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The Effect of Papaya Leaf Extract (*Carica Papaya L.*) to The Improvement of Platelet Count and Activity on Mice With Trombositopenia

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Abstract
Introduction: *Thrombocytopenia is a condition of platelet counts below 150,000/mm^3, this may cause bleeding which, if not handled properly, may lead to death. Some actions that can be done to overcome thrombocytopenia, one of which is with papaya leaf. This study aims to analyze the effect of giving papaya leaf extract to increased count and activity of platelets.* ***Methods:*** *This research design uses true experimental with design of post-test only control group design. The sample in this study were 30 male mice (Mus musculus) divided into five groups. The first group as the negative control group (CMC Na 0.5%), the second group as the positive control (CMC Na 0.5%), the third group was given ethanol 96% extract papaya leaf dose 0.5g/kgBW, the fourth group was given ethanol 96% extract papaya leaf dose 1g/kgBW, the fifth group was given ethanol 96% extract papaya leaf dose 2g/kgBW. Data collection using measurement sheet of platelet, bleeding time, clotting time and blood serotonin which were analyzed by MANOVA test.* ***Result:*** *The results of this study showed that platelet variable obtained p<0.0001 the highest average between treatment groups that is treatment group which given dose of 2g/kgBW (2±0,1). In the bleeding time variables p<0.0001 the fastest mean time occur between treatment groups that is treatment group with dose 2g/kgBW (2.74±0.14). In clotting time variables p<0.0001 the fastest mean time occur between treatment group that is treatment group with dose 2g/kgBW (2,24±0,03). On serotonin blood variable p<0.0001 the highest mean is between treatment group that is treatment group which given dose of 2g/kgBW (48,90±3,80). This result shows that there are significant differences between every groups.* ***Discussion:*** *The conclusion of this research is giving papaya leaf extract can increase the count and activity of platelets views of bleeding time, clotting time and blood serotonin*

Key Words: *papaya leaf; platelet; bleeding time; clotting time; serotonin blood.*

I. INTRODUCTION

Platelet are blood cells that play an important role in hemostatic, which is a cessation mechanism of bleeding from a damaged blood vessel (Ciesla, 2012). In the condition of platelet deficiency or thrombocytopenia, there is an increased risk of bleeding, which if not handled properly will worsen the condition of the patient as far as lead to death. The condition of thrombocytopenia is quite common in the Intensive Care Unit (ICU), which ranges from 8.3 to 66.6% in adult patients and 20-50% in neonates. Severe thrombocytopenia often occurs in the acute phase of Dengue Hemorrhagic Fever (DHF) and there is a dilemma in dealing with DHF patients because of the concerns about bleeding (Lei et al., 2009).

One plant that is often used in the community to treat Dengue Hemorrhagic Fever (DHF) is papaya plant. In the Pekanbaru, some communities use papaya plants by taking a papaya leaf and boiling the leaves that are drank it before bed as a therapy in DHF patients. The people of Java and Manado also use papaya leaves as medicinal plants to treat DHF disease (Purwanto, 2015). The use of papaya leaves can accelerate the healing process of DHF, but until now has never described the healing mechanism.

The number of Dengue Hemorrhagic Fever (DHF) cases are largely unreported and misclassified. Recent research shows 390 million dengue infections per year, 96 million manifest clinically with varying degrees. Other studies say, the prevalence of DHF is estimated to reach 3.9 billion people in 128 countries at risk of dengue virus infection. Ninety percent of DHF sufferers are children under 15 years of age and have decreased platelet (WHO, 2015). Data from the Ministry of Health mentioned as many as 511 districts / cities in Indonesia has the potential to be a place of DHF progression (CNN Indonesia, 2016). In early 2016, East Java provincial government recorded 2,027 cases of dengue fever spread in 38 districts / cities, 40 of them died. The death toll reached 40 people. A number of areas also experienced an increase in the number of cases more than doubled compared to the same month in 2015, namely Sidoarjo and Kota Probolinggo. Sidoarjo in January 2015 occurred 21 cases and in 2016 with 45 cases. Meanwhile, the city of Probolinggo in January in 2015 had as many as 20 cases and in 2016 with 47 cases (Health Office East Java, 2016).

Thrombocytopenia is a state of platelet count below 150,000 / mm^3, this can inhibit blood clotting and may even cause fatal bleeding. Thrombocytopenia is a hematological disorder characterized by a decrease in platelet levels in the blood which can also be caused by various factors such as drug induction such as cancer chemotherapy, heparin, quinidine, quinine, gold salts, valproic acid, sirolimus and sulfa antibiotics (Adnyana et al. 2012).

Treatment that can be done to overcome this bleeding problem is by Platelet Concentrate (TC) transfusion, both as a therapy and prophylactic action in thrombocytopenia patients with risk of bleeding. In addition, TC transfusion is also used as a therapy of platelet dysfunction (Blumberg et al., 2012). Regardless of the great benefits, TC transfusion can cause various side effects. Some of the side effects that may arise from the presence of contaminant leukocytes in these TC components include: Non-Hemolytic Transfusion Reactions (FNHTR), Transfusion Related Lung Injury (TRALI), aloimunization, increased risk of viral transmission (especially cytomegalovirus) and so on. These side effects occur due to the presence of plasma, erythrocytes and especially leukocytes in TC products (Gernsheimer et al., 2015).

There is no specific therapy for Hemorrhagic Dengue Fever (DHF), the main principle in the management of this disease is supportive therapy. The way that medical personnel do is through platelet transfusions to normal gradual platelet, provision of febrifuge and anticonvulsant drugs in case of seizures (Djunaedi, 2006). There are several other actions that can be done to overcome DHF and diseases caused by thrombocytopenia, one of them is by utilizing plants that are efficacious cure or prevent thrombocytopenia disease such as DHF. Utilization of nutritious plants is useful to encourage people to be back to nature, due to the high price of modern or synthetic drugs and the many side effects of synthetic drugs (Pramono, 2010). In the case of decreased platelet counts for example in DHF, medicinal plants often used to increase platelets include sweet potatoes, red guava extract, palm juice and black cumin (Djunaedi, 2006). In addition to these plants there is an alternative plant that is easily found, one of which is papaya (*Carica papaya Linn*) which is thought to affect platelet activity.

Based on Patil's study (2013), papaya leaf extract proved able to increase the number of platelet but its effect on platelet activity is not yet known. Papaya leaf is also capable of therapy for patients with Hemorrhagic Dengue Fever (DHF) who have thrombocytopenia. Papaya plant is a tropical plant that thrives in Indonesia so it is easy to get and the price is cheaper. Papaya leaf contain some secondary metabolite compounds, one of which is flavonoids and tannins (Milind & Gurditta, 2011). Based on research conducted by Soegijanto, et al (2010), total flavonoid compounds and total tannin is a marker compound in increasing the number of blood platelets in DHF patients. In addition, papaya leaves also contain alkaloids including carpain, pseudocarpain and dehidrocarpain I and II that can react to the bone marrow, preventing its destruction and increasing platelet production.

II. METHODS

This study used true experimental design with design of post-test only control group, conducted in February-March 2017. This research had been approved by the Ethics Committee of the Faculty of Veterinary Medicine of Universitas Airlangga.

The tools and materials in this study were plastic polypropylene enclosure for the size of 20 cm x 30 cm x 40 cm which was covered with gauze wire with 6 mm hole size, drinking bottle, feeding container, husk, sonde, scale, 1 ml syringe, sterile tube, blood analyzer, stopwatch, absorbent paper, scalpel, syringe, serotonin ELISA kit, elisa reader, ELISA washer, well plate, micropipette, ultrasonic tool, vacuum rotary evaporator, oven and petri bowl.

This study used mice (*Mus Musculus*) as experimental animals which are male criteria, 3 month age, 25-30 gram weight and in healthy condition. The sample in this study were 30 mice (*Mus musculus*) divided into five groups. Each group consists of 6 mice. The first group as the negative control group (CMC Na 0.5%), the second group as the positive control (CMC Na 0.5%), the third group was given ethanol 96% extract papaya leaf dose 0.5 g/kgBW, the fourth group was given ethanol 96% extract papaya leaf dose 1 g/kgBW, the fifth group was given ethanol 96% extract papaya leaf dose 2 g/kgBW. Mice were thrombocytopenia induced with cotrimoxazole at 249.6 mg/kgBW (p.o) for 8 days.

Fresh papaya leaf selected, washed, cut and dried by aerated, then powdered using a blender. A total of 200 grams of simplisa powder papaya leaf added ethanol 96% and to accelerate the extraction process used ultrasonic tool. Ultrasonic treatment is repeated for 3 times each treatment takes 2 minutes. The ultrasonic result at each treatment was filtered using filter and filter paper which is then stored in the bottle for evaporation for 12 hours. To obtain the viscous extract, the evaporated plant leaf solution was put in an oven / heater with a temperature of 39-40 ^{0}C

for 3 days. After obtained the viscous extract, then the rendemen analytical result measured with the analytical scales.

Papaya leaf extract as per dose of each treatment group was put into a reaction tube dissolved in CMC Na 0.5% until the volume was exactly 10 ml, stirred until homogeneous. Taken as much as 0.5 ml with 1 ml syringe and given to each animal test. Given ethanol 96% extract papaya leaves every day for 5 days, then examined the number of platelets, bleeding time, clotting time and the amount of serotonin in the blood in all groups.

Data collection using platelet measurement sheets, bleeding time, clotting time and blood serotonin. The results of the measurements in the study are numerical data, so referring to Nugroho (2014) in the first stage, the data are analyzed descriptively as mean and standard deviation. In the second stage, tested the normality of data using Kolmogorov-Smirnov test, and continued with the last stage of the Manova test.

III. RESULT

Table 1. Mean and Standard Deviation of Number of Platelet Variable (in 10^5)

Group	Mean ± SD
K1 (negative control)	$2.07^{a} \pm 0.16$
K2 (positive control)	$1.06^{b} \pm 0.09$
K3 (extract dose 0.5 gr/kgBW)	$1.63^{c} \pm 0.12$
K4 (extract dose 1 gr/kgBW)	$1.86^{d} \pm 0.1$
K5 (extract dose 2 gr/kgBW)	$2^{a} \pm 0.1$
ANOVA *p-value* < 0.0001	

* Different letters show a significant difference based on the LSD test at $\alpha = 0.05$

Table 1 shows that the highest average number of platelet counts was negative control group, which is normal mice group without treatment namely 2.07 ± 0.16 whereas the lowest average platelet count was found in the positive control group which is the untreated thrombocytopenia mice group namely 1.06 ± 0.09. In the table and the above figure also shows from the three treatment groups, which has the highest average platelet count is the treatment group with papaya leaf extract dose 2 g/kgBW namely 2 ± 0.1.

From the LSD test result it can be seen that the platelet value in K1 has significant differences with K2 (p<0,0001), K3 (p<0,0001) and K4 (p=0.003) and has no significant difference with K5 (p=0.284), Whereas K2 has a significant difference with K3, K4 and K5 (p<0,0001). K3 has significant difference with K4 and K5 (p<0,0001), while K4 has difference with K5 (p=0,042).

Table 2. Mean and Standard Deviation of Bleeding Time Variable (Minutes)

Group	Mean ± SD
K1 (negative control)	$2.76^{a} \pm 0.22$
K2 (positive control)	$3.64^{b} \pm 0.18$
K3 (extract dose 0.5 gr/kgBW)	$3.13^{c} \pm 0.15$
K4 (extract dose 1 gr/kgBW)	$2.95^{a,c} \pm 0.14$
K5 (extract dose 2 gr/kgBW)	$2.74^{a} \pm 0.14$
ANOVA *p-value* < 0.0001	

* Different letters show a significant difference based on the LSD test at $\alpha = 0.05$

Table 2 shows that the longest average bleeding time was negative control group which is normal group without treatment namely 3.64 ± 0.18 while the fastest bleeding time was found in the treatment group with papaya leaf extract dose 2 g/kgBW namely 2,74 ± 0.14.

The result of LSD test showed that the bleeding time at K1 had significant difference with K2 (p<0,0001) and K3 (p=0,001) but did not have significant difference with K4 (p=0,056) and K5 (p=0,826) whereas K2 has significant differences with K3, K4 and K5 (p<0,0001). K3 had significant differences with K5 (p<0.0001) but did not have significant difference with K4 (p=0,087) while K4 had significant difference with K5 (p=0,035).

Table 3. Mean and Standard Deviation of Clotting Time Variable (Minutes)

Group	Mean ± SD
K1 (negative control)	2.60 [a] ± 0.05
K2 (positive control)	2.84 [b] ± 0.06
K3 (extract dose 0,5 gr/kgBW)	2.48 [c] ± 0.05
K4 (extract dose 1 gr/kgBW)	2.33 [d] ± 0.03
K5 (extract dose 2 gr/kgBW)	2.24 [e] ± 0.03
ANOVA *p-value* < 0.0001	

* Different letters show a significant difference based on the LSD test at α = 0.05

Table 3 shows that the longest clotting time was negative control group which is normal without treatment i.e. 2.60 ± 0.05 while the fastest clotting time was found in the treatment group with papaya leaf extract dose 2 g/kgBW namely 2,24 ± 0.03.

The result of LSD test showed that the time value of clotting time on K1 had significant difference with K2, K3, K4 and K5 (p<0,0001) while K2 had significant difference with K3, K4 and K5 (p<0,0001). K3 has a significant difference with K4 and K5 (p<0,0001) while K4 has significant difference with K5 (p=0,002).

Table 4. Mean Value and Standard Deviation Variable Blood Serotonin Level (ng/ml)

Group	Mean ± SD
K1 (negative control)	42.34 [a] ± 2.26
K2 (positive control)	30.81 [b] ± 2.21
K3 (extract dose 0,5 gr/kgBW)	43.86 [a] ± 2.45
K4 (extract dose 1 gr/kgBW)	48.07 [c] ± 2.83
K5 (extract dose 2 gr/kgBW)	48.90 [c] ± 3.80
ANOVA *p-value* < 0.0001	

* Different letters show a significant difference based on the LSD test at α = 0.05

Table 4 shows that the lowest level of serotonin blood level was negative control group which is normal group without treatment namely 30,81 ± 2,21 while mean of highest serotonin blood level was found in treatment group with papaya leaf extract dose 2 g/kgBW i.e. 48.90 ± 3.80.

From the result of LSD tests it was found that blood serotonin levels in K1 had significant differences with K2 (p<0,0001), K4 (p=0.001), K5 (p<0,0001) and had no significant difference with K3 (p=0.353) whereas K2 had significant differences with K3, K4 and K5 (p<0,0001). K3 had significant difference with K4 (p=0,014) and K5 (p=0,004) while K4 had no significant difference with K5 (p=0,610).

IV. DISCUSSION

A. The Effect of Papaya Leaf Extract on The Platelet Levels

This study aims to see whether there is effect of papaya leaf extract to platelet count. The results obtained from this study showed that the treatment group which given papaya leaf extract showed an increase in platelet counts rather than the positive control group of thrombocytopenia. Treatment group given papaya leaf extract with dose of 2 g/kgBW showed highest platelet yields than other treatment groups with an average of 2 ± 0.1.

The results of this study are supported by Bajaj & Rekha research (2016) which states that in the group of papaya leaf extract showed an increase in platelet counts than the group that was not given papaya leaf extract. The number of platelets in mice has a wide range of between 150,000-400,000/mm^3. Platelets can increase or decrease, whereas a decrease in platelets called thrombocytopenia is a hematological disorder characterized by a decrease in platelet levels in the blood caused by bone marrow suppression or decreased platelet production by bone marrow, aggravated vascular endothelial platelet aggregation and increased destruction platelets and various factors such as drug induction and the presence of autoimmune processes in platelets (Marzuki, 2012). One of the plants that can be used to overcome thrombocytopenia is papaya (*Carica papaya L.*). Papaya leaf contain

flavonoid and tannin compounds that have activity in increasing the number of megakaryocytes in the bone marrow so as to increase the number of platelet s in the blood (Soegijanto, 2010).

The results of this study can be explained that the giving of papaya leaf extract with a dose of 2 g/kgBW can increase platelet levels to the limit equal to the negative control group (normal). This is due to the substances contained in the papaya leaf extract is more optimal in increasing the platelets in that dose.

B. The Effect of Papaya Leaf Extract on The Bleeding Time

This study aims to see whether there is an effect of giving papaya leaf extract to bleeding time. In the results of this study showed that the treatment group which given papaya leaf extract showed faster bleeding time than the positive control group thrombocytopenia. Treatment group given papaya leaf extract with dose of 2 g/kgBW showed the fastest bleeding time result compared to other treatment group with an average of 2.74 ± 0.14.

There are several compounds in papaya leaf that act as hemostatic agents. Some of these compounds are flavonoids and tannins. The mechanism of tannin in stopping the bleeding is through its astringent effects (Klatoe et al., 2012). Flavonoid compounds can stop bleeding by vasoconstriction mechanism in blood vessels (Dougnon et al., 2012). Flavonoids and tannins contained in the papaya leaf are thought to play a role in inhibition of local synthesis and production of prostaglandin I2 vasodilatation (prostacyclin) resulting in the process of wound contraction (vasoconstriction) becoming faster (Salawu et al., 2008). Tannin is one component responsible for the secretion of 5-hydroxytryptamin (serotonin) and thromboxane A2 (Sari et al., 2013).

C. The Effect of Papaya Leaf Extract on The Clotting Time

This study aims to see whether there is an effect of giving papaya leaf extract to clotting time. In the results of this study showed that the treatment group given papaya leaf extract showed a time of clotting time faster than the positive control group thrombocytopenia. Treatment group given papaya leaf extract with dose of 2 g/kgBW showed the fastest clotting time than other treatment group with an average of 2.24 ± 0.03.

The results above show that papaya leaf extract has an effect in affecting the time of blood clotting (clotting time). This is because there are several compounds in papaya leaf that act as hemostatic agents. Some of these compounds are flavonoids and tannins. Based on research by Rahmi et al. (2013) that flavonoids are one type of antioxidant that can inhibit platelet secretion because the flavonoid is able to inhibit the metabolism of arachidonic acid by cyclooxygenase. Tannin is one component responsible for the secretion of 5-hydroxytryptamine (serotonin) and thromboxane A2 thus affecting blood clotting (Sari et al., 2013).

D. The Effect of Papaya Leaf Extract on The Blood Serotonin Levels

This study aims to see whether there is effect of papaya leaf extract on blood serotonin levels. The results obtained from this study indicate that the treatment group given papaya leaf extract showed an increase in blood serotonin levels than the positive control group thrombocytopenia. The treatment group given papaya leaf extract with dose of 2 g/kgBW showed the highest serotonin blood level than the other treatment group with an average of 48,90 ± 3,80.

The results above show that papaya leaf extract has an effect in influencing the amount of serotonin in the blood. This is because there are several compounds in papaya leaf that act as hemostatic agents. Papaya leaf contain flavonoid and tannin compounds that have activity in improving the stimulation of proliferation and differentiation of megakaryocytes so that it can increase α platelet granules which resulted in an increase in the amount of serotonin in the blood. ADP and serotonin stimulate and recruit additional platelets (Barret et al., 2015).

The results of this study can be explained that the giving of papaya leaf extract with a dose of 2 g/kgBW can increase blood serotonin levels. This is due to the substances contained in the papaya leaf extract is more optimal in increasing the blood serotonin in that dose.

V. CONCLUSION AND SUGGESTION

A. Conclusion

Giving papaya leaf extract (*Carica papaya L.*) with dose 0,5 g/kgBW, 1 gr/kgBW and 2 g/kgBW can increase platelet count, accelerate bleeding time and clotting time and increase blood serotonin level. Papaya leaf extract with dose of 2 g/kgBW give maximum effect in increasing the count and activity of platelet seen from bleeding time, clotting time and blood serotonin level.

B. Suggestion

Further research is needed to find out the mechanism of increasing the number and activity of platelets and research related to the use of papaya leaf water extract as a treatment of thrombocytopenia in humans. In addition, toxicology testing and dose determination of papaya leaf extract is appropriate to be applied clinically as an alternative treatment in humans.

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