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Activities peels purple sweet potato (*Ipomoea batatas* (L.)Lam) on erythropoietic male white mice

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ABSTRACT

The peels sweet potato contains many compounds flavonoid, fhenolik and anthocyanins. In the state of the skin day yet optimally utilized by the community. In this study the effects seen on the peels extract of purple sweet potato (Ipomoea batatas (L.) Lam) on the hematopoietic process with the observation parameter number of erythrocytes, reticulocytes, hemoglobin and hematocrit values. Animals used white male mice that had anemia, devidedinto 4 groups: negative control (saline physiological solution), dosage of 10, 30 and 100 mg/kgbw. To make the anemi, white mice given chloramphenicol orally dosage of 130 mg/kgbw daily for 14 days. The continued with given extract ethanol peels purple sweet potato until 28 day. Observation number of erythrocytes, reticulocytes, hemoglobin and hematocrit valuesranging from days 0, 7, 14, 21, and 28 day. The research show at dosage 10, 30 and 100 mg/kgBB can increase the number of erythrocytes, reticulocytes, hemoglobin and hematocrit values male white miceanemia significantly (<0:01). From the research can results that extract peels of purple sweet potato (Ipomoea batatas (L.) Lam) can increased process eriythropoietic.

Keywords: Peels purple sweet potato *(Ipomoea batatas* (L.) Lam), erythrocytes, reticulocytes, Hematopoietic, hematocrit and hemoglobin.

INTRODUCTION

Anemia is a condition in reducing the ability of blood or red blood cells to carry oxygen which is usually caused by a decrease in the number of circulating red blood cells. Anemia also occurs when the hemoglobin level below 12 g / dL in women and 14 g / dL in men, a diagnosis of anemia is not only seen from the number of erythrocytes and hemoglobin levels but also be seen from the hematocrit and reticulocyte count. Calculate the value of hematocrit is important to see these kinds of anemi and the reticulocyte count is useful to look at the activity of the spinal cord, where the bone marrow where red blood cell production [1].

Indonesia is a potential biological diversity in the discovery of new compounds that have medicinal properties.skin of purple sweet potato *(Ipomoea batatas* (L.) Lam). Peels sweet potato are included in the category of organic waste, because these wastes can be degraded (decomposed or destroyed) naturally. According to data from the Ministry of Agriculture of the Republic of Indonesia in 2008, skin purple sweet potato expected in silkan ± 364 thousand tons per year. The peels of purple sweet potato has bioactive components name anthocyanin, where the anthocyanin can be categorized as an antioxidant [2].

The purple sweet potato contains 32.30 g carbohydrates, 0.40 g fat, 1.10 g protein, kalori136 cal per 100 g of sweet potatoes. The sweet potato also contains vitamin A by 900 SI, 0.0001 g of vitamin B, vitamin C 0.035 g per 100 g of sweet potatoes. While the mineral in sweet potatoes which are calcium 57.00 g, iron 0.0007 g and phosphorus 0.052 g per 100 g of sweet potato [3]. Nutrient content of purple sweet potato is very good and can be used as a food additive. Purple sweet potato has been reported that pregnant women who consume trismester 2 purple sweet potato

can increase the formation of new tissues such as placenta, amniotic fluid, enlarged breasts and increase the volume of blood throughout the body [4].

Has traditionally been used for the treatment of rheumatism, gout, stiff, and night blindness and leaves are used for the treatment of inflammation, fever, burns, cancer, and dengue fever as well as a medicinal herb skin diseases [5,6]. The antosianin contained in purple sweet potato can increase the secretion of insulin cells cell β-pancreatic [7]. The ethanol extract of tubers of purple sweet potato has a cytotoxic effect on breast cancer cells T47D and can inhibit the proliferation of breast cancer cells T47D *in vitro* [8], while the extract water can reduce levels of MDA in blood , liver, heart and intestines of mice and has been active as hemostatika [9].

The study of purple sweet potato peels extract has been proven as an immunomodulator and increase the number of leukocytes [10]. Subsequent research turns purple sweet potato peels extract can increase the activity and phagocytic capacity of macrophages, increase the weight of spleen relatife and lymphocyte cell count [11].

The based on the above, then further research is to see the activity of extract peels sweet potato to the formation of red blood cells. The parameters measured were the number of erythrocytes, reticulocyte count, hemoglobin concentration and hematocrit values. Research was conducted on male white mice.

MATERIALS AND METHODS

Toolsand materials

The tools used are haemocytometer (Newbauer), analytical balance (Satorius), microscopy (Olympus), micro pipette (Socorex), pipette, oral needles, surgical tool (Scissors), hematocrit centrifuge (Kubota 3100) and photometer (Perkin Elmer)

Materials used are bark extract of purple sweet potato *(Ipomoea batatas* (L.) Lam) [11], 96% ethanol (Brataco), aquadest (Brataco), Na CMC (Merck), a solution of Drabkins (Merck), a solution of Hayem (Merck), brilian cresyblu (Merck).

Animals used are male white mice weight 20-25 g, and the age of 2 months before use acclimatization one week at the Serolologi Laboratory of the Faculty of Pharmacy, University of Andalas.

Dosage Planning

1. Negative control group (K1), given physiological solution (0.9%).
2. Test group 1 (K2), by the suspension of purple sweet potato peels extract at a dosage of 10 mg/kgbw for 14 days.
3. Test group 2 (K3), given suspension of purple sweet potato peels extract at a dosage of 30 mg/kgbw for 14 days.
4. The test group 3 (K4), given the suspension of purple sweet potato peels extract at a dosage of 100 mg/kgbw for 14 days.

Determination the Number of Erythrocytes (Gandasoebrata, 1999)

a. Filling the pipette erythrocytes

Pipette erythrocytes first rinsed with Hayem solution to clean the remnants of dirt and blood that may still rest in erythrocyte pipette, then rinse discarded. Mice were given alcohol 70%, cut, wipe the blood on the tail were cut using a tissue let the blood out and then sucked using a pipette erythrocytes to mark the line of 0.5 mL. Excess blood attached to the pipette tip is removed with a tissue. Then enter the pipette into a solution of Hayem while holding 0.5 mL of blood on the line, then sucked Hayem solution was to line 101 mL. Pipette removed from the solution, cover the pipette tip by using fingers and rubber suction is released, the pipette shake for 30 seconds.

b. Rooms fill Count

The rooms were clean count with glass lid mounted horizontally laid on the table. Pipette filled was shaken for 3 minutes continuously, 1-2 drops of the first liquid contained in the pipette removed and immediately contacted the pipette tip on the surface of the room with offensive edge of the glass pe closed, let's count room slowly filled with fluid. Rooms count already filled it stand for 2-3 minutes for the erythrocytes settle.

c. Counting the number of erythrocytes

Derived condenser lens, the microscope table in an average position. The focus is set in advance by using a small objective lens (10x) to see the position of the erythrocyte room, then the lens is replaced with a large objective lens (40x), until the lines for the major areas of the center are clearly visible. Then count all the erythrocytes contained in five fields composed of 16 small fields (for example on the four corners plus a large field with a field in the middle). Start counting from the top left corner, keep to the right, then down to the bottom and from right to left and so on.

Sometimes there is a cell that is offensive line of a field, the offending cells left boundary line or the top line should be calculated. Conversely cells that offend the right and bottom lines should not be counted.

$$\text{Total erythrocytes} = N \times 10{,}000$$

Description: N = Number of erythrocytes were counted in 5 fields.

Determination of The Reticulocyte

Insert the tube into the blood and dyes (brilliant cresyl blue) with a ratio of 1: 1, mix well, let stand for 15 minutes for coloring perfectly. Make smear the mixture, let it dry in the air. Check under a microscope with a magnification of 100 x. Erythrocytes and reticulocytes appear light blue will be up as cells containing granules / filaments are colored blue. Count the number of reticulocytes in 1000 se l erythrocytes.

$$\% \text{ Reticulocyte} = \frac{\text{reticulocyte count}}{10} \times 100\ \%$$

Determination of Hemoglobin with Method Sianmethaemoglobin

Put 5 ml Drabkins solution into a test tube. Animal experiments included in tube by a cover that has a small hole to pull out the tail, the tip of mice cleared with 96% ethanol, then with scissors that have been sterilized mice were cut along the tail end of 5 mm from the tip of the tail. Collect blood comes out, pipette 20 mL of venous blood of mice with an automatic pipette and then enter in a test tube, pipette rinse several times with a solution drabkins thoroughly, shake until a homogeneous mix of both materials. Leave at room temperature for 3 minutes. Check absorbances at 5010 photometer at a wavelength of 546 nm.

Determination of Hematocrit Value

Animal experiments included in a tube by a cover that has a small hole to pull out the tail, the tip of mice cleared with 96% ethanol, then with scissors that have been sterilized mice were cut along the tail end of 5 mm from the tip of the tail. Fill pipette mikrokapiler with venous blood of mice ¾ by way of direct charging of which one end is closed with wax. Enter micro capillary tubes in centrifuges (centrifuges microhematocrit), then centrifuged at a speed of 16000 rpm for 5 minutes.

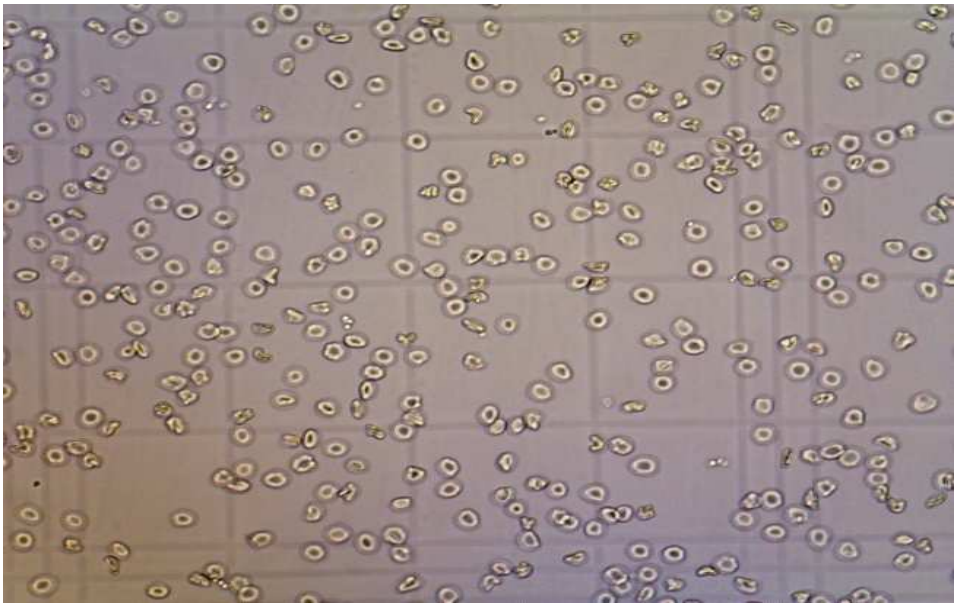
RESULTS AND DISCUSSION

Identification of purple sweet potato crop has been done in the herbariumANDA, Universitas Andalas Padang. The results of the identification of states that samples be employed in this study are sweet purple potato *(Ipomoea batatas* (L.) Lam), Potato photograph can be seen in picture 1.

Picture 1. Photo of purple sweet potato *Ipomoea batatas* (L.) Lam)

The preparation used in the form of purple sweet potato peels extract, which in previous studies has been proven as an immunomodulator and increase the number of leukocytes in male white rats [10] and increase the the total number of leukocytes, increased the percentage of neutrophil segment, increase lymphocyte percentage and relatively spleen weight)[11].

In this study the effect of extract ethanol peels purple sweet potato seen on some variants dosageto erythrocytes, reticulocytes, hemoglobin and hematocrit. Observation do on days 0, to see normal value before the given induction chloramphenicol for 14 days, then checks to 2 done at day 14, to see whether the inducer had an effect on experimental animals and cause anemia, checks to 3 as well as to 4 on day 21 and 28 after a given dosage of extractethanolpeels purple sweet potato, to see the effect of purple sweet potato peel extract.



Picture 2. Photo erythrocytes of male white mice after orally extrac ethanol peels purple sweet potatoviewed with a microscope enlargement 40

In this experiment the animals were divided into 4 groups of the first group which is dick positive only given chloramphenicol alone for 14 days at a dose of 130 mg/kgbw, the second, third and fourth is a group that was given treatment in induction with chloramphenicol for 14 days at a dosage 130 mg/kgBB and continued with the suspension the extractethanolpeels purple sweet potato with a dosage 10, 30 and 100 mg/kgbw for 14 days.

Result that varies from each individual of each group of experimental animals, this may because of physiological conditions in animal experiments, the influence of the environment and food. In this study, all animals is normal circumstances characterized by the number of erythrocytes, reticulocytes, hemoglobin and hematocrit levels are in the normal value. After given induction chloramphenicol at dosage 130 mg/kgbw for 14 days then to four parameters can be seen to decrease catagory anemi. After given a suspension extract ethanol peels purple sweet potato in 3 groups with a dosage that has been set, three groups experienced an increase which is marked by the increase in the number of erythrocytes, reticulocytes, hemoglobin levels and hematocrit values. On the positive controls were only given induction chloramphenicol 130 mg/kgbw for 14 days without extract ethanol suspension peels purple sweet potato, next 14 days also increased though not significantly, this increase occurred because the Traffic possible to stabilize ourselves back. While in the group given the extract ethanol peels purple sweet potato looks a higher increase in the appeal of the positive control. The result of the calculation of the number of erythrocytes can be seen in Table 1.

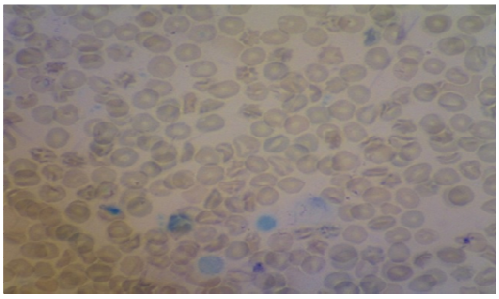
In each group of test animals, erythrocyte seen decreased after 14 days induction chloramphenicol at dosage of 130 mg/kgBB. This is because chloramphenicol works by suppressing the bone marrow and thereby inhibits the reproduction and proliferation of bone marrow stem cells to any component of blood cells cause aplastic anemi. Forms erythrocytes of mice can be seen in picture 2.

The results of analysis two-way ANOVA test,provedextract ethanol peels purple sweet potato can increase the number of erythrocytes were highly significant ($p < 0.01$). More high dosage extract, the number of erythrocytes is also growing. Duncans test show varian of dosage can gave influanceamount of erythrocytes. It turned out that the difference of treatment dosege of the extract (10, 30, 100 mg/kgbw) have different effects. The ability of an increasing number erythrocytes highest dosage indicated by extract ethanol peels purple sweet potato 100 mg/kgbw. While at doses 10 and 30 mg/kgbw effects similar to the positive control group.

Reticulocyte counting is done by making smears prior to mixing blood with a brilliant cresyblue reagent ratio of 1: 1, followed by counting the reticulocyte count under the microscope (picture 3). Reticulocyte cell will look like grains or a net with a dye brilliant blue colored cresyblue that will distinguish the erythrocytes colorless. Statistical test results showed that two-way ANOVA reticulocyte count after extractethanol peels purple sweet potato to the reticulocyte count was highly significant (p <0.01). The result of the calculation of reticulocytes can be seen Table 2. Comparing the effects of each dosage in increasing the number of reticulocytes, there is no significant effect (p> 0.04).

Table 1. Amount of erythrocytes white male mice that induction of chloramphenicol for 14 days and then given a suspension extract ethanol peels purple sweet potato various dosage of 14 days

Treatment	Animal	amount erythrocytes (million/µl)			
		0	14	21	28
Chloramphenicol 130 mg/kgbw	1	5.12	4.81	4.78	4.81
	2	5.31	4.75	4.82	4.91
	3	5.23	4.76	4.98	4.92
	4	5.18	4.35	4.55	4,86
	5	4.96	4.51	4.67	4.94
	$\dot{x}$	5.16	4.63	4.76	4.88
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 10mg/kgbw.	1	4.98	4.61	4.85	5.08
	2	4.85	4.56	4.32	4.91
	3	4,86	4.41	4.57	4.96
	4	5.11	4.91	5,05	5.31
	5	4.96	4.42	4.56	4.89
	$\dot{x}$	4.95	4.58	4.67	5.03
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 30 mg/kgbw.	1	5,13	4.55	5.21	5.47
	2	5,05	4.38	4.25	5.25
	3	4.97	4.59	4.81	5.31
	4	5.01	4.16	4.46	5.12
	5	5.17	4.05	4.71	5.38
	$\dot{x}$	5.06	4.39	4.68	5.30
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 100 mg/kgbw.	1	5.45	4.25	5.59	5.95
	2	5.38	4.34	5.57	5.73
	3	5.21	4.63	5.67	5.77
	4	5.51	4.65	5.91	6.51
	5	5.30	4.29	5.23	5.69
	$\dot{x}$	5.37	4.43	5.59	5.93



Picture 3. Photo reticulocytes of male white mice after induction extract ethanolpeels purple sweet potato seen with a microscope enlargement 100 x

The examination of hemoglobin levels by photoelectric (sianmethemolobin). Blood hemoglobin is converted into a form sianmethemoglobin using Drabkin reagent. Sianmetheglobin be measured using UV-VIS spectrophotometer at a wavelength of 546 nm which is the wavelength of maximum sianmethemolobin. Hemoglobin measurement results can be seen in Table 3. The results of the two-way ANOVA test shows that the hemoglobin levels after induction extract ethanol peels purple sweet potato with varian dosage and duration is increased significantly.

Further, Duncans test show difference effects of varian dosage was highly significant (p <0.01). Increased levels of hemoglobin maximum awarded by extract ethanol peels purple sweet potato with dosage100 mg/kgbw. The last parameter is observed hematocrit. In calculating the hematocrit value using micro method, this method is selected because the amount required is relatively less so it can be used for mice. The measurement results hematocrit value can be seen Table 4.

Table 2. The reticulocyte count white male mice that induction of chloramphenicol for 14 days and then given a suspension extract ethanol peels purple sweet potato various dosage of 14 days

Treatment	Animal	Total Reticulocytes (%)			
		0	14	21	28
Chloramphenicol 130 mg/kgbw	1	0.7	0.4	0.7	0.7
	2	0.7	0.4	0.6	0.7
	3	0.7	0.3	0.7	0.7
	4	0.6	0.4	0.6	0.6
	5	0.7	0.4	0.6	0.6
	$\dot{x}$	0.68	0.38	0.64	0.66
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 10mg/kgbw.	1	0.6	0.4	0.6	0.8
	2	0.6	0.5	0.7	0.8
	3	0.7	0.4	0.6	0.7
	4	0.8	0.4	0.7	0.8
	5	0.7	0.4	0.7	0.7
	$\dot{x}$	0.68	0.42	0.66	0.76
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 30mg/kgbw.	1	0.8	0.4	0.7	0.9
	2	0.8	0.4	0.7	0.8
	3	0.7	0.5	0.8	0.8
	4	0.8	0.4	0.7	0.8
	5	0.8	0.5	0.7	0.9
	$\dot{x}$	0.78	0.44	0.72	0.84
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 100mg/kgbw.	1	0.7	0.4	0.7	0.9
	2	0.8	0.4	0.8	0.9
	3	0.7	0.4	0.8	0.9
	4	0.7	0.5	0.8	1.0
	5	0.8	0.4	0.7	0.8
	$\dot{x}$	0.74	0.42	0.76	0.9

The results of a two-way ANOVA test shows that the hematocrit value after induction extract ethanol peels purple sweet potato with variation dosage and duration is increased significantly ($p < 0.01$). Dosage of 30 and 100 mg/kgbw showed the most significant improvement. Further Duncans test show, each group hematocrit values is increase. But dosage of 30 and 100 mg/kgbw increase in hematocrit values equal.

Table 3. Hemoglobin white male mice that induction of chloramphenicol for 14 days and then given a suspension extract ethanol peels purple sweet potato various dosage of 14 days

Treatment	Animal	Total Hemoglobin (g / dl)			
		0	14	21	28
Chloramphenicol 130 mg/kgbw	1	14.21	12.35	13.21	13.81
	2	14.96	11.81	13.93	13.79
	3	14.52	12.52	13.92	14.51
	4	15.01	12.35	13.01	14.34
	5	15,20	13.49	14,10	14.26
	$\dot{x}$	14.78	12.50	13.63	14.14
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 10mg/kgbw	1	15.31	12.45	14.79	15.61
	2	14.23	11.73	14.22	14.87
	3	14,15	12.81	14.41	14.37
	4	14.52	12.39	14.71	14.98
	5	14.51	12.59	14.58	14.19
	$\dot{x}$	14.54	12.39	14.54	14.80
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 30mg/kgbw.	1	15.12	13.18	14.48	14.83
	2	16.92	13.16	14.53	15.24
	3	15.12	13.04	14.72	15.23
	4	15,18	13.82	14.41	15.14
	5	15.64	12.27	14.87	15.03
	$\dot{x}$	14.54	13.09	14.60	15.09
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 100mg/kgbw	1	15.42	12.87	14.85	16.23
	2	15.46	13.86	14.51	15.27
	3	14.97	12.96	14.98	15.56
	4	14.76	12.92	14.92	15.69
	5	16.14	13.31	14.59	16.75
	$\dot{x}$	15.35	13.18	14.77	15.9

Table 4. Hematocrit value white male mice that induction of chloramphenicol for 14 days and then given a suspension extract ethanol peels purple sweet potato various dosage of 14 days

Treatment	Animal	Total hematocrit (%)			
		0	14	21	28
Chloramphenicol 130 mg/kgbw.	1	46.21	36.39	39.12	40.71
	2	46.75	37.46	38,71	39.52
	3	46.16	37.28	38.93	40.31
	4	47.18	38,12	38.88	39.80
	5	47.96	39.12	40.16	39.41
	$\dot{x}$	46.85	37.64	39.16	39.94
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 10mg/kgbw.	1	47.15	42.56	48.16	49.71
	2	48.13	39.72	48.17	48.42
	3	47.11	38.18	48.52	49.12
	4	48.06	38.16	48.83	48.73
	5	48.42	38.23	47.21	48.51
	$\dot{x}$	47.77	39.37	48.17	48.89
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 30mg/kgbw.	1	48.88	42.18	48.16	48.42
	2	47.76	39.72	48.75	49.02
	3	47.36	38.18	48.24	50.21
	4	47.12	38.16	48.42	51.36
	5	48.01	38.23	48.13	49.34
	$\dot{x}$	47.82	39.27	48.34	50.06
Chloramphenicol 130 mg/kgbw extract ethanol peels purple sweet potato 100mg/kgbw.	1	47.18	39.18	48.91	49.53
	2	49.08	40.16	49.23	50.67
	3	48,18	42.15	49.17	49.23
	4	48.12	41.02	49.79	49.36
	5	47.98	40.03	49.28	50.28
	$\dot{x}$	48,10	40.55	49.07	49.81

CONCLUSION

Based on research that has been done, the induction extract ethanol peels purple sweet potato *(Ipomoea batatas* (L.) Lam) to erythropoietic male white mice, it can make conclusion :

1. Extract ethanol peels purple sweet potato *(Ipomoea batatas* (L.) Lam) at dosage 10, 30 and 100 mg/kgbw can increase the number of erythrocytes and the results are shown in the maximum dosage 100 mg/kgbw at day 14 after induction extract ethanol peels purple sweet potato *(Ipomoea batatas* (L.) Lam).
2. Extract ethanol peels purple sweet potato *(Ipomoea batatas* (L.) Lam) at dosage of 10, 30 and 100 mg/kgbw can increase the number of reticulocytes and maximum results are shown in doses of 30 and 100 mg/kgbw at day 14 after induction extract ethanol peels purple sweet potato *(Ipomoea batatas* (L.) Lam).
3. Extract ethanol peels purple sweet potato *(Ipomoea batatas* (L.) Lam) at dosage of 10, 30 and 100 mg/kgbw can increase the hemoglobin levels and maximum results are shown in a dose of 100 mg/kgbw at day 14 after induction extract ethanol peels purple sweet potato *(Ipomoea batatas* (L.) Lam).

4 Extract ethanol peels purple sweet potato *(Ipomoea batatas* (L.) Lam) at dosage 10, 30 and 100 mg/kgbw can increase the hematocrit value and maximum results are shown in dosage 30 and 100 mg/kgbw at day 14 after Award bark extract preparation of purple sweet potato (Ipomoea batatas (L.) Lam).

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