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Mesua ferrea L. (Calophyllaceae) exerts therapeutic effects in allergic asthma by modulating cytokines production in asthmatic rats

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Abstract: In the present study, ethanolic extract of *Mesua ferrea* L. stamens was investigated for its potential to reverse some features of bronchial asthma in ovalbumin-induced murine model of asthma. *Mesua ferrea* commonly called nagakeshar (Family, Calophyllaceae) is a well-known antiallergic drug in the Asian folk system of medicines. In the present work, pharmacological studies are done to provide scientific evidence for therapeutic potential of plants in allergic asthma. Asthma was induced in experimental rats with allergen suspension of ovalbumin and aluminium hydroxide followed by treatment with dexamethasone (2.5 mg/kg, p.o) or *M. ferrea* stamen extract (3.75 and 15 mg/kg, b.w., p.o). Biomarkers of inflammatory response including cell counts, Immunoglobulin E, cytokines such as interleukin (IL)-4, -5, -1β, tumor necrosis factor (TNF)-α, leukotriene (LT)-D-4, and nitrite concentration in blood as well as bronchial (BAL) fluid were tested. Lung functions in asthmatic and treated animals were evaluated as breathing rate and tidal volume. Treatment with *M. ferrea* stamen extract (MFE) markedly ($p < 0.001$, $p < 0.01$ and $p < 0.05$) diminished infiltration of inflammatory cells, IgE, cytokines, and nitrites in blood/serum and bronchial fluid. Improvement in lung functions ($p < 0.05$) of asthmatic animals after MFE treatment also supports our findings. Results of the study suggest a therapeutic potential of *M. ferrea* in allergic asthma that can be related to the ability of plants to attenuate the response of inflammatory cells and thereby, the production of inflammatory and proinflammatory cytokines in airways.

Key words: *Mesua ferrea* extract, antiallergic, inflammatory cells, lung functions, airway remodeling

1. Introduction

Bronchial asthma is noncommunicable lung disorder affecting 5%-10% of the world population or an estimated 23.4 million people, including 7 million children and 250,000 deaths.The annuall increase in asthma prevalence by approximately, 3.6% makes disease a global issue of public health (Asher et al., 2020). Bronchial asthma is characterized by chronic inflammation, excessive mucus formation, reversible constriction, and hyper-responsiveness of airways usually triggered by allergens (WHO, 2020). Another pathological feature of asthma is airway remodeling, described as structural and functional changes in lungs attributed to persistent inflammatory response in airways (Roh et al., 2008; Zeiger et al., 2014; Fehrenbach, et al., 2017; Arora and Ansari, 2019). The classical dogma of asthma is central to allergen induced imbalance between T_H1 and T_H2 cytokines leading to excessive production of T_H2 cytokines, mainly, IL-4, IL-5, and IL-ß, TNF-œ. IL-4 activates B cells to produce IgE, in contrast, IL-5 has ability to activate migration and infiltration of eosinophils, inducing eosinophilic airway inflammation (Kuruvilla et al., 2019). Together with T_H2 cells, innate lymphoid group 2 cells (ILC2) also play a key role in augmenting T_H2 response and production of IL-5 in the airways (Hirose et al., 2017). All these cardinal features of bronchial asthma are observed in ovalbumin (OVA) exposed lung tissues of Wistar rats also.

Glucocorticoids, widely accepted as mainstay therapy in chronic asthma. These corticosteroids act by modulating T_H2 and nonspecific innate immune responses in individuals. Other therapies, available in the form of short-term and long-term relievers, have failed to control symptoms of asthma completely and even intensive treatment is found to be ineffective. Consequently, effort should be made to identify new remedies for mitigating this disorder. For decades, herbal drugs have been utilized

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as a source of new therapeutically active molecules, scaffolds, pharmacophores, and chemotypes.

Mesua ferrea Linn. also known as Ceylon iron wood or Nagakesara, is an evergreen medium to large size tree belonging to the family, Calophyllaceae. It is indigenous to Asia. Its flowers are highly attractive and beautiful and hence, generally cultivated for ornamental purposes. *M. ferrea* is ethnomedicinally used as an antipyretic, antiasthmatic, cardiotonic, carminative, expectorant, antiseptic, purgative, and blood purifier in many countries of the Asian continent (Burkill, 1966, Parukutty and Chandra, 1984; Rai et al., 2000; Anonymous, 2005). The plant is also used in the treatment of chronic diseases like gastritis, bronchitis, rheumatism, skin infections, piles and is used as an antidote against snake and scorpion string.

Various parts of *M. ferrea* tree like, flowers, leaves and seed kernels are attributed with medicinal properties. High medicinal importance of *M ferrea* is due to the presence of various bioactive phytocomponents including glycosides, flavonoids, triglycerides, resins, sesquiterpenes, fatty acids, steroids, tannins, and saponins (Figure 1) (Chahar et al., 2013; Keawsa-Ard et al., 2015, Asif et al., 2017; Sharma et al., 2017).

In several in vitro and in vivo studies, these phytocompounds have exhibited considerable pharmacological activities including antimicrobial, anticancer, antiinflammatory, antiulcer, antihistaminic, central nervous system (CNS) depressant, antiarthritic, anticonvulsant, immunomodulatory activity, antioxidant and hepatoprotective activity, analgesic activity, antispasmodic activity, antivenom activity, wound healing and spermicidal in preclinical studies (Chahar et al., 2013; Keawsa-Ard et al., 2015; Sharma et al., 2017; Asif et al., 2017).

The plant has been indexed as an important constituent of various traditional antiasthmatic herbal medicines (Arora and Ansari, 2015) (Table 1). However, the role of the plant in context to modulation of airway inflammation and airway remodeling in allergic asthma has never been reconnoitered. In our study, we have investigated the potential of *M. ferrea* standardized stamens extract (MFE) in ovalbumin-induced airway inflammation in asthmatic animals.

2. Materials and methods

2.1. Chemicals

All the reagents of analytical grade were purchased from commercial Sigma Aldrich suppliers and were used in the research work. Standard ELISA kits of interleukins, IL-4, IL-5, IL-1β, leukotriene, LTD4, immunoglobulin E (IgE), tumor necrosis factor TNF-α, and calorimetric kit of nitric oxide (NO) were used to estimate levels of these biomarkers in treated and control rat groups.

2.2. Preparation and standardisation of *M. ferrea* flower stamens extract

M. ferrea stamens were harvested from the University botanical and medicinal garden of Dibrugarh University, Dibrugarh, Assam, India. The harvested fresh flowers were collected, and stamens were handpicked by foreships and dried under shade. The plant sample was validated by Dr. Singh, NISCAIR, India, with voucher number NISCAIR/RHMD/consult/-2011-12/1752/52. The dried stamens (250 g) were coarsely grounded and thoroughly extracted with a mixture of ethanol and water (7:3) for 24 h at room temperature (32 ± 2°C) with periodic stirring. The extracted content was filtered under a vacuum. The filtrate was concentrated under reduced pressure to obtain semisolid crude extract. The crude extract was placed in lyophilizer to remove any residual solvent and dried at -40 °C to obtain yellow-brown colored dried powder. The lyophilized powdered extract was stored in a desiccator until further use.

The percentage yield (%w/w) obtained of the dried extract was 12.9 ± 1.64. All the quality control parameters were performed for standardization of extract as per the guidelines of Ayurvedic Pharmacopoeia of India (Anonymous, 2008). The standardized extract was stored at 2-8 °C till further use in experiments. For conducting pharmacological studies with MFE, 15 g of lyophilized dried powder was suspended in 100 mL of 0.1% carboxymethyl cellulose to make a stable suspension for oral administration in animals.

2.3. Study design for investigation of *M. ferrea* standardized extract (MFE) treatment in ovalbumin sensitized and challenged asthmatic rats

Thirty healthy male Wistar rats were weighed and divided randomly into five groups (6 rats per group). All the animals were shifted and allowed to acclimatize for 15 days under standard housing conditions (25 ± 2 °C/50 ± 5% with a 12 h light/dark cycle) before commencement of the experiment. The animals for the study were issued and approved by the Institutional Animal Ethics Committee (approval no. JHAEC/173/CPCSEA dt. 28.1.2000), Jamia Hamdard, New Delhi, India.

Group 1 (N) rats served as normal control and received vehicle (0.1% CMC w/v in purified water). Group 2 (S) rats served as asthma control; Group 3 (S+D), received standard drug, dexamethasone 2.5 mg/kg (D); Group 4 (S+M1) and Group 5 (S+M2) rats received MFE, 3.75 mg/kg (M1) and 15 mg/kg, b.w., (M2), respectively. Treatments were given daily through oral route from day 1 to 28 between 10.00 to 11.00 a.m. Administered dose of the *M. ferrea* stamens extract in rats was derived from the daily recommended dose of the drug recommended in Ayurvedic Pharmacopoeia of India (Anonymous, 2008). The standard reference drug used in the study was corticosteroid dexamethasone.

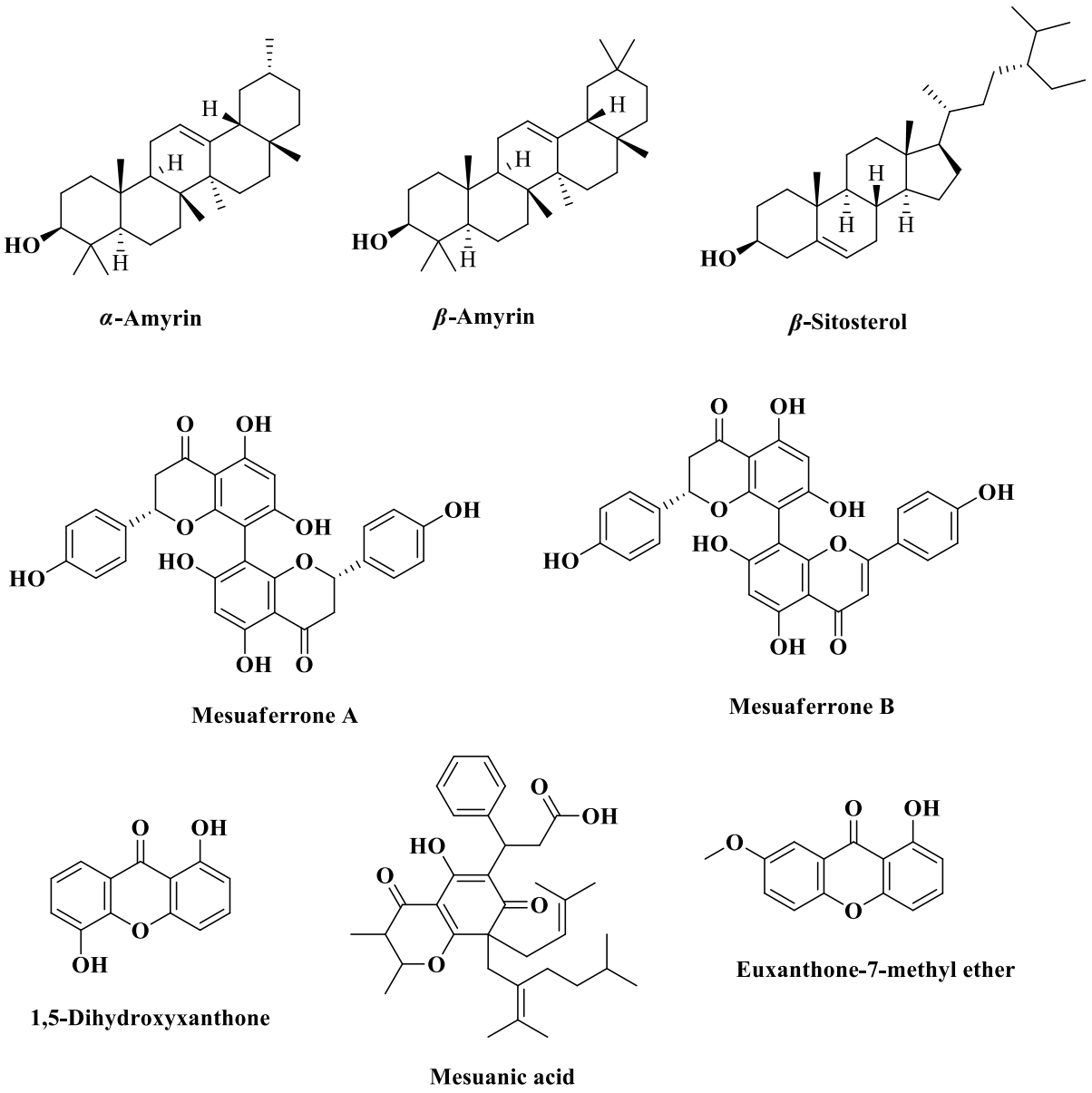

Figure 1. Major active phytoconstituents present in *M. ferrea*.

Asthma was induced in rats (group 2 to 5) by administering suspension of ovalbumin (40 mg/animal) and $Al(OH)_3$ (2 mg/rat) (Arora et al., 2017). The suspension of ovalbumin and aluminum hydroxide acts as an allergen that activates the allergic response in the rats; and makes them sensitized and susceptible to asthma.

After 15 days of i.p. administration of allergen suspension, sensitized rats were exposed to mist of 1% chick egg albumin (ovalbumin) suspension (1 mg/100 mL normal saline) for 20 min in a closed chamber regularly (once a day) for the next 8 consecutive days (day 15 to day 22) and thereafter, with a gap of 3 days i.e. on day 25 and day 28. In control group animals (Group I) animals that were not sensitized by allergen (ovalbumin and aluminum hydroxide), animals were exposed to a mist of normal saline solution for the above specified time period.

2.4. Lung function test

Lung function was gaged using two parameters (i) breathing rate and (ii) tidal volume. Rats were anaesthetized with sodium pentobarbitone (i.p.,105 mg/kg) on the 28th day, five min after challenging the OVA-sensitized rats. To record airflow signals of the rat's lung, the rat's trachea was connected with a spirometer (pneumotachograph) and differential pressure transducer device (Parasuraman and Raveendran, 2012). The transducer integrates the electronic airflow signals into lungs tidal volume (VT,

Table 1. List of some marketed formulation containing *Mesua ferrea* as an ingredient.

Marketed product	System of medicine	Marketing company	Uses/indications
Maharisi amrit kalash-4	Ayurveda	Maharishi Ayurveda	Immunomodulator
Dasamoolarishta	Ayurveda	Baidyanath, Zandu	Appetizer, indigestion, liver diseases, piles, jaundice, respiratory diseases
Shri gopal tail	Ayurveda	Baidyanath, Dabar	Erectile dysfunction, memory enhancer, neuromuscular dysfunction
Sarivadi vati	Ayurveda	Baidyanath, Divya Pharmacy (Patanjali)	Hearing problem, tinnitus and ear infections, epilepsy, bleeding disorder
Jatiphaladi churna	Ayurveda	Baidyanath	Digestive and respiratory conditions
Drakshadi churnam	Ayurveda	Zandu	Digestive and respiratory conditions
Vyaghrihareetaki avaleha	Ayurveda	Zandu, Baidyanath, Patanjali	Cough, cold, asthma, bronchitis
Brahma Rasayana	Ayurveda	Dabar, Shri Shri Ayurveda,	Immunity booster, memory enhancer
Eladi churna/ vati	Siddha	Baidyanath	Carminative, vomiting, indigestion, anorexia
Lavangadi churna/ vati/tablet	Siddha	Baidyanath, Dabar	Cough, diarrhea, dysentery, mouth diseases, dental caries, anemia, and fever
Jawarish Shehryaran	Unani	Hamdard	Stomach and liver tonic
Halwa-i-supari pack	Unani	Hamdard	General digestive tonic
Kapooradi churnam	Siddha	SKM	Anorexia, indigestion, vomiting

mL/s) and breathing rate (*f*, breaths/min). Physiological respiratory data were collected, analyzed with the help of Power Lab System and LabChart Programme software (spirometer Model no: FE141, ADInstruments, Pty, Australia). Before recording data in L/s, the spirometer was calibrated, and any drift in signals attributable to the instrument transducer was neutralized for high precision. Changes in *f* and VT were measured after intravenous (i.v.) administration of vehicle (in normal control group) and methacholine (1.5 mg/kg) in rats through femoral vein. Heparin (0.5%) was administered to prevent any chances of blood coagulation during study. One data point was generated by recording and averaging 10-12 respiratory cycles. To avoid disturbances arising due to spontaneous respiration, an i.v. injection of vecuronium bromide at the dose of 0.2 mg/kg was given to the anesthetized rats. The excessive bronchial secretion produced during the procedure was discharged by means of a small polyethylene tube so that the trachea was not disturbed throughout the procedure (Arora et al., 2017).

2.5. Collection of bronchial fluid (BAL) fluid

Bronchial fluid or bronchoalveolar fluid (BAL) fluid was collected as per the procedure described by earlier (Arora et al., 2017). Briefly, lungs were lavaged with 0.9% physiological normal saline solution. The whole procedure was repeated thrice with fresh 5 mL of physiological solution. All the collected BAL fluids were mixed together and centrifuged at 4500 rpm/4 °C. The supernatant was collected to analyse inflammatory biomarkers. The cell pellet obtained after centrifugation was suspended in normal saline (1 mL) and used for quantitative analyses of total and differential leukocyte count in BAL fluid as described earlier (Canning and Chou, 2008).

2.6. Estimation of cell count in blood and BAL fluid

The blood was collected via cardiac puncture using a 19-gauge sterile syringe. Two separate vials were used for blood collection. In one vial, an anticoagulant (heparin) was added and stored at 4 °C to determine total blood cells and differential cell count. In the second vial, blood was allowed to clot and serum was collected through centrifugation. Serum was stored at -80 °C and was used for estimation of IgE and cytokines levels.

2.7. Estimation of nitric oxide, nitrites, and cytokines in serum and BAL fluid

The levels of various key inflammatory biomarkers like IgE, IL-4, IL-5, IL-1β, LTD-4, and TNF-α in serum and BAL fluid, were quantitatively assessed through ELISA kits. The assays were performed according to the instructions supplied with the commercial kits. Automatic ELISA reader (Model no. ELX-80MS, Biotek, USA) was used to analyze the samples. The UV kinetic method was employed to quantify the concentration of nitrite and nitric oxide in serum and BAL fluid.

2.8. Statistical analysis

Pharmacological responses are represented as mean ± SEM. To validate the results statistically with statistical differences at 5% level of probability ($p < 0.05$), a one-way analysis of variance (ANOVA) followed by a post hoc Tukey's test was employed. All statistical calculations and analyses were done using Graph Pad Prism software (San Diego, CA, USA).

3. Results

3.1. Effect of MFE treatment on lung function parameters

Administration of methacholine caused a significant increase in breathing rate ($p < 0.001$) in asthma control animals (S) as compared to normal control animals (N). Treating asthmatic animals with *M. ferrea* stamens ethanolic extract (MFE), 3.75 mg/kg or 15 mg/kg, b.w. reduced breathing rate by 26.72% ($p < 0.05$) and 32.75% ($p < 0.01$) respectively, as compared to the asthma control group (S). Remarkable ($p < 0.001$) decrease in lungs tidal volume (VT) of the asthma control group (S) as compared to normal control group (N) was also observed. Treating asthmatic rats with MFE (3.75 mg/kg or 15 mg/kg, b.w.) significantly improved VT by 12.5% ($p < 0.05$) or 26.78% ($p < 0.01$), respectively (Figure 2). Oral treatment of asthmatic animals with a reference standard, dexamethasone, also improved lung functions, significantly ($p < 0.001$) in comparison to the asthmatic control group (S).

3.2. Effect of MFE treatment on circulating cell count in BAL fluid

Total leukocyte count (TLC) and differential cell count (DLC), including lymphocytes, eosinophils, and neutrophils in blood and BAL fluid samples of asthmatic rats increased considerably ($p < 0.001$) in comparison to normal control (N) group. However, in the study, a lesser number of lymphocytes as compared to that of normal group (N) were observed in the blood sample of OVA-sensitized and challenged rats. Treating asthmatic rats with MFE (15 mg/kg) or dexamethasone normalized the circulatory cell count in blood as well as BAL fluid in comparison to asthma control group ($p < 0.05$, $p < 0.01$ and $p < 0.001$) (Table 2). Reversal of OVA-induced altered levels of circulatory cells after MFE treatment was found to be comparable with that of dexamethasone in both body fluids analysed.

3.3. Effect of MFE treatment on serum cytokines levels

Rats of the asthmatic control group (S) had shown a dramatic increase in the concentration of various cytokines, IL-4, LTD-4, IL-1β, IL-5, and TNF-α in serum after exposure to ovalbumin in comparison to the nonasthmatic control group (N) with statistical significance of ($p < 0.001$).

Treatment of asthmatic rats with MFE at a dose of 3.75 mg/kg and 15 mg/kg, b. w. significantly decreased elevated serum levels of asthmatic biomarkers like IL-4, LTD-4, IL-1β, IL-5, and TNF-α. MFE treatment at a lower dose of 3.75 mg/kg, b. w. (S + M1) declined elevated serum levels of IL-4, IL-5, TNF-α, IL-1β, LTD-4, by 22.22%, 18.18%, 13.47%, 6.70%, and 13.13%, respectively, with confidence interval of $p < 0.05$ in comparison to asthmatic control group (S) (Figure 3).

At a dose of 15 mg/kg, b. w., MFE extract dampens the elevated cytokines storm of IL-4, IL-5, TNF-α, IL-1β, LTD-4, by 38.17% ($p < 0.01$), 29.32% ($p < 0.01$), 31.37% ($p < 0.01$), 25.46% ($p < 0.05$), and 31.22% ($p < 0.05$), respectively, in comparison to asthmatic control group (S) (Figure 3). A similar comparison for S + D group showed a significantly reduced concentration of all the cytokines ($p < 0.001$) tested in our study. Comparing the efficacy of MFE with standard reference dexamethasone, we found that herbal drug was comparable in preventing OVA-induced changes in serum cytokines in rats, however, the comparison was significant at both dose levels tested in our study.

3.4. Effect of MFE treatment on cytokine levels in BAL fluid

Level of all the inflammatory and proinflammatory cytokines in the bronchial fluid of asthmatic animals (S)

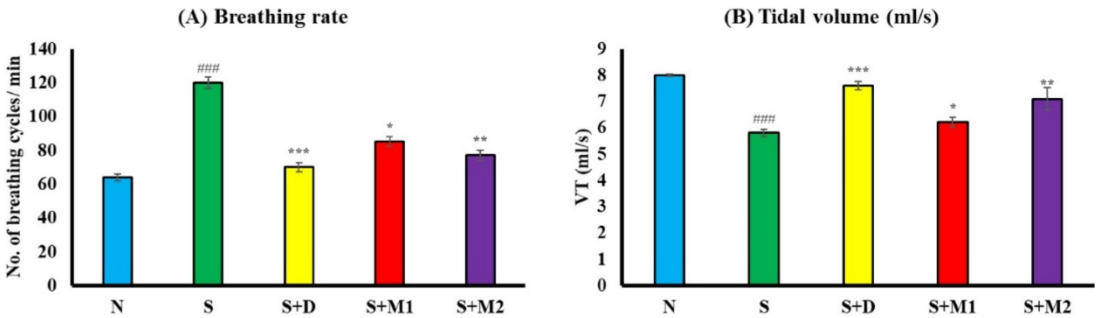

Figure 2. Statistical analysis of effect of MFE treatment on lung functions, breathing rate (A) and tidal volume (B), in different groups. Values are represented as mean ± S.E.M. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, compared to the asthmatic control (S) group; $^{###}p < 0.001$ compared to normal control (N) group.

Table 2. Estimation of total leucocyte count (TLC) and differential cell count in BAL fluid ($\times 10^5$ cells/mL).

Groups	N	S	S+D	S+M1	S+M2
Total cells	7.11 ± 0.46	13.37 ± 0.85$^{###}$	8.06 ± 0.45***	9.43 ± 0.22*	7.68 ± 0.22**
Eosinophils	0.54 ± 0.49	11.42 ± 0.42$^{###}$	3.48 ± 0.43***	7.83 ± 0.14^{ns}	6.59 ± 0.41*
Lymphocytes	9.83 ± 0.42	20.33 ± 0.75$^{###}$	10.37 ± 0.51***	14.09 ± 0.17*	13.12 ± 0.33*
Macrophages	5.29 ± 0.46	13.19 ± 0.76$^{###}$	6.72 ± 0.69***	10.34 ± 1.66^{ns}	8.44 ± 0.51*
Neutrophils	0.54 ± 0.08	5.69 ± 0.44$^{###}$	1.99 ± 0.08***	3.38 ± 2.06*	2.79 ± 2.06***

Values represent mean ± S.E.M ($n = 6$); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and ns (non-significant) compared to the asthmatic control (S) group; $^{###}p < 0.001$ compared to normal control (N) group.

were found markedly high ($p < 0.001$) in comparison to normal control group (N). Treatment with both doses of MFE (3.75 mg/kg and 15 mg/kg) elicited substantial reduction in cytokines level arises after ovalbumin exposure in asthmatic rats. MFE treatment at lower dose of 3.75 mg/Kg, b. w., (S + M1) declined elevated serum levels of IL-4, IL-5, IL-1β, and TNF-α by 26.54%, 13.44%, 13.79%, and 11.27%, respectively, ($p < 0.05$) in comparison to asthmatic control group (S) (Figure 3).

At a dose of 15 mg/kg, b. w., MFE extract inhibited elevated cytokines storm of IL-4, IL-5, IL-1β, by 42.97%, 24.85%, and 27.25%, respectively, with a confidence interval of $p < 0.01$ in comparison to the asthmatic control group (S) (Figure 3). It was very interesting that MFE treatment at 15 mg/kg, b. w. inhibits the TNF-α level in BAL fluid up to 27.37% with $p < 0.001$ in comparison to the asthmatic control group (S). MFE treatment (15 mg/kg) was able to produce considerable reduction (37.95%) in concentration of LTD-4 in BAL fluid after comparing with asthma control group (S) animals ($p < 0.05$). In comparison to standard reference dexamethasone, *M. ferrea* stamens extract was found to be significantly comparable in reducing BAL fluid levels of cytokines in OVA-sensitized animals.

3.5. Effect of MFE treatment on IgE levels of serum and BAL fluid

IgE levels of both serum and BAL fluid samples of animals increased significantly ($p < 0.001$) after OVA-sensitization and challenge. A significant reduction of circulating IgE levels in serum and BAL fluid by 13.96%, 29.67% was observed after administration of MFE (3.75 mg/kg, b. w.) in asthmatic challenged rats, respectively, in comparison to untreated asthmatic rats (S). In a similar fashion, 29.67% and 28.05% decrease in circulating IgE levels in serum and in BAL fluid was observed after administration of 15 mg/kg, b. w. of MFE, respectively, in comparison to untreated asthmatic rats (S). Treatment of asthmatic animals with standard drug, dexamethasone also elicited a significant ($p < 0.001$) reduction in IgE levels in both the body fluids analysed (Figure 4). Further, the effect of herbal drug treatment in attenuating serum and BAL fluid levels of allergen-induced IgE levels were found to be statistically significant when compared with the reference, dexamethasone.

3.6. Effect of MFE treatment on total nitric oxide and nitrite concentration

In asthmatic rats (S), total NO and nitrite concentrations in body fluids were increased significantly ($p < 0.001$) in comparison to normal group (N). Animals receiving MFE at a dose level, 15 mg/kg b. w., exhibited a reduction in nitric oxide in serum and BAL fluid by 28.77% and 31.15%, respectively, in comparison with the asthmatic control group (S). Animals receiving MFE, 15 mg/kg b. w., also exhibited a reduction in nitrite content in serum and BAL fluid by 39.49% and 58.75% in serum as well as BAL fluid ($p < 0.05$), respectively (Figure 5) in comparison with the asthmatic control group (S). Reference drug, dexamethasone, and higher dose of the extract also showed a significant reduction in elevated levels of both the analytes ($p < 0.001$). Reduction in serum and BAL fluid levels of total NO and nitrite after MFE treatment was comparable to standard drug treatment.

4. Discussion

The recent resurge in the interest of researchers to utilise plants as a source of novel therapeutically active interventions can be advocated for the presence of multi-dimensional chemical structures with varied biological functions. Due to the complex nature of chronic diseases e.g., asthma, the treatment strategy has shifted to a multi-target approach with combination agents. It is known that spasmogenic and contractile responses of airway smooth muscles are tightly regulated by neurotransmitters and other biological chemicals including acetylcholine, histamine, bradykinin, etc. (Canning, 2006). In sensitized individuals, allergen exposure activates naive T cells (T_H0) that lead to excessive production of T_H2 cells in comparison to T_H1. Activated T_H2 cells by producing cytokines and other biologically active molecules are

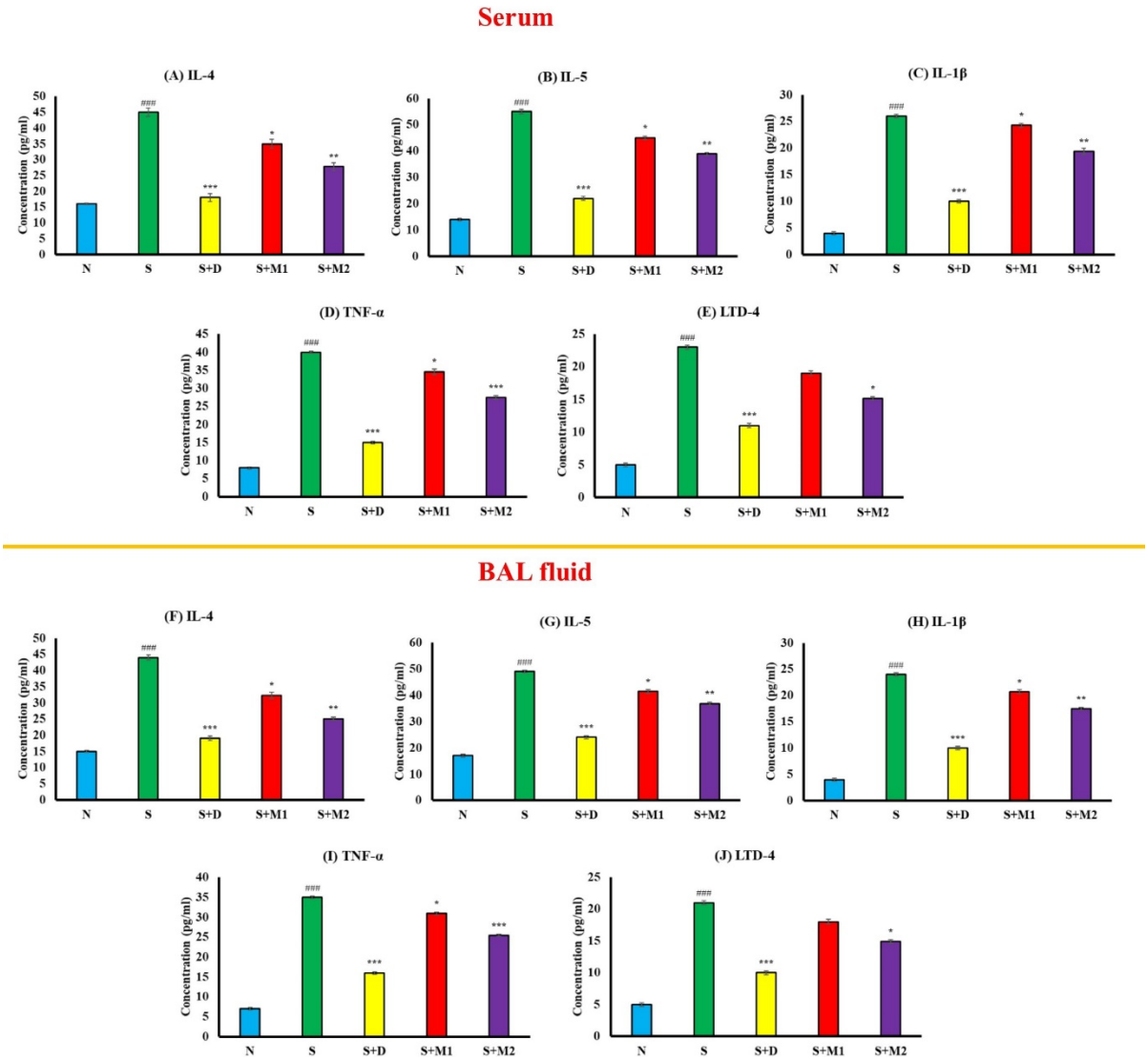

Figure 3. Statistical analysis of effect of MFE treatment on various estimations in different groups; serum cytokines levels (A-E) and BAL fluid cytokines levels (F-J) of different treatment groups. Data represent mean ± S.E.M. $^{*}p < 0.05$, $^{*}p < 0.01$ and $^{***}p < 0.001$, compared to the asthmatic control (S) group; $^{###}p < 0.001$ compared to normal control (N) group.

key components in the pathogenesis of asthma. Among these cytokines, IL-4 and IL-5 are mainly implicated in cardinal features of asthma, maturation of eosinophils and goblet cell, IgE mediated hypersensitive response, mucus secretion, bronchial constriction, and tissue remodelling (Lambrecht et al., 2019).

Upon allergen recognition, and cross-linking of IgE with high affinity receptors, FcεRI (Fc epsilon RI) present on mast cells and the low affinity receptors FcεRII or CD23 cells present on antigen presenting cells activate mast cell (Bax et al., 2012; Samitas et al., 2015). Activation results in the release of primary and secondary chemical substances (e.g., histamine, heparin, and proteases) and inflammatory mediators, such as cytokines and arachidonic acid metabolites (Heather et al., 2012). Lately, it has been found that the newly formed Ag-FcεRI-IgE (allergen-FcεRI-IgE) complex also accelerates the production of chemokine ligand 28 (CCL28), that selectively attracts T_H2 lymphocytes production (Matucci et al., 2018).

IL-4 is a key player in establishing the basis for IgE-mediated hypersensitivity reactions. IL-4 upregulates the transcription factor, GATA-3 in naive-T cells, thereby, regulating differentiation of allergen-specific T helper cells (Zhu et al., 2010). Excessive production of IL-5 in airways favours growth, survival, accumulation, and activation of eosinophils in the lungs (Roufosse, 2018).

In the present study, allergen sensitization elicited marked alteration in breathing rate and lung tidal volume.

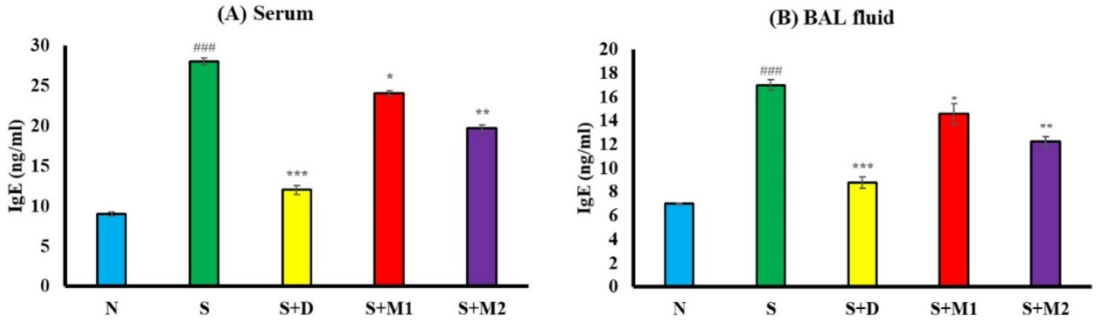

Figure 4. Statistical analysis of effect of MFE treatment on various estimations in different groups (i) serum IgE levels (A); (ii) BAL fluid IgE levels (B). Values are represented as mean ± S.E.M. (n = 6); ###represents $p < 0.001$ compared to normal control (N); *represents $p < 0.05$, ** represents $p < 0.01$ and ***represents $p < 0.001$, compared to the asthmatic animals (S).

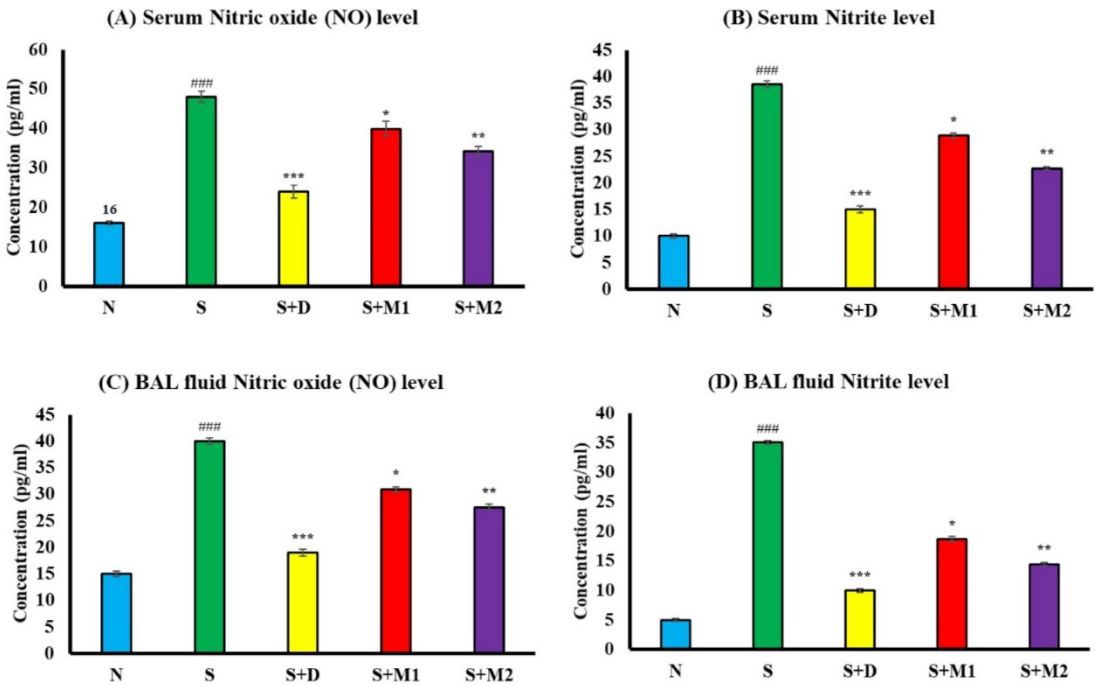

Figure 5. Statistical analysis of effect of MFE treatment on various estimations in different groups nitric oxide (NO) and nitrites serum (A and B) and BAL fluid levels (C and D). Values are represented as mean ± S.E.M. (n = 6); ###represents $p < 0.001$ compared to normal control (N); *represents $p < 0.05$, ** represents $p < 0.01$ and ***represents $p < 0.001$, compared to the asthmatic animals (S).

Further, all the asthmatic control group animals (S) have shown all the symptoms and manifestations of asthma that include coughing, irritability, and breathing difficulty. Persistent inflammation of airways in asthma obstructs air flow resulting in bronchoconstriction and dyspnoea. However, treatment of OVA-sensitized animals with MFE and dexamethasone prevented methacholine-induced bronchoconstriction. Broncho-relaxant effects of the drugs in the current study can be advocated antiinflammatory effects of MFE on airways.

In the study, allergen provocation of sensitized rats altered the normal count of inflammatory cells, including eosinophils, lymphocytes, and neutrophils in blood as well as BAL fluid of experimental animals when compared with normal control group. Eosinophils are directly associated with T_H2 immune response in asthma and have pleiotropic effects on various inflammatory cells (Petsky et al., 2012). Several pieces of studies demonstrate that controlling eosinophil count leads to decreased asthma exacerbations in allergic patients. Oral administration of MFE (3.75 and

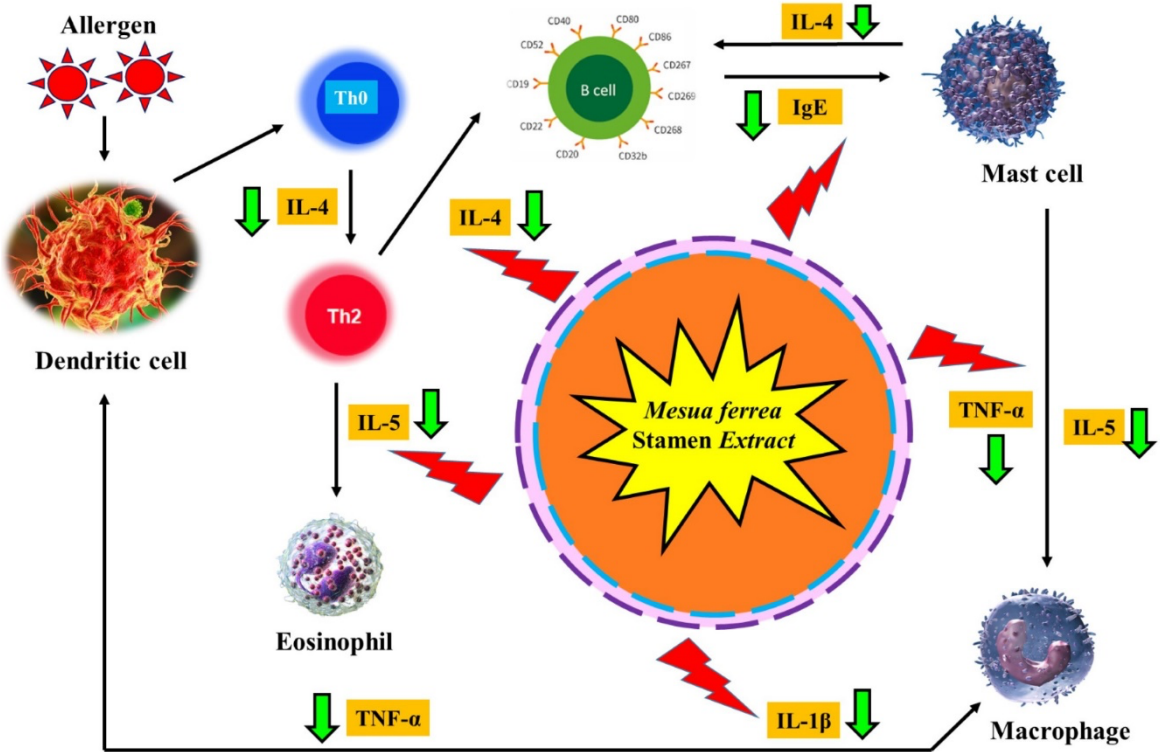

Figure 6. Proposed mode of action of *Mesua ferrea* stamen extract. IL, Interleukin; TNF-α, tumor necrosis factor; LT-D-4, leukotriene D, IgE, Immunoglobulin E; B cells, B lymphocytes (cells), T_H0, Naive T helper cells; T_H2, T helper type 2 cells.

15 mg/kg, b.w.) for 28 days in asthmatic rats efficiently suppressed allergen-induced inflammation cascade and inhibited migration of immune cells particularly of eosinophils in rat's lung, demonstrating the prospective role of the herbal extract in bronchial asthma.

Neutrophils are the first inflammatory respondent that directly or indirectly activates other inflammatory mediators to release a storm of cytokines, myeloperoxidase, COX-2, and LOX-5 enzymes and accelerate and augment the inflammatory response (Tanaka et al., 2006, Dileepan et al., 2019). Neutrophils secrete an enzyme known as elastase that acts as a potent secretagogue in airways and cause goblet cell hypersecretion (Nadel et al., 1999). In our studies, treating asthmatic animals with MFE at both doses (3.75 and 15 mg/kg), resulted in reduced neutrophil count in both blood as well as BAL fluid of treated animals. Significant reduction in neutrophils can be owed to the presence of bioflavonoids, rhusflavanone or mesuaferrone B, and pentacyclic triterpenoids, such as α-amyrin or β-amyrin, present mainly in stamens of *M. ferrea*. These triterpenoids are reported to have multiple biological functions, including modulation of leucocyte migration and neutrophil infiltration in the asthmatic lungs, antihistaminic activity, antiallergic activity, mast cells stabilisation, myeloperoxidase (MPO) inhibitory activity (Oliveira et al. 2004, Okoye et al., 2014). Rhusflavanone and mesuaferrone B exhibit potent human leukocyte elastase inhibitory activity in in vitro studies (Zar Wynn Myint et al., 2021).

Another two sesquiterpenes, largely present in *M. ferrea* flowers are germacrene D and α-copaene. Germacrene D impedes production of reactive oxygen species in neutrophils and sojourn *N*-formylmethionyl-leucyl-phenylalanine (*f*MLF)-induced neutrophil Ca2+ mobilization. Thus, MFE has the ability to modulate *f*MLF-induced neutrophil migration and chemotaxis in dose dependent manner (Schepetkin et al., 2020). *α*-Copaene is reported as potential phosphatidyl-inositol 3-kinases (PI3Ks) inhibitor. In asthma pathology, PI3Ks play a vital central signallingrole. PI3Ks inhibition drops the mucus production, averts degradation of mast cells, assists bronchodilation and discourages immune cell conscription thus helps in the management of allergic asthma (Queiroz et al., 2014; Yoo et al., 2017).

T_H2 mediators, TNF-*α*, IL-5, IL-4, IL-1ß play a primary role in coordinating various inflammatory mechanisms in asthma. Therapeutic approaches targeting IL-4, IL-5, and TNF-*α* through their selective inhibitors are under clinical trials in management of asthma. In our study, presence of T_H2 cytokines in asthmatic animals indicates perpetuating airway inflammation in sensitized lungs. Oral treatment of asthmatic rats with MFE (3.75 or 15 mg/kg) reversed

(IL)-4, -5, and TNF-α levels in both body fluids (serum and BAL) of animals in comparison to the asthma group. Effects produced by MFE at higher doses were significant and comparable to that of corticosteroid, used as a standard drug in the study (2.5 mg/kg). Cysteinyl leukotrienes (cys LTs) represent biologically active arachidonic acid metabolites and account for clinical features of asthma (Laidlaw and Boyce, 2012). Cysteinyl leukotrienes mediate biological effects through G-protein receptors CysLTR (1-3). CysLT1R is mainly present in bronchial muscles and has a higher affinity to LTD4. Market available montelukast, zafirlukast specifically antagonise CysLT1R (Al-Azzam and Elsalem, 2020). In our study, standardized MFE extract treatment also significantly ameliorated OVA-induced increased levels of IL-1β and cysteinyl leukotrienes (LTD4) in serum and BAL fluid of asthmatic animals as compared to OVA-control group. Marked reversal of LTD-4 levels after treatment of asthmatic animals with MFE demonstrate possible antagonistic effects of herbal extract at CysLT1R as similar to market drugs.

Serum Immunoglobulin E (sIgE) levels are high in asthmatic individuals as compared to normal subjects and these levels increase with the severity of asthma making it a strong clinically predisposing factor in the bronchial asthma. Oral administration of MFE at both doses (3.75 and 15 mg/kg) was found to be effective in reducing IgE titer in both blood/serum and BAL fluid of treated animals. Decreased concentration of IgE can be related to suppression of histamine release from mast cells and demonstrates antiallergic role of the plant.

Inflammatory cells in asthmatic individuals are capable of producing several fold more free oxygen radicals and nitrites by autooxidation of nitric oxide molecule (NO) (Bhujbal et al., 2010). An increase in generation of NO, called 'nitrosative stress', contributes to ongoing process of inflammatory process in airways. The nitric oxide is produced during the production of the L-citrulline from L-arginine by *i*NOS (inducible nitric oxide synthase), which, in turn, inhibits the arginase activity. *i*NOS is also induced by biochemicals, such as TNF-α, IL-1ß and stimulates the formation of nitric oxide. Nitrate (NO3-) and nitrite (NO2-) radicals are metabolite products of NO oxidation (Zuo et al., 2014). Studies report that acidic pH in asthmatic airways may facilitate conversion of nitrite to NO. NO_2^-/NO_3^- and FENO (fractional exhaled nitric oxide) levels may be considered as biological markers to relate it with intensity of allergic sensitisation (Fernando et al., 2020). In the current study, investigating the role of MFE on NO and nitrites in blood and BAL fluid of allergen sensitized rats, the crude drug extract was found to reduce levels of both gaseous radicals in a significant manner. These results may be correlated due to the presence of antioxidant phytoconstituents, xanthones, flavonoids, α-amyrin, β-amyrin, germacrene D, and α-Copaene in the plant part.

Potential role of *M. ferrea* ethanolic (70%) extract observed in our study is assumed to be related to the presence of sesquiterpenes, phenolics, and steroidal compounds present in the plant as mentioned elsewhere. We had chosen 70% ethanol for extraction and pharmacological in our previous preliminary experiments, maximum %extractive yield of *M. ferrea* was obtained hot extraction using a solvent mixture of ethanol : water (7 : 3). In addition, maximum flavonoid and phenolic content (w/w) was also analysed in MFE. Though due to lack of analytic facilities in our lab, it was not possible to quantitatively standardize the crude herbal extract for specific biomarkers. However, collective piece of evidence shows that pentacyclic tripterpenoids, bioflavanoids (Rhusflavanone and mesuaferrone B), and phenolic compounds exhibit large physiological antiasthmatic and antiinflammatory activities in laboratory animals.

The phenolic compounds intervene in cell transduction pathways mediated via GATA-3 and RORγt (transcription factors of the RAR-related orphan nuclear receptor (ROR) family) mechanism, major pathways in maintaining balance of T_H1/T_H2 in allergic conditions (Nadif et al., 2014). In preclinical studies, gallic acid a phenolic compound present widely in plants, reduces airway resistance, eosinophil infiltration, interleukin -5, -13 levels in BAL fluid and IL-33 expression in lungs by inhibiting MyD88 expression and downregulating nuclear factor (NF)-κB signaling (Arora et al., 2011; Wang et al., 2018). IL-33 inhibition by gallic acid results in inactivation of type 2 innate lymphoid cells (ILC2) and Th2 mediated cytokine release. Quercetin ameliorates the stability of Th1/Th2 phenotype, histamine production, release of proinflammatory mediators and antigen-specific IgE antibody by B cells (Jafarinia et al., 2020). Kaempferol curbs the Tyk-STAT signalling mediated through eotaxin-1 and eotaxin receptor CCR3. It also inhibits TLR-4 activation and decreases the production of IL-8, IL-4, IL-5, IL-13, and TNF-α by inhibiting Akt phosphorylation (Gong et al., 2013; Chung et al., 2015). Similarly, alleviations in levels of different cytokines by luteolin treatment in animal as well as in human subjects are also documented (Das and Ghosh, 2003; Liang et al., 2020). Triterpenoid lupeol significantly modulates the level of type II cytokines (IL-4, IL-5 and IL-13) during in vivo experiments (Vasconcelos et al., 2008). Similarly, apigenin has ability to modulate levels of proinflammatory mediators IL-1β, TNF-α, NO, lymphocytes and neutrophils levels in vitro studies (Lago et al., 2014). Similar pharmacological effects in animal models of asthma have been observed with phytosterol β-sitosterol (Mahajan and Mehta, 2011). Accumulating all evidence, we can presume that presence of phenolic

and triterpenoids bioactives in *M. ferrea* stamens could be collectively responsible for therapeutic role of herbal drug in bronchial asthma.

Our findings suggest that MFE could exert antiasthmatic effects in experimental rats by regulating T_H2 related cytokines production in lungs, mainly IL4, IL5, and others. The suggested mode of action of *Mesua ferrea* stamens is depicted in Figure 6. Reduced levels of T_H2 derived cytokines could inhibit secretion of IgE and thereby, suppress degranulation of mast cells and release of several cytokines, chemokines that might ameliorate airway inflammation cascade and ROS generation in lungs (Mahajan and Mehta, 2011). These studies demonstrate multiple targets underlying preventive effect of our herbal drug in bronchial asthma. Despite promising results, the clinical approach towards use of *Mesua ferrea* stamens in asthma therapy needs detailed pharmacological studies at cellular and molecular levels.

5. Conclusion

The results of our study statistically proved and suggest the potential role of *Mesua ferrea* stamens extract in the treatment and management of allergic asthma by attenuating ongoing inflammatory processes, mediated via inhibiting subsequent infiltration of eosinophils, lymphocytes, neutrophils into lungs airways, and release of inflammatory and proinflammatory mediators. Further, studies on bioactives present in *M. ferrea* may be advised to investigate its mechanism of action at molecular and cellular levels.

References

Al-Azzam N, Elsalem L (2020). Leukotriene D4 role in allergic asthma pathogenesis from cellular and therapeutic perspectives. Life Sciences 260: 118452. doi: 10.1016/j.lfs.2020.118452

Anonymous (2005). Foundation of resuscitate and encourage Thai traditional medicine. Thai Pharmaceutical Book. Pikanate Printing Center Co-oporation, Bangkok.

Anonymous (2008). Ayurvedic pharmacopoeia of India. Ministry of health and family welfare, Government of India. The Controller of Publications, Civil Lines, Delhi, India, Part II, vol. II, (formulations) 1st English edition: 31-32.

Arora P, Ansari SH (2015). Quality standard parameters of an anti-asthmatic Ayurvedic formulation "*Kanakasava*". International Journal of Pharmacognosy and Phytochemical Research 6 (4): 983-987.

Arora P, Ansari SH (2019). Role of various mediators in inflammation of asthmatic airways In: Asthma-Biological Evidences; Celso Pereira Ed. IntechOpen. doi: 10.57720/intechopen.84357

Arora P, Ansari SH, Anjum V, Mathur R, Ahmad S (2017). Investigation of anti-asthmatic potential of Kanakasava in ovalbumin-induced bronchial asthma and airway inflammation in rats. Journal of Ethnopharmacology 197: 242-49. doi: 10.1016/j.jep.2016.07.08.

Arora P, Ansari SH, Nazish I (2011). Study of anti-obesity effects of ethanolic and water extracts of grapes seeds. Journal of Complementary and Integrative Medicine 8: 1-14. doi: 10.2202/1553-3840.1510.

Asher MI, García-Marcos L, Pearce NE, Strachan DP (2020). Trends in worldwide asthma prevalence. European Respiratory Journal 56: 2002094. doi: 10.1183/13993003.02094-2020

Asif M, Jafari SF, Iqbal Z, Revadigar V, Oon CE et al. (2017). Ethnobotanical and phytopharmacological attributes of Mesua ferrea: A mini review. Journal of Applied Pharmaceutical Science 7: 242-51.

Bhujbal S, Rabindra KN, Gayatri G, Shubangi J, Priti D et al. (2010). Protective effects of icosahydropicenic acid isolated from the roots of *Clerodendrum serratum* (L) Moon on experimental allergic asthma. Journal of Complementary and Integrative Medicine 7: 1-7. doi:10.2202/1553-3840.1401

Burkill I (1966). A dictionary of the economic products of the Malay Peninsula., 2nd Edition. Ministry of Agriculture and Co-Operatives, Kuala Lumpur, Malaysia.

Canning BJ (2006). Reflex regulation of airway smooth muscle tone. Journal of Applied Physiology 101 (3): 971-985. doi: 10.1152/japplphysiol.00313.2006

Canning BJ, Chou Y (2008). Using guinea pigs in studies relevant to asthma and COPD. Pulmonary Pharmacology and Therapeutics 21 (5): 702-720. doi: 10.1016/j.pupt.2008.01.004

Chahar MK, Sanjaya Kumar DS, Geetha L, Lokesh T, Manohara KP (2013). *Mesua ferrea* L.: A review of the medical evidence for its phytochemistry and pharmacological actions. African Journal of Pharmacy and Pharmacology 7: 211-9. doi:10.5897/AJPP12.895

Chung MJ, Pandey RP, Choi JW, Sohng JK, Choi DJ et al. (2015). Inhibitory effects of kaempferol-3-O-rhamnoside on ovalbumin-induced lung inflammation in a mouse model of allergic asthma. International Immunopharmacology 25 (2): 302-10. doi: 10.1016/j.intimp.2015.01.031

Das M, Ram A, Ghosh B (2003). Luteolin alleviates bronchoconstriction and airway hyperreactivity in ovalbumin sensitized mice. Inflammation Research 52 (3):101-6. doi: 10.1007/s000110300021.

Dileepan M, Rastle-Simpson S, Greenberg Y, Wijesinghe DS, Kumar NG et al. (2019). Effect of dual sEH/COX-2 inhibition on allergen-induced airway inflammation. Frontiers in Pharmacology 10: 1118. doi: 10.3389/fphar.2019.01118.

Fehrenbach H, Wagner C, Wegmann M (2017). Airway remodeling in asthma: what really matters. Cell and Tissue Research 367 (3): 551-569. doi: 10.1007/s00441-016-2566-8.

Fernando Y, Wickramasinghe P, De Silva U, Alahakoon M, Anuradha KWDA et al. (2020). Differences in serum markers of oxidative stress in well controlled and poorly controlled asthma in Sri Lankan children: a pilot study. Allergy Asthma and Clinical Immunology 16:66. doi.org/10.1186/s13223-020-00463-9.

Gong JH, Shin D, Han SY, Park SH, Kang MK et al. (2013). Blockade of airway inflammation by kaempferol via disturbing Tyk-STAT signaling in airway epithelial cells and in asthmatic mice. Evidence-based Complementary and Alternative Medicine 2013: 250725. doi: 10.1155/2013/250725.

Bax HJ, Keeble AH, Gould HJ (2012). Cytokinergic IgE action in mast cell activation. Frontiers in Immunology 6 (3):229. doi: 10.3389/fimmu.2012.00229.

Hirose K, Iwata A, Tamachi T, Nakajima H. (2017). Allergic airway inflammation: key players beyond the Th2 cell pathway. Immunological Reviews 278 (1):145–161. doi: 10.1111/imr.12540.

Jafarinia M, Sadat Hosseini M, Kasiri N, Fazel N, Fathi F et al. (2020). Quercetin with the potential effect on allergic diseases. Allergy, Asthma and Clinical Immunology 16: 36; 1-11. doi: 10.1186/s13223-020-00434-0.

Keawsa-Ard S, Liawruangrath B, Kongtaweelert S (2015). Bioactive compounds from *Mesua ferrea* stems. Chiang Mai Journal of Science 42: 185-95.

Kuruvilla ME, Lee FE, Lee GB (2019). Understanding asthma phenotypes, endotypes, and mechanisms of disease. Clinical Reviews in Allergy and Immunology 56 (2): 219-233. doi: 10.1007/s12016-018-8712-1.

Lago JHG, Toledo-Arruda AC, Mernak M, Barrosa KH, Martins MA et al. (2014). Structure-activity association of flavonoids in lung diseases. *Molecules* 19 (3): 3570-3595. doi: 10.3390/molecules19033570.

Laidlaw TM, Boyce JA (2012). Cysteinyl leukotriene receptors, old and new; implications for asthma. Clinical and Experimental Allergy 42 (9): 1313-1320. doi: 10.1111/j.1365-2222.2012.03982.x.

Lambrecht BN, Hammad H, Fahy JV (2019). The Cytokines of Asthma. Immunity 50 (4): 975-991. doi: 10.1016/j.immuni.2019.03.018.

Liang KL, Yu SJ, Huang WC, Yen HR (2020). Luteolin attenuates allergic nasal inflammation via inhibition of interleukin-4 in an allergic rhinitis mouse model and peripheral blood from human subjects with allergic rhinitis. Frontiers in Pharmacology 11: 291. doi: 10.3389/fphar.2020.00291.

Mahajan SG, Mehta AA (2011). Suppression of ovalbumin-induced Th2-driven airway inflammation by β-sitosterol in a guinea pig model of asthma. European Journal of Pharmacology 650 (1): 458-64. doi: 10.1016/j.ejphar.2010.09.075.

Matucci, A, Vultaggio A, Maggi E (2018). Is IgE or eosinophils the key player in allergic asthma pathogenesis? Are we asking the right question? Respiratory Research 19 (1): 113. doi: 10.1186/s12931-018-0813-0

Nadel JA, Takeyama K, Agusti C (1999). Role of neutrophil elastase in hypersecretion in asthma. European Respiratory Journal 13 (1):190-6. doi: 10.1034/j.1399-3003.1999.13a35.x.

Nadif R, Rava M, Decoster B, Huyvaert H, Moual NL et al. (2014). Exhaled nitric oxide, nitrite/nitrate levels, allergy, rhinitis and asthma in the EGEA study. European Respiratory Journal 44: 351-360. doi: 10.1016/j.anai.2010.03.013.

Okoye NN, Ajaghaku DL, Okeke HN, Ilodigwe EE, Nworu CS et al. (2014). Beta-Amyrin and alpha-amyrin acetate isolated from the stem bark of *Alstonia boonei* display profound anti-inflammatory activity. Pharmaceutical Biology 52 (11): 1478-86. doi: 10.3109/13880209.2014.898078.

Oliveira FA, Lima-Junior RC, Cordeiro WM, Vieira-Júnior GM, Chaves MH et al. (2004). Pentacyclic triterpenoids, α, β-amyrins, suppress the scratching behavior in a mouse model of pruritus. Pharmacology Biochemistry and Behaviour 78 (4): 719-25. doi: 10.1016/j.pbb.2004.05.013

Parasuraman S, Raveendran R (2012). Measurement of invasive blood pressure in rats. Journal of Pharmacology and Pharmacotherapeutics 3 (2): 172-177. doi: 10.4103/0976-500X.95521.

Parukutty B, Chandra GS (1984). Studies on the medicinal uses of plants by the Boro tribals of Assam-II. Journal of Economic and Taxonomic Botany 5: 599-604.

Petsky HL, Cates CJ, Lasserson TJ (2012). A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). Thorax 67 (3): 199-208. doi: 10.1136/thx.2010.135574.

Queiroz JC, Antoniolli ÂR, Quintans-Júnior LJ, Brito RG, Barreto RS et al. (2014). Evaluation of the anti-inflammatory and antinociceptive effects of the essential oil from leaves of *Xylopia laevigata* in experimental models. The Scientific World Journal 2014: 2014; 816450. doi: 10.1155/2014/816450.

Rai LK, Pankaj P, Sharma E (2000). Conservation threats to some important medicinal plants of the Sikkim Himalaya. Biological Conservation 93 (1): 27-33. doi.org/10.1016/S0006-3207(99)00116-0.

Roh SS, Kim SH, Lee YC, Seo YB (2008). Effects of radix adenophorae and cyclosporine A on an OVA-induced murine model of asthma by suppressing to T cells activity, eosinophilia, and bronchial hyper-responsiveness. Mediators of Inflammation 781425: 1-11. doi: 10.1155/2008/781425.

Roufosse F (2018). Targeting the Interleukin-5 pathway for treatment of eosinophilic conditions other than asthma. Frontiers in Medicine 5: 49. doi: 10.3389/fmed.2018.00049.

Samitas K, Delimpoura V, Zervas E, Gaga M (2015). Anti-IgE treatment, airway inflammation and remodelling in severe allergic asthma: current knowledge and future perspectives. European Respiratory Review 24 (138): 594-601. doi: 10.1183/16000617.00001715.

Schepetkin IA, Özek G, Özek T, Kirpotina LN, Khlebnikov AI et al. (2020). Chemical composition and immunomodulatory activity of *Hypericum perforatum* essential oils. Biomolecules 10 (6): 916. doi.org/10.3390/biom10060916

Sharma A, Sharma S, Parashar B (2017). *Mesua ferrae* Linn:-a review of the Indian Medical Herb. Systematic Reviews in Pharmacy 8 (1):19-23.

Tanaka D, Kagari T, Doi H, Shimozato T (2006). Essential role of neutrophils in anti-type II collagen antibody and lipopolysaccharide induced arthritis. Immunology 119 (2): 195–202. doi: 10.1111/j.1365-2567.2006.02424.x.

Vasconcelos JF, Teixeira MM, Barbosa-Filho JM, Lúcio AS, Almeida JR et al. (2008). The triterpenoid lupeol attenuates allergic airway inflammation in a murine model. International Immunopharmacology 8 (9): 1216-21. doi: 10.1016/j.intimp.2008.04.011.

Wang X, Zhao H, Ma C, Lv L, Feng J et al. (2018). Gallic acid attenuates allergic airway inflammation via suppressed interleukin-33 and group 2 innate lymphoid cells in ovalbumin-induced asthma in mice. International Forum of Allergy and Rhinology 8 (11): 1284-1290. doi:10.1002/alr.22207.

WHO (2020). https://www.who.int/news-room/fact-sheets/detail/asthma. Assessed on 9th November, 2021.

Yoo EJ, Ojiaku CA, Sunder K, Panettieri Jr RA (2017). Phosphoinositide 3-kinase in asthma: novel roles and therapeutic approaches. American Journal of Respiratory Cell and Molecular Biology 56 (6): 700-7. doi: 10.1165/rcmb.2016-0308TR.

Zar Wynn Myint K, Kido T, Kusakari K, Prasad Devkota H et al. (2021). Rhusflavanone and mesuaferrone B: tyrosinase and elastase inhibitory biflavonoids extracted from the stamens of *Mesua ferrea* L. Natural Product Research 35 (6):1024-8. doi: 10.1080/14786419.2019.1613395.

Zeiger RS, Schatz M, Li Q (2014). High blood eosinophil count is a risk factor for future asthma exacerbations in adult persistent asthma. The Journal of Allergy and Clinical Immunology: In Practice 2 (6): 741-50. doi: 10.1016/j.jaip.2014.06.005.

Zhu J, Yamane H, Paul WE (2010). Differentiation of effector CD4 T cell populations (*). Annual Review of Immunology 28: 445-489. doi: 10.1146/annurev-immunol-030409-101212.

Zuo L, Koozechian LS, Chen L (2014). Characterization of reactive nitrogen species in allergic asthma. Annals of Allergy, Asthma and Immunology 112 (1): 18-22. doi: 10.1016/j.anai.2013.10.007