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Hematology Effects of Purple Sweet Potato Pollen *(Ipomea batatas Var. Ayamurasaki)* on the White Male Mice Induced by Sodium Nitrite

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ABSTRACT

Purple sweet potato is one among many types of potatoes that maintain a lot of vitamins and minerals such as vitamin C, vitamin A, iron, calcium, phosphorus and anthocyanin that are useful in our body. The use of sodium nitrite as a food preservative affects the ability of erythrocytes for carrying oxygen, that cause anemia. This study has been carried out by measurement of blood components (hematology), especially for the number of leukocytes, erythrocytes, hemoglobin, hematocrit of mice that were previously induced by Sodium nitrite roomat causing of blood components of mice become abnormal and then were induced by pollen purple sweet potato (Ipomea batatas var. Ayamurasaki) with variations of volume of 0.4 ml and 0.2 ml for normalizing of blood components of mice. The obtaining data showed pollen purple sweet potato can normalize blood components of mice, but for given volume variation did not show any different effect.

Keywords: *Ipomea batatas var.ayamurasaki*, Anemia, Hematology, $NaNO_2$

INTRODUCTION

Indonesia is countries rich in food availability that contain are vitamins, minerals, and nutrients necessary for the metabolism of the human body, one of which is the sweet purple. Sweet purple potato contains vitamin C, vitamin A, iron, calcium, phosphorus, and anthocyanin that are important in the human body. Vitamin C in sweet purple potato can help to enhance the number of erythrocytes in blood. Which can repair of erythrocyte damage? Recently many diseases that occur in the blood and one of them are anemia. Anemia is a condition that occurs when the blood less hemoglobin level which is less than the normal 10.0-16.1 g/dL [1]. Such as lack of iron (Fe) which can lead to reduce hemoglobin levels. As a result of the it can cause a shortage of blood which is usually referred to as anemia [2]. Anemia is itself a state of quantity and quality of blood becomes abnormally that indicated by the red blood cell count and hemoglobin concentration in the blood is reduced. In addition, anemia is also influenced by vitamin C, because vitamin C serves as to reduce the ferri (Fe^{3+}) to ferrous (Fe^{2+}) so easily adsorbed. Vitamin C also acts to inhibit the formation of hemosiderin which is difficult in mobilized to free iron that is needed by the body [3]. The roles of hematologic examination in health sciences assist medical personnel in diagnosing a disease through examination of blood components. The important of hematologic examination is performed to determine any abnormalities early so that treatment can be done before caused severe illness, in addition, in doing examination can determine what kind of therapy is appropriate and effective as an alternative to medication. The main purpose of this examination is to determine the hematologic disease in the body detected through a blood comprehensively. Parameter state of hematologic examination includes the number of white blood cells, red blood cell count, hematocrit, hemoglobin concentration, platelet number and volume, and also red cell indices [4,5].

In this study, researchers want to know the effect hematology of sweet purple potato in mice that was induced by Sodium nitrite ($NaNO_2$) in advance, where is sodium nitrite widely used as a preservative in food and can affect red cells to carry oxygen, it will state that mice blood became abnormal (anemia) and subsequently induced mice with sweet purple potato essence and blood of mice are expected back normal. Hoppefully, sweet purple potato essence can normalize the blood that has been induced by $NaNO_2$, so that the sweet purple potato essence can be utilized to world health that is as a drug for anemia patients, and sweet purple potato itself is fairly easy to obtain and the price is quite affordable for all people.

MATERIALS AND METHODS

Chemicals, equipment and instrumentation

The materials used in this study is sweet purple potato *(Ipomea batatas var Ayamurasaki)* purchased in traditional markets of Bandar Buat, mice were white males mice, and distilled water, Sodium nitrite ($NaNO_2$), coffee powder, BP-2 (feed mice), rice husks, ascorbic acid.

The equipments used in this study are hematolizer, oral sonde, *juicer*, scales, and mice cages, eating utensils and drinking mice, Ethylenediaminetetraacetic Acid (EDTA) tubes and glassware, sterile scissors, 1 set of tools High Performance Liquid Chromatography (HPLC).

Preparation of experimental animals and acclimatization of white male mice

Test animals were used in this study were white mice males as much as 32 head, which is obtained from the Faculty of Pharmacy Andalas Padang with body weight of mice on average 20-30 g. Animals acclimatized in the laboratory for two weeks. The mice were then divided into four groups where is each one used as a blank control group, one group as a negative control and two other groups were treated with induction volume of 0.4 ml and 0.2 ml. Acclimatization animal experiments carried out for 2 w which was originally the weight of mice were weighed prior to use weighing animals, then placed the mice in each group with the provision of water, food, air, and laboratory condition. Feed mice are given during acclimatization is BP-2 feed and drink provided is tap water.

Preparation of sodium nitrite

A solution of sodium nitrite was made at a concentration of 3.75 mg/ml. Sodium nitrite weighed as much as 150 mg and then dissolved in 40 ml of distilled water. Then stored in bottles that had been cleaned and closed tightly. Dissolved given the sodium nitrite on male white mice for 14 days using a needle probe.

Making cider sweet purple

A total of 500 g of sweet purple cleaned first without skin removed and then cut into smaller sizes and after that mashed it using a juicer and obtained the sweet purple potato extract and measured the volume obtained (total 63 ml). Sweet purple essence is stored in an airtight glass jar and then stored in the refrigerator.

Treatment to test animals

Animal tests have acclimatized each group were treated as follows:

1. Group I (Blank controls): 8 mice were not given any treatment, only fed pellets and given a drink of water.
2. Group II (Induction $NaNO_2$): 8 mice fed with pellets, water, and induced a dissolved of $NaNO_2$ continuously during the study.
3. Group III (volume 0.4 ml): 8 mice fed with pellets, water, and a sweet purple essence with a volume of 0.4 ml.
4. Group IV (volume 0.2 ml): 8 mice fed with pellets, water and a sweet purple essence with a volume of 0.2 ml.

Blood animal testing

Animal testing that had been treated with induced $NaNO_2$ then performed mice blood sampling by cutting the tip of the mice tail. Mice were prepared to put in a bottle and then removed the tail and top of the tail is cutted using scissors sterilized until blood came out, the first drop of blood is discarded and subsequent blood collected in EDTA tubes. After the blood is in the EDTA tube, then closed the EDTA tube inverted 6 times. The tail mice that had been cut.

Determining the vitamin C content of sweet purple

Sweet purple potato extract that induced in mice during the study period of its vitamin C content determined using the method of HPLC. Measurement vitamin C with standard HPLC performed manufacture of vitamin C is ascorbic acid at a concentration of 10 mg. Making the standard solution made by dissolving ascorbic acid as much as 1 mg in a 100 ml volumetric flask. Followed by the manufacture of mobile phase by making a mixture of phosphate buffer solution pH 3.6 to acetonitrile ratio of 7: 3 (v/v). Then measuring the HPLC with UV detector at a temperature of 25°C with injection volume of 10 µl and flow rate of 0.5 ml/min^{-1}.

RESULTS AND DISCUSSION

Animal acclimatization

Acclimatization this animal is one of thing that have to do because in this study we use living creatures so this acclimatization is the adaptation by the animals to their new environment (Table 1).

Table 1: Weight of male acclimatized mice

Code animals	Ex. I (g)	Ex. II (g)	Ex. III (g)	Ex. IV (g)
A	30	29.1	26.3	25.4
B	31.8	29.2	29.3	28.5
C	29.4	29.2	26.6	30.3
D	31.4	31.2	30.6	25.4
E	28.9	21.8	28.6	34.4
F	28.1	28.9	25.4	27.7
G	31.7	32.5	28.4	21.0
H	26.6	23.2	28.8	24.6

Acclimatization is intended to adjust the state of the environment during experiment. Aacclimatization mice was conducted over 14 days in which mice were given only drinking water and fed the mice plain is BP-2. During these 14 days was observed whether the mice could adapt to his new environment, if mice cannot adapt to his new environment, so the mice will slowly die.

Profile blood of mice that were not given any treatment

For hematological measurements on samples taken one group is B, G, D (code samples). This decision is based of body mice weight the highest.

In Table 2, mice only fed and watered with ordinary water, the blood of mice as measured in a profile of blood, there is no change in the blood profile of mice this is because mice are not induced with $NaNO_2$ or induced by pollen sweet purple potato so that mice were only consuming food for mice and drunk water so that the value for the blood profile in mice remain within the normal range [5-8].

Table 2: Group one untreated

The sample code	WBC 2.6-10.1 × 10^3 ml^{-1}	RBC 6.5-10.1 million.ml^{-1}	HCT 21.8-48.0%	Hb 10.0-16.1 g.dL^{-1}
B	5.3	8.95	38.4	14.6
G	6.5	8.44	36.5	13.7
D	12.2	10.04	42.9	15.9

WBC: White Blood Cell; HCT: Hematocrit; RBC: Red Blood Cells; Hb: Hemoglobin

Blood profile of induced mice by sodium nitrite

Mice induced sodium nitrite solution with a volume of 0.3 ml each mouse by its way in orally induced by mice mouth.

Table 3: Profile of the induced mice blood by sodium nitrite

Code mice	WBC 2.6-10.1 × 10^3 ml^{-1}	RBC 6.5-10.1 Million.ml^{-1}	HCT 21.8-48.0%	Hb 10.0-16.1 g.dL^{-1}
Kel.II (G)	3.9	2.75	15.1	5.7
Kel.III (D)	7.3	4.92	18.5	4.6
Ex.III (B)	9.7	6,15	20.4	9.8
Ex.IV (E)	10.1	5.90	19.3	7.9
Ex.IV (C)	5.8	3.85	16.9	9.4

In Table 3 is the value of mice blood components after induction with $NaNO_2$ which looks after induction with $NaNO_2$ value of red blood cells, hemoglobin, and hematocrit is abnormal or lower than the reference value for normal mice blood profile values. Sodium nitrite could affect the work of erythrocytes in carrying oxygen, causing anemia [9]. Reduced ability to carry oxygen occurs because the erythrocytes in hemoglobin binds to NO of $NaNO_2$ and formed nitrosohemoglobin that hemoglobin levels in erythrocytes are reduced. The result of measurements on the number of leukocytes in mice induced orally $NaNO_2$ is still in the normal range it is caused due to an intolerance body against $NaNO_2$ are given. Age animal may also effect the production of leukocytes [6]. After induction with $NaNO_2$ mice were weighed to see the effect of $NaNO_2$ and the data can be seen in the Table 4.

Table 4: Weight of induced mice with $NaNO_2$

Code animals	Group II (g)	Group III (g)	Group IV (g)
A	27.2	24.6	25.4
B	28.4	25.5	24.5
C	29.0	26.1	27.3
D	29.2	29.6	25.4
E	20.6	26.6	32.4
F	27.9	22.4	27.2
G	30.5	28.4	20.4
H	21.0	26.8	22.6

Body weight of mice after induction with sodium nitrite occurrence of weight loss in mice it is due to the influence of sodium nitrite the body's metabolism of mice.

Blood profiles of mice induced with sodium nitrite during the study periode

Table 5 mice induced blood profile $NaNO_2$ continuously during the study. Where the mice will be measured of blood component was taken based on the weight.

Table 5: Profile of induced mice blood by $NaNO_2$ during the study

The sample code	WBC 2.6-10.1 × 10^3 ml^{-1}	RBC 6.5-10.1 (million ml^1)	HCT 21.8-48.0%	Hb 10.0-16.1 $g.dL^{-1}$
G	2.5	2,86	16.1	4.7
A	3.7	2.92	12.5	4.6
E	2.8	4,86	20.9	3.9

In Table 5, it is clear that with induction of $NaNO_2$ mice blood profile changes especially in the red blood cells, hematocrit and hemoglobin. This is because sodium nitrite into the body will break down into toxic nitrite compound because a toxic compound that can be decomposed into NO and O. Nitroxides (NO) will bind to the blood, and forming nitrosohemoglobin which is resulted in competition for binding of O_2 by hemoglobin with NO, so that O_2 is the lower bound and activate the hormone erythropoietin that triggered erythropoiesis [4].

Likewise with hemoglobin changes and this is because the necessary of O_2 in metabolism. It is also associated with high or low dose $NaNO_2$ induced due to the higher $NaNO_2$ induced, so the higher in O_2 demand as well as the higher competition O_2 and NO to bind hemoglobin demand of O_2 increased due to O_2 binding to hemoglobin is reduced [4]. Mice that have decreased the amount of blood components and then induced sweet purple potato essence with a variation of volume of 0.4 ml and 0.2 ml and below the data obtained.

Blood profiles of mice induced with purple potato juice volume of 0.4 ml and 0.2 ml

Table 6, profile blood of mice (group III) which has been induced with sweet purple potato essence with a volume of 0.4 ml were previously mice induced with $NaNO_2$ and decreased the number of blood components (erythrocytes, hemoglobin, hematocrit) of the normal range for a number of blood normal components.

Table 6: Profile of blood induced with sweet purple potato essence volume of 0.4 ml which had previously been induced by sodium nitrite

The sample code (Kel. III)	WBC 2.6-10.1 (10^3 ml^{-1})	RBC 6.5-10.1 million ml^{-1}	HCT 21.8-48.0%	Hb 10.0-16.1 g dL^{-1}
D	9.3	8.27	35.7	12.9
B	9.3	9.11	38.6	14.2
F	2.9	8.29	37.4	13.3

Table 7: Profile of induced blood with sweet purple potato essence of 0.2 ml which had been previously induced by sodium nitrite

The sample code (Ex. IV)	WBC 2.6-10.1 10^3/ml	RBC 6.5-10.1 million/ml	HCT 21.8-48.0%	Hb 10.0-16.1 g/dL
E	4.5	9.87	43.4	15.3
C	4.9	8.90	39.6	13.7
G	3.6	9.81	44.7	15.3

Table 8: Body weight of mice after being fed by pollen purple yam

Code animals	Ex. 1 (g)	Ex. 2 (g)	Kel. 3 (g)	Ex. 4 (g)
A	39.5	27.4	32.4	30.4
B	34	Die	32.8	31.8
C	35	27.6	30.6	32.3
D	36	Die	33.6	30.4
E	37	20.8	31.6	35.4
F	36.4	25.4	30.4	30.0
G	34.2	29.0	31.4	31.6
H	34	Die	33.8	31.2

The process of formation erythrocytes required iron (Fe) and vitamin C. Iron (Fe) plays a role in the formation and maturation of erythrocytes in the process vitamin C serves as a trigger iron substance. Vitamin C has more influence on the quality of red blood cells because, the benefits of vitamin C as antioxidants that neutralize free radicals as like Sodium nitrite form this vitamin C has more functions in the repair process of red blood cells [4,10]. Vitamin C serves to reduce the ferric (Fe^{3+}) to ferrous (Fe^{2+}) so easily adsorbed, vitamin C affect the absorption and release of iron from transferrin into the tissues of the body [4]. Other compounds contained in sweet purple potato itself are a protein. Protein in the body plays a role as a shaper of erythrocytes. Iron will bind to protein molecules that they form ferritin and in a state of transport will form a transferrin which serves to transport the iron to be used in the process of hematopoiesis or the formation drops of blood (Tables 7 and 8) [1].

The weight measured during the study in group I, III, IV, during the study period showed an increasing. This increasing in weight is related to the growth and development happens in mice, meanwhile the addition of sweet purple essence also resulted in mice weight are increasing due to the absorption of nutrients from the essence of sweet purple potato itself. The content of vitamin C from sweet purple potato.

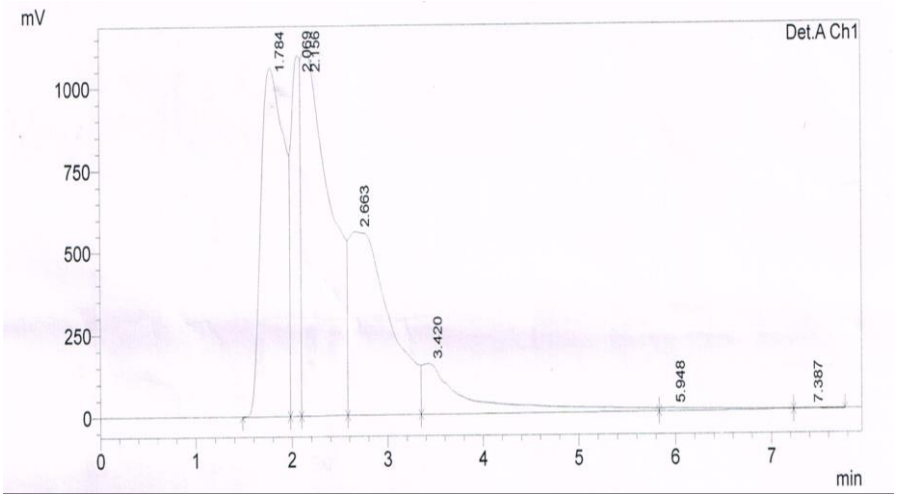

Figure 1: The sample of vitamin C

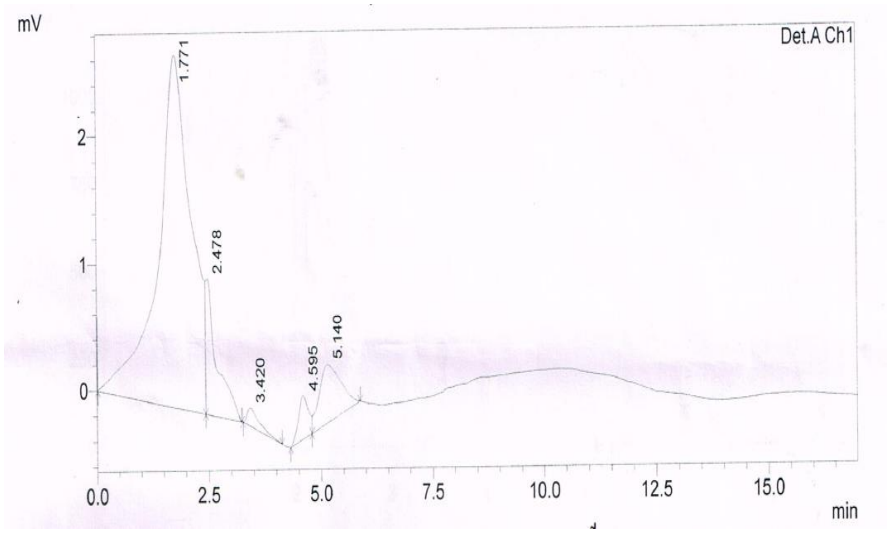

Figure 2: The standard of vitamin C

In Figure 1 the standard used for Vitamin C is ascorbic acid, the graph shows that vitamin C from a standard on the retention time of 1771 and compared with the Figure 2 the content of vitamin C from the sweet purple potato essence also be showed at a retention time of 1784, it is only a slight difference between samples and standards were used and it can be said as samples of sweet purple potato essence which contains vitamin C. this differences cause of the samples which are too concentrated, so the retention time produced quite different. In this study measured the amount of vitamin C of sweet purple essence in an amount sufficient could fight most of the factors that can inhibit the absorption of iron in the blood, so that the iron (Fe) and vitamin C are interconnected in the formation of erythrocytes [1,2].

CONCLUSION

Hematologic effects on mice induced by sodium nitrite made the number of blood mice are decreased, especially for the number of erythrocytes, hemoglobin and hematocrit. Giving sweet purple potato essence with volume induction of 0.4 ml and 0.2 ml are able to normalize blood components of mice, especially for the value of erythrocytes, hemoglobin, and hematocrit that have induced natrium nitrite. At the volume induction of 0.2 ml, is the value of blood components measured higher than the volume of 0.4 ml. Giving sweet purple potato essence are affect to body weight of mice in which it increased after giving sweet purple potato essence.

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