

# Antianaphylactic, mast cell stabilizing and antiasthmatic activity of AHR-1 (a polyherbal formulation)

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## ABSTRACT

**Objective:** This work was mainly aimed to study the anti-anaphylactic, mast cell stabilizing and antiasthmatic activity of AHR-1 (a polyherbal formulation) which contain various herbal extracts. **Methods:** The antianaphylactic activity of AHR-1 was evaluated in rats using active anaphylaxis model. Rats were then observed for onset of symptoms of anaphylaxis reaction such as increased respiratory rate, dyspnea, cyanosis and mortality. Serum IgE, leukocyte, eosinophil count and % polymorphs were calculated. Mast cell stabilizing effect was investigated by in vitro challenge of antigen sensitized rat intestinal mesenteries. Antiasthmatic effect was studied in guinea pigs using histamine-induced bronchospasm, in which occurrence of preconvulsive dyspnea (PCD) was noted as end point. **Results:** Anaphylactic shock caused by intravenous antigen challenge showed 83% mortality with a significant ( $P < 0.001$ ) increase respiratory symptom score. Treatment with AHR-1 (250 and 500 mg/kg) reduced the mortality and respiratory symptom score ( $P < 0.05, P < 0.001$ ), respectively. AHR-1 (250 and 500 mg/kg) significantly and dose dependently decreased Serum IgE ( $P < 0.05, P < 0.001$ ), AEC ( $P < 0.05, P < 0.001$ ), total leukocytes ( $P < 0.05, P < 0.01$ ) and % polymorphs ( $P < 0.01, P < 0.001$ ), respectively as compared to sensitized control group. Sensitized control rats were produced a significant (79%) mesenteric mast cell degranulation, but pre-treatment with AHR-1 (100 and 200  $\mu$ g/ml) produced in a significant ( $p < 0.001$ ) reduction in the number of degranulated mast cells when challenged with horse serum. AHR-1 significantly increased the time of PCD ( $P < 0.001$ ) as compared to control. **Conclusion:** From these finding, we concluded that AHR-1 is might be effective in treatment various hypersensitivity reactions like anaphylactic shock and asthma.

**Keywords:** Horse serum, AHR-1, IgE, Respiratory score.

## INTRODUCTION

Allergy is one of the common diseases that affect mankind with diverse manifestations. The prevalence of allergy and asthma has risen in recent years despite the general health improvement in the population.<sup>[1]</sup> Asthma affects about 300 million people worldwide and it has been estimated that a further 100 million will be affected by 2025.<sup>[2]</sup> It is known that asthma can be triggered by various factors: allergens, drugs, respiratory infection, dust, cold air, exercise, occupational stimuli, chemicals,

histamine, etc.<sup>[3]</sup> Anaphylaxis is mediated by histamine released in responses to cross-linking of IgE bound to  $Fc\epsilon R1$  on mast cells. Mast cell activation causes process of degranulation that result in releasing of mediators, such as histamine and an array of inflammatory cytokines.<sup>[4,5]</sup> In spite of the voluminous literature on the subject, the treatment of allergic diseases continues to be far from satisfactory. The disease statistics clearly necessitates the increasing need for drugs targeting the mechanisms involved in eosinophil and differential leukocytes activation and accumulations, for the management of asthma. The available treatment options for upper and lower respiratory tract allergic diseases have major limitations owing low efficacy, associated adverse events, and compliance issues.<sup>[6]</sup> Ayurveda, an ancient system of Indian medicines, has described several drugs from indigenous plant sources for the treatment of bronchial asthma and allergic conditions. AHR-1 is a polyherbal formulation containing herbal extract listed in (Table 1).

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**Table 1. Composition of each hard gelatin capsule of AHR-1**

Sr. No.	Common name	Latin name	Qty in mg/caps
1	Ardusi	<i>Adhatoda vasica</i>	63
2	Siris	<i>Albizzia lebeck</i>	25
3	Shallaki	<i>Boswellia serrata</i>	22
4	Devdar	<i>Cedrus deodara</i>	42
5	Bharangmool	<i>Clerodendron serratum</i>	25
6	Haldi	<i>Curcuma longa</i>	28
7	Pushkarmool	<i>Inula recemosa</i>	11
8	Tulsi	<i>Occimum sanctum</i>	28
9	Katuki	<i>Picrorrhiza kurroa</i>	28
10	Piper	<i>Piper longum</i>	28
11	Kantkari	<i>Solanum xanthocarpum</i>	31
12	Sharpunkha	<i>Tephrosia purpurea</i>	12
13	Guduchi	<i>Tinospora cordifolia</i>	25
14	Arkaparni	<i>Tylophora asthamatica</i>	11
15	Nagod	<i>Vitex negundo</i>	18

*Adhatoda vasica* is documented for its potent anti-inflammatory<sup>[7]</sup> antiallergic and antitussive activities<sup>[8]</sup>, bronchodilatory and smooth muscle relaxant activity.<sup>[9]</sup> *Albizzia lebeck* also known as tree of happiness is extensively used in various traditional medicines. In Chinese system of medicine it is used for relieving stress, anxiety and depression. Whereas in Indian system of medicine it is mainly used in allergic conditions such as allergic asthma, urticaria etc. It was reported that *Albizzia lebeck* having anti-histaminic and mast cell stabilizing property<sup>[10]</sup>, anti-inflammatory.<sup>[11]</sup> *Curcuma longa* (turmeric) contains curcumin, demethoxycurcumin and bisdemethoxycurcumin. The traditional uses of turmeric or natural curcuminoids in folk medicine are multiple, and some are based on their antioxidant, anti-inflammatory and antiallergic properties which have been confirmed by experimental study.<sup>[12]</sup> *Occimum sanctum* has been reported to protect against histamine as well as pollen induced bronchospasm in guinea pigs and inhibited antigen induced histamine release from sensitized mast cells.<sup>[13]</sup> *Solanum xanthocarpum* having strong bronchodilator effect along with anti-inflammatory activity.<sup>[14,15]</sup> *Clerodendron serratum* traditionally used for treatment of asthma, bronchitis, inflammation.<sup>[16]</sup> *Piper longum* has been shown to reduce the anaphylaxis in rats and protect guinea pigs against antigen induced bronchospasm.<sup>[17]</sup> *Vitex negundo* has been reported to possess mast cell stabilizing activity.<sup>[18]</sup> It was also reported that *Tephrosia purpurea* having mast cell stabilizing activity.<sup>[19]</sup>

*Boswellia serrata* is known as Salai Guggal, contain boswellic acid has been reported for antiasthmatic and anti-inflammatory activity by virtue of inhibition of leukotriene biosynthesis via 5-lipoxygenase.<sup>[20,21]</sup> It was demonstrated that *Cedrus deodara* having anti-allergic and anti-asthmatic property.<sup>[22]</sup> *Inula racemosa* is an ornamental plant of the Asteraceae family that is used both internally as well as externally in ayurveda. Externally it is used as antiseptic, antifungal activity and internally it is used for cough, bronchial asthma, dyspepsia, and amenorrhea as well as dysmenorrhea.<sup>[23]</sup> It was shown that *Picrorrhiza kurroa* had properties of preventing allergen and PAF induced bronchial obstruction in guinea pigs.<sup>[24]</sup> *Tinospora cordifolia* has been shown to produce anti-allergic and bronchodilator activity.<sup>[25]</sup> It was shown that *Tylophora asthamatica* had anti-asthmatic activity.<sup>[26]</sup>

There is lack of scientific data regarding the effect of AHR-1 (PHF) on asthma. Therefore, the aim of study was designed to investigate the effect of AHR-1, a polyherbal formulation on the active anaphylaxis, mast cell stabilizing in rats and histamine induced bronchospasm in guinea pigs.

## MATERIALS AND METHODS

### Drugs and chemicals

Histamine and horse serum were procured from sigma chemicals. Prednisolone, ketotifen and toluidine blue were procured from commercial source, Vadodara. Diphtheria tetanus and pertussis vaccine obtained from Serum institute of India ltd., Pune, India. All other chemicals used were of analytical grade.

### Experimental animals

Wistar rats (200-250g) and guinea pigs (400-600g) of either sex, housed in standard conditions of temperature (22 ± 2°C), relative humidity 60 ± 5% and light (12 h light/dark cycles) were used. They were fed with standard pellet diet and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethics Committee as per guidance of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

### Acute toxicity study

The procedure was followed as per Organization for Economic Cooperation and Development -425 (OECD-425) guideline. The acute toxicity study of ethanolic extract of AHR-1 was performed in Wistar rats of either sex. In this study, increasing doses of AHR-1 (100, 200, 500,

1000, 2500 mg/kg body weight) was orally administered to groups of 3 animals for each dose after a 12 h fast. The signs and symptoms associated with the AHR-1 administration was observed after 0, 30, 60, 120, 180 and 240 min and then once a day for the next 14 days.<sup>[27]</sup> We observed that there was no any sign of toxicity or mortality up to a dose of 2500 mg/kg.

### Horse serum induced active anaphylaxis model of rats

Thirty Wistar rats were sensitized by subcutaneous injection of 0.5 ml of horse serum followed by intraperitoneal injection of 0.5 ml Triple Antigen Vaccine containing 20000 million *Bordetella pertussis* organisms (Serum institute of India ltd., Pune, India). The sensitized animals were divided into 5 groups of 6 animals each. Group I served as a normal control and received distilled water, Group II served as sensitized control which received 0.5 % Sodium CMC (vehicle), groups III was treated with reference drug (Prednisolone 10mg/kg, p.o. ) and groups IV & V were administered AHR-1 at 250 and 500mg/kg, respectively, orally, once a day for 14days. On day 14, after 2 hr of treatment, all the animals were challenged with intravenous injection (tail vein) of 0.25 ml horse serum in normal saline except Group I. They were then observed for onset of symptoms such as increased respiratory rate, dyspnea, cyanosis and mortality for 1 hr by a blind observer. The severity of respiratory symptoms was scored: 0- No visual symptoms; 2- Increased rate of respiration; 4- Increased rate of respiration with immobility; 6- Dyspnea for 10 mins; 8- Cyanosis for 10 mins; 10- Dyspnea & cyanosis for 10 mins; 12- Death.<sup>[28]</sup> Blood sample was collected from the retro orbital plexus of rats under light ether anesthesia, using glass capillaries and stored in with or without disodium ethylene diamine tetra-acetate for biochemical parameter estimation. For serum separation, test tube (without EDTA) allowing to clot in open for 15 minutes, it was centrifuged at 5000 rpm for 20 minutes for separation of serum. Serum was stored at -20°C until further estimation. Serum IgE was quantified with an ELISA kit according to the manufacturer's instruction. Total leukocyte, eosinophil count, % polymorphs were calculated.<sup>[29]</sup>

### Degranulation of rat mesenteric mast cells

Adult male albino Wistar rats were sacrificed and pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out, wash with distilled water, placed in Ringer Locke solution (NaCl 0.9, KCL 0.42, CaCl<sub>2</sub> 0.024, NaHCO<sub>3</sub> 0.15 and Glucose 1 g/l of distilled eater) and then subjected to the following treatment schedules.

- Petri dish no.1: Ringer Locke solution (sensitized control)
- Petri dish no.2: 0.1 ml of Ketotifen (20 µg/ml)
- Petri dish no.3: 0.1 ml of AHR-1 (100 µg/ml)
- Petri dish no.4: 0.1 ml of AHR-1 (200 µg/ml)

Each petridish was incubated for 15 min at 37°C. The preparation was challenged with 5% v/v horse serum for 10 min and after that, all pieces were transferred to 10% formaldehyde solution containing 0.1% Toluidine blue and kept a side for 25 min. After staining and fixation of mast cells, the excess stain was washed with distilled water followed by dehydration by absolute alcohol. Finally slide were cleared with xylene and observed under the high power of light microscope. The Percentage intact and percentage degranulated mast cells were counted.<sup>[30]</sup>

### Histamine-induced bronchospasm in guinea pigs

Experimental bronchospasm was induced in guinea pigs by exposing them to histamine aerosol.<sup>[30]</sup> Guinea pigs of either sex were selected and randomly divided into two groups, each containing five animals. Group I and group II were exposed to 1% w/v of histamine aerosol in histamine chamber (Inco Ltd, Ambala, India) showed progressive dyspnea. The end point, preconvulsive dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion. As soon as PCD commenced, the animals were removed from the chamber and placed in fresh air. This time of PCD was taken as day 0 value. Group I and group II were treated with the AHR-1 at dose of 250 mg/kg and 500 mg/kg, orally, once a day for 5 day, respectively, after aerosol exposure on day 0. On day 5, 2 h after the administration of drug, the time for the onset of PCD was recorded as on day 0. The percentage increase in the time of PCD was calculating by this formula.<sup>[31]</sup>

$$\text{Percentage increase in the time of PCD} = \frac{(1 - T_1/T_2) \times 100}{100}$$

Where T<sub>1</sub> = time for PCD onset on day 0, T<sub>2</sub> = time for PCD onset on day 5

### Lung histology

After sacrifice, the lung tissue was rapidly dissected out and washed with saline immediately and fixed in 10% buffered formaldehyde. Paraffin-embedded specimens were cut into 5 µm-thick sections and stained with hematoxylin and eosin (H&E). The sections were examined under the light microscope (Olympus BX10, Japan) for histopathological changes and photomicrographs (Olympus DP12 camera, Japan) were taken. The pathologist performing

histopathological evaluation was blinded to the treatment as assignment of different study groups. The sections will be viewed under 10X and 40X magnifications.

### Statistical analysis

All the data are expressed as mean  $\pm$  SEM (n = 6). Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when  $p < 0.05$ .

## RESULTS

### Acute toxicity study of AHR-1

Acute toxicity study on the Wistar rats showed no mortality and morbidity up to 2500 mg/kg dose of AHR-1.  $1/5$  and  $1/10$  doses were taken for further study.

### Effect of AHR-1 on symptom score, IgE, AEC, total leukocytes and % polymorphs on horse serum induced anaphylactic in rats

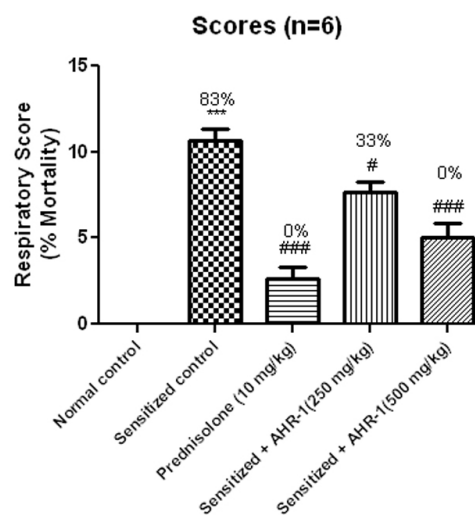
Horse serum sensitized rats when challenged with the same antigen (0.25 ml of horse serum, i.v.) after 2 weeks; induced anaphylaxis reaction i.e. increased respiratory rate, dyspnea, cyanosis and mortality. The treatment with AHR-1 (250 and 500 mg/kg, p.o.) showed a significant ( $P < 0.05$ ,  $P < 0.001$ ) decrease in anaphylaxis reaction, that was reflected in decreased respiratory score. Prednisolone (10 mg/kg, p.o.) showed a significant decrease a respiratory score (Fig. 1).

Serum IgE, AEC, total leukocyte count & % polymorphs were significantly ( $P < 0.001$ ) increased in sensitized control rats as compared to normal control group. AHR-1 (250 and 500 mg/kg, p.o. for 14 days) significantly and dose dependently decreased serum IgE ( $P < 0.05$ ,  $P < 0.001$ ), AEC ( $P < 0.05$ ,  $P < 0.001$ ), total leukocytes

( $P < 0.05$ ,  $P < 0.01$ ) and % polymorphs ( $P < 0.01$ ,  $P < 0.001$ ), respectively as compared to sensitized control group. Standard drug (Prednisolone 10 mg/kg, p.o. for 14 days) showed a significant ( $P < 0.001$ ) decrease in serum IgE, AEC, total leukocytes and % polymorphs as compared to sensitized rats (Table 2).

### Effects of AHR-1 on degranulation of mesenteric mast cells

Antigen challenge resulted in significant degranulation of the mesenteric mast cells. The treatment with AHR-1 (100 and 200  $\mu$ g/ml) or kitotifen (20  $\mu$ g/ml) showed a significant ( $P < 0.001$ ) protective effect on mast cells degranulation due to the challenge of antigen. The protective effect of AHR-1 on mast cell degranulation was dose dependant. Kitotifen (20  $\mu$ g/ml) treatment considerably a better protection compared to AHR-1 (200  $\mu$ g/ml) on mast cell degranulation (Table 3).



**Figure 1.** Effect of AHR-1 on respiratory score (% mortality) on horse serum induced anaphylactic in rats.

All the values are expressed as mean  $\pm$  SEM; n = 6, \*\*\* $p < 0.001$  compared to normal control; # $p < 0.05$ , ### $p < 0.01$ , ### $p < 0.001$  compared to sensitized control rats

**Table 2. Effects of AHR-1 on IgE, AEC, WBC, % polymorphs on horse serum induced anaphylaxis in rats**

Group	Serum IgE (ng/ml)	AEC(mm <sup>3</sup> )	WBC/ cmm	%Polymorphs
I	180 $\pm$ 16	89.00 $\pm$ 7.95	4900 $\pm$ 23	50.00 $\pm$ 1.6
II	320 $\pm$ 19***	201.6 $\pm$ 9.26***	10000 $\pm$ 64***	69.00 $\pm$ 3.5***
III	180 $\pm$ 9.7###	100.4 $\pm$ 11.21###	6000 $\pm$ 27###	39.00 $\pm$ 2.7###
IV	260 $\pm$ 9.1#	158.8 $\pm$ 6.31#	8100 $\pm$ 46#	51.00 $\pm$ 3.2##
V	220 $\pm$ 13###	111 $\pm$ 9.26###	6700 $\pm$ 30##	47.00 $\pm$ 3.1###

All the values are expressed as mean  $\pm$  SEM; n=6, \*\*\* $p < 0.001$  compared to normal control; # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  compared to sensitized control rats

### Effect of AHR-1 histamine-induced bronchospasm in guinea pigs

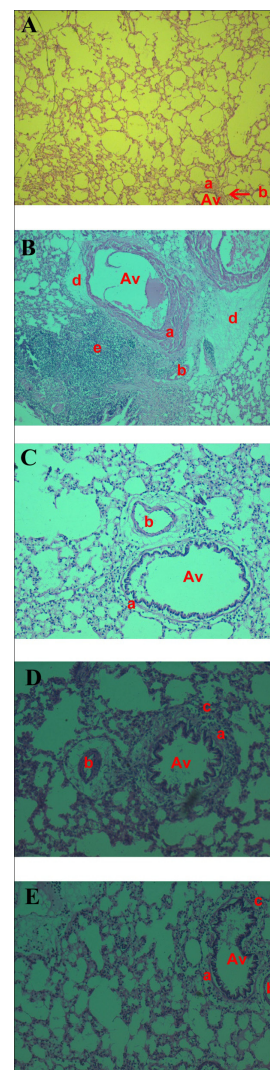
AHR-1 (250 and 500 mg/kg, p.o) significantly and dose dependently % increase in the time of PCD ( $P < 0.001$ ) as compared to control, following exposure to histamine aerosol on day 5 (Table 4).

### Lung Histology

Normal control rats showed normal lung histology, no mural inflammation, eosinophilic inflammation is absent. (Fig. 2A). In contrast, histological section of lung of sensitized control rats showed significant changes, airway (Av) lumen is plugged by mucus and an inflammatory cells, thickened pseudo-stratified columnary epithelium (a), destruction of pulmonary artery is observed (b), accumulation of lymphocytes is constantly high (c), eosinophilic edema fluid deposition is severe (d), Parenchymal inflammation is also observed (Fig. 2B). Treatment with Prednisolone and AHR-1 (250 and 500mg/kg, p.o.) produce significantly less inflammatory infiltrates, alveolar lumen (Av) is normal, less thickness of epithelial and sub-epithelial layers (a), pulmonary artery is normal (b) with moderate peri alveolar inflammation, very less accumulation of lymphocytes (c) and no parenchymal eosinophilic inflammation (Fig. 2C–E).

### DISCUSSION

Active anaphylaxis induced by triple antigen horse serum is a key model to study the symptomatic affect of type I allergy. The globulin fraction of horse serum acts as environmental allergen triggering the allergic responses.<sup>[32]</sup> In the present study, rats were sensitized by horse serum (0.5 ml, sc.) and then second exposure to the same antigen



**Figure 2.** Histological appearance of lungs. (A) Normal control; (B) Sensitized control; (C) Sensitized rat treated with Prednisolone (10 mg/kg, p.o.); (D) Sensitized rat treated with AHR-1 (250 mg/kg, p.o.); (E) Sensitized rat treated with AHR-1 (500 mg/kg, p.o.).

**Table 3. Effect of AHR-1 on mast cell degranulation**

Groups	Treatment	Dose	Intact mast cells %	Degranulated mast cells%
I	Sensitized control	–	20.54 ± 1.56	79.17 ± 2.63
II	Standard(Ketotifen)	20µg/ml	79.86 ± 2.76	20.67 ± 1.47 <sup>###</sup>
III	AHR-1	100µg/ml	62.64 ± 2.65	37.67 ± 0.80 <sup>###</sup>
IV	AHR-1	200µg/m	75.54 ± 3.54	26.33 ± 1.4 <sup>###</sup>

Values are expressed as mean ± SEM; n=6, <sup>###</sup> $p < 0.001$  compared to sensitized control rats.

**Table 4. Effects of AHR-1 (250 and 500 mg/kg) on histamine-induced bronchospasm in guinea pigs**

Group	Preconvulsive dyspnea time(sec)		
	Before treatment (control)	After treatment	% increase in the time of PCD
I-AHR-1 (250 mg/kg)	135.8 ± 6.64	361.8 ± 18.83 <sup>***</sup>	62.5
II-AHR-1 (500 mg/kg)	122.4 ± 6.83	391.6 ± 20.52 <sup>***</sup>	68.8

Values are expressed as mean ± SEM; n=5, <sup>\*\*\*</sup> $p < 0.001$  when compared with control guinea pigs

(0.25ml, iv.) caused acute anaphylactic shock mediated by histamine released in responses to cross-linking of IgE bound to Fc $\epsilon$ R1 on mast cells. Mast cell activation causes process of degranulation that resulting in the release of various mediators and cell infiltration. In our study, In sensitized control rats, intravenous antigen challenge caused anaphylactic shock in 83% of animals with mortality and significantly increased respiratory score, while in AHR-1(250 and 500 mg/kg, p.o.) treated rats, the onset of symptoms were delayed or symptoms were less severe with reduced the mortality by 33%, 0% respectively and significantly decreased respiratory score. We also found that antigen challenge resulted in significant increase in the numbers of eosinophil, lymphocytes, total leukocytes, serum IgE level. Treatment with Prednisolone and AHR-1 in antigen challenged animals significantly inhibited antigen induced hyper reactivity by decreasing the eosinophil count, % lymphocytes, infiltration of total leukocytes, serum IgE level. It was confirmed by histology of lung tissue, the section of lung tissue of sensitized animals showed airway plugging by mucus and cell, eosinophilic inflammation. Treatment with AHR-1 prevented the inflammation which led to normal lumen size and normal cellular structure.

In asthma, chronic inflammation is responsible for the bronchospasm which leads to airway narrowing and decrease in the lumen size of bronchiole.<sup>[3]</sup> It can be clearly seen in cross section of lung tissue. In the present study, treatment with AHR-1 was prevented the inflammation and bronchoconstriction as well as lead to cellular structure and normal lumen size and as compared to sanitized control rats.

It was reported that mast cell deregulation occurs when the tissues of sensitized animals brought into contact with antigen in vivo or in vitro, which is an important feature of anaphylaxis.<sup>[33]</sup> In the present study, we were finding that AHR-1 shown protection against the mast cell degranulation in antigen sensitized animals. AHR-1 (200 $\mu$ g/ml) protects the degranulation of mast cell comparable to kitotifen. This indicates that AHR-1 is effective in stabilizing the mast cell. Anti-anaphylactic and mast cell stabilizing activity of AHR-1 might be due to the presence of herbal extracts, which are known for their mast cell stabilizing property against antigen-antibody reaction or due to inhibition of IgE antibody production, which is accountable for mast cell degranulation.<sup>[13]</sup>

Asthma is chronic disorder characteristic by bronchospasm and inflammation. It is triggered by exposure of

allergen, irritants, cold air or exercise which releases the inflammatory mediator like histamine, acetylcholine and leukotrienes that directly causes the bronchospasm.<sup>[34,35]</sup> Exposure of histamine aerosol (1% w/v) to cause the bronchospasm in the form of PCD in guinea pigs. In our study, we observed that latent period of PCD was significantly increased in AHR-1 treated animals. These results suggested AHR-1 had bronchodilator activity.

## CONCLUSION

We observed that AHR-1 having antianaphylactic activity, stabilize antigen induced degranulation of mast cell and prolong the time of PCD. In conclusion, AHR-1 is might be effective in treatment various hypersensitivity reactions like anaphylactic shock and asthma.

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