent in itching and the number of itches. HK did not produce any significant inhibition in histamine induced contraction in guinea pig ileum. From the above findings we conclude that the HK possesses antiallergic activity mediated by reducing of the release mediators from mast cells and also by 5-HT antagonism without the involvement of histamine (H1) receptors.

Keywords: Herbex-kid – mast cell stabilization – Type I hypersensitivity reaction – antiallergic

Introduction

Herbex-kid (HK) a polyherbal syrup developed and marketed by Apex® Laboratories Ltd, India, contains herbal extracts standardized by HPTLC method. Each 5 ml of the syrup contains extracts of *Abies webbiana* (75 mg), *Alpinia chinensis* (150 mg), *Baleria perionitis* (75 mg), *Bacopa monniera* (75 mg), *Bryonia scabrilla* (100 mg), *Coleus aromaticus* (100 mg), *Ocimum sanctum* (200 mg), *Piper longum* (100 mg) and *Solanum trilobatum* (200 mg). All these herbs are traditionally in India for treating various allergic disorders such as cough, asthma, colds, purititis and other inflammatory diseases (1). Some of these herbs used in the formulation such as *A. chinensis, O. santum* and *P. longum* have been evaluated for their immunomodulatory (2–6), antitussive (7), anti-inflammatory properties (6,8,9). Type I or immediate hypersensitivity reaction consists of various allergic disorders, ranging form serious anaphylactic reaction to acute pruritus reaction and chronic conditions like allergic asthma. The basic components involved in the Type I allergic reaction are the mast cells and IgE antibodies (10). Complimentary alternative medicine (CAM) has been shown to be effective and worthy against several allergic disorders, where as conventional treatment has not shown to be effective including in cases of atopic dermatitis (11–15). Recently, evidence-based research, in CAM has been directed at best for establishing therapeutic evidence in CAM by stringent research, thereby

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integrating CAM into Western medicine. A need, therefore, has arisen to validate the claims of herbal medicine (16,17). Hence the present study was taken up to evaluate the formula in different animal models to assess Type I reactions involving mast cells.

Methods

Experimental Rats and Mice

Male Wistar rats (200–220 g), male Balb/C mice (18–22 g) and guinea pig (350–500 g) of either sex were obtained from the Central Animal House Facility of JSS College of Pharmacy, Ootacamund. They were maintained under controlled conditions at temperature of $22 \pm 2°C$, humidity $60 \pm 10\%$ and a 12/12 light/dark cycle. They had free access to standard rodent pellet and water. All experimental protocols were approved by the Institutional Animal Ethics Committee prior to beginning the experiments.

Drugs and Chemicals

Compound 48/80 (C 48/80), histamine acid phosphate and ovalbumin (OVA) were purchased from Sigma Chemicals Co., USA, horse serum from Hi-Media, India, o-Toludine blue and other solvents from S.D. Fine Chemicals, India, cetrizine hydrochloride, cyproheptadine and predinisolone as gift samples from Tablets (India) Pvt. Ltd, disodium cromoglycate (DSCG) from Cipla, India, triple antigen from Serum Institute of India and Herbex-kid syrup (HK) was a generous gift from Apex® Laboratories, Chennai, India.

Mast Cell Stabilization Activity

The overnight fasted male Wistar rats were sacrificed with excess dose of anesthetic ether and the abdomen was cut open to expose the intestine. Pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out and cut into small pieces and placed in a beaker containing Ringer Locke (mM concentrations of NaCl 154, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 6.0 and dextrose 5.5) solution for 30 ± 1 min. Different dilutions of HK (1.07, 10.7 and 107.5 mg ml^{-1}) were prepared in Ringer Locke solution. Then the tissues were incubated with C 48/80 (0.8 µg ml^{-1}) for a period of 30 ± 1 min. The pieces of mesentery were then placed on a clean slide. Excess fatty layers and adhering tissues were carefully removed. The trimmed tissue was placed in 4% formaldehyde solution containing 0.1% o-Toludine blue for 20–30 min and the tissue was then de-stained with acetone and xylene (two changes each) for 5 ± 1 min. The stained mesentery pieces were examined under a digital light microscope (M/s. Motic, Japan) at 100× magnification and 100 mast cells were counted, starting from the left

hand side of the field and then proceeding clockwise. The number of intact, fragmented or disrupted mast cells were counted. A mast cell was considered disrupted if four or five granules were observed around the mast cells (18). The percentage of fragmented or disrupted and intact mast cells was calculated. Six pieces of mesentery were used for each concentration of the test sample.

Systemic Anaphylaxis Model in Mice

The overnight fasted male Balb/C mice were administered orally with various doses of HK (1.07, 10.75 and 107.5 mg kg^{-1}) and after 1 h, 8 µg kg^{-1} of C 48/80 dissolved in normal saline was administered i.p. into each animal. Mice were placed in an observation cage (30 × 30 cm) made of perplex glass 10 min before the injection of C 48/80, for acclimatization and after injection they were put back in the observation cage (one mouse per cage) and the death time (in min) was noted (19).

Active Anaphylaxis Model using Rat Mesentery

Male Wistar rats were weighed and randomly selected. All rats were given 0.5 ml of horse serum along with 0.5 ml of triple antigen containing 20 000 million *Bordetella pertusis* organisms by subcutaneous route (20). Later rats were orally administered with HK (1.07, 10.75 and 107.5 mg kg^{-1}) or prednisolone acetate (10 mg kg^{-1}) once daily for 14 days. They were sacrificed on day 14, 1 h after the administration of the test substance. The abdomen was cut open to expose the intestine. Pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out and cut into small pieces and placed in a beaker containing 5 ml of Ringer Locke solution for 30 ± 1 min. The mesenteric pieces were then shifted to a beaker containing 5% v/v horse serum diluted in Ringer Locke solution. After an incubation period of 10 min, the tissues were removed, trimmed and stained with 0.1% o-Toludine solution and placed on a microscopic slide and the numbers of intact and fragmented/disrupted mast cells were counted under a digital microscope. From each rat, three mesenteric tissues were used for counting mast cells and the average from three observations were used to calculate the percentage of mast cells disrupted/fragmented and intact cells.

Passive Anaphylactic Model using Rat Mesentery

The vehicle control rats in the active anaphylactic model, used in the previous experiment were used for the study. On day 14, before sacrificing them, blood was withdrawn from each animal by sino-orbital puncture and serum separated aseptically. The serum thus obtained was administered (1 ml per rat; i.p.) to selected animals of all groups. After 1 h, HK (1.07, 10.75 and 107.5 mg kg^{-1})

or prednisolone ($10 \, \text{mg kg}^{-1}$) was administered orally, and on the following day as well. Forty-eight hours after the administration of rat serum, animals were challenged with 1 ml of horse serum by i.p. injection (20). Ten minutes later were sacrificed and the intestinal mesentery was collected and incubated in Ringer Locke solution for 30 min. Later pieces of mesentery were placed on slide and stained with 0.1% o-Toludine blue and the number of intact and disrupted mast cells were counted from each tissue. From each animal, three mesenteric tissue pieces were used for counting mast cells and the average of three observations were taken for calculating the percentage.

Nasal Allergy Model in Sensitized Rats

Male Wistar rats were weighed and randomly selected. The animals were injected intraperitoneally with 1 mg of OVA and 10 mg of alum prepared in 1 ml of normal saline for 7 days (21). On day 14, the overnight fasted were orally administered with HK (1.07, 10.75 and $107.5 \, \text{mg kg}^{-1}$) or cetrizine ($1 \, \text{mg kg}^{-1}$). After 1 h, animals were sensitized locally with OVA in saline solution ($10 \, \mu\text{g} \, 10 \, \mu\text{l}^{-1}$ per nostril). This was carried out by dropping OVA solution into the bilateral nasal cavities by a micropippete. Ten minutes before instilling OVA into the nostrils, rats were placed in an observation cage ($30 \times 30 \, \text{cm}$), made of perplex glass, for acclimatization then placed back into the observation cage (one rat per cage) and the number of sneezes and the nasal rubbing were counted for 30 min by a trained observer unaware of the treatment given earlier.

Skin Itch Induced by C 48/80 in Mice

Male Balb/C mice numbering 36 were divided into six groups with six in each group. Itching or scratching was induced in them by intradermal injection of $20 \, \mu\text{l}$ containing $100 \, \mu\text{g}$ of C 48/80 (dissolved in normal saline) on the rostral part of the back of shaved skin (22). Ten minutes before the injection, each mouse was placed in the observation chamber ($30 \times 30 \, \text{cm}$) made of perplex glass. The number of scratches and the time spent in scratching was visually noted for 60 min. Scratching behavior was characterized by vigorous scratching of the injected area with the hind limb.

Histamine Induced Contraction in Guinea Pig Ileum

Guinea pigs of either sex were starved overnight with free access to water. A segment of the ileum (2-cm-long) was suspended in a 30 ml organ bath containing Tyrode's solution (mM concentration of NaCl 136.9, Glucose 5.6, $NaHCO_3$ 11.9, KCl 2.68, MgSO4 1.05, $CaCl_2$ 1.8, NaH_2PO_4 0.37), gassed with air and maintained at $37°C$. The tissue was allowed to equilibrate for

45 min and the Tyrode's solution was replaced every 15 min intervals. Dose response curves were obtained for histamine acid phosphate at various concentrations. Later the Tyrode solution was replaced by HK prepared in Tyrode solution containing various concentrations (0.1, 1 and 10% v/v) and the contractile response of the ileum was obtained in the presence of histamine. Contact time between the agonist and the tissue was between 30 and 45 s. Experiments were carried out in triplicate ($n = 3$). The IC_{50} was calculated for histamine, in the presence and absence of HK, at various concentrations (23).

Statistical Analysis

Results were expressed as mean $\pm$ SEM. The intergroup variation was compared statistically using One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test or with Tukey's multiple comparison test wherever applicable. Statistical significance was considered at $P < 0.05$. The analysis was carried out using GraphPad Prism software V.4.

Results

HK Stabilizes Mast Cell Degranulation Induced by C 48/80

C48/80, a known mast cell degranulating agent, produced a significant ($P < 0.001$) increase in degranulation in rat mesenteric mast cells (92.7 ± 1.3), when compared with the mesentery exposed to Ringer Locke's solution alone (18.5 ± 3.4). The C 48/80 treated mesentry when exposed to HK produced a dose dependent reduction in the number of degranulated mast cells. HK at $1.07 \, \text{mg ml}^{-1}$ showed 74.0 ± 3.5 number of degranulated mast cells per 100 mast cells counted, while 10.75 and $107.5 \, \text{mg ml}^{-1}$ showed significant reduction ($P < 0.01$) in the number of degranulated mast cells namely, 25.2 ± 4.6 and 10.7 ± 3.9, in the presence of $0.8 \, \mu\text{g ml}^{-1}$ C 48/80. Disodium cromoglycate ($1 \, \text{mg ml}^{-1}$), a known mast cell-stabilizing agent also brought significant ($P < 0.01$) reduction in degranulated mast cells (Fig. 1).

Survival Time Improved in Systemic Anaphylaxis Model

In vehicle treated mice, systemic anaphylaxis was observed within $19.3 \pm 1.5 \, \text{min}$. Oral pre-treatment with HK, 1 h before the administration of C 48/80, showed a dose-dependent increase in latency to death time. Only HK at $107.5 \, \text{mg kg}^{-1}$ produced significant increase ($P < 0.01$) in latency to death time, namely, $49.5 \pm 9.6 \, \text{min}$ when compared with controls (Fig. 2).
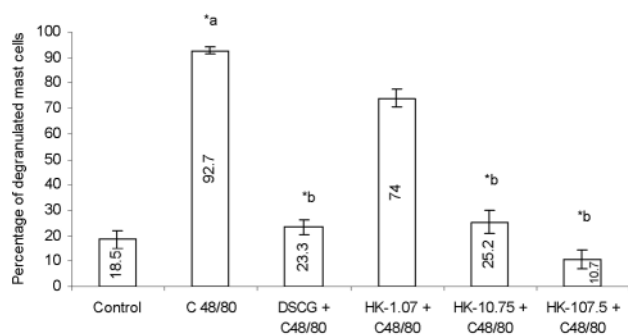
Figure 1. Effect of Herbex-kid (HK) on mast cell degranulation induced by C 48/80 in rat mesentery tissue. Values are mean ± SEM. Number of tissues per group is six. [*a]$P < 0.001$ vs control; [*b]$P < 0.001$ vs C 48/80; One way ANOVA followed by Tukey's multiple comparison test. DSSG: disodium cromoglycate; C 48/80: Compound 48/80.
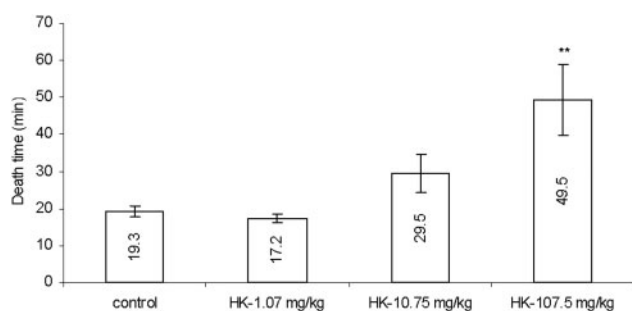

Figure 2. Effect of Herbex-kid (HK) in C 48/80 induced systemic anaphylaxis in Balb/C mice. Each bar represents mean ± SEM of six mice each. [**]$P < 0.01$ vs control. One way ANOVA followed by Dunnett's multiple comparison test. C 48/80: Compound 48/80.

Mast Cell Stabilization Property of HK in Active Anaphylaxis Model

Fourteen days of treatment with HK to presensitized rats produced dose dependent reduction in the degranulation of mast cell. HK at both 10.75, 107.5 mg kg^{-1} produced significant increase ($P < 0.01$) in the number of intact mast cells, namely, 58.9 ± 3.7 and 68.1 ± 2.8, when compared with vehicle control upon exposure to 0.5% horse serum, 30.1 ± 4.4. Prednisolone (10 mg kg^{-1}), used as a positive control also produced significant ($P < 0.01$) increase in intact mast cells, 73.6 ± 3.5 (Fig. 3).

Only HK at High Dose Increased the Number of Intact Mast Cells

In passive anaphylaxis model, only HK at 107.5 mg kg^{-1} produced significant ($P < 0.01$) increase in the number of intact mast cells, 64.2 ± 2.7 when compared with control namely 40.8 ± 4.5, while at all other doses, HK did not produce significant increase in the number of intact mast cells when compared with vehicle control (Fig. 3).
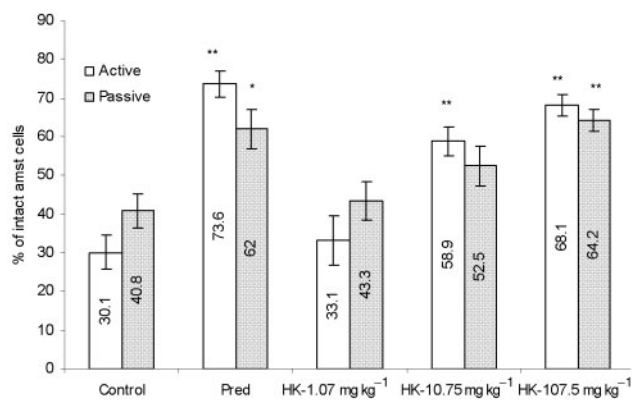

Figure 3. Effect of Herbex-kid (HK) in active (plain bars) and passive anaphylaxis (shaded bars) model in rats. Each bar represents mean ± SEM of six rats each. [*]$P < 0.05$, [**]$P < 0.01$ vs the experiment respective controls; One way ANOVA followed by Dunnett's multiple comparison test. Pred: Prednisolone (10 mg kg^{-1}).

Nasal Rubbing Suppressed after Induction by OVA

The vehicle treated OVA sensitized mice, produced 17.3 ± 2.8 number of sneezes and the time spent in nasal rubbing was 245.3 ± 10.7 s. The onset of sneezing was at 156.5 ± 48.3 s. Pre-treatment of HK to OVA sensitized Balb/C mice produced dose–dependent reduction in the number of sneezes and time spent in nasal rubbing. At all dose levels, HK produced significant reduction ($P < 0.01$) in time spent in nasal rubbing. HK at 1.07 mg kg^{-1} showed 184.3 ± 16.6 s ($P < 0.05$) of nasal rubbing while 10.75 and 107.5 mg kg^{-1} treated animals showed significant reduction ($P < 0.01$) in the time spent in nasal rubbing, namely, 160.5 ± 10.3 s, 84.7 ± 15.5 s. Only HK at 107.5 mg kg^{-1} showed significant reduction ($P < 0.01$) in number of sneezing, 4.5 ± 0.8, when compared with control. Moreover, both the standard drug, cetrizine and the herbal formulation, HK, however, did not produce any significant changes in onset of sneezing (Table 1).

Itching Induced by C 48/80 Reduced

In vehicle treated mice, intradermal injection of 20 μl of 100 μg of C 48/80 produced significant ($P < 0.001$) increase in the number of itches (181.7 ± 14.1) and the time spent in itching (267.7 ± 18.7 s) when compared with the vehicle treated mice injected with 20 μl of normal saline intradermally, namely, 13.0 ± 6.0 s and 6.2 ± 3.2 s. Pre-treatment with HK produced dose-dependent reduction in both these parameters. HK at 1.07 mg kg^{-1} showed non-significant reduction in the numbers of itches (135.7 ± 4.4) and significantly ($P < 0.001$) reduced the time spent in itching, namely, 165.3 ± 4.4 s when compared with C48/80 control. HK at 10.75 and 107.5 mg kg^{-1} produced significant reduction ($P < 0.01$) in time spent in itching, namely, 146.2 ± 8.4 and

Table 1. Effect of Herbex-kid in nasal allergy in antigen sensitized rats

Treatment ($n = 6$)	Dose (mg kg^{-1}; p.o.)	Number of sneezes	Time spent in nasal rubbing (s)	Onset of sneezing (s)
Control	–	17.3 ± 2.8	245.3 ± 10.7	156.5 ± 48.3
n-Cetrizine	10	$4.7 \pm 0.9**$	$121.2 \pm 17.9**$	97.5 ± 19.4
Herbex-kid	1.07	14.5 ± 3.7	$184.3 \pm 16.6*$	63.2 ± 17.5
	10.75	9.2 ± 1.6	$160.5 \pm 10.3**$	74.3 ± 16.1
	107.5	$4.5 \pm 0.8**$	$84.7 \pm 15.5**$	245.5 ± 58.9

Values are mean $\pm$ SEM; n = number of rats per group.
*$P < 0.05$; **$P < 0.01$ vs control; One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test.
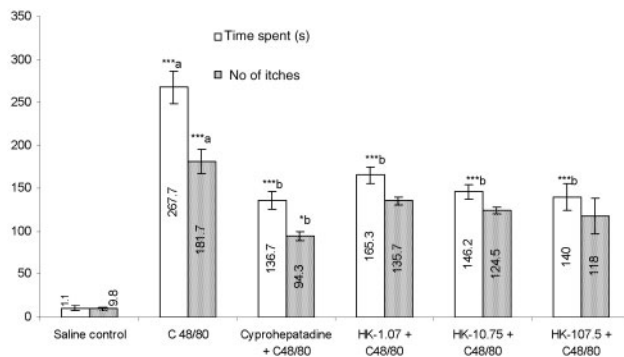

Figure 4. Herbex-kid (HK) at various doses in C 48/80 (Compound 48/80)-induced itching in Balb/C mice. Each bar represents mean of six mice each. ***^{a}$P < 0.001$ vs vehicle control; *^{b}$P < 0.05$, ***^{b}$P < 0.001$ vs C 48/80; One way ANOVA followed by Tukey's multiple comparison test.

140.0 ± 15.8, and the same dose levels also reduced the number of skin itches, namely, 124.5 ± 3.6 and 118.0 ± 20.6, when compared with C 48/80 control. The standard cyproheptadine at $1 \, \text{mg kg}^{-1}$ also showed significant reduction in the number of itches ($P < 0.05$) and time spent in itching ($P < 0.001$) induced by C48/80 (Fig. 4).

Non-Antagonism of Histamine

HK at concentrations of 0.1, 1 and 10% did not have any effect on histamine induced guinea pig ileum contraction (data not shown).

Discussion

Allergies are one of the common disorders affecting mankind. The basic components involved in Type I hypersensitivity allergic reactions are the mast cells and IgE antibodies (10). Presently most of the antiallergic agents are of H$_1$-antagonist (24). Recently rupatadine dual histamine (H$_1$) and platelet activated factor (PAF) has been developed (25). It is becoming clear that antihistaminics alone do not provide the full therapeutic value for most of the Type I allergic reaction (26).

From the histamine induced guinea pig ileum contraction, we conclude that HK does not possess prominent antihistaminic properties. Degranulation of mast cells, causes release of pre-formed and newly formed mediators leading to acute and late phase allergic reactions depending on the allergic disease (24). From the present study, HK possesses significant mast cell stabilization activity. Nevertheless certain types of allergic reactions like perennial rhinitis, atopic dermatitis and even in allergic asthma, several immune cells along with inflammatory mediators play an important role. By active and passive anaphylaxis model, it has been established that HK possess a certain degree of immunological property, which may be due to the herbs such as *O. sanctum* or *A. chinensis* which possess immunomodulatory properties (3,4).

Allergic rhinitis is a very common condition, characterized by early and late phase immune response (27). Antihistamines are commonly used for the relief of allergic rhinitis which may be beneficial in relieving sneezing but not other symptoms of allergic rhinitis (26). In the present study, OVA sensitized rat animal model of nasal allergy was used, as this model closely corresponds to the allergic rhinitis in humans, involving both early and late phase of immune response (28). In the early phase, the principal component is mast cell and histamine and is characterized by sneezing, and the late phase is characterized by nasal rubbing, wherein other inflammatory components are involved (21). The OVA model for nasal allergy involves both these phases. HK significantly reduced the time spent in nasal rubbing (late phase) at all the three dose level employed, but only at high dose the number of sneezes (early phase) was reduced significantly. Based on these observations, HK's involvement with the immunological component along with mast cell stabilization property would be the major feature in attenuating the allergic response without the involvement of histamine receptors. Based on the above fact, C48/80 induced itch model in mice, was, therefore employed, as antihistamines do not provide protection against C48/80-induced itching. Itching behavior is thus independent of histamine though mast cells are degranulated (29). Lipid mediators

also do not play important roles and 5-HT seems to participate in induction of itching (30). From the results of this experiment, it was evident that of all the dose level employed, HK produced significant reduction in number and time spent in itching, suggesting that HK has potent antipruritic activity probably mediated by mast cell stabilization along with 5-HT antagonism.

On the whole, the antiallergic property of HK could be attributed to the presence of immunomodulatory herbs such as *A. chinensis* (3), *O. sanctum* (4), *P. longum* (5) and antiallergic herbs such as *B. monniera* (31) and *P. longum* (32) used in the formulation, which further strengthens the concept of synergistic healing potential of polyherbal formulations (33).

In conclusion, the antiallergic property of HK could be through the involvement of mast cell stabilization, immunomodulatory activity and 5-HT antagonism without the involvement of histamine receptors. From the encouraging results obtained from this study, further experiments are being conducted to evaluate the potential of HK in experimental allergic asthmatic model and its immunological activity.

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