Original Article

Anti-inflammatory and Immunomodulatory Effects of *Albizia lebbeck* in Experimental Model of Bronchial Asthma

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Abstract

Background and objective. Bronchial asthma is a heterogeneous disease, usually characterised by chronic airway inflammation, reversible airway narrowing, airway hyperresponsiveness (AHR) and airway remodelling. The study was designed to evaluate the anti-inflammatory and immunomodulatory potentials of *Albizia lebbeck* in experimental model of bronchial asthma in rats.

Methods. Wistar rats were immunised with ovalbumin (OVA) on day 0 and were challenged with 1% OVA aerosol for 20 minutes daily, from 15th to 22nd day to induce inflammatory model of bronchial asthma. Standardised aqueous extract of *Albizia lebbeck (bark)* was administered orally for 22 days at doses of 100, 200 and 400 mg/kg, in separate groups. Rats were anesthetised and blood and bronchoalveolar lavage (BAL) fluid were collected and analysed for markers of inflammation (inflammatory cell counts, tumour necrosis factor-alpha [TNF-α], interleukin-6 [IL-6]) and immunomodulation [Ova sIgE], IL-4 and interferon-gamma [IFN-γ]).

Results. Pre-treatment with *Albizia lebbeck* significantly attenuated the levels of eosinophils, neutrophils, OVA sIgE, TNF-α, IL-6, IL-4 whereas, elevated the levels of IFN-γ in both blood and BAL fluid, thus validating the anti-inflammatory and immunomodulatory effect of the extract.

Conclusion. Taken together, the results showed that standardised extract of *Albizia lebbeck* has anti-inflammatory and immunomodulatory activity as evident from the modulation of cellular and molecular markers of inflammation and immunity in the model of airway inflammation. **[Indian J Chest Dis Allied Sci 2018;60:147-152]**

Key words: Bronchial asthma, Ovalbumin, Inflammation, Immunomodulation and *Albizia lebbeck*

Introduction

Bronchial asthma is a chronic inflammatory disorder of the airways in which many immunological and inflammatory cells play a crucial role.[1] The inflammation causes symptoms which are usually associated with widespread variable airflow obstruction that is often reversible, either spontaneously or with treatment, and causes associated airway hyperresponsiveness (AHR) to a variety of stimuli.[2] Persistent airway inflammation further leads to structural changes in airways known as airway remodelling.[3] The clinical features of asthma include dyspnoea, wheezing and coughing. Drugs used for bronchial asthma are mainly aimed at controlling and/or relieving asthmatic attacks, i.e., anti-inflammatory agents and bronchodilators, and are totally steroid dependent.[4,5] Various local and systemic side effects of these drugs are a major reason for non-compliance.[6] Hence, medicinal plants as therapeutics may be a viable alternative for the treatment of asthma.

In recent decade, complementary and alternative medicine approach using medicinal plants for the prevention and treatment of diseases have been gaining importance.[7] Yoga and Ayurveda complement each other in the treatment of many chronic diseases. Medicinal plants exhibit efficacy in treatment of a number of diseases which are not otherwise cured by synthetic drugs. Herbal drugs are rapidly emerging as safer alternatives/adjuncts in several chronic diseases and this has been shown in some inflammatory disorders. Ancient resources, such as, *Rig Veda, Atharva Veda, Charaka Samhita and Sushruta Samhita* can provide valuable guidelines to the selection, preparation and application of herbal formulation. A large number of medicinal plants have been used traditionally for the treatment of asthma and have been scientifically proven to have anti-asthmatic properties.[8] The literature has described various medicinal plants which have the potential to manage asthma. Out of these, *Albizia lebbeck* was selected on the basis of its importance in ayurvedic literature for the treatment of asthma and bronchitis

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and is traditionally being used. *Albizia lebbeck* (Sirish) is a deciduous plant found throughout India and many ayurvedic preparations like anti-asthma *kada*, *sirisa twakk vatha* contain *Albizia lebbeck*. Its leaves, seed, bark and roots are all used in traditional Indian medicine. The stem bark of this plant is used to treat asthma and bronchitis. It has anti-septic, anti-bacterial, anti-allergic, anti-dysentric and anti-dermatosis action.[9] The anti-inflammatory activity of *Albizia lebbeck* has been documented using petroleum ether, chloroform and ethanol extracts in acute and chronic animal model of inflammation.[10]

The study was designed to evaluate the effects of *Albizia lebbeck (bark)* extract in experimental models of airway inflammation, bronchial hyperreactivity and airway remodelling and possible cellular and molecular mechanisms involved therein. The hypothesis is that, if scientifically validated, *Albizia lebbeck* could have the potential to develop into an important lead compound for drug development and a pharmacological target could be identified in the process. In this study, we investigated the efficacy and pharmacodynamics of extract of *Albizia lebbeck* with reference to its potential therapeutic benefit in bronchial asthma.

Material and Methods

Wistar rats of either gender (150-200 g) were used for the study. Animals were maintained under standard laboratory conditions of natural light-dark cycle and temperature (22±2 °C), and had free access to food and water in the highly specialised experimental animal facility of the V.P. Chest Institute, Delhi. The study protocol was approved by the Institutional Animal Ethics Committee. The standardised *Albizia lebbeck* (bark extract) was procured from Natural Remedies, Bangalore. The doses of extract and prednisolone were selected on the basis of previous pilot studies and some preliminary studies that exhibit inhibitory activity in experimental models of inflammation.[10] All rats were actively sensitised on day 0 with an intra-peritoneal injection of a suspension containing 40mg of ovalbumin (OVA) and 2mg of aluminium hydroxide[11] and were divided into six groups (n=5 per group) as follows: (i) *normal control (NC) group*: 14 days after sensitisation, rats were challenged from 15th–22nd day with 0.9% saline through inhalation using nebuliser chamber for 20 minutes; (ii) *experimental control (EC) group*: after sensitisation on day 0, rats were dosed daily from 1st–22nd day, orally with distilled water. From 15th-22nd day, animals were challenged with 1% OVA through inhalation using nebuliser chamber for 20 minutes after one hour of administration of saline; (iii) *prednisolone (PDN) group*: after sensitisation, rats were dosed daily from 1st–22nd day, orally with 10mg/kg prednisolone. From 15th–22nd day, animals were challenged with 1% OVA through inhalation using nebuliser chamber for 20 minutes after one hour of administration of prednisolone; and (iv) *Albizia lebbeck (100-400 mg/kg) group*: after sensitisation on day 0, rats were dosed daily from 1st–22nd day, orally with 100-400 mg/kg in three *Albizia lebbeck* treatment dosage groups. From 15th – 22nd day, animals were challenged with 1% OVA through inhalation using nebuliser chamber for 20 minutes after one hour of administration of *Albizia lebbeck*.

After 24 hours of last OVA challenge, rats were anesthetised and blood was collected by cardiac puncture, centrifuged at 4 °C (3000 rpm) for 10 minutes and the serum was separated and stored at -80 °C. Bronchoalveolar lavage (BAL) fluid was collected by lavaging the lung through a tracheal cannula with 0.9% sodium chloride solution and centrifuged at 1500 rpm at 4 °C for 10 minutes. Supernatant was recovered and stored at -80 °C for assay of various biochemical markers. The precipitated pellets were re-suspended in 100μL of normal saline. Eosinophil and neutrophil counts in blood and BAL fluid were carried out using Neubauer's chamber.

Cytokine assay. Blood and BAL fluid samples were assayed for levels of tumour necrosis factor-alpha (TNF-α) (Diaclone, France), interleukin (IL)-4 (Diaclone, France), IL-6 (Diaclone, France), and Interferon-gamma (IFN-γ) (Diaclone, France) using commercially available enzyme-linked immunosorbent assay (ELISA) kits. The microtitre plate was pre-coated with an antibody specific to particular cytokines. Antigen and biotin-conjugated polyclonal antibody preparation specific for these cytokines were incubated for specified periods. Then, streptavidin horse-radish peroxidase and 3,32,5,52 tetramethyl benzidine (TMB) substrate were added to produce a colour reaction product. The absorbance was read at a wavelength of 450nm using ELISA plate reader.

Measurement of OVA specific immunoglobulin (Ig)E (OVA sIgE) levels. Blood and BAL fluid samples were assayed for levels of OVA sIgE using commercially available ELISA kits. The microtitre plate was pre-coated with monoclonal OVA sIgE antibody. Samples were pipetted into the wells with anti-rat IgE conjugated horseradish peroxidase and incubated. Any antibodies specific for the antigen present, bind to the per-coated antigen. After washing, a TMB substrate solution was added to each well to induce a colour reaction product. The reaction was stopped by the addition of stop solution and the intensity of the colour was measured using a microplate reader at a wavelength of 450nm.

Statistical Analysis

All data are expressed as mean ± SEM (standard error of mean) and analysed by using one-way analysis of variance (ANOVA) followed by Dunnett test. A p-value of at least 0.05 was used as the level of significance in all statistical tests.

Results

Effects of *Albizia lebbeck on* eosinophil and neutrophil counts in blood and BAL fluid

Sensitisation and challenge with OVA (for 22 days) in EC group resulted in significant ($p<0.05$) increase in eosinophil counts as compared to NC group, thus validating the experimental model of bronchial asthma. Results showed that pre-treatment with standardised aqueous extract (bark) of *Albizia lebbeck* (100, 200 and 400 mg/kg, per orally for 22 days) significantly ($p<0.05$) reduced the number of eosinophils in BAL fluid when compared to EC group of rats. Prednisolone used as positive control also reduced eosinophil numbers to a significant level ($p<0.05$) in both, blood and BAL fluid. Analysis of the data showed that there were significant differences in eosinophil levels across all the groups ($p<0.05$ in blood; and $p<0.05$ in BAL fluid). Further, exposure to OVA for 22 days in EC group resulted in significant ($p<0.05$) increase in neutrophil counts as compared to NC group. Result showed that pre-treatment with *Albizia lebbeck* (100, 200 and 400 mg/kg, per orally for 22 days) significantly ($p<0.05$) reduced the number of neutrophils in both blood and BAL fluid when compared to EC group of rats. Prednisolone used as positive control also reduced neutrophil numbers significantly ($p<0.05$) in blood and BAL fluid. Analysis of the data showed that there were significant differences in neutrophil levels across all the groups ($p<0.05$ in blood; and $p<0.05$ in BAL fluid). These results are shown in figure 1.

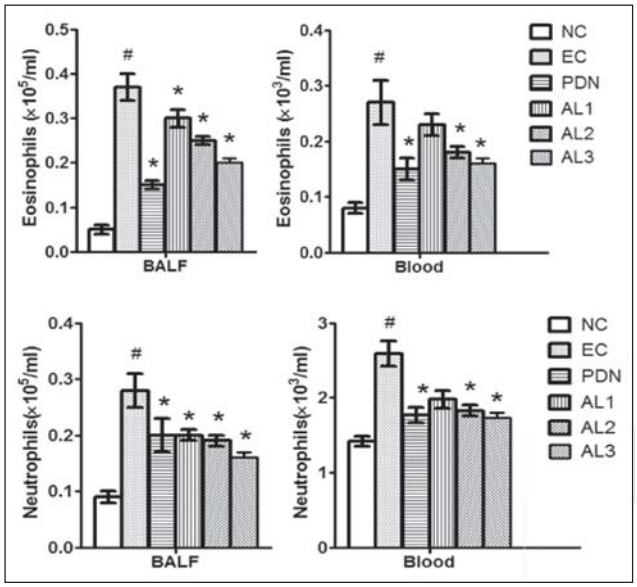

Figure 1. Effects of aqueous extract of *Albizia lebbeck* on eosinophil and neutrophil counts in BALF and blood in experimental model of airway inflammation.

#p<0.05 compared to NC and *p<0.05 compared to EC.

Definition of abbreviations: BALF=Bronchoalveolar lavage fluid; NC=Normal controls; EC=Experimental controls (sensitised and challenged with OVA), OVA=Ovalbumin; PDN=Prednisolone (10 mg/kg), AL1-AL3=*Albizia lebbeck* at dose 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively.

Effects of *Albizia lebbeck* on OVA sIgE levels in blood and BAL fluid

Rats challenged with OVA aerosols from 15th day to 22nd day in EC group showed significant ($p<0.05$) increase in IgE levels in comparison to NC rats. Treatment with *Albizia lebbeck* (100, 200 and 400 mg/kg) for 22 days, attenuated the levels of OVA sIgE in both blood and BAL fluid as compared to experimental control group with significant ($p<0.05$) reduction in the dose groups of 200 and 400 mg/kg in blood and at all three doses in BAL fluid. The results are more prominent in BAL fluid as compared to blood sample. Prednisolone also reduced OVA sIgE levels significantly ($p<0.05$) in blood and BAL fluid and the results were comparable with that of *Albizia lebbeck* treated groups. Analysis of the *Albizia lebbeck* data revealed that there were significant differences in OVA sIgE levels across all the groups ($p<0.05$ for OVA sIgE in blood; and $p<0.05$ for OVA sIgE in BAL fluid). These results are presented in figure 2.

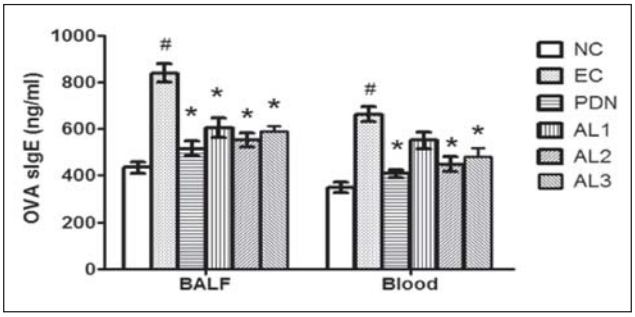

Figure 2. Effects of aqueous extract of *Albizia lebbeck* on ovalbumin (OVA) specific immunoglobulin (Ig)E in blood and BALF in experimental model of airway inflammation.

#p<0.05 compared to NC and *p<0.05 compared to EC.

Definition of abbreviations: NC=Normal controls; EC=Experimental controls (sensitised and challenged with OVA); PDN=Prednisolone (10 mg/kg); AL1-AL3=*Albizia lebbeck* at dose 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively; BALF=Brochonalveolar lavage fluid.

Effects of *Albizia lebbeck* on IL-6 and TNF-α levels in blood and BAL fluid

Post-hoc analysis showed IL-6 levels were markedly elevated ($p<0.05$) in both blood and BAL fluid in EC rats as compared to NC rats. Pre-treatment with *Albizia lebbeck* (100, 200 and 400 mg/kg) for 22 days, significantly ($p<0.05$) attenuated IL-6 levels at a dose 400 mg/kg in blood as compared to EC group whereas in BAL fluid, all three doses reduced the IL-6 levels significantly ($p<0.05$) in a dose related manner. Prednisolone also significantly ($p<0.05$) reduced IL-6 levels in blood and BAL fluid. Analysis of the *Albizia lebbeck* data showed that there were significant differences in IL-6 levels across all the groups ($p<0.05$ in blood; and $p<0.05$ in BAL fluid). Further, the results showed that TNF-α level were significantly ($p<0.05$) higher in rats sensitised and challenged (experimental control) with OVA as compared to normal control

group. Prior treatment with *Albizia lebbeck* for 22 days, significantly ($p<0.05$) attenuated the levels of TNF-α in blood (at 400 mg/kg dose) as well as BAL fluid (at all doses) as compared to experimental control group. Prednisolone also significantly ($p<0.05$) reduced IL-6 levels in blood and BAL fluid. Analysis of the data showed that there were significant differences in TNF-α levels across all the groups ($p<0.05$ in blood; and $p<0.05$ in BAL fluid).These results are highlighted in figure 3.

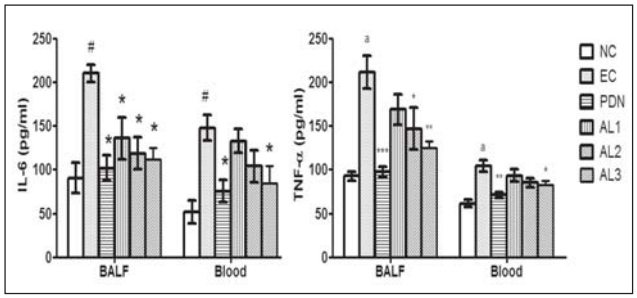

Figure 3. Effects of aqueous extract of *Albizia lebbeck* on IL-6 and TNF-α level in blood and BALF in experimental model of airway inflammation.

#p<0.05 compared to NC and *p<0.05 compared to EC.

Definition of abbreviations: IL=Interleukin; TNF-α=Tumour necrosis factor-alpha; BALF=Bronchoalveolar lavage fluid; NC=Normal controls; EC=Experimental controls (sensitised and challenged with OVA); PDN=Prednisolone (10 mg/kg); AL1-AL3=*Albizia lebbeck* at dose 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively.

Effects of *Albizia lebbeck* on IL-4 and IFN-γ levels in blood and BAL fluid

Analysis of data showed that the OVA immunisation followed by challenge in EC group resulted in significant ($p<0.05$) increase in the levels of IL-4 in comparison to NC group rats. Prior administration of *Albizia lebbeck* (100, 200 and 400 mg/kg) attenuated IL-4 levels in blood as well as BAL fluid as compared to that in EC group. Prednisolone also significantly ($p<0.05$) reduced IL-4 levels in blood and BAL fluid. Analysis of the data showed that there were significant differences in IL-4 levels across all the groups ($p<0.05$ in blood; and $p<0.05$ in BAL fluid). Furthermore, the effects of *Albizia lebbeck* (100, 200 and 400 mg/kg) were evaluated on IFN-γ levels in both blood and BAL fluid. The results showed that levels of IFN-γ were reduced significantly ($p<0.05$) in EC group of rats as compared to NC group. Administration of *Albizia lebbeck* reversed this effect by increasing IFN-γ levels in both blood and BAL fluid as compared to EC group of rats. The maximum response was observed at highest dose of *Albizia lebbeck* (400 mg/kg) in both blood and BAL fluid. Prednisolone also augmented the IFN-γ levels significantly ($p<0.05$) only in BAL fluid. Analysis of the data showed that there were significant differences in IFN-γ levels across all the groups ($p<0.05$ in blood; and $p<0.05$ in BAL fluid). These results are presented in figure 4.

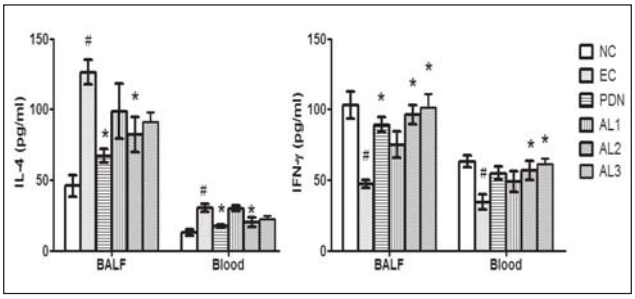

Figure 4. Effects of aqueous extract of *Albizia lebbeck* on IL-4 and IFN-γ level in blood and BALF in experimental model of airway inflammation.

#p<0.05 compared to NC and *p<0.05 compared to EC.

Definition of abbreviations: IL=Interleukin; BALF=Bronchoalveolar lavage fluid; IFN-γ=Intereferon-gamma; NC=Normal controls; EC=Experimental controls (sensitised and challenged with OVA); PDN=Prednisolone (10 mg/kg); AL1-AL3=*Albizia lebbeck* at dose 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively.

Discussion

Bronchial asthma is a chronic inflammatory airway disease of complex aetiology and is recognised as a Th2-mediated response with distinct tissue specific manifestations in the lungs. Allergen sensitisation and prolonged exposure results in asthma pathophysiology including airway inflammation, airway hyperresponsiveness, reversible airflow obstruction, and later, airway remodelling. Airway inflammation involves the interactions between various immunological mediators produced by inflammatory cells, such as mast cells, eosinophils, basophils, neutrophils, dendritic cells and lymphocytes.[12] Thus, aberrant inflammatory response exacerbates and propagates the disease symptoms of wheezing, cough, chest tightness and dyspnoea. The current approach to the pharmacological management of asthma includes conventional drug therapy with anti-inflammatory, *viz*, inhaled and systemic corticosteroids and bronchodilators *viz*, β2 adrenergic agonists and anti-cholinergics.[13] However, these drugs produce various local and systemic adverse effects, and this has led to search for safe and efficacious alternative therapeutic strategies from the traditional systems of medicine. Ayurveda and Unani are established traditional systems of medicine in India based on plant and plant-based products, with proven beneficial effects in some chronic intractable diseases. Medicinal plant-based treatments are rapidly emerging as viable alternatives/adjuncts by virtue of having efficacy with good safety profile and low cost of treatment. *Albizia lebbeck* (Sirish) has been mentioned in ayurvedic system of medicine for the treatment of various ailments, including bronchial asthma. Stem bark has been found to have active constituents possessing anti-asthmatic properties.[14] The study has been designed to evaluate and validate the effects of

Albizia lebbeck on markers of inflammation and immunity and their possible cellular/molecular mechanisms of action to assess their potential as an alternative/adjunct therapeutic agent for bronchial asthma.

Inhibition of the airway inflammatory response by reduction of the OVA-induced inflammatory cells and inflammatory cytokines production is a main therapeutic objective in the treatment of bronchial asthma. The precise mechanism of chronic airway inflammation is still not clear but it is considered to be dependent on sustained infiltration and activation of many inflammatory cells (eosinophils, neutrophils, basophils and macrophages) and various pro-inflammatory mediators and cytokines. Th2 cells play a key role in initiation and propagation of inflammation through release of Th2 cytokines, which in turn, cause the recruitment and activation of eosinophils and other effectors cells in airways in bronchial asthma. The experimental model for inducing allergic bronchial asthma in rats has been standardised and validated in our laboratory through estimation of various inflammatory and immunological markers.[15,16] The anti-inflammatory and immunomodulatory effects of *Albizia lebbeck* were evaluated both systemically (blood) and locally (airways-BAL fluid) in OVA sensitised and challenged rats. Peripheral blood eosinophil count is an indirect measure of airway inflammation which is one of the characteristic features of asthma.[17] In the present study, repeated inhalation of OVA in allergic asthma model raised the eosinophil counts in the blood and BAL fluid. Additionally, neutrophilic inflammation in the airways is recognised in acute exacerbation of asthma and status asthmaticus.[18] Neutrophils counts were also seen to be elevated with inflammatory changes in response to OVA exposure. Treatment with *Albizia lebbeck* bark extract, at different doses, attenuated the levels of eosinophils and neutrophils in experimental model of bronchial asthma. IgE is an important mediator in allergic asthma, particularly in the acute response to antigen and in the propagation of airway inflammation in bronchial asthma.[19] Antigen-specific IgE secretion from B cells results in the activation of mast cells and basophils which release various inflammatory mediators and further activate other inflammatory cells. Sensitisation and challenge with OVA induced release of OVA sIgE in serum and BAL fluid in experimental rats. Treatment with *Albizia lebbeck* resulted in reduction of OVA sIgE levels in both blood and BAL fluid, thus suggesting the anti-inflammatory effects of the herbal extract. TNF-α and IL-6 are important pro-inflammatory cytokines produced by primary lung epithelial cells and inflammatory cells in response to variety of allergens.[20,21] In the experimental model of bronchial asthma, sensitisation and challenge with OVA significantly increased the levels of IL-6 and TNF-α which were attenuated by the prior treatment with *Albizia lebbeck* bark extract. IL-4 is a Th2 cytokine and is a potent modulator of immune and inflammatory responses and acts on B-cells to facilitate IgE production and IgE-dependent mast cell activation.[22] IFN-γ is a Th1 type cytokine and plays an essential role in immune regulation.[23] OVA sensitisation and challenge in experimental control rats resulted in elevation of levels of IL-4 in both blood and BAL fluid *whereas* IFN-γ levels were reduced. Prior treatment with *Albizia lebbeck* (*bark* extract) attenuated the levels of IL-4 whereas, elevated the levels of IFN-γ in both blood and BAL fluid as compared to EC group of rats. Thus, the results of our experiment suggests that the anti-inflammatory and immunomodulatory effect of *Albizia lebbeck* as indicated by the reduction in the levels of eosinophils, neutrophils, pro-inflammatory cytokines (TNF-α, IL-6) and IL-4 which is important for Th2 cell activation and acts on B-cell to facilitate IgE production. Attenuation in the levels of OVA sIgE indicates that the plant extract inhibited the release of inflammatory mediators and cytokines from antigen presenting cells. The study also showed an increase in the levels of Th1 cytokine IFN-γ which indicates that *Albizia lebbeck* inhibits the functions of Th2 cells that promote the inflammation.

Conclusions

The results of the present study emphasise the anti-inflammatory and immunomodulatory role of *Albizia lebbeck*, and this could contribute to its possible therapeutic benefit in bronchial asthma. Taken together, the results showed that standardised extract of *Albizia lebbeck* has anti-inflammatory and immunomodulatory activity as evident from the modulation of cellular and molecular markers of inflammation and immunity in the model of airway inflammation and this could contribute to its possible therapeutic benefit in bronchial asthma.

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