

Research Article

Antiasthma Activities of *Moringa oleifera* Lam. Leaves Extract on the Eosinophil Count and Mast Cells in BALB/c Mice

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*Moringa oleifera***Abstract**

Asthma is a chronic inflammation of the respiratory tract. This study aimed to prove *Moringa oleifera* Lam. leaves extract's effect on reducing the eosinophil count and mast cells in asthmatic mice. Twenty BALB/c mice that met the inclusion criteria were divided into four groups of standard control (K1), negative control (K2), dexamethasone (K3), and *M. oleifera* leaves extract (K4). On days 0 and 7, intraperitoneal ovalbumin (OVA) was sensitized. On days 14, 16, and 18, mice were re-sensitized by inhalation using 1% OVA in sterile 0.9% NaCl. On days 18 to 25, K1 and K2 groups were given Na-CMC, the K3 group was given dexamethasone 1.3 µg/day, and the K4 group was given *M. oleifera* leaves extract 3.9 mg/day. On the 25th day, the mice were terminated to analyze the eosinophil count and stable bronchiolar mast cells. In conclusion, *M. oleifera* leaves extract was proven to decrease the eosinophil count with a p-value <0.05 and could stabilize bronchiolar mast cells with a p-value <0.05.

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INTRODUCTION

Asthma is a disease with an increasing prevalence rate, and there is a strong interaction between airway epithelial cells and the immune system against the pathogenesis of asthma¹. The most common immunopathology in asthma is inflammation of type 2 characterized by processes in the airway epithelium involving cytokines². Symptoms of asthma include wheezing, shortness of breath, heavy chest, and coughing at night and early morning. There is muscle contraction in the bronchial walls, swelling of the bronchial mucosa, and increased mucus production in the secretory cells of the bronchial mucosa, resulting in increased resistance to airflow through the narrowed bronchi. This condition occurs in shortness of breath^{3,4}. The increase in the eosinophil count in asthmatic patients

correlated with the severity of the respiratory failure and was predicted to increase the risk of sequential sub-exacerbations. Immunoglobulin E (IgE) is involved early in the inflammatory cascade and may be considered a cause of allergic asthma^{5,6}.

Moringa oleifera Lam. is considered a miracle plant with many benefits for humans and is credible for providing good nutrition and healing and preventing many diseases⁷. The pharmacologically reported effects include antibacterial, antifungal, antiviral, anti-inflammatory and analgesic, antioxidant, hypotensive, antiulcer, cardioprotective anesthetic, antiurolithic, and wound healing activity⁸⁻¹². The *M. oleifera* plant has various therapeutic properties. For example, the general nutritional content of *M. oleifera* to several specific properties, including anti-inflammatory, antimicrobial,

antihyperglycemic, antioxidant and anticancer properties¹³. The secondary metabolites of *M. oleifera* leaves are alkaloids, saponins, phenolics, tannins, flavonoids, and steroids¹⁴. Oral administration of *M. oleifera* aqueous extract has been shown to reduce symptoms of type I allergy in ovalbumin-induced rats. Aqueous extract of *M. oleifera* altered the T helper (Th)1/Th2 balance towards Th1 dominance in the allergy model in mice, resulting in suppression of mast cell activation, followed by histamine release¹⁵.

Treatment decisions must consider the biological background in this case of inflammation as they tend to predict the patient's response to asthma treatment¹⁶. Agrawal and Mehta¹⁷ studied fine powder dry seeds of 3 g on *M. oleifera* seeds for three weeks showing a significant increase in forced vital capacity, forced expiratory volume in one second, and peak expiratory flow rate values in patient subjects. *Moringa oleifera* leaves extract was proven to decrease the eosinophil count and could stabilize bronchiolar mast cells¹⁸. However, research on the anti-plasma activity of *M. oleifera* leaves extract reduces the eosinophil count, and the stability of the number of mast cells in asthmatic mice has not been reported. Therefore, this study aims to prove the effect of *M. oleifera* leaves extract on reducing eosinophils and mast cells in asthmatic mice.

MATERIALS AND METHODS

Materials

The materials used in this study were *M. oleifera* Lam. leaves extract (Deltomed Laboratories, Indonesia), dexamethasone (Cayman Chemical, US), ovalbumin (OVA) (Worthington Biochemical Corporation, US), and alhydrogel® (InvivoGen, US). The main instruments used in this study were electronic nebulizer, automated hematology analyzer XS1000i/800i (Sysmex, Japan), and Olympus digital microscope OptiLab Pro 6.1. The

software used for data analysis was IBM SPSS Statistics ®16.0.

Methods

Preparation of test animals

The test animals used were female BALB/c mice weighing 20-30 g, which has been acclimatized for a week. All test animals were given drink and standard feed ad libitum. Female BALB/c mice were used because they showed a better response to OVA than males¹⁹.

Research design

This study used a laboratory experimental quantitative approach with a post-test only control group design in asthmatic mice *in vivo*. The test group was divided into four groups: standard control (K1), negative control (K2), positive control (K3), and *M. oleifera* leaves extract group (K4). The treatment dose for *M. oleifera* leaves extract produced by Deltomed Laboratories was 1500 mg/day, with the conversion dose for mice was $1500 \text{ mg} \times 0.0026 = 3.9 \text{ mg/day}$. For the standard control group, BALB/c mice were given sterile 0.9% NaCl by intraperitoneal injection on days 0 and 7. On days 14, 16, and 18, inhalation was carried out with sterile 0.9% NaCl as much as 8 mL per treatment by inhalation using an electronic nebulizer for 20 minutes with airflow volume and volume nebulization on a scale of 1 on day 1. On days 18, 19, 20, 21, 22, 23, and 24, mice were treated with 0.5% Na-CMC orally 1 mL/day. For K2, K3, and K4 groups, all BALB/c mice have sensitized with 10 µg OVA + 1 mg alhydrogel® suspended in 0.5 mL of 0.9% sterile NaCl by intraperitoneal injection on days 0 and 7. On days 14, 16, and 18, inhalation was carried out with sterile 0.9% NaCl as much as 8 mL per treatment by inhalation using an electronic nebulizer for 20 minutes with airflow volume and volume nebulization on a scale of 1 on day 1. On days 18, 19, 20, 21, 22, 23, and 24, mice were treated with 0.5% Na-CMC orally 1 mL/day (K2), 1.3 µg dexamethasone orally 1 mL/day (K3), and 3.9 mg

M. oleifera leaves extract orally 1 mL/day (K4). The research design schematic was shown in Figure 1.

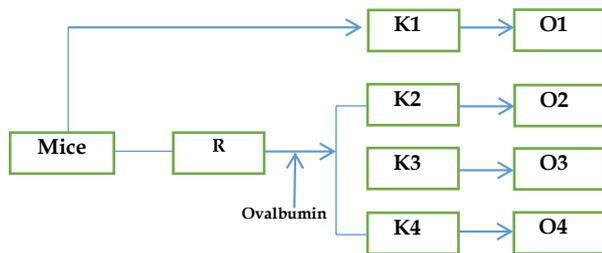


Figure 1. Research design. R: Randomization; O1-O4: Observation of the eosinophil count and bronchial mast cells

Examination of eosinophil count

Blood sampling was performed to the anesthetized mice by an intraperitoneal injection of ketamine 200 µg/g. The blood collection location was in the retro-orbital sinus/eye of mice using a hematocrit capillary pipette. The application could be made by inserting the pipette at an angle of 45°. This method could produce large volumes of blood, and samples could be obtained in both eyes alternately, accommodated in the EDTA blood tube²⁰. The examination of the blood eosinophils count was the eosinophils cell count x 10⁹/L of blood plasma, calculated by an automated hematology analyzer. This variable size scale was a ratio. Data analysis was performed using one-way ANOVA and analyzed by post hoc test to determine the difference between the two treatment groups. Data were presented as average ± SD. Significance was defined at the p <0.05 level.

Examination of bronchiolar mast cells

Mast cell examination was carried out by histopathology of the mice's lungs with Toluidine blue staining and observed with a 400x magnification software microscope Image OptiLab Pro 6.1²¹. This variable size scale was a ratio. Data analysis was performed using one-way ANOVA and then analyzed by post hoc test to determine the difference between the two treatment groups. Data were presented as average ± SD. Significance was defined at the p <0.05 level.

Ethical considerations

This research was conducted after obtaining approval from the health research biotech commission of the Faculty of Medicine, Universitas Islam Sultan Agung Semarang with Ethical Clearance No. 110/IV/2020/Bioethical Commission.

RESULTS AND DISCUSSION

Normality and homogeneity analysis of the eosinophil count and mast cells

The data normality test for the eosinophil count was performed with Shapiro-Wilk, with a p-value >0.05, indicating that the data were normally distributed as presented in Table I. As for the homogeneity of the data, the post hoc test results were obtained with a p-value >0.05, indicating that the data obtained for the eosinophil count and mast cells were homogeneous, as shown in Table II.

Table I. Normality of data with Shapiro-Wilk test

Groups	Variable	
	Eosinophil count (cell count x 10 ⁹ /L)	Mast cells
K1	0.777	0.119
K2	0.429	0.325
K3	0.697	0.119
K4	0.427	0.314

Table II. Data homogeneity by post hoc test

Parameter	Variable	
	Eosinophil count	Mast cells
p-value	0.117	0.509

Examination of eosinophil count

The statistical analysis of the test results for the average, SD, F value, and p-value of the eosinophil count in all groups were presented in Table III. The K2 had the highest average value of all other groups. This finding indicates that the high average value of eosinophils in the K2 could be reduced by administering dexamethasone or *M. oleifera* leaves extract. The average difference between the two groups analyzed by post hoc test showed that the average eosinophil count between the K2 was 2±0.71 x

$10^9/L$, and the K3 was $0.4 \pm 0.11 \times 10^9/L$, with a p-value of 0.002 indicate that there was a significant difference between K2 and K3. The average difference in the K3 was $0.4 \pm 0.11 \times 10^9/L$, and the K4 was $0.48 \pm 0.15 \times 10^9/L$, with a p-value of 0.503, suggesting no statistically significant difference between the K3 and K4 groups in reducing the eosinophil count. The analysis results of the average difference above resulted in an F value of 6.773 and a p-value of 0.001, suggesting that the eosinophil count was significantly different between each study group.

Eosinophils are promoters of type-2 inflammatory environment, which contribute to airway renovation in asthma, wherein eosinophils can increase airway hyperresponsiveness (AHR) and mucus hypersecretion^{22,23}. *Moringa oleifera* seed oil is proven to contain flavonoid compounds that have biological activity as anti-asthma by reducing the thickness of the bronchial epithelium of asthmatic mice²⁴. The subchronic toxicity effect of *M. oleifera* leaves extract based on liver histopathology shows mild reversible damage, and kidney histopathology shows that the renal filtration function of all treatment groups is still expected²⁵. *Moringa oleifera* leaves contain phenolic compounds that contribute to higher antioxidant activity. They can be used as a potential source of natural antioxidants in pharmaceuticals to improve the function of the endogenous antioxidant system and help reduce free radical levels in the body²⁶. Eosinophils contribute to asthma severity and may persist despite guideline-based treatment²⁷. The characteristic picture of inflammation is characterized by an increase in the number of activated eosinophils causing damage to the airway epithelium²⁸.

Table III. Average, SD, F value, and p-value of eosinophils count

Eosinophil count (cells $\times 10^9$)	Group			
	K1	K2	K3	K4
Average	0.38	0.9	0.4	0.48
SD	0.14	0.3	0.11	0.15
F value	6.773			
p-value	0.001			

Examination of bronchiolar mast cells

The test results for the average, SD, F value, and p-value of the mast cells in groups K1, K2, K3, and K4 were presented in **Table IV**. The analysis of the average difference above resulted in an F value of 10.062 and a p-value of 0.001, which means that the number of mast cells was significantly different between each study group. The K3 had the highest average number of stable mast cells than the K2 and K4.

The average difference between the two groups analyzed by post hoc test showed that the average number of mast cells that were stable between the K2 was 2 ± 0.71 and the K3 was 4 ± 1 , with a p-value of 0.003, indicates that there was a significant difference between the K2 and K3 groups. It could be assumed that dexamethasone could stabilize mast cells. The average difference between the K3 was 4 ± 1 , while the K4 was 3.2 ± 0.84 , with a p-value of 0.176, which means that statistically, there was no significant difference between the K3 and K4 groups in stabilizing mast cells.

Table IV. Average, SD, F value, and p-value of mast cells

Mast cells	Group			
	K1	K2	K3	K4
Average	5	2	4	3.2
SD	1	0.71	1	0.84
F value	10.062			
p-value	0.001			

The analysis of the number of stable bronchial mast cells in all groups was carried out by taking pulmonary organs. The histopathological test was carried out with Toluidine Blue staining, observed microscopically with a magnification of 400x. The histopathological picture of stabilizer mast cells was presented in **Figure 2**. In the lungs of patients with allergic asthma, mast cells accumulate in smooth muscle, bronchial epithelium, and alveolar parenchyma. This unique location of pulmonary mast cells can be replicated using a mouse model of allergic asthma²⁹. Mast cells migrate to inflamed tissue and release pro-inflammatory mediators by

degranulation upon activation of cell surface receptors such as high-affinity IgE receptor (FcεRI), prostaglandin E2 receptor (PGE2), or receptors for stem cell factor in human and mouse mast cells³⁰.

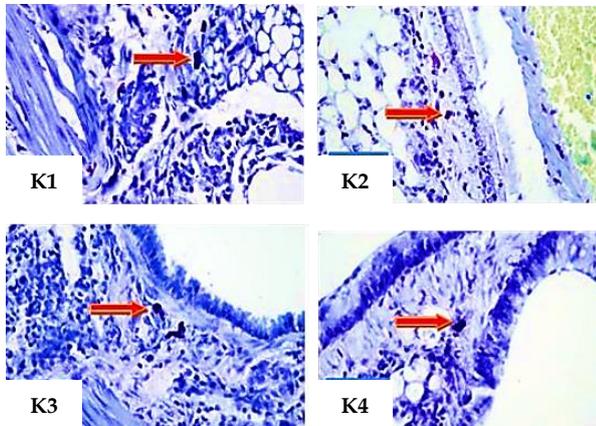


Figure 2. Comparison of stable mast cells in the standard control group (K1), negative control group (K2), positive control group (K3), and *Moringa oleifera* leaves extract group (K4), with toluidine blue staining and 400x magnification. Red arrows indicate stable mast cells

This study shows that *M. oleifera* leaves could reduce the eosinophil count and stabilize mast cells; in line with the research of Suresh *et al.*³¹, which reported that the methanol extract of *M. oleifera* leaves given to guinea pigs shows anti-plasma activity such as blocking the release of inflammatory mediators to local lung tissue and bronchospasm, mast cell degranulation, immune reactions, and anaphylactic reactions. This was found to inhibit the inflammatory mediator, histamine. *Moringa oleifera* extract can inhibit the release of β -hexosaminidase, histamine, and tumor necrosis factor (TNF)- α more actively than ketotifen fumarate. *Moringa oleifera* leaves has mast cell stabilizing activity and its potential to inhibit the final phase of the allergic response³².

CONCLUSION

Moringa oleifera Lam. leaves extract has been shown to reduce the eosinophil count and can stabilize the number of mast cells in asthmatic mice. There was no statistically significant difference between *M. oleifera* leaves extract.

Compared with positive controls given dexamethasone in reducing the eosinophil count, the p-value was 0.503, and there was no statistically significant difference between *M. oleifera* leaves extract. While compared with positive controls (dexamethasone) to stabilize mast cells, the p-value was 0.176.

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AUTHORS' CONTRIBUTION

Dian Arsanti Palupi: conceptualization, supervision, methodology, data curation, data analysis, validation, writing-original draft & editing. **Tri Wahyuni Prasetyowati:** conceptualization, supervision, data analysis, writing-review & editing. **Dwi Murtiningsih:** conceptualization, methodology, data curation, data analysis, writing-review & editing. **Dede Mahdiyah:** project administration, data curation, data analysis, writing-review & editing.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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