DOI: https://doi.org/10.53350/pjmhs2115102512

ORIGINAL ARTICLE

Carica papaya Leaf Extract modulates mRNA expression of Aquaporins in Mouse Model of Allergic Airway Inflammation

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ABSTRACT

Background: Asthma is a chronic inflammatory disease affecting smaller airways. Airflow obstruction leading to airway hyper-responsiveness and increased mucus production are salient features of asthma pathophysiology. Generally, Th2 cytokines are increased in allergic asthma.
Aim: To propose the molecular mechanisms by which *Carica Papaya* Leaves Extract (CPLE) alleviates pulmonary edema in animal model of allergic airway inflammation comparable to methylprednisolone.
Place and duration of study: Pharmacology Department, University of Health Sciences Lahore for 1 year.
Methods: We took twenty four male BALB/c mice and divided them equally into four groups. The control group was given PBS only, while Group II served as diseased group and induced airway inflammation by ovalbumin. Group III and IV were first induced with airway inflammation and side by side treated with *Carica papaya* leaf extract (CPLE) 100mg/kg body weight orally and methylprednisolone 15 mg/kg body weight intraperitoneally for seven consecutive days respectively. At the end of the experimental protocol, mice were euthanized and lung wet/dry ratio was measured. mRNA expression of AQP1 and AQP5 in lung tissue were also determined using RT-PCR.
Results: Ethanolic extract of *Carica Papaya* leaves decreased all markers of pulmonary edema in mouse model of allergic airway inflammation comparable to methylprednisolone by decreasing lung wet/dry ratio and enhancing AQP1 and AQP5 mRNA expression.
Conclusion: *Carica Papaya* leaves extract may diminish pulmonary edema in mice associated with allergic asthma.
Keywords: AQP1, AQP5 (Aquaporins), *Carica Papaya* Leaves Extract (CPLE), Pulmonary Edema, Th2 cytokines.

INTRODUCTION

Allergic asthma is initiated by a complex interplay of proinflammatory cytokines and inflammatory mediators in lung tissue causing airway narrowing, mucosal edema and airway hyperresponsiveness[1]. Leukotrienes, histamine and major basic protein are the main reasons for underlying bronchoconstriction. According to many studies, Aquaporins (AQP) are found to be responsible for pulmonary edema associated with bronchial asthma. These water channel proteins facilitate fluid transport in alveolar space and affect airway humidification, pleural fluid absorption, and submucosal gland secretion[2].

Out of 20 aquaporins, only AQP1, AQP3, AQP4 and AQP5 are expressed in lung tissue. AQP1 and AQP5 which are main water transport channels are located at apical membranes of capillary endothelial cells & type I alveolar epithelial cells. Some of the aquaporins belong to another subfamily which is mainly involved in glycerol transport across cell membranes[3]. Besides water transport, these protein channels are also found to be involved in cell migration, cell adhesion and inflammatory cascade. Aquaporins also play significant functions in various physiological and pathological manifestations of different lung diseases such as pleural effusion, bronchial asthma and lung cancer. Selective targeting of aquaporins can serve as new treatment modalities for these illnesses[4].

Pharmacological treatment of bronchial asthma includes the use of bronchodilators and anti-inflammatory drugs according to severity and frequency of asthmatic symptoms. Unfortunately, these groups of drugs neither provide long term relief, nor they are devoid of any adverse effects[5]. Keeping in mind these observations, scientists are now exploring to find more specific, potent, and safer drugs to decrease the burden of this prevailing disease[6] .

To date, medicinal plants have also gained attention in the field of pharmacology. Their potential against different diseases led us to investigate their active compounds and explore their proposed mechanism of action[7].

Carica Papaya belonging to the family *Caricacae* has been found to have anti-inflammatory, antioxidant, anticancer, antiviral and antibacterial properties. Alkaloids and flavonoids isolated from its fruit, pulp and leaves have been attributed towards these scientific findings[8]. Kovendin *et al.,* 2012 described that *Carica papaya* leaves have been traditionally used in the past for relief of asthmatic symptoms but very few studies have been carried out to confirm its anti-inflammatory or bronchodilator potential[9].

n our previous scientific research, *Carica papaya* leaves extract has been shown to reduce airway inflammation, goblet cell hyperplasia and mRNA

Received on 13-05-2021
Accepted on 17-09-2021

expression of proinflammatory cytokines including IL-4, IL-5, TNF-α, Eotaxin, iNOS and NF-κB in mouse model of allergic airway inflammation[10].

On the basis of above findings, we hypothesized and investigated that *Carica papaya* leave extract may relieve pulmonary edema by altering lung wet/dry ratio and modifying mRNA expression of lung aquaporins in mice.

MATERIALS AND METHODS

This experimental study had been carried out for a 1 year duration at the Pharmacology Department, University of Health Sciences, Lahore.

Preparation of plant extract (CPLE): Plant leaves extract (CPLE) was prepared following the methodology described in our previous publication[10]. *Carica papaya* leaves were collected and identified by a botanist of University of Punjab. After shade drying, the leaves were ground to powdered form. 400 g of powdered leaves were macerated with 2 L of ethanol for 7 days. This material was subsequently filtered and dried in an organ bath to yield 5% semisolid material which was stored in the refrigerator at 4° C for experimental usage[11].

Animals: Twenty-four Male BALB/c mice, weighing between 20-30 g, were purchased locally and divided equally into four groups by random balloting method. Mice were kept under normal conditions of temperature i.e., 22-24° C and humidity[12]. They were provided with animal feed and water ad libitum according to international standards approved by Ethical Review Board of University of Health Sciences, Lahore

Drugs:

1. Ovalbumin (Sigma Aldrich) 20 µg emulsified in 2 mg Alum[13]
2. Methylprednisolone (Sigma Aldrich) 15 mg/kg body weight[14]
3. *Carica papaya* leaves extract 100 mg/kg body weight[10]

Induction of Allergic Airway Inflammation: Airway inflammation was induced in mice by sensitizing and challenging them with ovalbumin till Day 21. Following allergic airway induction, treatment drugs CPLE and MP were given to animals of group 3 and 4 for 7 consecutive days. Methodology was followed as in our previous study[10]. Animals in control group were sensitized on Day 0 and Day 14 with Phosphate buffered saline (PBS) while group 2, 3 and 4 were sensitized with 20 µg of OVA mixed in 2 mg aluminium hydroxide and 0.1ml phosphate buffered saline (PBS) intraperitoneally on same days . PBS was given as intranasal challenge to control group animals for seven consecutive days i.e., from 21 to 27 days While animals of group II, III and IV were challenged intranasally with OVA, 1 mg/ml PBS for the same time period[15].

Group 2 which served as diseased group was not given any treatment while mice in group 3 were treated with *Carica Papaya* leaf extract, 100mg/kg body weight orally. Animals of group 4 were administered with standard drug, Methylprednisolone (MP) in a dose of 15mg/kg body weight intraperitoneally. All animals were sacrificed twenty-four after the last treatment, by giving light chloroform anesthesia[16].

Evaluation of lung wet/ ratio: We measured Lung wet/dry weight ratio to see effect on pulmonary edema development and subsequent amelioration after treatment with CPLE and MP. Lungs wet weight was measured immediately after excision of one lobe. Dry lung weight was measured after drying it in an oven at 56 °C for 15minutes[17].

Determination of mRNA expression level of Aquaporin 1 and 5 (AQP1, AQP5): Lung tissue was homogenized and total RNA was extracted by using standard TRIzol method. RNA was quantified by the Nanodrop spectrophotometer. It was then reverse transcribed into cDNA following sample and kit manufacturer's (Thermo Fisher Scientific, America). The chemicals and methodology were followed as described by Inam et al.,[10] cDNA was then amplified using polymerase chain reaction.

Briefly 1ul of forward and reverse primers were mixed with cDNA template and PCR (2X) master mix. Nuclease free water was added in the mixture to make a final volume of 20 ul. Cycles of denaturation, annealing, and extension were set in thermal cycler. Resultant PCR products were visualized through 2% agarose gel electrophoresis and finally quantified by Image J software[18]. Sequences of AQP1 and AQP5 primers were selected from a previous publication by Rana et al[14](Table 1).

RESULTS

CPLE significantly decreased lung wet/dry ratio: Lung wet/dry weight ratio of diseased group was significantly increased as compared to control group i.e., 0.0295 ± 0.01 vs 0.5017± 0.15 having P value < 0.0001. While Treatment with CPLE extract (0.050±0.01) and MP (0.06±0.02) showed a significant decrease in the lung wet/dry weight ratio (P < 0.0001) as compared to disease:

Fig. 1: Graphical representation of effect of CPLE and MP on lung wet/dry ratio

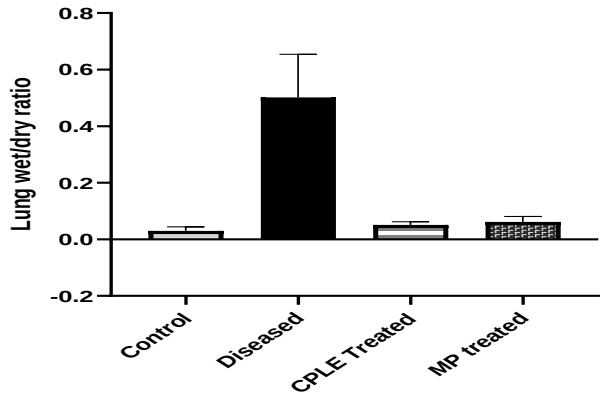


Table 1: Sequences of Primers (AQP1 and AQP5)

Primers	Forward & Reverse	Sequence	Product Size
AQP 1	(F) (R)	5' -AGAGCCCTGTCTGCATCCAT-3' 5' -CCTCGACTTAACCGCTGGAT-3' (Rana *et al*., 2016)	181
AQP 5	(F) (R)	5' -CCCAAGGCCACCATGAAGAA-3' 5' -TATGGCCAGGCCAAAGGCTA-3' (Rana *et al*., 2016)	171

Fig. 2: Graphical representation of effect of CPLE and MP on mRNA expression of AQP1

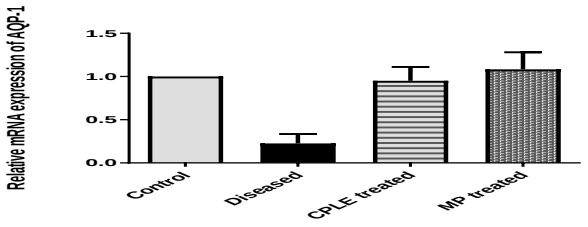
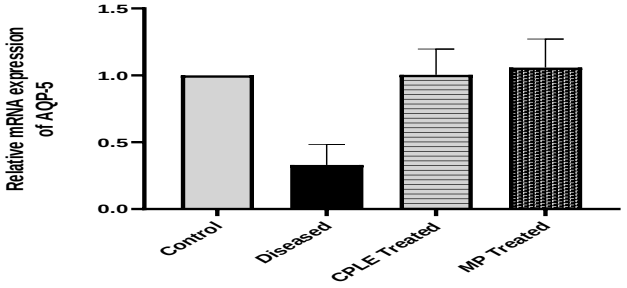


Fig. 3: Graphical representation of effect of CPLE and MP on mRNA expression of AQP5



We found that AQP1 mRNA expression level were significantly reduced in diseased group II (0.22±0.11) as compared to control group (1±0). While in Treatment group III and IV, AQP 1 expression was enhanced and measured to be (0.95± 0.16) and (1.09±0.19) respectively. Both CPLE and MP significantly increased the expression levels of AQP1 (all $P < 0.0001$) displayed in Fig.2,Table 3.

CPLE significantly increased AQP 5 mRNA expression levels in lung tissue: We observed a significant decrease in mRNA expression levels of AQP5 of diseased group as compared to control (0.33±0.15 vs 1±0). Group III treated with ethanolic extract of CPL showed a significant increase in mRNA expression of AQP5 as compared to diseased group (1.003±0.19 vs 0.33±0.15). Similarly, the Methylprednisolone treated group also showed a significant increase in mRNA expression of AQP 5 as compared to diseased group animals i. e. (1.06±0.21 vs 0.33±0.15) shown in Fig.3, Table. 3.

Statistical Analysis: Statistical analysis was done by using SPSS version 20. Mean and standard deviation was measured for all quantitative variables. One way ANOVA and post-hoc tukeys test was applied to see the difference among and within group mean[19].

Table 2: Lung wet/dry ratio in mice (n=6) P<0.0001

Parameters	Control group (I)	Diseased Group (II)	Treatment Group with CPLE (III)	Treatment Group with MP (IV)
Lung wet/dry ratio	0.029 ± 0.01	0.52± 0.15[a]	0.05±0.01[b]	0.06±0.02[b]

[a] Displays significant difference with group I [b] Displays significant difference with group I

Table 3: Relative mRNA expression levels of AQP1 and AQP 5 in all groups (n=6)

Aquaporins	Control group (I)	Diseased Group (II)	Treatment Group with CPLE (III)	Treatment Group with MP (IV)
AQP1	1± 0	0.22 ± 0.11[a]	0.95± 0.16[b]	1.09± 0.19[b]
AQP5	1± 0	0.33±0.15[a]	1.03±0.19[b]	1.06±0.21[b]

[a] Displays significant difference with group I [b] Displays significant difference with group II

DISCUSSION

Allergic asthma is characterized by airway inflammation, mucus hypersecretion, pulmonary edema and airway hyper-responsiveness[22]. Many studies have found a correlation between altered expression of Aquaporins and pulmonary edema. According to a study, mRNA expression levels of AQP1 and AQP5 are markedly reduced in pulmonary edema associated with airway inflammation[20]. These aquaporins are located in the apical membrane of the respiratory epithelial cells and microvascular endothelial cells and maintain water hemostasis, mucus secretion and eosinophil infiltration[21].

Although many pharmacological treatments including terbutaline, corticosteroids and montelukast are available to relieve asthmatic symptoms, all these drugs are not devoid of adverse effects[22]. There have been enormous studies showing clear indication of usage of phytochemicals as pharmacological treatment of airway inflammation as a better and safe choice. Present research has also been designed to explore plant based active compounds having anti inflammatory and immunomodulatory properties[23].

For this purpose, we chose *Carica papaya* leaf extract as a treatment drug in airway inflammation induced by ovalbumin in mice. *Carica papaya* leaves have been traditionally smoked to relieve cough in the past. CPLE contains many bioactive compounds including phenols, alkaloids, flavonoids, glycosides and saponins. Quercetin, kaempferol, caffeic acid and p-coumaric acid have been fractionated and isolated from CP leaves[9].

A previous study on allergic airway inflammation in mice concluded that CPLE shows promising immunomodulatory effects by downregulating Th2 cytokines, iNOS, eotaxin and NF-κB[10]. The current study has exhibited that ovalbumin induced airway inflammation is accompanied by pulmonary edema which manifests as increased lung wet/dry ratio, mucus hypersecretion and decreased mRNA expression level of AQP1 and AQP5 in lung tissue of diseased group animals. These findings are consistent with a previous study by Rana et al., 2016[14,24].

CPLE significantly reduced lung wet/dry ratio in the treatment group compared to methylprednisolone. The same results have been found in a previous study in a mouse model of allergic airway inflammation[14]. According to a research on animals of bronchial asthma, CPLE also attenuated pulmonary edema on histopathological evaluation[10]. Furthermore, to find the underlying mechanism of action of CPLE, we evaluated mRNA

expression of aquaporins which were decreased in diseased group as compared to control group. CPLE enhanced aquaporins expression in the treatment group comparable to methylprednisolone. In a past study, Quercetin which is a component of CPLE, has been found to enhance AQP5 expression in altered salivary secretion[24]. This is in accordance with the findings of our study. In light of these observations, we can conclude that quercetin may contribute to the anti-asthmatic effect of *Carica papaya* leaves extract, however further studies are required to isolate the active compound and compare its efficacy in clinical studies.

CONCLUSION

In conclusion, *Carica papaya* leaves extract (CPLE) demonstrated a significant reduction in biochemical and molecular markers of pulmonary edema associated with allergic inflammation mainly by inhibiting lung wet/dry ratio and enhancement of lung aquaporin's mRNA expression . However, further studies can be carried out to isolate the active compound from CPL extract to elucidate its exact mechanism, adverse effects and therapeutic dose.

Acknowledgments: We are very thankful to the Department of Immunology and Department of Biochemistry for providing technical and laboratory facilitation in this project.

Conflict of interest: The authors claim that we have not any conflict of interest. The study was purely done for research purposes.

Source of Support: Funding was granted by University of Health Sciences, Lahore.

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