

Anti-Asthmatic Potential of Blungsu Ciplukan (*Passiflora foetida* L.) Leaf Extract in an Asthma Model in Mice

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ABSTRACT

A report from the Ministry of Health of the Republic of Indonesia in 2018 revealed that the proportion of asthma recurrence within 12 months at all ages was still very high. It signifies that, so far, the therapy has not been effective. In fact, *Passiflora foetida* L. has the potential as an anti-asthma therapy since it contains apigenin, luteolin, and kaempferol and is easy to obtain. The method used in this research was quantitative experimental, with a post-test only design with a control group. The extraction technique employed was maceration. The test animals were made asthma models with lipopolysaccharide and ovalbumin for 17 days. Therapy was given on days 13-16. The negative control was given distilled water, while the positive control was given dexamethasone therapy. Then, lung histopathology was performed by assessing the degree of alveolar damage utilizing the Hansel and Barnes criteria. Alveolar edema, alveolar septal destruction, and inflammatory cell infiltration were then measured to demonstrate alveolar damage. The analysis results showed that the administration of Ciplukan Blungsu (*Passiflora foetida* L.) leaf extract gave a significant difference ($p < 0.05$) to the histopathological description of the lung organs of the test animals. In conclusion, Ciplukan Blungsu (*Passiflora foetida* L.) leaf extract has a good asthma therapy effect.

Keywords: Asthma, *Passiflora foetida* L., Maceration, Asthma Induction, Histopathology

1. RESEARCH BACKGROUND

Asthma is a chronic disease that attacks the respiratory tract [1]. In Indonesia, the number of people with asthma who died is estimated at 39,100 people. The highest incidence of mortality occurred at the age of more than 70 years, as many as 18,400, followed by the age of 60-69 years as many as 9,800 [2]. In addition, the proportion of asthma recurrence within 12 months in the population of all ages is still very high, namely 66.8-71.6%, with the highest incidence occurring at the age of 65-75 years, namely 61.7%-71.6%. It indicates that current treatment is not effective yet for treating asthma [3].

On the other hand, the drug classes of choice for asthma medications are corticosteroids, anti-leukotrienes, antimuscarinics, and β_2 -agonists [4]. Also, budesonide and beclomethasone dipropionate are inhaled corticosteroids used by patients in general. However, the side effects often complained of are dry throat, throat clearing, thirst sensation, and hoarseness [5]. Moreover, excessive use of SABA (Short-Acting Beta2 Agonist)

will cause dyspnea [6]. In addition, excessive use of SABA will actually lead to more frequent exacerbations and an increased risk of death [7].

If consumed in the long term, montelukast, a class of anti-leukotriene drugs, can cause side effects, such as anxiety, sleep disturbances, depression, suicidal ideation, fever, asthma exacerbations, and upper respiratory tract infections [8]. Meanwhile, the common side effects of using Ipratropium are inhalation bronchitis, nausea, dry mouth, dyspnea, dizziness, sinusitis, and dyspepsia [9].

More specifically, Indonesia has extraordinary biodiversity. Indonesia's biodiversity is second only to Brazil [42], with about 40,000 species; of the total number of species, about 1300 are used as traditional medicine [10]. In this case, *Passiflora foetida* is a tropical plant that has been known for its medicinal benefits. The flavonoid content in *Passiflora foetida* leaves also has been used as traditional therapy for insomnia, hysteria, asthma, and skin inflammation in India, Nigeria, and Brazil. In addition, the flavonoid content of *Passiflora foetida* has anti-hypotensive, anti-inflammatory, anti-spasmodic, and cytotoxic effects on breast cancer [11].

Previous studies have stated that *Passiflora foetida* L. has anti-allergic effects, especially asthma. However, these studies only briefly mentioned the anti-allergic effect [12]. Some quoted and mentioned active substances that can act as anti-asthma [11]. When citing the research conducted, the anti-asthmatic effect of *Passiflora foetida* L. refers to the book *the Useful Plants of India* written by Ambasta (1986). However, the book does not explain how the method is used and how to measure it. Therefore, the scientific discussion regarding the anti-asthmatic effect on *Passiflora foetida* L. is still unclear [13], so further research is needed to validate the therapeutic effect of *Passiflora foetida* L.

2. METHOD

2.1. Research Design

The research method used was experimental quantitative analysis, with a post-test only design with a control group. In this study, white male BALB/C 29-42 g were divided into four groups. Each group consisted of seven mice. Group 1 was as a non-asthmatic control. Group 2 induced asthma without any therapy. Group 3 induced asthma with dexamethasone therapy. Meanwhile, group 4 induced asthma with *Passiflora foetida* L. extract therapy.

2.2. Plant Determination

Plant determination was carried out at the Biology Laboratory, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta, by sending complete plant parts from leaves, flowers, stems, fruits, and roots.

2.3. *Passiflora foetida* L. Leaf Extract

Ciplukan Blungsu leaves were obtained from the Kp Puncak Suji area RT 01/04, Cinta Asih Village, West Bandung Regency - Cipongkor, West Java. Leaf drying was carried out with a food dehydrator (Tetra) at 50°C for 12 hours. Then, pollination was conducted using a stainless-steel electric dryer (Fomac).

The extraction technique used was maceration (cold extraction) at the Pharmacology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Surakarta. The fine powder of *Passiflora foetida* L. was macerated with 70% ethanol, with a ratio of 1/10 (w/v). The powder was macerated for 14 days with re-maceration on the 5th day. On the 14th day, the extract was filtered through filter paper and continued with evaporation using a rotary evaporator, set at 120 rpm at a temperature of 60-75°C. A water bath was also utilized to maximize the evaporation process at a temperature of 70-75°C so that a thick extract was formed. The viscous extract was then stored in a desiccator.

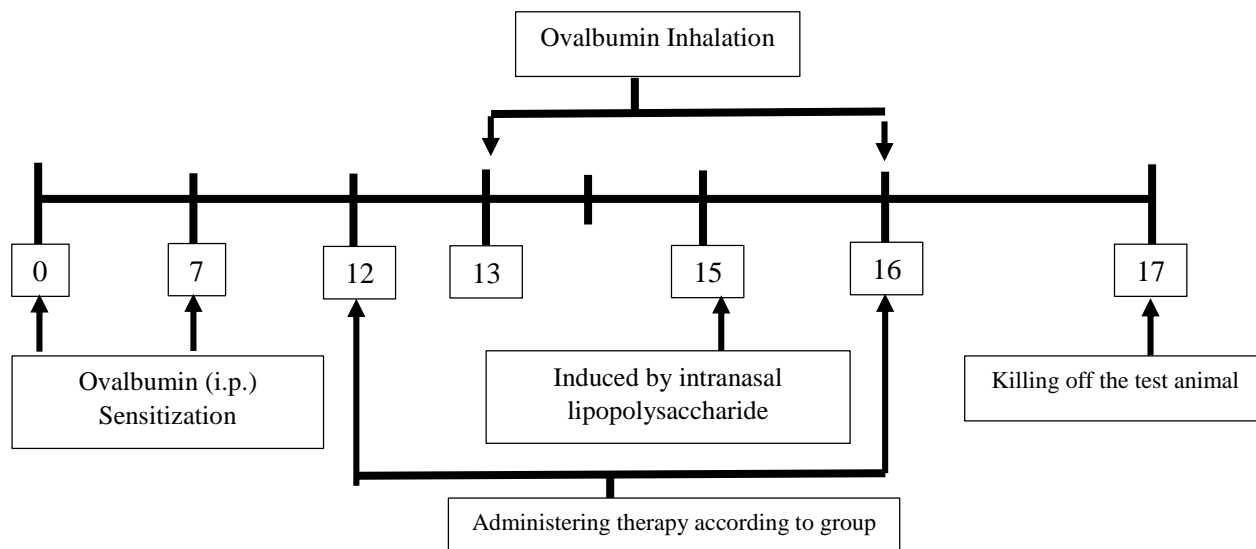


Figure 1. Timing of asthma induction, therapy administration, and termination

2.4 Asthma Induction

Asthma induction, therapy administration, and termination were carried out at the Pharmacology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Surakarta, for 18 days (Figure 1.). Mice were sensitized using ovalbumin (Worthington) 50 g in 5 mg aluminum hydroxide dissolved in PBS pH 7.4 200 l

intraperitoneally. The control group was sensitized with aluminum hydroxide in PBS. Induction of ovalbumin 1% in saline was administered by inhalation for 30 minutes utilizing a humidifier inserted into a can. Group 1 was given saline inhalation. Furthermore, lipopolysaccharide (LPS) induction from *Escherichia coli* O111:B4 (Sigma

Aldrich) 20 g dissolved in PBS was administered intranasally. Meanwhile, the control group was induced with saline. Before LPS induction, the test animals were anesthetized using ketamine. The induction process and therapy administration were conducted once a day. The negative control was given 0.1 ml/gBW distilled water, whereas the positive control was given 0.001 mg/gBW dexamethasone in distilled water. Meanwhile, the treatment group was given *Passiflora foetida* L. leaf extract at a 200 mg/kgBW mice dose. In addition, the test animals were killed by the cervical dislocation method.

2.5. Histopathology

Organs fixed in formalin solution for 6-72 hours were cut macroscopically and placed in labeled cassettes. The process of making histopathological preparations refers to Khristian & Inderiati (2017) on cyto-histotechnology [14]. Furthermore, pulmonary histopathological observations to assess asthma were carried out by assessing the degree of alveolar damage using the Hansel and Barnes criteria (Table 1). This observation was made under a light microscope in five views by looking at the four corners and the center of the slide at 400x magnification. For pulmonary histopathology scoring, it refers to Afiahama & Witjahyo (2014), which can be seen in Table 2 [15].

Table 1. Hansel and Barnes Criteria

Criteria	Description	Variation score
Normal	There was no histologic damage.	0
Minor damage	Alveolar damage is between 0% -30% of the entire visual field.	1
Moderate damage	Alveolar damage is between 30%-60% of the entire visual field.	2
Major damage	Alveolar damage is >60% of the entire visual field.	3

Table 2. Histopathological Scoring

Score	Pulmonary Histopathology of Alveolar Edema	Alveolar Septal Destruction	Inflammatory Cell Infiltration
0	No histologic changes occur.	No histologic changes occur.	No histologic changes occur.
1	Edema is less than a third of the entire visual field.	Alveolar septal destruction is less than one-third of the entire visual field.	Inflammatory cell infiltration is less than one-third of the entire visual field.
2	Edema is one-third to two-thirds of the entire visual field.	Alveolar septal destruction accounts for one-third to two-thirds of the entire visual field.	Inflammatory cell infiltration accounts for one-third to two-thirds of the entire visual field.
3	Edema is more than two-thirds of the entire visual field.	Alveolar septal destruction accounts for more than two-thirds of the total visual field.	Inflammatory cell infiltration accounts for more than two-thirds of the entire visual field.

3.2. Histopathology

Kruskal-Wallis test (Table 3) for groups 1-4 on the three indicators obtained $p < 0.05$. For the Mann-Whitney test in each group, the results can be seen in

2.6. Data Analysis

Histopathological results were tested with Kruskal-Wallis and followed by Mann-Whitney if there was a significant difference.

2.7. Ethical Approval

The ethical approval was issued by RSUD dr. Moewardi, with the number 717/VI/HREC/2021, stated that this research was ethical.

3. RESEARCH RESULTS

3.1. Determination Results

Plant determination was done to avoid mistakes in taking materials. The leaf of Ciplukan Blungsu (*Passiflora foetida*) was determined at the Biology Laboratory, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta, referring to the book *Flora of Java* by C.A. Backer and R. C. Bakhuizen van den Brink (1963) [16]. The determination results showed that the plant used in this study was the true species of *Passiflora foetida* L.

Table 4. Group 1 in the Mann-Whitney test was compared with groups 2, 3, and 4 with $p < 0.05$. It indicates that there was significant tissue damage in groups 2-4 in all three indicators, except in group 3 in the

septal destruction indicator, which was $p > 0.05$. Meanwhile, group 1 did not experience any damage. It means that the induction process of ovalbumin and lipopolysaccharide was successful. Meanwhile, in group 3, there was no significant damage.

Furthermore, the same pathogenesis starts from allergens stimulating T helper type 1 (Th1) cells and Th2 cells through APC (antigen-presenting cell). These two cells will stimulate a response, which will increase the proliferation and differentiation of neutrophil and eosinophil cells [17][18]. Test animals stimulated with ovalbumin will cause an inflammatory reaction through the activation of Th2 to produce inflammatory cytokines, such as IL-4, IL-5, and IL-13, which result in hyperresponsiveness in the respiratory tract. In addition, it leads to eosinophil recruitment, mucus production, and increased Ig-E, further exacerbating inflammation. Not only in the respiratory tract, but it can even trigger inflammation in the lungs [19]. Here, eosinophils act as APCs, apart from acting as a trigger for inflammation [41].

In addition, the lipopolysaccharide administration will induce the release of IL-1 β , IL-2, IL-6, IFN- γ , and TNF [20]. Lipopolysaccharides will also release protease

enzymes through the interaction complex between LPS-LPB (lipopolysaccharide-protein binding), which activates mast cells, macrophages, and neutrophils, causing a proteolytic effect. This enzyme is active through PAR (protease-activated receptor) and Th2 cells' activation through dendritic cells, causing damage to the lung epithelium, bronchioles, and cilia cells in respiratory bronchioles [21]. LPS will trigger an inflammatory reaction in the lungs by stimulating NCF (neutrophil chemotactic factor), leading to neutrophil recruitment in the inflammatory process [22].

Another mechanism that can trigger the occurrence of inflammatory cell infiltration and pulmonary edema is the induction of alveolar macrophages due to the binding of allergens to the TLR2, TLR3, TLR4, and NLRP inflammatory receptors [23]. These cells will recruit neutrophils and trigger the emergence of proteases, ROS (reactive oxygen species), eicosanoids, phospholipids, and cytokines, triggering inflammation [23]. Meanwhile, the destruction of the alveolar walls occurs due to the activation of pro-apoptotic protein molecules, such as Bax, caspase-3, and p53 [24]. The apoptosis process is active due to the stimulation of Th1 cells [23].

Table 3. Kruskal-Wallis Test Analysis

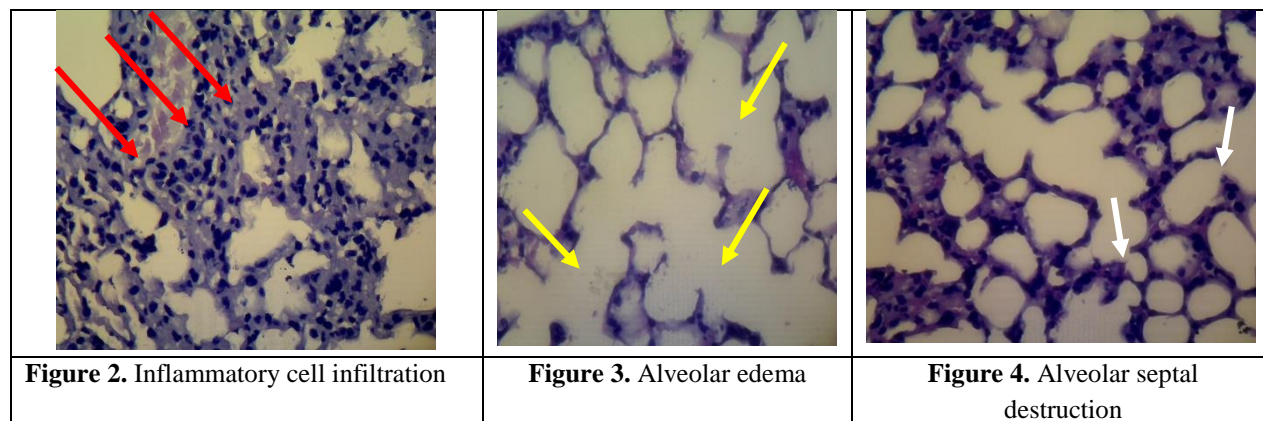
No.	Kruskal-Wallis	P-value
1.	Alveolar edema	0.001*
2.	Alveolar septal destruction	0.009*
3.	Inflammatory cell infiltration	0.001*

Description: * Significant ($P < 0.05$)

Table 4. Mann-Whitney Test Analysis

Uji Mann Whitney	P-value		
	Alveolar edema	Alveolar septal destruction	Inflammatory cell infiltration
P1 with P2	0.011*	0.006*	0.001*
P1 with P3	0.003*	0.081	0.007*
P1 with P4	0.001*	0.107	0.02*
P2 with P3	0.155	0.081	0.884
P2 with P4	0.019*	0.019*	0.015*
P3 with P4	0.475	0.593	0.07

Description: * Significant ($P < 0.05$); P1: Non-asthma; P2: Asthma without any therapy; P3: Asthma treated with dexamethasone; P4: Asthma treated with the extract



The anti-inflammatory effect of dexamethasone arises because of the GrA binding to the nucleus and thus affects gene transcription [25]. Meanwhile, GrB functions in GrA inhibition [26]. The GrA homodimer will be carried to the nucleus by importin- α and importin-13 and bound to the glucocorticoid response element (ERG), resulting in transactivation and transrepression. Transactivation occurs when the transcriptional co-activator triggers central histone acetylation associated with a glucocorticoid anti-inflammatory response in genes and the occurrence of gene transcription via RNA polymerase II. Meanwhile, the transrepression process occurs when CBP (cAMP-response element-binding protein) forms a complex with promoters of pro-inflammatory genes, such as NF- κ B and AP-1, which causes inhibition of transcription of pro-inflammatory genes and prevents DNA access to RNA polymerase II [26].

In this study, the group treated with dexamethasone did not experience a significant improvement in the three indicators, indicated by a $p > 0.05$. It is because corticosteroids, such as dexamethasone, are less reactive to the neutrophilic inflammatory response that originates from LPS stimulation [27]. Resistance to neutrophilic inflammation is due to reduced HDAC2, resulting in the dexamethasone failure to suppress the secretion of inflammatory proteins, such as CXCL8, matrix metalloproteinase-9 [28], PAI-1, TNF, and MIP-2 [29]. This condition can be seen in smokers, severe inflammation, cystic fibrosis, asthmatic patients who smoke, and severe asthma [30].

Inflammatory cytokines also have a strong involvement with corticosteroid-resistant (CR) asthma. The IL-17 cytokine can trigger the expression of GrB so that GrA inhibition will increase [31][32]. In addition, TNF, which synergizes with IFN- γ , bridges the occurrence of steroid resistance [33]. Steroid resistance in IL-33 and IL-5-induced eosinophil inflammation has also been identified [34].

In particular, *Passiflora foetida* L. is effective against inflammation since luteolin can suppress goblet cell number and mucus secretion through GABA inhibitory mechanisms [35]. In addition, luteolin can suppress IL-4, IL-13, and IL-5 [36]. Meanwhile, apigenin has a strong role in inhibiting inflammatory cytokines, such as IL-6, IL-1 B, and TNF- α [37]. Apigenin can also inhibit other pro-inflammatory cytokines, such as IgE, a cytokine produced by Th2 cells, such as IL-4 and IL-13 [38]. Suppressing IL-6, IL-1, IL-18, and TNF- α is also another effect of kaempferol [39]. Another study stated that the flavonoids of luteolin and apigenin also act as anti-inflammatory agents by inhibiting NO production through downregulation of iNos [40].

AUTHORS' CONTRIBUTION

R.P. oversaw the project. S.A.N, A.M.P.P, and E.D.P. experimented. N.N.S. led the manuscript writing with support from S.A.N, R.P, and E.D.P.

N.N.S organized the ideas presented, developed theories, analyzed data, and interpreted results. All authors provided critical feedback and helped shape the study.

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