



Immunomodulatory and anti-inflammatory effects of *Aerva lanata* in ovalbumin induced allergic asthmatic mice

Mariam Sharif^a, Irfan Anjum^{a,*}, Arham Shabbir^{a,b}, Muhammad Naveed Mushtaq^a

^a Department of Pharmacology, Faculty of Pharmacy, The University of Lahore, Pakistan

^b Institute of Pharmacy, Lahore College for Women University, Lahore, Pakistan

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ABSTRACT

Ethnopharmacological relevance: *Aerva lanata* Linn. (*A. lanata*) is traditionally used for cough, sore throat and asthma.

Aim of study: The aim of the present study was to investigate the immunomodulatory and anti-inflammatory potentials of *A. lanata* in allergic asthmatic mice.

Materials and methods: BALB/c mice were administered with three different (methanol, n-hexane and ethyl acetate) extracts of *A. lanata* two weeks after immunization with ovalbumin and continued for 7 days. Inflammatory cells count was estimated in blood and broncho-alveolar lavage fluid (BALF). RT-PCR was used to find out mRNA expression levels of inflammatory mediators. GC-MS analysis was also carried out.

Results: Among three extracts of *A. lanata*, ethyl acetate extract ameliorated ($p < 0.001$) count of inflammatory cells both blood and BALF remarkably. This study indicated that ethyl acetate extract of *A. lanata* lowered ($p < 0.001$) the level of inflammatory modulator TNF- α and IgE antibodies. *A. lanata* reduced ($p < 0.001$) interleukin 4, 5, 13 and enhanced ($p < 0.001$) expression levels of AQP1 and AQP5 in asthmatic mice. GC-MS analysis of ethyl acetate fraction indicated the presence of various anti-oxidant phyto-constituents. The groups treated with *A. lanata* improved inflammatory, goblet cells hyperplasia scoring and alveolar thickening.

Conclusions: The anti-asthmatic effect of *A. lanata* might be contributed by the suppression of edema, pro-inflammatory cytokines and IgE antibodies, and elevation of aquaporin expression levels, suggesting future study and clinical trials to propose it as a candidate to treat allergic asthma. The anti-oxidant phytochemicals present in *A. lanata* might be responsible for such potential.

1. Introduction

Asthma is a common inflammatory lung disease, exhibited by obstruction of airways and bronchial hyper responsiveness (Bassett et al., 2010). Asthma may be triggered by animals (pet hair or dander), dust mites, non-steroidal anti-inflammatory drugs, cold weather, exercise, mold, pollen, respiratory infections and tobacco smoke. Glucocorticoids, effectively used to treat airway inflammation, are considered as the first-line therapy for asthma (Abbas et al., 2005). However, glucocorticoids are not particular in their mode of actions and their use also lead to a number of side effects including reduce cortical secretions, decrease bone metabolism, and slow down development in children (Dong et al., 2012).

Allergic asthma is a prolonged lungs inflammation due to infiltration of eosinophils, neutrophils and leukocytes into the broncho-alveolar fluid

(BALF). As, inflammation is regarded the initial and lasting characteristic of asthma, treatment of asthma focuses on long-term inflammation management (Bochner and Busse, 2005; Yang et al., 2010). It has been found that infiltrating eosinophils in the lungs induce T-helper 2 (Th2) type immune reactions primarily by exposing antigens (Kool et al., 2011). Th2-type cytokines including interleukin (IL)-4, IL-5, and IL-13 perform a central role in the pathogenesis of asthma (Deo et al., 2010). Th2-type cytokines and tumor necrosis factor (TNF- α) are being produced by inflammatory cells, and their quantities are raised in asthmatic lungs (Yang et al., 2010).

Nowadays alternative therapies are being greatly accepted both by patients and physicians. Plants have been used as an important tool of therapeutic policy in old ages. The herbs have a variety of phytochemicals which show their biological activities. Herbal medicines can be preferred over conventional medical treatments due to their reduce

* Corresponding author. Department of Pharmacology, Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan.

E-mail address: anjum95@yahoo.com (I. Anjum).

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chances of adverse effects, increase efficacy, economically cheaper and easily available (Braman, 2006).

Aerva lanata (Linn.) is a medicinal plant bequeathed by nature. It is an erect, prostrate under shrub belonging to family, Amaranthaceae. Traditionally, *Aerva lanata* (*A. lanata*) is regarded as a valuable medicine for cough, sore throat and asthma (Gunatilake et al., 2012; Goyal et al., 2011). *A. lanata* has been documented for its antiurolithiatic, antimicrobial, cytotoxic, antidiabetic, nephroprotective, hepatoprotective, immunomodulatory, diuretic and anti-inflammatory activities (Goyal et al., 2011). The phytochemicals present in *A. lanata* include flavonoids, canthin-6-one and β -carboline alkaloids, steroids, terpenoids and phenolic acids (Paplińska-Goryca et al., 2016).

This study aimed to estimate the immunomodulatory and anti-inflammatory activities of *A. lanata* in allergic asthma.

2. Materials and Methods

2.1. Plant collection

The collection of whole plant was carried out from Mansehra, Khyber Pakhtunkhwa, Pakistan. The plant was identified by Department of Botany, Govt College University, Lahore (GC-Herb-Bot-3712).

2.2. Preparation of plant extracts

The whole plant dried under shade after washing and grinded to powder. The methanol extract of plant was prepared by cold maceration. The % yield was estimated (5.1%). The different fractions were prepared by using this methanol extract as described previously (Sharif et al., 2021).

2.3. Animals used

6–8 weeks old mice (BALB/c) of either sex (25–35 g) were used and kept in the animal house prior to the start of study for a duration of seven days in order to adjust them with normal condition of humidity (40–60%) and controlled temperature (24–26°C). 12 hr. dark/light cycles were maintained along with standard diet and tap water (Shabbir et al., 2014). The study was approved by the Institutional Research Ethics Committee Faculty of Pharmacy, The University of Lahore, Lahore (IREC#2017-39).

2.4. Drugs and chemicals used

For the present study, following drugs and chemicals were used;

1. Solu-Medrol 500 mg/7.8 ml injection (reference drug) manufactured by Pfizer Belgium
2. Methanol (Sigma Aldrich)
3. n-hexane (Sigma Aldrich)
4. Ethyl acetate (Sigma Aldrich)
5. Chloroform (Sigma Aldrich)
6. Isopropanol (Sigma Aldrich)
7. RNase free water (Sigma Aldrich)
8. Agarose (Sigma Aldrich)
9. TAE reaction buffer (50X) (Tris base, acetic acid and EDTA) (Sigma Aldrich)
10. Ethidium bromide (Sigma Aldrich)

2.5. Study design

Six groups of 36 mice were used ($n = 6$). The mice were sensitized intraperitoneal on days 0 and fourteen by ovalbumin (20 μ g) dissolved in an adjuvant $Al_2(SO_4)_3$ except control group. After 14 days, mice were exposed intranasal challenge of ovalbumin once a day for next week (days 15–21). Mice of control group were treated with phosphate buffer

solution (PBS) only (Yang et al., 2010; Kianmenher et al., 2016). The grouping and dosing schedules were same as described previously. The asthmatic mice were treated with standard drug (Methylprednisolone) (i.p. 15 mg/kg) (Sharif et al., 2021) and methanol extract of *A. lanata* (MEOAL), n-hexane extract of *A. lanata* (NHEOAL) and ethyl acetate extract of *A. lanata* (EAOAL) (800 mg/kg/day) orally from day 15 to day 21 (Sharma et al., 2011).

2.6. Blood and BALF inflammatory cells count

Dissection of mice was carried out under ether anesthesia at day 22 and blood samples were withdrawn in EDTA vacutainer. BALF was collected in sterile Eppendorf tubes by slow distillation with 0.5 ml PBS through bronchial route. DLC in BALF was measured by cyto-centrifugation on glass slide that was eventually fixed with methanol and Wright Giemsa staining to count on distinctive cell arrangement basis. TLC in blood and BALF was calculated by automated hemocytometer (Sysmax XT-1800i).

2.7. Lung wet/dry weight ratio

The lung wet/dry weight ratio was determined as previously performed (Sharif et al., 2021).

2.8. mRNA expression levels of pro-inflammatory cytokines

The mRNA expression levels of pro-inflammatory cytokines (IL-4, IL-5, IL-13, TNF- α , NF- κ B) and aquaporins (AQP1 and AQP5) in lung tissue were determined by Rt-PCR.

2.9. RNA isolation and RT-PCR

TRIzol method was used to isolate total RNA from the lung tissue. cDNA was synthesized and reverse transcription was performed as stated by Kit Thermo Fischer Scientific' protocols. For visualizing the PCR findings, agarose (2%) gel electrophoresis was performed as described previously. The results were semi-quantified by densitometry using ImageJ software (Khan et al., 2015).

2.10. ELISA

IgE level was measured by ELISA according to ELISA Kit protocols of Elab Science (SG-30383) as described previously (Sharif et al., 2021).

2.11. GC-MS analysis

GC-MS analysis was performed as described previously (Sharif et al., 2021).

2.12. Histopathological studies

The isolated lungs were fixed with formalin (10%). The tissues were embedded in paraffin wax and microtome was used to prepare 6 μ m thick sections. The Hematoxylin and Eosin (H&E) was used to stain tissues. To identify goblet cells, Periodic Acid Schiff (PAS) staining was performed (Quan et al., 2010). The data were semi-quantified using the histopathological scoring system, with 0, 1, 2, 3, and 4 representing none, minimum, mild, moderate, and severe changes, respectively (Khan et al., 2015). A blind independent histopathologic assessed the extent of goblet cell dysplasia and inflammatory cell infiltration.

2.13. Statistical analysis

The data were expressed as mean $\pm$ SEM. Blood and BALF inflammatory cell counts, lung histopathology, mucus cell hyperplasia, lung wet/dry ratio, serum IgE levels and mRNA expression levels of primers

were analyzed by One-way analysis of variance (ANOVA) followed by post hoc Tukey's test among groups. The value of $p \leq 0.05$ was accepted as statistically significant.

3. Results

3.1. TLC in blood and BALF

TLC was increased significantly ($p < 0.001$) in blood and BALF of asthmatic mice (12.633 ± 0.123 , 44.5 ± 0.763 , respectively). Pretreatment with different (methanol, n-hexane and ethyl acetate) extracts of *A. lanata* decreased ($p < 0.001$) TLC in blood (5.385 ± 0.078 , 4.733 ± 0.088 and 4.667 ± 0.133) and BALF (14.5 ± 0.763 , 12.5 ± 0.763 and 8.16 ± 0.477) as compared with mice treated with OVA only. Treatment with methylprednisolone also decreased TLC in blood (4.732 ± 0.132) and BALF (10.5 ± 0.763) as compared with diseased group Fig. 1 (A, B).

3.2. Neutrophil count

Neutrophil counts in blood and BALF were elevated ($p < 0.001$) in mice treated with OVA (1.608 ± 0.046 and 108.5 ± 5.213 , respectively) as compared with normal control group (0.321 ± 0.017 , 21.66 ± 2.472). Treatment with the methanol, n-hexane and ethyl acetate extracts of *A. lanata* remarkably reduced ($p < 0.001$) neutrophil count in blood (0.373 ± 0.021 , 0.382 ± 0.029 and 0.313 ± 0.012) and BALF (33.83 ± 5.861 , 40.83 ± 1.851 and 36.66 ± 5.981 respectively) as compared with group treated with OVA only Fig. 1(A and B).

3.3. Eosinophil count

In this study, it was found that eosinophil counts in blood and BALF were ameliorated in diseased group (5.64 ± 0.07 , 6.483 ± 0.135 , respectively) as compared with normal control group (3.20 ± 0.04 , 1.716 ± 0.140). Treatment with all three extracts (methanol, n-hexane and ethyl acetate) of *A. lanata* significantly restored ($p < 0.001$) (2.60 ± 0.094 , 2.62 ± 0.165 and 2.40 ± 0.139) eosinophil counts in blood and BALF (2.517 ± 0.174 , 2.683 ± 0.149 and 2.417 ± 0.083) as compared with diseased group Fig. 1 (A, B).

3.4. Lungs wet/dry ratio

There was a significant raise ($p < 0.001$) in lung wet/dry weight ratio of diseased group (0.627 ± 0.017) as compared with control group (0.306 ± 0.013). Pretreatment with different extracts of *A. lanata* (methanol, n-hexane and ethyl acetate) showed a significant decrease ($p < 0.001$) (0.313 ± 0.011 , 0.260 ± 0.014 and 0.215 ± 0.011) in lung wet/dry weight ratio in oval albumin induced asthmatic mice Fig. 1 (C).

3.5. Effect of *A. lanata* on serum IgE level

A significant rise ($p < 0.001$) in IgE levels in mice exposed to ovalbumin (287.666 ± 2.962) as compared with control (122.333 ± 2.375) was observed. After administration of different extracts of *A. lanata* (methanol, n-hexane and ethyl acetate), the serum level of IgE ($p < 0.001$) were restored (173.333 ± 2.928 , 169.5 ± 6.360 , 140 ± 3.596) as compared with diseased group Fig. 1 (D).

3.6. Effect of *A. lanata* on IL-4, IL-5 and IL-13 levels

The level of IL-4 had been ameliorated (1.430 ± 0.020) in diseased group as compared with normal control group (1 ± 0.030). Pretreatment with ethyl acetate extract of *A. lanata* ($p < 0.001$) remarkably restored (1.15 ± 0.036 , 1.23 ± 0.064 and 1.12 ± 0.094 respectively) IL-4 level in asthmatic mice Fig. 2 (A).

Similarly, the mRNA expression levels of IL-5 and IL-13 were found significantly elevated (1.736 ± 0.055 , 1.357 ± 0.062) in diseased group as compared with normal control group (1.000 ± 0.082 and 1.000 ± 0.054 , respectively). Pretreatment with different extracts (methanol, n-hexane ($p < 0.05$) and ethyl acetate extract ($p < 0.001$) of *A. lanata* reduced IL-5 (0.996 ± 0.0425 , 0.976 ± 0.049 and 0.647 ± 0.017) and IL-13 levels (1.022 ± 0.054 , 1.055 ± 0.035 and 0.993 ± 0.065) and in allergic asthmatic mice Fig. 2(A).

3.7. Effect of *A. lanata* on TNF- α level

The expression of mRNA of TNF- α was checked by gel electrophoresis. The level of TNF- α was raised significantly (1.773 ± 0.031 ; $p < 0.001$) in diseased group as compared with normal control group (0.977 ± 0.032). Pretreatment with the methanol, n-hexane ($p < 0.05$) and ethyl acetate ($p < 0.001$) extracts of *A. lanata* normalized (1.530 ± 0.026 , 1.435 ± 0.047 and 1.189 ± 0.034) the level of TNF- α in oval

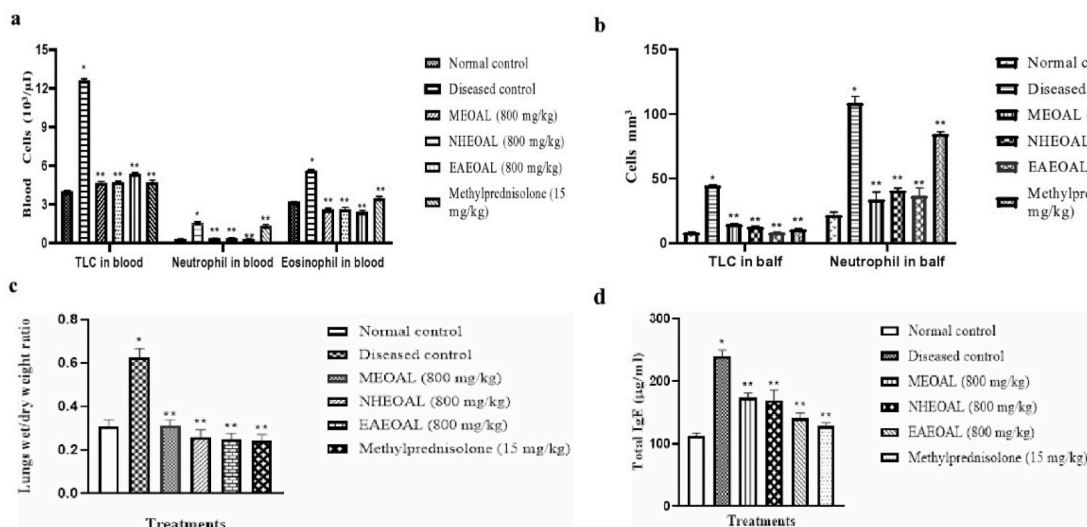


Fig. 1. Data have been presented as mean $\pm$ SEM. Effects of different extracts of *A. lanata* on TLC, neutrophils and eosinophils count in blood (A) BALF (B), lung wet/dry ratio (C), and level of total IgE (D) (* $p < 0.001$; compared to normal control group, ** $p < 0.001$ compared to diseased group $n = 6$, MEOAL; methanol extract of *A. lanata*, NHEOAL; n-hexane extract of *A. lanata*, EAOAL; ethyl acetate extract of *A. lanata*).

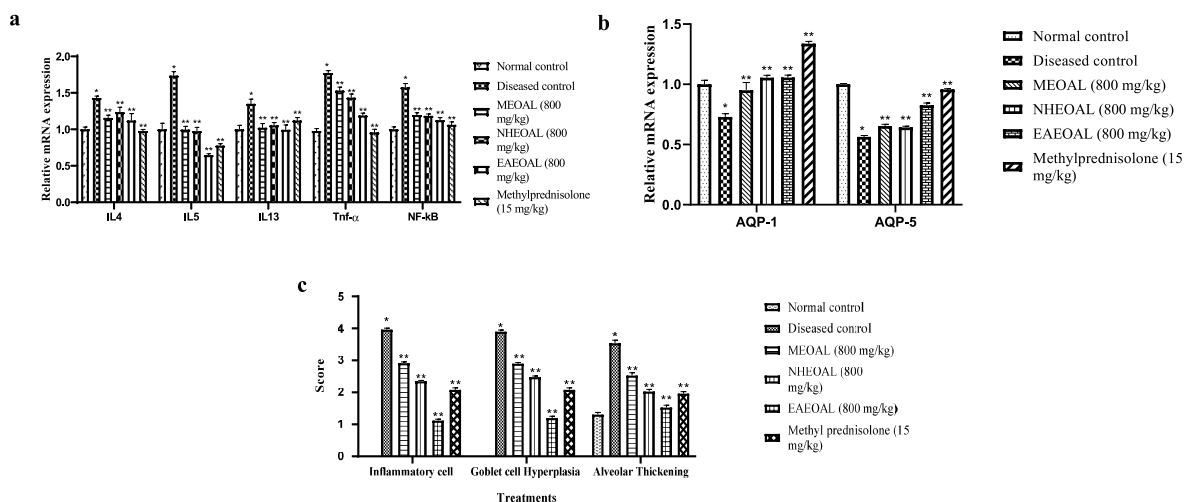


Fig. 2. Data have been presented as mean $\pm$ SEM. Effect of *A. lanata* on mRNA expression levels of IL-4, IL-5, IL-13, TNF- α , NF- κ B (A), AQP-1 and AQP-5 (B) and inflammatory cells and alveolar thickening amelioration by *A. lanata* (PAS Studies) (C). (* $p < 0.001$; compared to normal control group, ** $p < 0.001$ compared to diseased group; $n = 6$) (MEOAL; methanol extract of *A. lanata*, NHEOAL; n-hexane extract of *A. lanata*, EAEOL; ethyl acetate extract of *A. lanata*).

albumin induced allergic asthmatic mice Fig. 2 (A).

3.8. Effect of *A. lanata* on NF- κ B

The mRNA expression of inflammatory mediator NF- κ B was examined by gel electrophoresis. The level of NF- κ B had been raised (1.5 ± 0.05) in diseased group as compared with normal control (1.0 ± 0.03). Pre-treatment with different extracts of *A. lanata* (methanol, n-hexane and ethyl acetate) of *A. lanata* particularly ethyl acetate extract ($p < 0.05$) and ethyl acetate ($p < 0.001$) (1.198 ± 0.029 , 1.184 ± 0.027 and 1.126 ± 0.035) and methylprednisolone (1.0 ± 0.03) restored ($p < 0.001$) the level of NF- κ B in oval albumin induced allergic asthmatic mice Fig. 2 (A).

3.9. Effect of *A. lanata* on AQP1 and AQP5 levels

The mRNA expressions for AQP1 and AQP5 were found significantly reduced ($p < 0.001$) (0.774 ± 0.046 , 0.563 ± 0.0125) in diseased group as compared with normal control group (0.999 ± 0.032 and 1.000 ± 0.0076 , respectively). Pretreatment with all three extracts (methanol, n-hexane and ethyl acetate) of *A. lanata* particularly ethyl acetate extract ($p < 0.001$) restored (1.237 ± 0.029 , 1.163 ± 0.058 and 1.055 ± 0.019) AQP1 and AQP5 levels (0.653 ± 0.015 , 0.6416 ± 0.010 and 0.826 ± 0.018) in asthmatic mice Fig. 2 (B).

3.10. Effect of *A. lanata* on inflammatory cells infiltration and alveolar thickening score

Histopathological studies presented a high inflammatory cell infiltration and increased alveolar thickening in diseased group (3.967 ± 0.042 , 1.31 ± 0.061 ; respectively) as compared with control group. Treatment with different extracts (methanol, n-hexane; $p < 0.005$ and ethyl acetate; $p < 0.001$ of *A. lanata* (2.917 ± 0.040 , 2.35 ± 0.022 and 1.117 ± 0.048) significantly reduced the inflammatory cell infiltration and alveolar thickening (2.53 ± 0.08 , 2.03 ± 0.06 ; $p < 0.005$ and 1.53 ± 0.06 ; $p < 0.001$) as compared with the disease group Fig. 2 (C).

3.11. Effect of *A. lanata* on goblet cells hyperplasia score

A significant increase ($p < 0.001$) in goblet cell hyperplasia was found in diseased group (3.9 ± 0.052) as compared with control group (0). Treatment with different extracts (methanol, n-hexane; $p < 0.05$ and

ethyl acetate; $p < 0.001$) of *A. lanata* (2.9 ± 0.037 , 2.483 ± 0.031 ; $p < 0.05$; and 1.200 ± 0.058 ; $p < 0.001$) showed significant alleviation in goblet cell hyperplasia as compared with the diseased group Fig. 2(C).

3.12. Histopathological studies

The isolated lungs were stained with both H and E and PAS to observe morphological changes as shown in Figs. 3 and 4 respectively. Lungs from control group represented normal intact bronchioles with no inflammation while in diseased control group goblet cell hyperplasia in bronchiole (arrow), smooth muscle hypertrophy (asterix) and area of inflammation (oblong circles) was found. Pretreatment with MEOAL, NHEOAL, EAEOL and standard drug (methylprednisolone) depicted bronchioles with no/minimal goblet cell hyperplasia (asterix) and the alveoli in the intervening areas with minimal inflammation. A very thin peri-bronchiolar smooth muscle layer was appreciated. There were adjacent thin-walled blood vessels as well. PAS staining revealed bronchioles with few scattered goblet cells highlighted by PAS imparting magenta color to mucin goblet cells. However, ovalbumin treatment presented numerous goblet cells while rats treated with MEOAL, NHEOAL, EAEOL and methylprednisolone showed lungs with few scattered goblet cells highlighted by magenta staining of mucin in goblet cells (three bronchioles shown). All these observations supported the biochemical results of the present study.

3.13. GC-MS analysis of ethyl acetate extract of *A. lanata*

GC-MS analysis of ethyl acetate extract of *A. lanata* showed 15 different phyto-compounds including octadecadienoic acid, oxaandrosta-1,5-dien-3-one, 17-(acetyloxy)-(17- β), Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol, vitamin E, hexadecanoic acid, methyl ester, stigmasterol and phenol, 2-methyl-5-(1-methylethyl) accountable for anti-inflammatory and anti-asthmatic activities of *A. lanata* are shown in Fig. 5 (Table 1).

4. Discussion

Asthma is an inflammatory disorder of lungs comprised of mucus secretion, bronchial hyperactivity, spasm of bronchioles and edema. It leads to periodic occurrence of shortness of breathing, wheezing, chest tightness and coughing (Puthalapattu and Ioachimescu, 2014). The

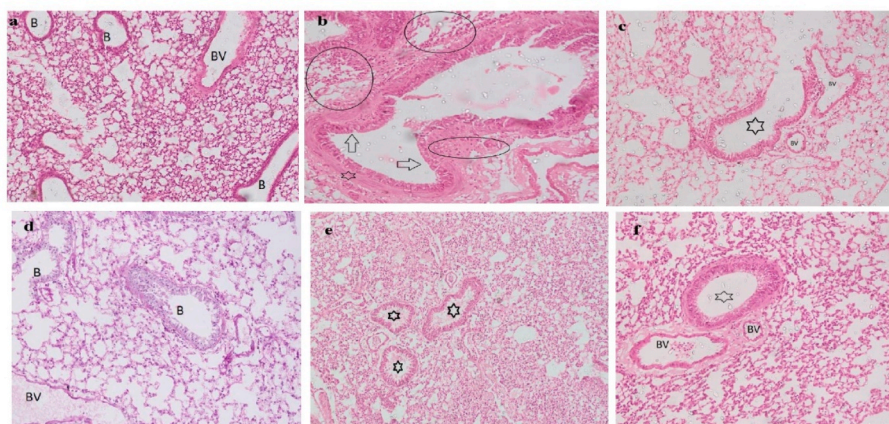


Fig. 3. Histopathological studies (H and E staining) (10X) of isolated lungs from Normal Control (a), Diseased Control (b), Methanol (c), n-hexane (d) and ethyl acetate (e) extracts treated groups of mice.

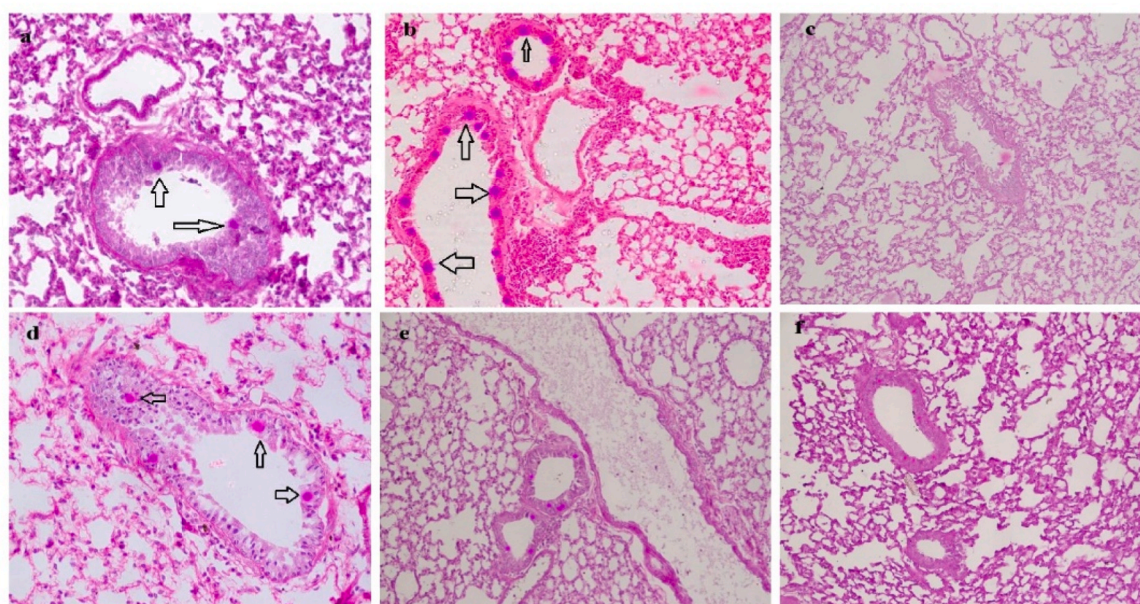


Fig. 4. Histopathological studies (PAS staining) (10X) of isolated lungs from Normal Control (a), Diseased Control (b), Methanol (c), n-hexane (d) and ethyl acetate (e) extracts treated groups of mice.

cause of asthma is not exactly known but various risk factors have been reported. Epigenetic variations, respiratory infections and airborne environmental exposures (tobacco smoke, pollutants, ozone) may be sources of asthma (Esposito et al., 2014). Asthma affects both children and adults. It may be incurable and approximately 250, 000 deaths occur due to asthma worldwide per year (Pawankar et al., 2012).

Medications used to treat asthma include corticosteroids, anti-leukotrienes, and monoclonal immunoglobulin inhibitors. Due to the high price, unavailability, unaffordability of drugs and development of a number of side effects from these therapies, it becomes mandatory that alternative sources of anti-asthma drugs should be scientifically characterized for cheaper and/or in combination with already in use anti-asthma drugs (Namsa et al., 2009). Hence, this study was aimed to validate traditional use of *A. lanata* in asthma and respiratory disorders. The anti-asthmatic potential of this plant has been determined in ovalbumin induced asthmatic model. BALB/c mouse effectively treated with ovalbumin provides a conventional animal model that is broadly utilized for researching different causes and therapeutic treatments of allergic asthma (Shahid et al., 2019).

Although glucocorticoids are the most essential anti-inflammatory medication for asthma, these are accompanied with a number of adverse effects and challenges (Abbas et al., 2005). The anti-inflammatory properties of *A. lanata* are equivalent to those of the glucocorticosteroid, methylprednisolone. In diseased group, total inflammatory cells (TLC), as well as eosinophils and neutrophils increased in both blood and BALF, but this was decreased after administration of *A. lanata* extracts. This is in line with recent findings, which showed an increase in inflammatory cell infiltration in the airways, collagen deposition, and goblet cell hyperplasia after ovalbumin exposure (Bousquet et al., 2000; Khan et al., 2015).

It has been well known that IL-3, IL-4, IL-5 and IL-13 participate actively in asthma associated inflammation. Interleukins 4, 5 and 13 are produced by Th2 cells which are associated with promotion of antibody, eosinophilic responses and provide phagocyte-independent protective mechanisms (Palmqvist et al., 2007). IL-13 is responsible for tracheal smooth muscle contraction, uncontrolled mucus production and accumulation of extracellular matrix proteins (Rael and Lockey, 2011). The animal studies have reported the role of IL-5 in airway smooth muscle

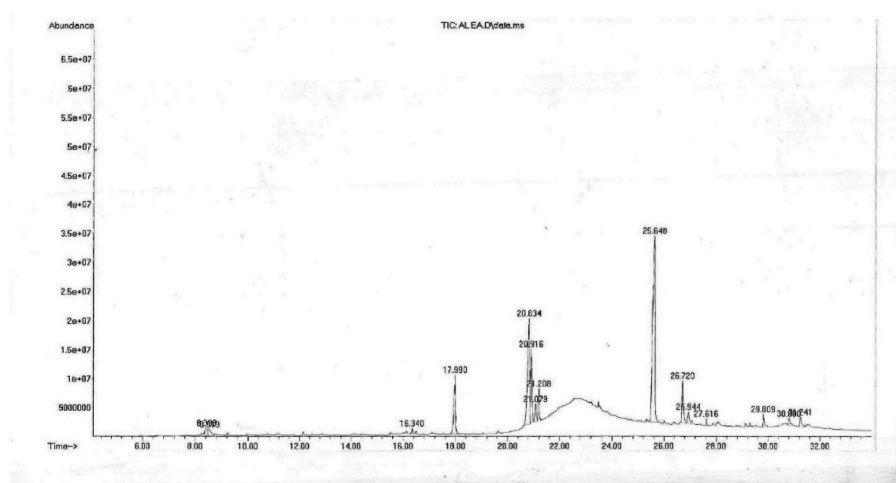


Fig. 5. GC-MS analysis of ethyl acetate extract of *A. lanata*.

Table 1

Phytocomponents identified in ethyl acetate extract of *A. lanata* by GC-MS.

Peak #	Retention Time (mins)	Area %	Molecular wt. (g/mol)	Molecular Formula	Phytocomponents
1	8.398	3.75	150	C ₁₀ H ₁₄ O	Phenol, 2-methyl-5-(1-methylethyl)-
2	8.513	3.60	150	C ₁₀ H ₁₄ O	Phenol, 2-methyl-5-(1-methylethyl)-
3	16.340	1.55	296	C ₂₀ H ₄₀ O	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4	17.990	21.99	270	C ₁₇ H ₃₄ O ₂	Hexadecanoic acid, methyl ester
5	20.834	53.23	294	C ₁₉ H ₃₄ O ₂	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
6	20.916	21.37	296	C ₁₉ H ₃₆ O ₂	9-Octadecenoic acid (Z)-, methyl ester
7	21.079	5.23	282	C ₁₈ H ₃₄ O ₂	Oleic Acid
8	21.208	7.17	298	C ₁₉ H ₃₈ O ₂	Octadecanoic acid, methyl ester
9	25.648	100.00	278	C ₁₆ H ₂₂ O ₄	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl)ester
10	26.720	13.16	328	C ₂₂ H ₃₂ O ₂	Pregn-4-ene-3,20-dione,6-methyl-, (6.alpha.)-
11	26.944	4.45	330	C ₂₀ H ₂₆ O ₄	4-Oxaandrost-1,5-dien-3-one, 17-(acetyloxy)-, (17.beta.)-
12	27.616	1.45	428	C ₃₀ H ₅₂ O	2,2,4-Trimethyl-3-(3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)-cyclohexanol
13	29.809	3.54	430	C ₂₉ H ₅₀ O ₂	Vitamin E
14	30.800	3.18	412	C ₂₉ H ₄₈ O	Stigmasterol
15	31.241	3.79	412	C ₂₉ H ₄₈ O	Stigmasterol

contraction and lungs remodeling suppression in allergic asthmatic mice (Cho et al., 2004). These outcomes supported our results which suggested that ethyl acetate extract of *A. lanata* ameliorated allergic asthma by attenuation of IL-4, 5 and 13 levels in asthmatic mice.

TNF- α , a pro-inflammatory cytokine, has been shown one of the main factor in asthma pathophysiology. Currently, anti-TNF- α therapy is

being preferred for treating asthma with high potential improvements in its signs and symptoms. The instigation of airway inflammation in allergic asthma is activated by TNF- α along with the increased hyper-reactivity of airways and stimulation of pro-fibrotic mechanisms in epithelium. It is also involved in the recruitment of neutrophils and eosinophils during asthma (Rana et al., 2016). Enhanced TNF- α has been observed in diseased control group that had been restored to normal with the ethyl acetate extract of *A. lanata* in our study.

Innate immunity responds to physical or physiological stress with inflammation of airways, which is linked to the activation of the NF- κ B. In the airway epithelium of humans and animals suffering from asthma, abnormal activation of NF- κ B has been found (Hoesel and Schmid, 2013). In comparison to the diseased group, the ethyl acetate extract of *A. lanata* significantly reduced the activation of NF- κ B triggered by OVA. Previous research has shown that active NF- κ B signalling can cause the production of pro-inflammatory cytokines, causing leukocytes to flock to the inflammatory areas (Kumar et al., 2004).

The down regulation of AQP5 and AQP1 levels are associated with pulmonary edema and inflammation in lungs. AQP1 plays a vital role in abnormal fluid and eosinophils movement in lung inflammation. Generally, anti-asthmatic drugs reduce the bronchial edema by increasing the AQP1 and AQP5 levels. The enhanced AQP1 and AQP5 levels in ovalbumin treated mice leading to development of pulmonary edema in this study correlated the previous findings. It has been reported earlier that suppression of AQP5 lead to changes in lung functions and increased mucus production (Ijaz et al., 2021). In our study, treatment with ethyl acetate extract of *A. lanata* and methylprednisolone restored AQP5 level as compared with diseased group.

A fall in lung wet/dry ratio was found by pre-treatment with *A. lanata* extracts indicated reduced lungs edema and obstruction. The inflamed lung tissue edema was estimated by lung wet/dry ratio (Sharif et al., 2021).

The IgE antibody is thought to be a defining feature of allergic asthma. It begins a series of chemical reactions after binding to basophils and mast cells, resulting in an increase in pro-inflammatory mediators responsible for allergic signs and symptoms (Sharif et al., 2021). In the current study, the diseased group had a higher level of IgE leading to an increase in allergic mediators. The IgE level was significantly lowered after treatment with ethyl acetate extract of *A. lanata*, which relieved clinical manifestations of asthma. These findings are consistent with those of a previous study (Khan et al., 2015). The GC-MS analysis of ethyl acetate extract of *A. lanata* determined the existence of previously reported anti-inflammatory phytochemicals including octadecadienoic acid, benzenedicarboxylic acid and linoleic acid ethyl ester, stigma-sterol and vitamin E which might be important for the

anti-asthmatic and immunomodulatory activity of *A. lanata* (Thangavel et al., 2014). It has been reported previously that antioxidant therapies including vitamins relieve asthma by decreasing oxidative stress (Arulselvan et al., 2016).

In present study, there was an increase in inflammatory cell score, alveolar thickening and goblet cell hyperplasia in diseased group and treatment with ethyl acetate extract of *A. lanata* significantly reduced all observed changes. Previous studies showed that epithelial hyperplasia and goblet cell hyper secretion were dependent on IL-4 (Palmqvist et al., 2007).

5. Conclusion

The present study supported the traditional use of *A. lanata* in asthma. The anti-asthmatic effect of *A. lanata* might be contributed by the suppression of edema, pro-inflammatory cytokines, IgE and elevation of aquaporin expression levels, proposing future study and clinical trials to establish its candidature in the treatment of allergic asthma. The anti-oxidants phytochemicals in the extracts of *A. lanata* might be responsible for its potential.

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Data availability

All data used during the present study are available from Dr. Irfan Anjum on request.

CRediT authorship contribution statement

Mariam Sharif: data collection, writing initial draft, Writing – original draft. **Irfan Anjum:** data interpretation, final draft, Supervision, Writing – review & editing, critical review of draft. **Arham Shabbir:** Methodology, Validation. **Muhammad Naveed Mushtaq:** Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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