

Pistacia integerrima ameliorates airway inflammation by attenuation of TNF- α , IL-4, and IL-5 expression levels, and pulmonary edema by elevation of AQP1 and AQP5 expression levels in mouse model of ovalbumin-induced allergic asthma



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ABSTRACT

Background: Natural products are considered as an essential source for the search of new drugs. *Pistacia integerrima* galls (PI) have been used for the treatment of asthma and cough in traditional system of medicine.

Aim/hypothesis: Current study investigates the immunomodulatory and anti-inflammatory activities of *P. integerrima* in mouse model of ovalbumin-induced allergic asthma.

Methods: Mice were intraperitoneally sensitized and subsequently challenged intranasally with ovalbumin to induce allergic asthma. Experimental group mice were treated with methanol extract of *P. integerrima* extract (200 mg/kg b. w.) and Methylprednisolone (MP) (15 mg/kg b. w.) for 07 consecutive days, alongside intranasal challenge. Lung tissues were stained with Hematoxyline and Eosin (H & E), and Periodic Acid-Schiff (PAS) stains for histopathological evaluation. Lung wet/dry weight ratio was measured as an index of lung tissue edema. Albumin was injected in the right ear 24 h before sacrificing the mice and difference of weight was taken as a degree of delayed type hypersensitivity (DTH). mRNA expression levels of TNF- α , IL-4, IL-5, Aquaporin-1 (AQP1), and AQP5 were evaluated using reverse transcription polymerase chain reaction (RT-PCR) followed by gel electrophoresis.

Results: The data showed both PI extract and MP significantly alleviated DTH and nearly normalized total leukocyte count and differential leukocyte count in both blood and BALF. We found significantly suppressed goblet cell hyperplasia and inflammatory cell infiltration after treatment with both PI extract and MP. Expression levels of TNF- α , IL-4, and IL-5 were also found significantly reduced after treatment with both PI extract and MP, which might have resulted in the amelioration of airway inflammation. Current study displayed that both PI extract and MP significantly decreased lung wet/dry ratio, suggesting reduction in pulmonary edema. RT-PCR analysis showed significant increase in AQP1 and AQP5 expression levels after treatment with both PI extract and MP, which might have caused the alleviation of pulmonary edema.

Conclusion: Our study displays that *P. integerrima* possesses significant anti-asthmatic activity which may be attributed to reduction in TNF- α , IL-4, and IL-5 expression levels, and increase in AQP1 and AQP5 expression levels.

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Introduction

Airway inflammation, mucus hypersecretion, infiltration of leukocyte, airway wall remodelling, and spasm of bronchial smooth

Abbreviations: (MP), Methylprednisolone; (AQP1), Aquaporin-1; (AQP5), Aquaporin-5; (BALF), Bronchoalveolar lavage fluid; (DTH), Delayed type hypersensitivity; (DLC), Differential leukocyte count; (TLC), Total leukocyte count.

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muscles are considered as the main features of asthma (Wang et al., 2008). Eosinophils and lymphocytes are the major cells which infiltrate the airway mucosa. Other types of different inflammatory cells which are involved in asthma are mast cells, macrophages, monocytes, neutrophils, and basophils (Cohn and Ray, 2000). The inflammatory mediators released during inflammation plays a central role in the pathophysiology of allergic airway inflammation. Most of the features of allergic airway inflammation are based on an excessive increase in Th2 mediated cytokines, such as, IL-4 and IL-5 (Deckers et al., 2013; Schuijs, 2013).

TNF- α is a pro inflammatory cytokine that is produced during allergic pulmonary inflammation by different cells, such as, neutrophils, eosinophils, macrophages, epithelial cells, and mast cells (Lee et al., 2010). IL-4 is another important cytokine that participates in the regulation of allergic airway inflammation. IL-4 plays critical role in the promotion of Th2 type immune response and production of IgE antibodies (Barnes, 2001). IL-5 influences the activation of eosinophils and adhesion, generates different inflammatory mediators, and causes chemotaxis and expression of membrane receptors (Kouro and Takatsu, 2009; Tomasiak-Lozowska et al., 2010). Aquaporins (AQPs) are the water channels that are part of the family of transmembrane water passages and are known to regulate cellular responses e.g. change in volume of fluid and osmolarity. Lungs and many other tissues require aquaporins for their routine secretory and absorptive functions (Krause et al., 2009). AQP1 and AQP5 contribute in the formation of major path for water transport by osmosis between capillary compartments and airspace (Chen et al., 2006). AQPs not only play a key role in normal physiology of transport, but also, they are involved in the pathophysiology of cerebral edema, pulmonary edema, secondary otitis media, and other processes which include abnormal transport of water (Bodis et al., 2001; Li et al., 2011). Anti-asthmatic therapies by increasing the levels of AQP1 and AQP5 in mouse lungs can reduce the pulmonary edema (Dong et al., 2012). Corticosteroids are known to improve the pulmonary edema by up regulating the expression of AQPs (Ben et al., 2012; Tran et al., 2010).

Corticosteroids are commonly used for the treatment of allergic asthma as anti-inflammatory agents. However, their use is associated with various undesirable adverse effects, such as, reduced bone metabolism, adrenal suppression, and reduced growth in children (Abbas et al., 2005). Both physicians and patients are now vastly considering the plant extracts as a source of alternative medicine (Markham and Wilkinson, 2004). *Pistacia integerrima* (Family: Anacardiaceae) is a deciduous plant which sheds its leaves during the dry season and is native to Asia (Vashist and Jindal, 2012). In traditional system of medicine, different parts of this medicinal plant, especially its galls, have been used for the treatment of asthma and cough (Bibi et al., 2015). Previously, Adusumalli et al. (2013) reported that aqueous extract of *P. integerrima* provided protection against histamine aerosol-induced bronchospasm in guinea pigs. The authors using isolated guinea pig tracheal preparation demonstrated that *P. integerrima* possessed spasmolytic activity against histamine-induced contraction. Current study investigates the anti-inflammatory and immunomodulatory activities of ethanol extract of *P. integerrima* gall using mouse model of ovalbumin-induced allergic asthma. Methylprednisolone, a commonly used drug for the treatment of allergic airway inflammation was used as a reference drug.

Materials and methods

Preparation of ethanol extract of *P. integerrima* galls

One kg of *P. integerrima* galls were purchased from the local market of Lahore, Pakistan. The galls were crushed using a grinder and powdered plant material was soaked in 3 L absolute ethanol for 14 days at room temperature. During maceration, plant material was subjected to occasional shaking on daily basis. After 14 days, vegetative debris was removed by passing through muslin cloth and subsequently Whatman No. 1 was used to filter the obtained liquid. The filtrate was then concentrated at 35°C under reduced pressure using rotary evaporator. Approximately, 7% yield was obtained after extraction process. The concentrated extract was kept in air tight jar until required for experimental use (Janbaz et al., 2014).

Experimental animals

24 healthy female BALB/c mice, aging of 6–8 weeks were weighed and placed into four groups having 6 mice in each group. All the animals were kept in experimental research laboratory, University of Health Sciences, Lahore at controlled room temperature (22–24 °C) and humidity (45–65%). The animals were kept under 12 h light and dark cycle and were given standard pellet diet and water *ad libitum*. Ethical Review Committee, University of Health Sciences, Lahore approved all the experiments of current study (Shabbir et al., 2014).

Induction of allergic asthma

Group-I (Control) mice were sham-sensitized by intraperitoneal injection of phosphate buffer saline solution (PBS) and challenged intranasally with the same solution. Mice from group-II, -III, and -IV were sensitized on day 0 and day 14 by intraperitoneal injection of 20 μ g of ovalbumin dissolved in 2 mg Al(OH)₃ (adjuvant) in a volume of 0.1 ml PBS. After the two weeks of second sensitization i.e. on 28th day, all the mice were intranasally challenged with 1% ovalbumin once daily for 7 consecutive days (El Gazzar et al., 2006; Yang et al., 2010).

Treatment protocol

The treatment was started at the same day when mice were challenged intranasally with ovalbumin i.e. at 28th day. All the groups were treated 30 min before intranasal challenge and therapy continued for 7 successive days. The group-III (PI) was treated with ethanol extract of *P. integerrima* at a dose of 200 mg/kg body weight. The group-IV (MP) was treated with Methylprednisolone at a dose of 15 mg/kg body weight. The group-I (control) and group-II (diseased) were given normal saline only. All the mice were sacrificed within 24 h of last challenge and samples were collected.

Delayed type hypersensitivity test

Delayed type hypersensitivity test is a measure of inflammatory response to antigen *in vivo*. All groups were injected with ovalbumin 24 h before sacrificing the mice in right ear and with PBS in left ear (as control) through intradermal route. Right and left ears were separated and weighed soon after sacrificing the mice. The difference of weight was represented as the degree of DTH (Shahzad et al., 2009).

Inflammatory cell count in blood and BALF

Total leukocyte count and differential leukocyte count i.e. eosinophils, neutrophils, lymphocytes, and monocytes level in both blood and BALF were evaluated using automated hemocytometer (Sysmax XT-1800i) (Shabbir et al., 2014). After euthanization the trachea with intact lungs were dissected out for the collection of BALF. The lungs were lavaged through trachea with 0.5 ml ice cold PBS by gradual instillation and withdrawal with blunt needle. The BALF was collected in 1.5 ml sterile eppendorf tubes (Li et al., 2014; Khan et al., 2015).

Histopathological evaluation of lungs

After sacrificing the mice, lungs were taken out and fixed in 10% neutral buffered formalin. After fixation, the 5- μ m thick paraffin sections were cut and stained with Hematoxylin and Eosin (H & E) for the assessment of inflammatory cell infiltration and alveolar thickening. Periodic Acid-Schiff (PAS) staining was used for the identification of goblet cell hyperplasia. The slides were observed

Table 1
Primer sequences along with product size.

Primers	Forward / Reverse	Sequence	Product Size
IL-4	Forward	5'-TCACTGACGGCACAGAGCTA-3'	154
	Reverse	5'-CCTTCTCCTGTGACCTCGTT-3'	
IL-5	Forward	5'-GGCTGGCCTCAAACCTGGTAA-3'	198
	Reverse	5'-CCCTGATGCAACGAAGAGGA-3'	
TNF- α	Forward	5'-TGGCCTCCCTCTCATCAGTT-3'	199
	Reverse	5'-ATCGGCTGGCACCCTAGTT-3'	
AQP1	Forward	5'-AGAGCCCTGTCTGCATCCAT-3'	181
	Reverse	5'-CCTCGACTTAACCGCTGGAT-3'	
AQP5	Forward	5'-CCCAAGGCCACCATGAAGAA-3'	171
	Reverse	5'-TATGGCCAGGCCAAAGGCTA-3'	

by a blind pathologist who graded the histopathological score on the following scale: 0, none; 1, mild; 2, moderate; and 3, severe (Khan et al., 2015).

Determination of mRNA expression levels of IL-4, IL-5, TNF- α , AQP1, and AQP5

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from lung tissue using the TRIzol method and quantified using nanodrop spectrophotometer. cDNA was synthesis using 1000 ng/reaction total RNA. Reverse transcription was conducted according to kit manufacturer's protocol (TOP Script cDNA synthesis kit, Enzynomics). Briefly, RNA template was mixed with oligo dT primer (80 μ M) and nuclease free water. The mixture was chilled on ice after heating it for 5 minutes at 65 °C. Then 1 μ l reverse transcriptase enzyme (200 U/ μ l), 2 μ l dNTP mixture (2 mM), 1 μ l RNase inhibitor (40 U/ μ l), and 2 μ l reaction buffer (10X) were added in reaction mixture. The volume was made up to 20 μ l with nuclease free water. The mixture was incubated at 42 °C for 60 min and resultantly produced cDNA was stored at -20 °C for further use as a template for amplification in PCR.

Polymerase chain reaction and gel electrophoresis

Briefly, 2 μ l cDNA was used as a template in all the reactions. 10 μ l (2X) PCR master mix (Thermo Scientific, America), 0.5 μ l forward and reverse primer each (10 μ M) (Table 1), and nuclease free water q.s. to 20 μ l were added in PCR tubes and thermal cycler (Bio-Rad, USA) was programmed for 35 cycles of denaturation (95 °C for 10 sec), annealing (58 °C for 20 sec), and extension (72 °C for 30 sec).

Agarose (2%) gel electrophoresis was used for visualization of PCR product. The PCR product was semi-quantified by densitometry using ImageJ software (Khan et al., 2015).

Lung wet/dry weight ratio

Lung wet/dry weight ratio was used as an index of lung tissue edema. One fresh lobe of right lung tissue was excised and wet weighed was measured immediately. Then the same lobe was dried in an oven at 56°C for 15minutes and dry weight was measured (Matsuyama et al., 2008).

Statistical analysis

One way ANOVA and Post hoc Tukey's test or student *t*-test was applied to determine the difference among groups. Mean $\pm$ Standard deviation (SD) was used to present the data and *P* value $\leq$ 0.05 was considered as statistically significant. Graph-Pad Prism v.6 software was used for analysis of data.

Results

Treatment with *Pistacia integerrima* nearly normalized the TLC and DLC in blood

Total leukocyte count was found significantly elevated in diseased group as compared with control group. Treatment with *P. integerrima* and MP significantly reduced TLC in blood as compared with positive control group.

We found a significant increase (all *P* < 0.001) in neutrophils, lymphocytes, eosinophils, and monocytes levels in blood of mice of diseased group as compared with control group. Treatment with ethanol extract of *P. integerrima* showed a significant decrease (all *P* < 0.001) in DLC as compared with diseased group. Similarly, MP treated group also showed a significant decrease (all *P* < 0.001) in DLC as compared with diseased group (Table 2).

Treatment with *P. integerrima* nearly normalized the TLC and DLC in BALF

Total leukocyte count was found significantly enhanced in diseased group as compared with control group. Treatment with *P. integerrima* and MP significantly attenuated TLC in BALF as compared with positive control group.

The data showed a significant increase (all *P* < 0.001) in neutrophils, lymphocytes, eosinophils, and monocytes levels in BALF of mice of diseased group as compared with control group. Treatment with ethanol extract of PI significantly decreased (all *P* < 0.001) DLC as compared with diseased group. Similarly, MP treated group also demonstrated a significant decrease (all *P* < 0.001) in DLC as compared with diseased group (Table 3).

Treatment with *P. integerrima* significantly attenuated histopathological score

Treatment with *P. integerrima* significantly decreased inflammatory cell infiltration

Inflammatory cell infiltration was found significantly elevated (*P* < 0.001) in diseased group as compared with control group. Treatment with both *P. integerrima* (*P* < 0.01) and MP (*P* < 0.001) significantly attenuated infiltration of inflammatory cells as compared with positive control group (Figs. 1 and 2B).

Treatment with *P. integerrima* significantly reduced goblet cell hyperplasia

We found significantly increased goblet cell hyperplasia in diseased group (*P* < 0.001) as compared with control group. Treatment with both *P. integerrima* and MP significantly alleviated (*P* < 0.01) goblet cell hyperplasia as compared with positive control group (Figs. 1 and 2C).

Table 2

Treatment with both PI extract and MP nearly normalized total leukocyte count and differential leukocyte count in blood. Mean $\pm$ SD is given to represent the data, where $n=6$. *** and ### Statistical difference as compared with diseased group and control, respectively at $P < 0.001$.

TLC and DLC in blood	Group I (Control)	Group II (Diseased)	Group III (PI)	Group IV (MP)
Total leukocyte count (10^3)	3.605 $\pm$ 1.333	5.930 $\pm$ 1.021###	3.742 $\pm$ 0.6363***	2.338 $\pm$ 0.857***
Lymphocytes	71.67 $\pm$ 4.76	83.83 $\pm$ 3.97###	68.67 $\pm$ 4.17***	68.50 $\pm$ 3.50***
Neutrophils	26.00 $\pm$ 3.63	38.00 $\pm$ 3.28###	19.52 $\pm$ 4.10***	16.33 $\pm$ 2.42***
Eosinophils	3.27 $\pm$ 0.49	5.60 $\pm$ 0.63###	2.60 $\pm$ 0.68***	3.05 $\pm$ 0.43***
Monocytes	0.128 $\pm$ 0.05	0.320 $\pm$ 0.05###	0.168 $\pm$ 0.04***	0.155 $\pm$ 0.04***

Table 3

Treatment with both PI extract and MP nearly normalized total leukocyte count and differential leukocyte count in BALF. Mean $\pm$ SD is given to represent the data, where $n=6$. *** and ### Statistical difference as compared with diseased group and control, respectively at $P < 0.001$.

TLC and DLC in BALF	Group I (Control)	Group II (Diseased)	Group III (PI)	Group IV (MP)
Total leukocyte count (10^3)	6.79 $\pm$ 0.61	12.64 $\pm$ 1.71###	5.09 $\pm$ 0.57***	4.68 $\pm$ 0.44***
Lymphocytes (%)	45.30 $\pm$ 7.54	87.38 $\pm$ 5.04###	56.95 $\pm$ 4.38***	60.83 $\pm$ 5.52***
Neutrophils (%)	5.33 $\pm$ 3.55	26.50 $\pm$ 4.68###	3.98 $\pm$ 1.21***	5.68 $\pm$ 1.86***
Eosinophils (%)	1.80 $\pm$ 0.46	6.61 $\pm$ 0.73###	2.25 $\pm$ 0.47***	2.58 $\pm$ 0.58***
Monocytes (%)	0.17 $\pm$ 0.02	0.32 $\pm$ 0.05###	0.13 $\pm$ 0.03***	0.20 $\pm$ 0.05***

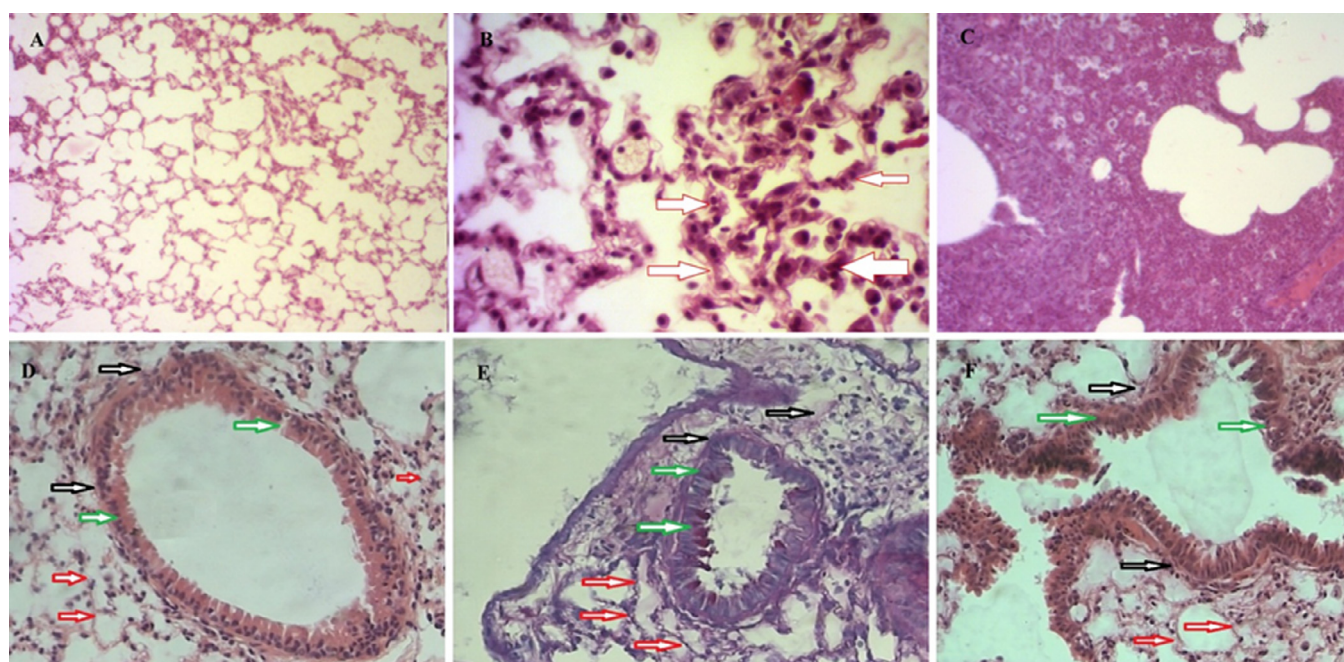


Fig. 1. H & E staining showing normal alveoli with no inflammation (A). Arrows showing alveolar thickening in diseased group (B). Severe inflammation was found in peribronchial and perivascular areas in diseased group (C). Treatment with PI extract significantly reduced alveolar thickening (red arrow), infiltration of inflammatory cells (black arrow), and goblet cell hyperplasia (green arrow) (D). PAS staining also showing reduction of alveolar thickening, inflammatory cell infiltration, and goblet cell hyperplasia in PI extract treated group (E). Treatment with MP significantly attenuated inflammation, goblet cell hyperplasia, and alveolar thickening (F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Treatment with ethanol extract of PI significantly attenuated DTH

The data showed a significant increase ($P < 0.001$) in DTH in diseased group as compared to control group (0.045 ± 0.013 and 0.006 ± 0.003 , respectively). Treatment with ethanol extract of PI (0.010 ± 0.005) and MP (0.020 ± 0.008) significantly attenuated ($P < 0.001$) DTH as compared with diseased group (Fig. 2A).

Treatment with PI extract significantly suppressed the mRNA expression levels of TNF- α , IL-4, and IL-5

The data showed significantly ($P < 0.001$) increased mRNA expression levels of TNF- α in diseased group as compared with control group (6.26 ± 0.49 vs 3.44 ± 0.44). Treatment with PI extract

(2.67 ± 0.37) significantly alleviated ($P < 0.001$) TNF- α expression levels, similar to the effect of MP (3.14 ± 0.32) (Fig. 3A).

We found significantly increased ($P < 0.001$) mRNA expression levels of IL-4 in diseased group as compared with control group (5.31 ± 0.51 vs 3.78 ± 0.45). Treatment with PI extract (3.63 ± 0.35) and MP (3.66 ± 0.35) significantly ($P < 0.001$) alleviated IL-4 expression levels (Fig. 3B).

IL-5 expression levels were also found significantly elevated ($P < 0.001$) in diseased group as compared with control group (6.40 ± 0.48 vs 3.37 ± 0.41). Treatment with PI extract (3.93 ± 0.40) and MP (4.18 ± 0.40) significantly attenuated ($P < 0.001$) IL-5 expression levels (Fig. 3C).

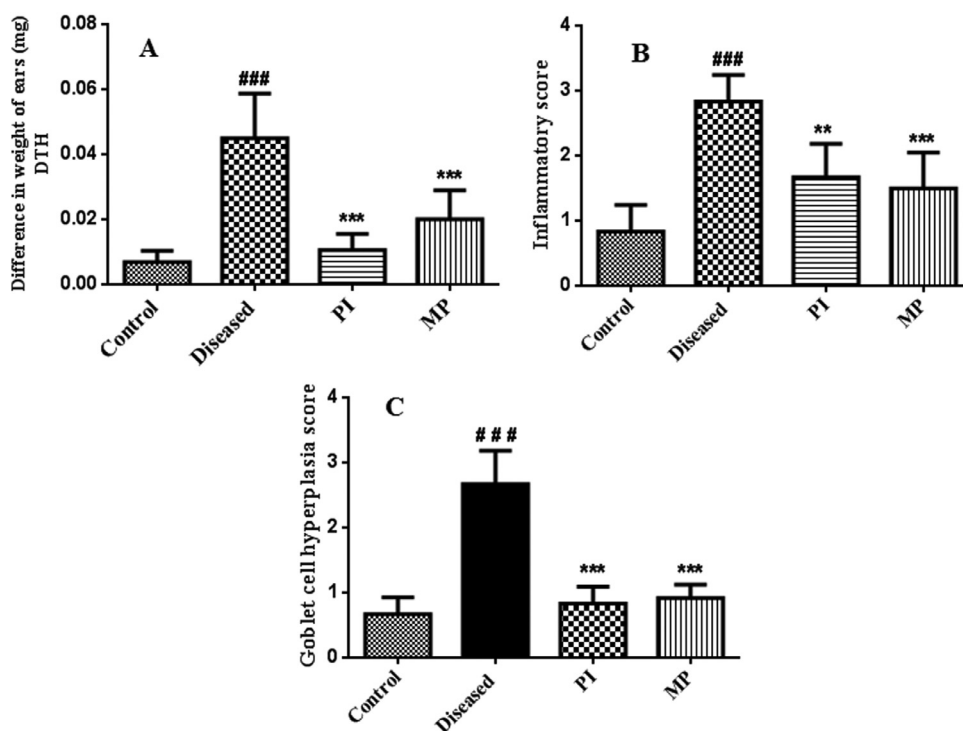


Fig. 2. Treatment with both PI extract and MP significantly attenuated DTH (A), inflammatory score (B), and goblet cell hyperplasia score (3). Mean $\pm$ SD is given to represent the data, where $n=6$. *** and ### Statistical difference as compared with diseased group and control, respectively at $P < 0.001$. **Statistical difference as compared with diseased group at $P < 0.01$.

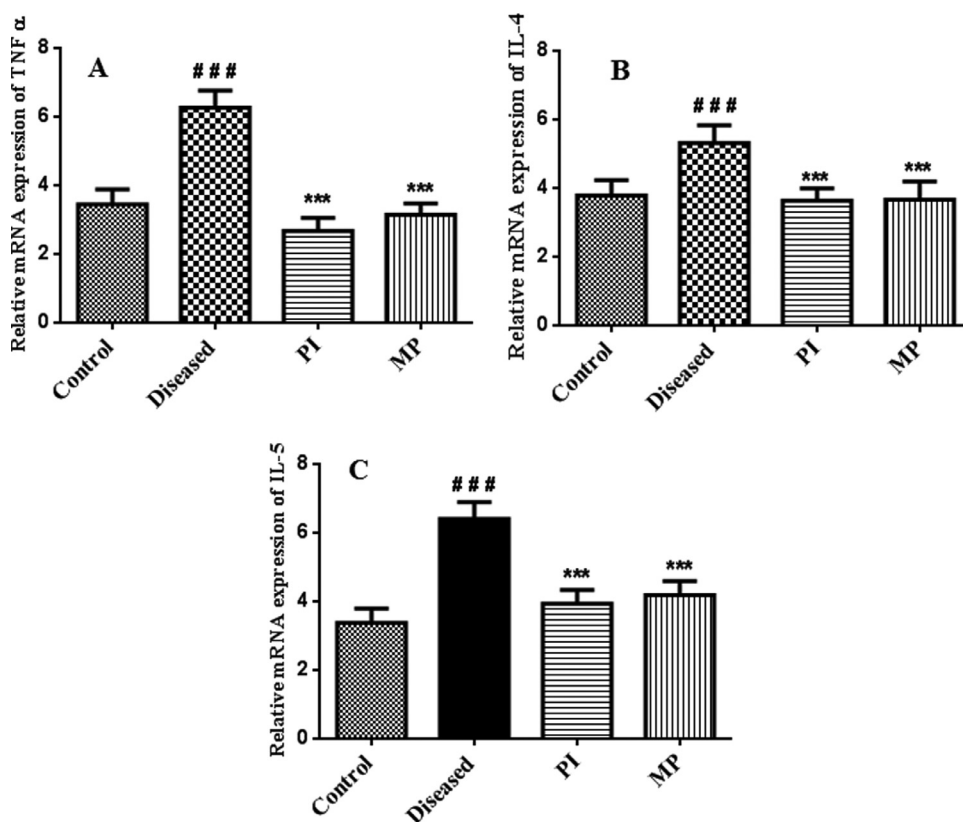


Fig. 3. Treatment with both PI extract and MP significantly suppressed mRNA expression levels of TNF- α (A), IL-4 (B), and IL-5 (C) as compared with diseased group. Mean $\pm$ SD is given to represent the data, where $n=6$. *** and ### Statistical difference as compared with diseased group and control, respectively at $P < 0.001$.

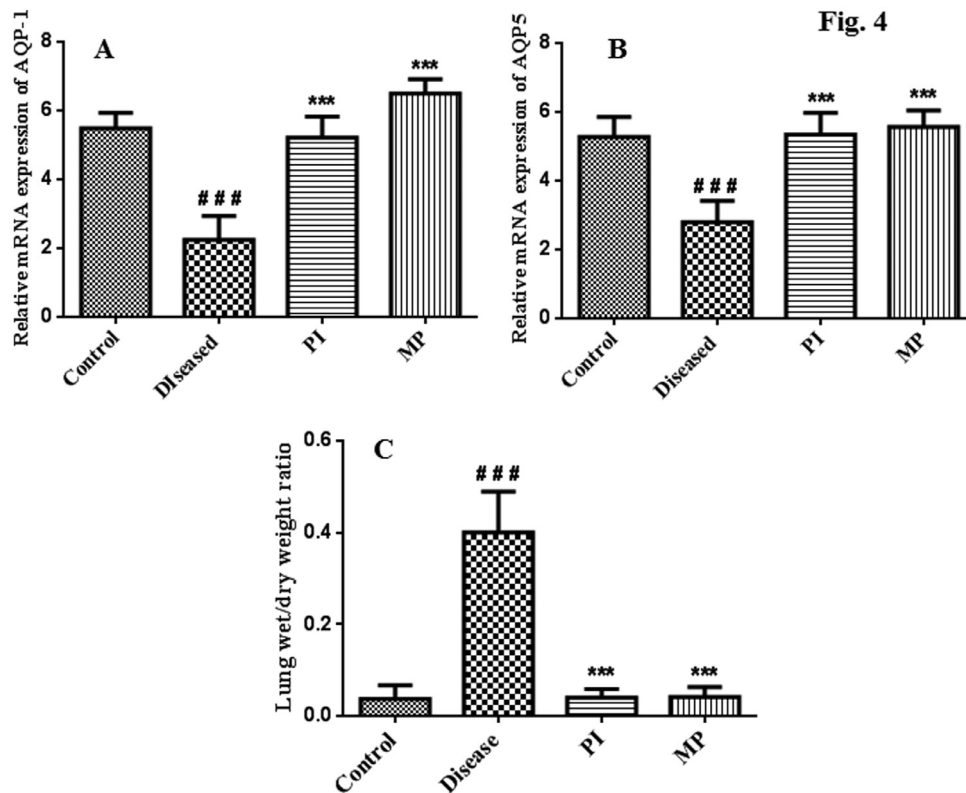


Fig. 4. Treatment with both PI extract and MP significantly elevated mRNA expression levels of AQP1 (A) and AQP5 (B) as compared with diseased group. Lung wet/dry ratio was also significantly reduced after treatment with both PI extract and MP (C). Mean $\pm$ SD is given to represent the data, where $n=6$. *** and ### Statistical difference as compared with diseased group and control, respectively at $P < 0.001$.

PI extract significantly elevated the expression levels of AQP1 and AQP5

AQP1 (2.24 ± 0.69) and AQP5 (2.79 ± 0.62) expression levels were found significantly reduced in diseased group as compared with control group (5.48 ± 0.4 and 5.26 ± 0.58 , respectively). Treatments with PI extract (5.21 ± 0.61 and 5.34 ± 0.63 , respectively) and MP (6.48 ± 0.42 and 5.56 ± 0.47 , respectively) significantly increased (all $P < 0.001$) the expression levels of AQP1 and AQP5 (Fig. 4A and 4B).

PI extract significantly reduced lungs wet/ dry weight ratio

Our results showed a significant increase ($P < 0.001$) in the lung wet/dry weight ratio of diseased group as compared with control group (0.400 ± 0.089 vs 0.036 ± 0.030). Treatment with PI extract (0.040 ± 0.018) and MP (0.041 ± 0.021) showed a significant decrease ($P < 0.001$) in the lung wet/dry weight ratio as compared with diseased group (Fig. 4C).

Discussion

Allergic asthma is characterized by temporary bronchoconstriction, elevated mucus production, and airway hyper-responsiveness. These changes result in the thickening of basement membrane, enhanced fibrosis, smooth muscle proliferation, and attenuation of total lung capacity (Walsh et al., 2010). In current study, histopathological evaluation revealed that goblet cell hyperplasia and inflammatory cell infiltration were significantly elevated in diseased group. Haematological evaluation also showed enhanced total leukocyte count and differential leukocyte counts in both blood and BALF. Treatment with PI and MP significantly ameliorated all the inflammatory markers. Previous studies demonstrated

that IL-4 has positive influence on goblet cell hyper-secretion and epithelial hyperplasia.

We found significantly increased TNF- α expression levels in diseased group as compared with control group. Asthma is considered as Th2 mediated disorder and is influenced chiefly by those cytokines which are associated with Th2 type profile, such as, IL-4, IL-5, and IL-13. However, various in vivo and in vitro studies have also implicated the role of TNF- α , a Th1 type cytokine, in asthmatic airway inflammation (Babu et al., 2004). TNF- α is abundantly found in asthmatic airways and is considered a pro-inflammatory cytokine (Renauld, 2001). Previously published data showed that TNF- α could facilitate the inflammatory cell immigration into the airways and up-regulate the adhesion molecules. It plays an important role of initiation of airway inflammation in allergic asthma, production of airway hyper-reactivity, and activation of pro-fibrotic mechanisms in the subepithelium. Recruitment of neutrophils and eosinophils in asthma is also considered under the influence of TNF- α (Thomas, 2001). In asthma, TNF- α blocking activity can be reflected as a possible therapeutic option in patients who are majorly dependent on corticosteroid therapy (Babu et al., 2004). The current study showed that treatment with PI extract significantly suppressed the expression levels of TNF- α as compared with positive control.

T cells can become polarized and restricted to generate Th2 type cytokines. Once the polarization of T cell population starts, it will generate the cytokines which are required to strengthen the polarization (Swain et al., 1995). IL-4 drives the polarization of T cells in favor of Th2 cells which further secrete IL-4, -5, -9, and -13 (Ashraf et al., 2015). The data showed significantly elevated IL-4 and IL-5 expression levels in diseased group as compared with control group. The increase in the levels of Th2 type cytokines

suggests the polarization of T cells in favor of Th2 cells which is a characteristic of allergic asthma.

IL-4 contributes in the pathogenesis of allergic asthma in various ways. It causes the hyper-secretion of mucus and stimulates the gene expression of mucin which contributes to airway obstruction. IL-4 plays essential role in IgE dependent mast cell activation, up-regulation of eotaxin expression, induction of vascular cell adhesion molecules on vascular endothelium, and migration of eosinophils, basophils, monocytes, and T lymphocytes to inflammatory loci. All these effects contribute to generation and promotion of immediate allergic response, inflammation, and lung remodelling. In addition, IL-4 prevents the apoptosis of T lymphocytes and eosinophils, which result in the fast proliferation and secretion of cytokines and promotion of eosinophilic inflammation, respectively (Moser et al., 1992; Doucet et al., 1998; Dabbagh et al., 1999; Hoontrakoon et al., 1999; Steinke and Borish, 2001). High levels of neutrophils have been found in patients of persistent or acute asthma. Neutrophils are also known to generate, store, and release IL-4. It was suggested that an autocrine stimulation of neutrophils is possible (Monteseirín, 2009). Our results demonstrated that treatment with PI extract significantly attenuated IL-4 expression levels as compared with diseased group.

Regulation of eosinophils, such as, induction, growth, differentiation, maturation, and survival is dependent on IL-5, which is majorly produced by T-lymphocytes. Eosinophils and basophils contribute to the high levels of cytokine release in asthma and also express IL-5 receptors on their surface. The clinical severity of asthma has also been correlated with mRNA expression of IL-5 in airways (Garcia et al., 2013). Interfering of eosinophilic function through inhibition of IL-5 is considered as an effective therapeutic approach in case of severe asthma. The data showed that PI extract significantly attenuated the increased eosinophil count which is in line with our finding of reduced expression levels of IL-5 after treatment with PI extract. Previously, we have shown that *Zingiber officinale* extract attenuated the expression levels IL-4 and IL-5 which caused the amelioration of allergic asthma (Khan et al., 2015). The attenuation of allergic airway inflammation by PI extract found in current study may also be, in part, attributed to the suppression of TNF- α , IL-4, and IL-5 expression levels.

We evaluated the effects of PI extract using lung wet/dry ratio, which is commonly used as an index of tissue edema (Shahzad et al., 2009). The results showed that both PI extract significantly attenuated the lung wet/dry ratio as compared with diseased group suggesting reduction in pulmonary edema after treatment with PI extract. We further evaluated the effects of PI extract on the expression levels of AQP1 and AQP5, both markers are known to be closely related with pulmonary edema (Dong et al., 2012).

We found significantly decreased expression levels of AQP5 in diseased group as compared with control group. Pulmonary edema and inflammation have been associated with a significant suppression of AQP5 and AQP1 expression levels in lungs (Li et al., 2011). AQP5 is highly expressed in acinar epithelial cell in large airway epithelia and submucosal glands, and in the type-1 pneumocytes in the distal lung (Shen et al., 2011). Previous studies have shown that compromised lung function and increase in mucus production in patient suffering from chronic obstructive pulmonary disease is correlated with suppression of AQP5 expression (Krane et al., 2009; Wang et al., 2007). Another study showed that mice deficient in AQP5 demonstrated a significant elevation in total lung resistance along with reduction in dynamic compliance, and also exhibited hyper-responsiveness to cholinergic stimulation (Krane et al., 2001). In current study, treatment with both PI extract and MP significantly elevated the expression levels of AQP5 as compared with diseased group. Our results are in line with the findings of Ben et al. (2008), who showed that treatment with dexamethasone

significantly increased the levels of AQP5. We also found that treatment with PI extract significantly elevated the AQP1 expression levels. AQP1 is largely found in mesothelial cells of parietal and visceral pleura, microvessels, and microvascular endothelium adjacent to alveoli and airways (Ben et al., 2008). Previously, Towne et al. (2000) demonstrated the role of AQP1 in the abnormal fluid fluxes during lung inflammation. AQP1 is also known to play important role in the movement of eosinophils (Lei et al., 2008). Anti-asthmatic agents are known to attenuate the pulmonary edema by increasing the expression levels of AQP1 and AQP5 (Dong et al., 2012). The findings of Dong et al. (2012) are in line with the results of our study, which suggests that increase in expression levels of AQP1 and AQP5 might have caused the attenuation of pulmonary edema.

As mRNA ultimately translated into protein, a correlation between the mRNA levels and that of protein levels might be assumed (Greenbaum et al., 2003). Some previous studies showed overall positive correlation between mRNA expression and protein levels and described that mRNA expression levels were useful in predicting protein levels; however, the predictions were not perfect (Guo et al., 2008). The results of Koussounadis et al. (2015) provided more optimism for the usefulness of inferences from mRNA expression in general. The possible effects of *P. integrerrima* extract on protein levels of TNF- α , IL-4, IL-5, AQP1, and AQP-5 require further studies.

Airway hyperresponsiveness is another characteristic of allergic asthma. Previously, Shirole et al. (2014) showed that *P. integrerrima* galls alleviated airway hyperresponsiveness in OVA-sensitized guinea pigs. *P. integrerrima* also exhibited anti-allergic activity by suppressing compound 48/80-induced mast cell degranulation. The results of Shirole et al. (2014) further complemented the inferences of this study.

Conclusion

Current study displays that *Pistacia integrerrima* possesses significant immunomodulatory and anti-inflammatory activities in mouse model of ovalbumin-induced allergic asthma, validating its traditional use in asthma and cough. The data displayed that PI extract significantly ameliorated airway inflammation, goblet cell hyperplasia, total leukocyte count, and differential leukocyte count, which might be attributed to the attenuation of TNF- α , IL-4, and IL-5 expression levels. Treatment with PI extract also significantly reduced pulmonary edema which might be attributed to the elevation of AQP1 and AQP5 expression levels. Further studies are required for the identification and isolation of active compounds responsible for anti-asthmatic activity.

Conflict of interest

The authors declare no conflict of interests

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