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Research Article

IMMUNOMODULATORY POTENTIAL OF ETHANOL EXTRACT OF HENNA LEAF (*Lawsonia Inermis L.)* AGAINST MACROPHAGE PHAGOCYTOSIS IN MALE MICE (*Mus Musculus*)

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ABSTRACT

Background: One of the herbal plants that can be used as an immunomodulator is henna leaves (Lawsonia inermis L.) that increase the function of the immune system. The purpose of this study was to determine the immunomodulatory potential of henna leaves on macrophage phagocytosis and to determine at what dose the ethanol extract of henna leaves has the potential for macrophage phagocytosis in male mice (Mus musculus).
Methods: The type of research used is analytical research using administering ethanol extract of henna leaves on increasing macrophage phagocytosis in male mice (Mus musculus) induced by Staphylococcus aureus, and to determine which dose is more effective in increasing phagocytosis using the phagocytosis test. The analysis method uses the ANOVA Statistical Test and the LSD Post Hoc Test.
Results: The study showed that the ethanol extract of henna leaves has an immunomodulatory effect based on the results of the One Way ANOVA statistical test and post hoc test (p-value <0.05). The dose of ethanol extract of henna leaves that has immunomodulatory potential is a dose of 100 mg/kgBW (34.33%), a dose of 200 mg/kgBW (46%), a dose of 300 mg/kgBW (74.66%) and a dose of 400 mg/kgBW (75.33%).
Conclusion: The study conclude that Ethanol extract of henna leaves has mmunomodulatory potential by increasing macrophage phagocytosis activity in male mice. Ethanol extract of henna leaves which is effective as an immunomodulator is 400 mg/kgBW which has a higher percentage of macrophage phagocytosis activity value compared to other dose variations, and is approximately the same as the positive control this is due to the number of phagocytic cells that actively carry out phagocytosis and is expressed as a percentage.

Keywords: *Immunomodulator, ethanol extract, henna leaves, macrophage phagocytosis*

INTRODUCTION

The immune system is a mechanism in the body that protects against pathogens such as bacteria, viruses, and parasites that can cause infections (Kresno, 2011). When the immune system does not function optimally, balance is maintained by specific and non-specific immune responses. Non-specific immune responses function as an initial defense when the body is exposed to antigens such as bacteria, viruses and harmful substances by increasing the phagocytosis process by macrophages. Phagocytosis is the process of particle absorption by cells, where macrophages are the main phagocytic cells that eliminate most foreign objects in the tissue.1

Immunomodulators are compounds that can restore the function of the immune system that is disturbed (immunorestoration), increase the function of the immune system (immunostimulant) or suppress the immune response (immunosuppression). Immunomodulators are drugs whose ability to prevent foreign substances from entering the body depends on the ability of the immune system to recognize foreign molecules or antigens on the surface of the foreign material and to make the right response to eliminate the antigen.2 Infectious diseases are one of the biggest public health problems in developed and developing countries. The World Health Organization (WHO) states that this disease is the main cause of death in children. According to WHO data in 2012, infectious diseases contributed 1-20% of deaths of children >5 years in Indonesia. Antibiotics are drugs used to treat infections caused by bacterial.3

Currently in Indonesia, the development of traditional medicine or often referred to as herbal medicine is popular because the side effects of synthetic medicine are greater than herbal medicine, making people more likely to choose herbal medicine, so that various studies on active plant and animal compounds so that they have antioxidants and are starting to develop, one of which is henna leaves (Lawsonia inermis L.) called henna or inai leaves in certain rural areas in Indonesia . Henna leaves (Lawsonia inermis L.) are one of the species of the Lawsonia genus and are included in the Lythraceae family. This plant also has properties as an antibacterial, antimicrobial, antifungal, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antiviral, antiparasitic.4

The active components of several secondary metabolites of plant immunomodulators are flavonoid compounds. Flavonoid compounds can increase the body's immune system and are able to fight irritation caused by viruses, bacteria, and other microbial infections. The mechanism of flavonoid compounds as immunostimulants is by increasing the stimulation of neutrophil oxidative activity, cellular phagocytosis and cytotoxicity.(Nielsen and Mice 2022). Phytochemical screening of henna leaf extract includes glycoside compounds, phytosterols, tannins, flavonoids and curcumin.5

Currently, there is no scientific data on the phagocytic activity of macrophage cells as one of the immunomodulatory parameters against the non-specific immune system of ethanol extract of henna leaves, so it is important to conduct further research. Therefore, researchers are interested in studying the immunomodulatory potential of ethanol extract of henna leaves (Lawsonia inermis L) against macrophage phagocytosis in male mice (Mus musculus).

METHODS

The type of research used is analytical research. The research plan is to study the effect of administering ethanol extract of henna leaves on increasing macrophage phagocytosis in male mice (Mus musculus) induced by Staphylococcus aureus, and to determine which dose is more effective in increasing phagocytosis using the phagocytosis test. The analysis method uses the ANOVA Statistical Test and the LSD Post Hoc Test.

RESULTS

Determination of henna nail leaf samples (Lawsonia inermis L.) was carried out at the Pharmacognosy-Phytochemistry Laboratory of Mandala Waluya University. The results of this determination were carried out to see that the plants used to guarantee the existence of their type or species. From the results of this determination, it is known that the plants used in this study are henna nail leaves (Lawsinoa inermis L.)

The results of the extraction of henna leaves (Lawsonia inermis L.) using the maceration method using 96% ethanol solvent and the percentage yield can be seen in table 1 below:

Table 1.Extraction Results of Henna Leaves (Lawsonia inermis L.)

Initial weight of simple substance (g)	Final extract feces (g)	Yield %
600	97	16.16

For immunomodulatory testing, mice were acclimated for 7 days to adjust to their environment. After that, the mice were divided into six groups, with each group consisting of 3 mice. Group I was given Stimuno® as much as 0.13 mg/kgBW as a positive comparison with the test sample. Stimuno® is formulated with maniram extract which has been proven to be an immunomodulatory agent by stimulating immune cell receptors and sending intracellular signals to cell receptors to further enhance cell function, is a herbal food supplement designed to strengthen the immune system.6 The second group was given 0.5% Na CMC. Na-CMC was chosen as a negative control because it does not contain active ingredients so it cannot provide pharmacological effects on experimental animals. Because Na-CMC has inert properties and provides a stable suspension, Na-CMC is also used as a suspension in the formulation prepared in this study. The third to sixth groups were given ethanol extract of henna leaves with different doses of 100 mg/kgBW, 200 mg/kgBW, 300 mg/kgBW and 400 mg/kgBW respectively for 7 days with oral administration. Oral administration of drugs is a common form of drug administration, and the oral administration method is used because it is easy to administer and relatively safe. The administration of the test preparation given for 7 days aims to increase the immune system of test animals against Staphylococcus Aureus bacterial infection.

On the 8th day, each mouse in this group was infected intraperitoneally with 0.5 ml of Staphylococcus Aureus bacterial suspension. Staphylococcus aureus was chosen because this bacteria is gram-positive and can bind giemsa dye clearly so that it is easy to count under a microscope. In addition, this bacteria does not have protein A, which acts as an antiphagocytic factor that prevents it from escaping phagocytosis by peritoneal macrophages (Hariyanti et al., 2015). The S.Aureus bacterial suspension used had a turbidity that met the McFarland turbidity criteria, after being infected with S.aureus bacteria and then waited for 1 hour,

all groups of test animals were dissected, peritoneal fluid was taken for macrophage phagocytosis testing. If a small amount of peritoneal fluid was detected, PBS solution was added to the peritoneal cavity of the mice. The purpose of this action is to help dissolve the large phagocyte cells attached to the organs of mice, which facilitates the process of taking the peritoneal fluid of the test animal, the aim is to encourage the macrophage cells attached to the organs of the test animal and to facilitate the collection of its peritoneal fluid.

After the peritoneal fluid is collected, apply a thin layer on the slide, fix with methanol for 5 minutes, color with 5% Giemsa stain, leave for 20 minutes, rinse with running water, and clean the slide with the peritoneal fluid. The fluid is dried and observed under a microscope with a magnification of 1000 x. Active macrophages and inactive macrophages can be distinguished by looking at Figure 1.

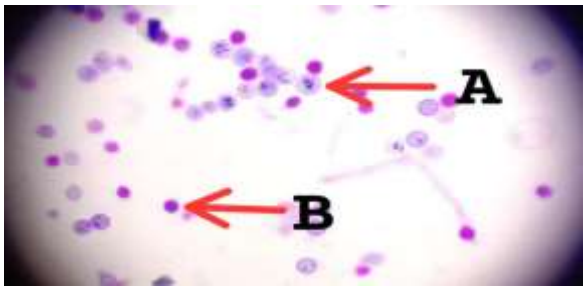

Figure 1. Active macrophages (A) and inactive macrophages (B)

Increased macrophage activity can be seen from changes in shape and increased size with very different pseudoplasma in the cytoplasm, a tortuous membrane, more liposomes, and an enlarged Golgi apparatus. In addition, the rough endoplasmic reticulum develops. The difference between active and inactive macrophages can be seen in Figure 11. This image shows a thin blood smear viewed under a microscope at 1000x magnification.

The immunomodulator test was carried out by calculating the phagocytic activity value of macrophage cells in the peritoneum of mice. The phagocytic activity of macrophages in the peritoneum of mice was calculated based on the percentage of the number of macrophage cells that actively phagocytose from a total of 100 cells (Fristiohady et al., 2020). After being expressed in percentage form, it can be seen in Table 2 and Figure 2

Table 2.Macrophage Phagocytic Activity Percentage

No.	Treatment	Average Activity Percentage (%)
1	Stimuno (Positive Control)	74.66 ± 3.51*
2	Na CMC (Negative Control)	28.33 ± 1.52
3	Henna Leaf Extract dose 100 mg/kg BW	34.33 ± 7.76*
4	Henna Leaf Extract dose 200 mg/kg BW	46 ± 14.42*
5	Henna Leaf Extract dose 300 mg/kg BW	74.66 ± 3.05*
6	Henna Leaf Extract dose 400 mg/kg BW	75.33 ± 6.65*

Values are expressed as mean ± SEM (n=3). P values were considered statistically significant against the negative control.

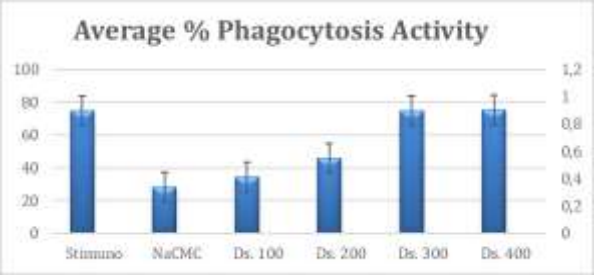

Figure 2.Average percentage of phagocytic activity data

Based on table 4, the average value of macrophage phagocytosis percentage in the positive group was 74.66%, the negative group was 28.33%, the 100 mg/kgBW dose group was 34.33%, the 200 mg/kgBW dose group was 46%, the 300 mg/kgBW dose group was 74.66% and the 400 dose group was 75.33%. It can be seen that phagocytosis activity increases along with the increase in the extract dose.

Based on phytochemical screening in research. that the ethanol extract of henna leaves (Lawsonia inermis.L) contains secondary metabolites in the form of glycosides, phytosterols, tannins, flavonoids and curcumin6. Flavonoid compounds can respond to lymphokines (Interferon γ) produced by T cells, thereby stimulating phagocytes to undergo phagocytic responses, encouraging lymphocyte proliferation, and increasing the number of T cells, can increase the secretion of interleukin-2. This is reinforced by research conducted which showed that galing extract significantly increased the phagocytic activity of macrophages in Balb/C mice, and galing plants do not have immune functions by causing proliferation is considered to have stimulating properties. Increasing the number of T lymphocyte cells can increase the proliferation of macrophage cells so that there is an increase in the secretion of cytokines such as IL-1, IL-6, IL-12, and TNF- -αso that bacterial phagocytosis occurs by macrophages.7

In addition, the phagocytosis activity data obtained were processed in SPSS version 23 using the one way ANOVA method. when testing with ANOVA (Analysis Of Variance) there are two requirements that must be met, namely the data must be normal and homogeneous. Regarding the normality of the data obtained using the Shapiro-Wilk Test, the data population is normally distributed if the Kolmogrov Sminov test results (p)> 0.05, because the data tested is less than 50..

The normality test shows that the data is normally distributed, and the homogeneity test also shows that the data is homogeneous. The purpose of the normality test is to determine whether the data is normally distributed or not. The level of normality is very important because for normally distributed data, the data is considered to be able to represent the population if the significance value obtained is P> 0.05 and the homogeneity test is one of the analysis requirement tests, in addition to the normality test is intended to show that two or more groups of sample data come from a population that has the same variation if the significance obtained is p> 0.05.

DISCUSSIONS

One Way ANOVA test was conducted with the aim of determining whether there is a significant difference between the average of more than 2 groups in this case the potential and effective dose of 4 variations in the dose of henna leaf ethanol extract (Lawsonia inermis L.) to increase the phagocytic activity of macrophage cells. The results of the one-way One Way ANOVA test obtained a significance value of p> 0.05, which is 0.000. This shows that the data for each concentration is significantly different, meaning that the HO hypothesis (No significant difference) is rejected and H1 (there is a significant difference) is accepted. To ensure that the administration of the extract at doses of 100 mg/kgBW, 200 mg/kgBW, 300 mg/kgBW and 400 mg/kgBW is effective in increasing the phagocytic activity of macrophage cells, statistical analysis was continued using LSD. If the p-value> 0.05 then there is no

difference or almost the same. If p <0.05 means there is a difference at each concentration.

The LSD test data in Table 6 shows a significant difference between the positive control group treated with doses of 100 mg/kgBW and 200 mg/kgBW, there is a significant difference which means that the effectiveness of the doses of 100 mg/kgBW and 200 mg/kgBW with positive controls is different, while 300 mg/kgBW and 400 mg/kgBW are not different or almost the same as positive controls. In addition, the negative treatment group showed a significant difference from various negative controls at doses of 200 mg/kgBW, 300 mg/kgBW and 400 mg/kgBW there was a significant difference which means that the effectiveness of the doses of 200 mg/kgBW, 300 mg/kgBW and 400 mg/kgBW with negative controls was different. Then the treatment group showed that the dose of the dose variant group had a significant difference between each other, which means that the effectiveness of each was different. This is because the average number of active macrophage cell phagocytosis at dose variations is different from each other. Based on the results obtained, the extract of henna leaves (Lawsonia inermis L.) can be used at doses of 100 mg/kgBW, 200 mg/kgBW, 300 mg/kgBW and 400 mg/kgBW has activity as an immunomodulator by increasing the phagocytic activity of macrophage cells where the doses of 300 mg/kgBW and 400 mg/kgBW have a higher average than other groups.

In the research 8 namely where the activity of macrophage phagocytosis from several groups that have an effect, namely groups A, B, C, D, F and G from the Hibiscus sabdariffa L. fraction contains flavonoid compounds that have activity as immunostimulants. Fraction A shows the best activity among all fractions. The results of the identification of chemical compounds from the Hibiscus sabdariffa L. fraction show that some of the compounds contained have the potential as immunomodulators. While group E does not have an effect like the other groups

The results of the study showed that the ethanol extract of henna leaves (Lawsonia inermis L.) has immunomodulatory potential, the effectiveness of which is 74.66% in the 300 mg/kgBW dose group and 75.33% in the 400 mg/kgBW dose group with a macrophage phagocytosis test using mice. This is supported by research(Mikhaeil et al. 2004), ethanol extract of henna leaves at a concentration of 1 mg/ml has shown immunostimulant action, which has been seen from the increase in the proliferation response of T lymphocytes. As many as seven compounds were isolated through fractionation guided by the lymphocyte transformation test (LTA) from the total methanol extract of henna leaves.

CONCLUTION

Based on the results of the research that has been done, Ethanol extract of henna leaves (Lawsonia Inermis.L) has immunomodulatory potential by increasing macrophage phagocytosis activity in male mice (Mus musculus). Ethanol extract of henna leaves (Lawsonia inermis.L) which is effective as an immunomodulator is 400 mg/kgBW which has a higher percentage of macrophage phagocytosis activity value compared to other dose variations, and is approximately the same as the positive control (Stimuno®) this is due to the number of phagocytic cells that actively carry out phagocytosis and is expressed as a percentage (%).

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