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Therapeutic potential of *Abelmoschus esculentus* seed extract in asthmatic mice: Immunological analysis and histopathological evidence

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Abstract: **Background**: Seeds from *Abelmoschus esculentus* have strong anti-inflammatory properties and may effectively treat inflammatory diseases. **Objective**: This study explores the therapeutic potential of *A. esculentus* seed extract on bioactive components, hematological parameters, liver function tests, leukocytes, cytokines and histology in asthmatic mice. **Methods**: Mice were divided into six groups: The normal group, groups treated with *A. esculentus* (10, 15 and 20 mg/mL), a dexamethasone group (3 mg/mL) and an asthmatic control group. On days 1 and 14 after acclimation, all groups except the normal control received intraperitoneal injections of ovalbumin, followed by oral doses of *A. esculentus* (10, 15 and 20 mg/mL) or dexamethasone (3 mg/mL) during 15th to 26th day of the experiment. All treated groups were exposed to ovalbumin inhalation for three days afterward. **Results**: The bioactive components in the acetonic extract were identified through Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The primary bioactive compounds identified were fatty acids, flavonoids and phenolics. Mice given the extract showed improved liver enzyme levels, reduced leukocyte infiltration, alleviated airway constriction, lower cytokine levels (IgE, IL-4, IL-5, IL-10, IL-13 and IL-17A) and normalized blood indices. **Conclusion**: In conclusion, the seed extract of *A. esculentus* has significant potential to reduce asthma symptoms in the asthmatic mouse model.

Keywords: *Abelmoschus esculentus*; Histology; Interleukins; Mice; Ovalbumin

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INTRODUCTION

Chronic airway inflammation, epithelial barrier dysfunction, subepithelial fibrosis and airway smooth muscle hypertrophy are the hallmarks of asthma, a complex, immunologically diverse respiratory disease that ultimately results in reversible tracheobronchial blockage. These pathological characteristics are responsible for the observed variation in clinical severity and response to treatment, as well as driving gradual airway remodeling. (Harker and Lloyd, 2023). The figure highlights the major pathological alterations involved in the pathophysiology of asthma by showing the anatomical differences between a normal and an asthmatic airway (Sinyor and Perez, 2023) (Fig. 1). The complications related to asthma pathogenesis are strongly influenced by the alterations in the natural balance of T helper 1 (Th1) and Th2 levels in the lung airways (Lee *et al*., 2024). Th2 interleukins i.e. IL-13, IL-5. IL-4 and Th1-mediated interleukin i.e. IL-10 work antagonistically in the progression and suppression of allergic symptoms, respectively (Shankar *et al*., 2022). Of these, IL-4 is primarily responsible for allergic reactions in asthmatic patients, while IL-10 functions as a basic anti-inflammatory cytokine that prevents the inflammatory cytokine (IL-5 and IL-4) secretion by the Th1 immune cells (Hammad and Lambrecht, 2021).

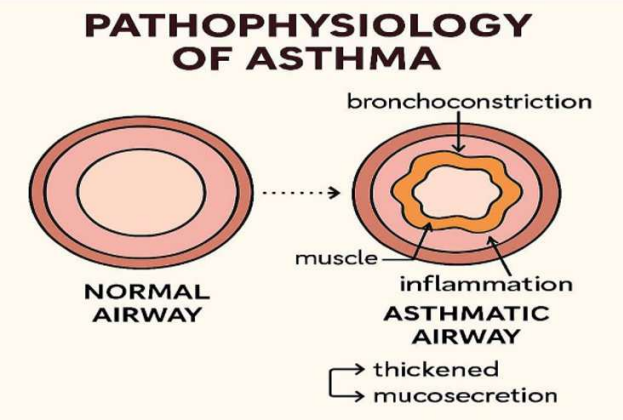

Fig. 1: Schematic representation of airway remodeling in asthma

Administration of oval-albumin on erythropoiesis-related tissues can result in erythrocyte damage or a decline in the RBC parameters. Anemia hypochromic disclosure is the result of unpaired erythrocyte formation or a reduction in hemoglobin or red blood cell counts. The elevated cytokine production that promotes inflammation, which results from OVA-induced reductions in hemoglobin and red blood cells, can lead to adverse effects on the liver and lungs (Hedayanti, 2023). Reduced concentrations of lung and liver toxicants correlate with the rise in white blood cells brought on by the hematological process of suppression (Lingas, 2023).

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Many drugs commonly used to treat asthma-related health issues can cause certain harmful side effects, such as delays in adolescence, growth retardation, decreased adrenal gland efficiency and immunodeficiency-related illnesses. These medications include immunosuppressants, mineralocorticoids, antihistamines and glucocorticoids (Beuschlein, 2024).

More cough is observed among patients during sleep and when awake in the morning. This disease affects 10% of the population. The mortality rate of asthma, morbidity and prevalence could be studied with the help of Epidemiology. Around the world, about 235 million people have been affected due to its high prevalence rate in many countries. Death rates from asthma continue to be a major global concern, with China and Malaysia reporting significantly lower mortality rates than other countries, while Australia, the United Kingdom and New Zealand record disproportionately higher death rates. There is a significant burden of asthma in the pediatric population, as evidenced by epidemiological data showing that the condition affects both adults and children, with adults accounting for about 7% of cases and children for a higher frequency of about 15% (Yang and Schwartz, 2012).

The focus of the recent study was to identify certain beneficial herbal products equipped with the natural capability of pharmacologically active compounds like phenolic acids, polysaccharides and flavonoids which could offer a safe remedial option by minimizing the hazardous side effects (Wang *et al*., 2023). The asthma-related complications of lung airways were reported to be mitigated significantly in the asthmatic mouse model by certain extract of medicinal herbs like *Trigonella foenum-Graecum* in Europe and Asia (Erten, 2025), *Urtica dioica* in Americas, Europe, Asia and Africa, *Erythronium japonicum* in Asia (Choi, 2018), *Echinodorus scaber* (Rosa, 2017) and *Samsoeum* in western hemisphere (Jeon *et al*., 2015). Despite increasing the quantities of specific constituents' chemical transformations, breakdown of molecules and functional components, fermented natural herbs have only been studied for their therapeutic effects in an asthma model (Tong *et al*., 2015).

Abelmoschus esculentus or ladyfinger, is an edible flowering plant distributed widely in Asia, America, Africa and Europe (Khomsug *et al*., 2010). The green seeds and okra pods are extremely useful as vegetables for the good health of exhausted, weak and depressed people. Additionally, reports have indicated that ladyfinger is effective in curing illnesses like irritable bowel, ulcers, sore throat, lung inflammation and asthma (Sengkhamparn *et al*., 2009). It helps to normalize cholesterol and blood sugar levels because of its fiber content and low glycemic in DEX (Sabitha *et al*., 2011).

The principal objective of the study was to determine the anti-asthmatic effect of *A. esculentus* extract on complete blood count (CBC) Indices, Liver function tests (LFTs) parameters, leukocytes, cytokines and lung histology in asthmatic mice.

MATERIALS AND METHODS

Collection and identification of Abelmoschus esculentus

The seeds of *A. esculentus* were procured from the local market of district Gujrat (Okra Advanta 828 F1 Ici Pakistan) and the species level identification was done by the expert penal of the Botany Department, University of Gujrat, Punjab, Pakistan (HS Codes 07099930). The seeds were allowed to dry in the shade after being cleaned with pure water. Panasonic's 1000W Mixer Grinder MX-AC300 was used to grind the plant materials into fine powder. Subsequently, the powder was centrifuged for 10 minutes at a speed of 4,000 rpm (Krajewska *et al*., 2024).

Drugs and chemicals

Seeds of *A. esculentus* (Okra Advanta 828 F1 Ici Pakistan), DEX sodium phosphate (Dexone®, Dorcas pharmaceutical laboratories, Sousse, Tunisia), Acetone (320110: Merck Sigma Aldrich, U.K), Ovalbumin were obtained from Sigma Aldrich, U.K. Ovalbumin (257-264) chicken.

Method of A. esculentus' extract and dose preparation

For the herbal extraction, 30mg seeds of *A. esculentus* were mixed with 3mL of acetone (procured from Merck Sigma Aldrich, U.K.: 320110) for 4-6 hours at 40- 50 °C by using the Soxhlet's method. Lastly, the extract was subjected to rotary evaporator until to use further and stored at 5 °C. The dried extract was reconstituted in 25% acetone for dose preparation, resulting in final concentrations of 10, 15 and 20 mg/mL. The literature supports the use of 25% acetone because it is non-toxic to organisms used in experiments (Agarwal and Upadhyay, 2023; Shah, 2025). The acetone due to its potential properties like great adaptability, polarity, safety and simplicity, is widely used as an extracting agent (Gil-Martín *et al*., 2022).

Experimental model

Randomly selected 36 mice (10-12 weeks old; weighing 20 g) were reared in an aseptic environment with a regular light/dark period of 12 hours, a humidity of 50% and a temperature of 20°C. For acclimation, all mice were provided food and water in addition to the cleaning of cages daily. The mice were treated with care to minimize their suffering and any pain (Faydaver, 2023).

Strategy of dose design and asthma induction

36 mature mice were classified into six groups (n=6) i.e., normal group, dexamethasone (DEX: D2915-100MG; Sigma Aldrich, U.K.) group, asthmatic group and three groups that received 500, 750 and 1000 mg of *Abelmoschus esculentus* seed extract dissolved in 50ml acetone per kg of bodyweight (Shah, 2025). The strategy of dose and the procedure of asthma induction were comprised of three

steps. Firstly, all the mice except the normal group were given 20µg OVA with 200µl PBS (phosphate-buffered saline) as a supplement and 1mg of aluminum hydroxide via intraperitoneal injection on the first and 14th day. In the second step, the positive control group received dexamethasone (3 mg/kg/day) suspended in 0.5% carboxymethyl cellulose (CMC) at the same volume and route as the treatment groups, which received oral gavage of *A. esculentus* seed extract at 500, 750 and 1000 mg/50mL (acetone)/kg/day from days 15-26 (corresponding to 10, 15 and 20 mg/mL acetone per 20 g mouse) except normal and asthma groups. In the third step, from 27-29 days, 100µg OVA with 200µl PBS solution was inhaled for 30 minutes with a nebulizer to all mice except the normal group (Fig. 2). In order to avoid error, groups were replicated (Kelly *et al*., 2023).

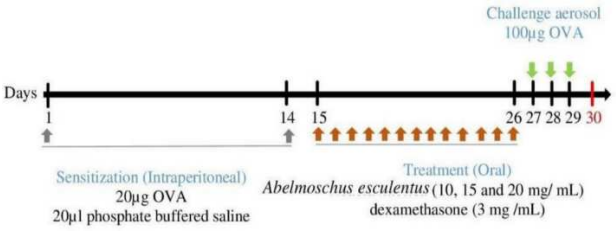

Fig. 2: Strategy of dose design and asthma induction

GC–MS evaluation of A. esculentus

An Rxi-5Sil MS capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 µm) was used in the Shimadzu QP2010S system for the GC-MS analysis. The carrier gas, high-purity helium, flowed steadily at a rate of 0.98 mL/min. At a pace of 10°C per minute, the oven temperature program was scaled up from 70°C to 280°C. The temperature for detector and injector was maintained at 300°C and 280°C, respectively. Splitless mode was used for the injection of the sample. The temperature of mass spectrometer in electron impact (EI) mode was set at 280°C. A pressure of 63.6 kPa was maintained for the carrier gas. In order to identify the compound, retention times (RT) were compared with reference data (da Silva, 2021).

Quantification of complete blood count (CBC) and liver enzymes

Medonic CBC Analyzer (M32m) and LFTs (Microlab 300) were used to assess the post-treatment levels of indicators of blood profile such as mean corpuscular hemoglobin (MCH), hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT) and platelets (Berta *et al*., 2023) and hepatic biochemical parameters i.e. albumin, alanine aminotransferase (ALT), globulin, alkaline phosphatase (ALP), total proteins, bilirubin, aspartate aminotransferase (AST) (Pandeya *et al*., 2024), respectively in the Hematological Lab, Department of Zoology, University of Gujrat.

Enumeration of leukocytes (Total cell count) in bronchoalveolar lavage fluid (BALF)

In order to collect BALF from the respiratory airways, the Alfaxan (Alfaxalone®, Jurox Pty Ltd., Hunter Valley, Australia) was used to anesthetized mice followed by lung treatment with cold 1 PBS (yield: 80%, total volume = 0.8 mL) thrice via tracheal pathway. The BALF thus collected was subjected to centrifugation (4000 rpm, 4°C) for five minutes. As a result, supernatant and pellets were formed that were used to perform ELISA analysis and cell examination, respectively. For total cell count, cytospin (500 rpm for 5 minutes) was used to mount pellets on the glass slide followed by fixation with methyl alcohol for half a minute. The slides were then immersed thrice in the solution of Giemsa stain (Sigma Aldrich, Germany) and May-Grunwald (Sigma Aldrich, Germany) for twelve and five minutes, respectively. After that, the coverslips were placed on the slides and the total cell enumeration was done at the magnification of 400x using light microscopy (Zheng, 2024).

ELISA for Interleukins and IgE in BALF

The level of interleukins i.e. IL-17A, IL-13, IL-10, IL-5, IL4 and OVA-specific IgE in the serum and BALF of mice was measured using an ELISA kit (BioLegend Inc., USA). The solution containing 50 µL BALF with anti-interleukin (IL-17A, IL-13, IL-10, IL-5 and IL4) antibodies and IgE-specific antigen was poured into a 96-well plate and placed for two hours at room temperature. Following the removal of any unbound proteins, each well was filled with the solution of detection antibody and Avidin-HRP D in equal volumes of 100 µL each followed by an hour incubation in the shaker incubator at room temperature. The enzymatic reactions were initiated and terminated through addition of substrate solution and $2MH_2SO_4$ solution, respectively. Consequently, the absorbance values were calibrated through the Versa Max plate reader at 450 nm (Zheng, 2024).

PCR analysis for interleukins in lung tissue

The RT-PCR was used to quantify the relative mRNA concentration for all the interleukin (IL-17A, IL-13, IL-10, IL-5 and IL4) genes. RNeasy mini kit (Qiagen, Hilden, Germany) was used to extract RNA molecules from frozen lung tissue. After quantification of RNA, an oligo-dT primer, dNTP (Invitrogen, Carlsbad, CA, USA) and reverse transcriptase mixture (Thermo Fisher Scientific, USA) was used to synthesize complementary DNA (cDNA). The cDNA template and 2-Power SYBR Green (TOYOBO, Japan) were used to perform RT-PCR as described previously and the level of mRNA expression was quantified using $2^{-\Delta\Delta Cq}$ method (Nur Husna *et al*., 2022). The primer sequences that we employed in our investigation are mentioned in (Table 1).

Table 1: The study's primers were utilized for PCR.

Primer		Primer sequences	Reference
IL-4	F	TCCGACCACCACTACAGCAA	(Edan Alsaimary, 2021)
	R	ATCTTTCAACACGCAGGACA	
IL-5	F	CTCTGTTGACAAGCAATGAGACG	(Pang *et al*., 2021)
	R	TCTTCAGTATGTCTAGCCCCTG	
IL-10	F	ACACATGGTATAGATGCAGC	(Edan Alsaimary, 2021)
	R	TTCCAAGACCTCAGGCAAGA	
IL-13	F	TGTGTCTCTCCCTCTGACCC	(Pang *et al*., 2021)
	R	CACACTCCATACCATGCTGC	
IL-17A	F	CAGAAGACCTACATGTTACT	(Du, 2016)
	R	GTAGCGCTATCGTCTCTCT	
β-actin	F	ACGGCCAGGTCATCACTATTG	(Livak and Schmittgen, 2001)
	R	CAAGAAGGAAGGCTG GAAAAGA	

Table 2: GC-MS analysis of bioactive compounds in seed extracts of *A. esculentus*

Peak No.	Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	% Area (approx.)	Pharmacological actions
1	4.83	Hexanoic acid	$C_6H_{12}O_2$	116.16	2.31	Antimicrobial, flavor compound
2	6.29	Octanoic acid	$C_8H_{16}O_2$	144.21	1.97	Antibacterial, anti-inflammatory
3	10.21	Palmitic acid	$C_{16}H_{32}O_2$	256.43	21.68	Antioxidant, anti-inflammatory, Antimicrobial
4	12.78	Linoleic acid	$C_{18}H_{32}O_2$	280.45	17.39	Hypocholesterolemic, anti-inflammatory
5	13.25	Oleic acid	$C_{18}H_{34}O_2$	282.47	14.11	Antioxidant, anticancer
6	14.62	Stearic acid	$C_{18}H_{36}O_2$	284.48	5.74	Emollient, stabilizer
7	15.93	Squalene	$C_{30}H_{50}$	410.73	4.55	Antioxidant, anticancer
8	17.12	β-Sitosterol	$C_{29}H_{50}O$	414.71	3.47	Anticholesterolemic, anti-inflammatory
9	18.36	Tocopherol	$C_{29}H_{50}O_2$	430.71	2.98	Antioxidant, skin protection
10	20.17	Campesterol	$C_{28}H_{48}O$	400.68	1.93	Antioxidant, cholesterol-lowering

Lung histology analysis

In order to measure the epithelial thickness of bronchial tree, the histological analysis of lung tissues was performed. In a nutshell, mice from a subset group had their right lungs removed and placed in 10% neutral buffered formalin for 2 days. The cutting of central lobes of frozen lung tissue was then precisely done into slices of 4mm thickness and implanted in paraffin wax to make paraffin blocks followed by staining of tissue sections with hematoxylin and eosin stain (H & E) (Sigma Aldrich, Germany). Consequently, histopathological characteristics were examined at 400x magnification using a light microscope.

In order to measure the thickness of epithelial and smooth muscles in bronchial tubes, Leica Application Suite was used (Leica Microsystems, Germany). A scale from 0 to 5 was used to grade the degree of peri-vascular and peri-bronchiolar inflammation (0 for no cells, 1 for few cells, 2 for a ring of cells one cell layer deep, 3 for a ring of cells 2-4 layers deep, 4 for a ring of cells and 5 for cells deep) (Lee *et al*., 2011). For the evaluation of peri-vascular and peri-bronchiolar inflammation, the average scores of randomly placed five sections of mouse lung airways were calibrated.

For the detection of mucus overproduction by goblet cells, Periodic Acid Schiff (PAS) was used. For this purpose, the tissue sections were deparaffinized and dehydrated followed by oxidation in the periodic acid solution for five minutes. After that, the samples were washed and placed in Schiff reagent for 15 minutes. Following a thorough cleaning, the tissue sections were stained for 30 seconds using the solution of hematoxylin stain (Sigma Aldrich, Germany). Subsequently, the sub-epithelial fibrosis and goblet cell hyperplasia were observed under the light microscope at 400x magnification. On the basis of a double-blind study analysis conducted on four distinct random sites by four independent researchers, the mucus score was determined and the results are as follows: No goblet cells are present at 0, 20% of the epithelium or less, 20%–40%, 40%–60%, 60%–80% and >80% of the epithelium, respectively (Uluer *et al*., 2025).

Statistical analysis

The mean ± SD is used to express all data. Before statistical analysis, datasets were evaluated for homogeneity of variance using Levene's test and for normality using the Shapiro-Wilk test. Using Statistix software (version 8.1), one-way ANOVA was used to compare differences between the treatment groups *A. esculentus* (10 mg/mL, 15 mg/mL and 20 mg/mL), dexamethasone, OVA-control and normal control for the parameters examined (CBC, LFTs, leukocytes, cytokines and lung histology) because the assumptions were met. Pairwise comparisons were conducted using Tukey's post hoc test at a significance threshold of $p \leq 0.05$. Where appropriate, effect sizes and precise p-values are provided and each figure legend specifies the number of animals in each group (n=6).

RESULTS

Identification of components

Using their distinctive mass spectra, bioactive components can be identified through GC-MS analysis. Abelmoschus esculentus's chromatographic profile showed 10 different peaks, each representing a different chemical (Fig. 3). The detailed records of these compounds include peak number, compound name, retention time (RT), molecular weight (g/mol), molecular formula, % area (approximate) and pharmacological actions (Table 2). This comprehensive chemical profiling demonstrates the complexity and potential therapeutic value of the chemicals present in the examined extract.

Administration of the extract of A. esculentus restored the elevated CBC values in asthmatic mice

To determine the pathological complications of diseases and the physiological status of the animal body, CBC is usually used as a biological indicator. In the asthma group, the values of WBCs (neutrophils, lymphocytes, monocytes and eosinophils) were higher than normal group and values of RBCs (MCV, HCT, MCH, hemoglobin and MCHC) and platelets were lower than the normal group. In case of herbal treatment (20mg/mL, 15mg/mL and 10mg/mL) and DEX groups, the values of all the CBC parameters were closer to the normal group. This indicates the effectiveness of herbal treatments in improving CBC (Table 3).

Administration of the extract of A. esculentus restored the elevated LFTs values in asthmatic mice

In order to find out the anti-asthmatic effect of *A. esculentus* on the biological hepatic activities in the OVA-induced mouse model, LFTs were performed. In the asthma group, the values of LFTs (AST, bilirubin, albumin, ALT, total proteins, ALP and globulin concentrations) were higher than the normal group. Whereas, in herbal treatment (20mg/mL, 15mg/mL and 10mg/mL) and DEX groups, the values of all the LFT parameters were closer to the normal group. This shows the role of herbal treatments in improving LFTs (Table 4).

Table 3: Anti-asthmatic potential of *A. esculentus* on CBC of male mice (Mean ± SD)

Treatments	Hemoglobin (g/dL)	WBC ($x10^3$ /uL)	RBC ($x10^6$ /uL)	MCV (fL)	HCT (%)	Platelets ($x10^3$ /uL)	MCH (pg)	MCHC (g/uL)	Neutrophils (x103 /uL)	Lymphocytes (x103 /uL)	Monocytes (x103 /uL)	Eosinophils (x103 /uL)
Normal	12.57 ± 0.25^{a}	3.76 ± 0.02^{e}	7.53 ± 0.05^{a}	41.23 ± 0.03^{a}	35.8 ± 0.17^{a}	922 ± 5.29^{a}	19.05 ± 0.05^{a}	38.47 ± 0.4^{a}	0.58 ± 0.01^{f}	0.87 ± 0.02^{f}	0.84 ± 0.02^{f}	0.54 ± 0.02^{c}
A. esculentus 10 mg/mL	8.73 ± 0.6^{c}	7.18 ± 0.03^{b}	5.13 ± 0.06^{e}	24.54 ± 0.05^{e}	24.2 ± 0.05^{e}	771 ± 2^{e}	11.91 ± 0.04^{d}	29.32 ± 0.03^{c}	2.29 ± 0.04^{b}	3.68 ± 0.03^{b}	4.28 ± 0.02^{b}	1.91 ± 0.02^{b}
A. esculentus 15 mg/mL	9.37 ± 0.03^{c}	7.07 ± 0.04^{b}	5.28 ± 0.03^{d}	27.22 ± 0.03^{d}	26.21 ± 0.02^{d}	788.33 ± 4.51^{d}	12.81 ± 0.55^{c}	29.85 ± 0.02^{c}	2.14 ± 0.02^{c}	3.49 ± 0.01^{c}	3.93 ± 0.03^{c}	1.9 ± 0.03^{b}
A. esculentus 20 mg/mL	10.53 ± 0.38^{b}	6.84 ± 0.05^{c}	6.31 ± 0.03^{c}	30.22 ± 0.04^{c}	28.53 ± 0.4^{c}	820 ± 5^{c}	16.91 ± 0.04^{b}	29.96 ± 0.04^{c}	1.69 ± 0.04^{d}	3.24 ± 0.05^{d}	3.67 ± 0.04^{d}	1.89 ± 0.04^{b}
DEX 3 mg/mL	10.60 ± 0.3^{b}	5.85 ± 0.07^{d}	6.93 ± 0.04^{b}	36.39 ± 0.13^{b}	34.77 ± 0.15^{b}	901.67 ± 3.06^{b}	17.12 ± 0.02^{b}	37.1 ± 0.6^{b}	1.06 ± 0.04^{e}	2.81 ± 0.05^{e}	2.14 ± 0.06^{e}	1.94 ± 0.05^{b}
OVA	5.84 ± 0.07^{d}	12.8 ± 0.1^{a}	3.2 ± 0.03^{f}	21.53 ± 0.01^{f}	19.52 ± 0.02^{f}	621 ± 2^{f}	8.22 ± 0.02^{e}	21.8 ± 0.02^{d}	4.33 ± 0.02^{a}	5.73 ± 0.03^{a}	6.3 ± 0.01^{a}	3.96 ± 0.04^{a}

Means showing different superscripts in a column have a difference of statistical significance whereas similar superscripts indicate that there is no significant difference among Mean values ($p \leq 0.05$) Tukey's Test

Table 4: Anti-asthmatic potential effects of *A. esculentus* on LFTs of male mice (Mean ± SD)

Treatments	Bilirubin (mg/dL)	ALT (U/L)	AST (U/L)	Alkaline phosphatase (U/L)	Total proteins (mg/dL)	Albumin (g/dL)	Globulin (g/dL)
Normal	0.21 ± 0.01^{e}	23.54 ± 0.03^{f}	57.33 ± 0.29^{f}	76.98 ± 0.01^{e}	5.3 ± 0.08^{c}	3.77 ± 0.12^{c}	2.1 ± 0.01^{e}
A. esculentus 10 mg/mL	0.36 ± 0.01^{b}	26.66 ± 0.04^{b}	61.96 ± 0.04^{b}	81.95 ± 0.04^{b}	5.56 ± 0.03^{b}	4.11 ± 0.01^{b}	2.59 ± 0.02^{b}
A. esculentus 15 mg/mL	0.33 ± 0.01^{c}	25.88 ± 0.01^{c}	61.28 ± 0.04^{c}	79.62 ± 0.02^{d}	5.43 ± 0.03^{bc}	4.11 ± 0.02^{b}	2.41 ± 0.01^{c}
A. esculentus 20 mg/mL	0.28 ± 0.01^{d}	25.7 ± 0.05^{d}	60.13 ± 0.07^{e}	79.37 ± 0.02^{d}	5.37 ± 0.02^{c}	4.05 ± 0.04^{b}	2.32 ± 0.03^{d}
DEX 3 mg/mL	0.34 ± 0.02^{bc}	25.57 ± 0.02e	60.55 ± 0.05^{d}	79.97 ± 0.04^{c}	5.29 ± 0.06^{c}	3.99 ± 0.01^{b}	2.37 ± 0.02^{cd}
OVA	1.43 ± 0.01^{a}	45.97 ± 0.02^{a}	89.52 ± 0.03^{a}	102.5 ± 0.26^{a}	7.54 ± 0.03^{a}	6.15 ± 0.04^{a}	3.6 ± 0.02^{a}

Means showing different superscripts in a column have a difference of statistical significance whereas similar superscripts indicate that there is no significant difference among Mean values (p≤0.05) Tukey's test

Table 5: Anti-asthmatic potential effects of *A. esculentus* on total cells of male mice (Mean ± SD)

Treatments	Total cells	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Normal	42.17 ± 2.02^{e}	2.26 ± 0.04^{c}	0.32 ± 0^{d}	35.58 ± 0.03^{b}	2.22 ± 0.04^{c}
A. esculentus 10 mg/mL	64.13 ± 0.05^{b}	7.54 ± 0.05b	1.54 ± 0.04^{b}	46.50 ± 0.03^{b}	7.14 ± 0.04^{b}
A. esculenus 15 mg/mL	62.15 ± 0.1b^{c}	5.94 ± 0.04b	1.28 ± 0.04^{bc}	46.34 ± 0.04^{b}	6.70 ± 0.02^{b}
A. esculentus 20 mg/mL	58.13 ± 0.2^{cd}	5.75 ± 0.04b	1.14 ± 0.04^{bc}	45.75 ± 0.05^{b}	6.23 ± 0.15^{b}
DEX 3 mg/mL	54.67 ± 3.06^{d}	5.5 ± 1.5b	0.75 ± 0.03^{cd}	44.67 ± 1.15^{b}	5.46 ± 0.31^{bc}
OVA	94.50 ± 4^{a}	12 ± 2a	2.58 ± 0.63^{a}	65.67 ± 6.11^{a}	13.33 ± 3.06^{a}

Means showing different superscripts in a column have a difference of statistical significance whereas similar superscripts indicate that there is no significant difference among Mean values (p≤0.05) Tukey's test

Table 6: Antiasthmatic potential effects of *A. esculentus* on real-time PCR (IL-4, IL-5, IL-10, IL-13 and IL-17A) of BALF male mice (Mean ± SD)

Treatments	IL-4	IL-5	IL-10	IL-13	IL-17A
Normal	0.34 ± 0.05^{e}	1.75 ± 0.04^{e}	0.92 ± 0.03^{f}	0.67 ± 0.03^{e}	0.35 ± 0.02^{c}
A. esculentus 10 mg/mL	1.95 ± 0.01^{b}	4.12 ± 0.02^{b}	2.94 ± 0.03^{d}	2.49 ± 0.02^{b}	0.43 ± 0.01^{b}
A. esculentus 15 mg/mL	1.5 ± 0.01^{c}	4.03 ± 0.01^{b}	3.15 ± 0.03^{c}	2.01 ± 0.01^{c}	0.43 ± 0.01^{b}
A. esculentus 20 mg/mL	0.49 ± 0.01^{e}	2.29 ± 0.02^{d}	5.43 ± 0.02^{a}	1.33 ± 0.03^{d}	0.4 ± 0.01^{b}
DEX 3 mg/mL	1.11 ± 0.02^{d}	3.12 ± 0.02^{c}	4.02 ± 0.03^{b}	1.88 ± 0.03^{c}	0.41 ± 0.01^{b}
OVA	3.23 ± 0.21^{a}	12.19 ± 0.36^{a}	2.67 ± 0.06^{e}	5.49 ± 0.34^{a}	1.55 ± 0.03^{a}

Means showing different superscripts in a column have a difference of statistical significance whereas similar superscripts indicate that there is no significant difference among Mean values (p≤0.05) Tukey's test.

Effect of A. esculentus on the activities of leukocytes from infiltrating the BALF of mice suffering from asthma induced by OVA

To analyze the anti-inflammatory impact of *A. esculentus* on asthma, the asthmatic mice were utilized as a model organism. In the asthma group, the values of total cells (monocytes, eosinophils, neutrophils and lymphocytes) in BALF were higher than normal group. In case of herbal treatment (20mg/mL, 15mg/mL and 10mg/mL) and DEX groups, the values of total cells were closer to the normal group. This indicates the effectiveness of herbal treatments in improving total cells (Table 5).

Effect of A. esculentus on cytokines (IL-17A, IL-13,IL-10, IL-5, IL4 and IgE) of BALF lung mice

ELISA was applied to determine the level of cytokines in asthmatic mice. When compared to the asthma group, the values of cytokines like IL-17A, IL-13, IL-5, IL4 and IgE were significantly higher, while the value of IL-10 was lower than normal group. Moreover, in treatment groups such as *A. esculentus* (10mg/mL), *A. esculentus* (15mg/mL), *A. esculentus* (20mg/mL) and DEX group, there were significantly lower levels of IL-17A, IL-13, IL-5, IL4 and IgE except IL-10. Conversely, the level of IL-10 was markedly elevated with the increase of the dose concentration. This shows the role of herbal extract in

reducing and enhancing the levels of IL-17A, IL-13, IL-5, IL4 and IgE and IL-10, respectively (Fig. 4-9).

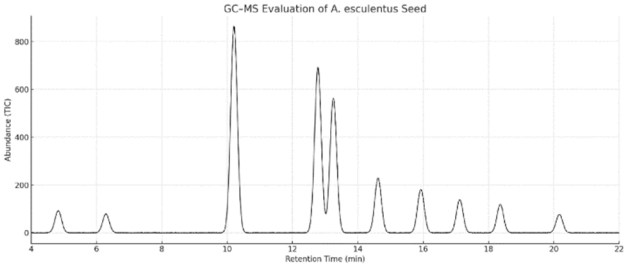

Fig. 3: Chromatogram obtained from the GC/MS with the extract of *A. esculentus* seed.

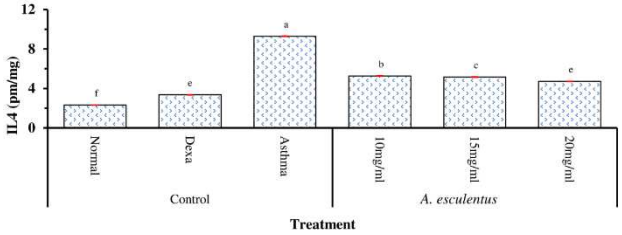

Fig. 4: ELISA: Anti-asthmatic impact of *A. esculentus* on IL-4 (pm/mg) of mice BALF. In BALF of mice lung, the amount of interleukin 4 (IL-4) was measured. Different alphabets present in different bars show significant differences from one another and the same alphabets show that parameters have no significant difference among themselves ($p \leq 0.05$) Tukey's test. The DEX group had the lowest level of IL-4 among the treated groups followed by the *A. esculentus* (20mg/mL), *A. esculentus* (15mg/mL) and *A. esculentus* (10mg/mL) groups.

Effect of A. esculentus on interleukins (IL-17A, IL-13, IL-10, IL-5 and IL4) of lung mice

Real-time PCR (RT-PCR) was used to find out mRNA expression of interleukins such as IL-17A, IL-13, IL-10, IL-5 and IL4. When compared to normal group, all the interleukins had significantly greater values in asthma group except IL-10. Furthermore, all treatment groups such as *A. esculentus* (10mg/mL), *A. esculentus* (15mg/mL), *A. esculentus* (20 mg/mL) and DEX group showed significantly decreased levels of IL-17A, IL-13, IL-5 and IL4. On the other hand, as the dose concentration increased, the levels of IL-10 increased significantly. This demonstrates the function of plant-based herbal extract in lowering and raising the levels of IL-17A, IL-13, IL-5, IL4 and IL-10, respectively (Table 6). ChemiDoc was used to measure the band intensities and the β-actin band strength was compared to five different protein expressions (Fig. 10).

Effect of A. esculentus on lung histology in asthmatic mice

To assess the anti-asthmatic impact on lung histology in OVA-induced mice, the histopathology was performed. The inflammatory changes including smooth muscle thickness, mucus overproduction, epithelial cell thickness, goblet cell hyperplasia, inflammatory cell infiltration and collagen deposition were observed in the asthma mice when compared to the normal. In addition, the thickness of the cells of epithelium and smooth muscles and mast cells in airways of mice treated with *A. esculentus* (20mg/mL, 15mg/mL and 10mg/mL) was significantly reduced. Moreover, the DEX group also showed a decrease in the inflammatory cell infiltration and a remarkable decline in the thickness of the airway epithelium when compared to the asthma mice. This reveals the role of the herbal extract of *A. esculentus* in reducing the thickness of smooth muscles and dilating the tracheal tracts of the respiratory system (Fig. 11).

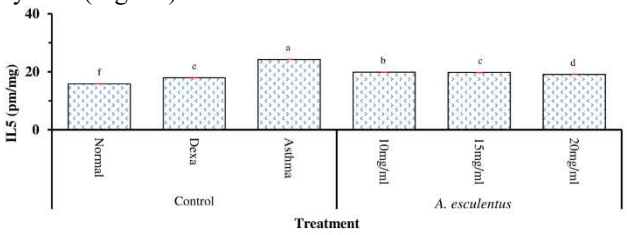

Fig. 5: Anti-asthmatic impact of *A. esculentus* on IL-5 (pm/mg) of mice BALF. In BALF of mice lung, the quantity of interleukin 5 (IL-5) was determined. Different alphabets present in different bars show significant difference from one another ($p \leq 0.05$) Tukey's test. The DEX group had the lowest level of IL-5 among the treated groups followed by the *A. esculentus* (20mg/mL), *A. esculentus* (15mg/mL) and *A. esculentus* (10mg/mL) groups.

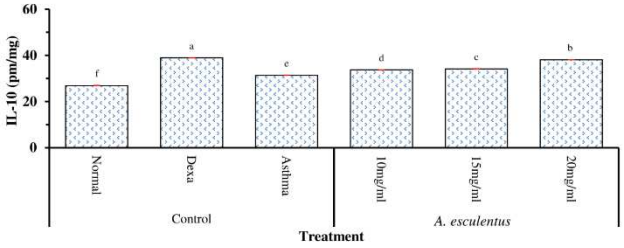

Fig. 6: ELISA: Anti-asthmatic impact of *A. esculentus* on IL-10 (pm/mg) of mice BALF. In BALF of mice lung, the amount of interleukin 10 (IL-10) was measured. Different alphabets present in different bars show significant difference from one another and same alphabets show that parameters have no significant difference among themselves ($p \leq 0.05$) Tukey's test. The DEX group had the highest level of IL-10 among the treated groups followed by the *A. esculentus* (20mg/mL), *A. esculentus* (15mg/mL) and *A. esculentus* (10mg/mL) groups.

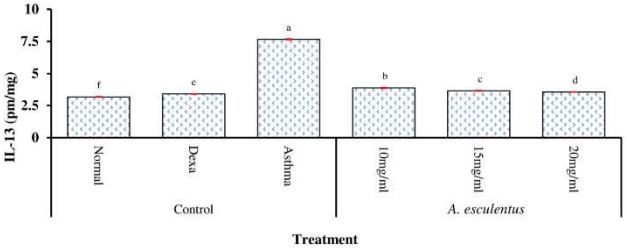

Fig. 7: ELISA: Anti-asthmatic impact of *A. esculentus* on IL-13 (pm/mg) of mice BALF. In BALF of mice lung, the

quantity of interleukin 13 (IL-13) was determined. Different alphabets present in different bars show significant difference from one another (p≤0.05) Tukey's test. The DEX group had the lowest level of IL-13 among the treated groups followed by the *A. esculentus* (20mg/mL), *A. esculentus* (15mg/mL) and *A. esculentus* (10mg/mL) groups.

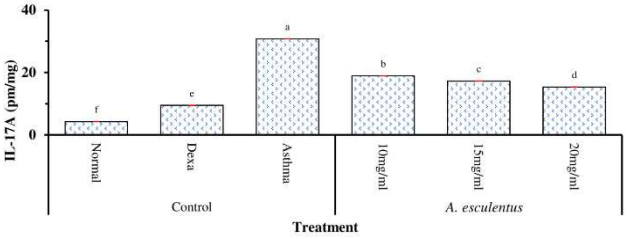

Fig. 8: ELISA: Anti-asthmatic impact of *A. esculentus* on IL-17A (pm/mg) of mice BALF. In BALF of mice lung, the amount of interleukin 17A (IL-17A) was measured. Different alphabets present in different bars show significant difference from one another (p≤0.05) Tukey's Test. The DEX group had the highest level of IL-17A among the treated groups followed by the *A. esculentus* (20mg/mL), *A. esculentus* (15mg/mL) and *A. esculentus* (10mg/mL) groups.

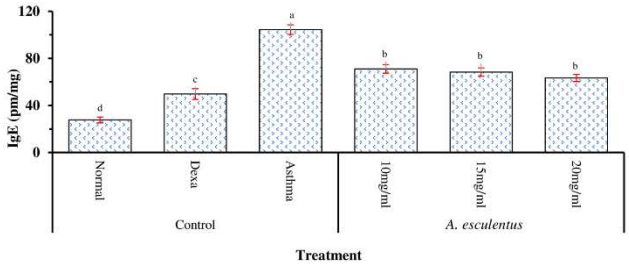

Fig. 9: ELISA: Anti-asthmatic impact of *A. esculentus* on IgE (pm/mg) of mice BALF. In BALF of mice lung, the quantity of IgE was determined. Different alphabets present in different bars show significant difference from one another and same alphabets show that parameters have no significant difference among themselves (p≤0.05) Tukey's Test. The DEX group had the lowest level of IgE among the treated groups followed by the *A. esculentus* (20mg/mL), *A. esculentus* (15mg/mL) and *A. esculentus* (10mg/mL) groups.

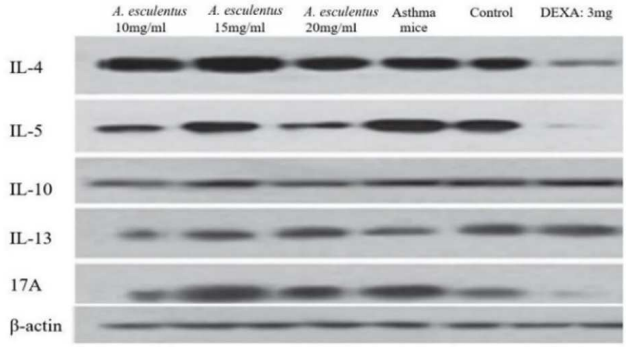

Fig. 10: Bands strength showed five interleukins' expressions compared to the strength of the β-actin bands.

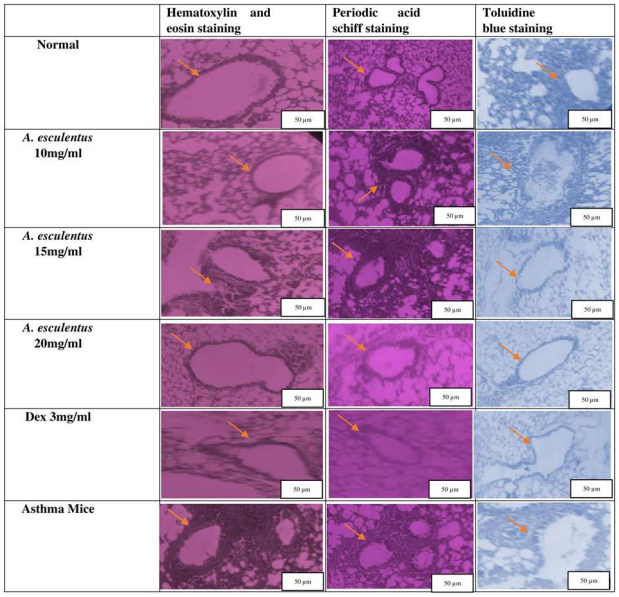

Fig. 11: Hematoxylin and eosin, Periodic acid-Schiff and Toluidine blue staining were used to examine the effects of *A. esculentus* extract on the structural changes in asthmatic mice pulmonary tissue. In contrast to normal mice, asthmatic mice pulmonary tissues showed substantial peribranchial infiltrates of cells called leukocytes and increased mucus production from goblet cell in the bronchial tubes. The enhanced recruitment of leukocytes and the excessive production of mucus were reduced by *A. esculentus* extract in a dose- dependent manner. In addition, *A. esculentus* extract substantially reduced the airway swelling as well. At a 400x magnification, lung tissue stained with toluidine blue (TB) revealed dysplasia, or aberrant cell development, and mast cell degranulation. In a double-blind screening, two separate researchers designed a scale to calculate the score by which the degree of cell infiltration in the airway can be measured. The level of peri-bronchiole and peri-vascular inflammation was measured on a 0–5 scale. Here are the results of the scores: 0, no cells; 1, a few cells; 2, a ring of cells one cell layer deep; 3, a ring of cells 2-4 layers deep; 4, a ring of cells; and 5, cells deep. Each mouse's lung's five randomly selected airway segments' average scores were calculated (score of the airways due to inflammation). These outcomes suggest that *A. esculentus* extract inhibits the growth of toxic inflammation in the lung tissue of asthma induced mice.

DISCUSSION

Asthma, a tracheal track disorder, can be distinguished mainly by the thickness of smooth muscles and the narrowing of the airways of the respiratory system. It was estimated that about 300 million people were affected by asthma in the globe annually (Azman *et al*., 2021). Various studies demonstrated that synthetic drugs such as corticosteroids (Abreu-Mendes *et al*., 2020; Scadding, 2017) and bronchodilators alleviated asthma symptoms,

which unfortunately had side effects such as depression, anxiety, suicidal thoughts, delayed puberty and immunodeficiency (Beuschlein, 2024). Hence, there is a need to discover therapeutic agents that have minimum side effects and are affordable and accessible to asthmatic people. Traditionally, herbal medicines have shown therapeutic potential in reducing allergic symptoms in various diseases. In this context, the ongoing study was conducted to alleviate asthma symptoms in asthmatic mice. The purpose of the analysis was to determine which bioactive components of the *A esculentus* extract are believed to be responsible for its cytoprotective, anti-inflammatory and antioxidant properties. Ten unique peaks were visible in the ensuing chromatogram, each of which represented a different phytochemical component. The existence of these components raises the possibility that these bioactive compounds are partially responsible for the therapeutic benefits noticed in the experimental groups. These results are consistent with earlier studies showing the pharmacological significance of long-chain alcohols, fatty acid esters and polyphenols frequently present in extract produced from plants (Solaiyappan *et al*., 2025).

Complete Blood Count (CBC) parameters help to diagnose and monitor various health issues, including infections, inflammation, anemia, leukemia and lymphoma (Seo, 2022). The purpose of the ongoing study was to find out the indices of CBC parameters after exposure to *A. esculentus* extract in asthmatic mice. When compared to the normal group, the levels of WBC parameters such as eosinophil, lymphocyte, monocyte and neutrophil were increased in the OVA group. On the other hand, the value of RBC parameters i.e., Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) and platelets were decreased in the OVA group as compared to the normal group. In the case of mice groups administered with different herbal doses of *A. esculentus* at 10mg/mL, 15mg/mL and 20mg/mL and DEX (3mg/mL), the levels of RBCs and WBCs were seen to be in the normal range and the level of platelets was also observed to be normal. However, the maximum recovery of these parameters was examined at the highest dose concentration, which indicates that the herbal extract worked to restore the CBC values in asthmatic mice in a dose-dependent manner. A toxicological study using 2000 mg/kg of okra extract in mice demonstrated no significant alteration in hematological parameters, indicating the pharmacological safety of okra (Chenoly, 2023). Similar responses to the herbal treatment with *okra (A. esculentus L.)* observed in the altered levels of biochemical parameters in the asthmatic mice model suggest the plant extract therapy as an effective remedy in alleviating allergic asthma (Salman *et al*., 2023).

Liver function tests (LFTs) parameters such as ALT, ALP, AST, bilirubin, total proteins, albumin and globulin help to diagnose, monitor and manage various liver-related health issues, including liver damage, liver dysfunction, bile duct disorder and fatty liver disease. The liver cell damage and bile duct obstruction are caused by elevated levels of ALT, bilirubin, ALP and AST. The higher levels of total proteins, albumin and globulin cause the reduction of protein synthesis in the liver, which maintains the blood volume, supports in the blood clotting process and the transport of hormones in target sites (Ekasari, 2022). The plant extract (*A. esculentus*) and DEX groups showed a remarkable decrease in the levels of LFTs' parameters than the OVA group. This demonstrates the role of plant extract in improving the LFTs' parameters in asthmatic mice. The okra fruit extract was reported to restore the biochemical parameters of the liver, i.e. transaminases and uric acid in pre-diabetic patients (Afsharmanesh *et al*., 2024). A toxicological study using 2000 mg/kg okra extract in mice demonstrated no significant alteration in biochemical parameters i.e. urea, total cholesterol, creatinine, AST, ALT, total protein and triglycerides indicating the pharmacological safety of okra (Alblihd *et al*., 2023; Chenoly, 2023).

Total cell counts such as neutrophils, lymphocytes, monocytes and eosinophils help to diagnose the asthma symptoms in the patient. Of these, eosinophils and neutrophils play a vital role in the development of allergic and non-allergic asthma. The elevated level of lymphocytes leads to airway inflammation and immune responses. Monocytes are only responsible for chronic inflammation (Yamasaki *et al*., 2022). Our results indicated that the growth of inflammatory cells and higher mucus production in the lungs were caused by ovalbumin-induced asthma in mice, both of which were decreased (led to normal range) by three concentrations of herbal extract and DEX treatment. In European sea bass, the okra seed extract has been found to enhance the combating capabilities of WBCs concerning respiratory burst activity, phagocytosis, bactericidal activity, and viability. An *in vitro* study on European sea bass pathogens i.e. *Vibrioharveyi* and *V. anguillarum* showed significant anti-microbial activity after exposure to okra seed extract (Guebebia *et al*., 2023).

The complications related to asthma pathogenesis are strongly influenced by the alterations in the natural balance of T helper 1 (Th1) and Th2 levels in the lung airways (Lee *et al*., 2024). Th2 interleukins i.e. IL-13, IL-5. IL-4 and Th1-mediated interleukin i.e. IL-10 work antagonistically in the progression and suppression of allergic symptoms, respectively (Shankar *et al*., 2022). Of these, IL-4 is primarily responsible for allergic reactions in asthmatic patients, while IL-10 functions as a basic anti-inflammatory cytokine that prevents the inflammatory cytokine (IL-5 and IL-4) secretion by the Th1 immune cells (Hammad and Lambrecht, 2021). The elevation in the values of IL-17A, IL-13, IL-5, IL4 and IgE was correlated with the increasing level of inflammation whereas the increasing level of IL-10 alleviates the inflammatory

symptoms in asthmatic mice. The decline in inflammatory cytokines and the elevation in anti-inflammatory cytokines of *A. esculentus* and DEX treatments suggest that herbal extract and DEX could play a vital role in alleviating allergic symptoms. These results showed that the plant extract acts as a therapeutic agent for reducing constriction, mucus overproduction and infiltration of cells in lung mice. It was reported that the mRNA expression of inflammatory mediators like TNF-α and IL-6 was significantly declined in response to *in vitro* treatment with okra leaf extract in human hepatoma cell line Huh7 (Panighel *et al*., 2022). The concentrations of inflammatory cytokines i.e. IL-13, IL-5, IL-4 and TNF-α were reported to decrease towards the normal level in an ovalbumin-induced asthma mouse model after treatment with herbal extract of *Lonicera japonica Thunb* (Lonicerin) (Lonicerin) (Dai, 2024). which is in harmony with our results. However, the level of anti-inflammatory cytokines like IL-10 was observed to rise after treatment with *A. esculentus L*. polysaccharides (Sheu ShyangChwen and Lai MeiHuei, 2012). An innovative study reported that *A. esculentus* pod extract modulates CD8+ cytotoxic T cells to protect against carcinogenic liver injury in mice (Hayaza *et al*., 2021). These findings suggest that plant therapy mitigates asthma-related symptoms by altering the cytokines levels.

Lung histology plays a crucial role in understanding the pathophysiology, diagnosis and management of asthma. Inflammation is caused by elevated levels of inflammatory cells such as neutrophils, lymphocytes and eosinophils. Increased smooth muscle thickness and goblet cell hyperplasia are developed by the excessive amount of mucus production in lung airways (Russell *et al*., 2024). The aforementioned asthma complications were restored to normal range with DEX treatment and the *A. esculentus* treatment groups in a dose dependent manner. These finding suggest that administering *A. esculentus* to asthmatic lung tissues can reduce inflammation and accumulation of mucus. Similar response was observed in a previous study conducted on ulcer rat model which showed a significant recovery of histological parameters after exposure to okra seed extract (Ortaç *et al*., 2018). A study on pharmacological safety of okra extract showed no significant variations in histological parameters of mice at dose of 2000 mg/kg (Chenoly, 2023). It has been revealed that the coffee of *A. esculentus* mast cell degranulation, collagen deposition, mucus production and bronchoconstriction towards normal range in an asthmatic mouse model (Anggraeni *et al*., 2022).

CONCLUSION

In conclusion, in an OVA-induced asthma mouse model, *A esculentus* seed extract exhibited dose-dependent protective effects, improved cytokine levels, lung histology, inflammatory cell infiltration and several other hematological and liver function indicators. These results suggest it may offer preventive benefits, but further translational, toxicological and mechanistic studies are needed to evaluate safety and efficacy. Okra mucilage can act as an allergen, so highly sensitive individuals should exercise caution.

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Authors' contribution
SHS and GM conceived the idea, and designed the study. SHS and MH performed experiments and prepared a draft. RA, SNA, BM, AY, IA SS and AZ analyzed data, contributed new methods or models, and SHS wrote the paper.

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Data availability statement
Data Availability upon reasonable request, the corresponding author will provide the data supporting the study's final findings.

Ethical approval
The University of Gujrat's Ethical Committee Examined and approved the experiment's use of animals (UOG/ORIC/2024/58) in the ongoing study and every experiment was performed following the institutional instructions (University of Gujrat, Gujrat, Punjab, Pakistan).

Conflict of interest
The authors have no conflict of interest.

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