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Assessment of Anti-Asthmatic Potential of *Cassia fistula* Bark in an Ovalbumin Induced Asthma in *Mus musculus*

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ABSTRACT

This study explored the anti-asthmatic potential of *Cassia fistula* bark using *Mus musculus* as model organism. The mice were treated with ovalbumin on day one and the 14th day and then herbal extract doses were given from day 15th to 26th to all groups except normal and asthmatic control. Then, oral inhalation of ovalbumin was provided from day 27th to 29th to all treatment groups except control. Mice were divided into six treatment groups i.e., normal, *C. fistula* (10mg/ml), *C. fistula* (15mg/ml), *C. fistula* (20mg/ml), DEX (3mg/ml), asthmatic (Control) and dose administered oral gavage. Blood serum analysis showed liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase) levels decreases in herbal and DEX treated mice. Similar trend was observed for bilirubin, total proteins, albumin and globulin. The results of the liver function test showed lower values serum parameters in all treatment groups except asthmatic control. In asthmatic control, all hematological parameters of red blood cells (MCV, HCT, MCH, hemoglobin, and MCHC) and platelets declined. While white blood cells (neutrophils, lymphocytes, monocytes and eosinophils) increased in asthmatic control. However, hematological parameters showed restoration in all *C. fistula* groups and DEX group. In all treated groups, total cells (neutrophils, lymphocytes, monocytes and eosinophils) of bronchoalveolar lavage fluid (BALF) also decreased as compared to asthmatic control. ELISA results confirmed that the levels of interleukins such as IL-4, IL-5, IL-13, IL-17A, IgE decreased in treated groups as compared to asthmatic control. However, over stimulation IL-10 was observed in treated groups than asthmatic control. Real-time PCR results confirmed that the relative mRNA expression of cytokines (IL-4, IL-5, IL-13, IL-17A) reduce in all treated groups as compared to asthmatic control. Moreover, over stimulation IL-10 (anti-cytokine) was seen in all treated groups as compared to asthmatic control. Histological studies revealed that airways constrictions (epithelium thickness, smooth muscle thickness and bronchiole) were reduced in all treated groups except asthmatic control. The study concludes that *C. fistula* has shown potential to reduce asthma, improved hematological parameters positively and suppresses and overshoots the cytokines and anti-cytokine, respectively. *C. fistula* reduces airways remodeling in asthmatic mice.

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Key words
Ovalbumin, *Cassia fistula*, ELISA, Asthma, Mice

INTRODUCTION

Asthma is a multifaceted chronic inflammatory disease that targets the airways of the lungs (Sinyor and Perez, 2023). Its pathogenesis is characterized by hyper-responsiveness, reversible obstruction, mucus hypersecretion and airwaey remodeling. Notably, it is associated with Th2-mediated overexpression of cytokines (Porter *et al*., 2011; Rosenberg, 2013; Endo *et al*., 2014). In asthmatic control, growth failure, immunodeficiency, adrenal insufficiency and delayed puberty are induced by asthma medications (glucocorticoids, antihistamines, and immunosuppressant) (Sousa *et al*., 2000; Sakuma *et al*., 2001). Consequently, the innovative research has focused to find out natural ingredients containing pharmacologically active elements to offer new treatments without undesirable aspect consequences (Cragg and Newman, 2013).

Several extracts of natural herbs, including *Samsoeum* (Jeon *et al*., 2015), *Erythronium japonicum* in East Asia (Seo *et al*., 2016), *Trigonella foenum-graecum* in Central Asia and Eastern Europe (Piao *et al*., 2017), *Echinodorus scaber* Rataj in the western hemisphere (Rosa *et al*., 2017), and *Urtica dioica* in Asia, Europe, Africa, and Americas (Zemmouri *et al*., 2017) have been studied. Mostly breakdown of molecules, and functional components, fermented natural herbs have only been studied for their therapeutic effects in an asthma model (Xiao *et al*., 2015).

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Fever, biliousness, heart diseases and snake bite are cured by the roots and fruits of *C. fistula*. Cough is treated by pod of *C. fistula* with honey (Bhalerao and Kelkar, 2012). The fruits and pulp of *C. fistula* are used to treat asthma and liver cancer, respectively (Chaerunisaa *et al*., 2018). Leaves, bark and fruits of *C. fistula* are used to treat anti-dysenteric, anti-bacterial, anti-fungal anti-pyretic, and anti-diabetic. Anthrax, leprosy, chest pain, eyes redness, cytotoxic and abdominal obstruction are also treated by leaves, bark and fruits of *C. fistula* (Sullivan and Turk, 2008). Stimulation of uterine function and inhibition of ovarian function in albino mice are induced by ethanoic flower extract of *C. fistula* (Chaerunisaa *et al*., 2018). The main objective of the study is to investigate the anti-asthmatic effect of herbal extracts of *C. fistula* using mice model.

MATERIALS AND METHODS

Preparation of extracts

Cassia fistula extraction by 1ml of acetone (Merck Sigma Aldrich, U.K) for 10 mg of bark (Kotze *et al*., 2002; Martini and Eloff, 1998). We added 3ml acetone in 30 mg of bark powder followed by centrifugation at 4000 rpm for 10 min. The extraction was taken in test tubes with 50 ml of polyester (Hettich Centrifuge, Rotofix32A, Labotec, Johannesburg, South Africa). With the help of Whitman filter paper, the supernatants were poured into pre-weighed test tubes and evaporated to dryness under a stream of cold air. The concentrated extracts were stored at 5 °C (Elisha *et al*., 2017).

Experimental animals

Eight weeks old healthy Swiss albino mice (males) weighing 30-35g were obtained from University of Veterinary and Animal Sciences, Lahore, Pakistan. Mice were acclimatized to the experimental environment for 2 weeks. Animal handling during the experiment was reviewed and approved by the ethical Committee of University of Gujrat. The mice had full access to the normal irradiated chow meal and water throughout the experiment. All mice were kept in a specified pathogen-free environment (SPF) with a rigorous lighting schedule (lights on at 08:00 and off at 20:00) (Jung *et al*., 2011; Zhou *et al*., 2014).

Asthma induction and dose design

Eighteen mature mice were selected randomly from the stock and divided into six treatment groups (n=3) i.e., control, OVA, dexamethasone (DEX), *C. fistula* (10mg/ml), *C. fistula* (15mg/ml) and *C. fistula* (20mg/ml). Asthma induction was divided into four main steps. In the first step, 200µl PBS (phosphate-buffered saline) containing 20µg OVA and 1mg aluminum hydroxide were inoculated in all groups of mice except control group of mice via intraperitoneal injection as a supplement on day 1 and 14. In the second step, the group i.e., *C. fistula* was treated with 10, 15, and 20 mg/ml (Elisha *et al*., 2017) and DEX group were treated with dexamethasone (3mg/ml) on the 15th-26th days. Moreover, in the third step, saline equivalent phosphate-buffered solution was administered to all the control mice. In the last step, mice were sensitized with 20µl phosphate-buffered saline solution along with 100µg OVA through intranasal inhalation on the 27th, 28th and 29th days except control group (Fig. 1). Moreover, the selected mice were administered orally. In order to control error, groups were replicated.

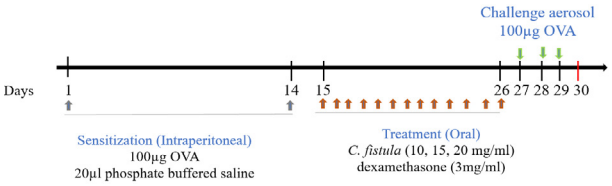

Fig. 1. Summary of asthma induction and dose design.

Detection of liver enzyme and CBC

The post-treatment levels of hematological parameters (Hb, HCT, MCV, MCH, MCHC, RBCs, WBCs, and platelets), liver enzymes (ALT, AST, ALP, and albumins, globulins, total proteins and bilirubin), CBC and LFTs were performed in the Department of Zoology Lab using the Micro-Lab Chemistry Analyzer (Park *et al*., 2009; Sagar *et al*., 2014).

Enumeration of total cells in bronchoalveolar lavage fluid (BALF)

Chloroform was used to anaesthetize the mice, lungs were washed thrice with cold 1 x PBS, resulting in 80% yield of BALF with volume of 0.8 ml. BALF was centrifuged at 2,000 rpm for five min at 4 °C, and the supernatant was then taken for ELISA analysis. While the pellets were used for cell examination. The total cells of the BALF pellet were attached to a sliding glass using a Cytospin for 5 min, with a speed of 500 rpm. Then, they were fixed in methanol for 30 sec. Slides were subjected to treatment with May-Grunwald solution (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 5 min, followed by Giemsa solution (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 12 min. The slides were covered after three rinses, and then immune cells were counted at a 400x magnification using light microscopy (Choi *et al*., 2018).

Table I. Details of primers developed in this study.

Primer	Primer sequences (5' to 3')	References
IL-4	F: TCCGACCACCACTACAGCAA	(EdanAlsaimary and Mezban, 2021)
	R: ATCTTTCAACACGCAGGACA	
IL-5	F: CTCTGTTGACAAGCAATGAGACG	(Pang *et al.*, 2021)
	R: TCTTCAGTATGTCTAGCCCCTG	
IL-10	F: ACACATGGTATAGATGCAGC	(EdanAlsaimary and Mezban, 2021)
	R: TTCCAAGACCTCAGGCAAGA	
IL-13	F: TGTGTCTCTCCCTCTGACCC	(Pang *et al.*, 2021)
	R: CACACTCCATACCATGCTGC	
IL-17A	F: CAGAAGACCTACATGTTACT	(Du *et al.*, 2016)
	R: GTAGCGCTATCGTCTCTCT	
β-actin	F: ACGGCCAGGTCATCACTATTG	(Livak and Schmittgen, 2001)
	R: CAAGAAGGAAGGCTG GAAAAGA	

Enzyme-linked immunosorbent assay (ELISA) for interleukins and IgE in BALF

The IL-4, IL-5, IL-10, IL-13, IL-17A and IgE in BALF were measured using ELISA kits (BioLegend, San Diego, CA, USA). Anti-IL-4, IL-5, IL-10, IL-13, IL-17A and IgE antibodies were reacted with on a 96-well plate for 2 h at room temperature using mixtures of BALF (or serum, 50 µL each) and assay buffer (50 µL). Unbound proteins were then removed from the wells, followed by the addition of detection antibody solution (100 µL) and avidin-horseradish peroxidase (HRP) D solution (100 L). The samples were then allowed to sit at room temperature for 30 min. The reaction was then stopped with blocking solution (100 µL) after these combinations were treated with substrate solution (100 µL) for 15 min. Using a Versa-max plate reader, the mixture's absorbance was then measured at 450 nm (Molecular Devices, San Jose, CA, USA) (Choi *et al*., 2018).

Analysis for cytokine gene expression

To assess the impact of *C. fistula* bark extract on the expression of interleukins (*IL-4, IL-5, IL-10, IL-13*, and *IL-17A*) genes in samples, we used quantitative Real-time PCR. By utilizing quantitative real-time PCR, expression of IL-4, IL-5, IL-10, IL-13, IL-17A, and β-actin mRNA levels were assessed (Kim *et al*., 2014). The primer sequences used in our study are provided in Table I.

Histological analysis

The histological characteristics were examined the epithermal thickness, smooth muscle thickness and bronchiole/ mast cells of mice lung. For this purpose, left lungs removed and fixed for 48 h in 10% neutral buffered formalin. The central lobes of the frozen tissues were then precisely cut off and implanted in the same orientation and position to create paraffin blocks. The lung section forms were gathered from the entire series (70%, 90%, 100% alcohol) of the section, after sectioning the block into 4 m thick slices. The sections were then stained with hematoxylin and eosin, periodic acid-Schiff, and toluidine blue stains (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). These were then microscopically evaluated for histopathological characteristics at 400x magnification. Histopathological characteristics like epithermal thickness and smooth muscle thickness, goblet cell hyperplasia and bronchiole, mast cells, and dysplasia.

The Leica Application Suite (Leica Microsystems, Wentzler, Germany) was used to measure the epithelial and smooth muscle thickness in the bronchial part. Additionally, based on a prior investigation, two independent researchers scored the level of cell infiltration in the airway in a double-blind screen (Lee *et al*., 2011).

A scale was used to grade the degree of peri-bronchiole and peri-vascular inflammation. The scale used (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). The average scores of the five airway sections that were randomly placed across the left lung of each mouse were calculated.

The formation of mucus was detected by goblet cell hyperplasia using periodic acid-Schiff (PAS) staining. After the lung sections were deparaffinized and dehydrated, the samples were oxidized in periodic acid solution for five min, washed, and deposited in Schiff reagent for 15 min (Lee *et al*., 2011).

Statistical analysis

Analysis of Variance (ANOVA) was applied to determine the effects of doses on different parameters and Tukey's post hoc was used for multiple comparisons when results showed statistical significance among treatment groups ($p< 0.05$).

RESULTS

CBC in asthmatic mice receiving the bark extract of C. fistula

The concentrations of hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, platelets and mean corpuscular hemoglobin concentrations in red blood cells were decreased in OVA-treated group. While the concentration of neutrophils, lymphocytes, monocytes and eosinophils in white blood cells were elevated in OVA-treated group (Table II). In all treated groups that received *C. fistula*, there were a noticeable rebuilding of altered levels of CBC (Table II).

Improved LFTs in asthmatic mice receiving the bark extract of C. fistula

The level of bilirubin ALT, AST, ALP, total proteins, albumin and globulin in blood serum were compared to OVA-treated group and non-treated group with under control set. The more toxicity that is caused by asthma was mitigated through the administration of *C. fistula*. With the help of concentration-based method, the targeted results of bilirubin, ALT, AST, ALP, total proteins, albumin and globulin were ensured from *C. fistula* results. The concentrations of bilirubin, ALT, AST, ALP, total proteins, albumin and globulin were enhanced in OVA-treated group. In all treated groups that received *C. fistula*, there were a noticeable restoring of these altered levels of LFTs (Table III). From the calm values of normal group, demonstrated non beneficial change. These results show that *C. fistula* treatment could potentially positively effect on LFTs in the immune system of the OVA-induced asthma model.

Table II. Anti-asthmatic effect (Mean ± STD) of *C. fistula* on CBC of male mice.

Parameters	Normal	OVA+ *C. fistula*			OVA+ DEX (3mg/ml)	OVA asthma control
		10mg/ml	15mg/ml	20mg/ml		
WBC ($x10^3/\mu L$)	3.76 ± 0.02^{e}	6.27 ± 0.03^{b}	5.79 ± 0.17^{c}	4.77 ± 0.02^{d}	5.85 ± 0.07^{c}	12.8 ± 0.1^{a}
RBC ($x10^6/\mu L$)	7.53 ± 0.05^{a}	5.29 ± 0.02^{e}	6.5 ± 0.11^{d}	7.12 ± 0.01^{b}	6.93 ± 0.04^{c}	3.2 ± 0.03^{f}
Platelets (x103/µL)	922 ± 5.29^{a}	872 ± 3^{d}	884.67 ± 4.51^{c}	912 ± 3.61^{a}	901.67 ± 3.06^{b}	621 ± 2^{e}
MCV (fL)	41.23 ± 0.03^{a}	30.15 ± 0.02^{d}	34.60 ± 0.16^{c}	36.20 ± 0.03^{b}	36.39 ± 0.13^{b}	21.53 ± 0.01^{e}
HCT (%)	35.8 ± 0.17^{a}	30.12 ± 0.04^{d}	32.4 ± 0.44^{c}	34.87 ± 0.06^{b}	34.77 ± 0.15^{b}	19.52 ± 0.02^{e}
MCH (pg)	19.05 ± 0.05^{a}	15.2 ± 0.05^{e}	15.68 ± 0.03^{d}	18.13 ± 0.03^{b}	17.12 ± 0.02^{c}	8.22 ± 0.02^{f}
Hemoglobin (g/dL)	12.57 ± 0.25^{a}	9.73 ± 0.15^{d}	10.34 ± 0.1^{c}	11.4 ± 0.10^{b}	10.6 ± 0.3^{c}	5.84 ± 0.07^{e}
MCHC (g/µL)	38.47 ± 0.4^{a}	34.52 ± 0.03^{c}	35.38 ± 0.41^{c}	37.87 ± 0.02^{ab}	37.1 ± 0.6^{b}	21.8 ± 0.02^{d}
Neutrophils ($x10^3/\mu L$)	0.58 ± 0.01^{d}	0.98 ± 0.01^{bc}	0.95 ± 0.15^{bc}	0.83 ± 0.02^{c}	1.06 ± 0.04^{b}	4.33 ± 0.02^{a}
Lymphocytes ($x10^3/\mu L$)	0.87 ± 0.02^{f}	2.65 ± 0.02^{c}	2.46 ± 0.1^{d}	2.02 ± 0.01^{e}	2.81 ± 0.05^{b}	5.73 ± 0.03^{a}
Monocytes ($x10^3/\mu L$)	0.84 ± 0.02^{c}	2.21 ± 0.53^{b}	2.08 ± 0.16^{b}	1.74 ± 0.03^{b}	2.14 ± 0.06^{b}	6.3 ± 0.01^{a}
Eosinophils ($x10^3/\mu L$)	0.54 ± 0.02^{e}	1.47 ± 0.08^{c}	1.29 ± 0.08^{d}	1.25 ± 0.03^{d}	1.94 ± 0.05^{b}	3.96 ± 0.04^{a}

Means with different superscripts in a row are significantly different from one another ($p\leq0.05$) Tukey's test.

Table III. Anti-asthmatic effect (Mean ± STD) of *C. fistula* on Liver function tests of male mice.

Parameters	Normal	OVA+ *C. fistula*			OVA+ DEX (3mg/ml)	OVA asthma control
		10mg/ml	15mg/ml	20mg/ml		
Bilirubin (mg/dL)	0.21 ± 0.01^{e}	0.34 ± 0.02^{b}	0.31 ± 0.01^{c}	0.23 ± 0.01^{d}	0.34 ± 0.02^{b}	1.43 ± 0.01^{a}
ALT (U/L)	23.54 ± 0.03^{d}	25.67 ± 0.02^{b}	25.62 ± 0.23^{b}	24.63 ± 0.45^{c}	25.57 ± 0.02^{b}	45.97 ± 0.02^{a}
AST (U/L)	57.33 ± 0.29^{d}	60.92 ± 0.02^{b}	60.86 ± 0.28^{b}	59.5 ± 0.58^{c}	60.55 ± 0.05^{b}	89.52 ± 0.03^{a}
Alkaline phosphatase (U/L)	76.98 ± 0.01^{e}	79.19 ± 0.37^{bc}	78.98 ± 0.02^{cd}	78.24 ± 0.48^{d}	79.97 ± 0.04^{b}	102.5 ± 0.26^{a}
Total proteins (mg/dL)	5.3 ± 0.08^{bc}	5.45 ± 0.04^{b}	5.36 ± 0.06^{bc}	5.26 ± 0.02^{c}	5.26 ± 0.08^{c}	7.54 ± 0.03^{a}
Albumin (g/dL)	3.77 ± 0.12^{c}	4.06 ± 0.03^{b}	4.04 ± 0.06^{b}	3.98 ± 0.01^{b}	3.99 ± 0.01^{b}	6.15 ± 0.04^{a}
Globulin (g/dL)	2.1 ± 0.01^{e}	2.48 ± 0.02^{b}	2.40 ± 0.02^{c}	2.30 ± 0.06^{d}	2.37 ± 0.02^{c}	3.60 ± 0.02^{a}

Means with different superscripts in a row are significantly different from one another ($p\leq0.05$) Tukey's test.

Table IV. Anti-asthmatic effect (Mean ± STD) of *C. fistula* on flame cells of BALF male mice.

Parameters	Normal	OVA+ *C. fistula*			OVA+ DEX (3mg/ml)	OVA asthma control
		10mg/ml	15mg/ml	20mg/ml		
Neutrophils (x10³/ml)	2.26 ± 0.04^{c}	4.95 ± 0.05^{bc}	4.8 ± 0.05^{bc}	4.25 ± 0.25^{bc}	5.5 ± 1.50^{b}	12 ± 2.00^{a}
Lymphocytes (x10³/ml)	0.32 ± 0.01^{b}	0.71 ± 0.03^{b}	0.69 ± 0.03^{b}	0.65 ± 0.05^{b}	0.75 ± 0.03^{b}	2.58 ± 0.63^{a}
Monocytes (x10³/ml)	45.58 ± 0.03^{b}	45.93 ± 0.03^{b}	45.74 ± 0.04^{b}	45.65 ± 0.13^{b}	46.67 ± 1.15^{b}	96.67 ± 6.11^{a}
Eosinophils (x10³/ml)	2.22 ± 0.04^{b}	4.69 ± 0.03^{b}	4.15 ± 0.05^{b}	3.55 ± 0.04^{b}	5.46 ± 0.31^{b}	13.33 ± 3.06^{a}
Total cells (x10³/ml)	42.17 ± 2.02^{d}	54.36 ± 0.31^{b}	50.37 ± 0.32^{bc}	46.23 ± 0.10^{cd}	54.67 ± 3.06^{b}	154 ± 4.00^{a}

Means with different superscripts in a row are significantly different from one another ($p \leq 0.05$) Tukey's test.

Table V. Anti-asthmatic effect (Mean ± STD) of *C. fistula on* real time PCR of male mice.

Parameters	Normal	OVA+ *C. fistula*			OVA+ DEX (3mg/ml)	OVA asthma control
		10mg/ml	15mg/ml	20mg/ml		
IL-4 (pg/ml)	0.34 ± 0.05^{d}	0.94 ± 0.02^{b}	0.71 ± 0.02^{bc}	0.45 ± 0.02^{d}	0.49 ± 0.01^{cd}	3.23 ± 0.21^{a}
IL-5 (pg/ml)	1.75 ± 0.04^{d}	3.27 ± 0.02^{b}	2.49 ± 0.02^{c}	2.23 ± 0.02^{c}	2.29 ± 0.02^{c}	12.19 ± 0.36^{a}
IL-10 (pg/ml)	0.92 ± 0.03^{f}	2.88 ± 0.11^{d}	3.9 ± 0.01^{c}	6.42 ± 0.03^{a}	5.43 ± 0.02^{b}	2.67 ± 0.06^{e}
IL-13 (pg/ml)	0.67 ± 0.03^{d}	1.77 ± 0.03^{b}	1.56 ± 0.04^{bc}	1.27 ± 0.02^{c}	1.33 ± 0.03^{c}	5.49 ± 0.34^{a}
IL-17A (pg/ml)	0.35 ± 0.02^{c}	0.41 ± 0.01^{b}	0.39 ± 0.01^{bc}	0.37 ± 0.01^{bc}	0.4 ± 0.01^{b}	1.55 ± 0.03^{a}

Means with different superscripts in a row are significantly different from one another ($p \leq 0.05$) Tukey's Test.

C. fistula *suppresses leukocytes inflow into BALF of OVA-induced asthma mice*

Leukocyte influx into the BALF of an asthma mice induced by OVA is suppressed by *C. fistula*. In contrast to the OVA group, the treated groups OVA + DEX, OVA + *C. fistula* (10mg/ml), OVA + *C. fistula* (15mg/ml) and OVA + *C. fistula* (20mg/ml) demonstrated a significant decrease in the number of total cells, neutrophils, lymphocytes, monocytes and eosinophils in BALF (Table IV). *C. fistula*'s suppressive effects were remarkably comparable to those of the positive control drug DEX. These results show that *C. fistula* therapy reduced the infiltration of leukocytes into the bronchoalveolar fluid following OVA inhalation.

Effect of C. fistula *on the production of cytokines of BALF of OVA-induced asthma mice*

The OVA treated group had greater levels of IL-4, IL-5, IL-13, IL-17A and IgE, except 1L-10, indicating that the asthma model's OVA induction was successful. The level of IL-10 was high in the DEX, *C. fistula* (10mg/ml), *C. fistula* (15mg/ml) and *C. fistula* (20mg/ml) treated groups as compared to OVA-treated group. On the other hand, in contrast to the treated group, DEX, *C. fistula* (10mg/ml), *C. fistula* (15mg/ml) and *C. fistula* (20mg/ml) treated groups show a drop in the levels of IL-4, IL-5, 1L-10, IL-13, IL-17A and IgE. As a result, the current findings show that *C. fistula* therapy successfully reduces the generation of IL-4, IL-5, IL-13, IL-17A and IgE except 1L-10 (Fig. 2).

Change in expression of key cytokines in OVA-induced asthma model

The OVA group had greater mRNA levels of IL-4, IL-5, IL-10, IL-13 and IL-17A except IL-10 Even though the reduction ratio varied, these levels significantly declined in the DEX, *C. fistula* (10mg/ml), *C. fistula* (15mg/ml) and *C. fistula* (20mg/ml) treated groups in comparison to the OVA group. The level of IL-10 mRNA increased in OVA-group as compared to un-treated group, and the level of IL-10 was increased in the DEX, *C. fistula* (10mg/ml), *C. fistula* (15mg/ml) and *C. fistula* (20mg/ml) treated groups as compared to OVA-treated group. Following *C. fistula* treatment, the five cytokines markedly and dose-dependently decreased in the lung tissue (Table V).

These findings show that in the OVA-induced model, *C. fistula* administration reduced the production of Th2-like and pro-inflammatory cytokines during airway inflammation. Using an image densitometer (ChemiDoc), band intensities were measured and four protein expressions were compared to the strength of the β-actin bands (Fig. 3).

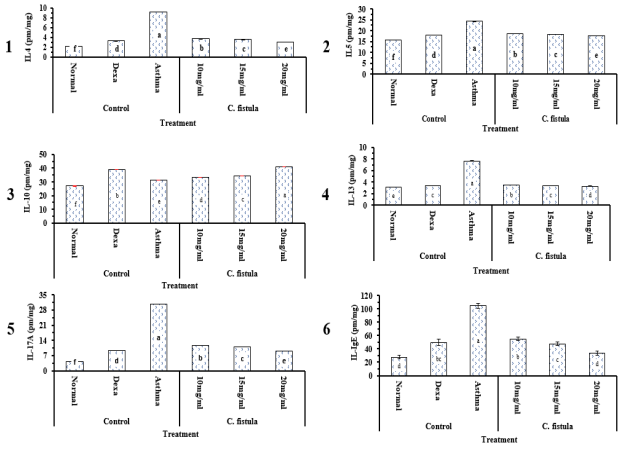

Fig. 2. **1,** An enzyme-linked immunosorbent assay was used to quantify the amount of IL-4. **2**, An enzyme-linked immunosorbent assay was used to quantify the amount of IL-5. **3,** An enzyme-linked immunosorbent assay was used to quantify the amount of IL-10. **4**, An enzyme-linked immunosorbent assay was used to quantify the amount of IL-13. **5,** An enzyme-linked immunosorbent assay was used to quantify the amount of IL-17A. **6,** An enzyme-linked immunosorbent assay was used to quantify the amount of IgE.

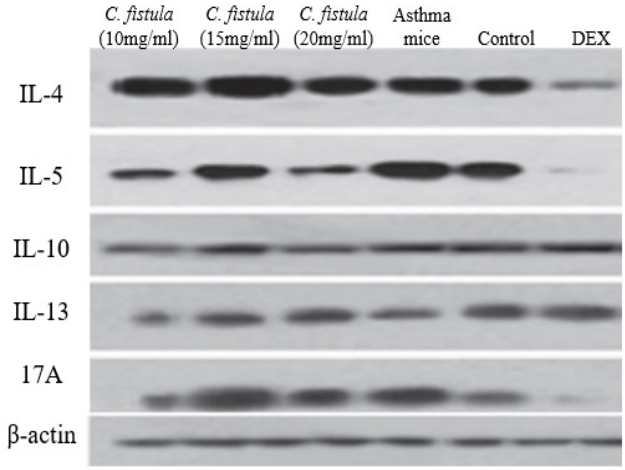

Fig. 3. Bands strength showed five interleukins' expressions with compared to the strength of the β-actin bands (internal control).

Changes in inflammatory cell infiltration, epithelial and smooth muscle thickness damage of OVA-induced asthma model

In lung tissue sections from mice that had been sensitized with OVA group, we noticed a thicker respiratory epithelium and smooth muscle thickness. In contrast to the OVA group, the thickness dramatically decreased in the DEX, *C. fistula* (10mg/ml), *C. fistula* (15mg/ml) and *C. fistula* (20mg/ml) treated groups. Moreover, in all groups that received *C. fistula*, there were a noticeable reduction in the infiltration of inflammatory cells in the peribronchiolar region (Table VI). In the bronchial airways of mice after OVA sensitization, the OVA treated group exhibited a higher mucus score than the No-treated group, demonstrating goblet cell hyperplasia (Fig. 4). These levels were, however, considerably lower in the DEX, *C. fistula* (10mg/ml), *C. fistula* (15mg/ml) and *C. fistula* (20mg/ml) treated groups as compared to the OVA treated group.

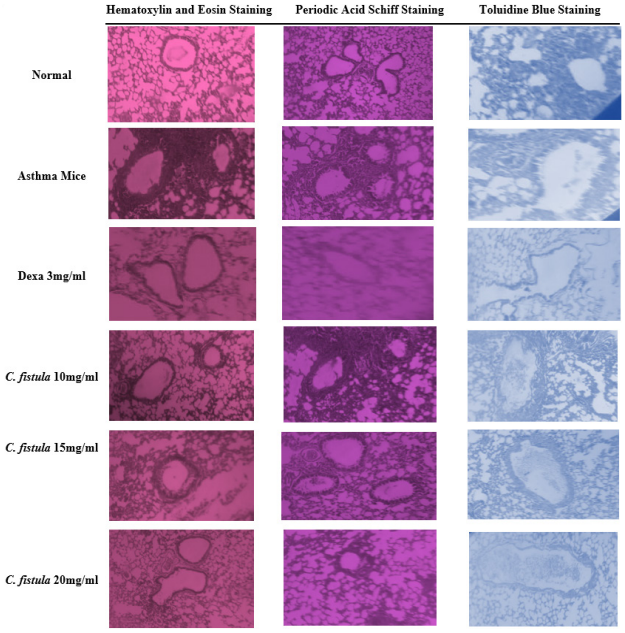

Fig. 4. Effect of *C. fistula* on airway inflammation in lung tissues. Lung tissue stained with H and E solution was examined under 400x magnification to note the thickness of the epithelium and smooth muscle. The Leica application suite (Bar scale 50µm) was used to measure changes in the epithelial and smooth muscle thickness of lung tissue. Periodic Acid-Schiff (pas) stained lung tissue was examined under 400x magnification and demonstrated mucus production (Hyperplasia of goblet cells). Examining lung tissue stained with toluidine blue at a 400x magnification revealed dysplasia (abnormal cell development within lung tissues) and mast cell degranulation.

DISCUSSION

The herbals medicines offer certain advantages including high potency, non-toxicity, low cost, greater tolerability, more protection and easy accessibility in the treatment of asthma. The main benefits of traditional herbal drugs include high potency, non-toxicity, low cost

Table VI. Anti-asthmatic effect (Mean±STD) of *C. fistula* on airways size of lung of male mice.

Parameters	Normal	OVA+ *C. fistula*			OVA+ DEX (3mg/ml)	OVA Asthma control
		10mg/ml	15mg/ml	20mg/ml		
Epithelium thickness (µm)	18.05 ± 0.18^{d}	19.35 ± 0.15^{b}	18.74 ± 0.04^{c}	18.18 ± 0.08^{d}	18.23 ± 0.1^{d}	34.27 ± 0.25^{a}
Smooth muscle thickness (µm)	2.94 ± 0.05^{e}	4.54 ± 0.06^{a}	4.08 ± 0.08^{c}	3.03 ± 0.06^{e}	3.33 ± 0.1^{d}	4.74 ± 0.04^{a}
Mast cells/bronchiole (0-5)	0.56 ± 0.04^{e}	1.75 ± 0.03^{b}	0.84 ± 0.04^{d}	0.76 ± 0.04^{d}	1.02 ± 0.07^{c}	3.8 ± 0.05^{a}

Means with different superscripts in a row are significantly different from one another ($p \leq 0.05$) Tukey's Test

(Angami *et al*., 2021), greater tolerability, more protection and easy accessibility (Vikas *et al*., 2013) for patient as compared to synthetic drugs.

In our study, administering *C. fistula* resulted in significantly reduced airway remodeling and inflammation of the lungs in OVA-induced asthma in model mice. This indicated that *C. fistula* may have therapeutic potential for asthmatic patient. Folic acid reduction, decrease of erythropoiesis, reduction in globin production that were observed during preparation of OVA-treated group. Oval-albumin on tissues of erythropoiesis can cause reduction in red blood cells count damage can be caused by administrational OVA. The unpaired erythrocyte formation or decrease of the numbers of hemoglobin or red blood cells is caused by anemia hypochromic disclosure. The amount of pro-inflammatory cytokines was caused by OVA which had negative effect on liver and lung activities (Yadav *et al*., 2010). The reduction in white blood cells along with reduction in kidney and liver toxicant concentrations due to suppression of hematopoietic process (Antai *et al*., 2009).

In our study, the values of platelets and RBC parameters (MCV, HCT, MCH, hemoglobin and MCHC) were markedly decreased in OVA-treated group as compared to all other groups including *C. fistula*, *A. nilotica, A. esculentus*, DEX and normal group. These results show that *C. fistula* treatment could potentially positively effect on blood profile in the immune system of the OVA-induced asthma model. The previous studies of *Morus indica* roots (Boro *et al*., 2022) and *Tinospora cordifolia* (Sharma and Pandey, 2010) were conducted on the blood profile in male mice. The CBC levels were returned to normal values.

On the other hand, the white blood cells (WBCs) like neutrophils, lymphocytes, monocytes and eosinophils showed a remarkable increase in OVA-treated group. In case of treated groups, the number of WBCs was closest to NC in *C. fistula* (20mg/ml) and DEX followed by *C. fistula* (15mg/ml) and *C. fistula* (10mg/ml). These results indicate that *C. fistula* has a potent role in the reduction of asthma symptoms. Effects of herbal extracts of *M. indica* roots (Boro *et al*., 2022) and *Avicennia marina* were demonstrated in previous studies. These authors also observed that the rebuilding of hematological parameters took place by the treatment with *M. indica* roots and *A. marina*. In the final version we can find out that some preventive measures need to be taken to avoid from immunological damages.

Bilirubin, ALT, AST, ALP, total proteins, albumin and globulin levels in the serum were important indicator for the analysis of hepatic functioning. The accurate cell damage was observed when the significant activities of liver were increased by the induction of OVA. The hepatoprotective activity was shown when the *C. fistula* is induced in mice with the reduction levels of bilirubin, ALT, AST, ALP, total proteins, albumin and globulin. Our study revealed that some of indices and enzymes were significantly normal with the dosage of *C. fistula*. The noticeable recovery of LFT parameters in *C. fistula* treated group correlated with reduction in symptoms of liver damage suggests that the *C. fistula* could serve as a potentially effective treatment in asthma related liver disease. Effect of herbal extracts of *Maytenus royleanus* (Shabbir *et al*., 2020) and *Cassia spectabilis* (Ekasari *et al*., 2022) were demonstrated in previous studies. The authors also observed a significant restoration of LFTs parameters i.e., ALT, AST, ALP and bilirubin in an ovalbumin induced asthma mouse model which is an indication of effectiveness of herbal treatment in treating asthma.

As eosinophils are the main regulators of airway remodeling, thus we also looked at changes in the inflow of leukocytes, including total cells, neutrophils, lymphocytes, monocytes and eosinophils (Alam and Busse, 2004). An important mechanism underpinning the pathogenesis of asthma, including airway remodeling and inflammation, is eosinophil-mediated damage (Camateros *et al*., 2007).

The total number of leukocytes (neutrophils, lymphocytes, monocytes and eosinophils) in the BALF of animals treated with *C. fistula* in the current investigation was significantly lower than that of animals treated with an OVA group. Our findings support a previous study that found various herbal products, including EM-X of unpolished rice, papaya, and seaweed, had therapeutic

effects in a mouse model of OVA-induced asthma. These findings reveal that BAW limits the inflow of leukocytes during airway inflammation (Higa and Ke, 2001).

Since the inflammation along with asthma was characterized by the invasion of Th2 cells and leukocytes (Ngoc *et al*., 2005) and was characterized by raised IL-4, IL-5, IL-13, IL-17A and IgE in BALF serum (Endo *et al*., 2014), changes in IL-4, IL-5, IL-13, IL-17A and IgE levels are thought to be important markers of anti-asthmatic effects. Th2 cytokines (IL-4, IL-5, IL-13 and IL-17A)/ Th1 (IL-10) had a variety of immunological effects, such as promoting/ demotion control of Ig class switching, promotion/demotion of mast cell proliferation/ non-proliferation, and Th2/Th1 lineage differentiation (Li-Weber and Krammer, 2003).

The elevation in the values of IL-4, IL-5, IL-13 and IL-17A, and IgE was correlated with increasing level of inflammation whereas the increasing level of IL-10 alleviate the inflammatory symptoms. The decline in inflammatory cytokinesis and the elevation in anti-inflammatory cytokines of *C. fistula* group suggest that *C. fistula* could play a vital role in alleviating the allergic symptoms in ovalbumin induced asthma mice model. Two earlier publications demonstrated the connection between fermented natural products and stimulation of anti-asthmatic characteristics. In the trachea and lungs of experimental asthmatic mice, therapy with *Artemisia princeps* Pampanini fermented with *Bifidobacterium infantis* K-525 revealed a decrease in IgE and cytokine levels (Bae *et al*., 2007). Another study revealed that the level of anti-inflammatory cytokines like IL-10 was observed to rise after treatment with *H. tiubae* (Mozzini Monteiro *et al*., 2016).

Real-time PCR, a nucleic acid sensitivity test, is used to quantify nucleic acids in biological samples. The expression and quantification of mRNA of inflammatory, anti-inflammatory cytokines in the lung tissues of treated and non-treated groups were assessed by application of Real-time PCR. Moreover, the mRNA expression level of IL-4, IL-5, IL-13, IL-17A and except IL-10 with real-time PCR was reduced by the administration of *C. fistula*. Conversely, it was found out that the mRNA expression of anti-inflammatory cytokines i.e., IL-10 was highest in *C. fistula* (20mg/ml) followed by DEX, *C. fistula* (15mg/ml), *C. fistula* (10mg/ml) and OVA group. These comparisons demonstrate that herbal extraction can be used to increase the anti-asthmatic properties of *C. fistula.* Band intensities were measured using a ChemiDoc, and the strength of the actin bands was compared to five protein expressions. After treatment with *M. indica* extracts, IL-13 expression was significantly lowered (Adesina *et al*., 2017), demonstrating the efficacy of herbal therapy in preventing the development of inflammation in asthma patients.

Histology in the lung tissue of an OVA-induced asthma model was observed after exposure to herbal extracts, DEX and OVA groups. For this purpose, the suppression of airway inflammation (including inflammatory cell infiltration and respiratory epithelium hyperplasia) and the inhibition of airway remodeling including excessive mucus production, collagen deposition, and angiogenesis were find out from OVA, DEX and *C. fistula* groups in lung tissue of asthmatic mice. *C. fistula* could mitigate inflammatory cell infiltration, epithelial and smooth muscle thickness damages in the airways. Moreover, groups who received *C. fistula* treatment showed a considerable improvement in goblet cell hyperplasia and collagen layer thickness. On comparing the results, it was found out that the highest airway remodeling occurred in *C. fistula* group (20mg/ml) followed by DEX, *C. fistula* group (15mg/ml) and *C. fistula* group (10mg/ml). These results suggest that *C. fistula* treatment could potentially alleviate the inflammatory response and associated epithelial and smooth muscle thickness damages in the airways of the OVA-induced asthma model.

These results are consistent with earlier *in vivo* research, where the activation of airway remodeling in OVA-treated BALB/c mice has been reported to be induced by a number of natural items, including *Vitex rotundifolia* L. (Bae *et al*., 2013), *Astragali radix* anti-asthmatic decoction (Xu *et al*., 2013), Bangpungtongseong-san (Lee *et al*., 2014), and Suhuang antitussive capsule (Zhang *et al*., 2016). These comparisons demonstrate that herbal extraction can be used to redcue the asthma symptoms.

CONCLUSION

Cassia fistula bark might reduce airway remodeling and inflammation in a mouse model of asthma caused by OVA. Overall, our findings indicate that *C. fistula* has the potential to alleviate asthma symptoms. Leukocyte inflow, OVA-specific IgE generation, Th2 cell activation, inflammatory cell infiltration, respiratory epithelial hyperplasia, goblet cell hyperplasia, angiogenesis, and collagen deposition are among the anti-asthmatic actions of *C. fistula*. The asthmatic patients treated with the extract of some herbal plant was advised to use with some protective measure to avoid from the chances of heamotoxicology and hepaticology. *C. fistula* has shown potential to reduce asthma, improved hematological parameters positively and suppresses and overshoots the cytokines and anti-cytokine, respectively.

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Ethical approval

The study was approved by Ethical Review Committee of the University of Gujrat (UOG/ORIC/2024/58).

Statement of conflict of interest

The authors have declared no conflict of interest.

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