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The Effects of Turmeric and Mangosteen Pericarp Ethanol Extract on Eosinophil Count, TNF-α and TGF-β1 Gene Expression in Asthmatic Rat Model

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Background: Asthma is a chronic respiratory disease that is characterized by inflammation, bronchial hyperreactivity, and airway remodeling. The long-term use of corticosteroids at high doses causes various side effects. Traditional herbal medicine has been suggested as an alternative therapy that is safe and effective in dealing with asthma. Natural plants such as turmeric and mangosteen are known to treat asthma and reduce inflammation.

Objective: The purpose of this study was to investigate the effects of turmeric and mangosteen pericarp ethanol extracts on the eosinophil counts, TNF-α and TGF-β1 gene expression, and inflammatory cell counts in the histopathology of an asthmatic rat model.

Methods: The preliminary study used 30 rats, which were divided into a normal group, negative control group (OVA-sensitized), turmeric normal group, mangosteen group, and positive control group. Blood samples were collected after the sensitization period to determine eosinophil counts. TNF-α and TGF-β1 gene expression, and histopathology were observed in the rat's lungs. The follow-up study used 30 rats divided into a normal group, negative control group (OVA-sensitized), combination of turmeric and mangosteen group (54m/200gr rats, 36mg/200gr rats, and 36mg/200gr rats), and positive control group. The examination procedures were the same as in the preliminary study.

Results: The administration of single ethanol extracts of turmeric and mangosteen significantly decreased eosinophils and improved the histopathological features of the lungs (inflammatory cell counts, bronchial inflammatory score, and bronchial smooth muscle thickness) ($p<0.05$). The combination of turmeric and mangosteen extracts at all doses significantly decreased eosinophils and improved the histopathological features of the lungs (inflammatory cell counts, bronchial inflammatory score, and bronchial smooth muscle thickness) ($p<0.05$). Both the single and combined administration of turmeric and mangosteen ethanol extracts did not cause significant changes in TNF-alpha and TGF-beta ($p>0.05$).

Conclusion: Turmeric ethanol extract and mangosteen pericarp ethanol extract have a reductional effect on the parameters of asthma based on the eosinophil counts, the inflammatory cell counts and score, and bronchial smooth muscle thickness.

Keywords: bronchial smooth muscle thickness, eosinophils, mangosteen, inflammatory cells, turmeric

Introduction

Asthma is a chronic respiratory disease that is characterized by inflammation, bronchial hyperreactivity, and airway remodeling.[1] This condition involves multiple cells, cytokines, and proteins, predominantly T helper 2 lymphocyte cells (Th2), eosinophils, basophils, mast cells, interleukins, and immunoglobulin E (IgE).[2,3] Asthma causes clinically significant symptoms such as wheezing, chest tightness, and coughing.[4] The prevalence and incidence of asthma are

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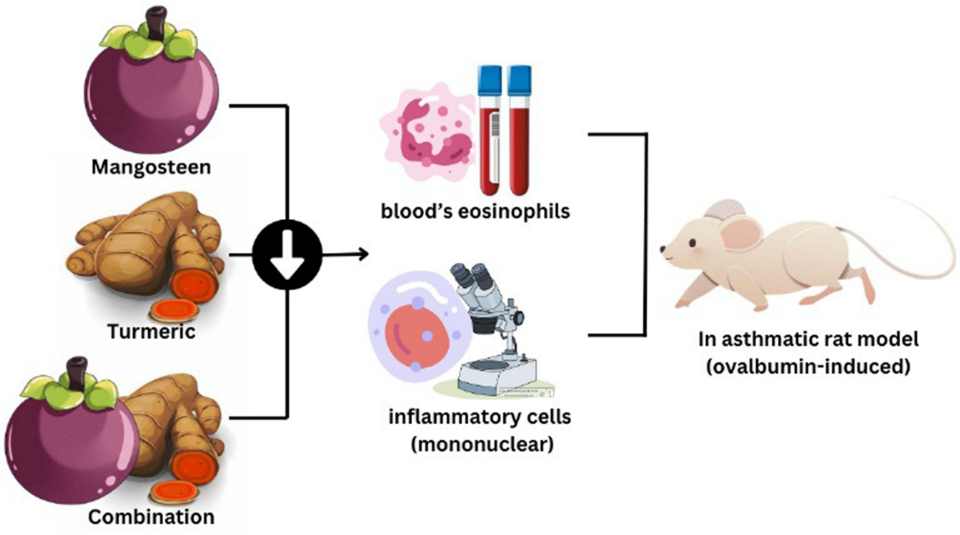
Graphical Abstract



increasing, and it has become a global public health issue.[5] There are three hundred million people worldwide who suffer from asthma.[6]

Environmental exposures, including allergens, pollution, and pathogens, can trigger asthma in susceptible individuals.[7] Allergens that enter the body are presented by dendritic cells to naive T cells and activated Th2 cells.[8] Apart from differentiation into Th2 cells, naïve T cells also differentiate into T helper 1 (Th1) cells and T helper 17 (Th17) cells.[9] Activated Th2 cells release pro-inflammatory cytokines, including interleukin (IL)-4, IL-5, IL-9, and IL-13.[10] The IL-5 and IL-13 produced by Th2 cells stimulate eosinophil differentiation.[11,12] IL-5 produced by Th2 cells, tumor necrosis factor α (TNF-α) and eotaxin produced by macrophages and epithelial cells trigger degranulation of eosinophil cells.[3,13–15]

Degranulation of eosinophil cells in the airways results in the release of cytotoxic major basic protein (MBP), eosinophil peroxidase (EPX), eosinophil cationic protein (ECP), galectin-10, eosinophil-derived neurotoxin (EDN), lipid mediators, chemotactic peptides, and other cytokines, such as IL-2, IL-4, IL-5, IL-6, IL-13, TGF-α, and TNF-α.[11,16,17] Degranulated eosinophil stimulates the release of histamine which activates neutrophil cells, alveolar macrophage cells, and type 2 inflammation.[16,17] Type 2 inflammation involves an innate immune response triggered by pollutants and infections through the involvement of lymphoid cells, and an adaptive immune response triggered by allergens through the involvement of Th2 cells.[18] TNF-α is an inflammatory mediator produced by Th2 cells, macrophages, epithelial cells, and eosinophil cells.[13,19,20] TNF-α plays a role in the inflammatory process by recruiting inflammatory cells, releasing inflammatory mediators, oxidizing the airways, and causing airway hyperresponsiveness.[20] Eosinophils produce Transforming growth factor-β (TGF-β) that takes effect in airway remodeling.[16] TGF-β is a cytokine that has a pro-inflammatory effect by increasing proliferation of the inflammatory cells, and an anti-inflammatory effect by increasing regulatory T cells as well as reducing the differentiation of helper T cells. It has a profibrotic effect that can result in lung remodeling by increasing the proliferation and differentiation of fibroblast cells.[21]

Generally, the treatment of asthma uses corticosteroids and beta-agonists.[22,23] However, the long-term use of corticosteroids at high doses causes various side effects, such as weight changes, increased blood glucose, and mood disturbances.[23] Traditional herbal medicine has been suggested as an alternative therapy that is safe and effective in dealing with asthma.[24] Such natural plants as turmeric and mangosteen are known to treat asthma and reduce inflammation.[24,25]

Turmeric (*Curcuma longa* L.) is a herbal plant found in India, China, and Southeast Asian countries, and is often used for the treatment of asthma and flu.[26] Turmeric reduces airway inflammation by decreasing inflammatory cells, such as neutrophils, eosinophils, and monocytes.[27] Curcumin, a substance in turmeric, reduces the proliferation of Th1 and Th2 cells and the production of cytokines IL-1, IL-6, TNF-α, and TGF-β.[28] Mangosteen (*Garcinia mangostana* L.) is a tropical plant found in Southeast Asia.[29] Xanthones are active substances in mangosteen and have anti-inflammatory, anti-allergic, antioxidant, anti-bacterial, anti-fungal, and anti-tumor effects.[26,30] Mangosteen inhibits eosinophils activation and reduces oxidative stress and inflammatory mediators, such as TNF-α, IL-1, IL-6, IL-8, cyclooxygenase-2 (COX2), prostaglandin E2 (PGE2), TGF-β, while increasing apoptosis and inhibits acetylcholinesterase activity.[30] The combination of turmeric and mangosteen can produce a synergistic effect that enhances their anti-inflammatory effects.[31]

The purpose of this study is to compare the effects of single administration of turmeric and mangosteen pericarp with their combination on eosinophil count, TNF-α and TGF-β gene expression, and histopathological features in an asthmatic rat model.

Materials and Methods

Asthma Induction in Rats

Asthmatic and treatment-positive control groups were induced with three intraperitoneal (i.p.) injections of 0.2 mL ovalbumin (OVA) 10% and 100 µg of $Al(OH)_3$ as an adjuvant on days 7,14, and 21. These animals were then exposed to aerosolized 1% OVA for 30 min/day for 21 days. The animals were obtained from PT. Biofarma (Bandung, Indonesia) and kept in a temperature-controlled (20–24°C), humidity 55–60%, 12:12 light–dark cycle with light on at 07.00 a.m., and free access to food and distilled water.[32] The animals were separated into 6 cages with wood shavings as bedding material. The 3Rs (Replacement, Reduction, and Refinement) principles were used in this study. The timeline of the study can be seen in Figure 1.

Plant Materials

The turmeric ethanol extracts and mangosteen pericarp ethanol extracts were acquired from PT Industri Jamu dan Farmasi Sido Muncul Tbk (Yogyakarta, Indonesia). The extraction procedure begins by soaking dried simplicia in 70% ethanol. The resulting residue is then concentrated at a temperature of 50°C until it becomes a condensed extract. This extract is further transformed into a powdered form.

Experimental Animal Groups

Preliminary and follow-up research was conducted. The preliminary research aimed to observe whether turmeric and mangosteen peel extracts affect the eosinophils counts, TNF-α and TGF-β, and histopathological changes in the lungs of asthma model rats. In the preliminary study, there were 5 treatment groups. The animals were randomly divided using a completely randomized design (CRD) into the following groups (n=5 in each group):

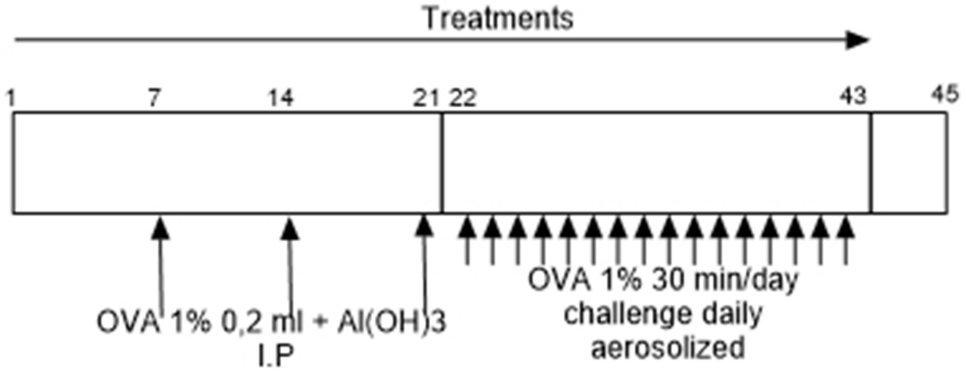


Figure 1 Timeline of the study.

N: Normal group of saline-treated rats

NC: Asthmatic rats (Ovalbumin-sensitized) as a negative control group

T: Asthmatic rats treated with turmeric ethanol extract 36 mg/200gr rats: conversion from adult dose (2g/day)

M: Asthmatic rats treated with mangosteen pericarp ethanol extract 36 mg/200gr rats: conversion from adult dose (2g/day)

PC: Asthmatic rats treated with 0.2 mL budesonide at a concentration of 0.5 mg/mL as a positive control group.

The number of animals used in this study was determined regarding Federer's formula. Federer's formula[33] (n=5 each group):

$$t(r-1) > 15$$

$$5(r-1) > 15$$

$$r > 15/5 + 1$$

$$r > 4$$

$$r \approx 5$$

Where:

t = number of treatments

r = number of replications, defined as the number of animals in each group

The follow-up study aimed to determine the effects of the turmeric and mangosteen combination at various doses and was conducted on 6 treatment groups. The animals were randomly divided using a completely randomized design (CRD) into the following groups (n=5 in each group):

N: Normal group of saline-treated rats

NC: Asthmatic rats (Ovalbumin-sensitized) as a negative control group

TM1: Asthmatic rats treated with the combination of turmeric and mangosteen pericarp ethanol extract 54 mg/200gr rats (Group TM1)

TM2: Asthmatic rats treated with the combination of turmeric and mangosteen pericarp ethanol extract 36 mg/200gr rats (Group TM2)

TM3: Asthmatic rats treated with the combination of turmeric and mangosteen pericarp ethanol extract 18 mg/200gr rats (Group TM3)

PC: Asthmatic rats treated with 0.2 mL budesonide at a concentration of 0.5 mg/mL as a positive control group.

The number of animals used in this study was determined regarding Federer's formula. Federer's formula[33] (n = 5 each group):

$$t(r-1) > 15$$

$$6(r-1) > 15$$

$$r > 15/6 + 1$$

$$r > 3.5$$

$$r \approx 4$$

with drop out 10% r = 5

where

t = number of treatments

r = number of replications, defined as the number of animals in each group.

Evaluation of Blood Eosinophils Count

On Day 43, blood samples of 1 mL were collected from the orbital sinus of the rats and stored in vials containing EDTA. The Eosinophils count was measured using a Sysmex hematology analyzer (cytochemical reaction, followed by analysis using fluorescence flow cytometry) and expressed in units of cell/mm^3.

Evaluation of TNF-α and TGF-β1 Genes Expression in Lungs

The day after blood collection, the rats were deeply anesthetized with an injection of 75 mg/kg ketamine. Total rat's lung RNA was isolated and purified according to manufacturer's protocol (My Taq One-Step RT-PCR Kit Meridian Bioscience Inc). The reaction was carried out in a Thermal Cycler (TC9639 Thermal Cycler Benchmark Scientific Inc). The primer sequence (Integrated DNA Technology, Inc.) can be seen in Table 1. DNA visualization tool (DNA blupad Bio-Helix co.) was used to visualize DNA.

Histopathological Analysis of the Lungs

The left lungs were excised and preserved in 10% buffered formalin. Following fixation, Hematoxylin and Eosin (H&E) staining was performed on cross-sectional lung slices. The samples underwent sequential dehydration, first immersed in 70% ethanol for 2 hours, followed by 80%, 90%, and 96% ethanol, each for 2 hours. Subsequently, the tissues were cleared by immersing twice in xylol for 2 hours per immersion and then embedded in paraffin after two dips, each lasting 2 hours. Sections of 5 µm thickness were prepared and mounted onto glass slides using enallene as an adhesive, with a coverslip applied. Histological observations were performed under a light microscope. Inflammatory cells in the bronchial region were quantified in three fields under 400x magnification, and inflammation was graded based on peribronchial inflammatory cell presence as follows: 0 (no inflammation), 1 (occasional inflammatory cells), 2 (a thin layer of 1–5 cells), and 3 (a thick layer with >5 cells).[34] Inflammatory scoring was conducted in three fields at 400x magnification. Additionally, bronchial smooth muscle thickness was measured using ImageJ software at five distinct points around the circumference.

Statistical Analysis

Statistical analysis was performed using SPSS (IBM SPSS Statistics for Windows, version 22, IBM Corp., Armonk, New York). The data were tested using the normality test Shapiro–Wilk, followed by Levene homogeneity test, and analyzed one-way analysis of variance (ANOVA) (parametric test) and Kruskal–Wallis (non-parametric test). The Mann–Whitney test was used to determine the significance of differences for the non-parametric test. P-values <0.05 were considered significant and P-values >0.05 were considered not significant.

Table 1 Primer Sequence for PCR (TGF-β1, TNF-α, and GAPDH)

Gene	Primer Sequence Forward 5'–3' Reverse 5'–3'	Product Size (bp)	Annealing (°C)	Cycle	References
TGF-β1	GCTAATGGTGGACCGCAACAAC	229	60	40	NM_021578.2
	CAGCAGCCGGTTACCAAG				
TNF-α	GTCGTAGCAAACCACCAAGC	187	58	40	NM_012675.3
	TGTGGGTGAGGAGCACATAG				
GAPDH	GTTACCAGGGCTGCCTTCTC	177	58	40	NM_017008.4
	GATGGTGATGGGTTTCCCGT				

Abbreviations: TGF-β1, Transforming Growth Factor-β; TNF-α, Tumor necrosis factor; GAPDH, Glyceraldehyde 3-Phosphate Dehydrogenase.

Ethics Approval and Consent to Participate

The Ethics Committee of the Faculty of Medicine at Maranatha Christian University has approved this study (022/KEP/IV/2022). This approval is based on adherence to the guidelines outlined in the 2020 Edition of the American Veterinary Medical Association (AVMA) for the Euthanasia of Animals and the Eighth Edition (2011) of the National Research Council of the National Academies' Guide for the Care and Use of Laboratory Animals.

Results

Preliminary Study

This study investigated the effects of turmeric and mangosteen pericarp ethanol extracts from Indonesia for an asthmatic rat model. Eosinophil Counts: Based on this study, Figure 2 shows a significant increase in the eosinophil counts ($p = 0.004$, $p<0.05$) in the negative control group (162 cells/mm^3) compared to the normal group (82 cells/mm^3). Additionally, there is a significant decrease in the eosinophil counts in the turmeric group ($p = 0.005$) (50 cells/mm^3), the mangosteen group ($p = 0.004$, $p<0.05$) (56 cells/mm^3), and the positive control group ($p = 0.004$, $p<0.05$) (36 cells/mm^3) compared to the negative control group.

Genes Expression: This study examined gene expression related to asthma using asthmatic rat models such as TNF-α and TGF-β1. Figure 3A shows that there is no significant difference ($p>0.05$) between the groups. The lowest average of TNF-α gene expression was in the mangosteen group, with 50.07. The negative control group had the highest average of the TNF-α expression gene, which was 56.03. Figure 3B shows that there is no significant difference ($p>0.05$) between the groups. The lowest average of TGF-β1 gene expression was in the turmeric group, which was 53.07, while the positive control had 65.17.

Lung's Histopathology: In addition to biological tests, lung histopathology was investigated in asthmatic rat models. Figure 4 shows that the histopathology of the lungs in the negative control group had more inflammatory cells in the bronchial epithelium than the normal, turmeric, mangosteen, and positive control groups.

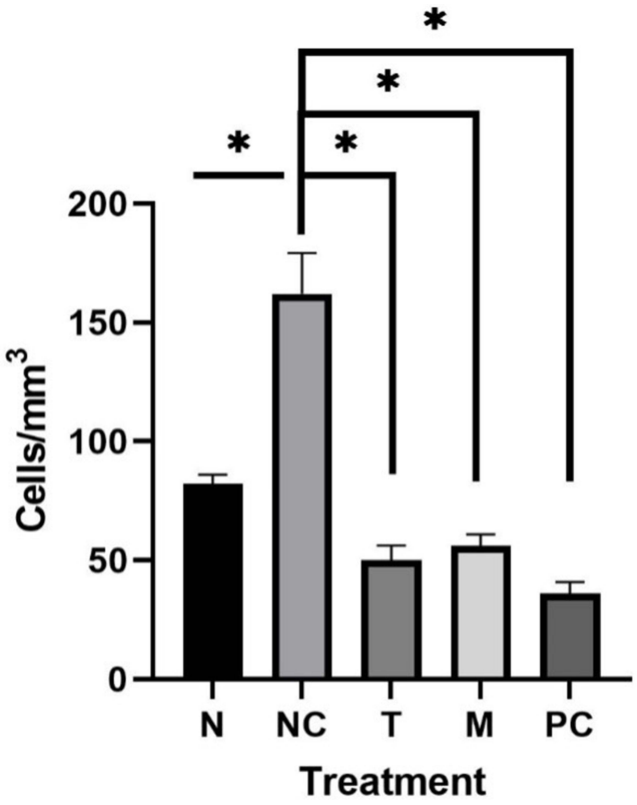


Figure 2 The effect of turmeric and mangosteen pericarp on eosinophil counts (preliminary study). The *denotes a significant difference ($p<0.05$). Treatment groups: N (normal), CN (negative control), T (turmeric), M (mangosteen), and CP (positive control).

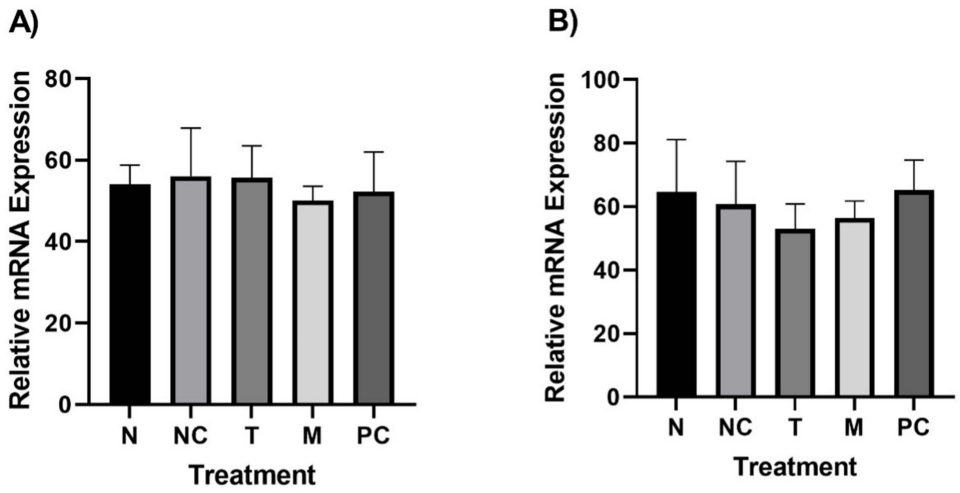


Figure 3 The effect of turmeric and mangosteen pericarp on TNF-α gene expression (**A**) and TGF-β1 (**B**) gene expression (preliminary study). Treatment Groups; N (normal), CN (negative control), T (turmeric), M (mangosteen), and CP (positive control).

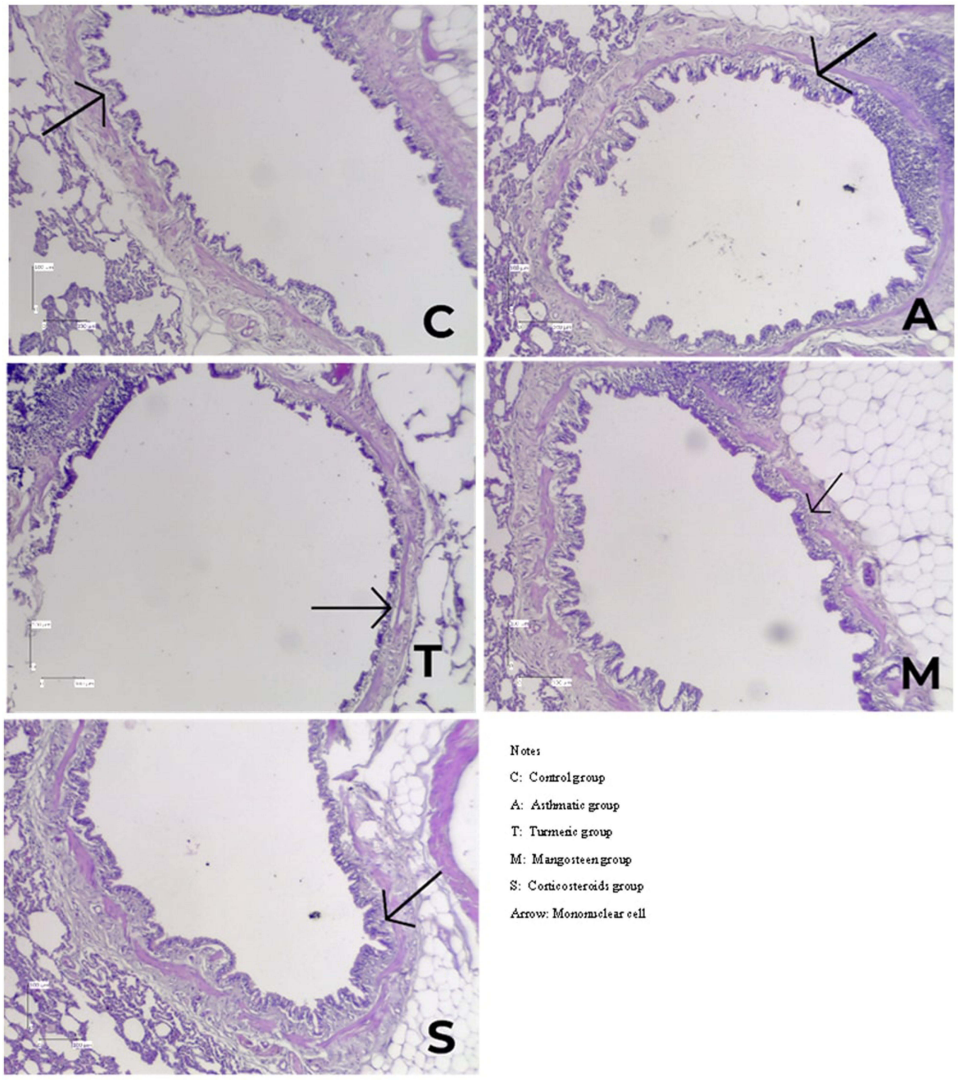


Figure 4 Histopathology of the lungs (preliminary study). The arrow shows mononuclear cells.

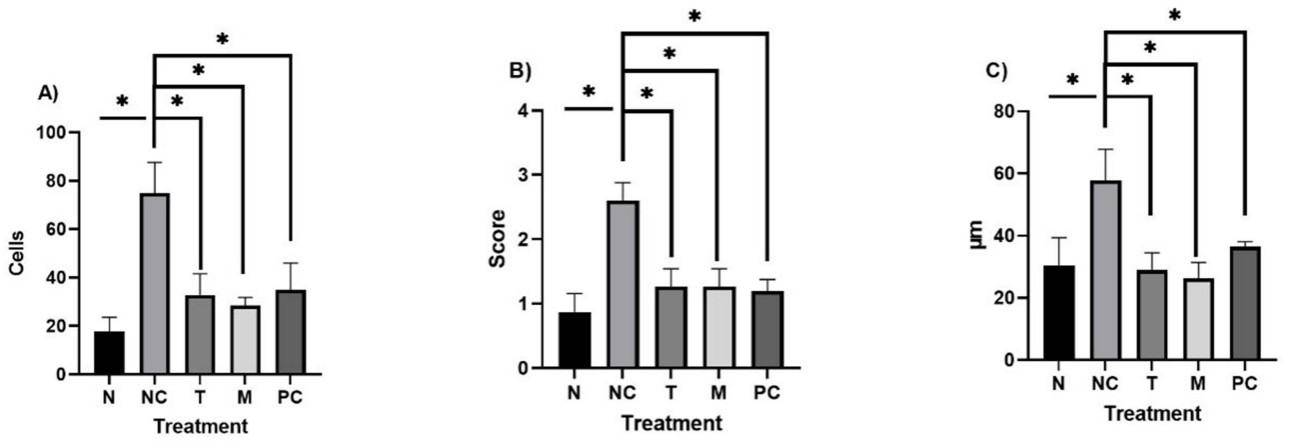


Figure 5 The effect of turmeric and mangosteen pericarp on inflammatory cell counts (**A**), bronchial inflammatory score (**B**), and bronchial smooth muscle thickness (**C**). Treatment groups: N (normal), CN (negative control), T (turmeric), M (mangosteen), and CP (positive control). The *denotes a significant difference (p<0.05).

Furthermore, Figure 5A shows a significant increase of inflammatory cells (p = 0.00, p<0.05) in the negative control group (75 cells) compared to the normal group (17.8 cells). Additionally, there is a significant decrease in the turmeric group (p = 0.00) (32.8 cells), the mangosteen group (p = 0.00, p<0.05) (28.4 cells), and the positive control group (p = 0.00) (35 cells) compared to the negative control group. Figure 5B shows a significant increase in bronchial inflammatory score (p = 0.008, p<0.05) in the negative control group (2.6) compared to the normal group (0.87). Additionally, there is a significant decrease in the turmeric group (p = 0.008, p<0.05) (1.27), the mangosteen group (p = 0.008) (1.27), and the positive control group (p = 0.008, p<0.05) (1.2) compared to the negative control group. Figure 5C shows a significant increase in bronchial smooth muscle thickness (p = 0.00, p<0.05) in the negative control group (57.82 µm) compared to the normal group (30.45 µm). Additionally, there is a significant decrease in the turmeric group (p = 0.00) (29.02 µm), the mangosteen group (p = 0.00, p<0.05) (26.30 µm), and the positive control group (p = 0.001, p<0.05) (36.52 µm) compared to the negative control group.

Follow-Up Study

This study examines the effects of the combination of ethanol extracts of turmeric and mangosteen peel at various doses on eosinophil count, TNF-α gene expression, TGF-β gene expression, and histopathological changes.

Eosinophil Counts: Based on this study, Figure 6 shows a significant increase in eosinophil counts in the negative control group (p = 0.000, p<0.05) (228 cells/mm^3) compared to the normal group (104 cells/mm^3). Additionally, there is a significant decrease in the eosinophil counts in the TM1 group (p = 0.000, p<0.05) (104 cells/mm^3), TM2 group (p = 0.000, p<0.05) (106 cells/mm^3), TM3 group (p = 0.000) (102 cells/mm^3), and positive control group (p = 0.000) (108 cells/mm^3) compared to the negative control group.

Gene Expression: Based on this study, Figure 7A shows a significant increase of TNF-α gene expression in the negative control group (0.72) compared to the normal group (0.46) (p = 0.032). Apart from the normal and negative control groups, there were no significant differences between the other groups. The highest average TNF-α gene expression was found in the TM3 group, with (0.88), while the lowest TNF-α gene expression was found in the normal group (0.46). Figure 7B shows that there is no significant difference between the normal group and the negative control group (p = 0.841, p>0.05). The highest average TGF-β gene expression (0.58) was observed in the Negative control group, while the lowest average (0.36) was found in the TM2 group.

Histopathology: Figure 8 shows that the histopathology of the lungs in the negative control group had more inflammatory cells in the bronchial epithelium than the normal, TM1, TM2, TM3, and positive control groups. Based on this study, Figure 9A shows a significant increase in the inflammatory cell count in the negative control group (59.12 cells) compared to the normal group (16.28 cells) (p = 0.000, p<0.05). Additionally, there is a significant decrease in the TM1 (21.64 cells) (p = 0.000, p<0.05), TM2 (16.04 cells) (p = 0.000, p<0.05), TM3 (12.44 cells) (p = 0.000, p<0.05),

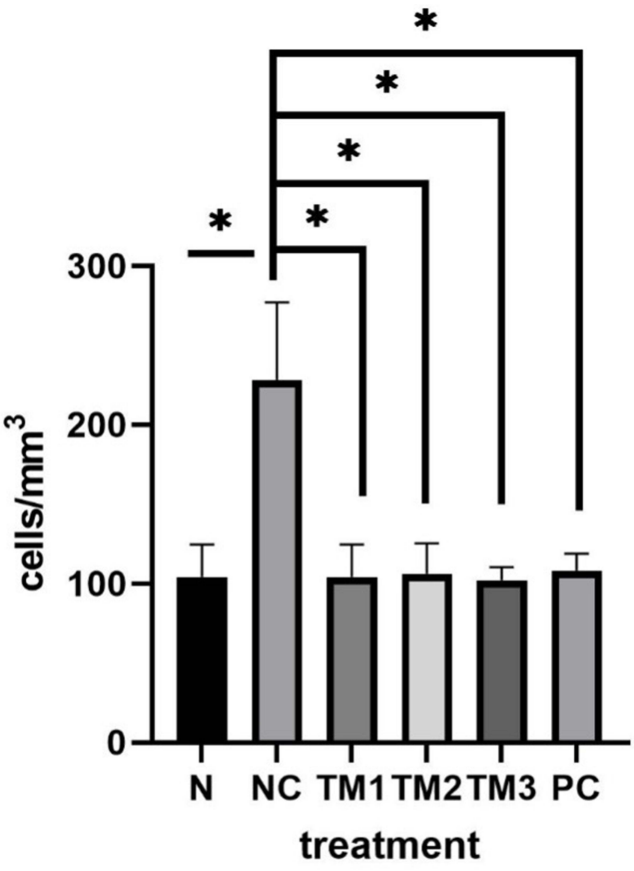


Figure 6 The effect of turmeric and mangosteen pericarp on eosinophil counts (follow-up study). The *denotes a significant difference ($p<0.05$). Treatment groups: N (normal), CN (negative control), TM1 (combination 54 mg/200gr rats), TM2 (combination 36mg/200gr rats), TM3 (combination 18mg/200gr rats), and CP (positive control).

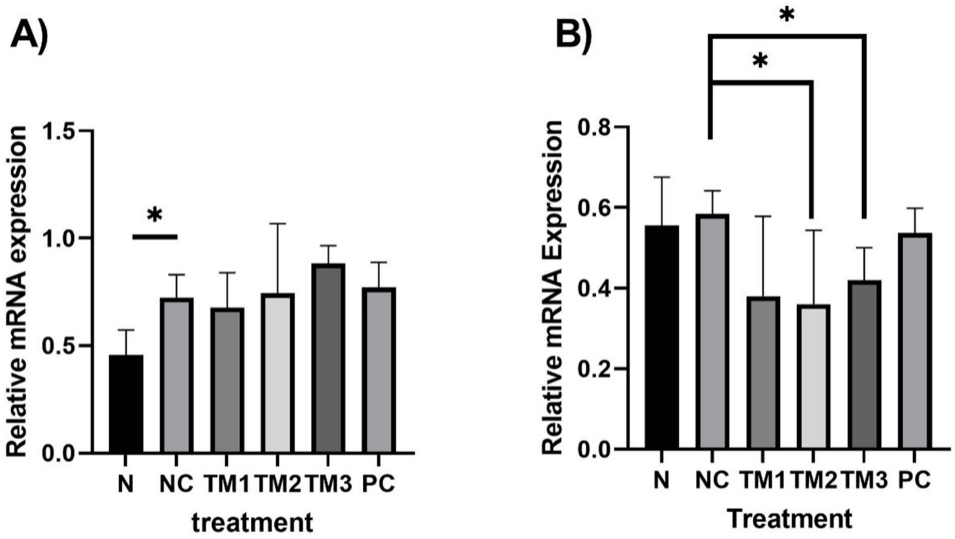


Figure 7 The effect of turmeric and mangosteen pericarp on TNF-α gene expression (**A**) and TGF-β1 gene expression (**B**) (follow-up Study). Treatment groups: N (normal), CN (negative control), TM1 (combination 54 mg/200gr rats), TM2 (combination 36mg/200gr rats), TM3 (combination 18mg/200gr rats), and CP (positive control). The *denotes a significant difference ($p<0.05$).

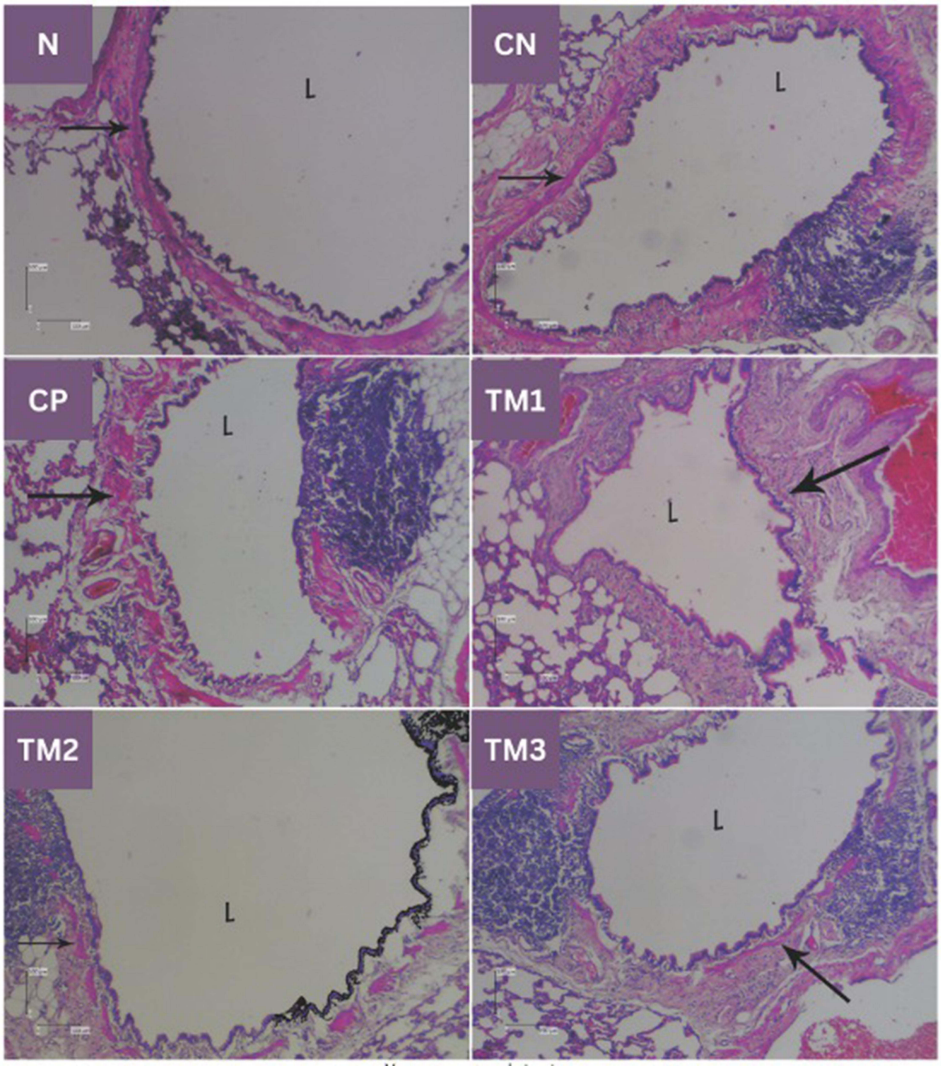


Figure 8 Histopathology of the lungs (follow-up study). The arrow shows smooth muscle.

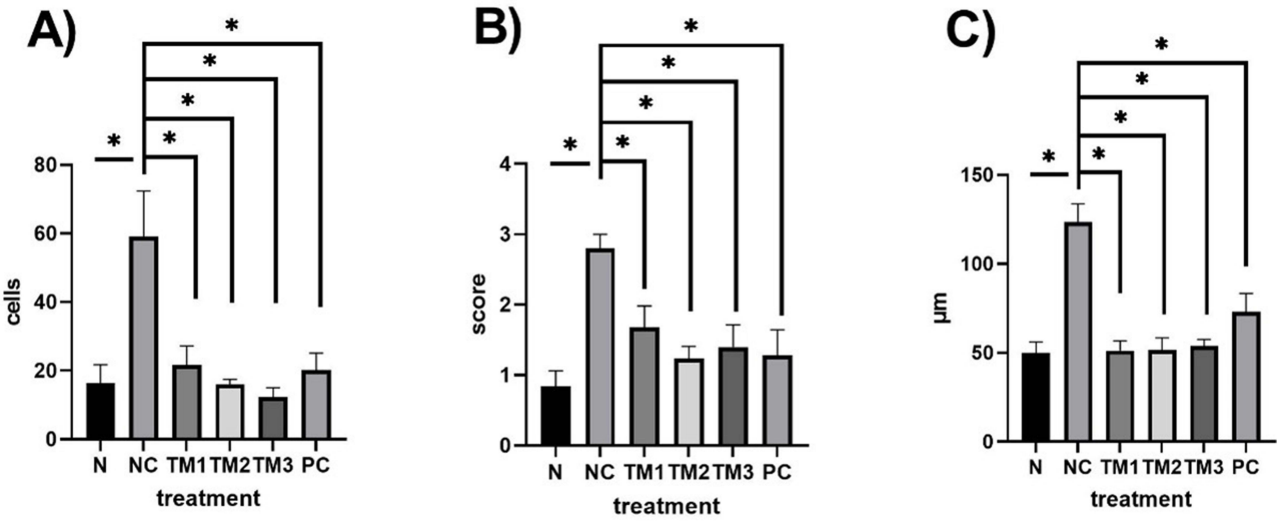


Figure 9 The effect of turmeric and mangosteen pericarp on inflammatory cell counts (**A**), bronchial inflammatory score (**B**), and bronchial smooth muscle thickness (**C**). Treatment groups: N (normal), CN (negative control), TM1 (combination 54 mg/200gr rats), TM2 (combination 36mg/200gr rats), TM3 (combination 18mg/200gr rats), and CP (positive control). The *denotes a significant difference ($p<0.05$).

and positive control groups (20.24 cells) ($p = 0.000$, $p<0.05$) compared to the negative control group. Figure 9B shows a significant increase in the bronchial inflammatory score in the negative control group (2.8) compared to the normal group (0.84) ($p = 0.008$, $p<0.05$). Additionally, there is a significant decrease in the TM1 (1.68) ($p = 0.008$. $p<0.05$), TM2 (1.24) ($p = 0.008$, $p<0.05$), TM3 (1.4) ($p = 0.008$, $p<0.05$), and positive control group (1.28) ($p = 0.008$, $p<0.05$) compared to the negative control group. Figure 9C shows a significant increase in the bronchial smooth muscle thickness in the negative control group (123.49) compared to the normal group (50.03) ($p = 0.000$, $p<0.05$). Additionally, there is a significant decrease in the TM1 (51.23) ($p = 0.000$, $p<0.05$), TM2 (51.74) ($p = 0.000$, $p<0.05$), TM3 (53.71) ($p = 0.000$, $p<0.05$), and positive control group (73.03) ($p = 0.000$. $p<0.05$) compared to the negative control group.

Discussion

In the preliminary study, based on preliminary results, a significant decrease in eosinophils was observed. Turmeric has an anti-inflammatory effect because it decreases histamine production, while increasing endogenous cortisol hormone, and improving circulation to remove toxins from the body quicker.[27] Curcumin, a substance contained in turmeric, inhibits eosinophil activation and its recruitment in the airways.[35] Mangosteen has antioxidant and anti-inflammatory effects by reducing inflammatory cells such as lymphocytes, neutrophils, eosinophils and reducing inflammatory mediators, such as TNF-α, IL-1, IL-6, IL-8, COX-2, NF-κβ, MAPK, α-SMA, TGF-β, along with improving histological damage.[30]

The results of preliminary study also showed a decrease in the average expression of the TNF-α gene in the group given turmeric and mangosteen, although it was not significant. This study's less-than-significant results in the turmeric and mangosteen groups might have been influenced by several factors, such as individual factors, inadequate dosage, more concentrated active materials, and insufficient duration of treatment.

The findings indicate a decrease in TGF-β gene expression in the groups administered turmeric and mangosteen; however, this decrease was not significantly different. The different results in this study might be influenced by multifactorial concerns, such as individual factors, inadequate dosage, more concentrated active materials, different methods, as well as insufficient duration of treatment. This study shows that turmeric ethanol extract and mangosteen ethanol extract significantly reduce eosinophil counts and inflammatory cell counts (histopathology) ($p<0.05$) but not significantly reduce TNF-α gene expression and TGF-β1 gene expression ($p>0.05$).

The result of the inflammatory cell counts and score in histopathology of preliminary study showed that there was a significant decrease in the normal group compared to the negative control group, which implies that characteristics of the asthmatic rat group were successfully induced. The inflammatory cell counts and score in the turmeric normal group were also significantly different from that of the negative control group, which implies that turmeric does effectively reduce inflammation in asthma.

The bronchial smooth muscle thickness of the preliminary study in the turmeric and mangosteen groups was significantly different from that of the negative control group. Curcumin reduces extracellular matrix (MMP-9) which is associated with the progression of airway hyperresponsiveness, inflammatory cell trafficking, and airway remodeling.[36] α-mangostin and γ-mangostin reduce inflammatory cell recruitment to the airway and airway hyperresponsiveness.[37]

The preliminary study results indicated that both turmeric extract and mangosteen extract have effects on eosinophil counts and histopathological changes. Consequently, a follow-up study was conducted using a combination of turmeric and mangosteen extracts at doses of 54 mg/200gr rats, 36 mg/200gr rats, and 18 mg/200gr rats. There was a significant increase in eosinophil results after treatment in the negative control group compared to the normal group, indicating that asthma induction with ovalbumin was successful. The TM1, TM2, and TM3 groups of rats showed significant reductions in blood eosinophil counts compared to the negative control group. The treatment groups showed comparable results to the positive control group. The findings are consistent with Boskabady's study, which showed that turmeric significantly reduced eosinophil counts in bronchoalveolar lavage in rats induced with ovalbumin.[26] Shahid's study also aligns with these findings, showing a significant decrease in eosinophil counts in a mouse asthma model treated with curcumin.[38] Jang's study reported similar results, with significant reductions in bronchoalveolar lavage eosinophil counts following the administration of α-mangostin and γ-mangostin.[39] The results are also consistent with

Higuchi's research, which showed a reduction in eosinophil counts in a mouse model of atopic dermatitis treated with ethanol extract of mangosteen peel.[40] Curcumin can inhibit the activation of the NF-κB transcription factor, which plays an important role in the inflammatory process in the airways by decreasing the proliferation and survival of immune cells.[41–43] Curcumin can balance Th1 and Th2 cell responses by suppressing the Th2 cell response through inhibition of IL-4, IL-5, and IgE.[44] IL-5 is a cytokine that plays a key role in eosinophil differentiation and activation.[13] Mangosteen has anti-inflammatory effects through the inhibition of free radicals, reduction of inflammatory cells (lymphocytes, neutrophils, eosinophils), and reduction of inflammatory mediators.[30]

The study results showed a significant increase of TNF-α gene expression in the negative control group compared to normal group. The treatment (TM2, TM3) and positive control groups experienced a decrease in TNF-α gene expression compared to group negative control, but the results were not statistically significant. These findings do not align with Shahid's study, which showed a significant decrease in TNF-α gene expression with the administration of curcumin and corticosteroids intraperitoneally in a mouse model of asthma induced with ovalbumin.[38] The findings also contrast with Shakeri's study, which reported a significant reduction in TNF-α gene expression in the group treated with curcumin compared to the asthma-induced group in a mouse model of asthma induced with ovalbumin.[45] Curcumin contained in turmeric can reduce pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α.[46] Curcumin inhibits TNF-α synthesis through the inhibition of acetyltransferase, leading to transcription factor suppression.[47] The TNF-α gene expression results in this study do not align with Tewtrakul's findings, which indicated that mangostin can moderately inhibit TNF-α in RAW264.7 macrophage cells.[48] Mangosteen has antioxidant effects that can reduce free radicals and are associated with reductions in pro-inflammatory cytokines such as TNF-α and IL-6.[49] The lack of significant results in this study regarding the effect of turmeric and mangosteen peel ethanol extracts on TNF-α may be influenced by several factors such as individual variation, inadequate concentration of active compounds, and insufficient treatment duration.

The results showed no difference between the negative control group and normal group. The lack of significant results may be due to factors such as inadequate drug dosage, insufficient frequency of drug administration, and individual variation. The TGF-β gene expression in the TM1, TM2, and TM3 groups showed a significant decrease compared to the negative normal group induced with asthma. This significant result does not fully address the research objective because a difference between the normal and negative control groups is needed to demonstrate the effect of the test substances on the asthma model rats. Overall, the average TGF-β gene expression in the groups treated with turmeric and mangosteen peel decreased compared to the negative normal group. These findings do not align with Shahid's study, which showed a significant decrease in gene expression with curcumin administration in a mouse asthma model.[38] The findings also do not align with Ammar's study, which reported a significant reduction in TGF-β gene expression in an asthma model treated with curcumin.[50] Jang's study conclusions also differ from this research, as they reported a significant reduction in TGF-β with the administration of α-mangostin and γ-mangostin in an asthma model.[39] TGF-β plays a role in cellular responses, differentiation, apoptosis, and cell proliferation.[51] TGF-β, a profibrogenic cytokine, is involved in asthma remodeling.[52] Curcumin, the active compound in turmeric, can reduce pro-inflammatory cytokines like TNF-α, TGF-β, and IL-6 due to its antioxidant effects and its ability to inhibit transcription factors.[53] γ-mangostin can inhibit NF-κB, and α-mangostin can inhibit PI3K activity, which plays a role in TGF-β regulation.[39]

There was a significant increase in inflammatory cell counts and inflammatory score on histopathology results after treatment in the Negative control group compared to the Normal group. The TM1, TM2 and TM3 groups of rats showed significant reductions in inflammatory cell counts and inflammatory score compared to the Negative control group. These results are consistent with Sharma's study, which showed a reduction in inflammatory cells (eosinophils and lymphocytes) after curcumin administration in the lungs of a mouse model of allergy induced by ovalbumin.[54] The combination of curcumin and dexamethasone administered intranasally can reduce inflammatory cells in the airways, prevent lung structural changes, and reduce the expression of TLR-4, NF-κB, NOD-like receptor pyrin domain containing 3 (NLRP3), IL-1, and IL-17 mRNA in a mouse model of asthma induced by ovalbumin.[55] Yang's study is consistent with this research, showing a reduction in inflammatory cell infiltration in a rat model of acute lung injury

treated with α-mangostin.[56] Flavonoid and xanthone components have the potential as anti-inflammatory agents, which can reduce inflammatory cell infiltration.[57]

The limitations of the study include individual variations and the short duration of the treatment, and the lack of perspective through other parameters. For future research, studies on the effects of turmeric and mangosteen on IL-4, IL-6, IL-13, and other inflammatory parameters are needed.

Conclusion

The administration of single ethanol extracts of turmeric and mangosteen significantly decreased eosinophils and improved the histopathological features of the lungs (inflammatory cell counts, bronchial inflammatory score, and bronchial smooth muscle thickness). The combination of turmeric and mangosteen extracts at all doses significantly decreased eosinophils and improved the histopathological features of the lungs (inflammatory cell counts, bronchial inflammatory score, and bronchial smooth muscle thickness). Both the single and combined administration of ethanol extracts of turmeric and mangosteen did not cause significant changes in TNFα and TGF-β.

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Disclosure

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