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Research Article

Immunostimulant Activity of Pegagan Embun Herbs Extract (*Hydrocotyle sibthorpioides* Lam.) With Carbon Clearance Method Towards Male White Mice

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ABSTRACT

Pegagan embun (*Hydrocotyle sibthorpioides* L.) has been used traditionally to cure various diseases such as anti-inflammatory, neutralizing toxins, dysentery, and others. This study aims to determine the immunostimulant effect of pegagan embun herbs extract using the carbon clearance method, the number, and the percentage of leukocyte cells. In this study, 25 male white mice were used, which were divided into five groups, groups 1, 2, and 3 given pegagan embun herbs extract in a dose of 10 mg/kgbw; 50 mg/kgbw; 200 mg/kgbw, group 4 was given 0,5% Na CMC, and group 5 were given stimuno 50 mg/kgbw. The preparation is given orally for seven consecutive days. On the eighth day, the phagocytosis index, number, and percentage of leukocyte cells were determined. The results showed that pegagan embun herbs extract has immunostimulant activity. The phagocytosis index of the 10 mg/kgbw dose group is 1.0211, 1.27796 for the 50 mg/kgbw, and 1.5250 for the 200 mg/kgbw. Then, the number of leukocytes of the 10, 500, and 200 mg/kgbw are 8.820/µL, 9.906/µL, and 10.790/µL, respectively. The percentage of lymphocyte cells are 58,5%, 60,6% and 62,8%, and for segment neutrophil cells were 26%, 27,6% and 30% for group 10, 50, and 200 mg/kgbw extract. Based on the results, that the pegagan embun herbs extract has immunostimulant activity against male white mice.

Key words: *Hydrocotyle sibthorpioides* Lam., Immunostimulants, Carbon clearance, Leukocyte, Eosinophil, Neutrophil, Lymphocytes.

INTRODUCTION

The body uses the immune system to maintain the integrity and protect against harm caused by various foreign objects or antigens. The immune system is a combination of cells, molecules, and tissues that have a role in resistance to infection. For this reason, the immune system is needed to maintain the integrity of the body against the dangers that can arise from various materials around the environment.[1]

The defense consists of non-specific (natural / innate) and specific (adaptive/acquired) immune systems. The non-specific immune system is the body's first line of defense against various microorganisms and can respond directly to antigens. Meanwhile, the specific immune system takes time to recognize the antigen before it can react.[1]

To repair or restore the body's immune system, compounds that are immunomodulators are used. At this time in the world of medicine, immunomodulators have a crucial role. That's because immunomodulators can help the body optimize the function of the immune system, which plays a role in the main defense system. There are three ways of working of immunomodulators, namely as immune restoration, immunostimulation, and also as immunosuppression. Immunomodulators can be sourced from nature or artificial (synthesis). Plants that are immunomodulators generally have specific and non-specific immunity-boosting activities.[1-3]

Indonesia is well known as a country rich in natural ingredients. The existence of natural materials in Indonesia itself has not been fully used as well as possible by the community. There are still many natural ingredients that are not known for their function and usefulness for the health of the body. Pegagan embun has the activity to cure fever, relieve swelling, diuretics, expectorant, anti-inflammatory, antibiotic, and neutralize toxins (detoxifications). The research conducted by Yolanda showed that pegagan embun extract could increase the activity and phagocytic capacity of macrophage cells, total leukocyte cell count, increase the percentage of lymphocyte cells.[3,4]

MATERIALS AND METHODS

Tools

This study used maceration bottles, funnels (Pyrex), 1 mL syringe (One Med), measuring pipettes (Pyrex), surgical scissors (Bertamed), beaker glass (Pyrex), animal scales (SF-400), analytical scales (Ohaus), stopwatch (Sewan), micropipette (Eppendorf), volumetric flask (Pyrex), sonde instrument, hemacytometer (Assistent), rotary evaporator (Buchi), UV-Vis spectrophotometer (Genesys 10S UV-VIS), Optilab microscope (Motic), animal cages, desiccators, ovens (Memmert), centrifuges (Gemmy PLC), slides (Slides), and TLC vessels.

Materials

The materials used were pegagan embun (*Hydrocotyle sibthorpioides* Lam.), distilled water (Andeska Laboratory), 70% ethanol (Andeska Laboratory),

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80% ethanol, silica gel 60 F_{254} (Sentana), physiological saline (Andeska Laboratory), rutin, $AlCl_3$, Na acetate 1 M, NaCMC 0.5%, Giemsa stain (Merck), china ink (Yamura), 1% acetic acid (Merck), Turk reagent (St. Reagensia), Mg, 2N HCl, $FeCl_3$, Liberman Burchard reagent, Mayer's reagent, Dragendorff's reagent, and stimuno (Dexa Medica, Batch No.: 462619).

Place of collection and identification of samples

The sample used for this study was pegagan embun taken in Batu Gadang Village, Lubuk Kilangan District, West Sumatra, Padang City. The plants were identified in Herbarium Andalas (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Andalas University (UNAND) Padang, West Sumatra.

Extract preparation

250 g of pegagan embun Simplicia powder has been finely ground, macerated using 70% ethanol as solvent. Put the dry Simplicia powder into the macerator, add 2.5 L of ethanol. Soak for the first six hours, stirring occasionally, then let stand for 18 hours and strain. Repeat the filtering process twice with the same type and amount. Collect all the macerate, then evaporate with a rotary evaporator until a thick extract is obtained.

Extract standardization

Non-specific and specific characterization of pegagan embun herb extract was carried out, non-specific characterization in the form of drying loss and total ash content. In contrast, specific characterization was the organoleptic test, identity parameter, the chemical content test of extract, and thin-layer chromatography.

Dosage

Using 3 variant doses of pegagan embun extract 10, 50, and 200 mg/kgbw.[4]

Immunomodulatory activity testing

Mice divide into five groups. The negative control group was given 0.5% Na CMC, and another three groups were given pegagan embun herb extract. The preparation of the test was carried out orally once a day for seven days. On the eighth day after administration, blood was taken in the tail capillary. Mice blood was taken in the 3rd, 6th, 9th, 12th, and 15th minutes after carbon injection, 25 µl of blood was taken and 4 ml of 1% acetic acid was added. Absorption was measured at a wavelength of 650 nm. The mice that have been tested for carbon clearance were sacrificed.[5]

Percentage of leukocyte types

One drop of blood is dripped on the object-glass and leveled with another object glass, then dried. After drying, add the methanol to coat the whole blood smear and leave it for 5 minutes. Add one drop of Giemsa solution, which has been diluted with distilled water (1:20), left for 20 minutes, rinsed with distilled water, dried. Calculate the number of eosinophil cells, stem neutrophils, segment neutrophils, lymphocytes, and monocytes under a microscope at 100X.[6]

Total leukocyte cell with haemocytometer

Fresh blood is taken with a leukocyte pipette up to 0.5 points. Add Turk solution to 11 points, shaken for 15-30 seconds, three drops discarded, next drop entered in the count room. Leave it for 3 minutes and then count the total leukocyte cells in the microscope in 40X.[7]

Data analysis

The data was obtained from the immunostimulant activity, leukocyte cell count, and total leukocyte cell count of the sample. The data was then statistically analyzed using one-way analysis of variance analysis (ANOVA) and followed by Duncan Multiple Range Test using IBM SPSS 26 version.

RESULTS AND DISCUSSIONS

The plant used in this research is pegagan embun. The plant identification was carried out at the Laboratory Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University (ANDA).

The process of pegagan embun herb extract begins with the collection of fresh samples. Then the sample was washed with running water to remove the dirt attached to the sample. Then the sample was dried by aerating. The dried samples were mashed using a grinder to obtain simplicia as much as 1037,0565 grams, then extracted using the maceration method using 70% ethanol as solvent. The maceration process produces a thick extract of 170,74 grams with a yield of 16.4646%. The extraction results were in accordance with the specifications in the Indonesian Herbal Pharmacopoeia.

The organoleptic examination was carried out, which showed that the viscous extract had a characteristic odor, dark brown color, and a bitter taste. The chemical content test of the extract or phytochemical test showed that the pegagan embun herb extract contained positive flavonoids, phenolics, saponins, and terpenoids.

In Thin Layer Chromatography, the comparison compounds used was rutin, for the stationary phase used was silica gel F_{254} and the mobile phase used were n-butanol: acetic acid: distilled water (4:1:5). TLC profile examination of the extract was carried out under UV light with a wavelength of 366 nm and using $AlCl_3$ stain viewer. From the results of the examination, the Rf value was 0,6.

Immunomodulatory activity of the extract of pegagan embun (*Hydrocotyle sibthorpioides* Lam.) herbs was test used experimental animal 25 male white mice (Mus musculus L.) BALB/c strain, aged 2-3 months, were divided into five groups. Groups 1, 2, and 3 were given pegagan embun herbs extract at doses of 10, 50, and 200 mg/kgbw, group 4 was given 0.5% Na CMC, and group 5 was given Stimuno at 50 mg/kgbw.

Mice were chosen because they are easy to obtain, relatively inexpensive, easy to handle, and physiologically similar to humans. To reduce deviations from the results of the study, mice with the same strain and sex, age, and weight were relatively the same. The immune system is also influenced by estrogen and testosterone, so male mice were chosen because they have more stable hormones than female mice.[8] The experimental animals used have met the ethical approval by the competent ethics committee. In this study, ethical approval was carried out by the Research Ethics Committee of the Faculty of Medicine, Andalas University, with approval number 274/UN.16.2/KEP-FK/2021.

The carbon clearance method was used to measure the activity of phagocytic cells in killing pathogenic organisms that enter the body. The carbon used as a marker is administered intravenously. Carbon clearance is seen at the times of testing at 3, 6, 9, 12, and 15 minutes. Carbon levels in the blood will decrease over time due to phagocytic events by leukocytes, especially by monocytes, neutrophils, eosinophils, and macrophages. The use of carbon as a marker has the advantage that the particle size is smaller and more stable so that the carbon does not cause blockage of blood vessels and lungs. Carbon also has characteristics as an antigen because of its isolation which is not normally found in the body.[9,10]

Carbon suspension was made using Na CMC with a concentration of 0.5% (w/v) and added with physiological saline to obtain a 64 mg/

ml concentration. The use of physiological saline in the manufacture of suspensions aims to ensure that the condition of the carbon suspension preparation (Chinese ink) is the same as that of the test animal body (isotonic). To see the phagocytic effect of the pegagan embun herbs extract, a standard curve was made between the carbon content in the blood and the absorbance value measured by a UV-Vis spectrophotometer. The carbon standard curve was determined with concentration series 40, 60, 80, 100, and 120 ppm. Measurement of the maximum absorption length is carried out running from a wavelength of 600-700 nm. The results of running show that the maximum wavelength of the carbon standard is at a wavelength of 636.5 nm. From the results of the determination of carbon obtained standard curve regression equation $y = 0{,}0056x + 0{,}1034$ and value of R^2 is 0,9959.

The value of the regression equation showed that there was a linear relationship between the carbon concentration in the blood of mice with the absorbance. The immunomodulatory pegagan embun herbs extract results were seen in the decrease in carbon absorbance every minute in the blood of male white mice that were given the test preparation for seven consecutive days. The reduced levels of carbon in the blood of the test animals at every minute of the test showed that the carbon concentration in the blood of mice was getting lower. It also showed an increase in the phagocytic activity of carbon in each group of extracts.

Carbon in the blood will stimulate the formation of a non-specific immune system in the form of phagocytic cells. Phagocytic cells that are active due to non-specific stimulants can quickly recognize the type of foreign antigen that enters the body and then destroy and clear the foreign antigen from the bloodstream. From the absorbance data, the value of the phagocytosis constant can be calculated.

The phagocytic constant is one of the parameters that show the speed of phagocytosis. The greater the rate of phagocytosis, the higher the phagocytic carbon clearance. After obtaining the value of the phagocytosis constant, the value of the phagocytosis index can be obtained. The average value of the phagocytic index is greater than 1, indicating that the test substance has immunostimulatory activity. However, if the average value of the phagocytic index is below 1, the test substance has immunosuppressant activity.

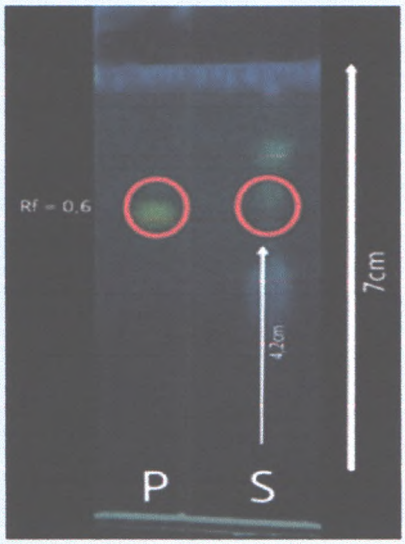

Figure 1: Thin chromatography profile of pegagan embun herbs. (S) pegagan embun extract, (P) rutin.

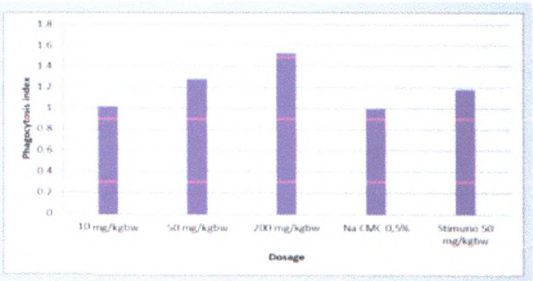

Figure 2: Graph of phagocytosis index value in male white mice given pegagan embun herbs extract for seven days.

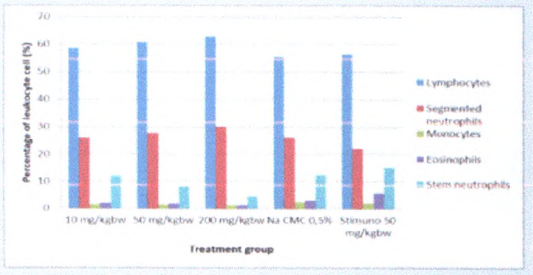

Figure 3: Graph of the percentage of leukocytes in male white mice given pegagan embun herbs extract for seven days.

Table 1: The results of the total leukocyte count in male white mice given pegagan embun herbs extract for seven days.

Total leukocyte (/µL blood)	
Doses	Mean ± SD
Na CMC 0,5%	7750 ± 554,5
10 mg/kgbw	8820 ± 148,3
50 mg/kgbw	9906 ± 658,9
200 mg/kgbw	10790 ± 624,9
Stimuno 50 mg/kgbw	9710 ± 653,2

From the data (Figure 2), it can be seen that the average phagocytosis index for pegagan embun herbs extracts 10, 50, and 200 mg/kgbw is greater than one (IF>1). So it can be said that the extracts of pegagan embun herbs at doses of 10 mg/kg, 50 mg/kg, and 200 mg/kg are immunostimulants. The highest phagocytic index value was indicated by the dose group of 200 mg/kg body weight, with a value of 1,5250. It can be concluded that the group with pegagan embun extract at a dose of 200 mg/kgbw had the best phagocytosis ability among the other dose groups. The results of the one-way ANOVA test obtained a significant result of 0.009 ($p<0.05$). These results indicate that there are substantial differences between the five groups in the phagocytosis index. Then proceed with Duncan's test analysis to see the effect of each treatment group. Duncan's test analysis results showed that the phagocytosis index at a dose of 10 mg/kgbw, stimuno, and a dose of 50 mg/kgbw had no significant difference. For the dose of 10 mg/kgbw and stimuno, there was a significant difference with the treatment group at a dose of 200 mg/kgbw. And for a dose of 50 mg/kg with a dose of 200 mg/kgbw, there is no significant difference.

The results of the total leukocyte cell count of the one-way ANOVA test obtained a significant result of 0.000 ($p<0.05$). These results indicate that there are significant differences between the five groups in total leukocyte cells and suggest that the hypothesis has been proven that

administration of pegagan embun herbs extract can increase whole leukocyte cells. The results of Duncan's test analysis showed significant differences in total leukocyte cells in all treatment groups. Still, between the stimuno treatment groups at a dose of 50 mg/kgbw, there was no significant difference. The highest total leukocytes count was found in the treatment group given the pegagan embun herbs extract at a dose of 200 mg/kgbw. Leukocytes play a role in fighting various germs that cause infection and foreign objects that enter the body. The total number of normal leukocytes in mice ranged from $6–15x10^3/m^3$. An increase in the number of leukocytes indicates a response to the body's resistance to disease-causing agents and is also associated with a stimulating effect on immune function and phagocytic capacity.[11-13]

The results of the calculation of the percentage of leukocyte cell types in male white mice after the administration of pegagan embun herbs extract. The highest percentages are lymphocytes, and the least are stem neutrophils. One-way ANOVA test showed that the pegagan embun herbs extract doses of 10, 50, and 200 mg/kgbw affected the percentage of lymphocytes, segment neutrophils, eosinophils, and stem neutrophils significantly ($p<0.05$), but did not show a significant difference ($p<0.05$). $p> 0.05$) to the percentage of monocytes.

The results of the study and data analysis showed that the pegagan embun herbs extract at doses of 10, 50, 200 mg/kgbw increased immunostimulant activity, increased the total leukocyte cell count, increased the percentage of lymphocyte cells, segment neutrophil cells and decreased the percentage of stem neutrophil cells. The immunostimulant activity of the pegagan embun herbs extract depends on the amount of concentration given, where the higher the concentration of the extract, the higher its potential as an immunostimulator. The greater the concentration of pegagan embun herbs extract given, the greater the content of active ingredients that can affect immunostimulant activity. The active compound which is thought to be immunostimulant in pegagan embun extract is flavonoids. The mechanism of flavonoids as immunomodulators is by increasing the activity of IL-2 (interleukin 2) and lymphocyte proliferation. CD4+ cells will affect lymphocyte proliferation and then cause Th-1 cells to be activated. Activated Th-1 cells will affect IFN- which can activate macrophages which are characterized by increased phagocytic activity quickly and more efficiently in killing antigens[14,15]

CONCLUSION

The administration of pegagan embun (*Hydrocotyle sibthorpioides* Lam.) herb extract at doses of 10 mg/kgbw, 50 mg/kgbw, and 200 mg/kgbw has immunostimulant activity, can increase the number of leukocytes, and the percentage of leukocytes in the lymphocyte and neutrophil segments in male white mice.

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CONFLICTS OF INTEREST

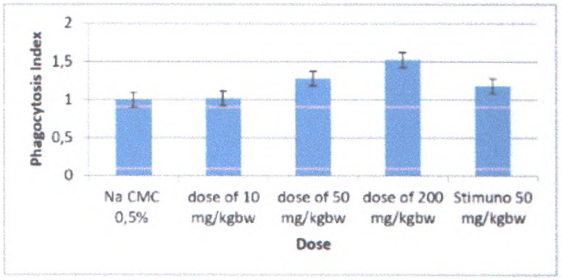
The authors declare that there is no conflicts of interest.

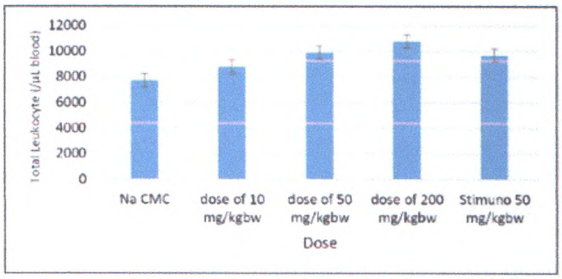
REFERENCES

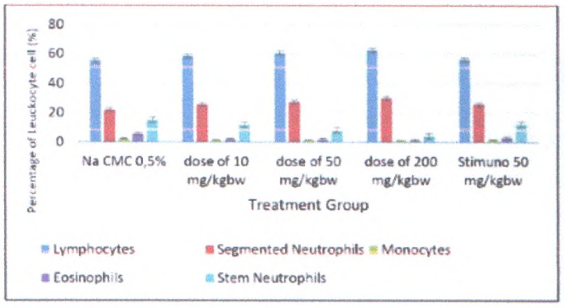
1. Bratawidjaja KG & RI. Imunologi Dasar. 2014.
2. Badrunasar A SH. Tumbuhan Liar Berkhasiat Obat. (Rachman E, Siarudin, eds.) Jawa Barat: Forda Press; 2016.
3. Setiawati A DY. Efek Sitotoksik Ekstrak Dietil Eter Herba Pegagan Embun (*Hydrocotyle sibthorpioides* Lmk.) terhadap Sel Kanker Payudara MCF-7. Farm Sains dan Komunitas. 2015;12(1):1-5.
4. Afriwardi, Aldi Y, Dillasamola D, Larakhansa YA, Badriyya E. Immunostimulatory activities of pegagan embun (*Hydrocotyle sibthorpioides* Lam.) in white male mice. Pharmacogn J. 2021;13(2):368-375.
5. Aldi Y, Dillasamola D, Yanti GR. Immunomodulator activity of ethanol extract of tapak liman leaves (*Elephantopus scaber* Linn.). Pharmacogn J. 2019;11(6):1419-1427.
6. Aldi Y, Dewi ON, Uthia R. Uji Imunomodulator Dan Jumlah Sel Leukosit Dari Ekstrak Daun Kemangi (*Ocimum basilicum* L.) Pada Mencit Putih Jantan. Sci J Farm dan Kesehat. 2016;6(2):139.
7. Aldi Y, Dillasamola D, Florina T, Friardi D. Test Immunomodulatory Effects of Ethanol Extract Skin of Purple Sweet Potato (*Ipomoea batatas* (L.) Lam) with Carbon Clearance Method and the Number of Leukocytes. Res J Pharm Biol Chem Sci. 2016;7(5):1192-1200
8. Aldi Y, Amdani A, Bakhtiar A. Aktivitas Senyawa Skopoletin dari Buah Mengkudu (*Morinda citrifolia*, Linn.) terhadap Respon Fisiologi Makrofag Mencit Putih Jantan. Sci J Farm dan Kesehat. 2016;6(1):25-35.
9. Aldi Y, Ogiana N, Handayani D. Uji Imunomodulator Beberapa Subfraksi Ekstrak Etil Asetat Meniran (*Phyllanthus niruri* [L]) pada Mencit Putih Jantan dengan Metoda Carbon Clearance. B-Dent, J Kedokt Gigi Univ Baiturrahmah. 2018;1(1):70-82.
10. Dillasamola D, Aldi Y, Fakhri M, Diliarosta S, Biomechy Oktomalio P, Noverial. Immunomodulatory Effect Test From Moringa Leaf Extract (*Moringa oleifera* l.) with Carbon Clearance Method in Male White mice. Asian J Pharm Clin Res. 2018;11(9):241-245.
11. Djaelani, M. A., Kasiyati dan S. Jumlah leukosit, persentase limfosit dan persentase monosit ayam petelur jantan setelah perlakuan penambahan serbuk daun kelor pada pakan. NICHE J Trop Biol. 2020;3(1):45-49.
12. Septianto RD, Ardana IBK, Sudira IW, Dharmayuda AAGO. Profil Hematologi Mencit Pasca Pemberian Jamu Temulawak Secara Oral. Bul Vet Udayana. 2015;7(1):34-40.
13. Pujastuti INE, Lestari SR, Gofur A. Gambaran Hematologi Mencit (Mus musculus) Model Toksisitas Subkronis. Scripta Biologica. 2017;4:75-78.
14. Lestari NLGD, Wiadnya IBR, Dewi LBK. Pemberian Filtrat Buah Pepaya (*Carica papaya* L.) Terhadap Titer Imunoglobulin G (IgG) Pada Kelinci Jantan (Orytolagus cuniculus) Dengan Teknik Hemaglutinasi. J Anal Biosains. 2017;4(1):6.
15. Putra B, Azizah RN, Nopriyanti EM. Efek Imunomodulator Ekstrak Etanol Herba Krokot (*Portulaca oleracea* L.) terhadap Tikus Putih (Rattus norvegicus) Jantan dengan Parameter Delayed Type Hypersensitivity (DTH). Jurnal Farmasi Galenik 2020;6(1):20-25.

GRAPHICAL ABSTRACT

Pegagan Embun
(*Hydrocotyle sibthorpioides* Lam.)







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