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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.

Introduction

Airway inflammation, mucus hypersecretion, infiltration of leukocyte, airway wall remodelling, and spasm of bronchial smooth 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; Schuijis, 2013).

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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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 secretary and absorptive functions (Krane 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 pallet 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 μg 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-μm 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