

ORIGINAL ARTICLE

Antiallergic effect of piperine on ovalbumin-induced allergic rhinitis in mice

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Abstract

Context: Allergic rhinitis (AR) is a global health problem that affects a large number of population. Piperine (PIP) has been reported to exhibit anti-inflammatory, anti-histaminic, and immunomodulatory activities; however, its antiallergic profile has not been studied.

Objective: The objective of the study was to investigate the antiallergic potential of PIP in ovalbumin (OVA)-induced AR, mast cell degranulation (MSD), and OVA-induced paw edema.

Materials and methods: Mice were sensitized with OVA alternately on 1, 3, 5, 7, 9, 11, and 13th day. They were treated with either vehicle, PIP (10, 20, and 40 mg/kg, p.o.), or montelukast (10 mg/kg, p.o.) from the 14th to 20th day. On the 21st day, intranasal (OVA: 5% μ l) challenge was done. Animals were evaluated for physiological parameters, biochemical parameters, spleen weight, expression of interleukins (IL-6 and IL-1 β), and immunoglobulin-E (IgE). Histopathology of nasal mucosa, lungs, and spleen was carried out. MSD and paw edema studies were made to understand the mechanism of action.

Results: PIP (10, 20, and 40 mg/kg, p.o.) showed a significant dose-dependent protection with respect to nasal rubbing, redness of nose, and sneezing ($p < 0.001$) following nasal challenge. PIP dose dependently reduced histamine, NO concentration ($p < 0.001$), as well as reduced expression of IL-6, IL-1 β , and IgE ($p < 0.001$) as compared with the control group. Histopathology showed inhibition of infiltration of eosinophils and hyperplasia. It dose dependently reduced MSD and paw edema ($p < 0.001$).

Discussion and conclusion: PIP acts by mast cell-stabilizing activity, exhibits immunomodulatory and anti-inflammatory activity, thereby providing an effective treatment for AR.

Keywords

Antihistamines, degranulation, mast cells, montelukast, OVA

History

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Introduction

Allergic rhinitis (AR) is a global health problem that causes major illness and disability worldwide (Ozdoganoglu & Songu, 2012). Patients from all countries, all ethnic groups, and of all ages suffer from AR. It affects social life, sleep, school, and work. The economic impact of AR is often underestimated because the disease does not induce elevated direct costs. However, the indirect costs are substantial (Brožek et al., 2010).

AR, an inflammatory condition of the nasal mucosa mediated by an IgE-associated response to indoor and outdoor environmental allergens, has traditionally been classified as being seasonal or perennial, depending on whether an individual is sensitized to cyclic pollens or year round allergens, such as dust mites, pets, cockroaches, and moulds (Greiner & Meltzer, 2006). Patients with AR have symptoms such as sneezing, watery nose, marked increase in eosinophils in the nasal submucosa, and epithelium as well as chronic

inflammation of the nasal mucosal membrane (Okubo et al., 2011; Wang & Clement, 2000).

Antihistamines, corticosteroids, and mast cell stabilizers are used mainly in the treatment of AR but they produce potentially dangerous effects (Okubo et al., 2011; Ramírez et al., 2011; Walter et al., 2011). Commonly used first-generation antihistamines (diphenhydramine, brompheniramine, and chlorpheniramine) have poor selectivity for the H1-receptor and, as such, inhibit muscarinic receptors, which might result in various degrees of mucous membrane drying, vision blurring, constipation, urinary retention, tachycardia, and sedation (Okubo et al., 2011). Specific H1 antagonists like rupatadine, although provide benefit, but interfere with the metabolism of many drugs (Saint-Martin et al., 2003). The use of glucocorticosteroids (Al Suleimani & Walker, 2007) and anticholinergics is questionable due to long-term adverse effects (Nathan, 2007). Prophylactic use of mast cell stabilizers has the disadvantage of frequent administration due to the redundancy, synergy, and pleiotropism that exists for the mediators of AR (Al Suleimani & Walker, 2007; Gelfand, 2004).

Piper nigrum L. (Piperaceae) fruit is traditionally used for upper respiratory tract disorders such as cough, AR, sinusitis, and bronchitis (Agarwal & Agarwal, 2004;

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Szelenyi & Brune, 2002). PIP, an active alkaloid present in *Piper nigrum*, exhibits a wide variety of biological effects including anti-tumor (Lai et al., 2012; Makhov et al., 2012), anti-apoptotic (Muthukumar & Vanisree, 2011; Pathak & Khandelwal, 2009; Shrivastava et al., 2012), antibacterial (Chen et al., 2010; Karsha & Lakshmi, 2010), antioxidant (Kumar et al., 2010; Mehta et al., 2012), immunomodulatory (Alamgir & Uddin, 2010; Chuchawankul et al., 2012; Pathak & Khandelwal, 2009), anti-inflammatory (Bang et al., 2009), and antihistaminic activities (Gohil et al., 2011; Mehmood & Gilani, 2010). PIP has also been reported to reduce T helper cell type 2 (Th2) cytokines (IL-4 and IL-5). It has also been reported to lower the levels of eosinophil, expression of interleukin-13 mRNA in lung tissue as well as histamine and IgE production in serum (Kim & Lee, 2009). Therefore, the present study was aimed to evaluate the potential of PIP against OVA-induced AR in mice.

Materials and methods

Drugs and chemicals

PIP and OVA (grade V) were procured from Sigma Aldrich (St. Louis, MO), histamine (Loba Chemie, Pune, India), cetirizine-HCl, and montelukast sodium (Cipla Pvt. Ltd, Aurangabad, India), clonidine (Neon Laboratories Ltd., Mumbai, India), and disodium cromoglycate (DSCG) (Ahlcon Parenterals, Pune, India) were obtained as gift samples. *O*-Thalaldialdehyde (Thomas Baker Ltd., Mumbai, India), IL-6 kit (Quantikine, R&D Systems, Minneapolis, MN), and RPMI (Sigma Aldrich, St. Louis, MO) were purchased. *Bordetella bronchiseptica* (Cat. no. NCIM2267, batch no. ATCC-14455) was obtained from the National Collection of Industrial Micro-organisms (NCIM), National Chemical Laboratory (NCL), Pune, India.

Experimental animals

Swiss albino mice (25–35 g) were procured from the Sinhgad Institute of Pharmaceutical Sciences, Lonavala, Pune, India. Animals were housed in diurnal lighting conditions (12 h/12 h) and allowed free access to food and water for 7 d prior to the experiment. The experiments were performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethical Committee (IAEC) of Sinhgad Institute of Pharmacy, Pune, India (approval no. IAEC/SIOP/2012/30).

Experimental protocol induction of rhinitis

Experiments were carried out in rooms free of noise and strong odors. The temperature and the relative humidity were also controlled in the range of $23 \pm 2^\circ\text{C}$ and $55 \pm 5\%$, respectively. The animals were placed 40 min in advance in the perspex observation boxes ($26 \times 26 \times 26\text{ cm}^3$) for acclimatization. Observation boxes were thoroughly cleaned after each observation for every new animal.

Sensitization protocol

The sensitization solution (SS) consisted of 50 mg of ovalbumin, 1000 mg of aluminum hydroxide dissolved in 500 ml of

saline, and 0.5 ml of 1×10^{10} *Bordetella bronchiseptica* (Alcaligenaceae). Briefly, on days 1, 3, 5, 7, 9, 11, and 13, mice were sensitized by i.p. injection of 500 μl of SS, except Group I – normal (vehicle). The remaining mice were then divided into five groups ($n=6$). Group II – AR control; Group III – standard drug, montelukast (10 mg/kg, p.o.); Groups IV, V, and VI – PIP (10, 20, and 40 mg/kg, p.o.), followed by treatment with PIP or montelukast up to the day 20th. On 21st day, all groups were challenged with intranasal OVA (0.5 μl of SS) administration. Animals were evaluated for physiological and biochemical parameters, spleen weight, and histopathology (Wang et al., 2007).

Evaluation of parameters

Body weight

To monitor the effect of test drug on body weight, animals were weighed periodically on 0th, 7th, 14th, and 21st day.

Physiological parameters

Symptoms of rhinitis were observed and scored as following: the number of sneezing and rubbing/unit times was counted continuously in a randomized blind fashion directly for 40 min following nasal challenge. Sneezing is characterized by an explosive expiration just after a deep inspiration and nose rubbing is characterized by an external perinasal scratch with either one or both forelimbs of animal (Zhao et al., 2005). Redness of the nose was evaluated by observing the intensity in redness of nostrils and scoring them as the following: 0 – normal, 1 – faint, 2 – medium, and 3 – dark (Suleimani et al., 2008).

Biochemical parameters

Estimation of nitric oxide [nitrite concentration (NO)]

Nitric oxide is one of the important mediators in regulating patency of airways and its concentration increases in allergies related to the upper respiratory tract. The nitric oxide concentration in plasma samples was measured by the Griess reagent method, the absorbance was measured at 548 nm using the UV-visible spectrophotometer (V630, Jasco Analytical Instrument, Lakewood, NJ). The concentrations of nitrite were determined from standard curves constructed with serial dilutions of sodium nitrite (Iijima et al., 2001).

Measurement of histamine concentration

Histamine is a biogenic amine, noted for manifestation of certain allergic reactions and implicated as a mediator of hypersensitivity. Thus, histamine concentration in serum by an OPA spectrofluorometric procedure was estimated. The fluorescence intensity was measured at 460 nm (an excitation at 355 nm) using a spectrofluorometric method (Patange et al., 2005).

Measurement of IL-6, IL-1 β , and IgE

IL-6, IL-1 β , and IgE are the important inflammatory mediators released to stimulate immune response. The estimation of IL-6, IL-1 β , and IgE in the serum was carried out immediately after challenge. The concentrations were

estimated as per the instructions and procedure given in ELISA kit (Quantikine ELISA kit, R&D Systems, Minneapolis, MN).

Spleen weight

After 24 h, followed by the last intranasal challenge, animals were sacrificed and their spleen was separated, moisture was removed by blotting with the filter paper, and the organ weight was taken using a digital weighing balance.

Histopathological evaluation

Mice were euthanized after the intranasal OVA challenge test. The lung, spleen, and nose tissues were immersed in the freshly prepared 10% neutral buffered formalin for a period of 48 h. Following this, the tissue was rinsed in running tap water and decalcified in 10% nitric acid solution. After rinsing in tap water, the tissue processed for dehydration through graded alcohols and embedded in paraffin, sectioned, and stained with hematoxylin–eosin stains. Sections were examined by a light electron microscope ($40\times$) (Motic digital microscope with the image plus 2.0 software, Olympus Corporation, Tokyo, Japan). The intensity of inflammatory cell recruitment in nasal mucosa was graded using an arbitrary scale by the histopathologist: Grade 0 – not present or very slight, Grade 1 – mild, Grade 2 – moderate, and Grade 3 – severe disruption of epithelial observed in individual slides were also noted. All slides were coded before analysis and read blind to avoid observer bias (Zhao et al., 2005).

Measurement of paw edema

In the second set of animals, OVA-induced paw edema was carried out to study the antihistaminic activity of PIP. The procedure described by Medeiros et al. (2008) was followed with slight modification. Swiss albino mice were divided in following groups. Group I, normal: vehicle treated without challenge; Group II, control: vehicle treated and challenged; Group III, the standard group treated with cetirizine (10 mg/kg); and Groups IV, V, and VI were treated with test drug PIP (10, 20, and 40 mg/kg, p.o.). The standard and test drugs were given orally for 4 d. On the fourth day, all mice except Group I were challenged with SS-containing OVA (10 μ g/0.1 ml), by sub-plantar injection in the hind paw. The collateral paw received only vehicle. Paw thickness was measured at 1, 3, and 24 h after challenge using the plethysmometer (VJ Instruments, Karanja, India). The inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \left\{ \frac{(\text{paw volume of control animal} - \text{paw volume of test animal})}{\text{Paw volume of control animal}} \right\} \times 100$$

Mast cell degranulation (MSD)

MSD study was carried out using the reported method (Nirmal et al., 2012; Venkatesh et al., 2009). The Swiss albino mice were divided into five groups ($n=6$), the groups were divided as the following: Group I: control, Group II: standard, DSCG (50 mg/kg, p.o.), Groups III, IV, and V treated orally

with PIP (10, 20, and 40 mg/kg, p.o.). The treatment was given for 4 d, on the fourth day, all groups were injected with 5 ml of cold phosphate-buffer i.p. After gentle abdominal massage for 10 s, the peritoneal fluid was collected in centrifuge tubes, containing RPMI-1640 media (pH 7.2–7.4). The cell viability was checked using trypan blue dye (0.4%). Cells were washed three times with RPMI-1640 media by centrifugation at a low speed (500–600 rpm), after discarding the supernatant, the pellets of mast cells were resuspended in the medium. Mast cells were incubated with a mast cell degranulating agent, clonidine (50 μ g/ml), at 37 °C for 10 min in the incubator. Then the cells were stained with toluidine blue (1%) and the numbers of degranulated and intact mast cells were counted from the total number of 100 cells observed under high-power microscope. The percent protection of mast cells was calculated using the following formula:

$$\% \text{ of intact mast cells} = \left\{ \frac{(\text{total number of mast cells} - \text{total number of granulated cells})}{\text{total number of mast cells}} \right\} \times 100$$

Statistical analysis

Data for each parameter were analyzed by one-way ANOVA followed by Dunnet's *post hoc* test, body weight was analyzed using two-way ANOVA followed by the Bonferroni *post hoc* test using a GraphPad, prism software, version 4.03 (GraphPad Software, Inc., La Jolla, CA).

Results

Effect of PIP on body weight

Figure 1 shows the periodic observation in body weight of normal, AR control, standard drug montelukast (10 mg/kg), and test drug PIP (10, 20, and 40 mg/kg) treated groups. There was a significant decrease in the body weight from the seventh day onwards in all the treatment groups ($p<0.05$ and $p<0.001$) except normal. Treatment with montelukast as well as PIP (10, 20, and 40 mg/kg) significantly attenuated the loss in body weight as observed on day 21.

Effect of PIP on nasal symptoms

Table 1 shows the effect of PIP (10, 20, and 40 mg/kg) on OVA-induced nasal symptoms in mice. The AR control group showed a significant increase in sneezing, nasal rubbing, as well as nasal redness. PIP dose dependently reduced sneezing and nasal rubbing ($p<0.001$). The effects were comparable with that of the standard. Nasal redness as observed by the unbiased observer was very high in the AR control group. The redness was significantly attenuated by PIP as well as by montelukast.

PIP ameliorates allergic and inflammatory mediators

Figure 2(A) shows the marked increase in NO level in the AR control group as compared with the normal value ($p<0.001$). PIP (10, 20, and 40 mg/kg) as well as montelukast (10 mg/kg) dose dependently reduced the level of NO in serum samples. The reduction in NO level was significant ($p<0.001$) as compared with the AR control group.

Figure 1. Effect of piperine (10, 20, and 40 mg/kg) and montelukast (10 mg/kg) treatment on body weight in allergic rhinitis-induced mice ($n = 6$). Values are expressed in mean $\pm$ SEM. # $p < 0.05$, ### $p < 0.001$ compared with the normal group, *** $p < 0.001$ compared with the AR control group. Figure in parentheses indicates dose in mg/kg, p.o. PIP, piperine; Monte, montelukast.

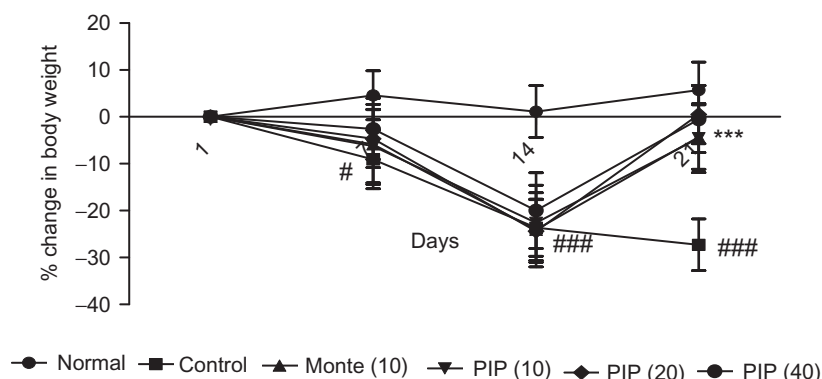


Table 1. Effect of piperine (10, 20, and 40 mg/kg, p.o.) and montelukast (10 mg/kg, p.o.) on sneezing, nasal rubbing, and nasal redness in allergic rhinitis-induced mice.

Group	Treatment	Sneezing/unit time	Nasal rubbing/unit time	Nasal redness
I	Normal	15.83 $\pm$ 1.86	19.33 $\pm$ 1.44	0.66 $\pm$ 0.21
I	AR control	79.17 $\pm$ 3.17###	71.67 $\pm$ 3.29###	2.66 $\pm$ 0.21#
III	Montelukast (10 mg/kg)	29.50 $\pm$ 2.99***	29.83 $\pm$ 1.92***	1.50 $\pm$ 0.42
IV	PIP (10 mg/kg)	29.83 $\pm$ 1.24***	29 $\pm$ 2.25***	1.5 $\pm$ 0.22
V	PIP (20 mg/kg)	26.27 $\pm$ 2.82***	23.83 $\pm$ 1.83***	1.83 $\pm$ 0.40
VI	PIP (40 mg/kg)	17.83 $\pm$ 1.66***	19.67 $\pm$ 1.11***	1.33 $\pm$ 0.21

Values are expressed in mean $\pm$ SEM. # $p < 0.05$, ### $p < 0.001$ compared with the normal group, *** $p < 0.001$ compared with the AR control group. Figure in parentheses indicates dose in mg/kg, p.o. PIP, piperine.

Stimulation by nasal allergen induces sneezing and nose rubbing due to the release of histamine. In the present study, significant increase in histamine level was observed in the AR control group ($p < 0.001$). PIP (10, 20, and 40 mg/kg) significantly reduced the histamine level ($p < 0.001$) as compared with the AR control. Montelukast also decreased the histamine level to a slight extent ($p < 0.05$) (Figure 2B). Serum IL-1 β and IgE were significantly increased in the AR control group as compared with the normal mice ($p < 0.001$). Treatment with PIP (20 and 40 mg/kg) and montelukast significantly reduced it ($p < 0.01$ and $p < 0.001$). We further investigated the ability of PIP to inhibit the production of Th2 cytokine, IL-6, in order to understand its role in the last phase of AR. Significant rise in serum IL-6 was observed in the AR control group indicating inflammatory immune response. PIP (10, 20, and 40 mg/kg) as well as montelukast (10) decreased the IL-6 level in a dose-dependent manner ($p < 0.001$) (Figure 2D–F).

PIP normalized spleen weight

The spleen is a part of the mononuclear phagocyte system and its enlargement usually characterizes infection or immune response. Sensitization with OVA has shown to cause a marked increase in the spleen weight in the AR control group as compared with the normal. Animals treated with PIP (10, 20, and 40 mg/kg) as well as montelukast (10 mg/kg) have shown significant ($p < 0.001$) decrease in the spleen weight as compared with the AR control group (Figure 3C).

Anti-histaminic activity of PIP

Prior treatment with PIP (10, 20, and 40 mg/kg) as well as standard drug cetirizine (10 mg/kg) showed significant

inhibition ($p < 0.001$) of paw edema induced by OVA as compared with the control group at 1 h (Figure 3) suggesting anti-histaminic activity of PIP.

PIP stabilizes mast cells

Table 2 and Figure 4 show the protective effect of PIP (10, 20, and 40 mg/kg) on mast cell degranulation. The control group exhibited a higher number of degranulated cells. PIP (20 and 40 mg/kg) ($p < 0.001$) showed maximum protection against degranulation by clonidine than PIP (10 mg/kg) ($p < 0.05$). The results of the higher dose of PIP were comparable with that of DSCG (50 mg/kg).

Histopathology

Photomicrograph representative sections of the lungs are shown in Figure 5. The normal group showed intact alveoli, bronchioles, and epithelial cell linings. Treatment with OVA resulted in the degeneration of the alveoli and inflammation of the bronchial epithelium. Treatment with test as well as standard drug showed protection as there was comparatively less damage to alveoli, bronchioles, and epithelial cell linings. Prominent effects were obtained with PIP (40 mg/kg). Figure 6 shows histopathology of the spleen. The normal group showed intact red and white pulps. Red pulp is a reservoir of monocytes and induces an innate response while white pulps are rich in lymphocytes and induce an immune response through humoral cell-mediated pathways. The AR control group showed complete degeneration of white and red pulps, and protection was offered by all the treatment groups in a dose-dependent manner. Figure 7 represents photomicrograph of nasal mucosa. The nasal mucosa of the AR control group showed increased infiltration of eosinophils and

Figure 2. Effect of piperine (10, 20, and 40 mg/kg) and standard drug montelukast (10 mg/kg) on nitric oxide ($\mu\text{g/ml}$) (A), histamine concentration ($\mu\text{g/ml}$) (B), average spleen weight (mg) (C), IL-1 β concentration (pg/ml) (D), Ig-E concentration (ng/ml) (E), and IL-6 concentration (pg/ml) (F) in allergic rhinitis-induced mice ($n=6$). Values are expressed in mean $\pm$ SEM. ### $p<0.001$ compared with the normal group, * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ compared with the AR control group. Figure in parentheses indicated dose in mg/kg, p.o. PIP, piperine; Monte, montelukast.

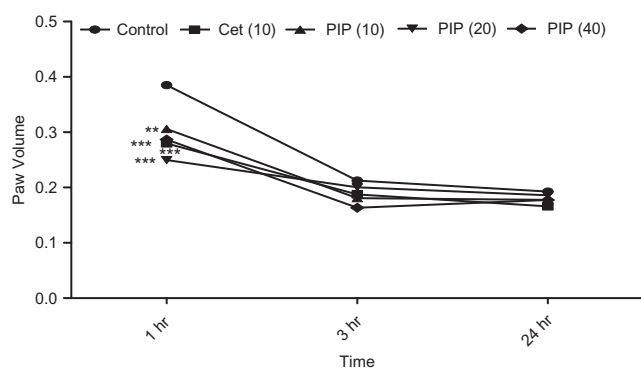
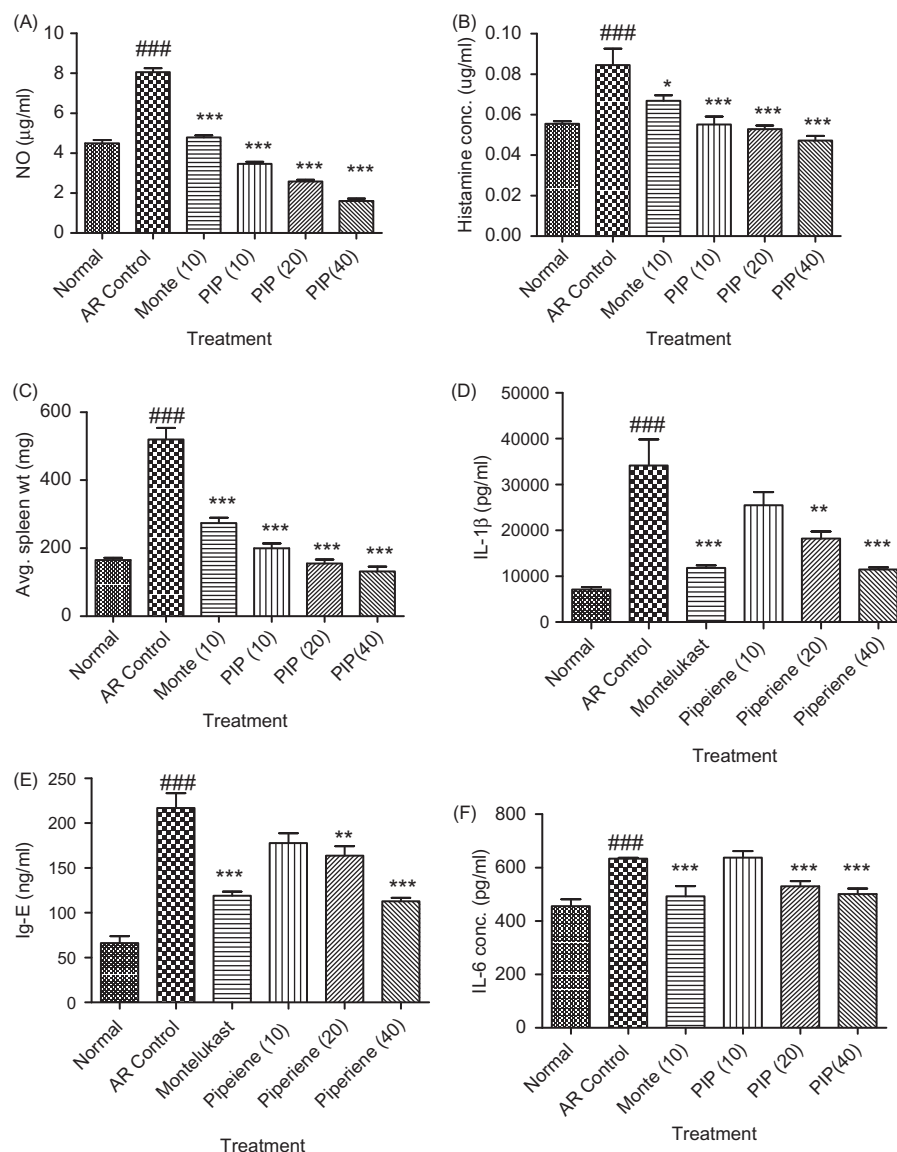


Figure 3. Effect of piperine treatment (10, 20, and 40 mg/kg) and cetirizine (10 mg/kg) on OVA-induced paw edema ($n=6$). Values are expressed in mean $\pm$ SEM. ** $p<0.01$ and *** $p<0.001$ compared with the control group. Figure in parentheses indicated dose in mg/kg, p.o. PIP, piperine; Cet, cetirizine.

disruption of nasal epithelium as compared with the normal mice (Grade 3). PIP (10 and 20 mg/kg) showed more intact epithelium and reduced eosinophil infiltrations (Grade 2) while montelukast and higher dose of PIP showed intact epithelium and very few eosinophil infiltrations (Grade 1).

Table 2. Effect of piperine (10, 20, and 40 mg/kg, p.o.) and disodium chromoglycate (50 mg/kg, p.o.) treatment on mast cell degranulation.

Group	Treatment	% Degranulated mast cells	% Intact mast cells
I	Control	57.33 $\pm$ 5.13	42.67 $\pm$ 5.13
II	DSCG (50 mg/kg)	9.66 $\pm$ 1.64***	90.33 $\pm$ 1.64***
III	PIP (10 mg/kg)	44.33 $\pm$ 1.64*	55.67 $\pm$ 1.64*
IV	PIP (20 mg/kg)	31.50 $\pm$ 2.04***	68.50 $\pm$ 2.04***
V	PIP (40 mg/kg)	16 $\pm$ 2.39***	84 $\pm$ 2.39***

Values are expressed in mean $\pm$ SEM. * $p<0.05$, *** $p<0.001$ compared with the AR control group. Figure in parentheses indicates dose in mg/kg, p.o. PIP, piperine; DSCG, disodium chromoglycate.

Discussion and conclusion

AR is characterized by symptoms such as sneezing, itchy nose, rhinorrhea, lacrimation, and nasal congestion. It is presumed to be triggered by multiple mediators released from the mast cells and other inflammatory cells (Al Suleimani & Walker, 2007). AR comprises two phases of allergic response. Prior phase involves the activation of mast cells and basophiles resulting in the production of histamine,

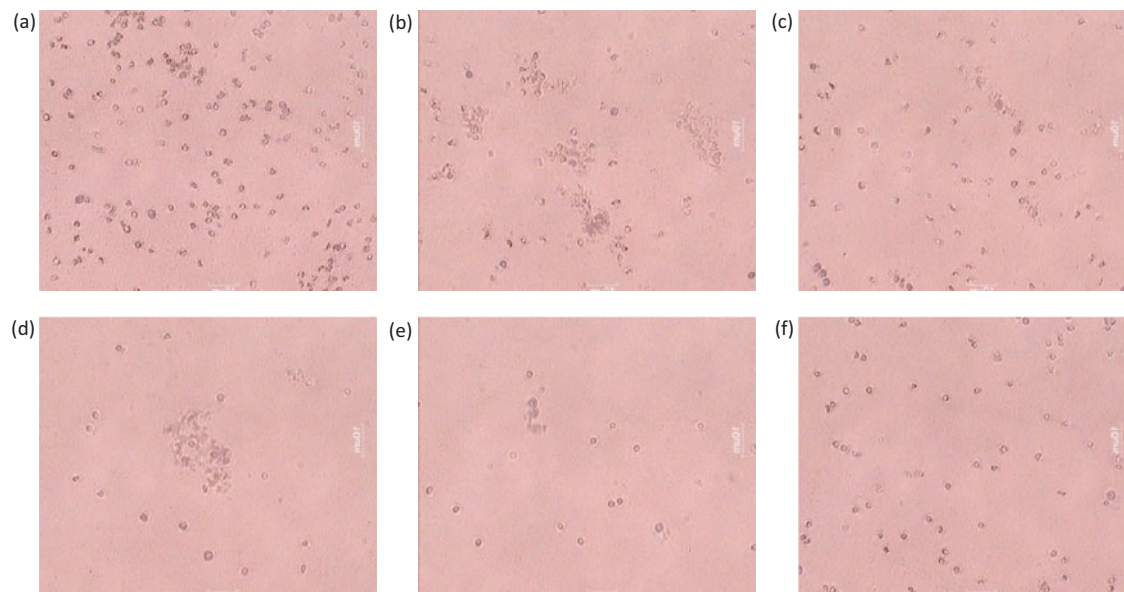


Figure 4. Effect of piperine treatment (10, 20, and 40 mg/kg) and disodium cromoglycate (50 mg/kg) on mast cell degranulation. (a) The normal group showing intact mast cells, (b) the AR control group showing degranulation of mast cells, (c) montelukast (10 mg/kg) treated group section showing partial cell degranulation, (d) PIP (10 mg/kg), (e) PIP (20 mg/kg), and (f) the PIP (40 mg/kg) treated group shows stabilization of mast cells in a dose-dependent manner.

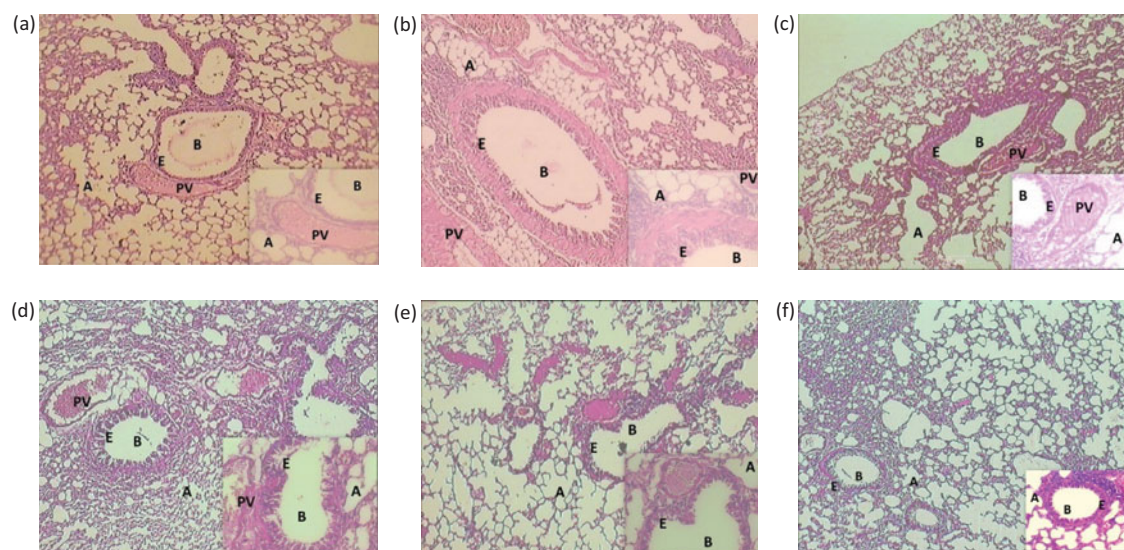


Figure 5. Histological alteration in lung tissue of mice: photomicrograph of lung sections stained with hematoxylin and eosin: (a) the normal group shows alveoli and bronchioles having normal architecture. Epithelial cells linings are intact and inflammation is absent. (b) The AR control group section shows abnormal bronchiole epithelium and degenerated alveolar sacs. (c) Montelukast (10 mg/kg) treated group section shows partial cell disruption in alveoli and bronchioles. The epithelial cell linings show less damage. (d) The PIP (10 mg/kg) treated group demonstrates intact epithelial cell linings; partial protection of bronchioles and alveoli, with minimum Inflammation. (e) The PIP (20 mg/kg) treated group shows moderate intact alveoli and bronchiolar structures. (f) The PIP (40 mg/kg) treated group sections showed more protective effect on alveoli and bronchiolar epithelial membrane. A, alveoli; E, epithelium; B, bronchiole; PV, pulmonary vein; PIP, piperine; Monte, montelukast. Figure in parentheses indicates dose in mg/kg, p.o. (40 ×).

prostaglandins, leukotriens, and cytokines, which causes sneezing, nasal itching, nasal congestion, and redness. The later phase involves migration of eosinophils, mast cells, and basophiles into the nasal tissue (Wang *et al.*, 2007). The available treatment intends either to reduce the effect of release of mediators from activated cell or end organ effects of released mediators. Commonly used pharmacological agents are H1 receptor antagonist, mast cell stabilizer, corticosteroids, anti-leukotrienes, and anticholinergic agents (Greiner *et al.*, 2012).

Piperine present in the long and black pepper has been used as a dietary factor in herbal medicine for centuries in the world. In India, it is widely used traditionally either in powdered or in decoction form, as a remedy for sore throat, cough, and nasal congestion. Piperine has already been reported for antihistaminic, immune-modulatory, anti-inflammatory, anti-apoptotic, and antioxidant activities (Meghwal & Goswami, 2013).

OVA-induced AR in mice shows similar nasal allergic symptoms as observed in humans (Wang *et al.*, 2007). The

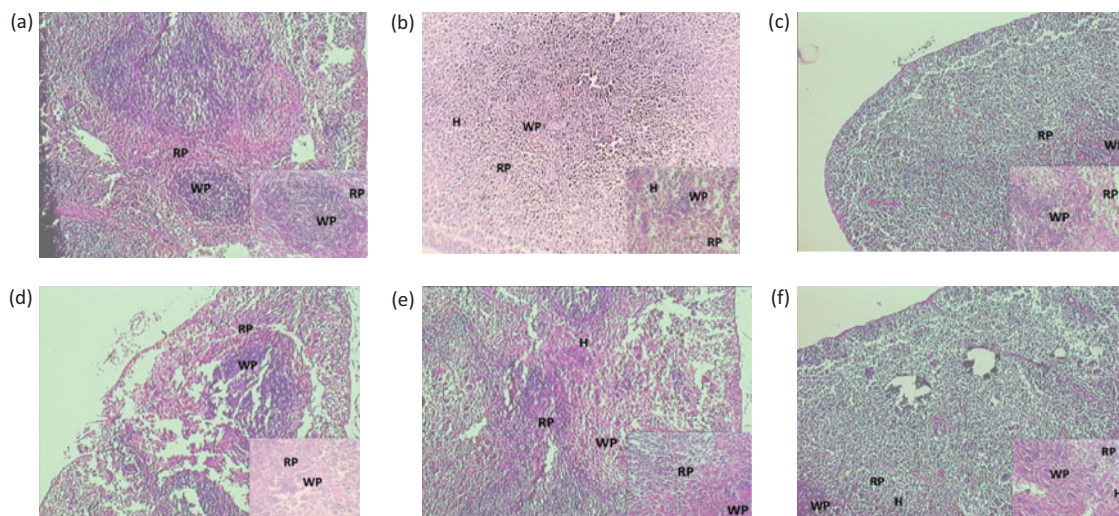


Figure 6. Histological alteration of spleen in mice: (a) the normal group shows intact white and red cell pulps. (b) The AR control group shows the complete disruption in white and red pulp cells, hyperplasia is observed. (c) Montelukast (10 mg/kg) group shows more protective effect on red and white pulps and less hyperplasia is observed. (d) PIP (10 mg/kg) shows protective effect on red and white pulp cells. (e) PIP (20 mg/kg) shows more protection of both cells and intact structure with less hyperplasia. (f) PIP (40 mg/kg) total protection of both pulp and hyperplasia is not observed. RP, red pulp; WP, white pulp; H, hyperplasia; PIP, piperine; Monte, montelukast. Figure in parentheses indicates dose in mg/kg, p.o. (40 ×).

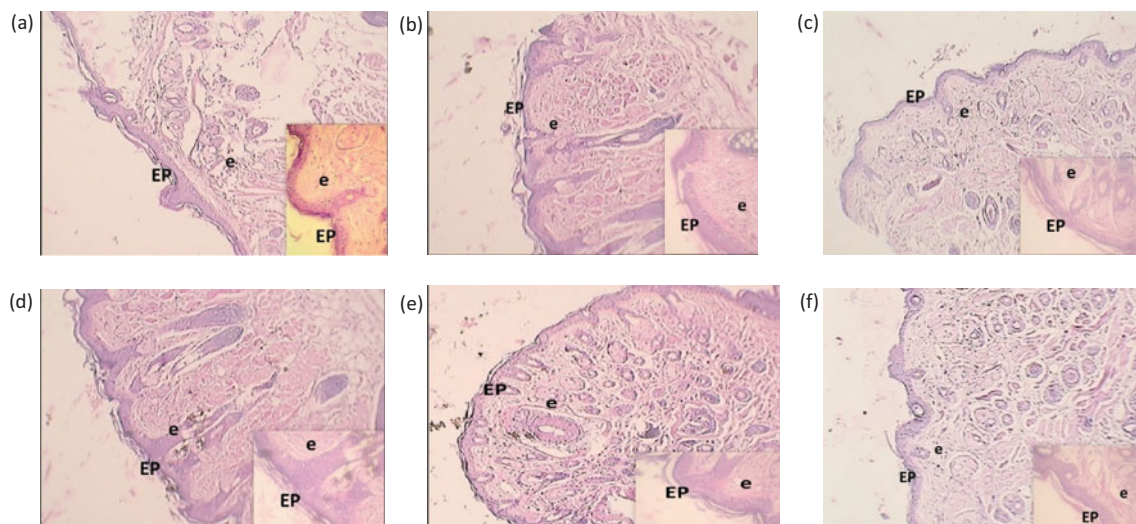


Figure 7. Histological alteration of nasal mucosa in mice: (a) the normal group shows intact epithelium and very few eosinophil infiltrations. (b) The AR control group shows abnormal epithelium, thickened subepithelial smooth muscle layer, and infiltration of eosinophil. (c) The montelukast (10 mg/kg) group shows intact epithelium and reduced eosinophil infiltration. (d) PIP (10 mg/kg) shows less intact epithelium and moderate number of eosinophil infiltrations. (e) PIP (20 mg/kg) shows more intact epithelium and reduced eosinophil infiltration. (f) PIP (40 mg/kg) shows intact epithelium and few eosinophil infiltration. EP, epithelium; e, eosinophiles; PIP, piperine; Monte, montelukast. Figure in parentheses indicates dose in mg/kg, p.o. (40 ×).

objective of the present study was to evaluate antiallergic profile of piperine using OVA-induced AR, OVA-induced paw edema, and clonidine-induced mast cell degranulation. Administration of SS for 14 d showed induction of rhinitis in mice as observed by the episodes of nasal irritation on the challenge. Piperine significantly ameliorated sneezing, rubbing, and redness induced by sensitization of nerve endings resulted from histamine released in response to antigen-antibody reaction. Histamine being the major mediator of early-phase allergic response was also analyzed in the serum of sensitized mice. The level of histamine in sensitized animals was higher when compared with normal animals. Treatment with piperine dose dependently reduced histamine

level in serum. The experiment demonstrated that piperine had antiallergic potential.

The cascade leading to AR includes production of IgE on antigen exposure. The acquisition of IgE by mast cell and basophils via the high affinity IgE receptor (FcεR1) promotes cross linking of bound IgE-FcεR1 complex with multivalent allergen. This results in release of proinflammatory and inflammatory mediators such as IL-6 and IL1β. The proinflammatory cytokine IL-6 has been recognized as an important mediator of allergy as it is secreted by cells of innate immunity. IL-6 has been reported to be released on allergen challenge in patients (Ohkubo et al., 1998). It is an important Th2 type of cytokine involved in the induction of

IgE synthesis as well as in mast cell proliferation and maturation (Bachert et al., 1998; Guzmán et al., 2010). The beneficial effects of piperine can be ascribed to its attenuating effect on IgE, IL-6, and IL-1 β concentration in serum. Our result corroborates with the previous study (Kim & Lee, 2009). The reduced level of these cytokines and immunoglobulin suggests a potential use of piperine as antiallergic, due to its potent anti-inflammatory and immunomodulatory activities.

AR is associated with increased production of nitric oxide (Arnal et al., 1997; Yamada et al., 2012). Iijima et al. (2001) have reported eosinophils as a measurable source of nitric oxide. In present study, exposure to OVA increased NO in AR control mice. There was significant reduction in the NO level in the entire treatment group; moreover, large numbers of eosinophils were observed in histopathological section of nasal mucosa in AR control mice. Piperine-treated group showed comparatively less migration of eosinophil into epithelial tissue. A similar effect was observed in histopathology of lung and spleen. The lung tissue of the AR control group showed complete disruption of alveoli and bronchiole morphology, inflammation, and redness in epithelial cell linings. Montelukast as well as piperine showed protection in epithelial cell lining and partial protection in the structure of bronchioles and alveoli. It also showed less inflammation and redness, thus the histological results obtained corroborate with the physiological, biochemical, and immunological findings.

The local injection of OVA into the paw of the animal results in acute allergic response due to the release of histamine from mast cells, release of proteoglycans (heparin and chondroitin sulfate E), proteases, and other inflammatory mediators (e.g., prostaglandins and leukotrienes) at the later hours. In the present study, piperine inhibited the paw edema at first hour indicating its efficacy in attenuating histamine; moreover, the anti-inflammatory effects of PIP (40 mg/kg) were sustained till the third hour advocating its anti-inflammatory potential at higher dose. Earlier reports also support the potent anti-inflammatory activity of piperine (Bang et al., 2009; Sudjarwo, 2005).

The mast cell degranulation study was designed to understand the mechanism of action of piperine. Mast cells are important mediators of inflammatory response such as allergy and anaphylaxis (Kim et al., 2013), where histamine remains the best characterized mediator implicated in the acute phase of allergic response (Petersen et al., 1996). Mast cell degranulation can be induced by clonidine, a mast cell degranulator which leads to histamine release. The results showed increased degranulated cells in the control group. Piperine-treated animal showed prevention of granulation of mast cells when exposed to clonidine. Piperine dose dependently acted as an antiallergic by stabilizing the mast cells.

In conclusion, the above results showed that piperine prevented allergic responses by mast cell stabilization thereby inhibiting the release of mediators like histamine, IL-6, IL-1 β , and IgE. The anti-inflammatory activity obtained provides additional benefits. The present study reveals the protective role of piperine, in OVA-induced AR, therefore, it is anticipated that piperine can be developed as a drug for clinical use.

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Declaration of interest

The authors report that they have no conflicts of interest.

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