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Anti-Inflammatory Effectiveness of *Sungkai* Leaf Extract (*Peronema Canescens*) Against Lung Histopathological Appearance, Polymorphonuclear Cell (PMN) Number, and Macrophages in Inflammatory Model Mice with Covid-19 Vaccine Induction

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ABSTRACT

Inflammation of the respiratory tract can occur when the respiratory tract tissue, especially the lungs, is injured, infected with bacteria and viruses, exposed to toxins, or heat. In the case of Covid-19 sufferers, when the virus first enters the body, macrophage cells will respond by producing cytokines to the infected tissue. One type of plant that can be used as an alternative treatment for inflammatory symptoms is the sungkai plant. Flavonoid compounds, saponins, alkaloids and phenols have anti-inflammatory activity. Where the content of secondary metabolites, tannins and flavonoids has activity as an antioxidant so that it can prevent damage due to oxidative stress. This type of research is an experimental laboratory research with a posttest group only design where the test is carried out at the end of the treatment period to see the relationship between the independent variables, namely the administration of sungkai leaf extract at graded doses to the independent variables in the form of pulmonary histopathology, the number of polymorphonuclear cells (PMN), and mouse macrophage (ratus norvegicus) inflammatory model by induction of covid-19 vaccine. Based on the One way ANOVA test, it was found that there was a significant effect of multi-dose sungkai leaf extract on the infiltration of lung inflammatory cells (p value 0.00), the number of polymorphonucleates (p value 0.00) and the number of macrophages (p value 0.00). The conclusion in this study was that the administration of multi-dose sungkai leaf extract had an anti-inflammatory effect which was reflected in the number of histopathological inflammatory cell infiltrates, the number of polymorphonuclear cells, and macrophages.

Keywords: Sungkai Leaf; Pulmonary Histopathology; PMN cells; macrophages; Experimental Animals;

INTRODUCTION

Inflammation of the respiratory tract can occur when the respiratory tract tissue, especially the lungs, is injured, infected with bacteria and viruses, exposed to toxins, or heat. In the case of Covid-19 sufferers, when the virus first enters the body, macrophage cells will respond by producing cytokines to the infected tissue. These cytokines then bind to the receptors of the infected cells, initiating endothelial release of molecules to attract leukocytes. Simultaneously, polymorphonuclear leukocytes (PMNs) are activated and release molecules that cause PMNs to accumulate and restrict vascular endothelium. The release of mediators by PMNs at the infected site is responsible for the cardinal signs of local inflammation in the form of local vasodilation and hyperemia and increased microvascular permeability

resulting in edema (Wong, 2021). Based on available data and statistics, of all Covid patients, 80% of them will experience mild symptoms, 15% of them will experience moderate-severe symptoms, and 5% of them will experience very severe or critical symptoms (Ali et al, 2021). In patients with mild or moderate symptoms, the duration of action of cytokines will be short (Quirch et al, 2020).

Various therapies for the treatment of inflammation include types of drugs from the NSAID group, such as ibuprofen, naproxen, diclofenac potassium, to aspirin. Another drug that is often used as therapy in suppressing the effects of inflammation is a drug from the corticosteroid class. However, long-term consumption of these two classes of drugs has been shown to cause side effects, such as stomach ulcers, kidney disease, visual disturbances, high blood pressure, and osteoporosis. With various side effects of using anti-inflammatory drugs, people tend to look for alternative treatments where alternative treatment options are in the form of phytopharmaceutical therapy by utilizing various types of plants that have active substances in suppressing inflammatory effects. One type of plant that can be used as an alternative treatment for inflammatory symptoms is the sungkai plant. Based on previous research, several secondary metabolite compounds in Sungkai (Peronema canescens Jack) leaves were reported, namely flavonoids, alkaloids, saponins, tannins, steroids and phenolics (Ramadenti et al., 2017). Flavonoid compounds, saponins, alkaloids and phenols have anti-inflammatory activity. Where the content of secondary metabolites, tannins and flavonoids has antioxidant activity so that it can prevent damage due to oxidative stress (Latief et al., 2020). Sungkai leaves (Peronema canescens Jack) contain lC50 of 44.933 ppm and are included in the category of very active antioxidants (Yunus & Zubaidah, 2015). Antioxidant compounds are one of the initial screenings for bioactive compounds that may have anti-inflammatory activity, by playing an active role in inhibiting NO (Nitric Oxide) which is a gaseous free radical (Tarigan, Lumbantoruan, et al., 2020).

Considering the high variety of anti-inflammatory agents found in sungkai plants, previous studies only looked at the effect of sungkai plants on reducing disease degrees without looking at how their effects were on immunocellular. This is proven by the absence of research that specifically proves how the influence of sungkai plants on the work of cellular modulators, especially polymorphonuclear cells (PMN) and macrophage activity in terms of the histopathology of inflamed lung tissue. Therefore, researchers are interested in proving how "The Anti-inflammatory Effects of Sungkai Leaf Extract (Peronema Canescens) Against Lung Histopathology, Polymorphonuclear Cell Number (PMN), and Rat Macrophages (Ratus Norvegicus) Inflammation Model With Covid-19 Vaccine Induction"

Aim And Objective Of Study

The purpose of this study was to determine the anti-inflammatory effectiveness of Sungkai Leaf Extract (Peronema Canescens) Against Lung Histopathology, Polymorphonuclear Cell (PMN) Number, and Macrophages in Inflammatory Model Mice With Covid-19 Vaccine Induction.

MATERIALS AND METHODS

This type of research is an experimental laboratory research with a posttest group only design where the test is carried out at the end of the treatment period to see the relationship between the independent variables, namely the administration of sungkai leaf extract at graded doses to the independent variables in the form of pulmonary histopathology, the number of polymorphonuclear cells (PMN), and mouse macrophage (ratus norvegicus) inflammatory model with covid-19 vaccine induction.

Lung tissue was fixed using 10% formalin solution. Each lung tissue was made one preparation containing three transverse

incisions and stained with hematoxylin-eosin stain. The

preparations were observed with a light microscope at 100x and 400x magnification assisted by veterinary experts. For each incision, five fields of view were selected, namely the four corners and the center of the preparation so that 10 fields of view were observed. Observation of lung histopathology was analyzed descriptively qualitatively. The score of the degree of damage obtained from 10 fields of view was taken with the highest score or the mean score.

STATISTICAL ANALYSIS

Hypothesis testing with computerized techniques using the SPSS version 17 application using the One Way ANOVA test. This test is also used to test the effect of independent variables on independent variables where the total group is more than two treatment groups.

RESULTS AND DISCUSSION

Table 1. Normality test

Variable	Shapiro-Wilk		
	Statistic	df	Sig.
Inflammatory cell infiltration	.935	24	0.128
PMN	.933	24	0.114
Macrophage	.923	24	0.067

Based on the results of the normality test using the Statistical Packages for Social Sciences (SPSS) Release 20.00 program, using the Shapiro Wilk test (sample < 50) it is known that all variables are normally distributed with p value > 0.05 with a p value of inflammatory cell infiltration with a significance of 0.12, the number of polymorphonucleates cell 0.114, and the number of macrophage cells 0.067

Because all variables were normally distributed, all variables met the assumption to be able to continue using the One Way ANOVA test to determine the effect of sungkai leaf administration on inflammatory cell infiltration, the number of polymorphonucleates and the number of macrophage cells. To make it easier to analyze the data, the test results are presented in table 2.

Table 2. One way ANOVA

Variable	N	F	Sig.
Inflammatory cell infiltration	24	81.214	0.000
PMN		117.853	0.000
Macrophage		163.624	0.000

Based on table 2. It is known that there is a significant effect of multi-dose sungkai leaf extract on the infiltration of lung inflammatory cells (p value 0.00), the number of polymorphonucleates (p value 0.00) and the number of macrophages (p value 0.00). The effect of this administration was manifested in histopathological analysis of lung tissue and blood smears of experimental animals, where there were differences in the number of inflammatory cell infiltrates, PMNs, and macrophages found in these preparations. An overview of the results of histopathological analysis and blood smears can be seen in the following figure:

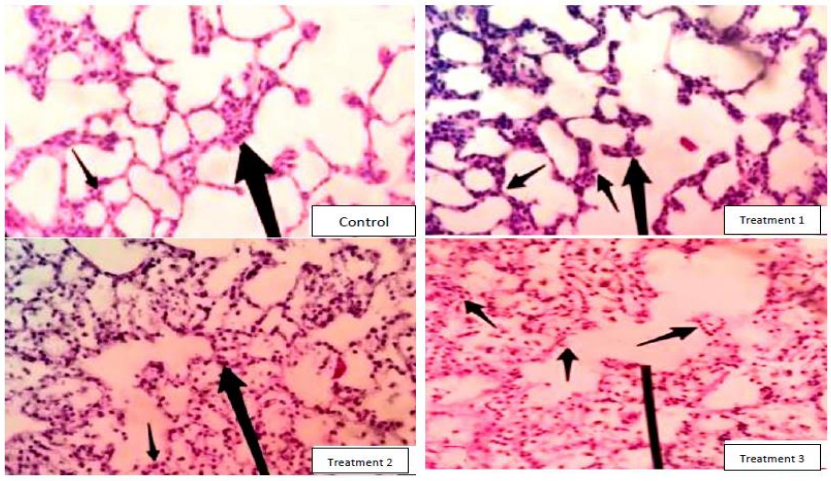

Figure 1. Histopathological description of experimental animal lungs, with inflammatory cell infiltration indicated by arrows

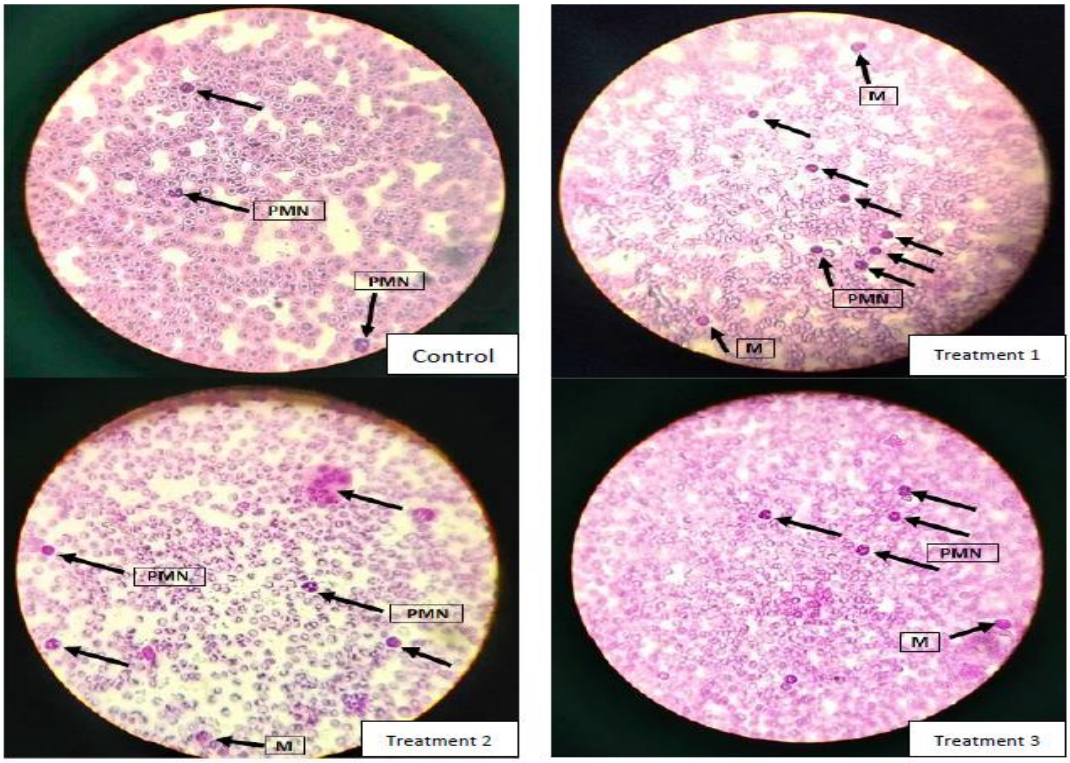

Figure 2. Number of Polymorphonuclear (PMN) and Macrophages (M) in experimental animal blood smears

DISCUSSION

Based on the results of the One way ANOVA test, it was found that there was a significant effect of giving sungkai leaf extract in graded doses to the infiltration of lung inflammatory cells (p value 0.00), the number of polymorphonucleates (p value 0.00) and the number of macrophages (p value 0.00). The significant effect of sungkai leaf extract on all variables was because the phytochemical screening showed that the sungkai plant had a fairly high amount of flavonoids. Flavonoids are known to have activity as immunostimulants by increasing: oxygen and nitrogen radicals, antibody production, cytotoxic activity against tumors by increasing activating receptors and downregulating inhibitory receptors. Therefore, flavonoids are potentially useful for the treatment of infectious diseases and cancer. Mechanism of Action In vitro, flavonoids and their derivatives inhibit various transcription factors, which modulate differentiation, proliferation, activation of immune cells and increase the formation of regulatory T cells. Several flavonoids exert anti-inflammatory effects through: NF-B blockade, and the NLRP3 inflammasome, inhibition of pro-inflammatory cytokine production, IL1β, IL-2, IL-6, TNF-, IL-17A, downregulation of chemokines, and species reduction reactive oxygen and nitrogen (Martínez et al., 2019).

This study proved that sungkai leaf has the ability as an anti-inflammatory, especially in the event of Covid-19, this is evident from the decrease in the number of immunocellular agents in the form of polymorphonuclear cells and macrophages. Research conducted by Latief et al., (2021) stated that the ethanolic extract of Sungkai leaves has anti-inflammatory activity marked by a decrease in the average number of neutrophils. The decrease in the average number of neutrophils could be caused by the presence of flavonoid bioactive compounds in the ethanol extract of Sungkai leaves. The ethanolic extract of Sungkai leaves used is a crude extract so that it is not possible to determine the specific metabolite compounds that work in providing anti-inflammatory effects. Reduction of

inflammatory mediators such as COX and NO will indirectly lead to inhibition of leukocyte accumulation in the area of inflammation, where under normal conditions, leukocytes move freely along the endothelial wall, but during inflammation various mediators cause leukocyte adhesion to the endothelial wall and cause immobile leukocytes.

The decrease in exudate volume can be caused by the content of active compounds in Sungkai leaves. One of them is flavonoids that can play a role by binding to the active site on the COX-2 enzyme so that it can interfere with the transcription process (Maleki et al., 2019), so that it can interfere with COX-2 expression through inhibition of binding to the NF-kB transactivator and blocking the entry of the p300 coactivator. which functions as a COX-2 promoter. Prostaglandins are synthesized by the COX-2 enzyme so that if there is a disturbance in the transcription and expression of COX-2, their production will be reduced. In addition, flavonoids can prevent the production of Inducible Nitric Oxide Syntase (iNOS), when inflammation occurs pro-inflammatory cytokine products that induce iNOS and are capable of producing large amounts of NO. NO has an effect as a vasodilator, so that when the process is inhibited, the edema will decrease (Karim et al., 2019)

Systemic changes in experimental animals after injection can be seen from the results of histopathological analysis of the lungs. It is known that there was damage to the alveolar septum and edema in some lung tissues in experimental animals in treatment group 1 with the lowest dose of sungkai leaf extract, so that the systemic recovery process was slow. In accordance with research conducted by Mouffak et al., (2020) stated that a continuous increase in viral load can cause hyperinflammation in the lungs which will result in damage to lung epithelial cells and cause fluid accumulation in the lung interstitials. This condition can be caused by a situation where there is an acute increase in cytokine activation called a cytokine storm. At this stage, the inflammatory process that occurs is due to an exaggerated immune response and can contribute to acute respiratory distress syndrome (ARDS) in people infected with Sars-CoV-2. Another study by Kurnia & Effendi (2019) In COVID-19, hyperinflammation can occur which results in damage to lung tissue which can lead to ARDS to death in infected people. The severity of cases in COVID-19 itself is divided into asymptomatic, mild, moderate, severe and critical symptoms.

CONCLUSION

The conclusion in this study was that the administration of multi-dose sungkai leaf extract had an anti-inflammatory effect which was reflected in the number of histopathological inflammatory cell infiltrates, the number of polymorphonuclear cells, and macrophages.

Conflict of Interest: None
Ethical Approval: Approved

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