COMPARING THE EFFECT OF RED YEAST RICE, DATE PALM, AND GUAVA LEAF EXTRACT ON THROMBOCYTE AND MEGAKARYOCYTE COUNT IN THROMBOCYTOPENIC WHITE RATS

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ABSTRAK

*Trombositopenia merupakan parameter penting pada Demam Berdarah Dengue (DBD). Terapi herbal telah digunakan oleh masyarakat untuk meningkatkan jumlah trombosit dalam kasus DBD, yaitu angkak, kurma (Phoenix dactylifera L), dan ekstrak daun jambu biji (Psidium guajava folia). Tujuan dari penelitian ini adalah untuk membandingkan efek angkak, kurma, dan ekstrak daun jambu biji terhadap peningkatan jumlah trombosit dan megakariosit sumsum tulang pada tikus yang trombositopenik. Dua puluh delapan tikus Wistar jantan yang dibagi menjadi 4 kelompok, diberikan trombositopenia dengan pemberian heparin 0,1 ml/100g BB tikus/hari, dengan injeksi subkutan selama 3 hari. K1 dilanjutkan dengan pemberian plasebo selama 3 hari (kelompok kontrol), K2 dengan suspensi ekstrak angkak 108 mg/kg tikus BB/hari, K3 dengan ekstrak kurma, dan K4 dengan ekstrak daun jambu biji. Jumlah trombosit dan megakariosit tertinggi adalah K2, K3 diikuti, K4, dan K1. Uji MANOVA menunjukkan perbedaan signifikan (p <0,05). Berdasarkan uji LSD, ekstrak angkak adalah obat yang paling efektif untuk meningkatkan trombosit pada tikus trombositopenik. Ketiga ekstrak menunjukkan kencenderungan peningkatan jumlah megakariosit, tetapi perbandingan tersebut tidak signifikan secara statistik.**(FMI 2013;49:82-87)***

Kata kunci: *ekstrak angkak, ekstrak kurma, ekstrak daun jambu biji, trombosit, megakariosit, trombositopenia*

ABSTRACT

Thrombocytopenia is an important parameter in Dengue Hemorrhagic Fever (DHF). Herbal therapies have been used by the community to increase the platelet count in DHF cases, i.e. red yeast rice, date palm (Phoenix dactylifera L), and guava leaf extract (Psidium guajava folia). The purpose of this study is to compare the effect of red yeast rice, date palm, and guava leaf extract on the increase of platelet and bone marrow megakaryocyte count in thrombocytopenic rats. Twenty eight male Wistar rats were divided into 4 groups, rendered thrombocytopenia by administering 0.1 ml/100g rat BW/day of heparin by subcutaneous injection for 3 days. K1 was continued with administration of placebo for 3 days (control group), K2 with suspension of 108 mg/kg rat BW/day red yeast rice extract, K3 with date palm extract, and K4 with guava leaf extract. The highest platelet and megakaryocyte count were K2, followed by K3, K4, and K1. MANOVA test showed significant differences (p < 0,05). Based on the LSD test, the red yeast rice extract is the most effective remedy to increase platelet in thrombocytopenic rats. All three extracts showed increasing trend of megakaryocyte count but the comparisons are not statistically significant. ***(FMI 2013;49:82-87)***

Keywords: *red yeast rice extract, date palm extract, guava leaf extract, platelet, megakaryocyte, thrombocytopenia*

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is a health problem in Indonesia and every year an increase in incidence (Nasronudin 2007). Health Department data shows in 2008 there were 953 deaths (117 830 cases of DHF) and increased to 1,013 deaths (121 423 cases of DHF) in January-October 2009 (Ministry of Health, Republic of Indonesia 2009). Complications that caused the death was shock and haemorrhage due to plasma displacement caused thrombocytopenia, so that thrombocytopenia is an important parameter in the DBD. The cause of thrombocytopenia in the early phase is decreased production of platelets due to virus attack megakaryocytes in the bone marrow. In the next phase, thrombocytopenia primarily due to destruction of platelets in the circulation (Nasronudin 2007). Prevention of bleeding therapy through improved levels of blood platelets needed in cases of thrombocytopenia but to date effective chemical drugs is questionable overcome thrombocytopenia (Rahajuningsih 2007). Therefore, people turn to herbal remedies, including red yeast rice (red yeast rice) is the product of fermentation of rice by *Monascus purpureus* mold, date palm

(*Phoenix dactylifera* L), and guava (*Psidium guajava folia*) (Purbani 2007, Rudiansyah 2010).

Several studies have shown that platelet levels can be increased by administration of red yeast rice (Triana & Nurhidayat 2006, Rahmi 2009, Wiyasihati 2011), dates (Zahroh 2010), and guava leaves (Soegijanto et al 2010). *Angkak* dates contain flavonoids and isoflavones (Tisnadjaja 2006), which can inhibit the activity of the enzyme hyaluronidase to degrade hyaluronic acid (Sahasrabudhe & Deodhar 2010, Salmen 2003). Hyaluronic acid in the extracellular matrix (ECM) of bone marrow to stimulate the release of interleukin-6 (IL-6), which stimulates megakariopoiesis (Khaldoyanidi et al 1999) and maturation of megakaryocytes (Matsumura & Kanakura 2002). While guava leaves contain flavonoids quercetin can increase granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) which can stimulate megakariopoiesis (Soegijanto et al 2010). With the increase in megakariopoiesis, will increase the number of blood platelets.

Currently *angkak*, dates and guava leaves are used extensively by the community for the treatment of patients with DHF (Rudiansyah 2010). This study aimed to compare the effect of red yeast rice extract, dates and guava leaves to the increased number of platelets and megakaryocytes in mice thrombocytopenia.

MATERIALS AND METHODS

Experimental animals used were white rats (*Rattus norvegicus*) Wistar many as 28 tails are male, aged 2-4 months, 150-200 g body weight, and physical good health. Acclimatization performed for 1 week, then randomly divided into 4 groups: K1: thrombocytopenia + placebo treatment group (control), K2: The treatment group thrombocytopenia + red yeast rice extract, K3: The treatment group thrombocytopenia + extracts of dates and K4: treatment group thrombocytopenia + guava leaf extract. Injection of heparin was administered subcutaneously at a dose of 0.1 ml/100g BB rat/day for three days as 1x/day Zahroh based research (2010) and Ekawati (2012). The dose is two times the adult human dose have dikonvesikan that all animal groups experienced thrombocytopenia (platelet count <50%).

Extract *Angkak*, dates and guava leaves is given in the form of a suspension of 0.5% CMC Na 1 ml/100 g BW rat/day through the sonde as 2x/day. The dose used was 108 mg/kg rat/day in accordance with the converted adult human dose and treatment was given for 3 days. Blood samples were taken from rat heart as much as 3 ml Vacutainer tubes with EDTA for platelet counts examination. Right femur was taken for examination of bone marrow smears. Femur which had been cleared of surrounding tissue is cut transversely, and bone marrow specimens were taken using a fine brush and applied to glass objects made preparations for a bone marrow smear with Wright-Giemsa staining method (Hedrich 2006).

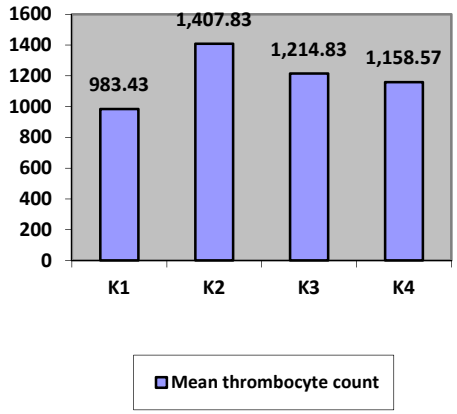
Platelet counts were determined by the method of automated hematologic analyzer using a Sysmex KX-21. The results of the calculation in units of the number of platelets/mL. Rat bone marrow smear preparations examined with semiquantitative analysis method per field of view using a fine brush smear preparations of bone marrow 400x magnification with a light microscope. The result of the calculation in units of the number of megakaryocytes/HPF (High Power Field).

RESULTS

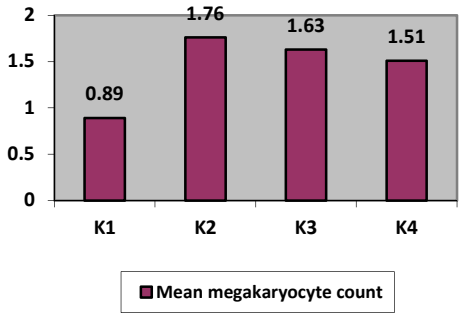
Description of the data from the variable mean platelet count and the number of megakaryocytes were obtained as follows :

Table 1. The mean value and standard deviation of the average variable platelet count and megakaryocyte

Groups	N	Thrombocyte count ($x10^3/\mu l$)	Megakaryocyte count (/HPF)
K_1	7	983.43±144.627	0.89±0.25119
K_2	6	1.407.83±238.098	1.76±0.22230
K_3	6	1.214.83±112.288	1.63±0.32506
K_4	7	1.158.57±146.737	1.51±0.18192



a)



b)

Figure 1. Diagram rod a) the average number of platelets and b) the average number of megakaryocytes

Test for normality with the Kolmogorov-Smirnov test showed normal distribution of data. Testing the correlation between the mean platelet count variable with a variable number of megakaryocytes performed using Pearson's test. The result shows $p < 0.05$ so that it can be concluded that there is a significant correlation between the variables and the number of megakaryocytes in platelet count (Table 2).

Table 2. Results of Pearson correlation test between variables mean platelet count and megakaryocyte

Statistics	Variables	Thrombocyte count ($x10^3/\mu L$)	Megakariocyte count (/HPF)
Pearson Corr.	Thrombovyte count ($x10^3/\mu L$)	1	0,521
P			0,006
Pearson Corr.	Megakaryocyte count (/HPF)	0,521	1
p		0,006	

MANOVA testing was conducted to determine differences in all variables depending on the group 1-4. The result shows the value of $p < 0.05$ so it can be concluded that there were significant differences (Table 3). To find out where the dependent variables are different in each group, then followed by LSD (Least Significant Difference) (Tables 4 and 5).

DISCUSSION

Red yeast rice, dates and guava leaf is a natural substance that is used by the public as herbal therapy to increase platelet counts in dengue fever (Rudiansyah 2010, Purbani 2007). This study aimed to compare the effect of red yeast rice extract, dates and guava leaves to the increased number of platelets and megakaryocytes in mice thrombocytopenia.

Table 3. Results of MANOVA variable mean platelet count and the number of megakaryocytes

Parameters	Value	F	Sig.
Pillai's Trace	0.860	5.534	0.000
Wilks' Lambda	0.214	8.142	0.000
Hotelling's Trace	3.334	11.112	0.000
Roy's Largest Root	3.226	23.660	0.000

The chosen animal is a white rat (*Rattus norvegicus*) as used in some previous studies related and mice never vomiting (Kusumawati 2004). Male gender and due to the homogeneity of the sample on the estrous cycle of female rats are suspected to affect the hematological profile of mice due to an increased number of megakaryocytes in the spleen (Lord & Murphy Jr. 1972). Injection of heparin was given to make a model of thrombocytopenia. Injection was administered subcutaneously at a dose of 0.1 ml/100g BB rat/day for three days as 1x/day Zahroh based research (2010) and Ekawati (2012). Extract treatment was given for 3 days and blood sampling for platelet counts examination performed on day 4 due to dengue fever is an infectious disease caused by a virus that can basically heal itself and generally improve within 7 days. The degree of thrombocytopenia in dengue cases tend to be associated with disease severity (Soegijanto et al 2010), therefore an increase in the number of platelets faster will provide many benefits, including reducing the need and cost for supportive therapy, and reduce the risk of fatal bleeding due to severe thrombocytopenia.

Table 4. Results of LSD test variables mean platelet count

Groups	K_1	K_2	K_3	K_4
K_1	-	0.000*	0.020*	0.060
K_2	0.000*	-	0.055	0.013*
K_3	0.020*	0.055	-	0.547
K_4	0.060	0.013*	0.547	-

Note: * = significantly different

Table 5. Results of LSD test variable average number of megakaryocytes

Groups	K_1	K_2	K_3	K_4
K_1	-	0,000*	0,000*	0,000*
K_2	0,000*	-	0,392	0,091
K_3	0,000*	0,392	-	0,397
K_4	0,000*	0,091	0,397	-

Note: * = significantly different

Descriptive data showed that the average number of platelets present in most red yeast rice extract treatment group (K2) followed by palm extract group (K3) (Table 1). Based on the results of statistical calculations there are significant differences between the control group

(K1) with red yeast rice extract treatment group (K2) and dates (K3) (Table 4). The results are consistent with the hypothesis of the study also support previous research that red yeast rice and dates can increase platelet counts in mice (Triana & Nurhidayat 2006, Rahmi 2009, Wiyasihati 2011, Zahroh 2010).

Angkak and dates contain flavonoids (Lin et al 2005), namely the isoflavones (Tisnadjaja 2006), which has been reported to inhibit the activity of the enzyme hyaluronidase (Sahasrabudhe & Deodhar 2010, Salmen 2003). Hyaluronidase is an enzyme that degrades hyaluronic acid (Sahasrabudhe & Deodhar 2010). When hyaluronidase activity was inhibited, the hyaluronic acid is not degraded. Hyaluronic acid in the extracellular matrix (ECM) of bone marrow plays a role in stimulating the release of interleukin-6 (IL-6). While it is known that IL-6 can increase hematopoietic progenitor cell proliferation and stimulates megakariopoiesis (Khaldoyanidi et al 1999). In addition, IL-6 also acts to stimulate the maturation of megakaryocytes cells (Matsumura & Kanakura 2002). As a end result, the number of platelets in the blood will increase.

The mean number of megakaryocytes also contained the largest in the group treated extract of red yeast rice (K2) followed by palm extract group (K3) (Table 1) and there is a significant difference between the control group (K1) with a group of red yeast rice extract (K2) and dates (K3) (table 5). These results are consistent with the research hypothesis that red yeast rice (Wiyasihati 2011) and dates can increase the number of megakaryocytes in the bone marrow. This increase is allegedly due to the isoflavone content of flavonoids in red yeast rice and dates, which through a series of reactions in the bone marrow, can stimulate an increase in hematopoietic progenitor cell proliferation and stimulates the formation of megakaryocytes (Khaldoyanidi et al 1999, Matsumura & Kanakura 2002, Sahasrabudhe & Deodhar 2010).

The descriptive analysis of the average number of blood platelets showed that the average number of platelets in the treatment group guava leaf extract (K4) is greater than the control group (K1) (Table 1). These results indicate that there is an increase in the number of platelets with guava leaf extract, but the increase was not significant (Table 4). Guava leaves contain flavonoids quercetin can increase the number of cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3)(Soegijanto et al 2010). GM-CSF and IL-3 is a factor that can stimulate megakariopoiesis (Matsumura & Kanakura 2002) so as to increase the number of platelets as evidenced by preclinical testing in mice (Sutikno 2009), mice, and clinical trials that have been performed on patients with DHF (Soegijanto et al 2010).

Guava leaf extract in this study did not show an increase in platelet counts significantly. This may be due to differences in research methods used. Sutikno (2009) using guava leaves in the form of infusion, whereas in this study using the extract form. Flavonoid content can vary depending on the processing factors (Manach et al 2004) so that the alleged differences in dosage form also affects the content of flavonoids.

The mean number of megakaryocytes in the treatment group guava leaf extract (K4) is greater than the control group (K1) (Table 1). Based on the results of the statistical calculations obtained significant differences (Table 5). These results are consistent and support the research hypothesis research Kusumawati(2004) (as cited by Soegijanto et al 2010) that the guava leaf extract can increase the number of megakaryocytes in the bone marrow. The increase in the number of megakaryocytes is due to the flavonoid quercetin, which increases the amount of cytokines GM-CSF and IL-3 (Soegijanto et al 2010) which can stimulate megakariopoiesis (Matsumura & Kanakura 2002).

This is in contrast with the effect of guava leaf extract on platelet count does not increase significantly (Table 4). As it is known that platelets are formed from megakaryocytes and is a process that involves massive reorganization of membrane and cytoskeletal components of megakaryocytes (Kaushansky 2008). This study was a cross-sectional sample that is suspected to have been formed when cells of megakaryocytes in the bone marrow, but has not been followed by the release of platelets into the circulation.

Extract of red yeast rice (K2) and dates (K3) can increase platelet counts were significantly (Table 4). Extract of red yeast rice (K2), dates (K3), and guava leaves (K4) can significantly increase the number of megakaryocytes, but there was no significant difference among the three groups (Table 5). This suggests that either the extract of red yeast rice, dates, guava leaves and equally effective in increasing the number of megakaryocytes. However, in increasing the number of platelets, red yeast rice extract and dates better than guava leaves. This is presumably due to differences in flavonoid content. *Angkak* dates contain flavonoids and isoflavones (Tisnadjaja 2006) while the guava leaves contain flavonoids quercetin (Kamath et al 2008).

Isoflavones are absorbed faster than that of quercetin concentration in plasma was also higher (Manach et al 2004). This allows *Angkak* and dates to cause a response of the body faster than the guava leaves. Isoflavones are

also thought to stimulate the release of IL-6 were not only instrumental in stimulating megakariopoiesis (Khaldoyanidi et al 1999), but also one of the factors that can stimulate the maturation of megakaryocytes (Matsumura & Kanakura 2002). While GM-CSF and IL-3 were increased after administration of quercetin (Soegijanto et al 2010) only plays a role in stimulating megakariopoiesis (Matsumura & Kanakura, 2002). Because isoflavones are also stimulates the maturation of megakaryocytes, it can be faster mature megakaryocytes, platelets will be released into circulation faster and faster platelet count will increase.

CONCLUSION

Red yeast rice extract dose 108 mg/kg rat/day for 3 days most effectively increase platelet counts in mice thrombocytopenia. Extracts of red yeast rice, dates and guava leaves increase the number of megakaryocytes in mice thrombocytopenia but no difference in effect between them significantly. Further research is needed to determine the effect of red yeast rice and dates on hematopoiesis in bone marrow and peripheral platelet function.

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