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Test immunomodulatory effects of ethanol extract skin of purple sweet potato (*Ipomoea batatas* (L.) Lam) with carbon clearance method and the number of leukocytes.

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ABSTRACT

The purple sweet potato skin (*Ipomoea batatas* (L.) Lam) has a bioactive component is anthocyanin. Anthocyanins are categorized as antioxidants, which improves the immune system of the human. This study aims to determine the immunomodulatory activity of the ethanol extract of purple sweet potato skin with carbon clearance method. Animals used in this study were female white mice. Extract dose used is 10 mg / kg BW, 30 mg / kg BW, 100 mg / kg BW administered orally for 6 days on 3 group of treatment and normal saline as a control. On day seven mice were injected intravenously with colloidal carbon of 0.1 ml / 10 g BW. Blood was taken at minute 3, 6, 9, 12 and 15 from the tail as much as 25 µl and calculated the absorbance. Then determined the absorbance obtained phagocytic index. Blood sampling time 0 is to determine the percentage of leukocytes. After taking blood samples for 15 minutes, the mice were killed and calculated the relative weights of the spleen. The results of the study showed the ethanol extract of purple sweet potato skin dose of 10 mg / kg BW, 30 mg / kg BW and 100 mg / kg BW have immunostimulatory activity. Phagocytic index increase significantly at a dose of 100 mg / kg BW ($p<0.05$). The increase in the number of leukocytes showed a significant difference in the control group ($p<0.05$). The increase in the number of lymphocytes and neutrophils segment relative spleen weights showed significant differences in the dosage of 30mg / kg bw and a dose of 100 mg / kg bw.

Keywords: Ipomoea batatas (L) Lam, Antosianin, Imunomodulator

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INTRODUCTION

Every time the body is always exposed to microorganisms that can cause infection and the body are usually resistant to infection because their immune system protects the body [1]. The body has a defense known as the immune system, it is very important in protecting the body against entry into the body and immune system disorders (hypersensitivity) [2].

Immunomodulating is a method to improve immune system function is impaired or to suppress excessive functions. Drugs that can restore the immune system called immunomodulators [3].

Skin purple sweet potato has bioactive components anthocyanin, anthocyanin is a coloring agent that can be categorized as an antioxidant [4]. The results showed that the anthocyanin content in the skin of purple sweet potato more than in the flesh [5] [6] [7] [8]. It has been reported also that the four varieties of purple sweet potato has anthocyanin content ranged from 24 to 53 mg in 100 g and 2 purple sweet potato varieties have anthocyanin content ranged from 3 to 7 mg in 100 g [9].

Anthocyanin compounds act as antioxidants and binding of free radicals, prevention of aging, cancer, and degenerative diseases. Anthocyanins have as antimutagenic and anticarcinogenic activity, preventing the liver dysfunction, antihypertensive, and decrease blood sugar levels [10]. It has been reported that the ethanol extract of purple sweet potato has a cytotoxic effect on T47D breast cancer cells by inhibiting cancer cell proliferation in vitro [11]. Giving of water extract of purple sweet potato can reduce levels of malondialdehyde (MDA) in the blood, the liver, the heart and the intestine of mice [12]. The extract of purple sweet potato can also be as exogenous antioxidants and can inhibit the growth of S180 cancer [13].

Based on data from previous studies, then to investigate the activity of the ethanol extract of the skin of purple sweet potato (Ipomoea batatas (L.) Lam) as immunomodulator, the test methods used to determine the immunomodulatory effects are methods of Carbon Clearance, by measuring the activity of phagocytic cells in phagocytosis foreign objects that enter the body. Besides, it also determined the total leukocyte cell numbers and percentages and relative spleen weights after the ethanol extract of purple sweet potato skin.

METHOD

Materials

The materials used are skin purple sweet potato (*Ipomoea batatas* (L.) Lam), 96% ethanol (BRATACO®), 12% formic acid (BRATACO®), methanol (BRATACO®), norit, the distilled water, normal saline NaCl 0, 9%, Mg powder, HCl (Merck®), H2SO4 (Merck®), chloroform 0.05 N ammonia (Merck®), KCl (Merck®), 0.2 N HCl (Merck®), Na acetate (Merck®), acetic acid anhydride (Merck®), EDTA, turk solution, emersi oil, reagents mayer, FeCl3, CMC Na 0,5%, 1% acetic acid (merck®), Giemsa dye (100-darstadt D6), Chinese ink (Faber Castel Drawing ink GmBH and Co. D-90 546). Animals used were female white mice weighing 20-30 grams.

Preparation of Ethanol Extract Skin Purple Sweet Potato (*Ipomoea batatas* (L.) Lam)

Purple sweet potato skin is cleaned, crushed, then macerated with a solvent mixture of 12% formic acid in 1 L of ethanol 96% to reach a pH of 3-4. Macerated during the first 6 hours while occasionally stirring, then let stand for 18 hours. Separate maserat with in filtration. Repeat the process three times maceration with the same kind of solvent. The filtrate obtained from each container collected and evaporated by using a rotary evaporator at a temperature of 40-60 ℃ to obtain a crude extract. The extract obtained is then weighed [14].

Animals preparation

Animals used were female white mice aged 2-3 months, weighing between 20-30 grams of 20 mice. Before the treated mice were acclimatized for 7 days with given food and drink sufficiently. It is for the adjustment of environment, health and weight control. Animals that are sick sign hairs standing, motor activity

and body weight decreased, so it is not used for research. Animals used are healthy mice that BW during acclimatized not change more than 10% and visually indicates normal behavior [15][16].

Dosage

A total of 20 mice were divided into 4 treatment groups consisting of 5 mice:

1. The negative control was given normal saline orally as 1 times a day for 6 days.
2. Group of purple sweet potato skin extract dose of 10 mg / kg BW, as orally 1 time a day for 6 days.
3. Group of purple sweet potato skin extract dose of 30 mg / kg BW, as orally 1 time a day for 6 days.
4. Group of purple sweet potato skin extract dose of 100 mg / kg BW, as orally 1 time a day for 6 days.

Carbon Clearance Method

During the 6 days of experimental animals were given suspension of the ethanol extract of purple sweet potato skin is orally once a day. On day 7, mice were moistened with ethanol so that the veins dilate and then tail of mice were cut and blood dripped on the plate drops that have been given a bit of EDTA. Blood is used as a blank sample (minute 0). Then injecting the carbon colloidal 0.1 ml / 10 g BW intravenous, mice blood was taken 25 mL at minute 3, 6, 9, 12 and 15 after the injection of carbon. Each 4 ml of blood lysis with 1% acetic acid and then the absorbance was measured with a UV-Visible spectrophotometer at 650 nm [17] [18].

Leukocyte Cell Calculation with Blood smears method

Blood used were minutes-0 in carbon clearance method. Blood dripped on the object glass and flattened with another object glass to obtain a homogeneous layer of blood (blood smear), and then dried. After drying, drops of methanol to cover the blood smear, let stand 5 minutes. Add a drop of Giemsa solution diluted with aquadest (1:20) and leave for 20 minutes. Wash with aquadest, drain and add oil emersi and look under the microscope. then count the number of eosinophils, neutrophils rod segment, neutrophils, lymphocytes and monocytes at a magnification of 10-100 times [19].

Calculation of total leukocyte cells with Haemocytometer

Blood that has been added EDTA pipette with pipette leukocytes to figure 0.5 then pipette the turk solution until the number 11 next shaken for 3 minutes. By means of the pipette 1-2 drops discarded and the haemocytometer counting chamber shed one drop. Let the liquid for 2 minutes to allow the leukocytes to settle. The number of white blood cells counted in the four corners of the room count.

RESULTS AND DISCUSSION

Fig 1. Purple sweet potato (*Ipomoea batatas* (L.) Lam)

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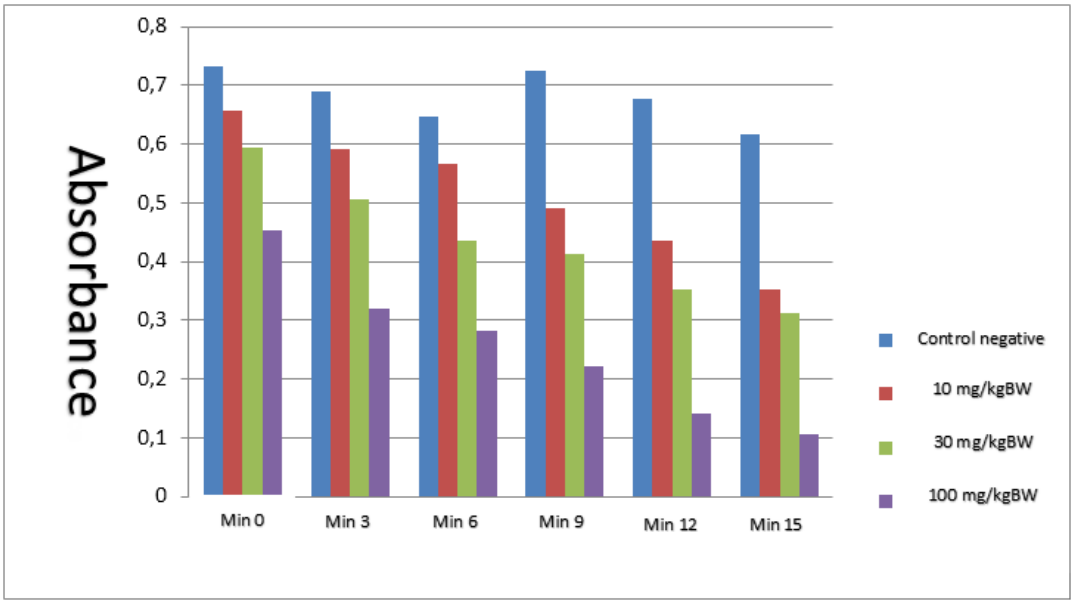
Determining the total amount of anthocyanin with differential pH method that is used at pH 1 buffer used KCl and pH 4.5 with buffer Na acetate buffer, it is known that the total anthocyanin content of the ethanol extract of purple sweet potato skin of 550.56 mg / l.

Based on the examination of the two-way chromatographic profiles that have been made against the skin ethanol extract of purple sweet potato (Ipomoea batatas (L.) Lam). Eluent used was butanol: acetic acid: water (BAA) with a ratio of 4: 1: 5 and 1% HCl, in which BAA is saturated one night and then taken the upper layer and HCl 1% pipette then inserted into the chamber and closed. Test solution of the ethanol extract of the skin of purple sweet potato (*Ipomoea batatas* (L.) Lam) weighed as much as 50 mg in water and ethanol. Then test solutions spotted on the plate at the bottom. Space of the upper and lower limit is 20 cm, and the space to the eluent with the lower limit is 4 cm. After the plate is inserted into the chamber which already contains the eluent (BAA), then cover and wait eluent move to the upper limit. Then inserted into the chamber which already contains the eluent HCl 1%, then cover and wait until the eluent move marks the upper limit, then removed and dried and measuring under UV, so it can be seen stains formed and mark. Stains formed at a distance of 11.2 cm with Rf 0.7 and at a distance of 1.5 cm with Rf 0.09.

Based on the results of a study of the absorbance decrease at all dose groups skin ethanol extract of purple sweet potato compared to the negative control group. The biggest decrease in absorbance at a dose of 100 mg / kg BW, and the dose of 30 mg / kg BW and then a dose of 10 mg / kg BW. Decrease of absorbance means the carbon concentration in the blood of mice slightly. This shows that an increase in phagocytic activity in each dose group.

Fig 2. Suspension of carbon absorbance against time on female white mice after adminstration of ethanol extract of the skin Purple Sweet Potato (Ipomoea batatas (L.) Lam.) For six days



Min 0 Min 3 Min 9 Min 12 Min 15

From the results obtained absorbance can be calculated constant phagocytosis of each dose of the extract. Phagocytosis constant is one parameter which indicates the rapidity of phagocytosis, the greater the constant speed the higher the carbon clearance phagocytic. Means there are effects of extracts tested are ethanol extract of purple sweet potato skin on the rate of elimination of carbon from the blood. The mean of the phagocytosis constant obtained based on the calculation that the negative control absorbance 0.025814, at a dose of 10 mg / kgBW 0.04228, at a dose of 30 mg / kgBW 0.054546, at a dose of 100 mg / kgBW 0 , 09 304.

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Phagocytosis Constant				
Time (min)	Contol Negative	dose 10 mg/kgBW	dose 30 mg/kgBW	dose 100 mg/kgBW
3	0,0539	0,0758	0,0986	0,1657
6	0,0315	0,0410	0,0599	0,0915
9	0,0156	0,0344	0,04278	0,0726
12	0,0141	0,0301	0,03775	0,0707
15	0,01397	0,0301	0,0337	0,0647
Mean	0,025814	0,04228	0,054546	0,09304
±SD	1,404333842	0,019261541	0,026581019	0,041838594

Table 1. Phagocytosis constant of female white mice blood after administration of ethanol extract Skin Purple Sweet Potato (Ipomoea batatas (L.) Lam.) For 6 days.

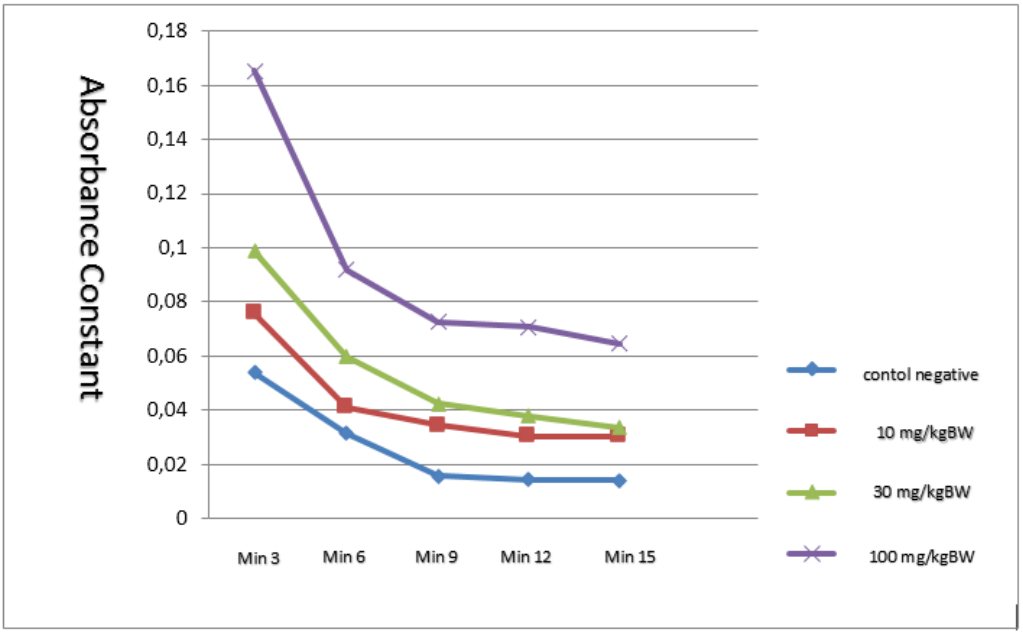

Figure 3. Constant phagocytosis compared to the time of the blood of female white mice after administration of ethanol extract Skin Purple Sweet Potato (Ipomoea batatas (L.) Lam.) For six days.

Phagocytosis index			
Time (min)	dose 10 mg/kgBW	dose 30 mg/kgBW	dose 100 mg/kgBW
3	1,4063	1,8293	3,0742
6	1,3015	1,9015	2,9047
9	2,2051	2,7423	4,6538
12	2,1347	2,6773	5,0141
15	2,1654	2,4123	4,6313
Mean	1,8426	2,31254	4,05562
±SD	0,4483	0,4272	0,9871

Table 2. phagocytosis index of female white mice blood after administration of skin extract Purple Sweet Potato (Ipomoea batatas (L.) Lam.) for six days.

Phagocytic index can be calculated once known constants phagocytosis. The larger the constant and phagocytic index means faster phagocytic cells that make the process of phagocytosis. Based on the

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calculations have been done, obtained an average index of phagocytosis showed phagocytic activity of phagocytic cells to carbon particles that serve as a markers due to the influence of sweet potato peel extract. If the mean index of phagocytosis more than one (IF> 1) indicates that the test substance has the ability immunostimulatory [20] [21]. The mean index of phagocytosis obtained by calculating the value of the constant is at the dose of 10 mg / kg 1.8426, at a dose of 30 mg / kg 2.31254, at dose of 100 mg / kg 4.05562.

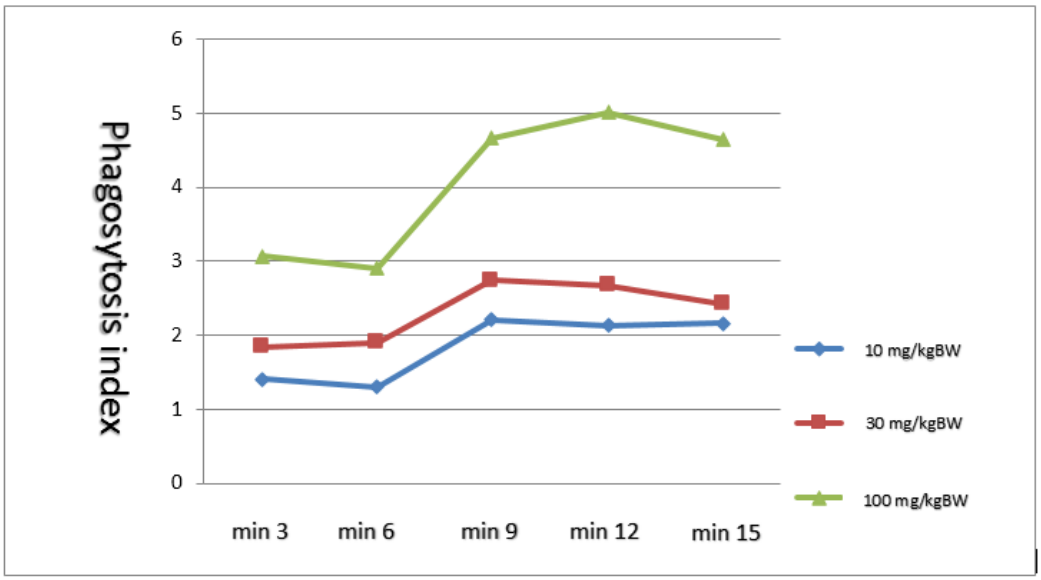

Figure 4. Comparison of phagocytosis index against time on female white mice after administration of skin extract Purple Sweet Potato (*Ipomoea batatas* (L.) Lam.) For six days.

The results of the mean fagoitosis index that indicates the test substance is as immunostimulatory seen at doses of 10 mg / kgBW, 30 mg / kgBW and 100 mg / kgBW. In statistical test phagocytic index by using one-way ANOVA test dose showed significant differences in the dose of 100mg / kgBW (P <0.05).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.592	2	6.796	15.012	.001
Within Groups	5.432	12	.453		
Total	19.024	14			

Table 3. Test one-way ANOVA phagocytic index on female white mice after administration of skin extract of purple sweet potato (*Ipomoea batatas* (L.) Lam.) For six days.

Then test specific immune response. It can be seen from the increase in relative spleen weights of each treatment with different doses. The results showed that the negative control relative spleen weights 0,37686g, at the dose of 10 mg / kg BW 0,38396g, at doses 30 mg / kg BW 0,54258g, at dose of 100 mg / kg BW 0,60302g. The meaning is the higher the weight of spleen more phagocytic cells resulting in the formation of antibodies.Based on the weight of spleen, lymph optimal weight increase was in the group of 100 mg /kgBW.

After statistical analysis, the increased weight of spleen showed significant differences in the dosage of 30mg / kgBW and 100mg / kgBBW compared with controls (P <0.05). Lymph is known that the secondary lymphoid organs containing B lymphocytes and T lymphocytes that contribute in the process of a specific immune response. In addition, the spleen also contained dendritic cells and macrophages act as APCs (Antigen Presenting Cell) that serves present antigens to lymphoid cells. Increased immune cells is correlated with spleen weight. The increase in relative spleen weights showed that the effect of the ethanol extract of purple sweet potato skin on immunostimulatory activity [21].

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Dose	Animals	BW (g)	LW(g)	Relative weigh spleen (%)
Control negative	1	26,5	0,1399	0,5279
	2	26	0,1200	0,4615
	3	25,5	0,1133	0,4443
	4	24	0,0998	0,4158
	5	23	0,0800	0,0348
			Mean	0,37686
10 mg/kg BW	1	25	0,0889	0,3556
	2	25	0,0889	0,3556
	3	25,5	0,1033	0,4051
	4	24,5	0,0850	0,3469
	5	27	0,1233	0,4566
			Mean	0,38396
30 mg/kg BW	1	26,5	0,1427	0,5385
	2	28	0,1430	0,5128
	3	25,5	0,1355	0,5314
	4	24,5	0,1400	0,5714
	5	25,5	0,1425	0,5588
			Mean	0,54258
100 mg/kg BW	1	27	0,1793	0,6641
	2	27,5	0,1795	0,6527
	3	28	0,1799	0,6425
	4	28,5	0,1800	0,6316
	5	29,5	0,1893	0,6417
			Mean	0,60302

Table 4. The weight of the spleen relative female white mice after administration of ethanol extract Skin Purple Sweet Potato (*Ipomoea batatas* (L.) Lam.) For six days.

In the calculation of lymphocyte cells by a method using blood smear Giemsa solution as a dye, then emersi oil as explanatory form of lymphocytes. Total lymphocyte cells in the negative control mean 23.2, 10 mg / kg BW, 24 and 30 mg / kg BW of 30.8, at a dose of 100 mg / kg BW 40.8. Lymphocyte cells can also be used as the test parameters to see the activity of the ethanol extract of purple sweet potato skin on female white mice. The increase in the number of lymphocytes in the lymph cells means also an increase in the specific immune response. Cell lymphocytes consist of B lymphocytes and T lymphocytes. B lymphocytes will proliferate and differentiate to form plasma cells and memory cells. This plasma cells that produce antibodies formed after contact with the antigen. To form antibodies, plasma cells need to be cooperation with T lymphocytes (T-helper cells) [21].

In the calculation of the total number of leukocytes by using a haemocytometer found the number of leukocytes mean the negative control 6389 / mL of blood, a dose of 10 mg/kgBW 11 690 / uL of blood, a dose of 30 mg/kgBW 13,450 / uL of blood, a dose of 100 mg/kgBW 15.350 BB / mL of blood. In statistical test total number of leukocytes using one-way ANOVA test dose showed significant differences in each group to the control ($P < 0.05$).

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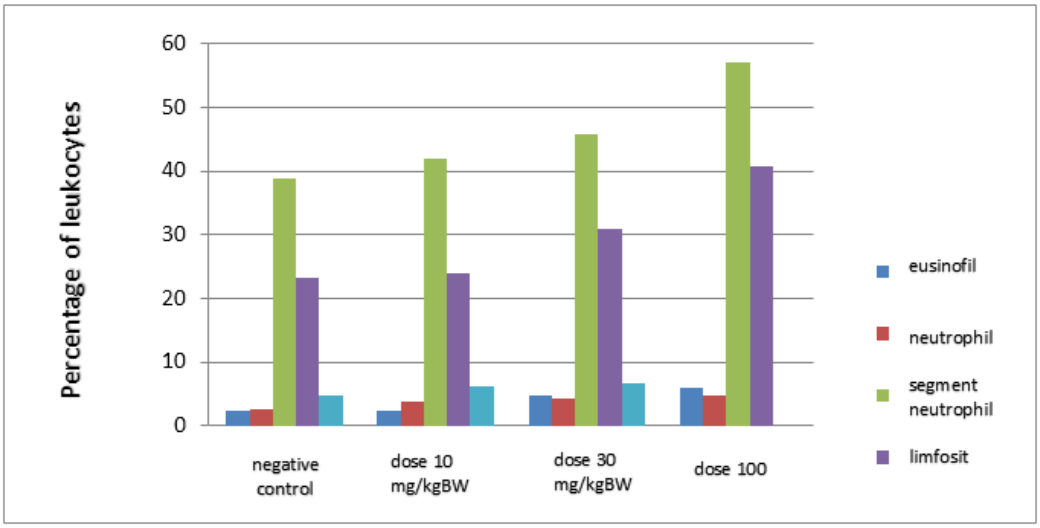

Figure 5. Percentage of leukocytes

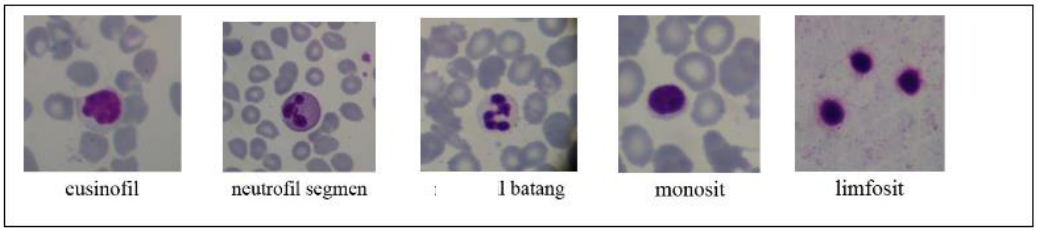

Figure 6. leukocyte cells in mice with Giemsa.

The total of leukocytes				
Negative control		10mg/kgBW	30mg/kgBW	100mg/kgBW
6700/µL		11.000/µL	13.900/µL	15.700/µL
6000/µL		11.700/µL	13.500/µL	15.000/µL
6500/µL		12.000/µL	12.900/µL	14.900/µL
6400/µL		12.500/µL	13.950/µL	15.500/µL
6345/µL		11.250/µL	13.000/µL	15.650/µL
mean	6389/µL darah	11.690/µL	13.450/µL	15.350/µL

Table 5. Total Number of leukocytes in the blood of female white mice after administration of ethanol extract Skin Purple Sweet Potato (*Ipomoea batatas* (L.) Lam.) For six days with Hemocytometer.

CONCLUSION

From the research that has been done can be concluded is bark ethanol extract of sweet potato (Ipomoea batatas (L.) Lam) were given to female white mice for 6 days to have activity as an immunostimulant, increasing the total number of leukocytes ($p < 0.01$), increase the percentage of neutrophils and lymphocytes segment ($p < 0.01$) and increase the the relative weight of lymph ($p < 0.01$).

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