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Reseacrh Article

Effect of Leuhang Therapy on Lung Histopathology, Leukocytes, and Bronchoalveolar Lavage Fluid (BALF) in Asthma Rat Model

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ABSTRACT

The prevalence of asthma is increasing, especially in developing countries due to lifestyle and air pollution. In addition, asthma medications can cause serious side effects, such as the use of the asthma medication LABA, which causes the side effects of tachycardia, tremor, and epicardial elevation, which is considered quite dangerous for the elderly. Sundanese people from West Java have a traditional therapy called Leuhang therapy. This therapy is believed to have various properties, including anti-asthma. In addition, this therapy is also safer and has minimal side effects. This study aims to obtain an optimal Leuhang therapy formula for preventing asthma recurrence and determine the relationship between plant extract vapor in Leuhang and its effects on the organs of asthma-induced mice. The study was conducted by dividing the mice into several groups, namely normal control, positive control, comparison, test I, and test II. Each mouse was given treatment according to its respective group. Based on the results obtained from this study, leuhang therapy with a combination of formulas focusing on anti-asthmatic properties showed anti-asthmatic activity against OVA-induced allergic asthma. The combination treatment decreased the total and differential cell counts in blood and BALF and affected the thickness of mouse lung mucosa and muscle, thus demonstrating its anti-inflammatory properties. Leuhang therapy also reduced the infiltration of neutrophils, lymphocytes, and plasma, showing its immunomodulatory properties. Statistical tests using One-Way ANOVA showed a p-value of <0.05, meaning that the four groups' mean lung histopathology scores were significantly different. Thus, Leuhang therapy has been shown to have promising potential in the alternative treatment of asthma.

Keywords: anti-asthma; asthma; histopathology; leuhang; lung; traditional

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INTRODUCTION

Asthma is a chronic airway disease associated with airway narrowing due to specific triggers such as allergic (dust, mites, animal dander, and pollen) and non-allergic (viral infections and cold air) stimuli characterized by recurrent episodic symptoms such as wheezing, shortness of breath, chest tightness, and coughing (Quirt et al. 2018). Based on data from the 2018 Indonesian Basic Health Research, asthma in Indonesia can occur at all ages, and the highest number of asthma sufferers are elderly patients, 4.5-5.1% (Kementerian Kesehatan RI 2019). Asthma is a non-communicable disease, but it is a disease with high morbidity and mortality and is classified as a severe case (Vennera, Sabadell, and Picado 2018).

Based on the Global Initiative for Asthma (GINA) guidelines, corticosteroid and long-acting beta agonist (LABA) drugs are the first line in managing asthma. However, there is some evidence of adverse side effects from the use of corticosteroids, such as slowing growth in children, risk of osteoporosis, and risk of fractures in adults, especially the elderly (Marques and Vale 2022). In addition, the use of LABA drugs can also cause significant side effects such as tachycardia, tremors, and epicardial in the elderly (Billington, Penn, and Hall 2015).

The Sundanese community in West Java has a traditional therapy called Leuhang therapy. Leuhang therapy utilizes steam from the decoction of spice plants that can empirically overcome various diseases. The ingredients used in Leuhang therapy are betel leaves (*Piper betle*), bay leaves (*Syzygium polyanthum*), lime leaves (*Citrus aurantiifolia*), clove leaves (*Syzygium aromaticum*), pandan leaves (*Pandanus ammaryllifolius*), ginger (*Zingiber officinale*), turmeric (*Curcuma domestica* Vall), galangal (*Alpinia galanga*), and Himalayan salt. Based on interviews with Leuhang therapy providers in Bandung, Leuhang therapy can nourish the lungs and prevent asthma recurrence with 14 days of therapy. However, these claims have not been scientifically supported, so further research is needed to prove the effectiveness of Leuhang therapy.

Based on these problems, it is necessary to test the effectiveness of Leuhang therapy in preventing asthma recurrence. However, its effectiveness in treating asthma through inhalation or steam therapy has not yet been established. Therefore, an inhalation test method was conducted that was adapted to the actual conditions during Leuhang therapy. Therefore, the formulation of the problem in this study is the effectiveness of Leuhang therapy on the morphology of leukocyte cell standards, the number of leukocytes in BALF, and histopathology in asthma-induced rats.

MATERIALS AND METHODS

Materials

Bay leaf, lime leaf, betel leaf, fragrant pandan leaf, fresh slices of red ginger, fresh slices of turmeric, and fresh slices of galangal. 0.9% NaCl, phosphate-buffered saline (PBS), distilled water, budesonide nebules, egg white, aluminum hydroxide, formalin, Giemsa dye, methylene blue dye, 1 mL syringe, micropipette tip, alcohol swab, 70% ethanol, 98% methanol, and centrifuge tube.

Methods

Preparation of Extract

In formula I, 50 grams of each simplisia were boiled in a pressure cooker containing 5 L of distilled water for 30 minutes. Meanwhile, in formula II, 0.02 mg of Himalayan salt was added.

Animals

Healthy Wistar rats with a weight range of 180-200 g were procured from the animal laboratory of SITH ITB. The animals were fed standard food pellets and drank water ad libitum. Before starting the experiment, the animals were acclimatized for 5 days. All experimental protocols were reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Bandung Islamic University. Approval number 038/KEPK-Unisba/VI/2024.

Induction of Asthma

The sensitization and aerosol challenge procedures were performed according to the reported procedures with some minor changes. The negative control group was sensitized by i.p. injection of normal saline (1 mL/200 g rat body weight). Except for the negative control group, all other groups were sensitized by intraperitoneal (i.p.) injection of 100 mcg OVA and 4 mg. Sensitization was performed on days 0, 7, and 14. On day 8, animals were injected subcutaneously (SC) with an aluminum-OVA injection in all mentioned groups except the negative control. Inhalation administration of the test preparation was given for 14 days according to each group. The control group was given normal saline (1 mL/200 g rat body weight). The standard group was given budesonide 0.5 mg/10 mL distilled water. Formula group I was given 10 mL extract, and formula group II was given 10 mL extract with 0.02 mg Himalayan salt. The preparation was first saturated in the container for 15 minutes, then the rats were put into the container and given the test preparation for 15 minutes. On day 28, OVA was administered by inhalation for 10 minutes, except for the normal control group, which was administered saline by inhalation. On day 29, the animals were dissected for blood

and Bronchoalveolar Lavage (BAL) fluid for biochemical evaluation (Nair and Prabhavalkar 2021).

Determination of Cell Count in Blood

A 1 mL blood sample was taken from the heart and put into a tube. Blood was dripped on a microscope slide into a blood smear, then 1 drop of methanol (3 minutes) and 1 drop of Giemsa solution (20 minutes) were added. Then, it was flushed with distilled water (2x1 min) and dried for 3 min. The preparations were prepared for morphology and cell count analysis.

Collection and Analysis of Bronchoalveolar Lavage Fluid (BALF)

PBS (0.5 mL) was introduced into the lungs and BALF (triplo) via tracheal cannulation. Each BALF was centrifuged, and the supernatant was taken. The supernatant was taken as 0.2 mL, and 1 drop of methylene blue was added and diluted with PBS to 1 mL. BALF samples were taken as much as 50 µL in the hemocytometer, and then live cells and dead cells were observed in four hemocytometer boxes. BALF samples were also taken at 20 µL on a microscope slide, then analyzed for the count of eosinophils and basophils (Nair and Prabhavalkar 2021).

Calculation of Rat Lung Organ Index

Organ index observations were made by analyzing the lung organs of the test animals. The weight of animal organs was weighed and compared with body weight to obtain the organ index. The organ index of the test dose group was compared with that of the control group and the standard group.

$$\text{Organ Index} = \frac{organ\ weight\ (gr)}{mouse\ body\ weight\ (gr)} \text{ x } 100$$

Histopathological Examination

In the histopathological examination, lung tissues were cut and put into tissue cassettes, then immersed in formalin for 3 days. The tissues were immersed in 70%, 80%, and 96% alcohol; ethanol (duplo); ethanol: xylene (1:1); xylene (duplo); and liquid paraffin (duplo), each for 30 minutes. Then, paraffin blocks were made by taking a mold, tissues were inserted, and liquid paraffin was added and allowed to harden. The finished paraffin blocks were cut with a microtome and stained with hematoxylin-eosin (HE). The stained preparations were dipped in xylene solution and dripped with a few drops of entelan, then the preparations were covered with a cover glass and dried.

Statistical Analysis

The data obtained were tested for normality and statistically analyzed by the ANOVA (one-way analysis of variance) method and continued with the LSD post-hoc method for parametric data. The presence of differences between groups was expressed by a significant value of $p < 0.05$ at the 95% confidence level.

RESULTS

Morphology of Leukocyte Cells in Blood

After dissection, blood cells were collected from the hearts of each group (Figure 1), then smears were made using glass slides and a microscope with 400x magnification.

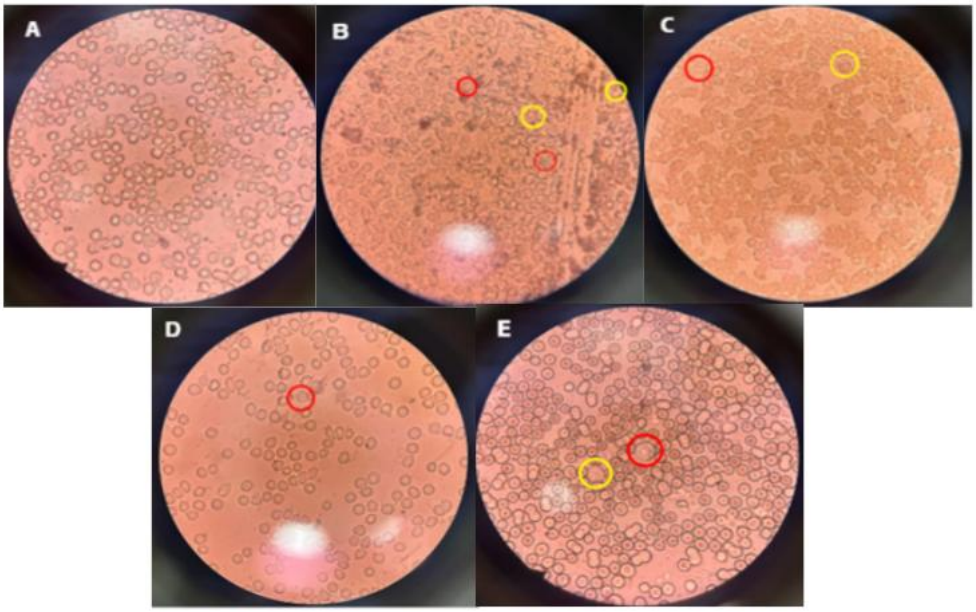

Figure 1. Cell Morphology on Blood Smear (400x magnification)
Information red circles = Eosinophils; Yellow circles = Basophils. (a) Normal control group, (b) Positive control group, (c) Comparison group, (d) Formula I, (E) Formula II.

The results showed that in the normal control group no eosinophils and basophils were found, but they were found in the positive control group. In the comparison group, few basophils were found. Then, in the formula I group, only eosinophils were found, and in the formula II group, samples of basophils and eosinophils were still found.

Collection and Analysis of Leukocytes Bronchoalveolar Lavage Fluid (BALF)

Then, BALF fluid was collected from the throat of each group (Figure 2) and examined using a microscope with 1000x magnification. The results of the study showed the presence of eosinophils and basophils in small amounts in normal

controls, while basophils were found in positive controls. Then, in the comparison group, formula I and formula II, there were eosinophils and basophils.

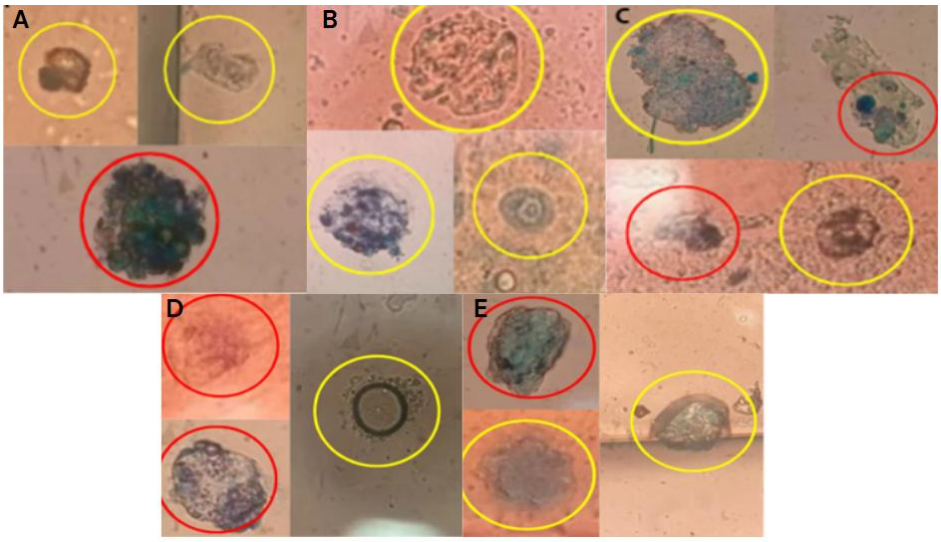

Figure 2. Morphology of BALF test animals (1000x magnification)
Information red circles = Eosinophils; Yellow circles = Basophils. (a) Normal control group, (b) Positive control group, (c) Comparison group, (d) Formula I, (E) Formula II.

Bronchoalveolar Lavage Fluid (BALF) in Hemocytometer

Then, BALF fluid was collected from the throat of each group (Figure 3) and examined using a hemocytometer on a microscope with 400x magnification. The results showed that there were small numbers of live and dead cells in all groups except the positive control.

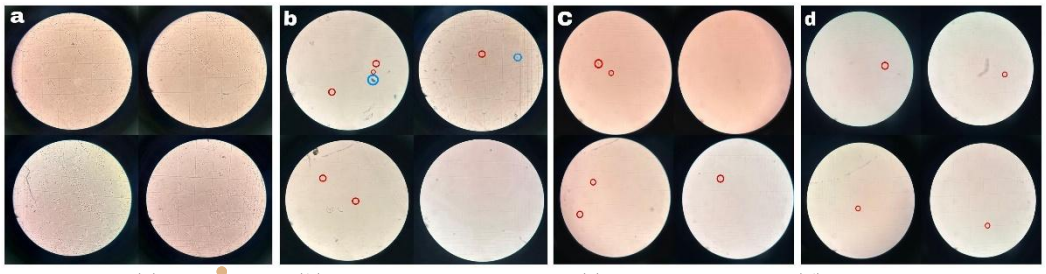

Figure 3. BALF Analysis on a Hemocytometer (400x magnification)
(a) positive control, (b) Standart, (C) Formula I, (E) Formula II.

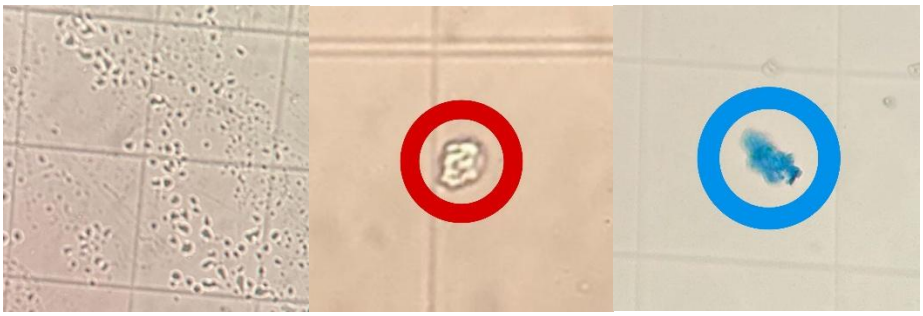

Figure 4. Zoomed View a Pile of Leukocyte in the Positive Control
(Red) alive cell, & (Blue) dead cells.

Calculation of Rat Lung Organ Index

Table 1. Effect of treatment on OVA-induced alterations in body weight and lungs weight.

Parameter	Negative Control	Positive Control	Standart	Formula I	Formula II
Body weight (g)	256,8 ± 31,6	268,8 ± 16,9	261,6 ± 9,5	279,6 ± 18,1	242,8 ± 44,6
Absolut lungs weight (g)	2,72 ± 0,64	2,6 ± 0,57	2,45 ± 0,6	2,26 ± 0,36	3,37 ± 1,10
Relative lungs weight (g)	1,06 ± 0,26	0,96 ± 0,21	0,93 ± 0,21	0,81 ± 0,11	1,46 ± 0,61**#

Description *: *different from negative control (p<0.05)*
**: *different from positive control (p<0.05)*
#: *different from the Standard (p<0.05).*

The results of the observation of rat pulmonary organ index showed no statistically significant differences between all groups except Formula II. In the formula II group, there was a decrease in body weight and an increase in lung organ index compared to the positive control group (**p<0.05) and the standard group (#p<0.05).

Histopathological Examination

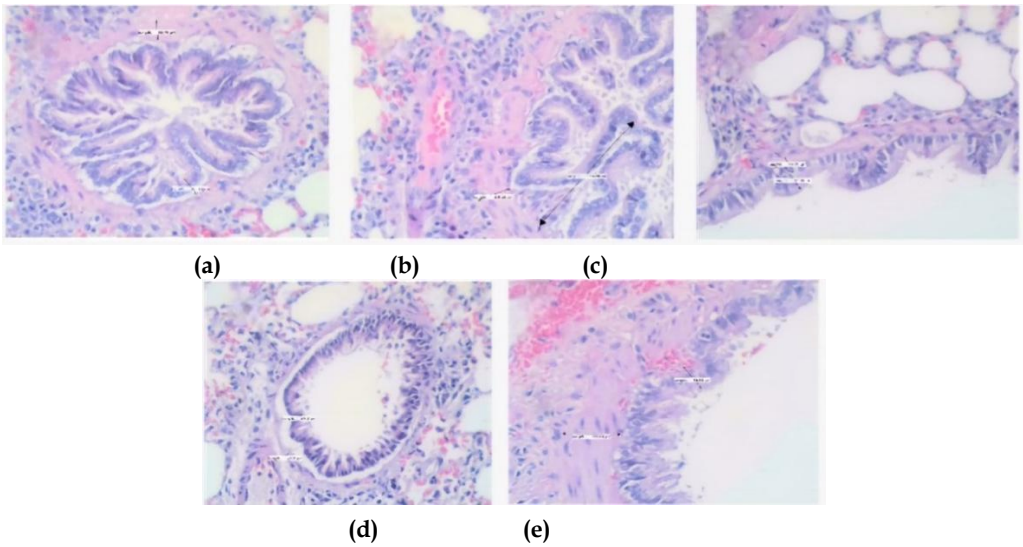

Figure 5. Rat lungs Histopathology (400x)
(a) Normal control group, (b) Positive control group, (c) Comparison group,
(d) Formula I, (E) Formula II.

Histopathological analysis revealed that the formula I and II groups exhibited type 1 emphysema, characterized by alveolar dilation exceeding 50% of the observed field. Mild infiltration of inflammatory cells. This pattern reflects a typical immune response in allergen-induced asthma without fibrosis. This finding suggests that Leuhang therapy may contribute to the regulation of airway inflammation.

Lung Histopathological Findings

Table 2. Lung Histopathological Findings

Parameter	Negative Control	Positive Control	Standart	Formula I	Formula II
Signs of pulmonary emphysema	Positive I*	Positive I**	Positive I	Positive I	Positive I
Interstitial pneumonia	Inflammatory infiltration#	Inflammatory infiltration	No inflammatory	Inflammatory infiltration	Inflammatory infiltration
Focal haemorrhagic	Haemmorrhage focus#	Haemmorrhage focus	Haemmorrhage focus	Haemmorrhage focus	Haemmorrhage focus
Eosinophil infiltration	Mild-Moderate	Mild-Severe	Negative	Mild	Mild
Lymphocyte infiltration	Mild-Moderate	Mild-Severe	Mild	Mild	Mild
Plasma cell infiltration	Mild-Moderate	Mild-Severe	Mild	Mild	Mild

Description:
(*) *if signs of emphysema (alveoli dilatation occurs in more than 50 percent of the field of observation;*
(**) *when signs of emphysema (dilated alveoli occur in less than 50 percent of the field of observation;*
(#) *in lung parenchyma;*
(*#) *if there is no/minimal focal hemorrhage in more than 50 percent of the field of view.*

These outcomes highlight the potential of Leuhang therapy—especially formula I—in alleviating asthma symptoms by influencing airway morphology and immune regulation.

Airway Histopathological Findings

Table 3. Airway Histopathological Findings

Parameter	Negative Control	Positive Control	Standart	Formula I	Formula II
Secretion of Mucus in the lumen	Negative	Positive	Negative	Negative	Positive
Goblet cell Proliferation	Negative	Positive	Negative	Negative	Positive
BALT Hyperplasia	Positive	Positive	Positive	Positive	Negative
Peribronchial cuffing	Negative	Positive	Negative	Positive	Positive

Edematous Mucosal layer	Negative	Positive	Negative	Negative	Positive
Inflammation of inflammatory cells	Negative	Positive	Negative	Negative	Positive

These results highlight the ability of formula I to suppress inflammatory responses and preserve the structural integrity of the airways in asthma-induced rats.

Table 4. Effects of the test on changes in rats' mucosal thickness and the lung muscle

Parameter	Negative Control	Positive Control	Standart	Formula I	Formula II
Mucosal Thickness (µm)	72,48 ± 35,16	216,77 ± 138,39*	155,85 ± 73,39	75,86 ± 13,11	130,93 ± 43,65
Muscle Thickness (µm)	50,35 ± 14,22	94,04 ± 52,93*#	50,55 ± 19,27**	24,57 ± 4,12**	84,09 ± 22,99

Description *: *different from negative control (p<0.05)*
**: *different from positive control (p<0.05)*
#: *different from the Standard (p<0.05).*

DISCUSSION

Morphology of Leukocyte Cells in Blood and Bronchoalveolar Lavage Fluid (BALF)

Asthma is characterized by inflammation accompanied by infiltration of eosinophils and basophils that are specific to asthma from other airway disorders. Basophils and eosinophils are leukocytes released by the body during asthma, which can narrow the respiratory tract. Basophils can release other inflammatory mediators and activate eosinophils, resulting in the release of toxic proteins that can damage lung tissue and trigger inflammation (Lokapirnasari and Yulianto 2014). The increase in eosinophils in the blood and bronchoalveolar fluid is evidence that inhalation exposure to allergens has been successful, resulting in a slow asthma reaction accompanied by inflammation. Lipid mediators produced by eosinophils are involved in allergic responses, causing contraction of smooth muscle in the airways, increasing mucus production, and helping eosinophil infiltration. Eosinophils are important in triggering asthma symptoms. There is a direct relationship between the number of eosinophils in the blood and bronchoalveolar lavage and bronchial hyperresponsiveness. However, eosinophils have another role, namely being able to neutralize inflammatory factors released by basophils (Lokapirnasari and Yulianto 2014).

Based on the results of morphological analysis of blood smears from mice that can be seen in Figure 1, in normal controls, no inflammatory activity and eosinophil cells were found. Eosinophils play an important role in assessing the severity and development of asthma (Lalrinpuia and P. 2019). This is in accordance

with the normal group without OVA induction. In the positive control group, eosinophils and basophils were found, indicating that the OVA induction method had been successfully carried out so that an inflammatory response occurred in asthma. Basophils play a role in hypersensitivity and increase inflammatory reactions in chronic allergies. While in the comparison group, only a few basophils were found in one field of view. The presence of basophils is related to the presence of an inflammatory response, but is counted as one cell in one field of view. In observations of blood samples in formula group I, I only found eosinophils, and in formula group II samples, there were still basophils and eosinophils. This concludes that test formula I produces the least number of leukocyte cells compared to test group II in the Leuhang therapy test.

Based on the results of Bronchoalveolar Lavage Fluid (BALF) morphology analysis, there was eosinophil and basophil cell activity in the normal control group test that was not given OVA induction. This shows that the presence of eosinophils and basophils in small amounts can occur in this case, so it can be concluded that formula I and formula II in the standard morphology of BALF show a decrease in asthma activity, and formula II has the potential to reduce asthma activity. Mice that do not suffer from asthma can be seen in Figure 2. Whereas in the positive control group given OVA induction, basophils indicated that the group consisted of rats suffering from asthma characterized by the presence of basophils, which resulted in morphological changes in lung tissue characteristic of asthma. In the comparison group test given the Pulmicort preparation (budesonide), there were basophils and eosinophils, which indicated that asthma recurrence activity decreased. This is because eosinophils can neutralize inflammatory factors released by basophils (Lokapirnasari and Yulianto 2014). Then, Formula group I contained eosinophils and basophils, indicating decreased asthma recurrence activity. Meanwhile, in formula group II there were eosinophils and basophils, indicating a decrease in asthma activity.

Bronchoalveolar Lavage Fluid (BALF) in Hemocytometer

Based on the results of the hemocytometer analysis on BALF to count the number of leukocyte cells (Figure 3), most of the leukocyte cells observed appear alive, with signs that the cells are not colored by methylene blue dye. When observed in more detail, visually the positive control group had a large number of leukocyte cells, scattered and piled up. Qualitatively, this finding indicates significant inflammation in the organ. In contrast, the comparison group given budesonide had relatively fewer leukocytes, with some dead leukocytes. This implies that the inflammation had been present for a long time but improved after administration of the comparator drug preparation. This is in line with the anti-inflammatory mechanism of corticosteroids in COPD cases, namely by reducing capillary permeability to reduce mucus and inhibiting the release of proteolytic enzymes from leukocytes (DiPiro et al. 2016).

Furthermore, in test group 1, the number of leukocytes observed was further reduced compared to the other groups. Comparison with the positive control group proves that test preparation 1 has an anti-asthmatic effect. Similarly, test group 2 showed almost equivalent leukocyte cell counts to test group 1. One of the plants in the Leuhang formula, betel leaf, is known to be able to reduce the total number of leukocytes and affect the types of leukocyte species, predominantly neutrophils and lymphocytes (Harahap and Zuhroh 2018). Based on this analysis, it can be concluded that both test preparation one and test preparation two showed anti-asthmatic effects. This conclusion is based on the established reference parameter, which is the anti-asthma effect produced by the drug budesonide in the comparison group.

Calculation of Rat Lung Organ Index

This study showed no significant difference between all groups except Formula II. In formula group II, there was a decrease in body weight and an increase in lung organ index compared to the positive control group (**$p<0.05$) and the standard group (#$p<0.05$) (Table 1). The significant difference between the positive control group and the formula group II can be caused by the inflammatory reaction that still occurs in the formula group II rats, which causes an increase in the relative organ index in the rat lungs. Whereas in the positive control group, the inflammatory process that occurred caused the rat lung tissue to be damaged, thus reducing the rat lung organ index. Therefore, these results indicate that formula II can prolong the inflammatory reaction in asthma-induced rats. Prolonged inflammation can be caused by the salt content (sodium chloride) in formula II, which can strongly induce IL-17 (Zieliński et al. 2022). Th17 cells release the proinflammatory cytokine IL-17, which has the ability to aggravate airway inflammation. IL-17 also increases the expression of α-smooth muscle actin in fibrocytes, which can worsen tissue contraction and narrow the airway (Gurczynski and Moore 2018).

Histopathological Examination

Asthma is a chronic inflammatory disease characterized by airway wall thickening, tissue remodeling, and infiltration of inflammatory cells such as eosinophils and macrophages, which contribute to bronchial hyperresponsiveness and airflow limitation (Patyk et al. 2020). Animal models, particularly asthma-induced rats sensitized with ovalbumin (OVA), are widely used to replicate key features of human asthma, including eosinophilic inflammation and structural airway changes (Thakur et al. 2019). Histopathological analysis of the lung tissues in these models reveals hallmark features such as goblet cell hyperplasia, mucus hypersecretion, BALT hyperplasia, and epithelial disruption, which are essential for evaluating disease progression and therapeutic efficacy. This information is crucial in understanding how asthma develops and how various therapeutic

interventions can affect the disease at the tissue level. The present study explores the potential of Leuhang therapy for its anti-asthmatic effects in modulating airway inflammation and preserving tissue integrity. The integration of such alternative therapies may offer insights into complementary strategies for asthma management.

When rats are affected by asthma from the environment, giving signs of emphysema in the lungs, pathophysiologically, emphysema is an immune response of neutrophils, macrophages, and lymphocytes in the lungs. Elastase, cytokines (including IL-8), and oxidants are released, causing epithelial injury and extracellular matrix (ECM) proteolysis. Thus, the incidence of emphysema is a normal condition and can be considered a result of inadequate wound repair (Kumar, Abbas, and Aster 2015). Based on the results of histopathological analysis, formula groups 1 and 2 had positive type 1 emphysema, meaning that dilated alveoli occurred in more than 50 percent of the observation field.

The assay rats had an infiltration of inflammatory (cellular) cells. The cellular pattern shows moderate interstitial inflammation, involving lymphocytes and some plasma cells in a diffuse and uneven distribution. This can be seen in the following parameter, namely in the formula I and II groups (Figure 5) experiencing mild infiltration of eosinophils, lymphocytes, and plasma cells. In the pathophysiology of asthma, this is a normal response of the body when an allergy is induced in the body to cause infiltration of immune cells (no fibrosis pattern) so that the generally shown symptoms are shortness of breath and cough (Kumar, Abbas, and Aster 2015). Interestingly, the standard group has not experienced eosinophil infiltration, so the method used is appropriate. Also, the condition of the formula I and formula II groups are close to the standard group in the parameters of lymphocyte infiltration and mild plasma cells. Based on the findings of lung histology in all groups experiencing focal bleeding (Table 2), this can occur due to lung inflammation. Continuous inflammation can damage small blood vessels and is prone to bleeding. In addition, frequent bronchospasm due to asthma attacks can increase the pressure in the capillaries of the lungs. This pressure causes capillary rupture and triggers bleeding (Corlateanu et al. 2021).

Another important histopathological observation is the condition of the airway. The airway affects how the muscle thickens and the presence of mucus in the lumen, as well as other vital parameters. The airway wall in asthmatics is characterized by increased mucosal gland hypertrophy and reduced airway caliber. Events result in airflow limitation. The effect on flow is enhanced by an increase in the amount of mucus and inflammatory exudates, causing an increase in surface tension so that it will close the airway Kumar, Abbas, and Aster (2015), as observed in the positive control group and formula II groups.

As it is known in the formula II group, there is an additional content of Himalayan salt, which is thought to have benefits. It is an initiative of several communities in adding components to Leuhang therapy. However, based on the

results of this study, it was found to worsen the incidence in asthma patients. This is reinforced by the findings of Musiol et al. (2024), that high salt consumption correlates with the incidence of asthma in adult patients with a history of allergies. While there was no mucus secretion in the formula I group in the lumen, indicating that formula I effectively reduced mucus secretion, this condition is the same as the standard and negative control groups.

To further evaluate the therapeutic effects of the Leuhang formulation on airway remodeling, histopathological parameters were assessed in each treatment group. Structural changes such as mucus secretion, goblet cell proliferation, and peribronchial thickening are characteristic of chronic asthma and are closely associated with disease severity and airflow limitation (Patyk et al. 2020). These characteristics reflect the underlying inflammatory and fibrotic processes contributing to airway obstruction and remodeling (Savin, Zenkova, and Sen'kova 2023). Therefore, comparative analysis of these parameters provides important insights into the extent of tissue repair or damage following therapeutic intervention.

In addition to signs of mucus secretion in the lumen, based on this overall observation, the parameters of goblet cell proliferation, edematous mucosal layer, and inflammation of inflammatory cells of the formula I group did not occur. This condition was the same as the standard group and even the negative control group. As for the parameters of bronchus-associated lymphoid tissue (BALT) hyperplasia, each of them showed that after the production of mucus secretion that causes shortness of breath had been reduced, the BALT response also occurred. BALT is a secondary lymphoid tissue that plays a role in maintaining and controlling lung mucosal immune homeostasis, inhibiting inflammation, and supporting immune clearance (He et al. 2019). Since BALT plays a role in immune response by regulating Th2 cell accumulation, eosinophil recruitment, and mucus production in pulmonary inflammation, it indicates the involvement of BALT in pulmonary immunological mechanisms (Hwang et al. 2020). BALT formation is associated with lung inflammation, if excessive, resulting in BALT hyperplasia. This manifests the immune system's attempt to cope with ongoing inflammation and is not an initial response.

Histological conditions have not decreased peribronchial cuffing in formula I and II groups. This could be because the Leuhang treatment for 14 days was not enough to decrease peribronchial cuffing. Peribronchial cuffing is a condition of bronchial thickening generally due to the accumulation of inflammatory cells and fluid around the bronchi (Bonser and Erle 2019). Such inflammation at the same time as the formation of lymphoid structures such as BALT occurs in structural changes in the bronchi. However, the Formula 1 group did not experience edema and inflammation from inflammatory cells. Based on this observation, it can be concluded that the formula I group can alleviate lung edema. The Zingiber officinale extract has active compounds such as shogaol, gingerdiols, and

proanthocyanidins, contributing to anti-inflammatory effects by inhibiting nitric oxide (NO) production. Furthermore, it can exhibit significant anti-asthmatic potential by reducing eosinophil counts and preventing mast cell degranulation, which is crucial in histamine release and allergic reactions (Palupi, Freistanti, and Apriliani 2021).

Statistical analysis using one-way ANOVA showed significant differences in airway muscle and mucosal thickness between treatment groups (Table 4). LSD post-hoc tests revealed that the Formula I group significantly reduced muscle thickness compared to the positive control group ($p = 0.001$), indicating a strong anti-remodeling effect. These findings are consistent with previous studies stating that airway smooth muscle hypertrophy is a characteristic of asthma and contributes to bronchial hyperresponsiveness and airflow limitation (Savin, Zenkova, and Sen'kova 2023; Elliot et al. 2018). Additionally, mucosal thickness in the Formula I group was nearly identical to that of the negative control group, suggesting that this therapy suppresses subepithelial fibrosis and epithelial hyperplasia, two key features of airway remodeling (Savin, Zenkova, and Sen'kova 2023). Conversely, the Formula II group exhibited significantly greater mucosal and muscle thickness compared to Formula I ($p = 0.03$ and $p = 0.003$), likely due to the addition of Himalayan salt. High sodium intake has been associated with increased production of Th2 cytokines and eosinophilic inflammation in asthma models (Adzani and Nova 2025). Observations based on airway mucosal and muscular thickness are strongly associated with asthma severity. Most studies suggest that this may result from increased reticular (mucosal) basement membrane thickness, supporting the role of sub-epithelial fibrosis resulting in airflow limitation in asthmatics. Meanwhile, muscle thickness is related to the degree of airway obstruction in asthma (Bonser and Erle 2019). These structural differences are clinically relevant, as increased airway wall thickness is associated with asthma severity and reduced lung function (Patyk et al. 2020). Overall, these results support the hypothesis that Leuhang therapy—particularly Formula I—may inhibit airway remodeling by reducing smooth muscle hypertrophy and mucosal thickening. These effects are likely mediated by active compounds such as gingerol and shogaol, which have been shown to inhibit the expression of IL-4, IL-5, and IL-13, suppress nitric oxide (NO) production, and prevent mast cell degranulation (Karaman et al. 2011; Adzani and Nova 2025).

CONCLUSIONS

Based on the research conducted, it can be concluded that Leuhang therapy shows anti-asthma activity that has been tested for 14 days based on leukocyte cell morphology parameters, organ index, and lung organ histopathology. In addition, formula I without Himalayan salt shows better anti-asthma activity than formula II with Himalayan salt.

These findings have implications for the potential development of Leuhang as a supportive inhalation therapy for asthma sufferers, especially in areas with medical limitations. Further research is recommended to identify the main active compounds of Leuhang therapy, determine the optimal dose and duration, and develop standardized and safe inhalation preparations for long-term use.

ETHICAL ISSUES

Submission of research ethics permits was made to the Research Ethics Committee of the Faculty of Medicine, Islamic University of Bandung. Faculty of Medicine, Islamic University of Bandung and the research began after obtaining a research ethics permit letter with number 038/KEPK-Unisba/VI/2024.

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