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by Yoni Astuti

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Effect of Angkak against Bleeding Anemia of Rat

Zulkhah Noor^{1*}, Yoni Astuti²

¹ Physiology Department of Medical and Health Sciences Faculty of Universitas Muhammadiyah
Yogyakarta

² Biochemistry Department of Medical and Health Sciences Faculty of Universitas Muhammadiyah
Yogyakarta

* Corresponding author, email: zulkhah@yahoo.com

Abstract

Mild and chronic anemia need different supplements that can stimulate the body to form blood cells quickly. Supplements for hemopoiesis are nutrients and other factors that can accelerate hemopoiesis. Nutrition and other factors are allegedly owned by the red yeast rice (a product of fermentation using *Monascus sp.*). The purpose of this study was to determine changes in the number of platelets, erythrocytes and blood hemoglobin levels of anemic mice. This research is experimental, pre-posttest control group design. The samples were 30 wistar rats aged 2 months divided into 6 groups. The research was conducted in UHP UMY and the LPPT laboratory LPPT of UGM. Dose of red yeast rice is 37 mg/200 g BW and 74mg/200g BW of rat. Data were analysed using a paired *t* test, one way ANOVA and post hoc. The results showed that administration of red yeast rice in normal mice did not cause changes in the number of blood cells significantly. Angkak can normalize platelet counts, erythrocyte counts, and hemoglobin concentration of bleeding anemia mice ($p < 0.05$).

Key words: Angkak, platelets, erythrocytes, hemoglobin.

INTRODUCTION

Various diseases and trauma cause vascular damage and bleeding. Acute blood loss will lower blood volume and oxygen supply to tissues. Sudden severe bleeding can lead to hypovolemic shock, cardiac failure, and death. Heavy bleeding may occur in peptic ulcer (Gralnek, *et al.*, 2008), dengue hemorrhagic fever (DHF) (Hadinegoro, *et al.*, 2001). Bleeding is the most common cause of maternal mortality, childbirth and postpartum (Pardosi, 2005). If bleeding occurs in chronic, hemoglobin levels can fall to the point where the oxygen needs of tissues are not adequate. Bleeding will reduce blood volume, blood cells and iron stores. Hidden bleeding from gastrointestinal in adults is a common cause of iron deficiency anemia, and in tropical countries is the most common cause of hookworm infection (Bakta, 1993;

Hoffbrand, 2001). Most often, hypovolemic shock is secondary to rapid blood loss (hemorrhagic shock). Acute external blood loss secondary to penetrating trauma and severe GI bleeding disorders are 2 common causes of hemorrhagic shock. Hemorrhagic shock can also result from significant acute internal blood loss into the thoracic and abdominal cavities.

Blood loss up to 5% of body mass did not decrease blood flow rate. The decline in blood flow rates with hemorrhage was due to decline pulse volume in amphibians (Hilman and Withers, 1988). Hypovolemia and reduction of erythrocytes due to blood loss caused a compensatory response and erythropoietic arrhythmias. Losing blood less than 20% of total blood volume causes vasovagal reactions and sometimes also anxiety. Losing blood as much as 20-30% causes orthostatic hypotension, tachycardia and

1 anxiety. Losing blood as much as 30-40% causes orthostatic hypotension, anxiety, tachycardia at the rest and syncope when sitting or standing. Losing blood more than 40% leads to hypovolemic shock, very low supine blood pressure (falling), tachycardia, damp and cold skin, anxiety, restlessness, confusion, short of breath (Hillman *et al.*, 2005).

Erythropoietic's response is the body's effort to restore the blood loss. Average production of erythrocytes varies depending on the number of erythrocyte destruction and the demand of tissue's oxygen. The decrease of oxygen is related to hemoglobin, hemoglobin levels, or the decreasing affinity of oxygen will increase the production of erythropoietin by renal interstitial cells. Erythropoietin was taken by blood flow to the bone marrow and bound to the erythropoietin receptor (EPOR) on the surface of erythroid precursors (Guyton & Hall, 2007). A few hours later there was an increase of DNA synthesis followed by the increase of proliferation cell and maturation of stem cell to form new red blood cells. Bone marrow response in full requires a few days. The increasing of reticulocyte can be detected 4-4 days, while the increasing of hematocrit will be seen after one week (Hoffbrand *et al.*, 2001)

Erythropoietic requires a variety of supplements that can stimulate the body to form blood cells quickly. Supplements that are needed for reforming Anemia are substances which are needed for the synthesis and hemopoiesis factors. Nutritional supplements are definitely needed. But it is necessary to have other factors that can accelerate hemopoiesis which may be found in angkak (red yeast rice).

Red yeast rice is fermented product which uses *Monascus sp* molds native to China. The first making was conducted by the Ming Dynasty who ruled in the 14th until the 17th century. In the traditional text of The Ancient Chinese Pharmacopoeia, it is stated that red yeast rice was

used as a remedy for digestion and blood circulation. Red yeast rice has also been used as a food supplement that can be consumed every day. Several species of fungi have been used to produce red yeast rice, among them are *Monascus purpureus*, *M pilosus*, and *M Anka*. Taiwan, Japan, Korea, and Hong Kong produce red yeast rice as a natural food coloring (Nurhidayat, 2002). Various testimonies said that red yeast rice might accelerate the production of platelets in patients with dengue fever and accelerate the synthesis of blood in anemia (Damisi dan Putritami, 2008). So far, there has not been found the effect of giving red yeast rice on hemopoiesis. Therefore, the purpose of this study was to determine the effect in the number of platelets, erythrocytes and hemoglobin levels of anemic mice.

MATERIAL AND METHODS

This study was an experimental study using pretest-posttest control group. The independent variable was the doses of angkak, which were 37 mg and 74 mg per 200 g of BW of rat and the dependent variable were the numbers of blood cells (platelets, erythrocytes and hemoglobin). The research subjects were 60 wistar rats, aged 2 months, divided into 6 groups:

- a. group 1: negative control (no treatment),
- b. group 2: positive control (rat bleeding),
- c. group 3: normal rat + 37 mg of angkak,
- d. group 4: normal rat + 74 mg of angkak,
- e. group 5: bleeding rat + 37 mg of angkak,
- f. group 6: bleeding rat + 37 mg of angkak

The research has been conducted at UPHP UMY for the maintenance and treatment. The collected blood was analyzed in the laboratory of LPPT UGM

Materials and research tools consisted of Wistar rats aged 2 months, red yeast rice (angkak), maintenance equipment and gastric sonde, blood sampling devices (disposable syringe, ependorf), and blood analyzer.

1 Stages of research done for data collection were as follows:

- a. Rats were grouped according to treatment
- b. Rats were acclimatized for 1 week
- c. The number of blood cells were measured before treatment (pretest). Blood samples were taken through the tail. Rat tail was smeared with balm to dilate the blood vessels of the tail. Approximately 1-2 minutes after being smeared with balm, the tail was cut approximately 0.5 mm long. The blood was mixed with anticoagulant in a tube.
- d. The treatment was carried out for 20 days
- e. Dose of angkak was based on the recommended dose for humans, i.e. 8 capsules per day, each capsule contained 250 mg, so a 2000 mg per day. The dose was converted to the rat body weight of 200 g with a ratio of 1:0.018, i.e. $0.018 \times 2000 \text{ mg} = 37 \text{ mg}$ per 200 g BW. The Second dose was doubled from the first dose, which was equal to 74 mg per 200 g BW rats per day.
- f. Bleeding was carried out 6 times in 20 days (4 times).
- g. Measurement of the number of blood cells (mm^3) was performed at the University of Gadjah Mada with automatic LPPT haemocytometer
- h. Data were tabulated and analyzed with a paired T test, ANAVA and continued with LSD.

RESULTS AND DISCUSSION

The influence of 6 times bleeding in 20 days affects the decreasing in Hb levels at the rate of 24.1% (from 14.33 mg/dl to 10.88 mg/dl). This was natural because chronic blood loss results in the loss of plasma and erythrocyte so that hemoglobin also decreases. Although the body's homeostasis compensates the loss of blood, the faster blood loss than the compensation of the bleeding will result in the reduced Hb levels of mice, which makes the mice

gets anemia. According to etiology, anemia can be classified into three kinds, i.e. the disorder of red blood cell production in bone marrow (hipoproliferation), disorder of red blood cell maturation (ineffective erythropoiesis), and the decreasing of the life span of red blood cells (blood loss or hemolysis). After bleeding, the body retains fluid immediately to increase blood volume. Anemia occurs when an increase in plasma volume is not comparable with the addition of blood cells and hemoglobin. In addition, it can be caused by an increased iron requirement and a lack of iron stores and iron intake in the diet. Iron is needed for eritropoesis (Atmarita, 2004 cit. Amirudin et al, 2007).

It seems clear that the effect of red yeast rice in helping of recovering the amount of hemoglobin due to bleeding process, so that Hb levels returned to normal values. Red yeast rice treatment has no effect on normal mice. This suggests that red yeast rice does not disturb homeostasis, so that the blood remained within normal limits. Although, previous researchers sad that the side effects of red yeast rice may increase the risk of bleeding. Caution is advised in patients with bleeding disorders or taking drugs that may increase the risk of bleeding. Dosing adjustments may be necessary. A metabolite of *Monascus* called mycotoxin citrinin (CTN) in fermentation may be harmful (Liu *et al.* 2005 *cit.* Red Yeast Rice, 2011). There is one report of anaphylaxis (a severe allergic reaction) in a butcher who touched meat containing red yeast rice (Red yeast Rice, 2007).

Red yeast rice has been known containing Fe (iron) (Damisi dan Putritami, 2008). This mineral is essential for the synthesis of hemoglobin. Heme is the iron core. Iron can get into the core activity of the enzyme heme because ferrochelatase. The enzyme activity is highly dependent on Fe mineral intake (Hoffbrand, 2001). Fe content in the red yeast rice is helpful to cover loss of hemoglobin due to bleeding.

1 The recommended dosage of Angkak is the best way to restore hemoglobin loss. Doubled dose of red yeast rice did not show a more rapid effect. So the recommended Angkak dose is enough to help the body restore the loss of Hb.

Table 2 shows that bleeding decreases erythrocyte number. These results indicated that the decreasing of erythrocyte was equivalent to 23.69% (751.7 ± 10.21 became 573.6 ± 26.65) million/mm³. It seems that the red yeast rice may accelerate the production of erythrocytes, so that bleeding rats which were given angkak did not have anemia. The mean number of erythrocytes in the normal range is 7.00-8.00 (million/ mm³), while that based on biological data of laboratory mice was 7.2-9.6 (million/mm³) (Arthur, *et al.*, 1928)). Red yeast rice can prevent the decrease in erythrocyte levels during hemorrhage because red yeast rice contains a number of materials needed for the synthesis of red blood cells. Red yeast rice contains a variety of B vitamins (B12, folic acid). This compound is necessary for the

synthesis of red blood cells. In addition, these substances have enough iron, so as to reduce the worsening of anemia due to blood loss.

Table 3 shows that the average number of platelets of rats was higher than the number of human platelets. The average number of platelets of various treatment groups showed improvement and reduction. The average number of platelets of bleeding group increased significantly ($p=0.0028$). This is estimated because of the whole blood mass decreased, especially red blood cells and hemoglobin. The decreasing in erythrocyte and hemoglobin will lead to hypoxia. This situation will spur the release of erythropoietin; a hormone functioned to lead the production of blood cells. Blood loss will stimulate the release of trombopoietin that will stimulate the production of platelets (Guyton and Hall, 2007). Blood loss makes iron deficiency. This will stimulate the release of trombopoietin that will stimulate the production of platelets. Extreme thrombo-

Table 1. The mean of hemoglobin levels (Hb) before and after treatment

Treatment	Hb -pretest	Hb - posttest	changes in Hb levels	T test (p)
Control	14 ± 0,2	14,86 ± 0,7	0.86	0.089
Bleeding control	14,33 ± 0,06	10,88 ± 0,69	-3.45*	0.000163
Angkak (37mg)	15,4 ± 0,71	13,75 ± 0,21	-1.65	0.087
Angkak (74 mg)	14,85 ± 1,34	13,85 ± 0,07	-1	0.403
Bleeding Angkak (37 mg)	15,05 ± 0,21	14,15 ± 0,92	-1.1	0.31
Bleeding Angkak (74 mg)	14,8 ± 0,14	13,4 ± 0,85	0.86	0.148
ANOVA		p=0.0001		

*significantly difference

Table 2. The mean of the erythrocytes number (EN) before and after treatment

Group Treatment	EN pretest (million/mm3)	AE postes (million/mm3)	changes	T test (p)
Control	737.3 ± 7.2	775.2 ± 71.3	35.2	0.410
Bleeding control	751.7 ± 10.21	573.6 ± 26.65	-178.1*	0.0001
Angkak 37 mg	730 ± 36.51	624.4 ± 111.39	-105.6	0.17
Angkak 74 mg	771.3 ± 25.48	726.8 ± 30.14	-44.5	0.078
Bleeding angkak 37 mg	715.7 ± 16.80	620 ± 33.38	-95.7	0.003
Bleeding angkak 74 mg	707.3 ± 35.70	482.4 ± 41.76	-224.9*	0.006
ANOVA		p= 0.03		

*significantly difference

Table 3. The mean of platelet number (PN) before and after treatment

Group Treatment	(PN) ($10^3/\text{mm}^3$)		Changes	T Tes p
	Pretes	postest		
control	1176,67± 166,54	1043,60 ± 46,11	-133.07*	0.2793
Bleeding control	1187,33 ± 50,01	1449,20 ± 82,82	261.87*	0.0028
Angkak 37 mg	1130,50±88,39	1104.00±93,34	-26.50	0.7981
Angkak 74 mg	1044.00±106,07	1222,50±95,46	178.50	0.2189
bleeding angkak 37 mg	872.00±32,53	1039.00±162,63	167.00	0.2905
bleeding angkak 74 mg	749.00±234,76	1084,50±44,55	335.5*	0.1855
ANOVA	p=0.005			

*significantly difference

cytosis can occur in cases of iron deficiency secondary to celiac disease (Voigt, et al., 2008).

Table 3 shows that administration of red yeast rice 37 and 74 mg dose in normal mice did not result in a significant change in platelet numbers. This suggests that red yeast rice did not inhibit or stimulate the production of platelets and did not cause damage to the circulating platelets. Giving Angkak 37 and 74 mg doses on bleeding group did not increase the significance of platelet numbers. The increasing in platelet was still within normal limits. Significant increase in platelet numbers occurred in the mice bleeding.

Factors that stimulate megakaryocyte (MK) are thrombopoietin (TPO) and cytokines. Circulating levels of thrombopoietin (TPO), the primary growth-factor for the megakaryocyte (MK) lineage, induce concentration-dependent proliferation and maturation of MK progenitors by binding to the c-Mpl receptor and signaling induction. Decreased platelet turnover rates results in increased concentration of free TPO, enabling the compensatory response of marrow MKs to increase platelet production. C-Mpl activity is orchestrated by a complex cascade of signaling molecules that induces the action of specific transcription factors to drive MK proliferation and maturation (Deutsch And Tomer, 2006). Cytokines are molecules such as a paracrine hormone that regulates the immune response. Cytokines are not only secreted by lymphocytes and macrophages, but also endothelial cells,

neurons, glia cells, and others. Interleukin-11 derived from bone marrow stromal cells serves to stimulate the production of acute phase proteins and stimulates megakaryocyte (Ganong, 2003).

The results of this study was different from the results of the research by Gunawan (2007) who conducted a study determining the levels of platelets of Swiss Webster mice. This study suggests that infusion of rice with red yeast rice 1.3 mg/kg BW and metabolite of yellow *Monascus purpureus* 6.6 mg /kgBW increased the level of blood platelets significantly after 7 days administration. Differences in these results are expected due to differences in sample species, dosage and the treatment of bleeding in this study.

This research should be developed to determine the effect of red yeast rice for the production of platelets in experimental animals that have trombocytopenia. This is a new challenge how to make the animal run trombocytopenia. Another factor to consider is the selection of experimental animals. Rabbits have a larger body. Venesection in rabbits is more likely to be done and make the bleeding more severe, and anemia and trombocytopenia will be more apparent. From that, we expect the effect of red yeast rice can be seen more clearly.

CONCLUSION

Angkak can normalize platelet counts, erythrocyte counts, and hemo-

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globin concentration of bleeding anemia mice ($p < 0.05$).

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