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Activity and Capacity Test of Macrophage Peritoneal Cell and Number Leukocyte of Ethanol Extract Purple Sweet Potato Peel *Ipomoea Batatas* (L.) Lam.

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ABSTRACT

Purple sweet potato peel *Ipomoea batatas* (L.) Lam have high levels of anthocyanins. Anthocyanins are a powerful antioxidants. This study aims to determine immunostimulatory effect of purple sweet potato peel, the observed parameter is the activity and capacity of peritoneal macrophages, the total number of leukocytes, the percentage of leukocytes and spleen weights relative. This study uses the female white mice which age about 3 month and divided into 4 groups; the control group was given 0.9% NaCl and dose group 10 mg/kgBW, 30 mg/kgBW, and 100 mg/kgBW. Each group was given the test preparation orally for 7 days. On day 8th, calculated the total number of leukocytes and leukocyte percentage, then the mice were injected intra peritoneal *Staphylococcus aureus*. After 1 hour, the liquid of peritonial was taken, then made preparations smear. The results showed the ethanol extract of purple sweet potato peel have the immunostimulatory effect by increasing the activity and capacity of peritoneal macrophage cells, increasing the total number of leukocytes, and increase the number of neutrophil segment ($P < 0.05$).

Keywords: Purple sweet potato peel *Ipomoea batatas* (L.) Lam, Activity, Capacity, macrophage, and leukocyte cell

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INTRODUCTION

The immune response is the body's response in the form of a sequence of the antigen complex process, to eliminate antigens. The immune response can involve to wide variety of cells and proteins especially, macrophages, lymphocytes, complement, and cytokines which forms a complex interaction. The defense mechanism of the body consists of a non-specific defense mechanisms and specific mechanisms. Non-specific defense mechanism called natural immunity, meaning that the defense mechanism is not intended for one type of antigen but for wide variety of antigens. While the specific mechanism (adaptive component) is a defense mechanism that are specific to one type of antigen. That non-specific defense mechanisms of the skin (gland), mucosal (enzyme), and phagocytic cells (macrophages, monocytes, polinuklear) and complement[1].

Phagocytosis in the body carried by phagocytic cells, mononuclear and polimorfnuklear. Phagocytic activity is determined by the cytokines produced by cells exposed to foreign substances, such as macrophages, lymphocytes and other phagocytic cells. Lymphocyte maturation process is the secondary limfoit (especially for T lymphocytes), such as the spleen. Basically, the blood flow through the lymph and contacts with a large number of macrophages and lymphocytes that trigger an immune response[2]. Macrophages are one of the non-specific immune system. The function of macrophages is phagocytosis and digest foreign substances and pathogens, stimulate lymphocytes and other immune cells to respond to pathogens. This cell is a professional phagocytic cells because the materials are destroyed without causing cell death[3].

One of the plants that have been proven to improve the immune system is purple sweet potato (*Ipomoea batatas* (L.) Lam). Dietary supplement containing a purple sweet potato that is given to the chickens can increase immune response of the chicken. This is evident from the relative weight of spleen and the total number of leukocytes were observed[4]. In addition, other studies show that the polysaccharide isolated from purple sweet potato can improve the immune system by increasing the function of phagocytes, increasing the haemolytic activity and increases the concentration of IgG[5].

Purple sweet potato has a high content of vitamins A and E. Vitamin A and E increase the production of the hormone melatonin produced by the pineal gland in the brain. Melatonin is an antioxidant that keep the health of cells and the immune system[6]. Besides the content contained on purple sweet potato is anthocyanin. The content of anthocyanins in purple sweet potato is higher than the black soybeans, black rice, eggplant purple, and blueberry[7]. The results showed that the anthocyanin content contained in the peel of purple sweet potato is higher than the meat[8].

Anthocyanins are a group of pigments that cause red-purple color in vegetables, fruits, and flowers. Anthocyanins are usually found in the epidermis and peripheral mesophyll cells of a plant[9]. Chemically all anthocyanin are derivatives of a single aromatic structure, sianidin, and all made of pigments sianidin with the addition or reduction of hydroxyl groups or by methylation or glycosylation[10]. Anthocyanins including powerful antioxidant compounds and also acts to prevent free radical oxidation[9]. Anthocyanins contained in purple sweet potato also has physiological functions, such as antioxidant, anticancer, antibacterial, protection against liver damage, heart disease and stroke prevention[6].

Purple sweet potato widely used in a home industry, but the peel was not utilized. Previous research has shown that the polysaccharide isolated from purple sweet potato and food supplements containing purple sweet potato can increasing the immune response. High anthocyanin content contained in the peel of purple sweet potato makes the author interested in conducting research using the ethanol extract of purple sweet potato peel as a substance that can enhance the immune system. The parameters will be observed that the activity and capacity of peritoneal macrophage cells as well as the number of leukocytes and spleen weights relative of white female mice.

MATERIALS AND METHOD

Materials

Purple sweet potato peel (*Ipomoea batatas* (L.) Lam), *Staphylococcus aureus* (SA), Ethanol 96% (Bratachem), methanol (Bratachem), aqua dest (technical), Giemsa dye, filter paper, physiological NaCl 0.9% , Na CMC, Na 2 EDTA, nutrient agar (NA), nutrient brooth (NB), Formic acid (Bratachem), pH paper and a solution

TURK, acetic acid (Merck), butanol (Bratachem), potassium chloride (Merck), sodium hydroxide (Merck), hydrochloric acid (Merck), ammonia (Merck), norit, sodium acetate (Merck), sulfuric acid (Merck), powder Mg (Merck), Feri chloride (Merck), acetic acid anhydride (Merck), chloroform (Merck).

Preparation of animals

Animals used in this study were 20 of female white mice. weighing about 25-30 grams, healthy, which age about 2-3 months, and had never received drug treatment. Before use, the animals acclimatized for one week and than devided into 4 groups. During acclimatization, the animals were giving food and drink adequatly. Healthy animals is not occur the weight loss and do not show significant abnormalities.

Method

Collection and preparation of simplisia

Purple sweet potato washed, then peeled the skin. tubers were separated and the skin that will be used as the test substance finely chopped.

Preparation of the ethanol extract of purple sweet potato peel

Peel of purple sweet potato that has been chopped, macerated with 96% ethanol (1:10). Formic acid is added to the solvent to achieve a pH of 3-4, and then measured with pH paper. Maceration during the first 6 hours while occasionally stirring, then let stand for 18 hours. Separate maserat by filtration. Repeat the process of maceration twice the kind and number of the same solvent. The filtrate obtained is collected and then evaporated using a rotary evaporator at a temperature of 40-60 ℃ to obtain a crude extract. The extract obtained were then weighed[11].

Determination of total anthocyanins with differential pH method

Determination of anthocyanins was conducted using pH difference. Ethanol extract of purple sweet potato peel dissolved KCl buffer pH 1.0 and pH 4.5 buffer $CH_3COONa.3H_2O$. Then a solution at different pH conditions was measured absorbance using a UV-Vis spectrophotometer (510 nm and 700 nm).

Measurement and calculation of total anthocyanin concentration

The ethanol extract of purple sweet potato peel dissolved in buffer pH 1.0 and pH 4.5, (1:4). Dilution factor appropriate to the sample determined in advance by dissolving the sample with KCl buffer pH 1.0 until the absorbance obtained Lamber beer. Then two of the sample solution is prepared, each sample is dissolved in a buffer solution based on the dilution factor predetermined. Absorbance is 510 and 700 nm. Total anthocyanin content was calculated using the molar extinction coefficient calculation is based on a molar extinction coefficient of sianidin-3 glucoside) and a molecular weight of 449.2 as follows:

$$\text{Anthocyanin total (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times L}$$

Note:

A = Absorb $(A_{510}\text{-}A_{700})pH_{1.0} - (A_{510}\text{-}A_{700})pH_{4.5}$
ε = 26900 molar extinction coefficient, in L x mol-1 x cm-1, for cyanidin-3-glucoside
L = Pathlength (1 cm)
MW = Molecular weight for cyanidin-3-glucoside (449.2 g/mol)
DF = dilution factor

Dosage

Dose ethanol extract of purple sweet potato peel are used to test the ability of phagocytosis and leukocyte cell numbers using 3 variations dose of 10 mg/kgBW, 30 mg/kgBW, and 100 mg/kgBW.

Preparation the suspension of the ethanol extract of purple sweet potato peel

Suspension of ethanol extract of purple sweet potato PEEL dose of 100 mg / kgBW made with as much as 250 mg of Na CMC was added to the mortar. Then add as much as 5 mL of hot water, allow 5 minutes for Na CMC expands. Purple sweet potato peel extract is added 700 mg, crushed until homogeneous. Then add 45 ml aqua dest. For a dose of 30 mg / kg and 10 mg / kg was made by diluting the suspension dose of 100 mg / kgBW.

Culturing *Staphylococcus aureus*

Staphylococcus aureus (SA) cultured on nutrient agar (NA) tilt. From one culture OSE SA NA slant were inoculated into the media after it was incubated at 37 ° C for 24 hours in an incubator. SA which grew on NA media slant on the move into nutrient brooth (NB), incubated 24 hours at 37 ° C, then centrifuged at 5000 rpm for 15 minutes and then formed pellets and suspended with physiological NaCl 0.9% which is equivalent to the transmittance 25 % T and measured at a wavelength of 580 nm[12].

Treatment to the animals

Mice were divided into 4 groups, group dose of 10 mg / KgBW, 30 mg / kg BW, 100 mg / kg BW, control (physiological NaCl 0.9%). Each group consisted of 5 mice. On day 1 to 7 mice were given test substance and control orally. On day 8 of each group were infected by the injection of 0.5 mL of *Staphylococcus aureus* in physiological NaCl 0.9% intra peritoneal, then calculate the total leukocyte count, percentage of leukocytes, activity and macrophage phagocytic capacity and relative spleen weights.

Analysis of macrophage phagocytic cells

On day 8, mice in each group were infected with Staphylococcus aureus injection of 0.5 mL of 0.9% in physiological saline intra peritoneal, then left to stand for 1 hour. After administration of Staphylococcus aureus, mice were killed and dissected, then added Na 2 EDTA in peritoneal fluid. Peritoneal fluid taken using a micro pipette. The peritoneal fluid is made smears on the slide and fixed with methanol for 5 minutes, then stained by Giemsa, allowed to stand for 20 minutes, rinsed and dried. Once dried preparations, preparations were observed under a microscope (10-100x) with emersi oil. Activities and macrophage phagocytic cell capacity is calculated. Phagocytic activity determined by the number of phagocytic cells are active phagocytosis in 100 phagocytic cells. Phagocytic capacity determined by the number of *Staphylococcus aureus* phagocytic by 50 active phagocytic cells[12].

Determination of the number of leukocytes

Fresh blood that has been given Na 2 EDTA sucked with the pipette leukocytes to numbers 0.5 and then added a turk solution to number 11 further shaken for 3 minutes. The solution of the pipette 1-2 drops first removed and the haemocytometer counting chamber is dripped a drop. Let the liquid for 2 minutes to allow the leukocytes to settle. The number of white blood cells counted on the four corners of rooms count[13].

The percentage of leukocytes

On day 8, mice were moistened using ethanol so that the blood vessels tail vein dilated and then the tip of tail vein of mice was cut and fresh blood is dripped as much as 1 drop on the slide, and then smooth it with a glass of other objects in order to obtain a layer of blood homogeneous (blood smear) , then dry. After drying drops of methanol, thereby coat the entire blood smear, allow 5 minutes. Add a drop Giemsa solution diluted with distilled water (1:20) and leave for 20 minutes. Wash with aqua dest, drain and add emersi oil and observed under a microscope. Count the number of eosinophils, neutrophils rods, segments neutrophils, lymphocytes and monocytes at magnification 10-100X[13].

Determining the relative spleen weights

After the mice were dissected and peritoneal fluid was taken, then taken its, spleen weights weigh one by one[14].Percent relative spleen weights can be calculated using the formula:

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% Relative spleen weights = (Weight of spleen) / (body weight) x 100%

RESULT AND DISCUSION

Fig 1. Purple sweet potato

The extraction of the sample obtained 500 g of dark purple colored viscous extract 24.28 g and the yield of 4.856%. Then extract characterized, the parameters observed were specific and non-specific parameter. The results of the non-specific parameter characterization of the ethanol extract of purple sweet potato skin can be seen in Table I. Observation of specific parameters include the observation of the organoleptic and chromatographic profiles. Organoleptic form of extract obtained is a viscous extract concentrated purple, smelling. From the results of a two-way paper chromatography using two different phases, BAA (Butanol: acetic acid: water 4: 1: 5) and HCl 1%, RF obtained in the two phases was 0.71 and 0.09 (Table II) , From the table anthocyanin RF (RF X 100) alleged that the anthocyanidins contained in the skin of purple sweet potato is peonidin, and anthocyanidins were bound with the sugar is peonidin-3-glucoside[15]. From the resulting color is suspected anthocyanin in the skin of purple sweet potato is sianidin and peonidin for producing color magenta. From the results of phytochemical screening purple sweet potato skin is known to contain anthocyanins, flavonoids and phenolic (Table III).

characterization	experiment	percentage
Drying decrease	1	11,76
	2	12,08
Ash content	1	13,37
	2	12,78
The content of acid	1	1,65
insoluble ash	2	1,38

Table I. The result of non-specific parameters

Mobile phase	The distance travelled by the solvent (cm)	The distance travelled by the pigment (cm)	Rf	Rf (x100)
BAA	16	11,2	0,70	70
HCl 1%	16	1,5	0,093	9,3

Table II. Rf value of *Ipomoea batatas* (L.) Lam

Class of compounds	Result
Alkaloid	(-)
Flavonoid	(+)
Saponin	(-)
Fenolik	(+)
Terpenoid	(-)
Steroid	(-)
Antosianin	(+)

* + = Presence of constituents; – = Absence of constituents.

Table III. Phytochemical screening on the ethanol extract of *Ipomoea batatas* (L.) Lam

The Skin of purple sweet potato has a high anthocyanin content. Therefore, it is the determination of total anthocyanins with the pH differential method. which used two solvents with different pH. The first solvent using KCl buffer pH 1.0 and a second solvent using Na acetate buffer pH 4.5. At pH 1.0 in the form of oxonium colored anthocyanins which represents the amount of anthocyanins and other compounds. While at pH 4.5 anthocyanin in the form hemiketal and decrease the intensity of color to colorless so as not to cause absorption. So uptake there is uptake which represents the amount of other compounds. Absorptions is measured at a wavelength of 510 nm and 700 nm. A wavelength of 510 nm is the wavelength of maximum absorption of anthocyanins while at a wavelength of 700 nm is a correction factor.

One mL of the crude extract reconstituted with 4 mL each of buffer solution. Then determined the appropriate dilution factor by making some concentration. From the five concentrations are made, the concentration of which got the maximum absorbance according to lamber beer is at a concentration of 2500 ppm. From the calculation of total anthocyanin purple sweet potato peel is 550.56 mg/L. The result of the calculation of total anthocyanins peel purple sweet potato is higher than the meat.

Test the immune response in terms of activities and capacity of peritoneal macrophages from female white mice that were given the preparation the ethanol extract of purple sweet potato skin orally for 7 days. The dose used is a dose of 10 mg / kgBW, 30 mg / kgBW, and 100 mg / kgBW. Physiological saline was used as control. Then on day 8 of each group in infection with the bacterium Staphylococcus aureus. The activity of macrophage cells and macrophage cell capacity was observed by making a smear preparation peritoneal fluid. Macrophage activity is the ability of macrophage cells were active phagocytosis in phagocytic cells 100, while the capacity is the number of macrophages in the phagocytosis of bacteria within 50 phagocytic cells active. Macrophages are doing phagocytosis can be seen in figure 2.

From the research, the percentage of macrophage activity and the capacity of macrophages dose groups, higher compared to the control. Statistical test results seen significant differences between the dose groups with control groups. The percentage of each dose group of the Duncan post hoc test results are significantly different. The higher the dose, higher the percentage of of activity and capacity. That can mean the ethanol extract of purple sweet potato peel dose of 100 mg/kgBW is the most effective dose in increasing capacities and activities of macrophages.

In the calculation of leukocytes by blood smear method using Giemsa solution as a dye, seemed cell neutrophils, eosinophils, monocytes, neutrophils cells and lymphocytes segment. While basophils alkaline cells can not be observed because these cells soluble in dye Giemsa. The percentage of blood leukocyte cell component female white mice after ethanol extract of purple sweet potato peel can be seen in Figure 4.

From the results the statistics by using one-way analysis of variance, it appears that the effects of different doses and the control ($P > 0.05$) for neutrophil segments. As for monocytes, neutrophils rod, and eosinophils although was not significant different, but see an increase with increasing the dose. For lymphocyte cell numbers the high dosage level, the smaller the percentage.

In the calculation of total leukocytes, the higher the dose given the increasing number of female white mice leukocytes cells. This increase is still in the normal range (Figure 5). The total number of leukocytes in the upper limit of normal showed the immune system produces sufficient total number of leukocytes in the blood circulation to fight the infection. Increasing the number of total leukocytes showed the ability of the immune system to fight infection or foreign substances. Leukocytes is in natural immune system protects the body from the invasion of microorganisms.

Tests on the relatively spleen weight, because the spleen is the organ that produces lymphocytes, it is estimated that more work in producing spleen lymphocyte cells can enlarge the size of the spleen. The results of the study illustrate that dose group had an average weight of the spleen relatively small compared to the control group. The higher the dose the smaller the relatively spleen weight. Accordingly, the use of the ethanol extract of purple sweet potato peel does not give effect to the relative weights of spleen and can be associated with the average number of lymphocytes. Statistical testing, the relative weights of spleen-dose group 10 mg / kgBW and 30 mg / kgBW and controls did not differ significantly. But a dose of 100 mg / kgBW differ significantly.

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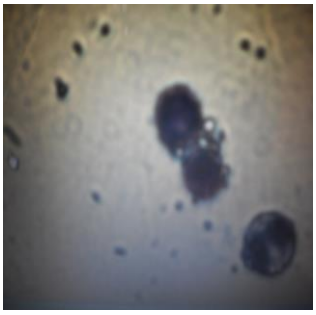

Figure 2. Phagocytosis by macrophage

Fig 3. Process to take the spleen

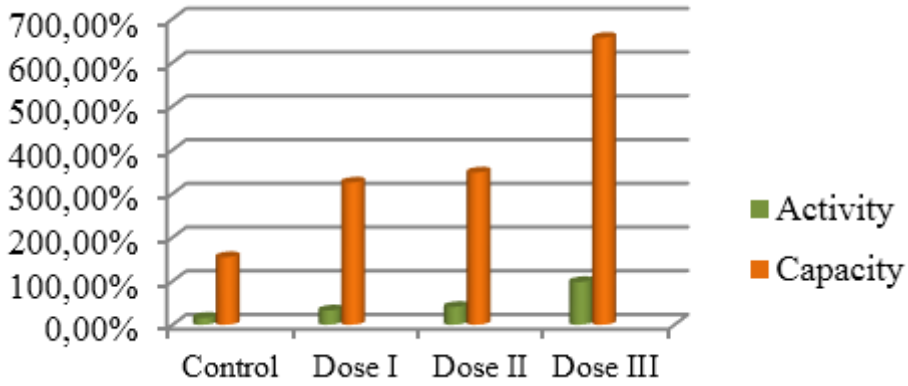

Figure 4. Graphic of activity and capacity of macrophage

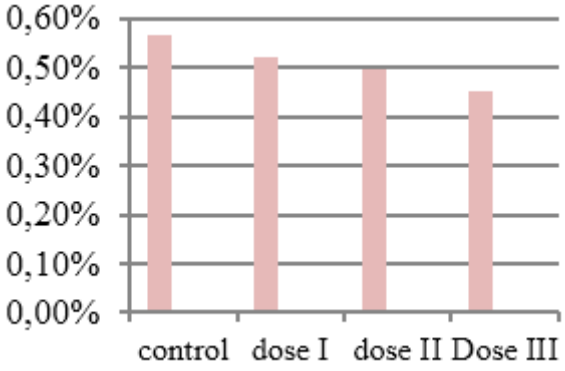

Figure 5. Graphic of relative spleen weight

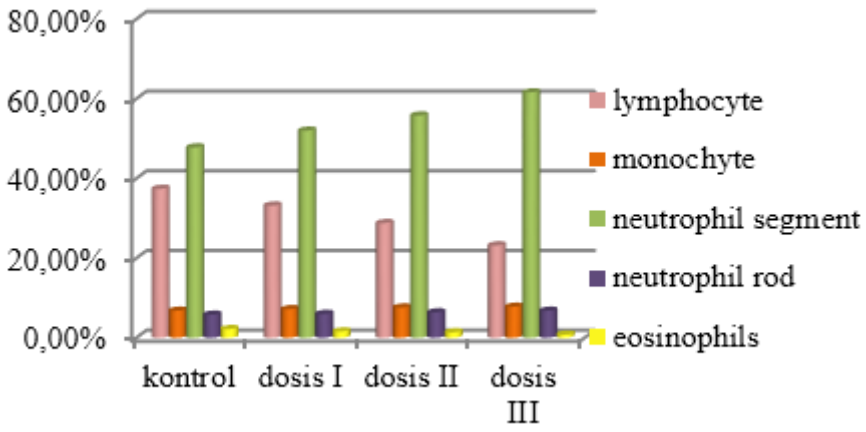

Figure 6. Graphic of leukocyte cell persentage

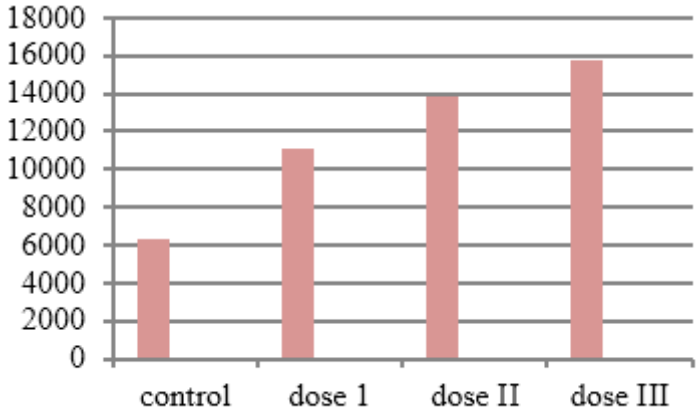

Figure 7. Graphic of Total number of leukocytes

CONCLUSION

The ethanol extract of peel of purple sweet potato Ipomoea batatas (L.) Lam dose of 10 mg, 30 mg, and 100 mg can increase the the total number of leukocytes (P <0.05), increased the percentage of neutrophil segment (P <0.05), increase lymphocyte percentage and relatively spleen weight.

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