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CARICA PAPAYA EXTRACTS REDUCE TH2 ASSOCIATED CYTOKINES AND ALLERGIC INFLAMMATION IN RAT MODEL OF ALLERGIC RHINITIS

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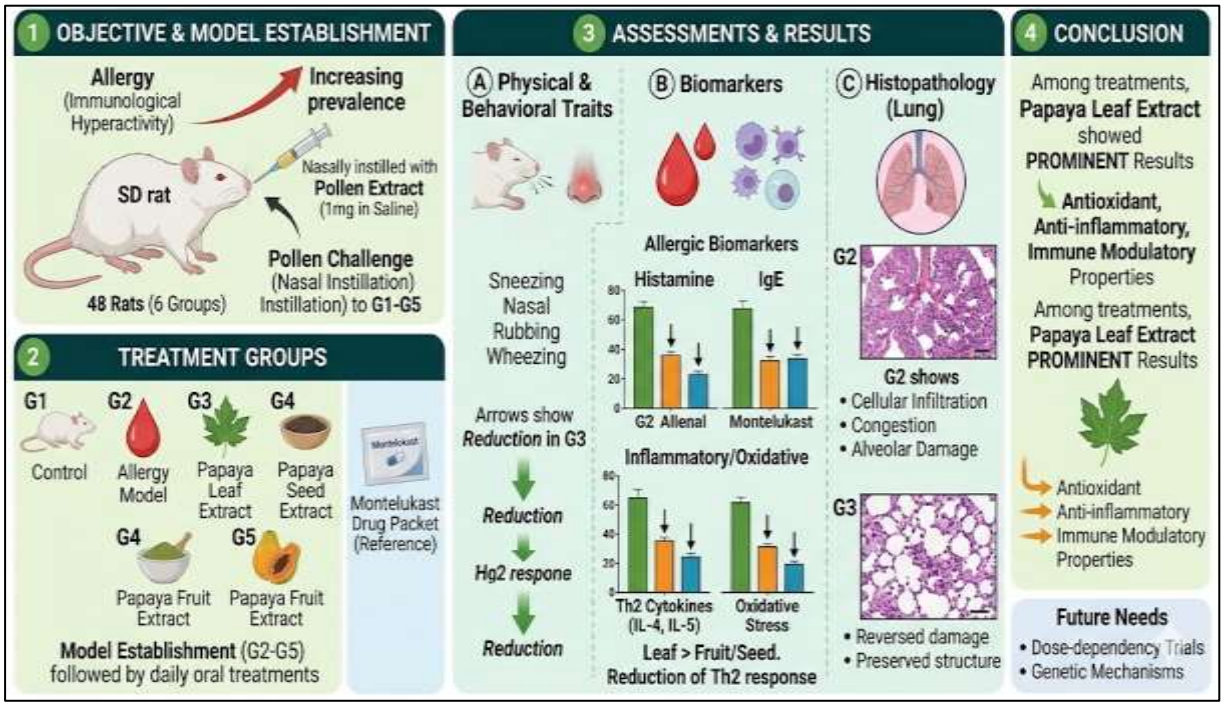
GRAPHICAL ABSTARCT



ABSTRACT

Allergy, termed as an immunological hyperactive disease, is increasing rapidly and carries a considerable global health burden. Considering papaya's medicinal properties, the current study aimed to assess the role of different parts of the papaya in relieving pollen-induced allergic rhinitis symptoms via animal models. Purposely, 48 Sprague Dawley rats were housed in the animal room and divided into six groups. To establish an allergic disease model, rats belonging to the G1 to G5 groups were nasally instilled with 1mg pollen extract mixed in a buffer saline solution. After model establishment, the G3 to G5 groups were given papaya-based extracts. Ultimately at the end of the animals were decapitated, and the results were elaborated under three major sections as per the effect of the papaya-based extracts on the physical and behavioral traits, inflammatory, oxidative, allergic biomarkers, and histopathology of the lungs. The leaf extract and drug-treated group showed a reduction in Th2 associated cytokines and other allergic inflammation biomarkers. The histamine, IgE, IL-4, and IL-5 markers were effectively lowered by the leaves extract compared to the fruit and seed extracts. Additionally, histological examination revealed that leaf extract and Montelukast reversed the cellular infiltration, congestion, and alveolar structural damage. Conclusively, among different treatments, the papaya leaf extract showed prominent results, highlighting its antioxidant, anti-inflammatory, and immune modulatory properties. Hence, more trials are needed in the future to ameliorate its dose-dependent effect and underlying genetic mechanisms.

KEYWORDS: Carica papaya, Anti-inflammatory, Immunemodulatory, Paper mulbery, Allergy Rhinitis

1. INTRODUCTION

Environmental pollution and increasing climatic changes are major contributors to the airborne allergies, whether they occur biologically or are produced by anthropogenic sources, ultimately affecting human health adversely (Lee et al., 2013; Selzle et al., 2017). Among different biological allergens, pollen, as an aeroallergen, is a major cause of allergies and asthma. Globally, approximately 400 million people are affected by allergic rhinitis, out of

which a large number remain undiagnosed and untreated. It affects about 10-30% of the adult population and up to 40% of children (Sulliva and Kushnir, 2020). Pollens produced from grasses, trees, and weeds act as aeroallergens and increase the rate of rhinitis allergy (Schmidt, 2016).

In Pakistan, the population has been at a greater risk of allergies and asthma due to rapid urbanization and environmental allergens. About 20% pediatrics population of Pakistan is already suffering from allergic diseases (Sabar et al., 2018). The highest rate of aeroallergen sensitization was observed in Karachi and Islamabad, where Paper mulberry (Broussonetia papyrifera) tree pollen is a major contributor to seasonal allergy. Its rapid growth and widespread dispersal further worsen the allergic symptoms such as nasal congestion, asthma, coughing, hives, and skin rashes (Aslam et al., 2015; Siddiqui et al., 2020).

Allergy is termed as immunological hypersensitivity leading to various multi-faced diseases (rhinitis allergy, asthma, anaphylaxis, and urticaria, and so on) via different pathomechanisms (Pinart et al., 2016). Allergen sensitization is initiated when susceptible individuals are exposed to allergens, triggering the immune system as if it were an invader, and in response, their body starts to release allergen specific immunoglobulin E (IgE) antibodies, leading to a cascade of inflammatory responses (Caraballo et al., 2020). Hence, in prolong phase, Th2-associated cytokines, particularly Interleukin 4 (IL-4) and Interleukin 5 (IL-5), worsen the disease by promoting increased production of IgE and eosinophils (Derendorf and Meltze, 2008).

For the treatment of rhinitis allergy, oral, intramuscular, or intranasal corticosteroids are mostly used (Petersen and Agertoft, 2016). Corticosteroids cause more adverse effects than inhaled corticosteroids. High doses of oral corticosteroids are linked with different side effects, as it retards the growth in children, glaucoma, cataracts, dysphonia, and osteoporosis, etc. (Dahl, 2006). Due to these adverse effects of pharmacotherapy, alternative therapeutic approaches are getting attention due to their lower side effects, particularly plant based compounds having antioxidant, anti-inflammatory, and immunomodulatory properties (Zhou et al., 2019). Recent studies have also explored various bioactive compounds and biologically derived interventions that may contribute to modulating immune responses and reducing inflammatory conditions (Butt et al., 2025a; Butt et al., 2025b; Butt et al., 2025c; Butt et al., 2024; Ahmed et al., 2024; Khan et al., 2024; Rashid et al., 2026). These findings collectively suggest that naturally derived and biologically active substances may offer supportive roles in managing immune-related disorders, thereby providing a rationale for exploring plant-based therapeutic agents in allergic conditions.

Carica papaya, also known as a tropical tree herb, is widely recognized for its therapeutic potential. Its different parts (leaves, fruit, seed, and latex) have many bioactive components, such as carotenoids, phenolics, polyphenols, flavonoids, vitamins (A, C & E), and papain. These offer significant health benefits ranging from digestion to immune modulation (Dave and Sunil, 2019). Previous research has reported that Carica papaya has immune-modulatory and anti-inflammatory potential. Therefore, it could be used against arthritis and rhinitis allergies (Inam et al., 2017). Hence, this study was designed to assess the immune-modulatory and anti-inflammatory effects of Carica papaya on allergic inflammation and Th2-associated cytokines in an allergic rhinitis rat model.

2. MATERIALS AND METHODS

2.1. Preparation of extracts

Extracts of papaya leaves, fruit, and seed were prepared according to the protocol of Elgadir et al. (2014) with the solvent's ethanol and water in 70:30 ratios, respectively. About 400 g of powder of each papaya part was extracted with 2 L of a solution of solvent by using the cold maceration method. Then the mixture was filtered, and the solvent was evaporated in a rotary evaporator, and the sample was stored at -18°C for further analysis.

2.2. Preparation of pollen extract

Extraction of pollen was done according to the method described by Çetereisi et al. (2019). About 10 g of pollen were suspended in the 125 mM NH4HCO3 at a 1:12 (w/v) ratio, for 12 hrs at 4°C with constant stirring. Sample was centrifuged at 13000*g for 1 hour at 4°C to remove the insoluble substances. Later, the extract was filtered through a Whatman No. 1 filter paper with 0.45 m pore size and a Millipore vacuum filtration system with 125 mm Whatman filter paper. The supernatant was dialyzed for 24 hrs at 4°C with a dialysis tube (43 mm) and distilled water. The completed dialysate was lyophilized and stored at -80 °C for later use.

2.3. Efficacy study

Animal Ethics Committee (Institutional Animal Care and Use Committee), University of Agriculture, Faisalabad, guidelines were followed for the efficacy study. Purposely, 48 Sprague Dawley rats of 150-200 g were purchased from the National Institute of Health and housed within the well-lighted and ventilated animal room. Animals were provided with diet and water ad libitum and were allowed to adjust to the environment for one week. The experiment was conducted for seven weeks by keeping experimental rats within six groups (n=8).

2.4. Treatment plan

For the experimental trial, about forty-eight rats were divided into six groups and kept in the animal room. The formulated groups were G0 (Normal diet (control negative), G1 (Normal diet + pollen-induced allergy (control positive), G2 (Normal diet + pollen-induced allergy + Montelukast (Standard), G3 (Normal diet + pollen-induced allergy + Papaya leaves extract (Treatment I), G4 (Normal diet + pollen-induced allergy + Papaya seeds extract (Treatment II), G5 (Normal diet + pollen-induced allergy + Papaya fruit extract (Treatment III).

2.3.1 Establishment of pollen allergy model

To establish an allergic disease model, rats belonging to the G1 to G5 were nasally instilled with 1mg pollen extract mixed in 20µL buffer saline solution for six consecutive days up to 2 weeks, while for four days in the third week (0-5, 7-12, and 14-17 days). Animals were intra-tracheally inflamed with pollen extract for three consecutive days for the final sensitization (Kato et al., 2014). After model establishment, G3 to G5 groups were given papaya-based extracts and nasally instilled pollen extract until the 7th week of trial. After that animal were sacrificed for further evaluation.

2.3.2. Physical parameters

Animals were weighed weekly to determine the changes within the rats (Burn et al., 2006).

2.3.3. Behavioral tests

In animals, runny nose, number of sneezes, and nasal rubbing were determined within a duration of 30 min of the final allergen dose within the experimental group (Yang et al., 2018). A recorded score >5 was considered a benchmark for allergy model establishment (Tu et al., 2020).

2.3.4. Haematological analysis

The effect of papaya extract on oxidative damage of cells caused by allergy was assessed by performing a complete blood count on an automated hematology analyzer (Li et al., 2015).

2.3.5. Serum IgE and anti-inflammatory biomarker analysis

Serum concentration of IgE was determined by ELISA assay as IgE and anti-inflammatory biomarkers such as histamine, interleukins associated with higher sensitization within the aeroallergen's allergies (Tiotiu, 2018; Vlaykov et al., 2020; Guo et al., 2021).

2.3.6. Stress biomarkers

Different antioxidant enzymes, such as SOD, CAT, and GSH, were determined in the serum as per the described protocols below.

2.3.6.1. SOD activity

Firstly, the serum sample was mixed with the 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride. Then xanthine oxidase was introduced into the solution, and the absorbance was recorded in the spectrophotometer at 505 nm. In the end, the SOD activity of the sample was expressed in the U/mL (Meca et al., 2021).

2.3.6.2. CAT activity

The CAT activity was measured by following Bahrami et al. (2016). The sample was incubated with the addition of H2O2 and buffer solution (Tris-HCl; pH=7) for 10 min. Then, 4% ammonium molybdate was introduced to terminate the reaction. The H2O2 and yellow complex of ammonium molybdate were measured at 410nm. The enzyme activity was expressed as U/mL.

2.3.6.3. GSH activity

The GSH activity was determined according to Ellman's reaction (Bahrami et al., 2016). Purposely, about 15 µL of the sample was well homogenized with 260 µL buffer assay (1m EDTA, 0.1M sodium phosphate, pH=8) and Ellman's reagent (5 µL). The sample was incubated for 15 min at 250 °C. The absorbance was taken at 412 nm.

2.3.7. Total antioxidant capacity and total oxidative stress

Total oxidant status and total oxidative stress were measured in serum according to the colorimetric method (Erel, 2004; Erel, 2005).

2.3.8. Histopathology of lungs

Lung histopathology was performed according to the method described by Inam et al., (2017). For this purpose, lungs after decapitation were kept in 40% formalin solution containers. The lung tissues were sliced into 5 µm thick sections with a microtome and mounted on slides. Then, the samples were stained with haematoxylin-eosin to evaluate the structural differences. The slides were viewed under a light microscope (MCX 100, Micros Austria).

2.9. Statistical analysis

The data generated from different assays were statistically analysed through Minitab 17. The two-way ANOVA under a completely randomized design was employed in some parameters of animal efficacy. However, one-way ANOVA was used in other parameters, followed by Tukey's test to evaluate the comparison between the groups with the significance level of $p>0.05$ and $p>0.01$ (Montgomery, 2008).

3. RESULT

3.1. Behavioral changes during allergy progression

A series of nasal inflammatory responses, such as sneezing, runny nose, congestion, and itching, characterize allergic rhinitis (AR). These changes were examined in the rats sensitized to pollen allergens to confirm the establishment of an allergic model. Furthermore, animals were examined daily to observe their progression and allergic symptoms. On day 0, no symptoms were observed in groups (G0-G5). On the 20th day (last instillation day), a gradual activity loss was observed as the frequency of nasal symptoms increased. As the treatments progressed, the nasal symptoms started declining, particularly in G3 (Papaya leaves extract) (**Figure 1a & b**).

3.2. Effect of treatments on the weight parameter

The physical growth parameter was measured on a daily basis and a weekly basis to determine the effect of pollen allergy induction and treatments. After the first week of pollen allergy induction, a significant decline in body weight was observed in the G1-G5 groups, as shown in Figure 2. However, the highest weight gain was recorded in the control group, about 138±7.69. Later, the weight decreased and then trended up to the third and fourth week of the trial, but from the fifth week, a gradual increase in the weight was observed for G2-G5, while the continuous

decline in the positive control group (G1) was noted. Among treatment groups, better weight gain results were depicted by the standard drug group (G2) and the papaya leaves extract group (G3).

3.3. Hematological indicators

3.3.1. Erythrocyte indices

Allergy induction significantly altered the erythrocyte indices, such as reducing Hb, HCT, MCV, MCH, and MCHC parameters in the positive control group as compared to the control ($p<0.01$). All treatment groups G2-G5 showed significant improvement in all these parameters, indicating restoration of RBC function. However, the best results were shown by the papaya leaf extract exhibiting a value closer to the control group (Figure 3. a-f).

3.3.2. Leukocyte's indices

The statistically significant effect on white blood cell (WBCs), lymphocytes (LYM), and eosinophil count (EOS) was seen in both treated and diseased groups ($p<0.01$). Mean results showed that an increase in the WBCs, eosinophils, and a decrease in the lymphocytes have been observed in the positive control group. Contrarily, after treatment, the reduction in the respective values of WBCs and EOS, and elevation in LYM were noted in the G2-G5 groups (Figure 4a-c). Among treatments, better outcomes were gained from papaya leaf extract.

3.4. Allergy inflammatory biomarkers

Allergy biomarkers were statistically determined in the allergy-induced and treated groups. Before the start of treatment, random sampling of rats was done from each group, G1-G5, and the IgE level in the samples was checked to confirm the establishment of the allergy model. The values were found to be high and similar in the G2-G5 for the respective allergy biomarker. However, after treatment, a significant difference was found for IgE and histamine, IL-4, and IL-5 among the G2-G5 treatments compared to the negative control at $p>0.01$, as shown in Figure 5a. The medicine and leaves extract had the same trend in decreasing the IgE level, whereas the fruit extract-treated groups depicted a minor effect Figure 5 b-d.

3.5. Antioxidants in serum

It is evident from the statistical analysis that oxidative stress biomarkers, including SOD, CAT, and GSH, showed significant variation among all the experimental groups ($p<0.01$). Figure 6a-c. The reduction in the level of respective antioxidant enzymes was observed in the G1 group exposed to allergens in comparison to the control rats. However, Montelukast, leaves, seeds, and fruit extract groups improve the antioxidant level in the serum, but more profoundly by the G3 (papaya leaves extract).

3.6. Total antioxidant capacity (TAC) and Total oxidative stress (TOS)

The TAC values were low in the G1 but high in the G0. Leaf extract and Montelukast-treated showed a similar trend in elevation of TAC. However, the seeds and fruit extract-treated groups showed the least effect compared to the control (Figure 7 a-b).

3.7. Histology of lungs

The histological examination of rat lung tissues (Figure 8) revealed that in the G0 group, no infiltration of inflammatory cells, no partial thickening of alveolar cells, and no tissue bleeding were observed. Unlike in the positive control group (G1), pollen extract significantly damaged the epithelium layer of the lungs, leading to the formation of an irregular alveolar cavity due to the onset of oxidative stress. Moreover, capillary congestion and thrombosis are also observed. In the Montelukast-treated rats (G2), recovery of the tissue was observed with intact alveolar structures, and the least inflammation was observed due to less infiltration of eosinophils and lymphocytes. In the papaya leaf extract-treated rats (G3), restoration of the epithelium layer of the lungs was observed, accompanied by mild thrombosis. While in the papaya seed (G4) and fruit (G5) extract-treated rats, results showed slow recovery as compared to G2 and G3, which was observed, as there is still alveolar wall thickening and capillary thrombosis present.

4. DISCUSSION

Exposure to allergens in allergic rhinitis initiates an inflammatory cascade in the nasal mucosa, resulting in classic symptoms including airway inflammation, obstruction, and nasal congestion (Min, 2010; Krishnamoorthy et al., 2020). In the current animal model to check the establishment and progression of AR nasal symptoms, such as nasal rubbing, continuous sneezing, and runny nose, were observed before and after treatment. A decline in nasal symptoms was observed after the papaya extract induction in rats after final sensitization, which supports the therapeutic potential of papaya extracts. The findings of the current study are in concord with those of Liao et al. (2021), who evaluated the effect of different herbal plant extracts against allergy rhinitis, and it was observed that extracts alleviate the ova-induced allergy in mice by balancing the T-helper cells in the lungs. Furthermore, episodes of sneezing, nasal rubbing, and nasal discharge were reduced in the treated mouse compared to the diseased group.

Body weight change another indicator of systematic allergic inflammation in the experimental rats. In the current study, weight loss is observed in the subjects during the sensitization period in G2-G5. In contrast, continuous decline was observed in the G1 (diseased group), but as the treatment onset with papaya leaves extracts and drug rats, the weight started to stabilize and improve. It might be attributed to reduce inflammation burden due to the onset of nasal symptoms (Yang et al., 2018).

Elevated haematological indicators such as WBCs and eosinophils confirm the establishment of an allergic model. Previous research has demonstrated that eosinophilia is linked with allergy sensitization and can be used as a sensitivity biomarker (Jung et al., 2011; Liu et al., 2012; Yang et al., 2018). Decline in these indicators by papaya leaves extract showed the suppression of inflammatory responses, which is well supported by Inam et al. (2017), who reported that C. papaya leaves extract significantly reduced the total and differential leukocytes in both blood and BALF.

On exposure to the allergens, IgE plays a role as the primary antibody in the transient hypersensitivity. Therefore, IgE is one of the crucial biomarkers in allergic rhinitis (Ren et al., 2018). The IgE antibody had high affinity towards the antigen-presenting, mast, and basophil cells (Gould and Sutton, 2008) and caused the release of inflammatory mediators such as histamines and interleukins (Gould and Sutton, 2008; Ren et al., 2018). It is evident from the recent study that an abrupt rise in the IgE was observed after the establishment of the allergic model and also in the positive control after the onset of treatment, which was later reduced by the leaves extract and the Montelukast. It might be due to phenols and flavonoids in the papaya leaves extract, which have anti-inflammatory roles. Some studies reported the polyphenols in the papaya leaves, such as kaempferol, quercetin, and their glycosides, etc. (Wang et al., 2015; Oloyede et al., 2016; Kadiri et al., 2017; Anjum et al., 2017; Soib et al., 2020), which are helpful in the reduction of IgE by suppression of extra signal-regulated kinase activation and chemokine release (Lee et al., 2010). Chen et al. (2011) also confirm the immunomodulatory effect of transgenic and native papaya in the mouse model. An oral dose of 1.6 g/kg body weight (for five weeks) of native green papaya fruit supplementation significantly decreased (0.04 μg/ml vs. 0.08 μg/ml in the control group) the OVA-specific IgE titer.

Histamine is another indicator released on the activation of mast and basophil cells and is an essential mediator in nasal allergy. This mediator causes an increase in vascular permeability, vasodilation, bronchial and visceral smooth muscles, and local inflammation (Rapanelli et al., 2018; Bosma et al., 2018). The leaves extract treated groups were found to have lower histamine levels. A well-studied impact of polyphenols such as quercetin on allergies is to inhibit the effect of degranulation, specifically histamine release from mast cells (Marzocchella et al., 2011; Weng et al., 2012). It has also been confirmed by a study conducted by Chaudhari et al. (2014) that Carica papaya leaf extract acts as a mast cell stabilizer and decreases the release of histamine.

In chronic allergic inflammation, activated mast cells start producing IL-4, IL-5, and TNF-α. The L-4 plays a crucial role in stimulating IgE production and differentiating naïve helper T cells into Th2 cells. Carica papaya leaves extract decreased the interleukins in the treated group, showing its immunomodulatory role. It was also confirmed by Inam et al. (2017) and Salim et al. (2014) that papaya leaves extract via downregulation of expression for IL-4, IL-5, TNF-a and iNOS improves the allergy condition in ova-albumin-induced mice.

The rats with pollen allergy sensitization showed low total antioxidant capacity. (Pointer et al., 2020). The current study's findings that Montelukast improves the total antioxidant/oxidation balance in rats were confirmed by Dilek et al. (2015). Moreover, papaya leaf extract attenuated the oxidative stress in the rats due to their high antioxidant capacity. These outcomes were supported by the investigation of Hublikar et al. (2023) that papaya aqueous leaves extract had an ameliorative effect against the oxidative stress produced by the acetic acid in albino Wistar rats via decreasing the MDA level, proving its antioxidant activity.

The histological observation of the current study is quite in corroboration with Wei et al. (2022), who reported epithelium layer damage, irregular alveolar cavities, and capillary thrombosis in the mouse model on the induction of pollen extract. Similarly, Kujur et al. (2015) also reported the augmentation of inflammatory cells in the lung tissue of ova-induced asthmatic mice. A study by Inam et al. (2017) reported the ameliorative effect of papaya leaf extract against an ovalbumen-induced allergic model. The histopathological examination showed that papaya leaf extract significantly reduces alveolar thickening by attenuating inflammatory cell infiltration into the lungs and goblet cell hyperplasia. Another study reported that phenolics (quercetin) treatment at 50 mg/kg BW hypoxia conditions the lungs by reducing inflammatory cell infiltration, blood cell, and edema (Tripathi et al., 2019). Administration of vitamin C and E also attenuated the airway inflammation in the airway mouse model, as evident from their histopathology through balancing the Th1/Th2 shift and restoration of Nrf2 levels (Jeong et al., 2010; Bansal et al., 2014; Quoc et al., 2021). Specifically, the current study provides a comparative analysis of different papaya parts extracts, highlighting that leaf extract showed superior efficacy compared to fruit and seed extracts. This supports its potential as a promising therapeutic agent for allergic rhinitis.

Future Perspectives

The findings of the present study highlight the immunomodulatory and anti-inflammatory potential of Carica papaya leaf extract in allergic rhinitis. However, translating these outcomes into broader health applications requires a One Health framework, which integrates human, animal, and environmental health systems. The increasing prevalence of allergic diseases is closely linked with environmental pollution, climate variability, dietary transitions, and microbial imbalances, necessitating interdisciplinary interventions rather than isolated therapeutic approaches.

From a human health perspective, future research should focus on clinical validation of papaya-derived bioactive compounds, particularly in relation to immune regulation, metabolic health, and chronic inflammatory disorders. Emerging evidence suggests that functional foods and nutraceutical interventions—such as probiotic-enriched formulations—can modulate immune responses and improve metabolic resilience (Ahmed et al., 2024; Rashid et al., 2026). The integration of papaya leaf bioactives into such functional systems may enhance therapeutic efficacy through synergistic microbiome–immune interactions.

From an animal health standpoint, the current rat model provides foundational evidence, but future studies should expand into translational animal systems and livestock models to evaluate long-term safety, dose optimization, and physiological adaptability. Nutritional intervention studies, including hybrid protein systems and dietary modulation strategies, have demonstrated systemic benefits in animal models (Butt et al., 2025a; Khan et al., 2024). These approaches could be extended to evaluate plant-based immunomodulators under varying physiological and environmental stress conditions.

The environmental dimension of One Health is particularly critical in allergic diseases. Climate-driven increases in aeroallergens, such as pollen, and environmental contaminants contribute significantly to immune dysregulation. Sustainable food systems and plant-based therapeutic resources—such as papaya—offer dual benefits by reducing environmental burden while improving health outcomes. Research on sustainable food innovations, including plant-based protein systems and alternative food products (Butt et al., 2025b; Butt et al., 2025c), supports the transition toward environmentally resilient nutrition strategies.

Moreover, food safety and environmental exposure risks must be considered within the One Health paradigm. Studies highlighting microbial contamination and heavy metal exposure in food systems (Riaz et al., 2026) emphasize the need for integrated surveillance systems that connect environmental quality with human disease outcomes. Incorporating plant-derived therapeutics into safer, controlled food systems could mitigate such risks while enhancing immune health.

At the molecular and genetic level, future research should explore epigenetic and gene-regulatory mechanisms underlying the immunomodulatory effects of papaya bioactives. Advances in CRISPR-based gene editing and epigenome modulation (Fatima et al., 2026; Jabeen et al., 2025) open new avenues for understanding how dietary phytochemicals influence gene expression related to inflammation and immunity. Similarly, nutritional epigenetics studies have shown that dietary components can regulate metabolic and inflammatory pathways (Butt et al., 2026b), suggesting a potential mechanistic basis for papaya's therapeutic effects.

Additionally, the integration of artificial intelligence and predictive modeling in health sciences offers opportunities to optimize personalized nutrition and treatment strategies. AI-driven health and decision systems (Kamal et al., 2026; Naeem et al., 2026) could be employed to predict individual responses to plant-based interventions and design precision therapeutics within a One Health ecosystem.

From a socio-economic and sustainability perspective, the One Health approach also emphasizes equitable access and environmental responsibility. Studies exploring sustainability performance and ESG frameworks (Khurshid et al., 2026) highlight the importance of aligning health innovations with broader societal and environmental goals. The utilization of locally available medicinal plants like papaya can support cost-effective healthcare solutions, particularly in developing regions.

Finally, future clinical translation should focus on multi-center human trials, formulation development (e.g., functional beverages, supplements), and regulatory validation. Integrating papaya-based therapies into holistic dietary patterns, alongside micronutrient optimization (e.g., zinc and immune regulation; Butt et al., 2026a), could provide comprehensive strategies for managing allergic and inflammatory diseases.

Extended One Health and Cross-Sectoral Future Directions (References 69–92 Integrated)

Beyond the core translational pathway of papaya-based therapeutics, broader One Health evidence further strengthens the need for integrative biological, agricultural, veterinary, and socio-environmental research systems. In veterinary and zoonotic health systems, parasitic and viral disease surveillance remains critical for ecosystem stability. The first report of Eimeria species in Japanese quails highlights emerging parasitic diversity in poultry systems (Tran Nguyen-Ho-Bao et al., 2025), while rotaviral enteritis in racing pigeons demonstrates the expanding burden of avian viral infections across geographic regions (Kalkanov, 2025). These findings reinforce the importance of immune-supportive nutritional strategies that may include plant-derived immunomodulators such as papaya.

Further supporting livestock immunobiology, dietary plant extracts have shown measurable improvements in animal growth performance, as demonstrated by Phyllanthus amarus supplementation in broiler strains (Mba et al., 2025). Similarly, AI-driven pest and disease prediction systems provide scalable tools for managing agricultural health threats under climate variability (Bukhari et al., 2025).

Microbiome and host–environment interactions are also central to One Health advancement. Regional microbiome variation in Kazakh horses (Anarkulov et al., 2026) and cytokine expression changes in rabbits under mycotoxicosis (Tarasova et al., 2026) highlight the complexity of immune regulation across species and environments. These findings align with the need to investigate plant-based immunomodulators in multi-host systems.

At the molecular plant and microbial interface, gene regulation and biotechnology are shaping future agricultural resilience. The HD-ZIP transcription factor family in drought-stressed plants provides insight into stress-adaptive genetic mechanisms (Khalid et al., 2025), while optimized fungal spawn production systems enhance sustainable food biotechnology applications (Awais et al., 2025). Likewise, arginine-mediated growth stimulation in jackfruit demonstrates how amino acid supplementation can enhance plant physiological performance (Nazir et al., 2025), complementing nutritional intervention strategies.

Environmental toxicology and aquatic health studies further expand One Health relevance. Copper toxicity in carnivorous fish highlights water-quality-linked metabolic disruptions (Iqbal et al., 2025), while grazing management practices are essential for maintaining pasture ecosystem integrity (Nasiyev et al., 2026). Similarly, rice husk biochar has demonstrated significant mitigation of salt stress through ion transport regulation in rice systems (Faiyue et al., 2026), underscoring the importance of sustainable agricultural inputs.

From a nutritional science and food safety perspective, nutraceutical research continues to expand. Grape seed extract exhibits strong bioactive potential in metabolic regulation (Wasim Sajid et al., 2025), while pesticide residue profiling underscores persistent chemical risks in food systems (Nadeem et al., 2025). Functional wheat processing approaches further demonstrate dietary modulation of gut health and glycemic control (Khan et al., 2026).

Human health epidemiology also supports the broader integration of One Health frameworks. Cervical dysplasia risk factors among HIV-positive and negative women highlight intersecting infectious and non-communicable disease burdens (Uzoh et al., 2026), emphasizing the need for immune-supportive nutritional interventions.

Agricultural innovation and sustainability studies provide additional structural support. Growth enhancement in Phyllanthus niruri under organic manure application demonstrates bioactive plant-environment interactions (Malik et al., 2026), while efficiency analyses of rice farming systems highlight the importance of optimized resource use in agricultural production (Tran et al., 2026). Similarly, drip irrigation adoption improves productivity and farmer livelihoods in South Punjab (Khan & Nadeem, 2025), while papaya seedling optimization using organic inputs reinforces its agricultural relevance (Wijyanti, 2025).

Socioeconomic determinants also influence health outcomes. Youth drug addiction is shaped by psychological and social factors (Liaqat et al., 2025), while large infrastructure projects such as CPEC significantly affect community health and socioeconomic stability (Shahbaz & Ali, 2025). These findings reinforce the need for integrated health–environment–society models.

Finally, sustainable land use and agricultural innovation are critical for long-term resilience. Agroforestry systems provide ecological restoration pathways (Hussain, 2025), while speed breeding technologies accelerate genetic gain for crop improvement under climate stress (Ahad, 2025). Together, these innovations support a resilient One Health framework that aligns food security, environmental sustainability, and human health.

CONCLUSION

The current study highlighted the antioxidant, anti-inflammatory, and immunomodulatory potential of papaya leaf, seed, and fruit extract in an animal model. Papaya leaf extract showed the best result in reducing inflammatory biomarkers and scavenging free radicals. Along with the biochemical analysis, histological examination of the lungs revealed a promising role for papaya leaf extract. It helped restore capillary thrombosis, congestion, and cellular infiltration. In addition, it had a strong impact on restoring the alveolar structure and the pulmonary epithelium layer. However, fruit and seed extract showed a slower recovery. Studies on the immunomodulatory role of papaya against allergy remain limited. Further exploration is required to identify the active component in papaya extract, establish dose-dependent trials, and elucidate the genetic mechanism of action.

Author Contribution:

Nehal Umar: Conceptualization; Methodology; Analysis; Data collection; Manuscript writing. **Allah Rakha:** Conceptualization; Methodology; Supervision; Review: Editing. **Masood Sadiq Butt**: Review and Editing, Conceptualization; Methodology. **Muhammad Naeem Faisal:** Review; Editing; Methodology; Conceptualization.

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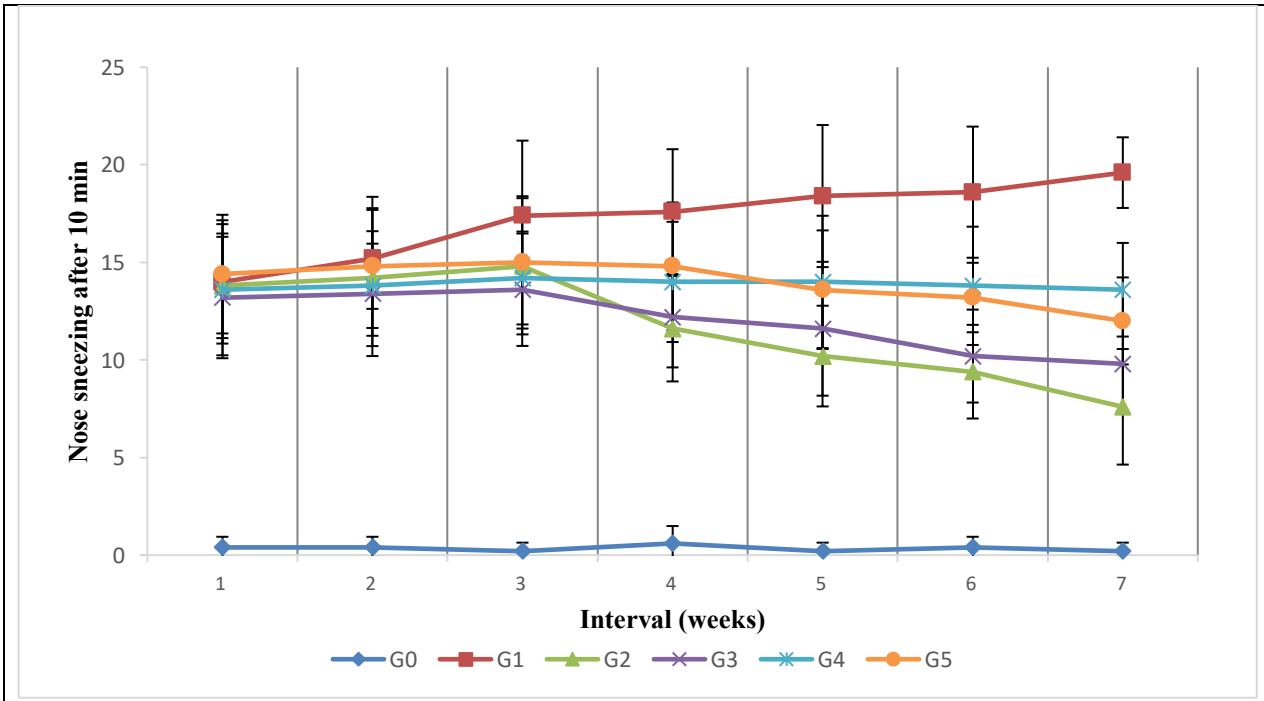
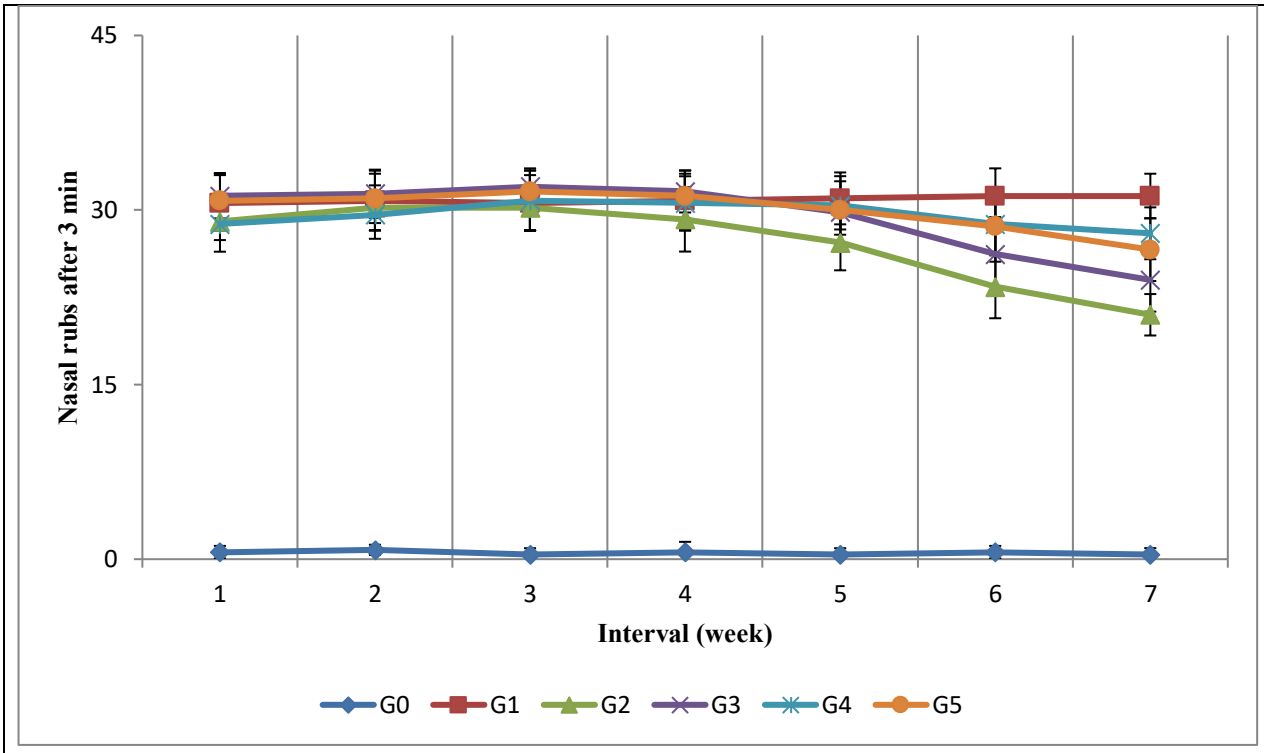
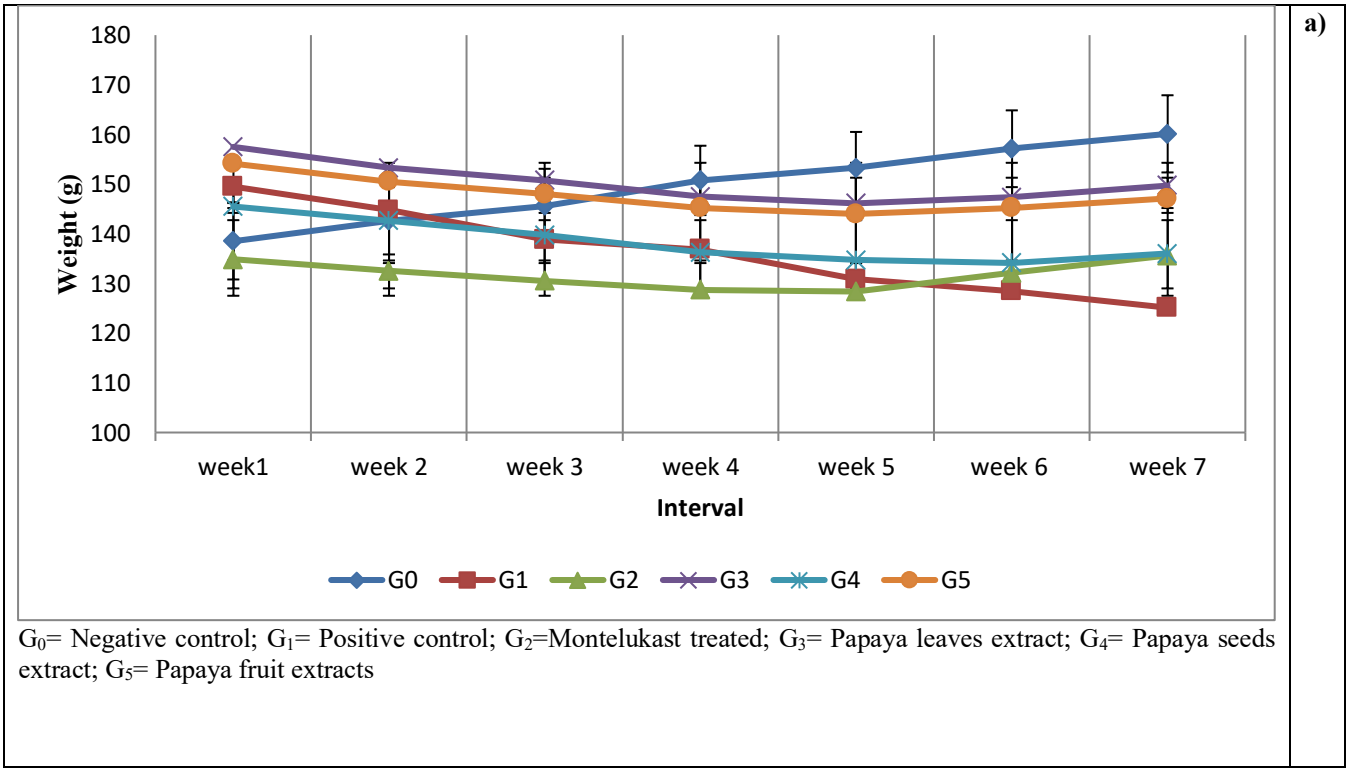
a)





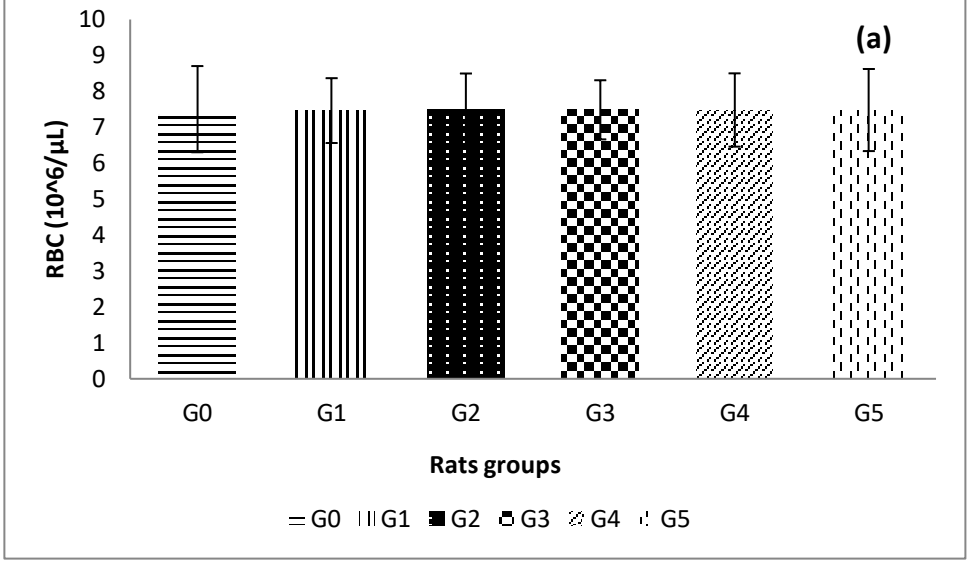
G_0= Negative control; G_1= Positive control; G_2=Montelukast treated; G_3= Papaya leaves extract; G_4= Papaya seeds extract; G_5= Papaya fruit extracts

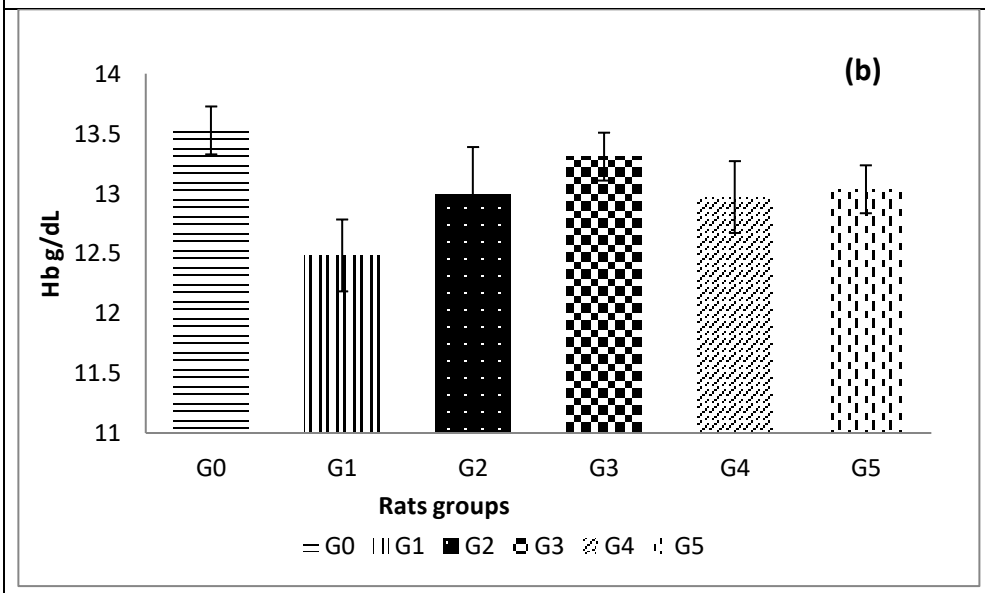
Fig 1. Mean camperison for nose sneezing and nasal rub score of experimental subjects

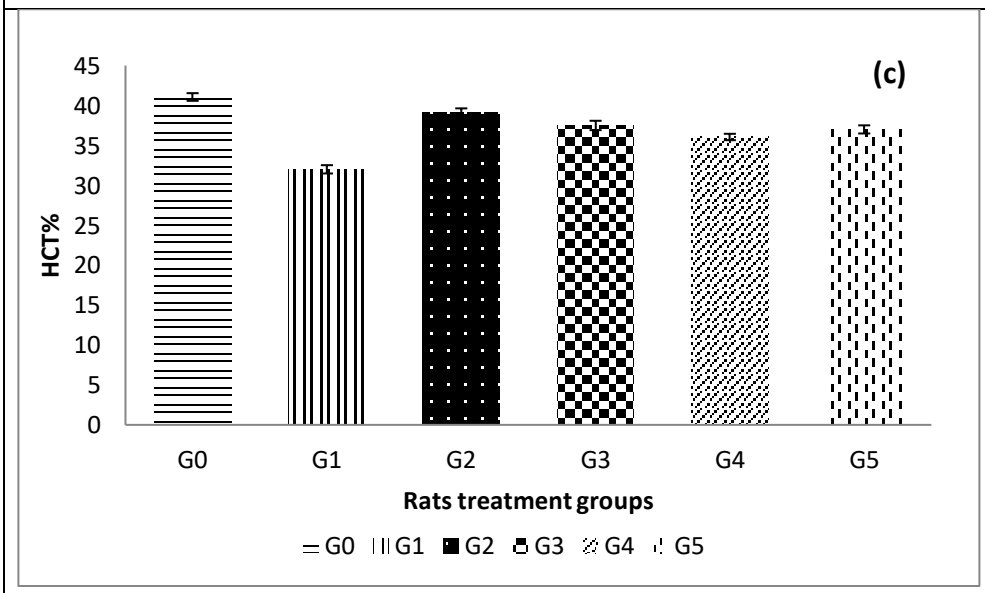


G_0= Negative control; G_1= Positive control; G_2=Montelukast treated; G_3= Papaya leaves extract; G_4= Papaya seeds extract; G_5= Papaya fruit extracts

Fig 2. Mean comparison for body weight of experimental subjects


(a)
RBC (10^6/µL)
10
9
8
7
6
5
4
3
2
1
0
G0
G1
G2
G3
G4
G5
Rats groups
G0 G1 G2 G3 G4 G5


(b)
Hb g/dL
14
13.5
13
12.5
12
11.5
11
G0
G1
G2
G3
G4
G5
Rats groups
G0 G1 G2 G3 G4 G5


(c)
HCT%
45
40
35
30
25
20
15
10
5
0
G0
G1
G2
G3
G4
G5
Rats treatment groups
G0 G1 G2 G3 G4 G5

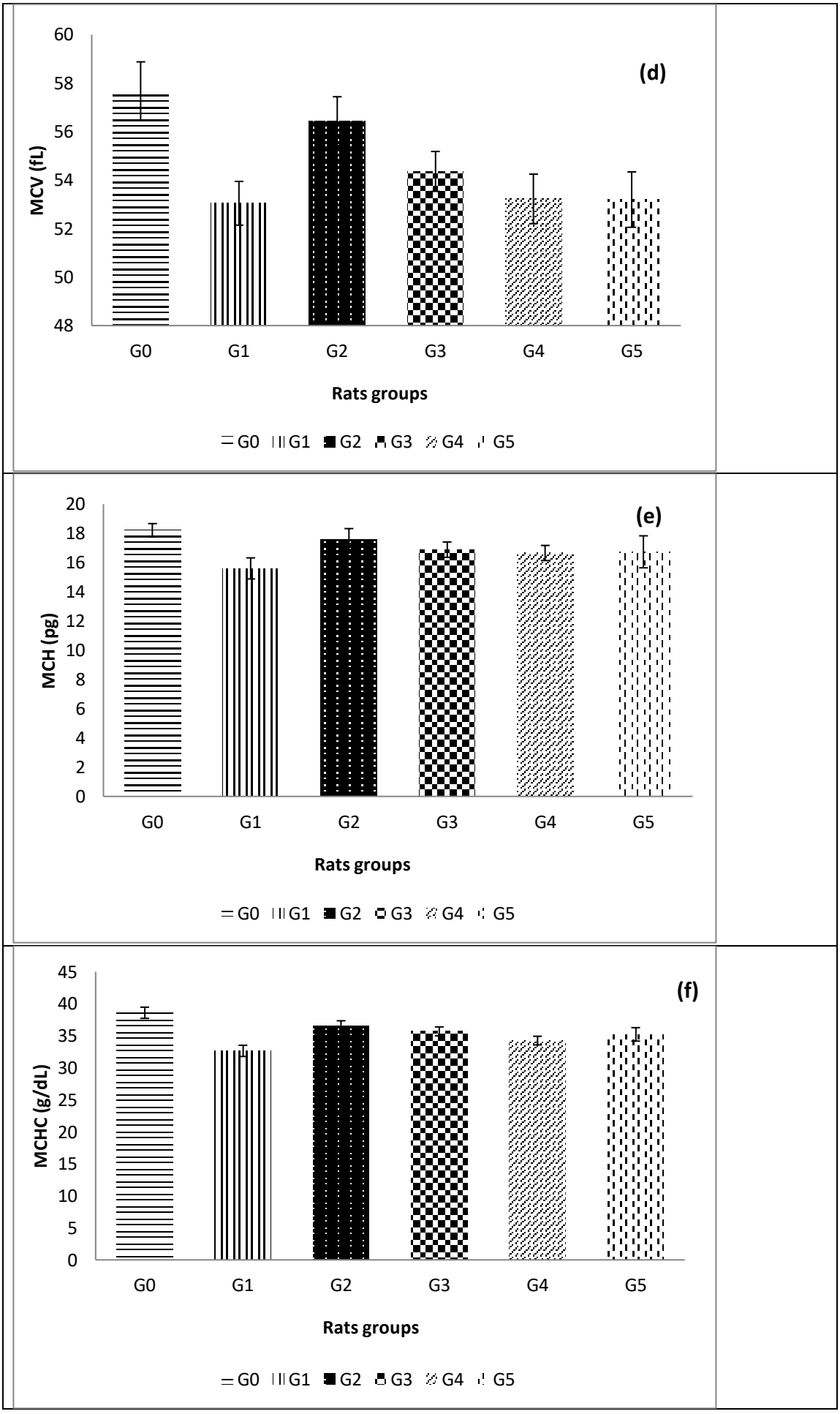
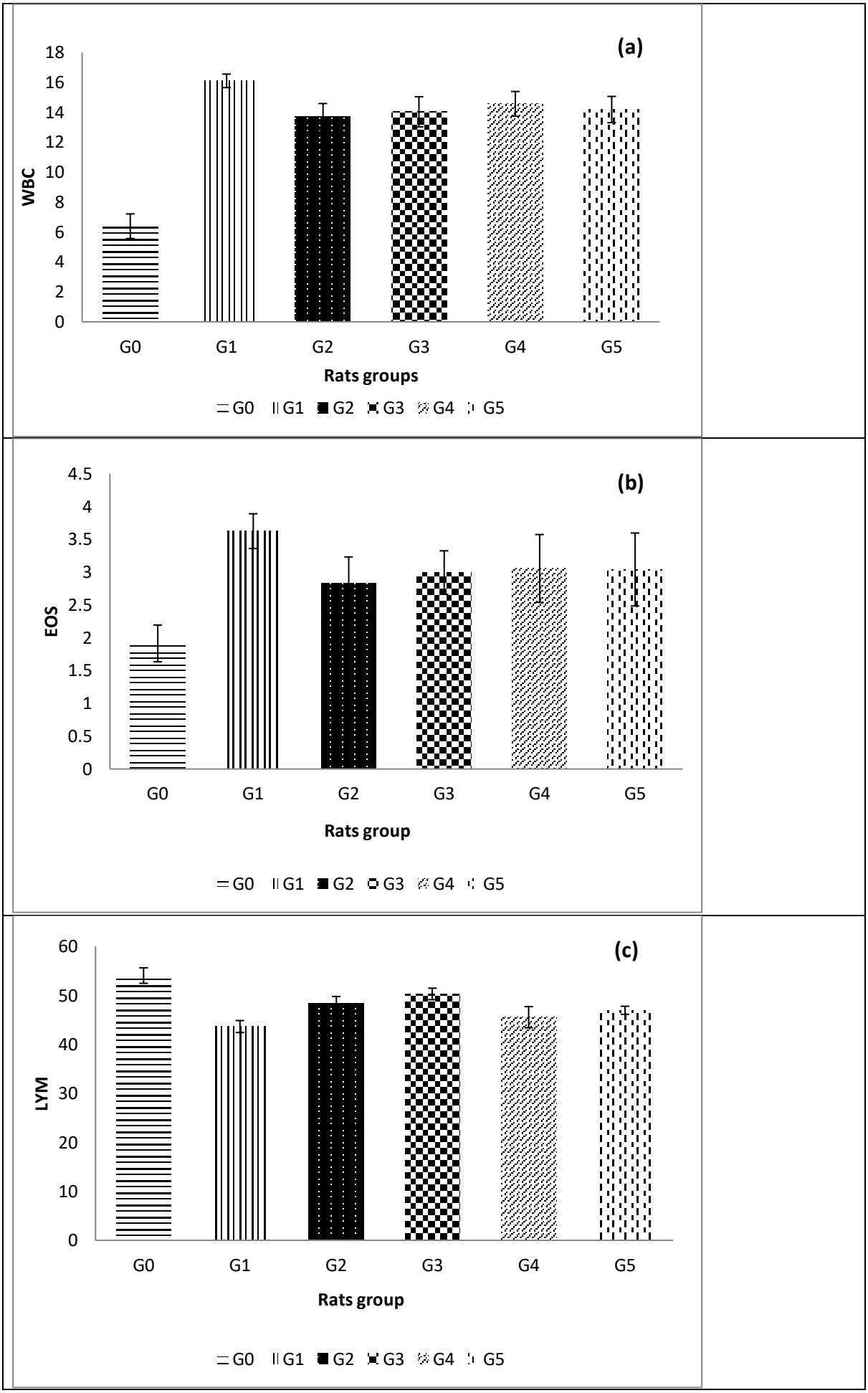
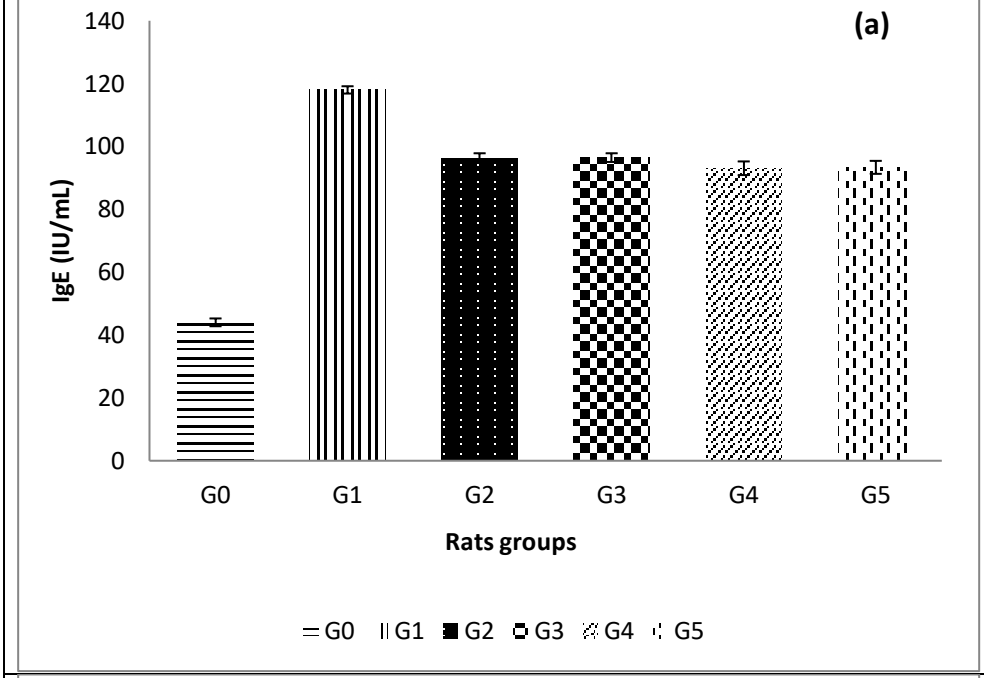


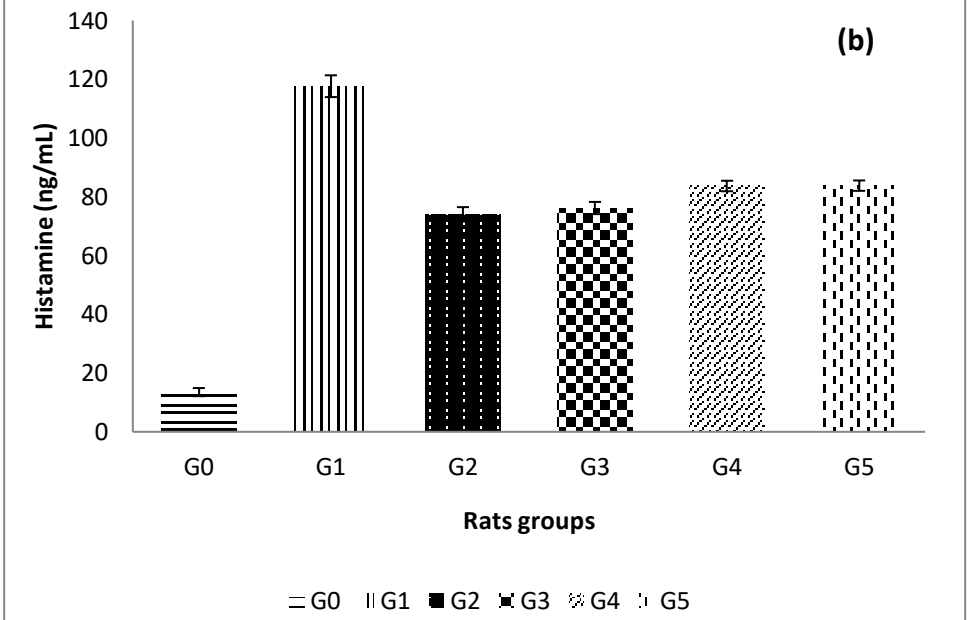
Fig 3 (a-f). Mean comparisons for erythrocyte's indices in the rats groups

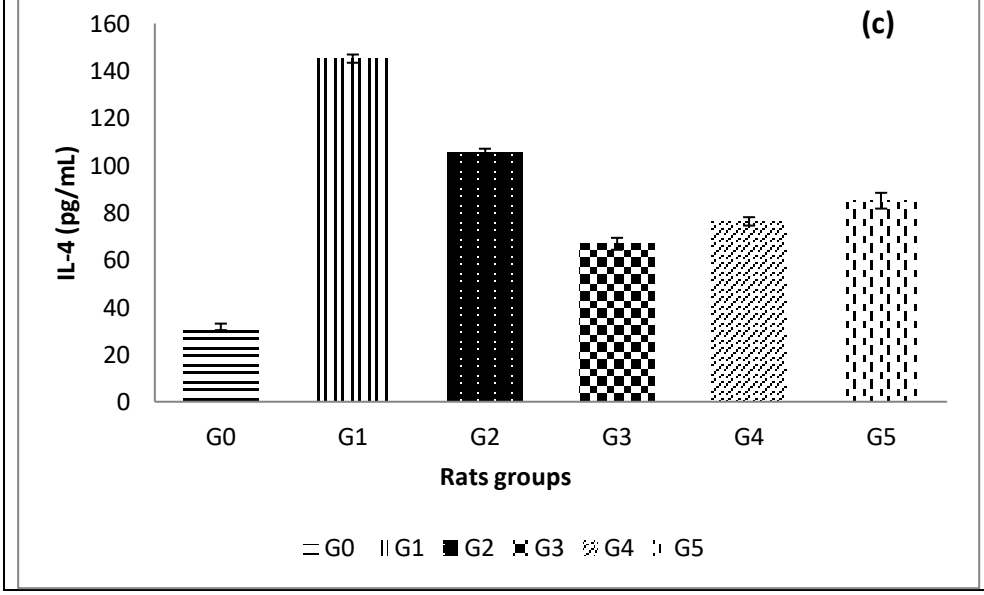


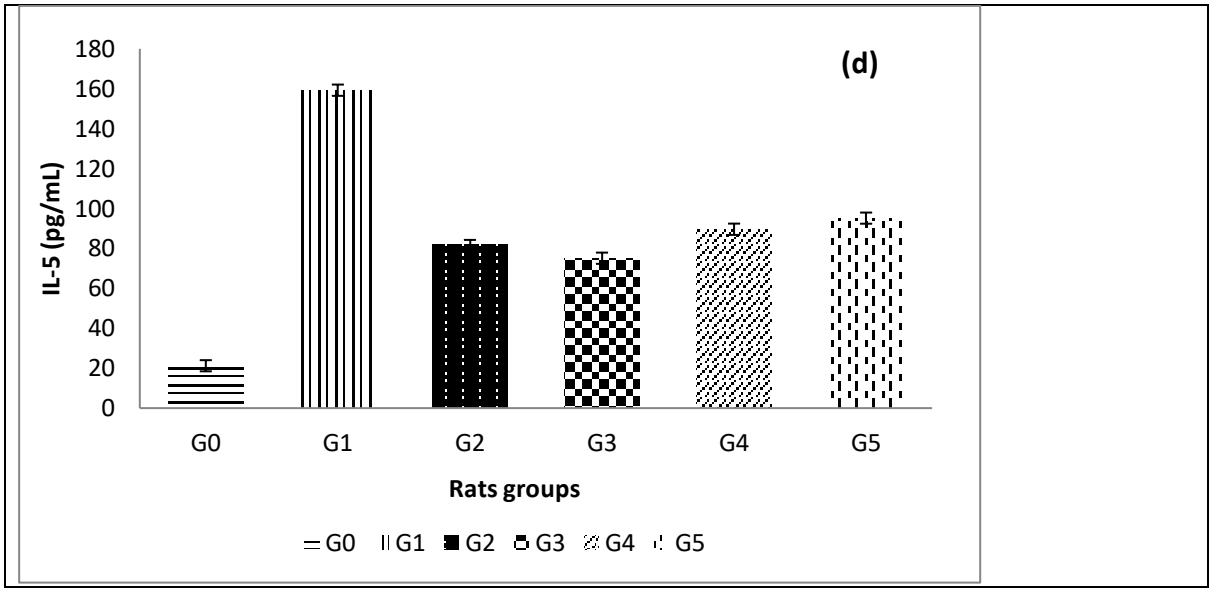
G_0= Negative control; G_1= Positive control; G_2=Montelukast treated; G_3= Papaya leaves extract; G_4= Papaya seeds extract; G_5= Papaya fruit extracts

Figure 4 (a-c). Mean comparisons for leukocytes indices in the rats groups


(a)
IgE (IU/mL)
140
120
100
80
60
40
20
0
G0
G1
G2
G3
G4
G5
Rats groups
G0 G1 G2 G3 G4 G5

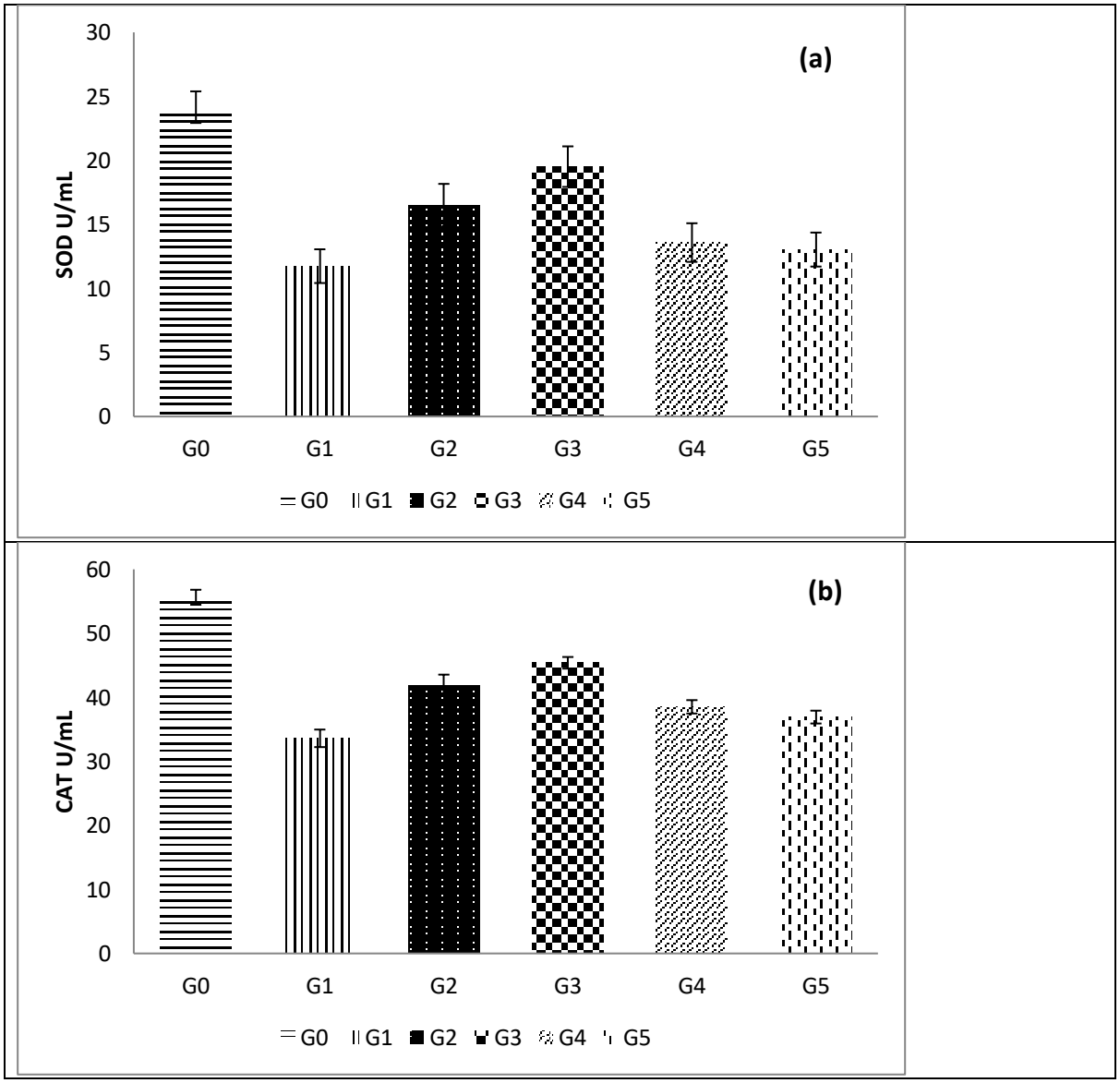

(b)
Histamine (ng/mL)
140
120
100
80
60
40
20
0
G0
G1
G2
G3
G4
G5
Rats groups
G0 G1 G2 G3 G4 G5

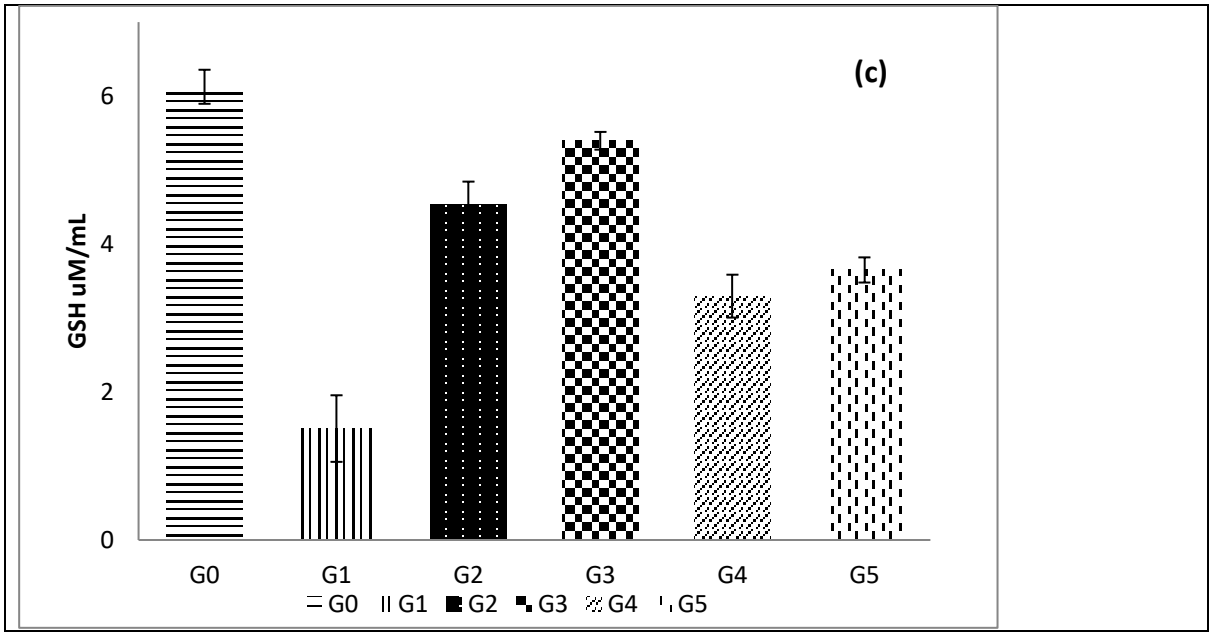

(c)
IL-4 (pg/mL)
160
140
120
100
80
60
40
20
0
G0
G1
G2
G3
G4
G5
Rats groups
G0 G1 G2 G3 G4 G5



G_0= Negative control; G_1= Positive control; G_2=Montelukast treated; G_3= Papaya leaves extract; G_4= Papaya seeds extract; G_5= Papaya fruit extracts

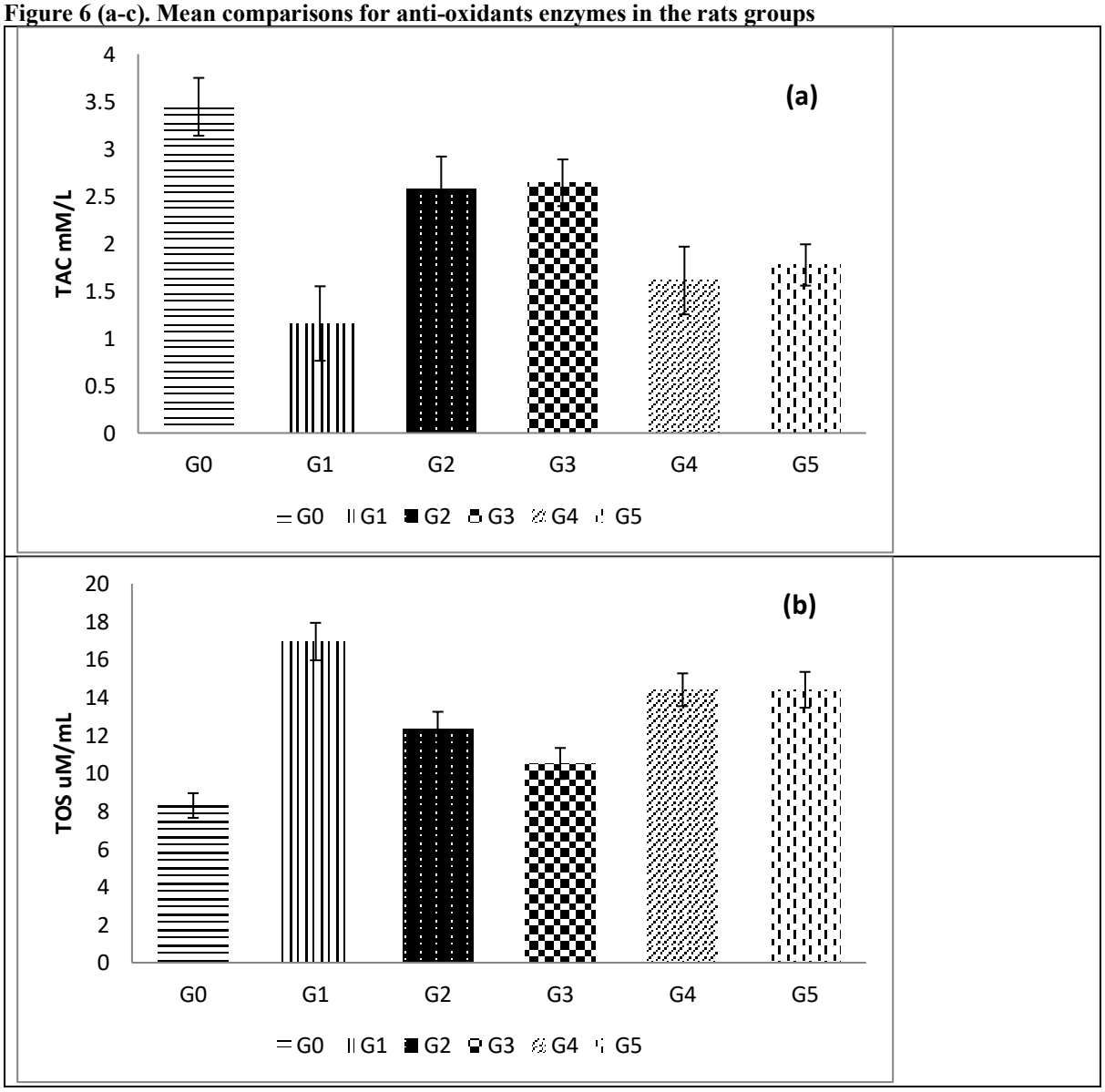
Figure 5 (a-d). Mean comparisons for inflammatory biomarkers in the rats groups





G_0= Negative control; G_1= Positive control; G_2=Montelukast treated; G_3= Papaya leaves extract; G_4= Papaya seeds extract; G_5= Papaya fruit extracts

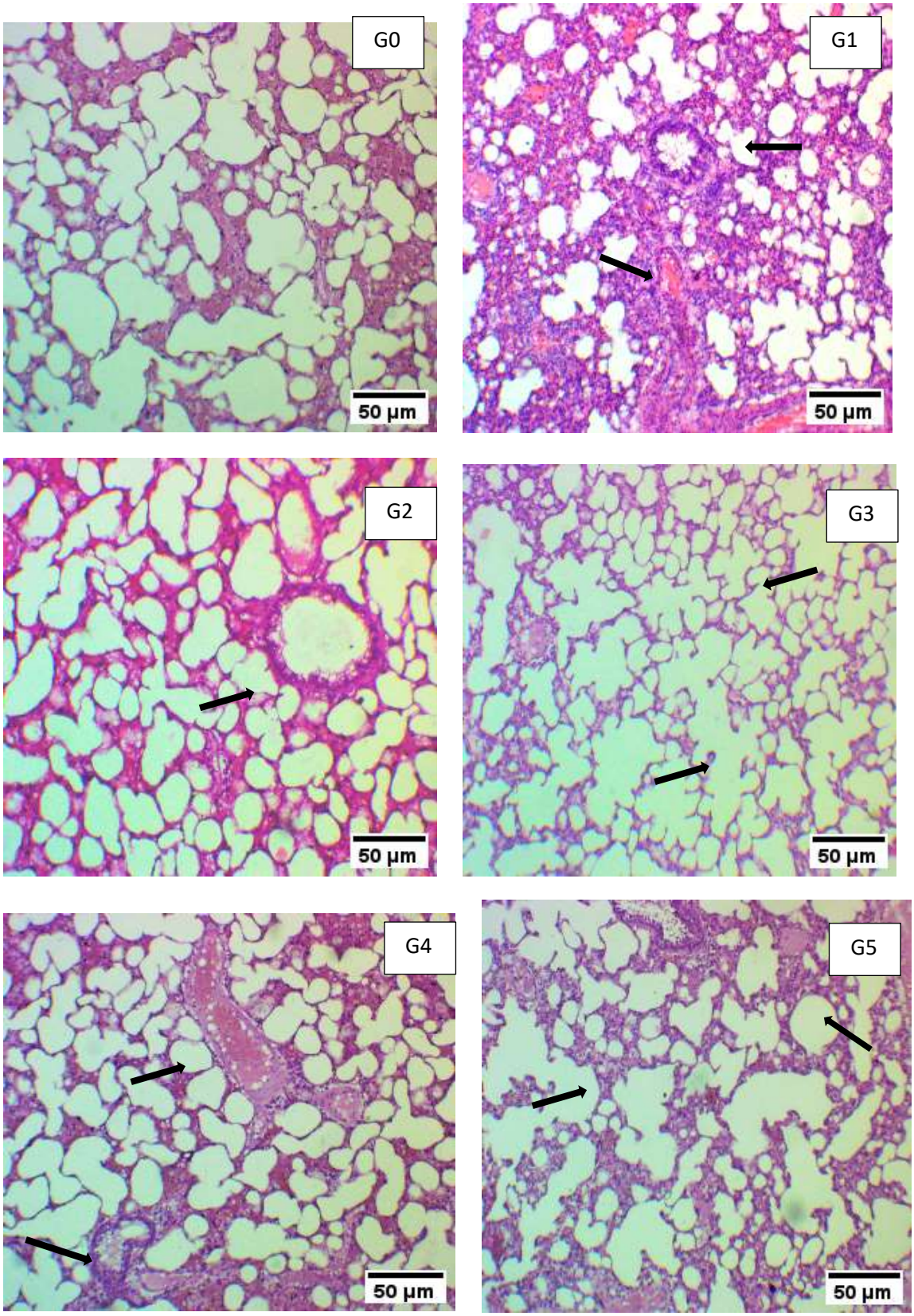
Figure 6 (a-c). Mean comparisons for anti-oxidants enzymes in the rats groups



G_0= Negative control; G_1= Positive control; G_2=Montelukast treated; G_3= Papaya leaves extract; G_4= Papaya seeds extract; G_5= Papaya fruit extracts

Figure 7 (a-b). Mean comparisons for TAC and TOS in the rats groups

Mega-dose vitamin C attenuated lung inflammation in mouse asthma model



G_0 (Negative control) = no infiltration of inflammatory cells, no partial thickening of alveolar cells with no tissue bleeding were observed; G_1 (Positive control)= Arrows showed pollen extract significantly damaged the epithelium layer of the lungs, leading to the formation of an irregular alveolar cavity due to onset of oxidative stress; G_2 (Montelukast treated) = Arrows recovery of the tissue was observed with intact alveolar structures and least inflammation was observed due to less infiltration of eosinophils and lymphocytes; G_3 (Papaya leaves extract)= Arrows showed restoration of the epithelium layer of the lungs accompanied by mild thrombosis; G_4 (Papaya seeds extract)= Arrows showed minimal effect as still alveolar wall thickening and thrombosis present; G_5 (Papaya fruit extracts)= Arrows showed minimal effect as some cellular infiltration, capillaries wall thickening and thrombosis exist

Figure 8. Histopathological analysis of allergy induced rat lungs