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Imunomodulator Activity of Three Types "Kayu Kuning" Borneo

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Abstract

The immune system is a system that plays a role in maintaining the integrity of the body against the dangers that various foreign objects can cause. Immunomodulators are substances or substances that can change/modify the immune system. Empirically, Kayu Kuning or Yellow Wood is used as a cleaner for post-partum conditions. Yellow wood in East Kalimantan is the name for three species, namely Arcangelisia Flava, Fibraurea tinctoria, and Coscinium fensteratum. With the approach of improving the immune system and metabolism, immunomodulatory tests carried out on three types of yellow wood found in Borneo Island. The immunomodulatory activity of Yellow wood steam extract in the non-specific immune response test used phagocytic index and organ index parameters where BALB/c female mice were randomly divided into 16 treatment groups which included a normal group, an immunostimulant comparison group (Levamisole 2.5 mg/kg body weight), an immunosuppressant comparison group (Methylprednisolone 40mg/kg BW), a test extract group with a dose of 100 mg/kg body weight, for the ethanolic extracts of A. Flava, F. tinctoria and C. fenestratum, respectively. Based on the study results, it means that the methanol extract of A. Flava methanol extract had immunostimulating activity, while the methanol extract of F. tinctoria and C. fenestratum showed an immunosuppressant effect.

Keywords: Immunomodulator, Arcangelisia Flava, Fibraurea tinctoria, and Coscinium fensteratum

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1 Introduction

The immune system is a system that plays a role in maintaining the integrity of the body against the dangers that various foreign objects can cause. Immunity refers to the body's ability to eliminate foreign substances (bacteria, viruses, parasites) or abnormal cells that are potentially harmful [1]. Some health problems detect due to disorders in the immune system. In the surrounding environment, there are

many agents or foreign objects that can expose the body. Many factors can weaken the immune system (viruses, bacteria, parasites, etc.) and unhealthy diet and lifestyle [2]. In contrast, conditions such as autoimmune diseases require agents that suppress the immune system [3]. Generally, various efforts are made to carry out treatment, one of which is by using immunomodulators.

Immunomodulators are substances or substances that can change/modify the immune system. In general, immunomodulators are categorized as immunosuppressants and immunostimulants because of their effects [4]. Immunostimulants are agents that can stimulate or enhance the immune system [5]. Immunosuppressants are agents that can suppress the immune response [6].

Currently, many immunomodulators have been developed, one of which is the use of natural materials. Empirically, Kayu Kuning or Yellow Wood is used as a cleaner for post-partum conditions. Yellowwood in East Kalimantan is the name for three species, namely Arcangelisia Flava, Fibraurea tinctoria, and Coscinium fensteratum [7]. With the approach of improving the immune system and metabolism, immunomodulatory tests carried out on three types of yellow wood found in Borneo Island

2 Materials and Methods

2.1 Collection and Preparation extract Yellow wood

The material used is three types of yellow wood plant stems. These three types of Yellow wood have been determined at the Samboja Natural Resources Conservation Center, East Kalimantan.

The stems of three types of Yellow wood were extracted by reflux method using methanol as solvent. The extract was concentrated using a rotary evaporator to obtain a thick extract. Non-specific Immune Response Test

2.2 Carbon Clearance Test

The immunomodulatory activity of Yellow wood steam extract in the non-specific immune response test used phagocytic index and organ index parameters where BALB/c female mice were randomly divided into 16 treatment groups which included a normal group, an immunostimulant comparison group (Levamisole 2.5 mg/kg body weight), an immunosuppressant comparison group (Methylprednisolone 40mg/kg BW), a test extract group with a dose of 100 mg/kg body weight, for the ethanolic extracts of A. Flava, F. tinctoria and C. fenestratum, respectively.

The grouped mice were given the test material once a day for seven days. On day 8, mice were injected with 1.6 ml carbon (pelican black ink B17) in 8.4 ml 1% gelatin [8]. Previously, blood was drawn from the mice via the tail vein (T0). After that, the mice were drawn blood at intervals of 3, 6, 9, 12, 15 minutes, calculated from the time of carbon injection. 20 l of blood was mixed with 2 ml of 1% acetic acid, and then the transmittance and absorbance values were measured using a spectrophotometer at a wavelength of 675 nm. The data obtained were further processed to determine the rate of elimination of carbon particles through the manufacture of a linear regression curve between 100-%T with respect to time [9]. The slope of the regression line indicates the rate of elimination of carbon particles. The phagocytic index is calculated by the regression line, which is the ratio between the line slope of the test and control groups [10].

2.3 Organ Index

After taking blood at 15 minutes, the test animals were then sacrificed using a chamber filled with CO_2 gas, and then surgery was performed to take the liver, spleen, and thymus organs which were then weighed to see the organ index [11] with the equation 1.

$$\text{Organ Index}(\%) = \frac{\text{Organ Weight (g)}}{\text{Body Weight (g)}} \times 100\% \quad \text{(Equation 1)}$$

3 Results and Discussion

The yellow wood plant used in this study came from the Wartono Kadri Forest, the Natural Resources Conservation Center (BKSDA), Samboja, East Kalimantan. Plant determination at the Samboja Herbarium Center for Natural Resources Conservation (BKSDA) Samboja with the results of Arcangelisia Flava, Coscinium fenestratum, and Fibraurea tinctoria. From the extraction results, the extracts for A. Flava, F. tinctoria, and C. fenestratum were 5.22%, 5.50%, and 8.07%, respectively.

3.1 Non-Specific Immune Response

Furthermore, the non-specific immune response test using the` carbon clearance method against the ethanolic extracts of A. Flava, F. tinctoria, and C. fenestratum. The results in % transmittance value were analyzed to obtain the phagocytic index. Phagocytosis is the primary non-specific defense mechanism against foreign antigens against pathogenic microorganisms and other antigens [12]. In this process, phagocytic cells will recognize and digest foreign substances. The carbon clearance test was carried out as an initial step to see the effect of the immunomodulator of the extract. The principle is of observing the phagocytic speed of the endothelial reticulum system in vivo by induction of carbon ink suspension as a foreign. The result of absorbance would read at 675nm.

Parameters in immunomodulatory effect were % transmittance (%T), elimination rate (k), and corrected phagocytic index (α). The elimination rate used for determine the rate of phagocytosis. The % Transmittance value in this study can be seen in table 1.

Table 1. Transmittance Value (%T) Methanol Extract 3 Types of Yellow Wood

Test Group	% T minute -				
	0,00	5,00	10,00	15,00	20,00
Ethanol Extract Arcangelisia flava 100mg/kgBB	38,85 ± 1,15	49,05 ± 1,15	49,69 ± 1,87	46,1 ± 1,66	33,80 ± 1,20
Fibraurea Ethanol Extract 100mg/kgBB	31,82 ± 5,37	29,05 ± 1,06	29,33 ± 2,27	33,59 ± 1,29	32,70 ± 5,76
Coscinium Fenestratum Ethanol Extract 100mg/kgBB	21,4 ± 8,20	24,65 ± 5,87	39,7 ± 1,61	38,1 ± 1,18	47,95 ± 0,35
Levamisole 2.5 mg/kgBW	27,92 ± 2,54	26,27 ± 1,80	35,58 ± 1,51	22,81 ± 1,67	33,72 ± 1,67
Methylprednisolone 40mg/kgBW	27,20 ± 0,22	28,47 ± 1,67	31,68 ± 1,61	28,01 ± 1,46	25,21 ± 8,08
Normal Control	35,93 ± 6,35	20,6 ± 7,62	27,3 ± 1,34	22 ± 5,20	30,97 ± 1,79

Calculate the value of R linear regression to 100%-Transmitter. This value describes the phagocytic speed of the body. The value of K(el) phagocytic velocity in this study can be seen in table 2.

Table 2. Phagocytic velocity Methanol Extract 3 Types "Kayu Kuning"

Test Group	K(el)
Ethanol Extract Arcangelisia flava 100mg/kgBB	0,28 ± 0,02
Fibraurea Ethanol Extract 100mg/kgBB	-0,62 ± 0,03
Coscinium Fenestratum Ethanol Extract 100mg/kgBB	-1,88 ± 0,13
Comparative Control Levamisole 2.5 mg/kgBW	0,62 ± 0,02
Comparative Control Methylprednisolone 40mg/kgBB	0,07 ± 0,02
Normal Control	0,27 ± 0,12

From the table 2, it can seem that the elimination rate of Levamisole is greater than the control and extract, so Levamisole has an effect as an immunostimulant. Meanwhile, Methylprednisolone as an immunosuppressant control was in a smaller number than the control. Then that was also seen that the methanol extract of A. Flava had immunostimulant activity, while the methanol extract of F. tinctoria and C. fenestratum showed an immunosuppressant effect with a lower phagocytic rate than the control.

Levamisole is an immunomodulator that works by influencing T cell levels to be higher than B cells. It was causing cutaneous reactivity to delayed-type hypersensitivity antigens and increasing the function of T-helper cells suppressor T cells and T-cytotoxic cells [13]. Methylprednisolone, a corticosteroid, has an immunosuppressant effect with the reduction of lymphocytes, inhibit T lymphocyte cell proliferation, cellular immunity, and gene expression on various cytokines (IL-1, IL-2, IL-6, IFN-alpha, TNF-alpha). Corticosteroids can also suppress the response of macrophages and

monocytes to reduce circulating macrophages and reduce the activity of microbicidal phagocytosis, digestive intracellular antigens, and the elaboration of plasminogen activation factor [14].

Furthermore, the value of phagocytic activity was analyzed based on the comparison between the slope of the linear regression line K(el) between 100%-Transmittance with respect to time in the test and control groups. The phagocytic index obtained from the results of the analysis can be seen in Table 3.

Table 3. Classification of Phagocytic Index of Methanol Extracts of 3 Types of "Kayu Kuning"

Test Group	Index Phagocytic	Classification Effect
Ethanol Extract Arcangelisia flava 100mg/kgBB	1,52	Immunostimulant
Fibraurea Ethanol Extract 100mg/kgBB	-3,68	Immunosuppressive
Coscinium Fenestratum Ethanol Extract 100mg/kgBB	-11,44	Immunosuppressive
Comparative Control Levamisole 2.5 mg/kgBW	3,31	Immunomodulator
Comparative Control Methylprednisolone 40mg/kgBB	0,36	Immunosuppressive
Normal Control	1,00	-

According to Wagner [15], if the phagocytic index of the test preparation has a value < 1, then the practice is not immunostimulating; a phagocytic index between 1-1.5 indicates a moderate immunostimulating effect, and a phagocytic index > 1.5 means a strong immunostimulating effect.

After the last minute of blood collection, the experimental animals that had been induced were then sacrificed for the supply of liver, spleen, and thymus organs. The liver has Kupffer cells that play an essential role in modulating cell differentiation and proliferation, and the spleen is the primary filter for pathogens and antigens consisting of T cells, B cells, dendritic cells [16], and the thymus is an organ where T cells maturation. The liver, spleen, and thymus are used as parameters in determining the phagocytic index. An increase in liver and spleen weight can indicate an increase in the proliferation of immune cells found in these organs.

Table 4. Index of Animal Organs Tested for Immunomodulator Test of Methanol Extracts of 3 Types of "Kayu Kuning"

Test Group	Indekx Organ		
	Spleen Organ Index	Liver Organ Index	Thymus Organ Index
Ethanol Extract Arcangelisia flava 100mg/kgBB	0,62 ± 0,13	5,43 ± 0,28	0,22 ± 0,02
Fibraurea Ethanol Extract 100mg/kgBB	0,50 ± 0,14	5,15 ± 0,40	0,18 ± 0,02
Coscinium Fenestratum Ethanol Extract 100mg/kgBB	0,40 ± 0,07	5,12 ± 0,52	0,18 ± 0,02
Comparative Control Levamisole 2.5 mg/kgBW	0,60 ± 0,06	5,92 ± 1,54	0,21 ± 0,08
Comparative Control Methylprednisolone 40mg/kgBB	0,48 ± 0,07	4,04 ± 1,78	0,14 ± 0,03
Normal Control	0,49 ± 0,003	5, 22 ± 0,38	0,20 ± 0,11

Based on the organ index values above, levamisole and methanol extract of A. Flava in the liver and thymus showed a significant increase compared to controls ($p<0.05$). Meanwhile, methylprednisolone group, methanol extract of F. tinctoria, and C. fenestratum showed a decrease organ index when compared to the control group.

4 Conclusions

Based on the study results, it means that the methanol extract of A. Flava methanol extract had immunostimulating activity, while the methanol extract of F. tinctoria and C. fenestratum showed an immunosuppressant effect.

5 Author Contribution

1. Dwi Hadi Setya Palupi and Riski Sulistiarini Conceptualization of Research and Manuscripts
2. Dwi Hadi Setya Palupi and Vita Olivia Analysis of Research Data
3. Dwi Hadi Setya Palupi and Riski Sulistiarini Methodology validation and research implementation
4. Riski Sulistiarini Writing and Compiling the Original Manuscript
5. Dwi Hadi Setya Palupi, Riski Sulistiarini and Vita Olivia Review & Editing the script

6 Conflicts of Interest

The authors declare no conflict of interest.

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