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Immunomodulator Activity of Puguntano (*Picria fel-terrae* Lour.) Extract in White Male Mice By Carbon Clearance Method

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Abstract. Poguntano Herb Plants (*Picria fel-terrae* Lour.) contains flavonoids compounds that have been potentially to developed into immunomodulators. The purpose of this study was to determine the immunomodulatory activity of Poguntano herb extract (*Picria fel-terrae* Lour.), with the method of carbon clearance. n-hexane extract puguntano herb(ENHHP), extract of ethyl acetate of Poguntano herb (EEAHP), and ethanol extract of puguntano herb(EEHP) Poguntano multistage results in the test of immunomodulatory activity with a method of carbon clearance. Results showed that the EEHP 200 mg/kg BB showed a strong immunostimulation effect, EEAHP 200 mg/kg BB showed strong immunostimulation effect, ENHP 200 mg/kg BB demonstrated strong immunostimulation effect. It is thought that extract N-hexane herb Poguntano (ENHHP), extracts of ethyl herb acetate Poguntano (EEAHP), and herb ethanol extract Poguntano (EEHP) Poguntano herb may increase phagocytosis activity. Extract of ethyl acetate Poguntano herb 200 mg/kg bw (EEAHP) has the best phagocytosis activity with a value of 2.376 which indicates there is a significant difference ($P < 0.05$) with another treatment group.

Keywords:, *Picria fel-terrae Lour*, immunodulator, carbon clearance,

Abstrak. *Tumbuhan herba poguntano (Picria fel-terrae Lour.) mengandung senyawa flavonoid yang berpotensi dikembangkan menjadi imunomodulator. Tujuan dari penelitian ini adalah untuk menentukan aktivitas imunomodulator ekstrak herba poguntano (Picria fel-terrae Lour.), dengan metode carbon clearance. Ekstrak n-heksana herba poguntano (ENHHP), ekstrak etil asetat herba poguntano (EEAHP) dan ekstrak etanol herba poguntano (EEHP) herba poguntano hasil maserasi bertingkat di uji aktivitas imunomodulator dengan metode carbon clearance. Hasil penelitian menunjukkan bahwa maka EEHP 200 mg/ kg bb menunjukkan efek imunostimulasi kuat ,EEAHP 200 mg/ kg bb menunjukkan efek imunostimulasi kuat , ENHP 200 mg/kg bb menunjukkan efek imunostimulasi kuat. Diperkirakan bahwa Ekstrak n-heksana herba poguntano (ENHHP), ekstrak etil asetat herba poguntano (EEAHP) dan ekstrak etanol herba poguntano (EEHP) herba poguntano dapat meningkatkan aktivitas fagositosis. Ekstrak etil asetat herba poguntano 200 mg/kgBB (EEAHP) mempunyai aktivitas fagositosis paling baik dengan nilai sebesar 2,376 yang menunjukkan terdapat perbedaan yang signifikan (p<0,05) dengan kelompok perlakuan lain.*

Kata kunci:, *Picria fel-terrae Lour, immunodulator, carbon clearanc*

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1. Introduction

Infectious diseases are caused by infective agents that attack and perform multiplication in our bodies so that the body needs to perform a defence system as a coordinated response to the introduction of foreign substances known as the immune response [1.2]

An immunomodulator is a compound that affects the immune system's work by enhancing or suppressing factors that play a role in the immune system [3]. it helping the body to optimize immune system function which is the main system that plays a role in body defense where most people are easily experiencing immune system disorders [4]. Based on how it works Immunomodulator is divided into 2 groups namely immunostimulator and immunosuppressor. Immunostimulator functions to improve immune system function and activity while immunosuppressor function suppresses immune system activity [5].

Picria fel-terrae or also known as Pugun Tanoh is known to have much efficacy and has been used empirically by the community in Karo district, Sumatera Utara, Indonesia. This herb contains flavonoid compounds, saponins, tannins, glycosides as well as steroids/terpenoids [6]. Flavonoids have various activities, such as immunostimulatory, anti-inflammatory, antioxidant, anti-inflammation, and preventing cancer growth.[7]. Flavonoids compounds have activity against lymphocytes (interferon ɤ), which is produced by T cells which can stimulate cells – phagocytic cells to activate phagocytosis response [8], One of the tests was done to determine the immunomodulatory of this Poguntano herbal extract by testing using the Carbon clearance method.

2. Materials and Methods

2.1 Sample Preparation and Extraction

Fresh Poguntano (*Picria fel-terrae* Lour.) herb obtained from the village of Tiga Lingga, Dairi Regency, Sumatera Utara Province. The plant identification was confirmed by Bogoriense Herbarium, LIPI, Jakarta Indonesia. The cleaned herb are dried and blended until they turn into powder. An amount of 500 g the dried of poguntano herb were exctracted with maceration method using 5 L ethanol, n-hexsan, ethyl acetate until discoloration. Then the ethanol, n-hexsan, ethyl acetate macerate was evaporated at ± 40 ° C in a rotary vacuum evaporator and thickened by heating in a water bath at ± 40 °C [9].

2.2 Animals

All procedures were evaluated by Animal Research Ethics Committees (AREC) Faculty of Mathematics and Natural Science, Biological Department, University of Sumatera Utara. Mice were divided into fourteen groups and each group obtained three mice. Before the experiment begins, the animals were acclimatized in the experimental room for 7-14 days with room temperature and conditions 12 hours of light and 12 hours of darkness.

2.3 Carbon Colloidal Suspension Setup

The manufacture of carbon suspensions is done in the following manner: suspension of 1.6 ml of the Chinese inking B-17 in 8.4 ml of 1% B/V gelatine in a physiological solution of NaCl [10].

2.4 Carbon Clearance Test

In this method used 3 mice each positive control group, negative control and treatment group. Each group is gave an oral imboost 32.5 mg/kg bw as a positive control group, Na-CMC suspension as a negative control and ENHHP, EEAHP and EEHP at a dose 25, 50, 100 and 200mg/kg bw as a treatment group extract 1 time a day for 7 consecutive days. On the 8th day after sampling the suspension of the samples in each group, the tail ends were cuted. It takes 1ml of blood and is inserted into a tube that contains Na citrate, then the blood is taken 25 µl and added 4 ml of 1% acetic acid to fill red blood cells, the first blood used as a Blanko (minute 0), then 0.1 ml carbon suspension is injected in I. V through the blood vessels on the tail, and in the 5, 10, 15 and 20 minutes after the carbon injection is carried out blood sampling, it is accommodated into tubes that have contained Na citrate, then the blood is taken as much as 25 µl, each added 4 ml of 1% acetic acid to a line of red blood cells, and then measured its absorption using UV-Vis spectrophotometer at a wavelength of 632.0 nm. After 12 hours of blood was taken, then the liver and lymph recorded weighing [11]. After taking the blood on the tail end of the mice is calculated a constant carbon elimination speed (K) and phagocytosis index (α) by using the formula [12].

$$\text{Constant carbon elimination speed (K)} = \frac{\log \mathrm{OD}_{20} - \log OD_5}{\mathrm{T}_2 - \mathrm{T}_1}$$

2.5 Statistical Analysis

Data from observations of immunodulatory activity testing were statistically analyzed by the One Way ANOVA method followed by Post Hoc Tukey HSD test using SPSS (Statistical Product and Service Solution) version 25.

3. Result and Discussion

The carbon clearance method is a test of non-specific immune responses to see the ability of phagocytosis by using carbon as a foreign substance administered intravenously. This method is used to measure the elimination rate of carbon particles in the blood based on measuring carbon particle absorbance by using a UV-Vis spectrophotometer at 640.5 nm wavelength. Carbon will be reduced in number in the blood over time due to elimination or phagocytosis events by primarily neutrophil cells, monocytes, macrophages, and eosinophils [13].

Based on the classification of the effect of immunostimulation, the EEHP 200 mg/kg bw showed a strong immunostimulation effect, EEAHP 200 mg/kg BB showed a strong immunostimulation effect, ENHP 200 mg/kg BB showed a strong immunostimulation effect (table 1). The lower the concentration of eating phagocytosis index values will drop also

Table 1. Results of phagocytosis Index of Herb Poguntano

No	Treatment	Phagocytosis Index (Mean ± SEM)
1	Control	1.0499± 0.045
2	ENHHP 25	1.4277± 0.003
3	ENHHP 50	1.7696± 0.008
4	ENHHP 100	1.9178± 0.017
5	ENHHP 200	2.1607± 0.009
6	EEAHP 25	1.3603± 0.014
7	EEAHP 50	1.7258± 0.013
8	EEAHP 100	2.1767± 0.003
9	EEAHP 200	2.3767± 0.014
10	EEHP 25	1.4096± 0.016
11	EEHP 50	1.6922± 0.012
12	EEHP 100	1.9578± 0.004
13	EEHP 200	2.0477± 0.016
14	Imboost	2.6933± 0.069

The stimulation index is the result of comparisons between test groups and control groups. An immunostimulatory substance if the stimulation index is greater than 1 and immunosuppressive if the stimulation index is smaller than 1 [14]. If the phagocytic index of the test dosage has a value of less than 1, indicating the dosage is non-immunostimulation, the phagocytic index of 1-1.5 indicates a moderate immunostimulation effect and a phagocytic index of more than 1.5 exhibits a strong immunostimulation effect. The increasing phagocytic index in clearance carbon tests showed an increase in phagocytosis activity of macrophages and an increase in nonspecific immunity [15].

The carbon clearance test is conducted to observe the activity of the reticuloendothelial system in eliminating the carbon colloidal suspension of blood circulation. The macrophages responsible for the elimination process are mainly in the liver, and the rest are in the spleen. In general, the process of opsonization plays an important role in the process of phagocytosis of

foreign matter by macrophages in the liver and spleen [16]. Macrophages play an important role in all levels of body defense in both immune and congenital immunity. When the pathogens successfully pass the epithelial barriers, pathogenic bacteria will be phagocytosis by macrophages and digest using the enzyme lysosomal [17].

4. Conclusion

N-hexsan Extract, Ethyl acetate, and poguntano herb ethanol may increase phagocytosis activity. Extract of ethyl acetate Poguntano herb 200 mg/kg bw (EEAHP) has the best phagocytosis activity with a value of 2.376 which indicates there is a significant difference ($P < 0.05$) with another treatment group.

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