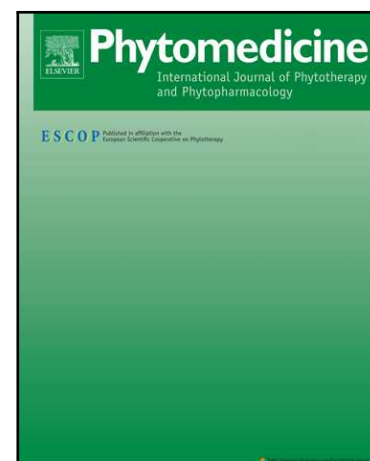


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***Carica papaya* ameliorates allergic asthma via down regulation of IL-4, IL-5, eotaxin, TNF- α , NF- κ B, and iNOS levels**

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ABSTRACT

Background: Natural products have a prime importance as an essential source for new drug discovery. *Carica papaya* leaves (CPL) have been used to treat inflammation in traditional system of medicine.

Aim/hypothesis: Current study evaluates the anti-inflammatory and immunomodulatory effects of CPL extract using mouse model of ovalbumin- (OVA) induced allergic asthma.

Methods: All the mice were intraperitoneally sensitized and subsequently given intranasal challenge with OVA except the control group. Group-III and -IV were treated for seven consecutive days with CPL extract and methylprednisolone (MP), respectively. At the end of study, histopathological examination of the lungs was performed and inflammatory cell counts were done in blood as well as bronchoalveolar lavage fluid (BALF). The mRNA expression levels of IL-4, IL-5, eotaxin, TNF- α , NF- κ B, and iNOS were measured using reverse transcription polymerase chain reaction (RT-PCR).

Results: Results showed significant attenuation of lung infiltration of inflammatory cells, alveolar thickening, and goblet cell hyperplasia after treatment with CPL extract. We also found significant suppression of total and differential leukocyte counts in both blood and BALF samples of CPL extract treated group. CPL extract also alleviated the expression levels of IL-4, IL-5, eotaxin, TNF- α , NF- κ B, and iNOS. Similarly, treatment with MP, used as a reference drug, also significantly ameliorated all the pro-inflammatory markers.

Conclusion: Current study shows that CPL extract possesses anti-inflammatory effect in mouse model of allergic airway inflammation by down-regulating IL-4, IL-5, eotaxin, TNF- α , NF- κ B, and iNOS expression levels.

Keywords: Airway inflammation; Cytokines; Immunomodulation; Th2-type immunity; Eosinophilia

Abbreviations:

Carica papaya leaves (CPL); Interleukin-4 (IL-4); Interleukin-5 (IL-5); Tumor necrosis factor- α (TNF- α); Nuclear factor-kappaB (NF- κ B); inducible nitric oxide synthase (iNOS); Total leukocyte count (TLC); Differential leukocyte count (DLC); Bronchoalveolar lavage fluid (BALF); T helper cells (Th); Ovalbumin (OVA)

Introduction

Allergic asthma is characterized by mucus metaplasia, bronchoconstriction, chronic inflammation, increased IgE levels, Th2 phenotype skewing, and airway hyperresponsiveness (Fanta, 2009; Willart and Lambrecht, 2009). Despite the fact that pathogenesis and treatment of asthma has been improved, its prevalence is increasing (Mahajan and Mehta, 2006). It affects approximately 300 million people throughout the world and its incidence is increasing in industrialized countries (Taur and Patil, 2011).

Extensive studies on the pathogenic mechanisms have shown that the allergic asthma is generally a T-helper (Th) type 2 mediated disease that leads to an imbalance between Th1 and Th2 cells and their cytokines (Kon and Kay, 1999). Exposure of allergen to the airways enhances the concentration of IL-4 and IL-5 by activating the inflammatory cells. IL-4 is key factor in the differentiation of T cells into Th2 type which generates a Th2 dominant immune response (Deckers et al., 2013; Schuijs et al., 2013). IL-5 plays a critical role in the enhanced generation of eotaxin and eosinophilia in the lung (Zhang-Hoover et al., 2005). Presence of eosinophils in

high concentrations produces some of the characteristic features of asthma and their recruitment is linked with the increased presence of eotaxin which serves as a potent chemoattractant for eosinophils (Conroy and Williams, 2001).

Upregulation of adhesion molecules causes the recruitment of eosinophils to the airways. TNF- α is known to increase the expression of adhesion molecules in the airway epithelium. The role of TNF- α in asthmatic airway inflammation has been established by various *in vivo* and *in vitro* studies (Babu et al., 2004). Strong and extended activation of NF- κ B has also been observed in the asthmatic patients. The activation causes the promotion of allergic sensitization to inhaled allergen (Ather et al., 2011; Kato and Schleimer, 2007). Inducible nitric oxide synthase (iNOS) drives the production of nitric oxide (NO) which affects many features of asthma pathophysiology. NO can be favorable in asthma as it may cause relaxation of airway smooth muscles. However, high concentration of NO produced by iNOS can result in mucus secretion in large quantity, vasodilation, edema, and in direct stimulation of Th2 immune response by recruiting eosinophils (Prado et al., 2011).

Inhaled corticosteroids are considered as first-line therapy of persistent asthma. The use of corticosteroids alone in high dose or in combination with long acting β_2 agonists and/or leukotriene modifiers has not been successful to achieve asthma control in all the patients. In addition to the inadequate asthma control associated with current anti-asthma therapy, there are concerns regarding efficacy and drug related adverse effects (Hanania, 2008). Plant extracts are vastly being considered as a source of alternative medicine by both physicians and patients (Markham and Wilkinson, 2004).

Carica papaya L. (family: Caricaceae), commonly known as papaya, is an ancient herbal medicinal plant. Leaves of the plant have been used to relieve asthma in traditional system of

medicine (Kovendan et al., 2012). Previously, *Carica papaya* leaves have been reported to possess anti-inflammatory properties, and it was proposed that disorders having inflammatory conditions, such as, asthma and arthritis can be treated using *Carica papaya* leaves extract (Owoyele et al., 2008). Present study investigates the anti-inflammatory and immunomodulatory effects of *Carica papaya* leaves extract using ovalbumin-induced allergic asthma model in mice.

Materials and methods

Preparation of the plant extract

Carica papaya L. leaves were collected from the botanical garden of University of the Punjab, Lahore, and were authenticated by Dr. Rasool Bakhsh Tareen, a botanist at University of Quetta. The leaves were cleaned from adulterants and shade-dried for 24 h. The dried leaves were ground to powdered form (500 g) and were macerated in absolute ethanol (2L) for 7 days with occasional shaking daily. The material was successively filtered through muslin cloth and Whatman No. 1 filter paper. The filtrate was evaporated in water bath (maintained at 40 °C) to obtain semi solid extract with approximate yield of 5%. The extract was then stored in a refrigerator at 4 °C for further use (Janbaz et al., 2013, 2014) .

Experimental animals

Male *BALB/c* mice (20-25 g) were purchased from National Institute of Health, Islamabad. All the animals were kept in Experimental Research Laboratory, University of Health Sciences,

Lahore, at controlled room temperature (22-24 °C), humidity (45-65%), and 12 h natural light/dark cycle. The mice were fed with standard pellet diet and water *ad libitum*. A prior approval of all the experiments was attained from the Ethical Review Committee, University of Health Sciences, Lahore.

Treatment protocol and induction of allergic airway inflammation

Mice were divided into four groups *i.e.* Group-I served as a control group; group-II as diseased group; while group-III and -IV served as treatment groups. Mice in group-III were treated with CPL extract orally (100 mg/kg b.w.) (Owoyele et al., 2008). Group-IV was intraperitoneally treated with methylprednisolone (MP) used as a reference drug (15 mg/kg b.w.).

20 µg of ovalbumin (OVA) (Sigma Aldrich, USA) was dissolved in 2 mg of aluminum hydroxide in a volume of 0.1 ml phosphate buffer saline (PBS). All the mice were intraperitoneally sensitized with OVA at day 0 and day 14 except the control group, which was sham-sensitized with PBS only. One week after the sensitization, the mice were given intranasal challenge with OVA (1 mg/ml PBS) once daily for seven consecutive days *i.e.*, from day 21 to day 27. The mice in the control group were challenged intranasally with PBS only. Treatment with CPL extract and MP was also started from day 21 and continued for 7 consecutive days. Twenty four hours after the last challenge and respective treatment (on day 28), all the mice were sacrificed (Khan et al., 2015).

Inflammatory cells in bronchoalveolar lavage fluid (BALF) and blood

At day 28, blood was collected in EDTA vacutainers using cardiac puncture technique. After taking trachea out with lungs intact, BALF was collected in 1.5 ml tubes by gradual instillation and withdrawal of 1 ml of ice cold PBS through trachea and lungs. Automated hemocytometer (Sysmex XT-1800i) was used to determine total leukocyte count (TLC) and differential leukocyte count (DLC) in BALF and blood (Shabbir et al., 2014).

Histopathological evaluation of lung tissue

Briefly, one lobe of the each mouse lung was fixed with 10% buffered formalin. The tissues were dehydrated with series of graded ethanol, and then embedded in paraffin wax. The blocks were made using paraffin and sections of 5 μ m thickness were cut for staining. Tissue sections were stained with Hematoxylin and Eosin (H & E) to observe infiltration of inflammatory cells and vascular congestion, while Periodic Acid Schiff (PAS) staining was used to evaluate goblet cell hyperplasia. According to following semi-quantitative scale, inflammatory score was graded: 0, none; 1, mild; 2, moderate; and 3, severe. Goblet cell hyperplasia was scored according to the percentage of PAS-positive cells among all bronchial epithelial cells: 0, none; 1, <25%; 2, 25 to <50%; 3, 50 to <75% (Quan et al., 2010).

Determination of mRNA expression levels of IL-4, IL-5, eotaxin, iNOS, TNF- α , and NF- κ B

Total RNA from the lung tissue was extracted by using standard TRIzol method (Ashraf et al., 2015). Nanodrop spectrophotometer was used to quantify the total RNA in each sample. cDNA was synthesized using 1000 ng total RNA/sample and kit manufacturer's protocol was followed (ThermoFisher Scientific, America). The concentrations of all the agents were according to the specifications mentioned in our previous publication (Shabbir et al., 2014). Amplification of the cDNA was performed using polymerase chain reaction. Briefly, cDNA template and PCR (2X) master mix (ThermoFisher Scientific, America) were mixed with each other. Sequences of IL-4, IL-5, and TNF- α primers were picked from our previous publications (Ashraf et al., 2015; Rana et al., 2016), while rest of the primers were designed manually or by using Primer3 Input (v. 0.4.0) software (Table 1). 1.0 μ l of forward and reverse primers (10 μ M) were added in the mixture and the volume was made up to 20 μ l with nuclease free water. Thermal cycler was programmed for 35 cycles of denaturation, annealing, and extension. The following cycle profile was used: 95 °C for 10 sec, 58-62 °C for 20 sec, and 72 °C for 30 sec. The annealing temperatures for different genes were set as following: 58 °C for IL-4, IL-5, and TNF- α ; 60 °C for eotaxin and NF- κ B; and 62 °C for iNOS. PCR product was visualized using 2% agarose gel electrophoresis. ImageJ software was used for densitometry and semi-quantification.

Statistical analyses

All the data were analyzed using GraphPad Prism v.5 software. One way ANOVA was applied to observe the difference among all groups. Post hoc Tukey's test or student t-test was applied to analyze the comparison between the groups, where applicable. The data was presented as Mean $\pm$ standard deviation (SD) and P-value $\leq$ 0.05 was considered as statistically significant.

Results

CPL extract nearly normalized the TLC and DLC in blood and BALF

Total leucocyte count and differential numbers of eosinophils, and lymphocytes in the blood of diseased group were found significantly elevated ($P < 0.001$) as compared to the control group. Treatments with CPL extract and MP decreased ($P < 0.001$) the counts of total leukocytes, eosinophils, and lymphocytes. Non-significant difference was found in neutrophil counts among all groups (Table 2).

Similarly, we also found significantly elevated counts of total leukocytes ($P < 0.001$), eosinophils ($P < 0.001$), neutrophils ($P < 0.05$), and lymphocytes ($P < 0.05$) in the BALF of the diseased group as compared to the control group. Administrations of CPL extract and MP decreased total leukocyte ($P < 0.001$), eosinophil ($P < 0.001$), neutrophil ($P < 0.05$), and lymphocyte ($P < 0.05$) counts as compared to diseased group (Table 3).

Treatment with CPL extract attenuated inflammation, alveolar thickening, and goblet cell hyperplasia in lung airways

H & E staining showed significantly increased ($P < 0.001$) inflammatory cell infiltration (2.500 ± 0.836) and alveolar thickening (2.417 ± 0.376) in the lungs of mice in diseased group as compared to control (0.333 ± 0.516 and 0.8333 ± 0.166 , respectively). CPL extract significantly decreased inflammatory cell infiltration (1.333 ± 0.516 ; $P < 0.05$) and alveolar thickening (1.333 ± 0.408 ; $P < 0.001$) as compared to diseased group. Similarly, treatment with MP significantly

reduced inflammation (1.333 ± 0.516 ; $P < 0.05$) and alveolar thickening (1.250 ± 0.418 ; $P < 0.001$) as compared to diseased group (Fig. 1A-B; Fig. 2).

Furthermore, lung tissue sections were stained with PAS to examine goblet cells hyperplasia which was found significantly increased (2.333 ± 0.516 ; $P < 0.001$) in the diseased group as compared to the control group (0.667 ± 0.516). Treatments with both CPL extract (1.333 ± 0.516 ; $P < 0.05$) and MP (1.167 ± 0.408 ; $P < 0.01$) significantly alleviated the goblet cells hyperplasia as compared with the diseased group (Fig. 1C; Fig. 3).

CPL extract decreased IL-4, IL-5, eotaxin, iNOS, TNF- α , and NF- κ B expression levels in lung tissue

We found increased ($P < 0.001$) expression levels of IL-4 (10.54 ± 0.605) in diseased group as compared to negative control group (1.000 ± 0.00). Treatment with CPL extract (4.957 ± 0.371) and MP (2.745 ± 0.416) significantly ($P < 0.001$) alleviated expression levels of IL-4 as compared to diseased group. IL-5 expression levels were also found elevated ($P < 0.001$) in diseased group (48.82 ± 3.36) as compared to negative control (1.000 ± 0.00). Treatments with CPL extract (21.44 ± 2.58) and MP (15.15 ± 1.49) significantly attenuated ($P < 0.001$) the IL-5 expression levels as compared to diseased group.

The data showed enhanced ($P < 0.001$) eotaxin expression levels in the diseased group (8.318 ± 0.801) as compared to control (1.000 ± 0.00) which were significantly ($P < 0.001$) reduced after treatments with CPL extract (3.060 ± 0.865) and MP (1.85 ± 0.434). Current study also demonstrated significantly enhanced ($P < 0.001$) expression levels of iNOS in diseased group

(7.873 ± 0.62) as compared to control (1.000 ± 0.00). Treatments with CPL extract (3.76 ± 0.712) and MP (2.873 ± 0.353) significantly abrogated the increased expression levels of iNOS. The results also showed significant elevation ($P < 0.001$) in the expression level of TNF- α in diseased group (6.355 ± 0.756) as compared to control group (1.000 ± 0.00). Treatments with CPL extract (2.775 ± 0.522) and MP (2.65 ± 0.524) significantly ($P < 0.001$) suppressed the TNF- α expression levels as compared to diseased group. The data demonstrated significant elevation ($P < 0.001$) in the expression levels of NF- κ B in the diseased group (21.01 ± 2.057) as compared to control group (1.000 ± 0.00). Both CPL extract (9.102 ± 1.536) and MP treated (6.252 ± 1.069) groups showed significant reduction ($P < 0.001$) in the expression levels as compared to diseased group (Fig. 4).

Discussion

Although, many pharmacological treatments are available for asthma management, yet they are not able to cure the disease rather they just provide symptomatic relief (Shahzad et al., 2009). Various medicinal plants have been evaluated for their anti-asthmatic potential using different animal models of asthma due to the adverse effects associated with current asthma therapy (Taur and Patil, 2011). Current study showed the anti-asthmatic effects of *C. papaya* leaves extract in mouse model.

The results showed significantly elevated expression levels of iNOS in diseased group as compared to control. Nitric oxide (NO) is a gaseous signaling molecule which is produced by T-lymphocytes and epithelial cells and plays crucial role in regulating different aspects of asthma. NO is produced through the action of iNOS in response to pro-inflammatory cytokines and

lipopolysaccharides. Increased expression of iNOS contributes significantly to both asthma and allergic rhinitis (Gabazza et al., 2000; Islam et al., 2010; Ricciardolo et al., 2006; Ricciardolo et al., 2004). Different animal based and genetic studies have shown that overexpression of iNOS increased the local production of NO in asthma which resulted in the damage of airways. Furthermore, elevated NO levels and iNOS expression have also been found in the exhaled air and pulmonary epithelial cells of the asthmatic patients, respectively (Batra et al., 2007). We found that therapy with CPL extract and MP significantly reduced the elevated expression level of iNOS. Our study showed a significant elevation in the total leukocytes, eosinophils, and lymphocytes counts in both blood and BALF of diseased group as compared to control. Treatment with *C. papaya* extract and MP significantly decreased the counts of inflammatory cells in both blood and BALF. Lymphocytes play crucial role in the pathogenesis of allergic asthma, especially T-helper type 2 lymphocytes which produce pro-inflammatory cytokines (Kay, 2006; Rana et al., 2016). IL-4 is the key cytokine which drives the T cell polarization in favor of Th2 cells. This results in the further secretion of IL-4, IL-5, IL-9, and IL-13 (Ashraf et al., 2015). IL-4 also facilitates the differentiation of B-lymphocytes which causes a switch to IgE generation. Thus, an allergic reaction can be seen as an allergen-specific generation of excess IL-4 (Deo et al., 2010). In current study, we found a significant increase in the expression levels of IL-4 in diseased group as compared to control. Treatment with both *C. papaya* extract and MP significantly reduced the elevated expression levels of IL-4. Previous studies have demonstrated the important role of IL-4 in the development of goblet cell hypersecretion and epithelial hyperplasia (Noben-Trauth et al., 1997; Voehringer et al., 2004). Amelioration of the histopathological parameters demonstrated in this study might be attributed to the reduction in IL-4 expression levels.

Eosinophils are circulating granulocytes which participate in the characteristic features of asthma, such as, bronchial hyper-responsiveness, airway remodeling, mucus hypersecretion, epithelial cell damage, and modulation of immune response (Possa et al., 2013). Previous studies, using allergen challenge IL-5 deficient mice, anti-IL-5 antibody treated mice, and IL-5 transgenic mice models, have firmly established the relationship between the development of airway eosinophilia and IL-5 (O'Byrne et al., 2001). IL-5 is considered as a crucial target that plays a major role in the beginning and sustenance of eosinophilic airway inflammation in allergic disease. IL-5 is also known to facilitate the eosinophil chemotaxis, enhance the expression of adhesion molecules on eosinophils, and increase their transmigration into the tissue from the blood vessels (Stein and Munitz, 2010). Thus, inhibition of IL-5 leads to interference of eosinophilic function and is considered as vital therapeutic strategy in case of severe asthma (Rana et al., 2016). Current study showed that CPL extract significantly alleviated the enhanced IL-5 expression levels, which is consistent with the result of decreased eosinophil counts after treatment with the plant extract.

Exposure to both IL-5 and CCR3 receptor ligands is required for the activation and degranulation of eosinophils. Together they produce a synergistic effect as compared with single stimulus (Ochkur et al., 2007). Eotaxin is a CCR3 ligand which contributes strongly along with IL-5 in the pathogenesis of asthma by inducing the eosinophil recruitment. Th2 lymphocytes regulate the recruitment of eotaxin in allergic reactions. Inhaled allergen causes the release of IL-4, IL-13, and TNF- α from Th2 lymphocytes and mast cells. The cytokines produce the eotaxin by stimulating smooth muscle cells, fibroblasts, and lung epithelial cells. Eotaxin selectively recruits eosinophils into the lung tissue by binding to the CCR3 receptors on eosinophils (Conroy and Williams, 2001). The data showed significant increase in the eotaxin expression levels in

diseased group as compared to control. Treatment with CPL extract and MP significantly suppressed the elevated expression levels of eotaxin.

Eosinophil recruitment is also indirectly linked with TNF- α , which increases eosinophil recruitment through upregulation of adhesion molecules (Babu et al., 2004; Yamamoto et al., 1998). We also evaluated the expression levels of TNF- α and NF- κ B, which are found elevated in diseased group as compared with control. Various evidences suggested a direct link between TNF- α and asthmatic complications. Increased expression of TNF- α is a characteristic of severe persistent asthma along with increased expression of IL-4 and IL-5. TNF- α has been shown to mediate airway inflammation and late-phase hyper-responsiveness in murine asthmatic model through activation of phospholipase A2 (Choi et al., 2005; Mukhopadhyay et al., 2006). Mast cells contain ample quantities of TNF- α , undergo degranulation in response to allergen challenge, and release TNF- α . The ability of TNF- α to increase its own release from the mast cells is mediated by NF- κ B (Babu et al., 2004). NF- κ B is an important inflammatory mediator which is known to regulate the expression of cell adhesion molecules, chemokines, and cytokines in airway pathology. The activation of NF- κ B happens in response to TNF- α and involves the dissociation of I κ B α . The degradation of I κ B α is catalyzed by I κ B α kinase and leads to the translocation of NF- κ B into the nucleus. NF- κ B binds to the promoter of responsive gene in the nucleus and induces gene transcription (Hinz and Scheidereit, 2014; Schuliga, 2015). Enhanced expression levels of NF- κ B in asthmatic tissue have been demonstrated by different studies (Edwards et al., 2009). We found that treatment with CPL extract and MP significantly reduced the elevated expression levels of TNF- α and NF- κ B. Previously, asthmatic patients who were treated with anti TNF- α therapy had shown relief in exacerbation frequency and an improvement in airway hyper-responsiveness, lung function, and quality of life (Desai and

Brightling, 2010). Inhibitors of NF- κ B activation have also demonstrated reduction in inflammatory cell infiltration and airway hyperresponsiveness triggered by allergic inflammation (Sheller et al., 2009).

Previously, *Carica papaya* leaves have been extensively characterized using various phytochemical assays. Yusha'u et al. (2009) described the presence of alkaloids, flavonoids, steroids, and tannins in the ethanol extract of the leaves, while glycosides, saponins, and reducing sugars were found absent. Juárez-Rojop et al. (2014) exhibited that tannins and alkaloids were existed in leaf ethanol extract only, while n-hexane and chloroform extracts were found devoid of these constituents. However, steroids and quinones were found present in all types of leaf extracts. The authors also quantified the phytochemical constituents and provided molar concentrations and percentages of the constituents. Canini et al. (2007) used gas chromatography-mass spectrometry analysis to identify and quantify phenolic compounds from the leaves of *C. papaya*. They identified phenolic acids and coumarins as the major constituents, such as, kaempferol, caffeic acid, *p*-coumaric acid, protocatechuic acid, chlorogenic acid, and quercetin. Aqueous extract of *C. papaya* leaves was subjected to liquid chromatography-mass spectroscopy analysis to conduct phytochemical profiling. The authors identified 21 constituents which included flavonoids, alkaloids, aminoacids, and phenolics. The list of 21 identified compounds including their molecular mass was presented by Akhila and Vijayalakshmi (2015).

Conclusion

The data showed that CPL extract possessed anti-inflammatory and immunomodulatory properties. The CPL extract significantly ameliorated infiltration of inflammatory cells, alveolar thickening, goblet cell hyperplasia, and total and differential leukocyte counts in mouse model of

OVA-induced allergic asthma. The anti-asthmatic activity might be attributed to the down-regulation of IL-4, IL-5, eotaxin, TNF- α , NF- κ B, and iNOS levels. Further studies are required to identify and isolate the active ingredients responsible for its anti-asthmatic activity.

Conflict of interest

The authors declare that they have no competing interests.

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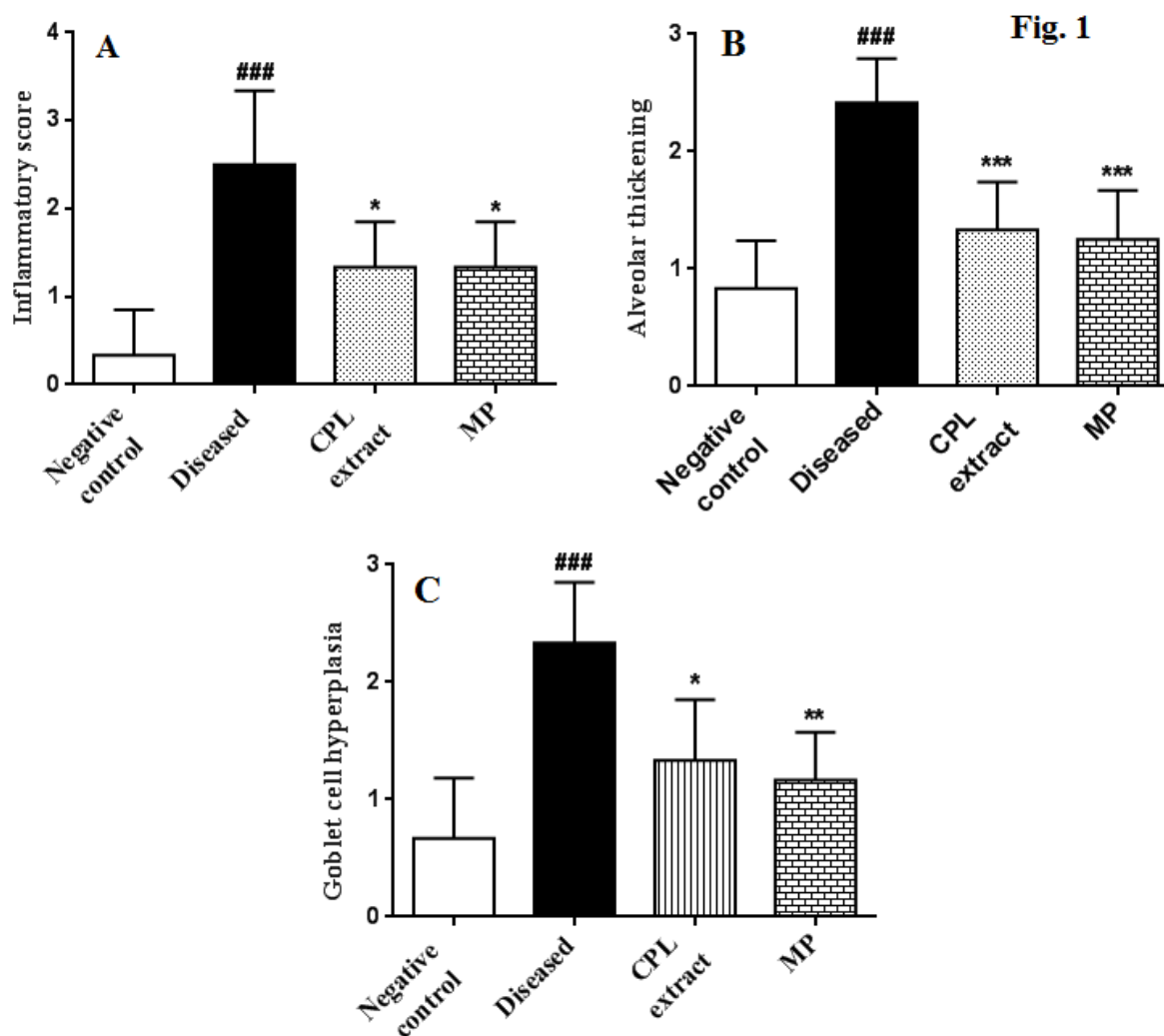


Fig. 1. Histopathological evaluation showed the amelioration of inflammation (A), alveolar thickening (B), and goblet cell hyperplasia (C) after treatment with both CPL extract and MP. The data was analyzed by One way ANOVA followed by Tukey's test, and is represented by Mean $\pm$ SD, where n = 6. # and * represent the comparison of diseased group with negative control and treated groups, respectively (*P < 0.05; **P < 0.01; ***P < 0.001).

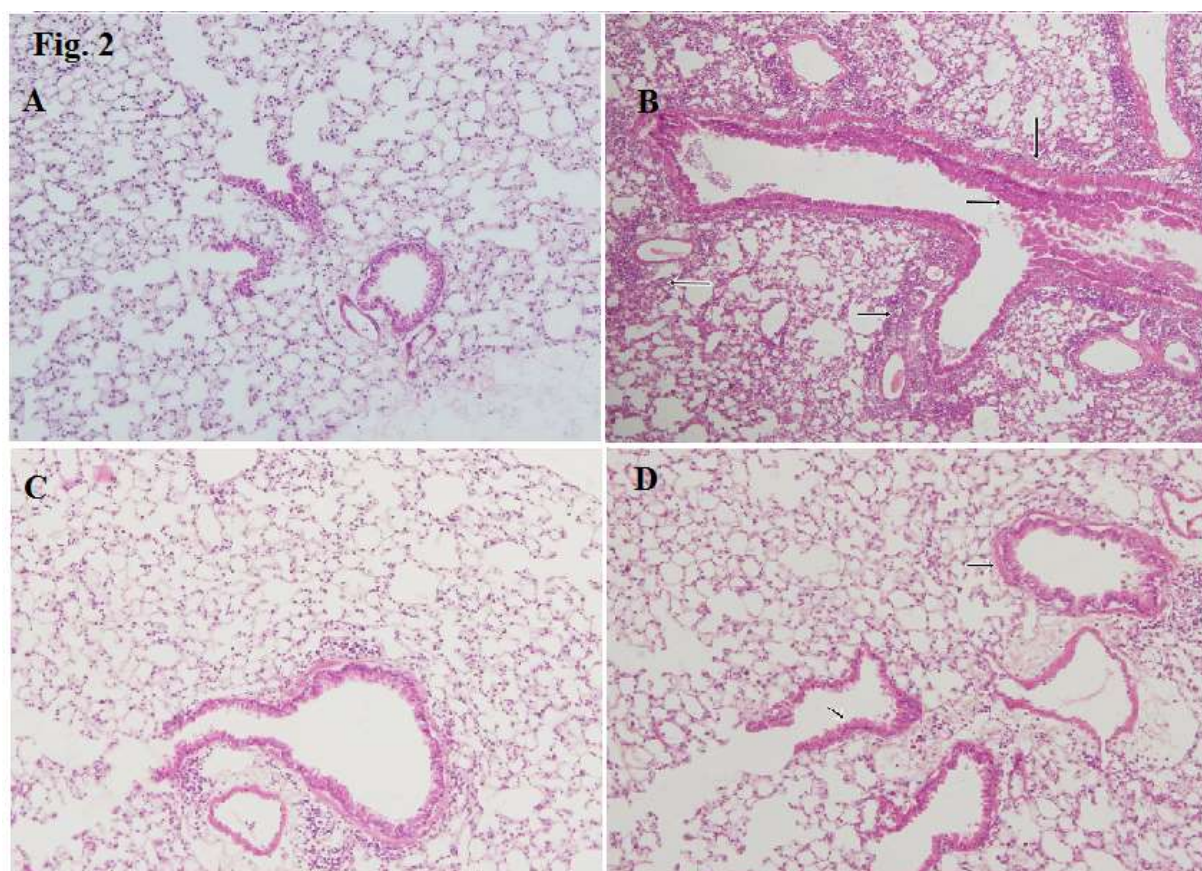


Fig. 2. H&E staining of lung sections of mice showing normal bronchial epithelium and alveolar lining in control group (A). A significant increase in inflammation and vascular congestion was found in diseased group (B). Treatment with both CPL extract and MP significantly attenuated the inflammation and vascular congestion (C and D, respectively).

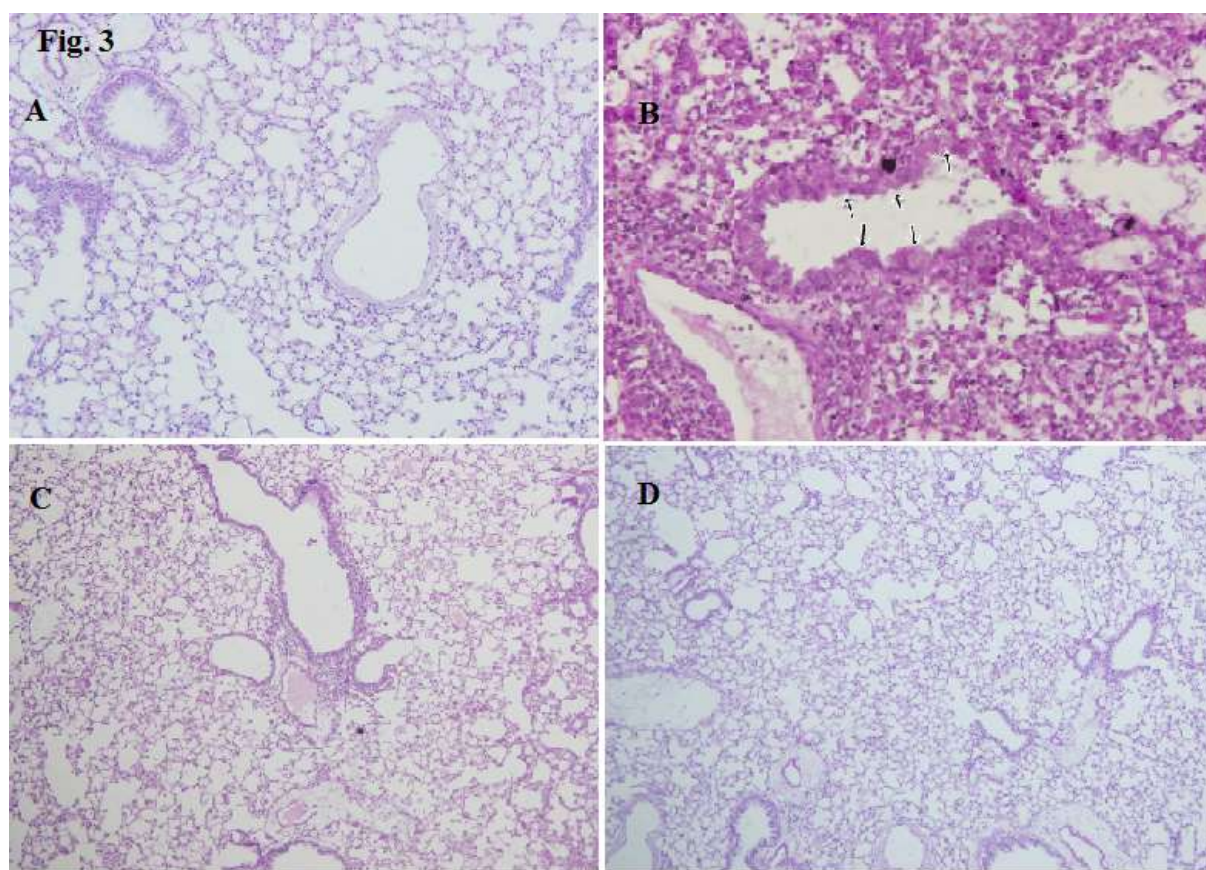


Fig. 3. PAS staining of lung sections of mice showing normal epithelium and alveoli in control group (A). We found significantly ($P < 0.001$) elevated goblet cell hyperplasia in diseased group (B), which was found attenuated after treatment with CPL extract ($P < 0.05$) and MP ($P < 0.01$).

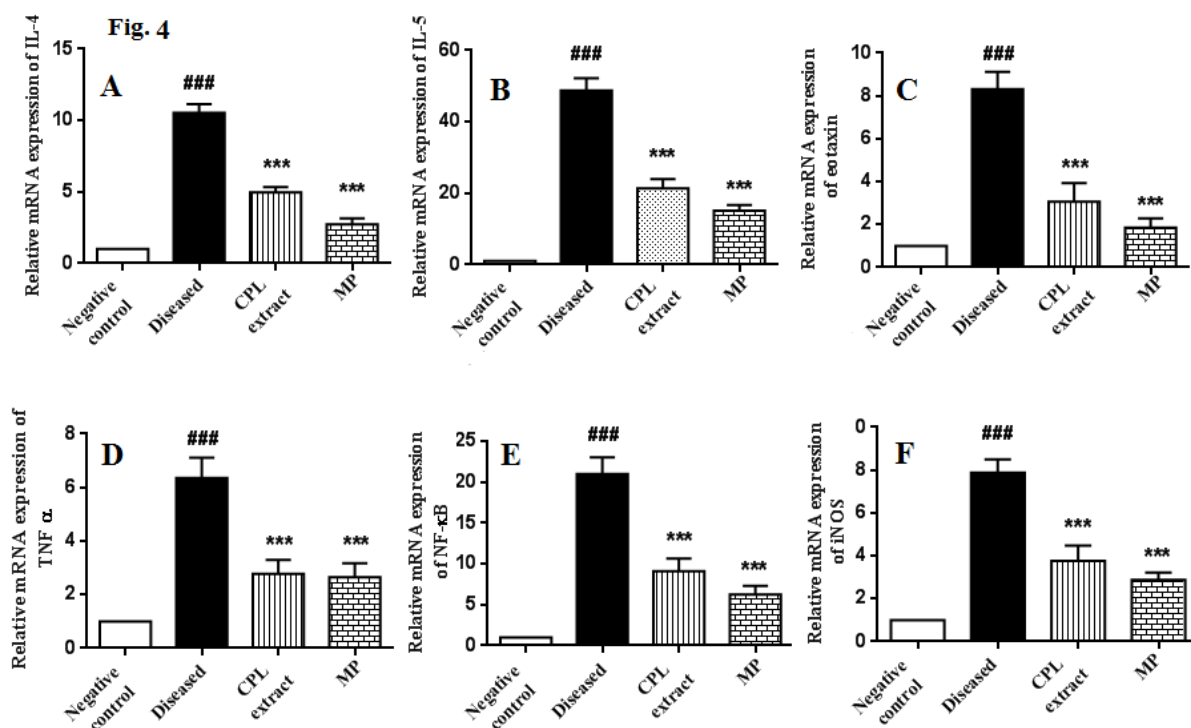


Fig. 4. Treatment with both CPL extract and MP significantly ($P < 0.001$) attenuated the increased mRNA expression levels of IL-4 (A), IL-5 (B), eotaxin (C), TNF- α (D), NF- κ B (E), and iNOS (F). The data was analyzed by One way ANOVA followed by Tukey's test, and is represented by Mean $\pm$ SD, where $n = 6$. # and * represent the comparison of diseased group with negative control and treated groups, respectively (** $P < 0.001$).

Table 1

Primer sequences along with product size

Primers	Forward / Reverse	Sequence	Product Size
Eotaxin	Forward	5'-CTGCTCACGGTCACTTCCTT-3'	198
	Reverse	5'-GGGGTCAGCACAGATCTCTT-3'	
iNOS	Forward	5'-ACCAGAGGACCCAGAGACAA-3'	121
	Reverse	5'-CCTGGCCAGATGTTCTCTA-3'	
NF- κ B	Forward	5'-GAGGCGTGTATTAGGGGCTA-3'	158
	Reverse	5'-ACGCTCAGGTCCATCTCCTT-3'	

Table 2

Both CPL extract and MP significantly alleviated total and differential leukocyte counts in blood. The data was analyzed by One way ANOVA followed by Tukey's test, and is represented by Mean $\pm$ SD, where n = 6. Statistical difference is shown by ### and *** which represents comparison with control and diseased group, respectively at P < 0.001.

TLC and DLC in Blood	Negative control group	Diseased group	CPL extract treated group	MP treated group
Total leukocyte count (10^3)	4.383 $\pm$ 0.491	14.61 $\pm$ 2.369###	6.492 $\pm$ 0.864***	5.695 $\pm$ 0.749***
Lymphocytes (%)	64.12 $\pm$ 1.023	72.86 $\pm$ 2.792###	67.39 $\pm$ 0.876***	67.13 $\pm$ 0.706***
Neutrophils (%)	14.84 $\pm$ 0.945	15.86 $\pm$ 1.343	15.71 $\pm$ 1.335	14.9 $\pm$ 1.107
Eosinophils (%)	0.613 $\pm$ 0.701	10.04 $\pm$ 1.49###	6.563 $\pm$ 1.114***	3.798 $\pm$ 1.489***

Table 3

Both CPL extract and MP significantly alleviated total and differential leukocyte counts in BALF. The data was analyzed by One way ANOVA followed by Tukey's test, and is represented by Mean $\pm$ SD, where n = 6. Statistical difference is shown by # and * which represents comparison with control and diseased group, respectively at $P < 0.05$, while ### and *** represents $P < 0.001$.

TLC and DLC in BALF	Negative control group	Diseased group	CPL extract treated group	MP treated group
Total leukocyte count (10^3)	1.17 $\pm$ 0.278	5.06 $\pm$ 0.787 ^{###}	1.74 $\pm$ 1.005 ^{***}	1.64 $\pm$ 1.110 ^{***}
Lymphocytes (%)	42.87 $\pm$ 8.379	70.63 $\pm$ 22.19 [#]	44.1 $\pm$ 17.27 [*]	45.58 $\pm$ 7.592 [*]
Neutrophils (%)	15.64 $\pm$ 1.12	17.45 $\pm$ 0.1332 [#]	15.85 $\pm$ 0.988 [*]	15.65 $\pm$ 1.213 [*]
Eosinophils (%)	1.18 $\pm$ 0.617	17.8 $\pm$ 2.299 ^{###}	8.95 $\pm$ 3.656 ^{***}	3.61 $\pm$ 1.28 ^{***}

Graphical abstract

Key:

Down-regulate: ↓

Effects of *Carica Papaya* on the mRNA
expression levels of the markers of
allergic asthma

