Effectiveness of Dose Concentration of Ethanol Extract of Iles-iles Tubers on Increasing Number of Macrophage Cells and Weight of Immune Organ Weight in White Rats Wistar Strain

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Abstract

This study was conducted to test the activity of macrophages, since macrophages play an important role in the immune response and to analyze the phagocytic activity due to the effects of flavonoids and polysaccharides iles iles on the immune response. The purpose of this study was to analyze the effectiveness of dose concentration of ethanol extract iles-iles to the increased number of macrophages and the immune organ weights heavy on white rats Wistar strain. This study is a laboratory experimental study of white wistar strains. This research was conducted at Pharmacology Laboratory of Padjadjaran University of Bandung and conducted in May-June 2017. Wistar strain of white rat of 30 tails is divided into 5 groups. Wistar strain white mice in each treatment were administered orally for 7 days on June 1 to 7, 2017. The effectiveness of the effect parameter dose concentration of ethanol extract iles-iles to the increased number of macrophages and the immune organ weights heavy on white rats Wistar strain microscopically are an increase in the number of macrophages and macroscopically is an increase in the weight of the immune organ, namely the liver, spleen and thymus. Microscopically through macrophage images assessed after 1, 3 and 6 h hour inoculation of S. aureus. The results showed that the number of macrophages in the group of ethanol extract iles-iles dose of 220 mg /kg on treatment after 1, 3 and 6 h higher than the group of ethanol extract iles-iles dose of 110 mg / kg and 55 mg/kg ( P <0.05), as well as comparison with the Tragakan and aqueduct negative control group (p <0.05). Thus, the ethanol extract of iles-iles (Amorphophallus variabilis Bl.) Effective for concentration dose of ethanol extract of iles-iles 220 mg/kg as an immunostimulant and as effective enhancement of immune response is better compared to the control comparator tragacanth 0, 3%.

Keywords: Ethanol Extract, Dosage, Number of macrophages, Weight of Immune Organ, Iles-iles Tubers

Introduction

Indonesia is known as the largest biodiversity mega centre in the world that consists of tropical plants and marine life. In the Indonesian territory there are about 30,000 species of plants and 7,000 of them are thought to have medicinal properties.

About 90% of medicinal plants in Asia, growing in Indonesia. In developed countries, biodiversity prospecting is a search for biological resources that have potential for the future, continue to be activated including research of various plants as a source of medicinal materials.1

Iles-iles (Amorphophallus variabilis Bl.) Belong to the Amorphophallus clan and belong to the taro tribe (Araceae). Iles-iles tuber plant is one of the plants used as a natural medicine. Iles-iles bulb is efficacious as a medicine for ulcers, irises and wounds from the bites of venomous animals. Iles-iles tubers contain chemicals: water, glucomannan, saponins, flavonoids, starch, protein, fat, crude fiber and calcium oxalate. While iles-iles leaf has a chemical content of tannins. Natural chemical ingredients found in iles-iles tubers that have anti-inflammatory activity and spur macrophage activity so that boost the immune system are flavonoids, saponins and polysaccharides.2 Given its wide-ranging spectrum especially in infectious diseases, it is suspected that the effects are positive effects immunostimulator.3
The efficacy of traditional medicine can be caused by chemical compounds contained therein; on the other hand can also cause toxic effects. To know the degree of safety of a substance, some studies are needed, among others, acute toxicity, sub chronic toxicity, carcinogenic, teratogenic and mutagenic. From the results of previous studies 70% of iles-iles tuber efficacious antihiperuresemia.4

Iles-iles bulbs contain several chemical elements, including flavonoids, saponins and polysaccharides.5 the effects of flavonoids on various organisms are very diverse, therapeutic effects caused by flavonoids include, among others, ant allergic, anti-inflammatory, anti-inhibiting tumor growth, the effect is caused by the effect flavonoids to arachidonic acid metabolism.6

Macrophages are one of the cells that play an important role in the immune response, both functional role in phagocytosis and its role as antigen presenting cells (APC). In doing both roles, the help of endogenous mediators such as cytokines is definitely needed. While the need for exogenous mediators such as carotenoids and flavonoids, still need in-depth research.7

The purpose of this study was to determine the effectiveness of dose concentration of ethanol extract of iles-iles bulb increase of macrophage cell number and weight of organ immunity weight in white rat of wistar strain.

Based on the results of the literature review above and the absence of research on ethanol extract of iles-iles tubers, it will be investigated the effectiveness of dose concentration of ethanol extract of iles-iles tuber (Amorphophallus variabilis Bl).

To macrophage cell number and weight of organ immunity in white rat Rattus norvegicus) wistar strains. The effectiveness of dose concentration of iles-iles bulb extract plays a role to see the immune response. Determination of immune response by measuring the number of macrophages and changes in the weight of the immune organ. This study was to analyze the amount of macrophages and changes in the weight of the immune organ due to the effects of flavonoids and polysaccharides on this immune response. The results of this study are expected to provide information and description to the public about the use of dosage concentration of iles-iles bulbs extract, so that can be used as a reference for the next use in the utilization of iles-iles tubers as medicine.8

Method

The research was experimental laboratory with post test design only control group design. This experiment using animal tried of white mouse strain wistar strain divided into 5 treatment groups. The subjects were 30 white rats (Rattus novergicus) strains of wistar obtained from Pharmacology Laboratory of Universitaas Padjadjaran Bandung. White mice should be given treatment of adaptation to laboratory conditions that will be used before being given treatment for 7 days on 25-31 May 2017, at Pharmacology Laboratory Faculty of Medicine, University of Padjadjaran. After adaptation period rats should not exceed 250-300 grams or sickness.

The mice must meet the following inclusion criteria: healthy mice with characteristics: clean rat hair and no injuries to the body, active mice, Wistar 2-3 month old rats and 250-300 gram weight, healthy condition and no physical abnormalities. The experimental exclusion criteria of the experimental animals were as follows: weight loss during adaptation in the laboratory for 7 days > 10% of initial body weight and ill appearance during adaptation period in the laboratory. The drop out criterion is a dead rat during adaptation and treatment.

The materials used in this study were: iles-iles ilanol ilanol extract. Iles-iles bulb, the result of plant determination at Herbarium Bandungense School of Life Sciences and Technology ITB stated that the plant is Amorphophallus variabilis Bl. of the Araceae tribe. Iles-iles tubers as test materials were taken from plantations in depok and majalengka areas, West Java. The selected bulbs are the adult iles-iles bulbs that are ready for harvest, then extracted at the Pharmacy Laboratory of Bandung Institute of Technology (ITB), sterile aqueduct (aquabidest), 0.9% pyrogen free NaCl, 70% alcohol and absolute methanol, EDTA anticoagulants and a turk solution containing 1% gentian violet in water and glacial acetic acid, 20% gyemsa modified
solution solution, 0.3% trace, an in vivo hank (Hank's balanced salt solution) growth medium solution, a phosphate buffer saline (PBS) solution and chloroform solution, as anesthesia at the time the animal attempts to be used.

The tools used in this research are: weight measuring devices (animal weights, Agis digital scales (0-2000 gram scale) with precision 0.01 grams), adaptation equipment of experimental animals (mouse cage, mouse drinking place, feeding tube FR-5), equipment of extracting iles-iles iles (eg, rotary evaporator, oven) ethanol extractor, orally (5 ml oral injection with 18 gauge bottle, for storing dose), culture equipment Staphylococcus aureus ATCC 25923 (ose/cruciate, reaction tube, Petri dish, fire bunsen, micro pipette/pipette Eppendorf, pipette, McFarland 0.5 turbidity gauge), equipment count leukocyte count (Eppendorf blood sample tube, hemosimeter, Sysmex XE 5000) peritoneal discharge equipment (minor surgical device, 500 ml Erlenmeyer, 1 cc, 3 cc, 5 cc and 10 cc injection syringe), makrofag smear making apparatus (glass and object glass cover, immersion oil, centrifugate r 2500 rpm), the tools for calculating and viewing macrophage cell activity are light microscopes (Olympus®) with 1000x magnification and LCD Notebook and documentation equipment are digital cameras.

The sample selection was done by simple random sampling which then divided into five groups. Minimum sample sizes are calculated by using the Federer formula (r: number of samples, t: number of treatments) (r-1) (t-1) ≥15. The minimum sample size for each treatment group should be greater than or equal to 5. The experimental animal must meet the criteria to be sufficient number of samples, then the animal required in this study is 30 heads.

The experimental animals used in this study were white rat strains of wistar strains aged 2-3 months, weight 250-300 grams. Prior to use as a trial animal, all white rats were nurtured for about one week for environmental adjustment, controlling health and weight and uniform foods.

Selected iles-iles tubers, cleaned with clean running water, then sliced as thinly as possible with a stainless steel knife, in order to avoid adverse chemical reactions. The iles-iles tube is then weighed to obtain the weight. Then iles-iles tubers are dried in a room that is not exposed to direct sunlight for 2 days. The special feature of the already dry plant is the weight of the tuber has been reduced by 70-80% of the fresh tuber's weight, and is called simplicia. The simplicia is then smoothed to a powder with a grinding tool, then weighed so that the weight is obtained before it is extracted.

The simplicia is then inserted into the maserator which has been given cotton pad and ethanol solvent, stuck for 24 hours, then removed from the maserator outlet, and is called aqueous extract. Add new solvent to the dregs present in the maserator, and so on until the solvent out of the maserator outlet is colorless (usually after 5-6 times the bath). The dilute extract obtained is then concentrated by means of a rotary evaporator until solid or until no more solvent is dripped in the rotary evaporator condenser. Concentrated extract is usually shaped pasta.

This dose obtained the result of the conversion of the usual dose in humans with the following calculations: Net weight simpilisia iles-iles 1500 gram bulbs; The net weight of ethanol extract of iles-iles bulb is 41.6 grams (± 2 wt% simplisia); The usual dose of simplicia in humans per day 3 x 1 tablespoon of simplicia powder = 3 x 10 grams = 30 gr simplicia; Dose of iles-iles ilanol extract on humans (70 kg) per day 2% x 30 gr simplicia 0.6 gr ethanol extract of iles-iles bulb; Dose of iles-iles ilanol ilanol extract in mice (200 mg) per day 0.018 x 0.6 gram = 0.0108 gram = 10.8 mg ≈ 11 mg = 55 mg/kg BW.

Twenty rats were divided into 5 treatment groups for sub-chronic toxicity tests. Then the mouse was adapted for 7 days with pellet giving, in order for healthy mice. The treatment group was performed for 7 days, to see macrophage phagocytosis activity by giving oral extracts with each treatment as follows: Group I (iles-iles iles-iles bulb extract dose 220 mg/kg BB); Group II (iles-iles tuber extract of dose 110 mg/kgBB); Group III (iles-iles ilanol extracts bulb extract dose 55 mg/kg BB); Group IV (positive control using comparison using 0.3% Tragakan and Group V (negative control using 5 ml aqueduct).

The dose series of ethanol extract of iles-iles tubers was made by dissolving a number of x mg of ethanol extract of iles-iles bulb added
solvent (aquadest solution of 5 ml/white mouse tail of wistar strain). Aquadest is used as both normal solvent and control controls. On day 7 the mice were injected with S. aureus intraperitoneally (i.p) with a dose of 108/mice in 0.5 ml of 0.9% physiological solution, then left in 1, 3 and 6 hour intervals.

After administration of ethanol extract for 7 days, then exudate the peritoneal fluid exudate of the mice by injecting the Hank's balanced salt solution medium into the mice's abdominal cavity by 3 ml, and after all its cells descend from the tube, the fluid was centrifuged 2500 rpm in 8-10 min, discarded his supernatant. Cells precipitated first after that discarded the supernatant that remains.

The supernatant suspension were dripped ± 0.5 ml over prepared glass prepared, prepared smear and fixed with methanol for 5 minutes, stained with 20% Gyemsa modified solution, rinsed with aquadest and dried. Preparations are seen under a light microscope with 1000x magnification. The activity of phagocytosis is determined by the percentage of phagocytes that perform phagocytosis from 100-200 macrophages.

The mice that had been extracted iles-iles etanol extract for 7 consecutive days were dissected and the mice were mutated and dissected, then the liver, spleen and thymus were isolated and weighed for a percentage of the organ index. The significance of the test group's organ index's to the control is determined based on statistical results.

After the research results collected, then performed data processing in the following order: Data obtained from the percentage of the number of active macrophages and total number of macrophages, index changes of weight of the immune organ, from each group has been collected, the initial analysis is tested data distribution; Data were analyzed using one-way ANOVA test to see whether there were differences in treatment effect between positive control group, negative control, and treatment group (iles-iles bulb ethanol extract); If the test results are significant, then it is continued with the Duncan test with a significance level of 5% (p <0.05).

To complement the results of the comparison between the five observation groups, graphs are drawn into bar charts and the mean of each variable.

In this experiment, we use the experimental animal of white strain of wistar strain to replace humans, because new substances or tools should not be used for the first time in humans, unless they have been tested in animals and have sufficient impression about their safety. The experimental animals used for health research for humans will mostly experience unpleasant things for the animals, such as animals will experience inconvenience, discomfort, distress, pain, and death (death). Later in this study applied the principles of animal treatment in accordance with the basic principles of ethical considerations of health research, namely the principle of 3R (Replacement, Reduction, and Refinement).

The research was conducted at Pharmacology Laboratory of Faculty of Medicine Universities Padjadjaran, Microbiology Laboratory of Medical Research Unit (UPK) of Faculty of Medicine UNPAD/Dr. Hasan Sadikin Bandung, and Department of Clinical Pathology of Dr. Hasan Sadikin Bandung. The study was conducted from May to June 2017.

Results

The subjects consisted of 30 rats of wistar strains that had passed the adaptation period in the laboratory for seven days, fulfilled the inclusion criteria, and did not meet the exclusion criteria. The results of the research on the effectiveness of dose concentration of ethanol extract of iles-iles tubers were done by comparing with positive control (Tragakan 0,3%) and negative control (Aquadest). After the research results collected, then performed data processing with the initial analysis of data distribution testing, then further analysis of ANOVA test to see whether there is difference of treatment effect given between positive control group and negative control group. If the test results are meaningful, then proceed with multiple comparison using Bonferroni Method (p <0.05). To complement the results of the comparison between the five observation groups, a graphical representation was made to the bar chart and the mean of each variable.

Extraction of 20 kg of iles-iles tubers by percolation method yielded 156,1297 gr of
pasta extract. The results of phytochemical screening show that iles-iles bulb extract contains proteins, carbohydrates, alkaloids, saponins and triterpenoids (table 1). Alkaloids and triterpenoids are low molecular weight compounds and polysaccharides which are components of carbohydrate compounds that could potentially be immunostimulants.

The results of phytochemical studies of ethanol extract of iles-iles tubers showed that the extract contained compounds that have the potential to stimulate the immune system. The main content of ethanol extract of iles-iles bulbs used in this study is carbohydrate that is 85%. The results of phytochemical screening in ethanol extract of iles-iles bulbs are alkaloids, saponins and triterpenoids.

The mean number of intergroup macrophages increased per hour, in which the number of macrophages from groups A (iles-iles bulb ethanol extract dose 220 mg/kg BW) was higher than the B and C groups as well as the positive control group (D) as well as the negative control (E). The mean number of intergroup macrophages, which can see significant results of significance (p <0.05) after 1, 3 and 6 hours of inoculation of *S. aureus*. Ethanol extract of iles-iles bulbs can significantly increase the number of macrophages (p = 0.001).

The analysis continued with the Bonferonni Test to see the differences in each group compared with the controls. The highest average difference was seen in the Tragakan group of 0.3% and significantly (p <0.05) compared with the controls. The third group of doses had a significant mean difference (p <0.05) compared with the controls. An increase in the number of macrophages can illustrate the effect of iles-iles bulb ethanol extract on the immune response. This is because it affects bone marrow synthesis to produce leukocyte cells as well as red blood cells.14

The effect of ethanol extract of iles-iles bulbs on the number of macrophages can be explained in the graph of the relationship between time and number of macrophages. When seen in the number of macrophages due to the extract of ethanol iles-iles tubers (Figure 1), the sharpest slope of the line occurred in groups A treatment (dose 220 mg/kg BB) and followed by groups B, C, D and E. This indicates that all doses of ethanol extract of iles-iles tubers can eliminate the amount of macrophages quickly enough in every time even though the velocity is still below grouped a (dose 220 mg/ kg BW). Combined in each group is shown in Table 1.

Table 1: Difference in the average number of active macrophages between groups after 1, 3 and 6 hours

<table>
<thead>
<tr>
<th>No.</th>
<th>Makrofag Total</th>
<th>Etanol Extract 220 mg/kgbb (A)</th>
<th>Etanol Extract 110 mg/kgbb (B)</th>
<th>Etanol Extract 55 mg/kgbb (C)</th>
<th>Tragakan 0.3% (D)</th>
<th>Aquadest (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Active Makrofag 1 jam</td>
<td>157.83</td>
<td>56.83</td>
<td>92.83</td>
<td>53.17</td>
<td>46.83</td>
</tr>
<tr>
<td>2</td>
<td>Active Makrofag 3 jam</td>
<td>163.33</td>
<td>160.67</td>
<td>157.5</td>
<td>159.17</td>
<td>56.83</td>
</tr>
<tr>
<td>3</td>
<td>Active Makrofag 6 jam</td>
<td>143</td>
<td>149.5</td>
<td>126.5</td>
<td>128</td>
<td>61.5</td>
</tr>
</tbody>
</table>

The average number of macrophages after 1 hour in the ethanol extract group of iles-iles tubers 220 mg/kg BW (A) were 157.83, the ethanol extract group of iles-iles tubers 110 mg/kg BB (B) was 56.83, the ethanol extract group iles-iles bulb 55 mg/kg BB (C) was 92.83, the positive control group (D) was 53.17, and the negative control group (E) was 46.83.

The average number of macrophages after 3 hours in the ethanol extract group of iles-iles tubers 220 mg/kgBB (A) were 163.33, the ethanol extract group of iles-iles tubers 110 mg/kg BW (B) was 160.67, the ethanol extract group iles-iles bulb 55 mg/kg BB (C) was 157.5, the positive control group (D) was 159.17, and the negative control group (E) was 56.83.

The average number of macrophages after 6 hours in the ethanol extract group of iles-iles tubers were 220 mg/kg BW (A) was 143, etanol extract group of iles-iles iles-iles 110 mg/kg BB (B) was 149.5, ethanol extract group of iles tubers 55 mm/kg BB (C) is 126.5, the positive control group (D) is 128, and the negative control group (E) is 61.5. Based on the data, it is seen that the average number of macrophages after 1, 3 and 6 hours in group A is bigger than group B, C, D and E. From the analysis results obtained difference of average number of macrophages in the five treatment groups. The results

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were then tested with a 95% confidence level (p < 0.05) and the results were significantly different after 1, 3 and 6 hours. Bonferroni tests results showed a significant difference in the number of macrophages among 5 groups. Phagocytosis activity of rat peritoneal exudate mouse was evaluated by looking at its ability to eat S. aureus as a test bacterium. The peritoneal exudates consist of 90% macrophages and the rest are monocytes and other cells. The effect of ethanol extract of Iles-iles bulb on the activity test of mouse peritoneal exudate macrophage phagocytosis of mice can be explained in table 3 below.

The mean inter-group phagocytic index, after 1, 3 and 6 hours of inoculation of S. aureus (p < 0.05). Ethanol extracts of Iles-iles bulbs can significantly increase phagocytic index (p = 0.001). An increase in the phagocytic index may provide an illustration of the effect of Iles-iles bulb ethanol extracts on the immune response.

This is due to the content of Iles-iles bulbs such as flavonoids and polysaccharides that can act as immunostimulants, thus increasing metabolic activity in macrophage cells. Increased metabolism in the cells will increase the enzymes and other substances that play a role in phagocytosis, so the ability of phagocytosis is increasing.14

Other results of this study are consistent with findings where the inflammatory process in peritonium fluid is closely related to the function of leukocyte work in the body's defense mechanisms, especially in marking with increasing PMN (Polymorphonukler). The flavanoid content of Amorphophallus variabilis Bl. on Iles-iles tubers have anti-inflammatory properties.

This is due to the bioactive component of Iles-iles tubers can increase the activity of macrophages to eat foreign objects in this case S. aureus, which will lead to the process of phagocytosis in the macrophages that cause lysis of bacterial cells. B-D-glucans may activate peritoneal macrophages such as lysosomal enzymes, phagocytic activity and H2O2 production. Macrophages recognized B-D-glucans through TLR-2 receptors or complementary receptor 3 (CR3, which is denoted as a combination of CD11 b / CD18, (M, 2 and MAC1) which is one part of Pattern recognition receptors (PRR). -D-glucans with CR3 directly will be the trigger for the phagocytosis process.15

Data on average difference in the number of active macrophages between groups. The difference can be seen in Figure 2 below:

<table>
<thead>
<tr>
<th>Time</th>
<th>Experimental Groups</th>
<th>N</th>
<th>Active Makrofag</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Hour</td>
<td>Iles-iles Extract Dose 220 mg/kg</td>
<td>6</td>
<td>(157.83 ± 40.98)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 110 mg/kg</td>
<td>6</td>
<td>(56.83 ± 27.09)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 55 mg/kg</td>
<td>6</td>
<td>(92.83 ± 36.00)</td>
</tr>
<tr>
<td></td>
<td>Tragakan 0.3%</td>
<td>6</td>
<td>(53.17 ± 28.83)</td>
</tr>
<tr>
<td></td>
<td>Aquadest</td>
<td>6</td>
<td>(46.83 ± 5.49)</td>
</tr>
<tr>
<td>3 Hours</td>
<td>Iles-iles Extract Dose 220 mg/kg</td>
<td>6</td>
<td>(163.33 ± 28.17)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 110 mg/kg</td>
<td>6</td>
<td>(160.67 ± 29.90)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 55 mg/kg</td>
<td>6</td>
<td>(157.50 ± 36.82)</td>
</tr>
<tr>
<td></td>
<td>Tragakan 0.3%</td>
<td>6</td>
<td>(159.17 ± 33.77)</td>
</tr>
<tr>
<td></td>
<td>Aquadest</td>
<td>6</td>
<td>(56.83 ± 12.35)</td>
</tr>
<tr>
<td>6 Hours</td>
<td>Iles-iles Extract Dose 220 mg/kg</td>
<td>6</td>
<td>(143.00 ± 12.00)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 110 mg/kg</td>
<td>6</td>
<td>(149.50 ± 12.04)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 55 mg/kg</td>
<td>6</td>
<td>(126.50 ± 19.52)</td>
</tr>
<tr>
<td></td>
<td>Tragakan 0.3%</td>
<td>6</td>
<td>(126.00 ± 20.93)</td>
</tr>
<tr>
<td></td>
<td>Aquadest</td>
<td>6</td>
<td>(61.50 ± 11.19)</td>
</tr>
</tbody>
</table>

Table 2: Average increase in number of active macrophages after 1, 3 and 6 hours of inoculation S. aureus
The experimental results showed a significant increase in the amount of macrophages that bacterial phagocytosis were significant (p <0.001) in the three doses of iles-iles ilanol ethanol extract, Tragakan group 0.3% (positive control) and aquadest group (negative control). The analysis was continued by Bonferroni method to see the difference of each treatment group compared with the control.

Percent weight of the liver, spleen and thymus gland can be used as one of the parameters to assess the immune response. Increased organ weight is expressed in percent against body weight (% of organ index). Elimination of S. aureus 90% occurs in the liver by kuffer cells. Increased proliferation of liver cells, spleen and thymus gland and the weight of these organs provide an indication of increased immune response, whereas decreasing the weight of these organs means a decrease in the immune response. The effect of ethanol extract of iles-iles bulbs on organ weight can be seen in Table 4 below.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Experimental Groups</th>
<th>N</th>
<th>Immune Weight After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepar</td>
<td>Iles-iles Extract Dose 220 mg/kg</td>
<td>6</td>
<td>(3.34 ± 4.26)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 110 mg/kg</td>
<td>6</td>
<td>(0.97 ± 0.33)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 55 mg/kg</td>
<td>6</td>
<td>(0.95 ± 0.35)</td>
</tr>
<tr>
<td></td>
<td>Tragakan 0.3%</td>
<td>6</td>
<td>(1.18 ± 0.38)</td>
</tr>
<tr>
<td></td>
<td>Aquadest</td>
<td>6</td>
<td>(1.24 ± 0.25)</td>
</tr>
<tr>
<td>Limp</td>
<td>Iles-iles Extract Dose 220 mg/kg</td>
<td>6</td>
<td>(1.07 ± 0.57)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 110 mg/kg</td>
<td>6</td>
<td>(0.79 ± 0.17)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 55 mg/kg</td>
<td>6</td>
<td>(1.45 ± 0.51)</td>
</tr>
<tr>
<td></td>
<td>Tragakan 0.3%</td>
<td>6</td>
<td>(1.24 ± 0.48)</td>
</tr>
<tr>
<td></td>
<td>Aquadest</td>
<td>6</td>
<td>(1.16 ± 0.50)</td>
</tr>
<tr>
<td>Thymus</td>
<td>Iles-iles Extract Dose 220 mg/kg</td>
<td>6</td>
<td>(7.11 ± 4.96)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 110 mg/kg</td>
<td>6</td>
<td>(9.59 ± 0.78)</td>
</tr>
<tr>
<td></td>
<td>Iles-iles Extract Dose 55 mg/kg</td>
<td>6</td>
<td>(9.29 ± 0.33)</td>
</tr>
<tr>
<td></td>
<td>Tragakan 0.3%</td>
<td>6</td>
<td>(8.78 ± 0.52)</td>
</tr>
<tr>
<td></td>
<td>Aquadest</td>
<td>6</td>
<td>(9.90 ± 0.83)</td>
</tr>
</tbody>
</table>

In table 4 it can be seen that the weight of organ immunity, liver, spleen and thymus gland in all groups of ethanol extract of iles-iles tubers is not significant compared to control. This occurs because of the body’s feedback mechanism on immune suppression so that the administration of the extract affects the production of kuffer cells and secondary lymphoid organs in the phagocytic reaction of foreign particles.\(^6\)

Mean weight of liver organ in etanol extract group of iles-iles iles-iles 220 mg/kg BB (A) was 3.34, etanol extract group of iles-iles iles-iles 110 mg/kg BB (B) was 0.97, ethanol extract group of iles tubers -ile 55 mg/kg BB (C) is 0.95, the positive control group (D) is 1.18, and the negative control group (E) is 1.24.

The average weight of spleen organ in etanol extract group of iles-iles iles-iles 220 mg/kg BB (A) was 1.07, etanol extract group of iles-iles iles-iles 110 mg/kg BB (B) was 0.79, ethanol extract group of iles tubers -ile 55 mg/kg BW (C) is 1.45, the positive control group (D) is 1.24, and the negative control group (E) is 1.16. The average weight of organs between groups increase every hour. The results were then tested with a 95% confidence level (p <0.05) and a significantly different result after inoculation of S. aureus was obtained.

The average weight of the thymus organ in the ethanol extract group of iles-iles tubers 220 mg/kg BW (A) were 7.11, the ethanol extract group of iles-iles tubers 110 mg/kg BW (B) was 9.59, the ethanol extract group of iles tubers -ile 55 mg/kg BB (C) is 9.29, the positive control group (D) is 8.78, and the negative control group (E) is 9.90. Based on these data, the average weight of liver, spleen and thymus in group A is greater than that of group B, C, D and E. The Bonferroni test results showed significant differences in thymus weight among 5 groups.

**Discussion**

Increasing the number of enzymes in the macrophages is related to the ability of intracellular digestion of phagocyte material, the development and maintenance of inflammatory reactions and the killing of germs.\(^5\) The lysozyme enzyme released will hydrolyze the peptidoglycan cell wall of germs. Other enzymes such as ribonuclease, protease, deoxyribonucleic, lipase and rafinose will hydrolyze the components of germs.
In the anova test seen the difference in each treatment group (p <0.05). Ethanol extract of iles-iles tuber dose 220 mg/kg BB had the highest number of macrophages, phagocytosis index and organ index compared to ethanol extract of iles-iles tuber dose 110 mg/kg BB, dose 55 mg/kg BB, comparison 0.3% Tragakan and control group (aquades).

The results showed that ethanol extract of iles-iles bulb dose of 220 mg/kg BB could increase the amount and activity of macrophage cell phagocytosis in inoculated mice S. aureus. Increased phagocytosis is caused by the number of antigens that act as inductors to improve the phagocytosis function of macrophages. Increased macrophage activity after administration of ethanol extract of iles-iles bulbs is due to increased secretion of cytokines produced by immunocompetent cells, interleukin 1 and interleukin 6. The presence of cytokines that constitute macrophage cell activator, macrophage phagocytosis activity can be increased.

Macrophages are activated by the presence of antigens that enter the body, flavonoid compounds and pure polysaccharides can be responded well by the body's immune system. The iles-iles ilanol bulb extract contains flavonoid and polysaccharide compounds that enhance the immune system, which increases the activity of macrophage cells.

S. aureus will stimulate macrophages to produce IL-6 which will activate NK cells, which then will secrete IFN-8 that will activate macrophages. Macrophages will also be stimulated to produce TNF-α that will activate other macrophages. Immunostimulant activity is indicated by increased weight of liver organ, spleen and thymus.

Microscopic picture in this study is macrophages derived primarily from precursor cells in the bone marrow, from promonosit that will divide produce monocytes circulating in the blood. In the second stage monocytes immigrate into the connective tissue where they mature and this is what is called macrophages. In macrophage tissue can proliferate locally to produce more similar cells. Macrophage system cells are present in the loose connective tissue of macrophages or histiocytes, in the blood of monocytes, in the liver coating sinusoidal cells known as kuffer cells, perivascular macrophages of the spleen sinusoid, lymph nodes and bone marrow and on the central nervous system in the form of microglia derived from mesoderm. The observation results show that microscopically at 100x objective enlargement visible peritoneal exudate macrophage cells that phagocytosis S. aureus (light colored in macrophages) after 1, 3 and 6 hours > 100 cells per field of view.

On macrophages on day 3 and day 7 there was no significant difference in mean. While on the 14th day the three groups experienced the highest increase in the ethanol extract group of iles-iles bulb 220 mg/kg bb and the lowest average in the control group. On day 14 to day 21, control and ethanol extract of iles-iles tubers 220 mg/kg bb increased macrophage value. While on ethanol extract of iles-iles tuber 110 mg/kg bb decreased.

A significant decrease in mean macrophage scores was observed on days 14 and 21 in the ethanol extract group of iles-iles tubers with a dose of 220 mg/kg bb. It can be concluded that the dose of 220 mg/kg bb has the highest leukocyte decrease ability than iles-iles iles-iles tuber ethanol dose 110 mg/kg bb and control.

Ethanol extracts of iles-iles bulbs contain flavonoids, saponins and polysaccharides. Flavanoid is an active ingredient that has anti-inflammatory and anti-bacterial effects. Flavanoids can block the pathway of cyclo oxygenase and lipooksgenase from arachidonic acid metabolism, this leads to the synthesis of inflammatory mediators. Like prostaglandins, thromboxane is inhibited so as to decrease inflammation. Low concentrations of flavanoid compounds only block the pathway of lipooksgenase, while high concentrations of flavanoids can block lipooksgenase and cyclooxygenase pathways. Flavanoids have the ability to inhibit the release of arachidonic acid and lysozyme enzyme secretion from neutrophil cells and endothelial cells and inhibit the phase of proliferation and exudation phases of the inflammatory process.

The decrease in monocyte migration caused by the blocking of the lipooksgenase pathway results in a decrease in the number of macrophages. These effects affect the duration of inflammation, so that it will be followed by the speed of the healing and
recovery process marked by decreasing the number of macrophages.

On observations of days 7, 14 and 21 enter the proliferation phase. The entire group averaged a decrease in scores for inoculation of S. aureus on the 7th and 14th days because at this stage the impact of topical use of ethanol extract of iles-iles tubers thereby encouraging the inoculation of S. aureus to accelerate the granulation process and the content of flavonoids itself that functions antioxidants and antimicrobials, thus helping to improve the immune system and prevent the development of microbes.

This suit the function of macrophages is to clean the wounds of bacteria, dead cells and debris by way of phagocytosis. The content of flavonoids and saponins extracts iles-iles tubers can inhibit tissue destruction and as an antimicrobial. Flavonoids can help wound healing by increasing collagen formation, decreasing macrophages and tissue edema and increasing the amount of fibroblasts. Cell necrosis is reduced by flavonoids by reducing lipid peroxidation. Inhibition of lipid peroxidation can improve the viability of collagen fibers, blood circulation, prevent cell damage and increase DNA synthesis.

Based on observation of macrophage cell phagocytosis activity inoculated S. aureus showed the difference of macrophage cell number which phagocytosis of bacteria from each treatment group dose 220 mg/kg BB, 110 mg/kg BB, 55 mg/kg BB, comparison 0.3% Tragakan and control group (aquadest). The results of statistical analysis showed that each of the extract given gave a significant effect on the increase of macrophage cell phagocytosis activity ($p < 0.05$). The smear preparations of each treatment were calculated phagocytic activity (SFA). The results obtained were compared with positive control (Tragakan 0.3%) and negative control (Aquadest). When the number of active macrophages of the treatment group is greater than the control group, it identifies the effects of stimulation or increased phagocytosis activity by the test material.15 Flavonoids, saponins and polysaccharides contained in the iles-iles ilanol extract have been shown to have anti-inflammatory effects. This is supported by previous research by Khan A., et al who saw iles-iles tubers as antibacterial, antifungal, cytotoxic and anti-inflammatory in white mice, which showed that iles-iles tubers had anti-inflammatory effects.3,4 Based on the results and discussion it can be concluded that iles-iles ilanol extract increases the number of white rat rat strand macrophage cells against S. aureus as test microbes, increases the phagocytic activity of mouse cell macrophages of wistar strains, and the higher concentration of ethanol extract of iles-iles, the increased number and activity of active macrophage cell phagocytosis in white mice Wistarr strains inoculated S. aureus.

References

