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The activity testing of kesambi leaves (Scheichera oleosa) ethanol extract on white mice of the Balb/C strain induced by ovalbumin

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Abstract

Background: Asthma is a chronic inflammatory disorder of the respiratory tract that involves many cells. The anti-inflammatory effect of kesambi leaves is due to the presence of secondary metabolites. Flavonoid compounds are known to have potential effects as anti-inflammatory and antioxidants
Method: This research uses the maceration method with 96% ethanol solvent. The test animals used were 26 white mice of the Balb/C strain which were divided into 6 treatment groups (N), (K-), (K+), (P1), (P2), (P3). Treatments on day 7 and day 14 were sensitized using OVA i.p. Next, on days 21, 23 and 25, 1% OVA was inhaled. On days 26 to 39, treatment was given, namely kesambi leaf extract (P1), (P2), (P3). On day 40, histopathologically discontinued. Data analysis using SPSS.
Results: The yield of kesambi leaf extract was 28.2%. Data were analyzed using the Mann- Whitney and Wilcoxon tests at T0, T1 and T2 to determine differences in eosinophil counts. The results of the analysis of the eosinophil count were 70.23. Meanwhile, mast cell data analysis uses Mann Whitney 52.57 µm. Analysis of bronchiolar epithelial thickness data 73.33 µm.
Conclusion: The effective dose of kesambi leaf ethanol extract is 400 mg/kgBW which can provide anti-asthma effects on blood eosinophils, mast cells and bronchiolar epithelial thickness in white mice induced by ovalbumin Balb/C.

Key words: Asthma; Eosinophils; Mast cells; Bronchiolar epithelium; Scheichera oleosa

INTRODUCTION

Asthma is a chronic inflammatory disorder of the respiratory tract that involves many cells. According to the World Health Organisation (WHO), there are an estimated 100-150 million people worldwide. The Asthma and Allergy Foundation of America reports that approximately 3-5% of adults have asthma (1). Meanwhile, according to the National Centre for Health Statistics, the prevalence of asthma in adults is 8.2% (2). Based on the 2018 Riskesdas, in Indonesia the prevalence of asthma in adults is around 2.2-3.4%(3). Indonesian people traditionally use natural ingredients to treat various diseases (4).

The ability of plants to overcome various diseases can be due to the synergistic effect between secondary metabolite compounds. In addition, secondary metabolite compounds also have polyvalent activity so that they can allow them to overcome various diseases. Based on this, asthma can be overcome by using natural ingredients (5). Because herbs have fewer side effects and greater tolerance than synthetic drugs, their use in traditional medicine is increasing (6). One of the potential alternative medicine plants is kesambi. The utilisation of kesambi has been known for a long time. Kesambi leaves contain flavonoids, phenolic compounds, alkaloids, and tannins as secondary metabolites. The most components in kesambi leaves are phenolics and flavonoids. Analysis conducted by thin layer chromatography (KLT) method shows quercetin is one of the flavonoid components in kesambi leaves (7). Research on kesambi leaf extract is known to have activity as an immunomodulator and anti-inflammatory which has a mechanism against inflammatory

mediators including histamine, serotonin and prostaglandins. Meanwhile, asthma can also be interpreted as a chronic inflammatory disease of the respiratory tract which can cause bronchial hyperactivity due to various stimuli (8)

The purpose of the study was to determine the anti-asthma effect of ethanol extract of kesambi leaves on the number of eosinophil cells, the number of mast cells and the size of the thickness of the bronchiole epithelium of white mice induced by ovalbumin and to determine the effective dose of ethanol extract of kesambi leaves on the number of eosinophil cells, the number of mast cells and the size of the thickness of the bronchiole epithelium of white mice induced by ovalbumin. The type of research conducted is experimental by extracting, testing specific and non-specific parameters and continuing the anti-asthma activity test which includes eosinophil test parameters, mast cell count and bronchiole epithelial thickness size.

METHOD

Materials

The main material in this study is the extract of kesambi leaves that grow in the East Nusa Tenggara and obtained kesambi leaf powder from Liliba. 96% ethanol, OVA induction. Asthma test are Balb/C mice aged 2-3 months, theophylline, carboxymethyl cellulosa (CMC 0,5%), haematolgy analyser. The test materials for compound content are silica gel GF_{254}, sulfuric acid anisaldehyde (H_2SO_4), citroboric acid (C6H8O7), ferric chloride ($FeCl_3$), Dragendrof reagent, Lieberman-Burchard reagent, formic acid (CH_2O_2). Aluminium hydroxide $Al(OH)_3$, natrium clorida (NaCl) 0.9%.

Method

Female Balb/C mice used in this study were 2-3 months old and weighed between 20 and 30 grams. A total of 26 mice were used and the mice were divided into 6 treatment groups, each consisting of 4 mice.

Extraction

Scheichera oleosa leaves as much as 7kg simplisia as much 500 gram were extracted for 5 x 24 hours at room temperature using 96% ethanol. Maceration is carried out by placing the powder in a macerator, then adding 96% ethanol solvent in a ratio of 5:2, then closing it and storing it for 5 days in a cool place, shaking it 3 times every 24 hours.

Identification of chemical compounds in kesambi extract

Flavonoid Identification. A total of 2 ml of ethanol extract of kesambi leaves was mixed with 0.5 ml of shaking and heated. Plus magnesium powder as much as 0.2 grams in a test tube, plus 3 drops of HCL. If positive it will change colour to red (9)

Identification of Tannins. A total of 2 ml of kesambi leaf extract dissolved with 2 ml of distilled water. Sprinkled with one or two drops of 1% FeCl3. If positive blue or dark green colour (10)

Alkaloid Identification. The ethanol extract of kesambi leaves was dissolved with 5 ml of HCL 2 N in three different tubes. The first tube was added 3 drops of mayer reagent with a marked change in the presence of a white precipitate. The second tube added dragendorf reagent 3 drops with a change in the presence of orange precipitate. The third tube added 3 drops of Wagner's reagent with a change in the presence of a brown precipitate (9)

Table 1. Grouping of test animal

Group	Treatment
N	Normal Group
N(-)	Ovalbumin and CMC Na induction negative group
P(+)	Positive group of ovalbumin and theopl induction
K1	Ovalbumin induction and kesambi leaf extract 200 mg/kg
K2	Ovalbumin induction and kesambi leaf extract 400 mg/kg
K3	Ovalbumin induction and 800 mg/kg portabella leaf extract

Identification of Saponins. A total of 2 ml of ethanol extract of kesambi leaves plus 5 ml of hot water, sprinkled with 2 drops of HCL 2 N and then shaking. The presence of changes is indicated by the presence of foam or foam (9)

Grouping of test animals

The test animals used are mice, randomly grouped

Modelling allergic asthma

Treatment day 0 to day 7 mice were acclimatised for one week while still being fed and drank. Day 7, the 1st sensitisation with ovalbumin induction intraperitoneally with a mixture of 10 µg + 1 mg $Al(OH)_3$ dissolved in 0.5 ml of normal saline (NaCl 0.9%) as much 0.5ml intraperitoneally. Day 14, 2nd sensitisation with ovalbumin induction intraperitoneally by injecting a mixture of 20 µg ovalbumin + 2 mg $Al(OH)_3$ dissolved in 1 ml normal saline (NaCl 0.9%) 1 ml intraperitoneally. Day 21, 23 and 25 were re-sensitised by inhalation by giving 1% ovalbumin in normal saline (NaCl 0.9%) as much 8 ml using a philips nebuliser for 20 minutes. On day 25 animal model of allergic asthma, the blood count of eosinophils from each treatment group was measured. Day 26 to day 39 drugs in each treatment group, namely for the negative control treatment group given CMC Na solution, the positive control treatment group given theophylline solution. Each treatment was given 1 time a day as much as 1 ml orally. As for the treatment group of kesambi leaf extract 200 mg / kgBB, 400 mg / kgBB and 800 mg / kg, each treatment group was given 0.5 ml orally every day with 1 administration. On the 40th day, the test animals were determined, the neck was dislocated, the lung organs were taken and immersed in 10% formalin PBS.

Eosinophil

The place for blood collection is the retroorbital sinus or eye of the test animal using a hematocrit capillary pipette inserted at 45ºC from the body. This process can produce a lot of blood. Samples were taken alternately from each eye and placed in an EDTA blood tube. An automated hematology analyzer was used to count the blood eosinophil cell count, which was measured as eosinophil cell count × 10^9/L blood plasma (Sysmex-XS-800i, Japan). The normal value of eosinophils in general is 1-3%.

Mast cell

Using Toluidine Blue staining followed by fixation in 10% formalin, the count of mast cells in the lungs of test animals was examined histopathologically. Specimens were stained with toluidine blue for 2-3 minutes, then rinsed with distilled water and dehydrated gradually with 70%, 80%, 95% and 100% ethanol concentrations, and examined with 400× *Image Optilab* Pro6.1 software.

Bronchioles epithelial thickness.

Hematoxylin-Eosin staining was used to determine the thickness of bronchiolar epithelium in the lungs of test animals. The preparations were then immersed in xylol I, II, and III for three minutes, soaked in Mayer's hematoxylin solution for seven minutes. The preparation is soaked in Eosin solution for approximately half a minute.Clearing was carried in xylol I, II for 2 minutes. The preparation was given 1 drop of glass object and then histopathologically observed in the lungs of the test animals using 400 x Image Optilab Pro6.1 software.

Statistical analysis

The data was processed using statistical methods, namely the Statistical Package for the Social Sciences (SPSS). At this analysis stage, a data normality test was carried out with the aim of seeing the normality of the data using the Shapiro Wilk test because the number of samples was small (<50). The homogeneity test was carried out with the aim of finding out whether the variance in the final results of the two groups was the same or not. The statistical test used is homogeneity of variance using the One Way Anova test. Post Hoc further tests used the Tuckey method with the aim of comparing the treatment group.

RESULTS

The conclusion of the analysis showed that the sample was scientifically recognised as Scheichera oleosa from kesambi leaves. The aim is to ensure and verify the accuracy of the type of plant to be used and whether the plant is really the desired plant. This study used 500 grams of simplisia powder with 96% ethanol solvent with the resulting extract of 141 grams, the percentage yield of 28.2%. Organoleptically the extract of kesambi leaves obtained is

blackish green, thick consistency and has a distinctive smell of kesambi leaves. Drying shrinkage is done by using a drying tool, namely moisture balance. The results of determining the drying shrinkage rate of kesambi leaves with an average value of 10.3%, it does not meet the standard limits of the determination of drying shrinkage < 10%. The cause of the drying shrinkage rate exceeding that is likely due to the simplisia storage process that is too long, causing the presence of microbes. The method used to determine the water content of simplisia is distillation with the tool used, namely Sterling Bidwell. A good simplicia water content has a maximum limit of <10%. The results of the percentage of water content of simplisia, namely 7.00%, meet the predetermined requirements of <10% (11)

The results of phytochemical screening of kesambi leaves can be seen in table 2.

Tabel 2. Phytochemical screening results of kesambi leaves

Chemical content	Description
Flavonoids	+
Tanins	+
Saponins	+
Alkaloids	+
	-
	+

Description: (positif) +; (negatif) -

Identification of phytochemical compounds in kesambi leaves includes flavonoids, tannins, saponins and alkaloids. In the results of the phytochemical screening test of kesambi leaves, compounds that gave positive results were flavonoids, tannins, saponins and alkaloid compounds with mayer and wagner reagents, while for dragendorf reagent negative results. The results of phytochemical screening are reinforced by KLT testing to determine the content of compounds in ethanol extracts of kesambi leaves. Flavonoid KLT test results on kesambi leaf extract with chloroform: acetone: formic acid (7: 3: 0.4). The stationary phase used is silica gel 60 F_{254} . Flavonoid compounds are characterised by yellow or orange coloured spots. Quersetin as a comparator because quersetin is the most widespread compound and 25% is found in plants.

Eosinophils are inflammatory cells that are highly prevalent in allergy-related inflammation. Eosinophils are examined using *haematology analyser*. Eosinophil examination uses blood samples collected in haematocrit tubes with anti-coagulant ethylene diamine tetraacetic acid (EDTA) with a minimum volume of 0.5 mL. Calculation of the average number of eosinophils T2 where the number of eosinophils T2 is the average result of the number of eosinophils that each treatment group has been given treatment except the normal group. The results of the average number of eosinophils T2 are in the table 3.

Table 3. Eosinophil Count

Treatment Group	Average eosinophil count
Normal	0.10 ± 0.01^{b}
Negative Control	3.15 ± 0.05^{ac}
Positive Control	0.63 ± 0.02^{ab}
200 mg/kgBB extract dose	0.91 ± 0.02^{ab}
400 mg/kgBB extract dose	0.71 ± 0.01^{ab}
800 mg/kgBB extract dose	0.80 ± 0.02^{ab}

a ; Significantly different from the normal group b; Significantly different from the negative group c; Significantly different from the positive group.

Mast cell analysis was performed by first removing the lung organ, colouring the preparation with toluidine blue, then using an Image Optilab pro6.1 microscope with 400x magnification software to observe it.

Table 4. Mast cell

Treatment Group	Mast Cell Average
Normal	19.75 ± 5.22^{bc}
Negative Control	78.89 ± 15.97^{ac}
Positive Control	41.58 ± 7.70^{ab}
200 mg/kgBB extract dose	76.08 ± 4.76^{ac}
400 mg/kgBBd extract dose	52.67 ± 1.94^{ab}
800 mg/kgBB extract dose	62.83 ± 9.15^{ac}

a: Significantly different from the normal group; b: Significantly different from the negative group ; c: Significantly different from the positive group.

Analysis of bronchiole epithelium in all treatment groups was carried out by taking lung organs, followed by histopathological tests using Hematocyllin Eosin staining and observed with an Image Optilab Pro6.1 software microscope magnification of 400 x. The results of the analysis can be seen in the table 5.

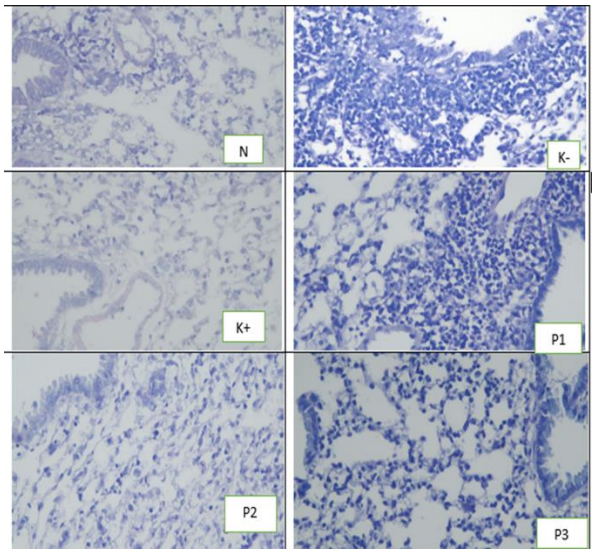

Figure 1. Differences in mast cells in the treatment groups by staining using toluidine blue observed at 400 x magnification microscope.

Table 5. Bronchiole Epithelial Thickness

Treatment group	Average thickness of Bronchiolar Epithelium (um)
Normal	65.00 ± 7.64[b]
Negative Control	92.50 ± 4.93[ac]
Positive Control	75.00 ± 7.64[b]
200 mg/kgBB extract dose	81.67 ± 7.64[a]
400 mg/kgBB extract dose	73.33 ± 8.50[b]
800 mg/kgBB extract dose	76.67 ± 8.50[b]

a: Significantly different from the normal group; b: Significantly different from the negative group; c: Significantly different from the positive group.

DISCUSSION

Based on the results of the average number of eosinophils in table 3, shows the average number of eosinophils and the percentage of eosinophil reduction in all treatment groups. The normal group (N) showed a mean eosinophil count of 0.10. The average result is the lowest compared to other treatment groups. The negative group (N-) found an increased number of eosinophils 3.15. The average result was the highest compared to the other treatment groups. Repeated administration of ovalbumin can stimulate B cells to produce Ig E with the help of T helper (Th) cells. The positive group (P +) showed an average of 0.63 eosinophils. Theophylline administration in allergic asthma model mice is expected to suppress inflammatory reactions due to allergies in the positive group as a positive control, so as to reduce mast cell and eosinophil counts (12). The results of the study showed that theophylline was able to reduce mast cell and eosinophil counts but not significantly. This may be due to the effect of the drug that has not reached the intestinal tissue.

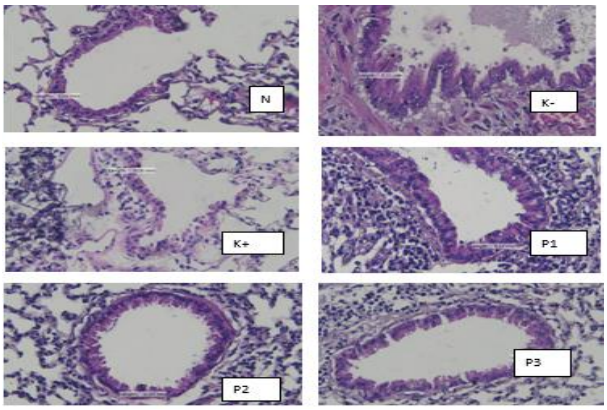

Figure 2. Differences in bronchiolar epithelium in the treatment groups by staining using HE observed at 400 x magnification microscope. Description: N (Normal); K- (CMC Na); K+ (Theophylline); P1 (200 mg/kgBB extract); P2 (400 mg/kgBB extract); P3 (800 mg/kgBB extract).

The extract group dose of 200 mg/kgBB (P1) showed a decrease in the average eosinophils 0.91. The 400 mg/kgBB dose group (P2) showed a decrease in the average number of eosinophils 0.71. The 800 mg/kgBB dose group showed a decrease in the mean number of eosinophils 0.80. The high increase in the number of eosinophils is caused by hypersensitivity due to the response of a disease. This is the same as research that has been done by (13) related to anti-inflammatory analgesic activity which says the 400 mg/kgBB dose has an anti-inflammatory mechanism against inflammatory mediators. Mediators that play a role in eosinophils are TNF-α, granulalocyte macrophage-colony stimulating factor (GM-CSF), IL-3 and IL-5).

Doses of 200 mg/kgBB, doses of 400 mg/kgBB and doses of 800 mg/kgBb are significantly different from the negative control, this is because the doses of 200, 400 and 800 mg/kgBB give the effect of a decreased reaction to eosinophils. The effective dose in the eosinophil parameter is at a dose of 200 mg / kgBB because the dose

is the smallest dose that has the effect of reducing the allergic reaction of eosinophils.

The ability of kesambi leaf extract to have an anti-inflammatory effect is thought to be due to the secondary metabolites that flavonoid molecules may have antioxidant and anti-inflammatory properties. It has been shown that flavonone chemicals, including flavonols, quersetin, and cathechin, prevent liposaccharides from activated macrophages from producing TNF-α and nitric oxide. Flavonoids suppress TNF-α by preventing NFkB activation. Although suppression of nitric oxide synthase occurs during the transcriptional phase, inhibition of TNF-α occurs during the post-transcriptional phase (14).

Based on the results in table 4 and figure 1. The normal group (N) showed an average number of mast cells of 19.75. The average result is the lowest compared to other treatment groups because the mice were not exposed to ovalbumin or allergens, only fed and drank so that the mice were in a healthy condition. The negative group (K-) showed the highest mean number of mast cells 78.89 compared to the other treatment groups because the mice were given ovalbumin but without being treated or treated.

Repeated administration of ovalbumin can stimulate B cells to produce Ig E with the help of T helper (Th) cells. Immunoglobulin E will then attach to the surface of mast cells and cause a bond between ovalbumin (as an antigen) with Ig E (as an antibody) on the surface of mast cells. This bond can stimulate the degranulation of mast cells so that the mediators contained in mast cells are released, namely histamine leukotrin, Eosinophile Chemotactic Factor-A (ECF-A) and cytokines (TNF- α, IL-1, IL-4, IL-5, IL-6, IL-13) and enzymes (chymase and tryptase).

The positive group (K+) showed an average number of mast cells that was significantly different from the normal and negative groups, because theophylline administration to allergic asthma model mice can suppress inflammatory reactions due to allergies in the positive group, thereby reducing the number of mast cells and eosinophils (12). There is a significant difference in the results of the 400 mg/kgBB extract dose group with the negative control group because the 400 mg/kgBB dose is the smallest dose that has an effect on mast cells so that it is said to be an effective dose.

The dose of 200 mg/kgBB is not significantly different from the negative control, this is because the dose given is too small so that the effect of the drug has not reached the instetinal tissue and has not given the effect of an allergic reaction. The dose of 800 mg/kgBB is not significantly different or comparable to the negative control because the dose given is the largest dose that has given the effect of an allergic reaction but the effect produced exceeds so as not to reduce the number of mast cells.

The kesambi plant has anti-inflammatory activity. Proinflammatory cytokines including TNF- α, IL-3, and IL-4 from mast cells, as well as leukotrienes and prostaglandins, are released when there is an increase in inflammatory cells, particularly eosinophils, mast cells, and lymphocytes. The cytokines involved will activate and attract additional inflammatory cells to the airway through local and systemic actions. As for the activity as an immunodulator due to the presence of flavonoid and alkaloid compounds that produce cytokines as an induced response due to invasion of pathogenic bacteria, cell damage and cell regeneration (14). The flavonoid group has the ability to strengthen the immune system and protect the body from infection by bacteria, viruses, and other microorganisms. In addition, by promoting the growth and activity of T and B lymphocytes, secreting a variety of specific cytokines such as interferon-gamma, tumour necrosis factor-alpha, and other interleukins, activating the complement system, and promoting phagocytic cells such as monocytes and macrophages, flavonoid compounds can affect the immune system (8). Because toluidine blue staining of mast cells would result in a purple violet (metachromacy reaction) on a blue background, it is used in histological investigations of mast cells. The acidophilic metachromacy dye toluidine blue, also referred to as tolonium chloride, selectively colors the phosphate, carboxylic,

and sulphate radicals that make up acidic tissues. Fundamentals of mechanisms metachromacy is the basis for toluidine blue staining, in which the dye combines with the tissue to produce a color distinct from the dye's initial color(15).

Based on the data from the statistical test results, there are significant test results where the sig. value of each group is> 0.05 so that it is declared that the data is normally distributed, and continued with the homogeneity test. The homogeneity test results show a significant value of 0.001 <0.05, meaning that the data is not homogeneous. So that the Nonparametric Test is carried out using Mann-Whitney to find out if there is a difference in each treatment group with a sig value <0.05.

Based on the data from the Mann-Whitney statistical test, the results obtained were <0.05 so that there were differences from each treatment group, namely between the normal group (N), the negative group (CMC Na), the positive group (Theophillin), the 200 mg / kgBB dose group (P1), the 400 mg / kgBB dose group (P2) and the 800 mg / kgBB dose group (P3). The normal group was significantly different from all treatment groups. The negative group was significantly different from the positive control group and the 400 mg/kgBB dose treatment group. The positive group was significantly different from the negative group and the 200 mg/kgBB dose treatment group, the 800 mg/kgBB dose treatment group. While the 400 mg / kgBB dose treatment group had no difference with the positive group.

Table 5 and Figure 2 show the average value of bronchiole epithelial thickness in all treatment groups. The purpose of staining with Hematocyllin is to give a blue color to the cells and eosin as a balance, namely to give a red color to the cytoplasm, connective tissue and other tissues (16)

The normal group (N) showed a mean bronchiolar epithelial thickness of 65.00 µm. The average result is the lowest compared to other treatment groups because the mice are not exposed to ovalbumin or allergens, only given food and drink so that the mice are in a healthy condition. The negative group (K-) found damage, namely the occurrence of bronchiolar epithelial thickening of 92.50 µm. The average result is the highest compared to the other treatment groups because the mice were given ovalbumin but without being treated or treated. Ovalbumin treatment will encourage the synthesis of Th-2 cells and IgE by dendritic cells. Th-2 causes the release of inflammatory cells by increasing IgE levels on the mast cell surface, which in turn triggers mast cell degranulation and the release of inflammatory mediators.

The positive group (K+) showed improvement with a decrease in the average thickness of bronchiole epithelial thickness of 75.00 µm. The administration of theophylline to mice with allergic asthma models is expected to suppress inflammatory reactions due to allergies in the positive group (K+) as a positive control, so as to reduce the thickness of bronchiolar epithelium. Theophylline drugs can prevent human and guinea pig sensory neurons involved in the response to different cough stimuli, by expanding calcium-activated potassium channels. Theophylline is an antitussive drug for various diseases, according to clinical trials. According to clinical research, theophylline works as an antitussive for a number of ailments. Theophylline increases the opening of calcium-activated potassium channels, which prevents sensory nerves in humans and guinea pigs from activating in response to different cough stimuli (17). According to research by (12) asthmatic mice's bronchiolar smooth muscle thickness can be decreased by injecting aminophylline, a theophylline derivative.

The dose of 200 mg/kgBB is not significantly different from the negative control, this is because the dose given has the same activity so that the bronchioles epithelial thickening occurs. The extract dose group that is significantly different from the negative control group is the dose of 200 mg/kgBB.

The dose of 400 mg/kgBB extract because it is the smallest dose that has an effect on bronchiole epithelial thickness. The 800 mg/kgBB extract dose was significantly different from the negative control but the 800

mg/kgBB dose was not the smallest dose that had an effect. Inflammation is associated with airway hyperresponsiveness based on the varied asthma symptoms associated with brochus inflammation. Asthma is characterised by inflammation, which includes eosinophil and neutrophil infiltration, mast cell degranulation, subbasal thickening, and loss of epithelial integrity(18). As for immunopathology in the general bronchiolar epithelium that occurs in asthma is type 2 inflammation involving specific cytokines for the epithelium including IL-33. Type 2 cytokines, which are mostly produced by CD4+ T cells and ILCs, become more active in the airway when epithelial cytokines are present (18)

Bronchiolar epithelial thickening may occur due to the rejection effect of the epithelial sloughing mechanism. Bronchiolar epithelial hyperplasia and metaplasia, subepithelial fibrosis, goblet cell hyperplasia, smooth muscle hyperplasia and hypertrophy, and increased vascularity (angiogenesis) are structural changes that occur during airway remodelling. Asthma severity, resistance to treatment, and airway hyperresponsiveness (AHR) are assumed to be associated with this airway wall thickening(6). Airway obstruction associated with narrowing of the bronchioles lumen diameter is a hallmark of asthma. Chronic inflammation of the airway wall accompanied by oedema, plasma extravasation and an influx of inflammatory cells such as mast cells, neutrophils, eosinophils, lymphocytes and macrophages is one of the main causes of airway narrowing. Bronchial hyperresponsiveness, the tendency of airway smooth muscle to contract in response to inhaled stimuli (such as histamine or cholinergic agonists), and the potential for temporary airway narrowing that can be resolved with bronchodilator therapy are physiological characteristics of asthma (19)

The test animal model is mice induced intraperitoneally with ovalbumin which introduces OVA-specific Th2 cells that produce IL-4, IL-5, IL-10, and IL-13 as well as antigen-specific IgE and IgG1 synthesis. Emulsion with aluminium hydroxide, a Th2 cell adjuvant. In corticosteroid-resistant mice, ovaalbumin induction can increase IgE levels and neutrophil numbers (6). Then the sensitised mice will be given ovalbumin by inhalation and the lungs of the test animals will show certain clinical and physiological characteristics of asthma along with increased cytokines in the mediastinal lymph nodes and respiratory tract. On the other hand, rats exposed to ovalbumin in previous studies had thicker bronchiolar epithelium (20).

Data normality test for bronchiole epithelium obtained p-value > 0.05 means that the data is normally distributed. The results of the bronchioles epithelium homogeneity test with p-value of 0.418> 0.05 means that the bronchioles epithelium data is homogeneous. Continued ANOVA statistical test shows p-value of 0.008 there is a difference between treatment groups.

CONCLUSION

Kesambi leaf extract can provide anti-asthma effects on the eosinophil count cells, mast cells and the size of the thickness bronchioles epithelium in white mice of Balb/C strain induced by ovalbumin. The effective dose of ethanol extract of kesambi leaves that can have an effect on the eosinophil count, mast cells and the size of the thickness of the bronchioles epithelium in white mice Balb / C strains induced by ovalbumin is a dose of 400 mg / kgBB.

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