Combination of Angkak (Red Yeast Rice), Red Guava (Psidium guajava Linn) Leaf Extract and Red Guava Fruit Juice Increase Thrombocyte in Quinine-Exposed Rats

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Abstract: Dengue fever leads to decrease in the number of thrombocytes. Objective of this research was to study the effect of combination of angkak (red yeast rice), Psidium guajava fruit juice, and P. guajava leaves extract toward blood hematology profile of the quinine-induced rats (number of thrombocyte, erythrocyte, and hematocrit). Male Sprague-Dawley rats were divided into 6 groups (n=5), consisting of normal group (aquadest), positive control group (Remufit® 0.17 g/kg p.o), negative control group (aquadest), treatment of angkak (400 mg/kg p.o) and P. guajava extract (50 mg/kg p.o) combination, treatment of angkak (400 mg/kg p.o) and P. guajava juice (10 g/kg p.o) combination, and treatment of angkak (400 mg/kg p.o), P. guajava juice (10 g/kg p.o), and P. guajava extract (50 mg/kg p.o) combination. Each group, exclude normal group, was induced by 100 mg/kg quinine on day 1-14 and were administered by specific treatment for each group until day 28. Observation was done on day 0, 14, and 28. Administration of quinine, angkak, P. guajava juice, and P. guajava leaves ethanol extract showed no affect to body weight of animal models (P>0.05) compared to that of normal group. The highest increase in thrombocyte (up to 127%) was achieved by group that administered with 400 mg/kg bw/day of angkak combined with P. guajava juice 10 g/kg bw/day (P<0.05). Combination of angkak and P. guajava leaves ethanol extract significantly increased erythrocyte number and hematocrit value.

Keywords – Angkak, Psidium guajava, thrombocyte, rat

I. INTRODUCTION

Quinine is a drug used to treat malaria and muscle cramp. Quinine can cause thrombocytopenia, neutropenia, anemia, kidney failure, and liver intoxication. Thrombocytopenia is an abnormally low number of thrombocyte in blood. In this study, the animal model is administered to quinine to reduce its thrombocyte level, which is a similar symptom to dengue fever.

Angkak is a fermentation product of rice by mold Monascus purpureus. Angkak is reported to increase thrombocyte level of Sprague Dawley rat model for 152.20% compared to negative control, exceeding its normal maximum level¹. Angkak also showed the highest platelet and megakaryocyte count compared to date palm and guava leaf extract in thrombocytopeni mice². Angkak can normalize platelet counts, erythrocyte counts, and hemoglobin concentration of bleeding anemia mice³. Angkak increased thrombocyte count in dengue infected patients due to its anti-inflammatory effect or stimulation of thrombopoiesis in bone marrow⁴. Lovastatin were active constituents in angkak, beside sterols, isoflavones, and monosaturated fatty acids⁵. Lovastatin and vitamin B₁₂ as secondary metabolites is presumably the bioactive compound responsible for the thrombocyte-increasing effect. Besides, is able to retain the erythrocyte, hemoglobin, and hematocrit level within their normal level range. Angkak also reported to contribute in liver and kidney rehabilitation.

A guava contains 260 mg vitamin C, 2-5 times as much as that of an orange. Vitamin C is highly correlated to body immune system against infection, including dengue virus infection⁶. Extract of guava leaves is proven to reduce the incident of vascular permeability that lead to plasma leakage and stress which can be lethal to the patients. The extract can also improve the synthesis of antibody, both IgG and IgM. Guava leaf extract in dose of 108 mg/kg BW also increased thrombocyte number of thrombocytopenic rats, but lower than angkak in the same dose⁷.
Many efficacy tests have been done before on angkak and guava as a separate extract, especially their metabolic test toward blood hematology. Angkak produces lovastatin as its secondary metabolite, which can increase thrombocyte number, while guava leaf extract is known for its ability to induce thrombocyte synthesis. But, currently there is no scientific study on the effect of angkak and guava leaves as a mixture formula toward the figure of blood hematology. Chou\(^8\) stated that drug combination could improve the effectiveness of the drugs, reduce the dosage to prevent toxicity, prevent drug resistance, and give a selective synergy effect toward the target.

This research aims to study the effect of combination of angkak and guava, as well as combination of angkak and ethanol extract of guava leaves toward blood hematology profile of the quinine-induced rats (number of thrombocyte, hemoglobin, erythrocyte, leucocyte, and hematocrit).

### II. METHODS

#### 2.1 Research design

This study used randomized post-test only control-group design. Research was carried out at Department of Biochemistry, Bogor Agricultural University.

#### 2.2 Experimental animals

Number of animals was determined by Federer equation: \((t-1) \frac{(n-1)}{t} \geq 15\), in which \(t\) represents the number of treatments (3 treatments, 2 controls, and 1 normal group) and \(n\) represents the minimum number animals within group.

A total of 30 male Sprague Dawley rats, ± 3 month-old weighing 250-350 g obtained from Biofarmaka research center, Bogor Agricultural University. The rats were adapted for 14 days to adjust the eating and life pattern. Those animals were kept in individual cages with 12h light-dark cycle and water ad libitum.

Five of the animals were administered with water along the treatment period and served as normal group (CO), while the others received quinine 100 mg/kg bw/day p.o for 14 days. Quinine induced rats were divided equally into 5 groups and receive respective treatments from day 15 until day 28. PC was the positive control group in which the rats were administered using Remufit\(^®\) 170 mg/kg bw/day; KC was the negative control groups in which the rats receive no treatments.

Angkak was given as much as 0.4 g/ kg bw/ day\(^1\) to optimally increase the number of thrombocyte of Sprague Dawley rats. Prabawati\(^7\) and Agustinus\(^8\) reported that 10 g/kg bw/ day of guava juice (ratio 1:1) given orally can increase the number of thrombocyte of Sprague Dawley rats significantly.

Formulation of angkak and guava juice was done based their optimum dosage, in which 400 mg/kg bw/day of angkak was mixed with 10 mg/kg bw/day of guava juice and given orally to the rats (AL group). Formulation of angkak and guava leaves extract was comprised of 400 mg/kg bw/day of angkak and 54.05 mg/kg bw/day of guava leaves extract given orally (AJ group). Lastly, formulation of angkak, guava juice, and guava leaves extract was done by mixing them in each optimum dosage and given orally (ALJ group).

#### 2.3 Collection and analyses of blood

Blood was collected on day 0, 7, 14, 21, and 28 for blood hematology analysis. Blood obtained from tail vena was put into a tube previously filled with EDTA of 1 mg/mL blood. Blood was analyzed for its hematology profile including number of thrombocyte, erythrocyte, hemoglobin, leucocyte, and hematocrit. During the adaptation and treatment period, the rats’ appetite, body weight, eye condition, and behavior were observed every day. Blood hematology profile of the rats includes number of thrombocyte, erythrocyte, hemoglobin, leucocyte, and hematocrit. Hematocrit value (Ht) was determined using microhematocrit\(^7\), hemoglobin level (Hb) using Sahli method based on acid hematin development\(^10\), and total leucocyte\(^11\), thrombocyte, and erythrocyte\(^9\) were determined using Hayem solution.

#### 2.4 Preparation of plant materials

\(P.\ guajava\) juice was prepared by cutting clean fruit pulp, crushed using a blender, and sieved to separate the seed and other bigger parts. Subsequently, the fruit pulp was dip into boiling water for about 3 minutes. Aquadest was added with ratio 1:1 (b:v). \(P.\ guajava\) juice was kept in 4 °C storage.

The \(P.\ guajava\) leaf extract was made using maceration\(^{12,13}\). \(P.\ guajava\) leaves were washed on flowing water twice and leave them to let the water drip. The clean leaves were sun-dried to reach moisture content of ±10% and were crushed in a disc mill until 40-mesh-size powder.

As much as 50 g of guava leaf powder was macerated with 250 ml of ethanol 70% as the solvent for 24 hours in an orbital shaker of 200 rpm speed to accelerate the extraction process. It was done thrice until we get a nearly clear filtrate.
The supernatant was filtered using Whatman No. 1 filter paper and the filtrate was then concentrated in a spray dryer. The extract obtained was put in a closed bottle and stored in a refrigerator of 4-8°C.

2.5 Data Analysis

Data in graphs represents the average of 5 measurements, while data in table were represents the average and deviation standard of 5 measurements. Results were analyzed using one-way ANOVA. Values of \( p<0.05 \) was considered statistically significant.

III. RESULTS

Data of the rats’ body weight from adaptation until the end of treatment period are presented on Figure 1. Statistical analysis showed that the average body weights of each groups on day 0 were not significantly different (\( P<0.05 \)). During adaptation, an increase in average body weights was observed in all groups. But, they tend to decrease during treatment from day 0-14.

The decrease in body weight for all groups were not significantly different (\( P>0.05 \)) compared to control group (CO). Administration of 100 mg/kg bw/day of quinine did not significantly affect the body weight decrease. The increase in body weight observed in group AL, AJ, and ALJ from day 14-28 of treatment period were also not significantly different compared to control group (\( P>0.05 \)) (Fig. 1). These changes in body weight of the animal models were still in control in compliance to that of control group.

Figure 1. Body weight of male Sprague Dawley rats during adaptation (day 0-14) and treatment period (quinine, angkak, guava leaf extract, and guava juice) from day 15-42. CO (■), PC (■), NC (■), AL (■), AJ (■), dan ALJ (■).

Figure 2. Thrombocyte number of male Sprague Dawley rats.
Data of thrombocyte number of the animal models are presented on Figure 2. Average thrombocyte number of all rats on day 0 before receiving treatment was $377.3 \pm 115.79 \times 10^3$ mm$^3$ (Fig. 1). Thrombocyte number of a normal rat ranges from 150-460 $\times 10^3$ mm$^3$.

The analysis showed a decrease in thrombocyte number in-group AL, AJ and ALJ after the administration of 100 mg/ kg bw of quinine compared that on day 0. But, the decrease wasn’t significant ($P > 0.05$) compared to that of group CO on day 14.

On day 28, the thrombocyte number of group PC (given 0.17 g/ kg bw/ day of commercial drug) was higher than that of day 14, increasing from $241.375 \pm 77.241 \times 10^3$ mm$^3$ to $361.13 \pm 94.26 \times 10^3$ mm$^3$. These increases were observed in-group AL, AJ, and ALJ during 14-day treatment compared to group NC.

![Figure 3. Erythrocyte number of male Sprague Dawley rats.](image)

The low erythrocyte number on day 0 corresponded to average hematocrit value on day 0, which is $25 \pm 2.52 \%$. The erythrocyte number of all groups tends to increase during the administration of quinine although in-group NC, AL, AJ, and ALJ it wasn’t as high as that of group CO (Fig. 3). The highest increase was found in-group CO with erythrocyte number of $8.37 \pm 1.08 \times 10^6$ mm$^3$. On the last day of treatment period (day 28), the erythrocyte number of group AL was found highest compared to that of group PC, AJ, and ALJ.

![Figure 4. Hematocrit value of male Sprague Dawley rats](image)

Alongside with the erythrocyte number, the highest hematocrit value was also found in-group AL, significantly different ($P < 0.05$) compared to that of NC and CO (Fig. 4). This increase in hematocrit value was found in-group with combination treatment (AL and AJ).

**IV. DISCUSSION**

The 14-day quinine induction with dosage of 100 mg/kg bw/ day reduced the rats’ appetite, resulting in a decrease in body weight. Queasy, vomiting, stomachache, and diarrhea are several symptoms of local irritation caused by quinine$^{14}$. After administration of quinine, some rats were found to have diarrhea and bleeding on
their nose and ears. Quinine is known to have a toxic effect called thrombocytopenia, causing bleeding on the animal models\textsuperscript{14}. Howard et al.\textsuperscript{15} reported that rashes on skin and bleeding on mucocutaneous might also happen because of quinine induction.

During treatment from day 14-28, group NC, AL, AJ, and ALJ showed an increase in body weight compared to group CO and PC (Fig. 1). This is because of angkak administration to group AL, AJ, and ALJ which can improve the rats’ appetite. Angkak contains some unsaturated fatty acid such as oleic acid, linolenic acid, and linolenic acid, as well as vitamin B complex like niacin\textsuperscript{6}. Vitamin B complex consists of vitamin B\textsubscript{1} (thiamin), B\textsubscript{2} (riboflavin), B\textsubscript{3} (niacin), B\textsubscript{6} (pyridoxine), and B\textsubscript{12} (cobalamin). Vitamin B supports and improve body metabolism. Vitamin B\textsubscript{1}, B\textsubscript{3}, and B\textsubscript{12} improve eating appetite and body growth, thus result in increasing rat’s body weight. Reduction of thrombocyte number by quinine administration was purposefully done to get the similar symptom as dengue fever. Thrombocytopenia is one of non-specific laboratory criteria for dengue fever\textsuperscript{4}. A chemical bond between quinine and molecule on thrombocyte’s plasma membrane surface will alter the structure and result in the new form of membrane cell’s molecule, becoming a foreign substance for immune system\textsuperscript{16}. Although thrombocyte number did increased in group PC, it’s still lower than that of group AL, AJL, and NC, indicating that once quinine administration was stopped thrombocyte number will increase naturally (Fig. 2). Administration of angkak, guava leaf extract, and red guava juice induced the thrombocyte synthesis better. After 14-day treatment with each combination, the thrombocyte number of group AL, AJ, and ALJ increased for 121\%, 127\%, 64\% respectfully compared to that of group NC. Here group AJ showed the highest increase out of all groups. In the previous study, the same treatment done for 15 days by Rombe\textsuperscript{1} showed 63\% increase in thrombocyte number.

Red guava juice didn’t significantly affect thrombocyte synthesis on the animal models, but it improved body immune and reduced cell membrane damage because of its high content of quercetin and vitamin C. Guava contains 260 mg vitamin C per 100 g of fruit, 2-5 times as much as that in orange. Ascorbic acid is an important compound in activating enzyme prolyl hydroxylase that functions in collagen synthesis. Without ascorbic acid, collagen fiber won’t be perfectly developed and weak.

Vitamin C is really crucial for growth and tissues’ strength in subcutaneous tissue, cartilage, bone, and teeth. Quercetin activity can lower damage of neuronal cell membrane caused by dengue virus\textsuperscript{17}. A study by Agustinus\textsuperscript{1} showed that dengue fever patients administered with red guava juice increased their thrombocyte number better than control. Aster\textsuperscript{17} reported that quinine affected thrombocyte and also granulocyte, erythrocyte, and other tissues. Increase in erythrocyte number on day 14 was followed by an increase in hematocrit value, it happened on all groups. But, the increase of hematocrit value was still within the normal limit of Sprague Dawley rats. This result is in agreement with study by Wolford et al.\textsuperscript{18} in which hematocrit value of male Sprague Dawley rats will increase as they grow older.

When the blood liquid leak out of blood vessel while the solid part remains inside, the hematocrit value will increase. As the liquid leaks, the percentage of solid toward the liquid will increase thus the hematocrit value will also increase. On day 28, the erythrocyte number of group AL was found as the highest compared to that of group PC, AJ, ALJ. Angkak contains vitamin B\textsubscript{12} that promotes synthesis and maturation of red blood cells. In addition, lovastatin in angkak will also contribute in providing ubiquinone and hemeA which are important in cells’ energy improvement and red blood cells recovery\textsuperscript{19}. Hematocrit (Ht) is a comparison value of solid part of the blood (blood cells) and the liquid part (plasma). Higher hematocrit value means higher blood cells compared to blood plasma. Guava juice contains a considerable amount of ascorbic acid that may increase random migration of neutrophil, monocyte, and eosinophil in blood vessel\textsuperscript{17}. Administration of angkak and red guava juice to group AJ allowed the hematocrit value to increase within the normal limit.

V. CONCLUSION

The results showed that combination of angkak and ethanol extract of guava leaves were able to increase erythrocyte number and hematocrit value significantly and might be further evaluated as a treatment for thrombocytopenia.

REFERENCES

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Combination of Angkak (Red Yeast Rice), Red...