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Exploring the Therapeutic Potential of Aqueous Psidium Guajava Extract: Anti-Anaphylactic and Anti-Asthmatic Efficacy in Experimental Models

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KEYWORDS

Anti-Anaphylactic Activity Anti-Asthmatic Activity; Psidium Guajava; Oxidative Stress; TNF-A.

ABSTRACT:

The medicinal plant Psidium guajava L, which is known for its high content of tannins, triterpenes, flavonoids, quercetin, pentacyclic triterpenoid, and ursolic acid, demonstrates a variety of pharmacological effects, including antioxidant, anti-inflammatory, anti-cough, anti-diabetic, and antimicrobial properties. Animals were used to assess the anti-anaphylactic and anti-asthmatic capabilities of the aqueous extract of Psidium guajava (Myrtaceae). Psidium guajava extracts were orally fed to rats at 125, 250, and 350 mg/kg bw for 14 days. Modulation of experimentally produced airway inflammation, oxidative stress, TNF- levels, and lung histopathology were evaluated. Using a milk-induced eosinophilia and leukocyte count model, the anti-asthmatic action was further examined. The reduction of eosinophils and leukocytes indicated the efficiency of Psidium guajava in preventing anaphylaxis and asthma. These findings reveal the therapeutic potential of Psidium guajava as a helpful natural cure for illnesses linked with airway inflammation and immunological responses, promoting additional research into its applications in the treatment of anaphylactic reactions and asthma.

1. Introduction

Bronchial asthma is a complicated inflammatory condition marked by airway constriction, alterations in eosinophil count, mast cell activation, lymphocyte proliferation, and cytokine production [1]. By secreting interleukins (IL)-4, IL-5, and IL-13, allergen-specific Th2 cells play a vital role in initiating and perpetuating this inflammatory cascade. In particular, the recruitment and activation of eosinophils by tumor necrosis factor-alpha (TNF-) contributes to airway inflammation, with eosinophil cationic protein (ECP) emerging as an important clinical diagnostic [2,3].

During asthmatic responses, the production of reactive oxygen species (ROS) worsens lung damage in affected animals by exacerbating inflammation. ROS-induced airway hyperactivity causes mast cells to produce histamine and epithelial cells to secrete mucus [4].

The medicinal plant Psidium guajava L., widely known as Guava and a member of the Myrtaceae family, assumes relevance in this perspective. Psidium guajava is widely distributed in tropical and subtropical countries

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and functions as both an essential food crop and a medicinal plant [5]. Tannins, triterpenes, flavonoids, quercetin, guajanoic acid, saponins, carotenoids, lectins, ellagic acid, and a variety of other beneficial substances have been identified through phytochemical investigation of Psidium guajava. This study was conducted to investigate the anti-asthmatic potential of the aqueous extract of Psidium guajava. Using in vivo experimental animal models for allergy and milk-induced eosinophilia, this study aims to determine the therapeutic potential of Psidium guajava in reducing asthma-related pathophysiology [6].

2. Material and Methods

2.1 Materials

The horse serum was procured from HIMEDIA Chemicals (Mumbai, India), whilst the Triple antigen (DPT Vaccines) was purchased on the local market from the Serum Institute of India Pvt. Ltd. Sigma-Aldrich, India supplied the essential chemicals 5,5-dithiol-bis (2-nitrobenzoic acid) (DTNB), Nitro blue tetrazolium (NBT), hydroxylamine, and Triton-X. To ensure precision and dependability, all chemicals and reagents used in this investigation were of analytical quality. In addition, Cytokine ELISA Ready SET-Go kits for TNF- (Catalog Number: 837324-22, Batch Number: E09479-1645) were utilized, contributing to the standardized and accurate detection of cytokine concentrations during the experimental procedures.

2.2 Collection of plant material and Extraction

Fresh plant *Psidium guajava Linn (Myrtaceae)* was collected from Betawad, Dist. Dhule (Maharashtra) town and was authenticated by Dr S. R. Kshivsagar (Assistant professor, Department of botany) from SSVPS Late Kramveer Dr P. R. Ghogrey Science College, Dhule. A sample of 100 g guava leaves in 1.5 L distilled water was boiled for 4 h [7]. The sample was then filtered using Whatman filter paper No. 4. The filtrate was concentrated by using a hot plate at 60°C and dried using a freeze drier. The resulting extracts were stored at -18°C until the analysis [8,9].

2.3 Preliminary phytochemical screening

Psidium guajava Linn (Myrtaceae) leaf extract was submitted to a complete phytochemical screening to assess the existence of significant bioactive components. During the examination, flavonoids, alkaloids, saponins, and carbohydrates were identified. The presence of flavonoids was determined by exposing the extract to diluted hydrochloric acid and magnesium ribbon, which yielded an orange-yellow hue [10]. To determine the presence of alkaloids, Wagner's reagent was used to produce a reddish-brown precipitate from the extract. The presence of saponins was identified by the production of foam when shaking the extract with distilled water. The formation of a brick-red precipitate indicated the presence of reducing sugars when Fehling's solution was applied to analyse carbohydrates [11]. This extensive screening not only confirmed the existence of these phytochemicals, but also provided the framework for understanding the possible therapeutic properties of the Psidium guajava extract. To ensure the accuracy and reproducibility of the data, each test was conducted three times [12].

2.4 Animals and Ethics statement

Wistar rats (180-250 g) of either gender were used for the Active Anaphylaxis Model, while Swiss Albino mice (25-30 g) of either gender were used for the Milk-Induced Eosinophilia Model. Animals were obtained from the Central Animal House at RCPIPER, Shirpur. They were housed in ordinary polypropylene cages under regulated environmental conditions, including room temperature of 25 1 °C, relative humidity of 60 5 percent, and a light-dark cycle of 12:12 hours. Animals got free access to commercial food pellets (Neutrivet Life Science, Pune) and ad libitum water. The Institutional Animal Ethical Committee examined and approved all experimental methods (Approval No. IAEC/CPCSEA/RCPIPER/2017-2022), assuring accordance with established rules and principles for the humane treatment of animals in scientific research.

2.5 Milk induced eosinophilia

Six sets of six Swiss Albino mice were used for the experiment. Group I served as the control and received a Tween-80 solution containing 1 percent. Group II, the control group, was given boiled and cooled milk (4 mL/kg, subcutaneously). Psidium guajava was supplied at doses of 125, 250, and 350 mg/kg bw to Groups III, IV, and V, respectively [13]. Group VI received 50 mg/kg i.p. dexamethasone. All groups, with the exception of the control, were injected with boiling and chilled milk (4 mL/kg, subcutaneously) 30 minutes after their individual treatments. Blood samples were drawn from the retro-orbital plexus, and the total eosinophil count was evaluated prior to the administration of the test chemicals and 24 hours after the administration of milk. For each group, the difference between the total eosinophil count before and after 24 hours of drug administration was measured, simplifying the evaluation

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of Psidium guajava's effect on milk-induced eosinophilia [14].

2.6 Triple antigen and horse serum-induced active anaphylaxis in rats

Twenty-five albino Wistar rats of either gender weighing between 180 and 250 grammes were sensitised for active anaphylaxis induction [15]. Sensitization consisted of the subcutaneous injection of 0.5 mL of horse serum, followed by 0.5 mL of a triple antigen vaccination containing 2 * 1010 Bordetella pertussis organisms per millilitre. The sensitised animals were then separated into six groups of five rats apiece. Group I acted as the normal control (NC), consuming a conventional diet and consuming water at will. Group II served as the sensitised control (SC), receiving horse serum and then the triple antigen vaccine. Group III was supplied conventional prednisolone (10 mg/kg b.w., p.o.), whereas Groups IV, V, and VI got Psidium guajava at doses of 125, 250, and 350 mg/kg b.w. orally, once daily for 14 days. After 14 days of treatment, all animals, with the exception of Group I, were challenged intravenously with 0.25 mL of horse serum in saline through the tail vein, and the start of anaphylactic symptoms was monitored for 1 hour, beginning 2 hours after treatment. The objective of this research was to assess the potential protective benefits of Psidium guajava against active anaphylaxis produced by triple antigen and horse serum in sensitised rats [16].

Collection of Broncho alveolar lavage fluid (BALF)

Following the treatment protocol, bronchoalveolar lavage fluid (BALF) was collected on the fourteenth day. A tracheal cannula was placed through a mid-cervical incision, and 1 mL of cold phosphate-buffered saline (PBS) with a pH of 7.4 was used for lavage. The collected BALF was then centrifuged for 10 minutes, and the resultant supernatant was used for biochemical marker analysis. In the supernatant, the levels of tumour necrosis factor-alpha (TNF), glutathione, catalase, malondialdehyde, and superoxide dismutase were measured. This methodical procedure was designed to determine the effect of the medication on the inflammatory and oxidative stress parameters in the bronchoalveolar milieu [17,18].

2.7 Determination of Catalase content

Hydrogen peroxide (H2O2) decomposition at 240 nm was used to determine catalase concentration as part of a catalase activity determination method. Briefly, the assay combination contained 3 mL of H2O2, phosphate buffer, and 0.05 mL of tissue homogenate supernatant (10 %). Using a UV-visible spectrophotometer, the absorbance change at 240 nm was measured. The results obtained were reported as micromoles of H2O2 decomposed per milligramme of protein per minute. This enzymatic assay gave useful insights on the catalase activity of the examined tissue, reflecting its ability to scavenge hydrogen peroxide, a crucial marker of oxidative stress [19,20].

2.8 Determination of Superoxide Dismutase

Superoxide dismutase (SOD) activity was determined via an assay system containing 0.1 mM EDTA, 50 mM sodium carbonate, and 96 mM nitro blue tetrazolium (NBT). 2 mL of the aforementioned components, 0.05 mL of hydroxylamine, and 0.05 mL of the sample's supernatant were mixed in the cuvette [21]. The auto-oxidation of hydroxylamine was then observed for two minutes at 30-second intervals by measuring absorbance at 560 nm with a microplate reader. This method enabled the determination of SOD activity by measuring its ability to prevent the auto-oxidation of hydroxylamine, hence offering vital information into the antioxidative capability of the sample under study [22,23].

2.9 Determination of Reduced Glutathione

A 1.0 ml aliquot of the supernatant (10 percent) was precipitated with 1.0 ml of sulfosalicylic acid for the determination of reduced glutathione (GSH) levels (4%) [21]. Following a minimum of one hour of incubation at 4 C, the samples were centrifuged at 1200 rpm for 15 minutes at the same temperature. The resultant supernatant was incorporated into the assay mixture, which contained 0.1 ml of supernatant, 2.7 ml of phosphate buffer (0.1 M, pH 7.4), and 0.2 ml of 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, 0.1mM, pH 8.0) in a total volume of 3.0 ml. GSH levels were determined using the molar extinction coefficient of 1.36 x 104 M(-1) cm(-1), expressed in micromoles per milligramme of protein, and the yellow colour generated was measured at 412 nm using a microplate reader. This test gave a quantitative evaluation of the sample's reduced glutathione concentration, hence revealing the sample's antioxidant capacity [22,23].

2.10 Determination of Lipid Peroxidation

The quantitative assessment of lipid peroxidation was performed by measuring thiobarbituric acid reactive substances (TBARS) according to the Wills method (1966) [24]. Malondialdehyde (MDA), a marker for lipid peroxidation, was quantified using a microplate reader and its interaction with thiobarbituric acid at 535nm. 100l of the homogenate's supernatant was

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combined with 600l of thiobarbituric acid and 20l of glacial acetic acid in this process. After 15 minutes of centrifugation at 5000 rpm, the mixture was heated for 45 minutes until a pink hue developed. After adding 4l of butanol, the mixture was vortexed for 5 minutes [21]. Using a microplate reader, the absorbance of the organic layer was measured at 535 nm. This method enabled the accurate assessment of lipid peroxidation levels, hence shedding light on the oxidative stress present in the examined materials [25].

2.11 Determination of TNF-alpha

The TNF-alpha concentration was evaluated using a sandwich enzyme-linked immunosorbent assay (ELISA) as per the manufacturer's instructions. Briefly, the assay consisted of trapping TNF-alpha with antibodies immobilised on a solid phase, followed by detection with a secondary antibody and quantification with a standard curve. Based on the standard curve, the final TNF-alpha concentration was determined and expressed as picograms per milligramme of protein. This method enabled a sensitive and specific detection of TNF-alpha levels, thereby giving vital data to the evaluation of the inflammatory response in the samples investigated [26,27].

2.12 Histopathology Study

Lungs were carefully isolated from rats and kept in 10% formalin after BALF collection. After embedding the lung tissues in paraffin, a microtome cut them into 5 mm slices [28]. These thin sections were stained with hematoxylin and eosin (H&E). Stained slices were examined under a microscope to document histological changes. Changes in the airway lumen, columnar epithelium, lymphocyte accumulation, and parenchymal inflammation were assessed. This histopathological study revealed structural and cellular alterations in lung tissues, helping to understand how experimental therapies affected respiratory disease [29,30].

3. Statistical analysis

All results are shown as mean standard error of the mean (Standard Error of the Mean). The biochemical data were analysed statistically using one-way analysis of variance (ANOVA) and Bonferroni's test for post hoc comparisons. The statistical analyses were conducted using Graph Pad Prism Programme. A p-value less than 0.05 was regarded as statistically significant, indicating that differences were not likely due to random chance. This strong statistical method provided a thorough examination of experimental results and the identification of significant differences between research groups.

4. Results and Discussion

4.1 Preliminary phytochemical screening

The preliminary phytochemical analysis of Psidium guajava revealed the presence of several beneficial chemicals, as listed in Table 1. Alkaloids, flavonoids, saponins (including steroids and triterpenoids), and carbohydrates all returned positive results during the qualitative examination. Each discovered component was subjected to at least one additional confirmation test to increase the result's reliability.

The existence of these phytoconstituents in Psidium guajava is indicative of its potential therapeutic usefulness, setting the groundwork for future research into its pharmacological actions and medicinal applications.

4.2 Effect of *Psidium guajava* on horse serum-induced oxidative stress

As shown in Fig. 1, the level of Catalase content was significantly lower in horse serum-challenged rats compared to normal rats in the context of oxidative stress (6.426 0.686, #P 0.001). Catalase levels decreased after oral administration of Psidium guajava at doses of 125 and 250 mg/kg, although the decrease was not statistically significant. However, Psidium guajava showed a substantial rise in Catalase levels at a dose of 350 mg/kg. The Catalase levels of rats treated with Prednisolone were significantly higher than those of rats treated with horse serum (P 0.001). Catalase activity was significantly increased at the highest dose of Psidium guajava, to the point where it was comparable to the positive control Prednisolone, suggesting a possible dose-dependent protective action against oxidative stress generated by horse serum.

4.3 Effect of *Psidium guajava* on horse serum-induced release of TNF-*α*

Psidium guajava's effect on the TNF- release generated by horse serum shown in Figure 2. TNF- levels were significantly increased in horse serum-challenged rats compared to normal rats, suggesting an inflammatory response (Fig. 2, P 0.001). Treatment with Psidium guajava at oral doses of 125 and 250 mg/kg resulted in a dose-dependent, albeit non-significant, decrease in TNF- release. Psidium guajava showed no effect on TNF- levels at 100 or 200 mg/kg, but it significantly reduced TNF- levels at 350 mg/kg, indicating a possible anti-inflammatory action at this higher dose. In addition,

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when comparing Prednisolone-treated rats to those exposed to horse serum, there was a statistically significant reduction in TNF- production (P 0.001). These results suggest that Psidium guajava has a promising modulatory effect on the serum-induced production of TNF- in horses, with the highest dose exhibiting a statistically significant anti-inflammatory benefit comparable to Prednisolone.

4.4 Effects of *Psidium guajava* on Histological Changes in horse serum-induced Asthmatic rat

Lung histopathology studies helped reveal how Psidium guajava affected inflammatory responses prompted by horse serum. The experiment showed that the sensitised mice group had significantly higher levels of inflammatory cell infiltration and epithelial cell thickening than the control group. Figure 3 shows that when sensitised mice were exposed to Psidium guajava, the infiltration of inflammatory cells into their airway epithelium decreased dramatically.

4.5 Effect of *Psidium guajava* on milk induced eosinophilia (in vivo):

Figure 4 shows that the total number of eosinophils and leukocytes in the blood of sensitised control mice increased significantly after receiving a dose of boiled and cooled milk (4 ml/kg, s.c.). There was a significant and dose-dependent reduction in eosinophil and leukocyte cell count after treatment with Psidium guajava (125, 250, and 350 mg/kg). When Dexamethasone, a common medicine, was given at a dose of 1 mg/kg, the number of white blood cells, or leukocytes, dropped dramatically. These results demonstrate that Psidium guajava has an anti-eosinophilic impact, indicating its potential as a therapeutic agent in reducing milk-induced eosinophilia in a way that is equivalent to the positive control, Dexamethasone.

5. Conclusion

This study examined the anti-anaphylactic and anti-asthmatic capabilities of an aqueous extract of Psidium guajava in animal models of active anaphylaxis and eosinophilia. Rats treated with Psidium guajava extracts displayed a dose-dependent reduction of active anaphylactic responses, including decreased mortality, in response to anaphylactic shock caused by triple antigen and horse serum. In addition, in a mouse model of milk-induced eosinophilia, administration of Psidium guajava at various doses markedly lowered eosinophil and leukocyte counts, similar to the results found with the conventional medication Dexamethasone. Notably, Psidium guajava therapy displayed anti-inflammatory effects in both models, as seen by decreased levels of the pro-inflammatory cytokine TNF- and decreased oxidative stress in bronchoalveolar lavage fluid. Its promise as an anti-anaphylactic and anti-asthmatic drug is strengthened by Psidium guajava's preventive effect on histological alterations. In conclusion, Psidium guajava exhibits promise anti-allergic and anti-asthmatic activity, warranting further investigation into its clinical application.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

Rahul Patil: Supervision, Validation, Methodology, Data Curation **Anil Pawar:** Investigation, Writing – original draft, **Alka Zade:** Conceptualization, Administration, Funding.

Abbreviations

Interleukin: IL
Eosinophil cationic protein: ECP
Release of reactive oxygen species: ROS
Nitro blue tetrazolium: NBT
Thiobarbituric acid reactive substances: TBARS
Malondialdehyde: MDA
Broncho alveolar lavage fluid: BALF

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Table 1: Preliminary phytochemical study

Sr.no	Chemical test of extract	Result
1	Alkaloid test	+
2	Flavonoids test	+
3	Saponin test	+
4	Carbohydrate test	+

Where, + indicate the test is positive and – indicate the test is negative

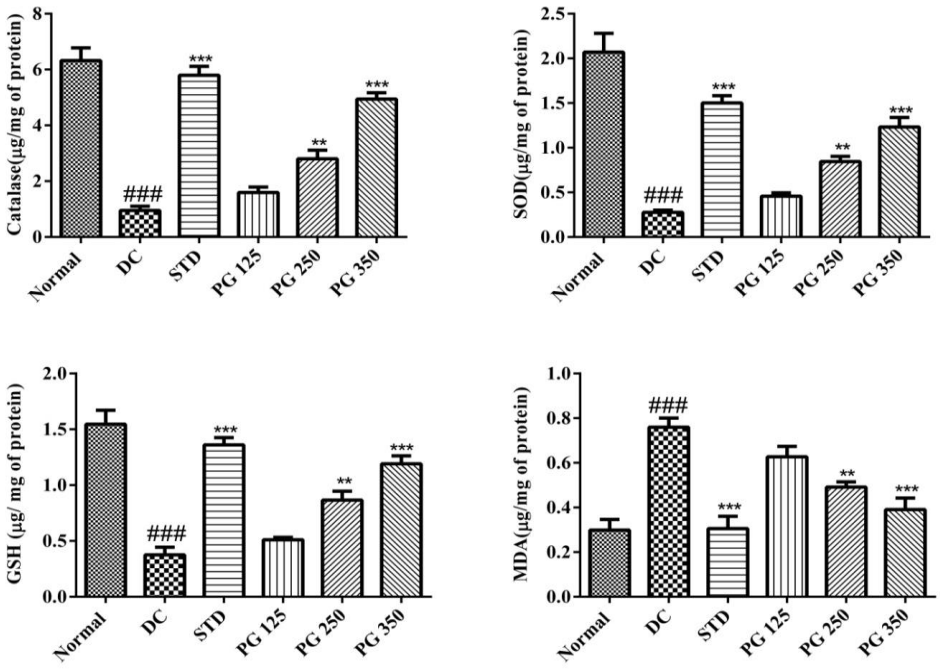

Fig.1: Effect of *Psidium guajava* on horse serum-induced oxidative stress

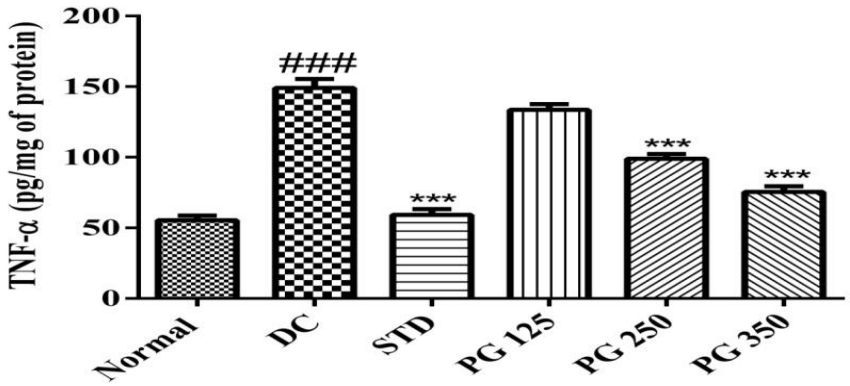

Fig.2: Effect of *Psidium guajava* on horse serum-induced release of TNF-α

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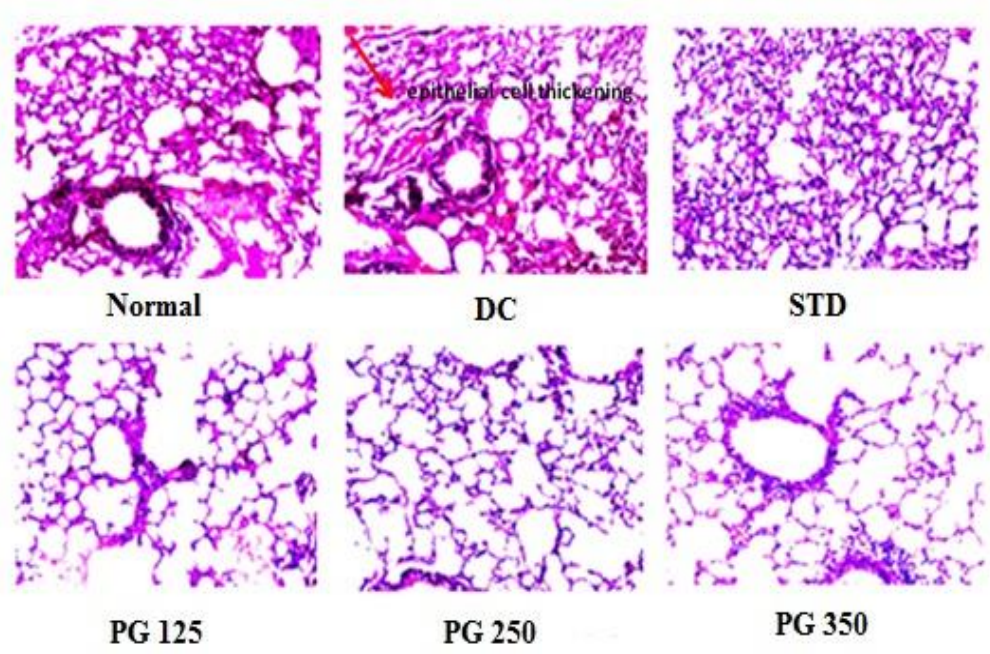

Fig.3: Effects of *Psidium guajava* on Histological Changes in horse serum-induced Asthmatic rat

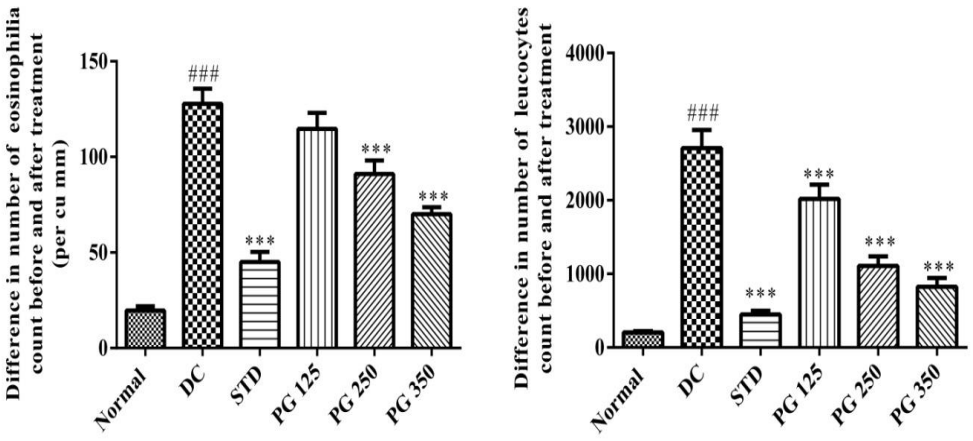

Fig.4: Effect of *Psidium guajava* on milk induced eosinophilia