Unveiling the Antioxidant Potential of Carica Papaya Leaf Extract in Ovalbumin-Induced Asthma

Asma Inam,[1] Shazana Rana,[2] Sabeen Irshad,[3] Sadia Majeed,[4] Muhammad Saqib Musharaf,[5] Sadia Ikram[6]

Abstract

Objective: The main objectives of this study were to determine the antioxidant potential of Carica papaya leaf extract (PLE) in a mouse model of bronchial asthma by measuring superoxide dismutase SOD, Catalase, Glutathione peroxidase, and Malondialdehyde (MDA) levels in bronchial tissues.

Materials & Methods: Male albino mice weighing 20-25g were randomly divided into three groups. Group 1 (control group) was treated with 1% phosphate-buffered saline (PBS). Groups 2 and 3 were sensitized with ovalbumin intraperitoneally (I.P) on days 0 and 14. Animals of these group were subsequently challenged with ovalbumin intranasally from day 21 to 27. The animals of Group 3 were treated with Carica papaya leaf extract (PLE) orally at 100mg/kg body weight on days 21-27. After sacrifice, the lung tissue of animals was isolated and stored at -80 C for measuring superoxide dismutase (SOD), Catalase, malonaldehyde (MDA), and Glutathione peroxidase (GPx).

Result: The administration of Carica papaya leaf extract resulted in a significant decrease of MDA levels in treatment group as compared to diseased group (7.9±0.38 Vs 8.06±0.2 nmol/mg protein). Treatment with PLE also increased activities of antioxidant enzymes such as glutathione peroxidase (115.3±2.7 Vs 106.±3.6 u/mg protein) catalase (0.16±0.01 Vs 0.14±0.01Ku/mg protein) and Superoxide dismutase SOD (24.1±0.35 Vs 21.7±0.54 u/mg protein) in the treatment group as compared to diseased group $p \leq 0.05$.

Conclusion: Carica papaya leaf extract demonstrates promising antioxidant properties in the context of ovalbumin-induced asthma, offering a novel avenue for natural-based therapeutics for respiratory conditions.

Keywords: Carica papaya leaf extract, catalase, glutathione peroxidase (Gpx), Malondialdehyde (MDA) oxidative stress, ovalbumin (OVA), phosphate-buffered saline (PBS), superoxide dismutase (SOD).

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Introduction

Asthma is a chronic respiratory disease that affects individuals of all ages. It is often characterized by episodes of wheezing, coughing, chest tightness, and dyspnoea.[1] Approximately 339 million people suffer from asthma around the globe, with prevalence rates continuing to rise in urban areas particularly. This escalating burden requires the urgent need for effective therapeutic interventions that can reduce symptoms, improve lung function, and enhance the overall quality of life in asthmatic patients.[2]

The pathogenesis of allergic asthma involves a complex interplay between environmental factors and immune dysregulation directed by genetic predisposition.[3] The development of airway inflammation involves various immune cells, including eosinophils, mast cells, T lym-

1. *Department of Pharmacology, Azra Naheed Medical College, Lahore.*
2. *Department of Pharmacology, Dental College, HITEC, IMS Taxilla, Cantt.*
3. *Department of Pharmacology, Allama Iqbal Medical College, Lahore.*
4. *Department of Pharmacology, Continental Medical College, Lahore.*
5. *Department of Pulmonology, Al Aleem Medical College, Lahore.*
6. *Department of Pathology, HBS Medical & Dental College, Lehtrar road, Islamabad.*

Correspondence:
Dr. Asma Inam, *Associate Professor Pharmacology, Azra Naheed Medical College, Lahore. Asma.inam@superior.edu.pk*

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phocytes, and dendritic cells.[4] Production of ROS also plays an important role in accentuating airway inflammation. These reactive oxygen species include superoxide anion (O2•−), hydrogen peroxide (H2O2), and hydroxyl ions (•OH). These highly reactive molecules can cause oxidative stress and ultimate damage to cellular components including lipids, proteins, and DNA.[5]

Multiple stimuli such as allergens, pollutants, and respiratory viruses can lead to the development of oxidative stress. ROS upregulates nuclear factor kappa B (NfkB) which stimulates the production of nitric oxide and prostaglandins. As a result, inflammatory cascade in airways involving recruitment and activation of eosinophils and neutrophils occurs.[6] The release of pro-inflammatory mediators, cytokines, and chemokines perpetuates a cycle of inflammation, causing tissue damage in bronchial tissues of asthmatic patients.[7] Airway hyperresponsiveness and remodeling can also happen as a result of oxidative stress. Multiple studies have shown that reactive oxygen species can induce structural changes in airways such as goblet cell hyperplasia, subepithelial fibrosis and hypertrophy of bronchial smooth muscles. These pathological processes are responsible for airway obstruction and exacerbation in symptoms subsequently.[8]

Conventional treatment of bronchial asthma involves the use of bronchodilators and anti-inflammatory medications to control symptoms and reduce bronchial inflammation. However, these pharmacological treatments have their own side effects.[9] Alternatively, there is increasing interest in exploring complementary approaches for asthma management. Natural herbs and plants possessing antioxidant properties have been tried in past. Research in biomedical sciences have identified multiple bioactive compounds having antiasthmatic effects owing to their antioxidant properties.[10]

Carica papaya, commonly known as papaya, is one of the emergent tropical fruit-bearing plant which has been cultivated in many parts of the world. Various parts of the papaya plant including the leaves, seeds, and latex, have been utilized in traditional medicinal system for decades. Papaya fruit has been used as digestive, carminative, expectorant and sedative agent. The seeds of the unripe fruit have been used as anti dysentery and abortifacient.[11]

Carica papaya leaves extract (PLE) has been found as a rich source of bioactive compounds such as flavonoids, phenols, carotenoids and vitamins. This extract displays multiple pharmacological properties including its antioxidant, anti-inflammatory, antiviral, and immunomodulatory activities.[12]

In recent years, Carica papaya leaf extract has attracted considerable attention due to its antioxidant potential. Studies have shown that Carica papaya displayed hepatoprotective, neuroprotective and nephroprotective role in various animal models via its antioxidant activity.[13] Given the supportive evidence of role of oxidative stress in asthma pathophysiology, there is a need for exploring the therapeutic potential of natural antioxidants, such as Carica papaya leaf extract, in ameliorating oxidative damage in asthmatic individuals.

The aim of present study is to investigate the antioxidant potential of Carica papaya leaf extract in ovalbumin-induced asthma. By harnessing the therapeutic potential of PLE, we endeavor to contribute to the growing body of knowledge on natural antioxidants and their potential applications in respiratory health and disease.

Materials & Methods

It was an experimental study conducted at Hide here text from editor for a duration of 6 months from July 2015- January 2016. All the experimental procedures were carried out after approval by Review board and Ethical Committee of the institute (Ref No. UHS/ education/126-14/874). Six to eight weeks old male BALB/c mice (20-25 grams) were obtained from the animal house of University of Health Sciences Lahore. The animals were kept under standard laboratory conditions i.e., 12 hours of light-dark cycle and 22-25°C. Animals were given free access to standard food and water.[14]

Carica papaya leaves were collected locally and shade-dried. powdered form was yielded in pestle and mortar (400g total). This sample was extracted with 2 liters of ethanol by cold maceration. At 50°C, the extracted material was filtered and allowed to evaporate in a water bath. There were 20 grams of semi-solid extract produced. After that, the extract was refrigerated at 4°C for further use.[15]

Fifteen albino mice were equally divided into following three groups (n= 5 per group) by sample random sampling technique. Group 1 served as control group (received PBS 1ml/kg) while Group 2 was diseased group which was sensitized and challenged with Ovalbumin (OVA). Group 3 was treatment group which was given Papaya leaf extract (PLE).[15]

Asthma was induced using the established ovalbumin

(OVA) sensitization and challenge protocol. Briefly, the control group received PBS, 1ml/kg body weight (I.P) on Days 0 and 14 and subsequently given challenge with PBS on Days 21 to 27 while animal in groups 2 and 3 were intraperitoneally (I.P) sensitized with 20 µg of OVA emulsified in alum (2 mg) on Days 0 and 14. All animals of group 2 & 3 were challenged with aerosolized 1% OVA for 30 minutes daily on Days 21 to 27.[16] Mice in Groups 3 received daily oral administration of PLE (100 mg/kg body weight) throughout the challenge period (Days 21 to 27). The control group and OVA-challenged group (Group 2) received PBS orally (Fig. 1).

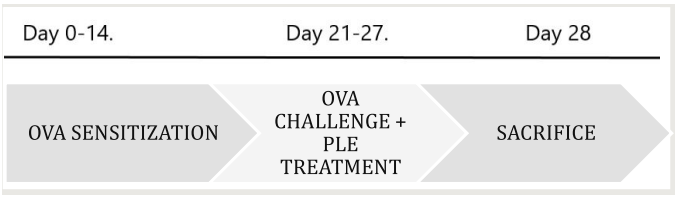

Fig 1. *Experimental protocol of asthma induction and treatment in albino mice*

On Day 28, all mice were euthanized by giving light ether anaesthesia. Lung tissues were collected immediately, washed with saline and stored at -80°C until further analysis.[16]

1% Lung tissue homogenates (w/v) were prepared using PBS. The homogenates were then centrifuged at 600 rpm for 10 minutes to obtain the clear supernatant for enzymatic assays. The activity of SOD, catalase (CAT) and Glutathione peroxidase (GPx) enzymes in the lung tissue homogenates was measured according to the kits protocol. The level of MDA in the lung tissue homogenates was determined using the thiobarbituric acid reactive substances (TBARS) assay. The absorbance was measured by a spectrophotometer at specific wavelengths for each assay. Protein concentration in the homogenates was evaluated using the Bradford assay.[17,18]

Data was interpreted as mean ± standard deviation (SD). Statistical analysis was carried out by using SPSS version 22. One-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test was applied to compare differences among the groups. P value less than or equal to 0.05 was considered statistically significant.[19]

Results

Determination of superoxide dismutase (SOD Activity): Our study demonstrated that superoxide dismutase (SOD) activity in U/mg protein) in diseased group was decreased significantly (21.7 ± 0.54) as compared to control (25.9 ± 0.4) and treatment group (24.1 ± 0.35) respectively (P ≤ 0.05). Fig 2, Table 1.

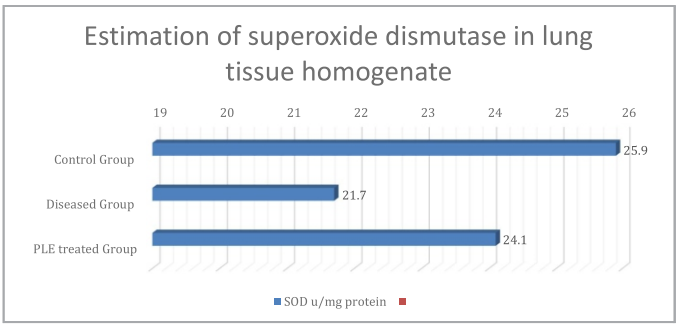

Fig. 2. *Evaluation of Antioxidant enzyme (SOD) in mouse model of allergic airway inflammation*

2. Evaluation of Catalase Activity: This research reflected a typical raised level of catalase activity in lung tissue in control and PLE group (0.17 ± 0.02 and 0.16 ± 0.01) respectively, in comparison to asthmatic mice of group 2, having significant decreased catalase level (0.14 ± 0.01) Ku/mg protein (P ≤ 0.05). Fig 3, Table 1.

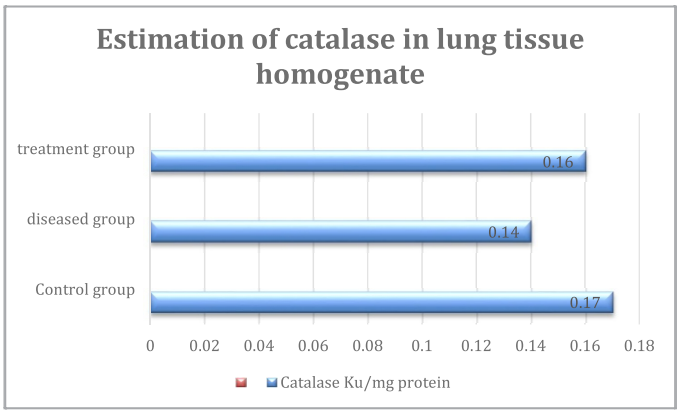

Fig. 3: *Evaluation of Antioxidant enzyme Catalase (CAT) in mouse model of allergic airway inflammation*

Our study indi-cates a significant decrease in glutathione peroxidase in diseased group as compared to control group (120.5±2.4 Vs 106.1±3.6 u/mg protein). However, treatment with PLE significantly increased GPx level in lung homogenate to 115.3±2.7 (p ≤ 0.05). **(Fig-4, Table-1)** Estimation of Malondialdehyde (MDA) Level: Regarding MDA level, our research indicated a signi-ficant decrease in MDA levels of treatment group (8.06±0.2 nmol/mg protein) as compared to diseased group (10.7±0.79), suggesting reduced oxidative stress (P ≤ 0.05). These findings of treatment group were also com-parable with

control group (8.06 ± 0.2 Vs 7.9 ± 0.38) Fig 4, Table 1.

Fig. 4 *Evaluation of Antioxidant enzyme (GPx) and lipid peroxidation marker (MDA) in mouse model of allergic airway inflammation*

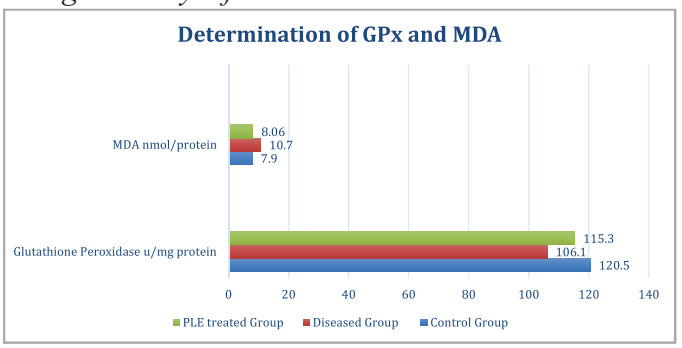

Table 1: *Depicting effect of PLE on SOD, Catalase, GPx and MDA level in lung tissue homogenates of mice.*

Parameters	Control Group (Mean±SD)	Diseased Group (Mean±SD)	PLE treated Group (Mean±SD)
SOD (U/mg protein)	25.9 ± 0.4	21.7 ± 0.54	24.1 ± 0.35
Catalase (kU/mg protein)	0.17± 0.02	0.14 ± 0.01	0.16 ± 0.01
Glutathione Peroxidase (U/mg protein)	120.5 ± 2.4	106.1± 3.6	115.3 ± 2.7
MDA (nmol/mg protein)	7.9 ± 0.38	10.7 ± 0.79	8.06 ± 0.2

Discussion

Asthma is a prevalent chronic inflammatory airway disease. Airway hyperresponsiveness, inflammation, and mucus production are underlying mechanisms involved in asthma pathogenesis. An increasing body of research indicates that oxidative stress is a major factor in the onset of asthma.[1] During asthmatic attacks, inflammatory cells generate excessive amounts of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide. These ROS can ultimately damage bronchial cells and contribute to airway inflammation and hyperresponsiveness.[4,5] Carica papaya is a native plant which possess many biological activities proven by scientific researches in the past. Numerous bioactive substances found in Carica papaya leaves, including rutin, papain, chymopapain, cystatin, ascorbic acid, α-tocopherol, ρ-coumaric acid, and caffeic acid, might be involved in the plant's anti-oxidant activity.[12]

The present study explored the antioxidant potential of Carica papaya leaf extract (PLE) in a murine model of asthma by evaluating the activity of antioxidant enzymes and the marker of oxidative stress (SOD, catalase, glutathione peroxidase and MDA). We confirmed that PLE administration enhances antioxidant enzyme levels along with a moderate decrease in lipid peroxidation marker in lung tissue homogenates. These findings are consistent with a research conducted by Nisa et al., whereby the antioxidant activity of papaya leaf extract was verified by the DPPH and FRAP test.[20]

Our findings are also aligned with previous studies that have documented the antioxidant properties of PLE. In vitro studies have shown that PLE can scavenge free radicals and inhibit lipid peroxidation. A previous study by Salla et al., proved antioxidant and apoptotic activity of papaya peel extract in HepG2 cells via increasing catalase, SOD and glutathione peroxidase activity.[21] Additionally, research in animal models of other inflammatory conditions have also demonstrated PLE's ability to enhance antioxidant enzyme activity and reduce oxidative stress markers.

The precise mechanisms by which PLE exerts its antioxidant effects are not fully comprehended, still several pathways have been proposed in this context. PLE is rich in various antioxidant compounds which can directly scavenge free radicals, preventing them from damaging cells. Additionally, PLE may upregulate the level of antioxidant enzymes like SOD, catalase, and glutathione peroxidase, further bolstering the body's defense against oxidative stress. In our previous study, PLE exhibited it's anti-inflammatory activity in murine model of asthma by downregulating IL-4, IL-5, TNF alpha, Eotaxin and NFkb.[15] Modulation of NFkb by papaya leaf extract may lead to attenuation of oxidative stress in bronchial tissues. The current study's findings add to the growing body of knowledge suggesting that targeting oxidative stress could be a promising strategy for asthma management. PLE, with its demonstrated antioxidant potential, might offer a complementary approach to traditional asthma medications. While this study provides encouraging results, some limitations need to be considered. Firstly, the investigation was conducted in a murine model, secondly, the optimal dosage and formulation of PLE for therapeutic use in asthma require exploration. It is therefore suggested to translate these findings to humans through clinical trials.

Conclusion

This study demonstrates the potential of PLE as an

antioxidant agent in an animal model of asthma. The observed increase of antioxidant enzyme activity and decrease of MDA levels suggest its ability to mitigate oxidative stress, a crucial factor in asthma pathogenesis. Future research is warranted to explore the clinical applications of PLE and its role in asthma management.

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Conflict of interest: *None*

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Authors Contribution

AI: Conceptualization of Project
SR, SI: Data Collection
: Literature Search
SM, SI: Statistical Analysis
SI: Drafting, Revision
SR, MSM: Writing of Manuscript