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The effect of papaya leaf extract (*Carica papaya l.*) To the bleeding time on mice with trombositopenia

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ABSTRAK

Latar belakang: *Trombositopenia merupakan suatu keadaan jumlah trombosit di bawah 150.000/mm³, hal ini dapat menyebabkan perdarahan yang apabila tidak ditangani dengan baik dapat berujung pada kematian. Beberapa tindakan yang dapat dilakukan untuk mengatasi trombositopenia, salah satunya adalah dengan tanaman daun pepaya.*
Tujuan: *untuk menganalisis pengaruh pemberian ekstrak daun pepaya terhadap bleeding time (waktu perdarahan).*
Metode : *Desain penelitian ini menggunakan true experimental dengan rancangan post test only control group design. Sampel dalam penelitian ini sejumlah 30 ekor mencit jantan (Mus musculus) yang dibagi menjadi lima kelompok. Kelompok pertama sebagai kelompok kontrol negatif (CMC Na 0,5%), kelompok kedua sebagai kontrol positif (CMC Na 0,5%) , kelompok ketiga diberi esktrak etanol 96% daun pepaya dosis 0,5g/kgBB, kelompok keempat diberi esktrak etanol 96% daun pepaya dosis 1g/kgBB, kelompok kelima diberi esktrak etanol 96% daun pepaya dosis 2g/kgBB. Pengumpulan data menggunakan lembar pengukuran bleeding time, yang dianalisa dengan uji ANOVA.*
Hasil: *Hasil penelitian ini menunjukkan bahwa didapatkan nilai p<0.0001 dengan rerata waktu paling cepat antara kelompok perlakuan yaitu kelompok perlakuan dengan dosis 2g/kgBB (2.74±0.14). Hal ini menunjukkan bahwa terdapat perbedaan signifikan antar semua kelompok.*
Kesimpulan: *Kesimpulan dari penelitian ini adalah pemberian ekstrak daun pepaya (Carica papaya L.) dapat mempercepat bleeding time (waktu perdarahan).*

Kata kunci: daun pepaya; *bleeding time,* trombositopenia

ABSTRACT

***Background*:** Thrombocytopenia is a condition of platelet counts below 150,000/mm³, 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.
Objectives: This study aims to analyze the effect of giving papaya leaf extract to bleeding time.
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 bleeding time which were analyzed by ANOVA test.
Results: The results of this study showed that p<0.0001 the fastest mean time occur between treatment groups that is treatment group with dose 2g/kgBW (2.74±0.14). 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). This result shows that there are significant differences between every groups.
Conclusion: The conclusion of this research is giving papaya leaf extract can increase bleeding time.

Keywords: leaf of papaya, bleeding time, thrombocytopenia.

INTRODUCTION

Platelets are blood cells that an important role in the process of stopping bleeding from a damaged blood vessel (1). In the condition of thrombocytopenia, there is an increased risk of bleeding which if not handled properly will worsen the patient's condition and cause death. One disease that is characterized by bleeding is dengue hemmoraghic fever which has become a serious disease in Indonesia, but until now there has not been found a drug or vaccine that can cure the disease, as well as the lack of alternative drugs to increase platelet counts (2).

One of the plants that is often used in the community to treat dengue hemmoraghic fever (DHF) is the papaya plant. In the Pekanbaru area, some people use papaya by taking a papaya leaf and boiling the leaves taken before going to bed as therapy for DHF patients. The Javanese and Manado people also use papaya leaves as a medicinal plant to treat DHF disease (3).

Data from the Ministry of Health states that as many as 511 districts / cities in Indonesia have the potential to become places for developing DHF (4). In early 2016, the East Java Provincial Government noted that there were 2,027 cases of dengue fever in 38 districts / cities, 40 of whom died. The death toll reached 40 people. A number of regions also experienced more than double the number of cases compared to the same month in 2015, namely Sidoarjo and Kota Probolinggo. Sidoarjo in January 2015 there were 21 cases and 2016 with 45 cases. Meanwhile, the city of Probolinggo in January 2015 amounted to 20 cases and 2016 with 47 cases (5).

Thrombocytopenia is a state of platelet count below 150,000 / mm3, 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 (6). The normal hemostatic mechanism of the body is sufficient to repair damage and stop the release of blood from these fine microcirculation vessels. The mechanism of the body in stopping bleeding involves three main steps: vascular spasm, platelet blockage formation, and blood coagulation (7). If there is a wound in a blood vessel, vasoconstriction of the blood vessels will occur immediately so that blood flow to the injured blood vessels decreases. Then the platelets will gather and attach to the part of the injured blood vessel to form a platelet plug. Activated blood clotting factors will form fibrin threads that will form a platelet plug to become non-permeable so that bleeding will stop. There are several systems that play a role in hemostasis, namely the vascular system, platelets and blood clots (8).

Handling that can be done to overcome this bleeding problem is by Thrombocyte Concentrate (TC) transfusion, both as a therapy and prophylactic action in thrombocytopenia patients with the risk of bleeding. (9). TC transfusion can cause various side effects. Some of the side effects that can arise due to the presence of leukocyte contaminants in this TC component include: non-hemoglobin transfusion reactions (FNHTR), transfusion related lung injury (TRALI), alloimmunization, increased risk of viral transmission (especially cytomegalovirus) and so on. (10).

In the case of a decrease in platelet counts, for example in DHF, medicinal plants that are often used to increase platelets include sweet potatoes, guava extracts, dates and black cumin extracts (11). 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. In Patil's research (2013), papaya leaf extract was shown to be able to increase platelet counts but its effect on bleeding time is unknown. Papaya leaves are also able to treat Dengue Hemmoraghic Fever (DHF) sufferers who have thrombocytopenia. Papaya plants are tropical plants that thrive in Indonesia, making them easy to obtain and the price is more economical. Papaya leaves contain several secondary metabolites, one of which is flavonoids and tannins (12). Based on research conducted by Soegijanto, et al (2010), total flavonoid compounds and total tannin are marker compounds in increasing blood platelet counts in DHF patients. In addition, papaya leaves also

contain alkaloids including carpain, pseudocarpain and dehydrocarpain I and II which can react to the bone marrow to prevent their destruction and increase platelet production (13).

MATERIALS AND METHODS

This study uses true experimental design with a post test only control group design. This research was conducted in February-March 2017. This research has been submitted to the Research Ethics Commission of the Faculty of Veterinary Medicine, Airlangga University number 662-KE by applying the principle of 3R (Replacement, Reduction, Refinement) and has been declared ethical. The tools and materials in this study are polypropylene plastic cages for mice measuring 20 cm x 30 cm x 40 cm which are covered with wire mesh with a hole size of 6 mm, drinking bottles, places to eat, husks, scales, sonde, syringes 1 ml, sterile tubes , blood analyzer, stopwatch, absorbent paper, scalpel, syringe, serotonin ELISA kit, Elisa reader, Elisa washer, well plate, micropipette, ultrasonic device, vacuum rotary evaporator, oven and petri dish.

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

Papaya leaves are selected which are still fresh, washed, cut and dried by aerating, then pollinated using a blender. As much as 200 grams of simplisa papaya powder are added with 96% ethanol and to accelerate the extraction process ultrasonic devices are used. This ultrasonic treatment is repeated for 3 times, each treatment takes 2 minutes. The ultrasonic results of each treatment were filtered using a filter and filter paper which was then stored in a bottle for evaporation for 12 hours. To get a thick extract, then the solution of plant leaves that have been through evaporation is put in an oven / heater with a temperature of 39-40 C for 3 days. After the thick extract is obtained, then measure the yield.

Papaya leaf extract according to the dosage of each treatment group was put into the test tube dissolved in CMC Na 0.5% until the volume was exactly 10 ml, stirred until homogeneous. 0.5 ml taken with 1 ml syringe to be given to each test animal. Given 96% ethanol extract of papaya leaves every day for 5 days, then bleeding time was examined in all groups.

Mice that have fulfilled the criteria are given injuries by means of the mouse mice being marked 2 cm long from the tip of the tail. The hair on the tail is cleaned using a hair shaver then cleaned using 70% ethanol, anesthetized topically using ethyl chloride around the tail to be injured, then given a 3 mm wound using a minor surgery. The outgoing blood is absorbed using filter paper every 30 seconds without touching the wound surface. Time begins to be measured using a stop watch when the blood is absorbed for the first time until the blood stops with no blood being absorbed on the filter paper. The time interval when the blood comes out first until the blood stops coming out is the bleeding time.

Data collection uses a bleeding time measurement sheet. Data processing and analysis by using SPSS and One Sample Kolmogorov Smirnov normality test ($p > 0.05$) followed by Anova test. Data that has been processed and analyzed is presented in table form.

RESULTS

Table 1 shows that the longest average bleeding time was negative control group, ie the group of normal mice without treatment was 3.64 ± 0.18 while the fastest bleeding time was found in the treatment group with papaya leaf extract dose of 2g/kgBB which is 2.74 ± 0.14. The difference in bleeding time values between the control group and

Table 1 Mean Value and Standard Deviation of Variable Bleeding Time (minutes).

Group	Mean ± SD
negative control	2.76^{a} ± 0.22
positive control	3.64^{b} ± 0.18
extract dose 0,5 g/kgBB	3.13^{c} ± 0.15
extract dose 1 g/kgBB	$2.95^{a,c}$ ± 0.14
extract dose 2 g/kgBB	2.74^{a} ± 0.14
ANOVA p<0.0001	

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

the treatment group was carried out by the ANOVA test with a 95% confidence level obtained by p-value = 0,000, which means that at least a pair of groups had a difference in the average bleeding time.

Table 2 Differences in Bleeding Time Values in the Control Group and Treatment

Group	p-value
negative control	
positive control	
extract dose 0,5 g/kgBB	0.000
extract dose 1 g/kgBB	
extract dose 2 g/kgBB	

Based on **Table 2**, p-value = 0,000 is obtained so that at least there is a pair of groups that have different mean bleeding time. To find out which groups are different, then proceed with the Post Hoc test using LSD.

From the results of the LSD test, it was found that the bleeding time at negative control group had significant differences with positive control group (p <0,0001) and extract dose 0,5 g/kgBB group (p=0.001) but did not have a significant difference with extract dose 1 g/kgBB group (p=0,056) and extract dose 2 g/kgBB group (p=0.826) while positive control group has significant differences with extract dose 0,5 g/kgBB group, extract dose 1 g/kgBB group and extract dose 2 g/kgBB group (p <0.0001). extract dose 0,5 g/kgBB group has a significant difference with extract dose 2 g/kgBB group (p<0,0001) but does not have a significant difference with extract dose 1 g/kgBB group (p=0,087) while extract dose 1 g/kgBB group has a significant difference with extract dose 2 g/kgBB group (p = 0.035).

DISCUSSION

This study aims to see whether there is an effect of giving papaya leaf extract to bleeding time. The results of this study indicate that the treatment group given papaya leaf extract showed a faster bleeding time than the positive thrombocytopenia control group. The treatment group given the papaya leaf extract at a dose of 2g/kgBB showed the fastest bleeding time compared to the other treatment groups with an average of 2.74 ± 0.14.

There are several compounds in papaya leaves that function as hemostatic agents. Some of these compounds are flavonoids and tannins. The mechanism of tannin in stopping bleeding is through its astringent effect (14). Flavonoid compounds can stop bleeding with vasoconstriction mechanisms in blood vessels (15). Flavonoids and tannins contained in papaya leaves are thought to play a role in inhibiting local synthesis and production of vasodilatory prostaglandin I2 (prostacyclin) which causes the process of wound contraction (vasoconstriction) to be faster (16). Tanin is one component that is responsible for the secretion of 5-hydroxytryptamin (serotonin) and thromboxane A2 (17).

The flavonoids and tannins they contain are thought to play a role in inhibiting local synthesis and production of vasodilating prostaglandin I2 (prostacyclin) so that the process of wound contraction (vasoconstriction) becomes faster (16). Tanin is one component that is responsible for 5-hydroxytryptamin (serotonin) secretion and thromboxane A2 (17).

Serotonin and thromboxane A2 are compounds secreted due to the response to platelet activation that attaches to the walls of damaged blood vessels. Serotonin functions as a strong vasoconstrictor, while thromboxane A2 also functions as a vasoconstrictor, contributes to the process of the activation of adjacent platelets and because of the sticky nature of these additional platelets, it will cause it to attach to previously active platelets (platelet aggregation). This platelet activation cycle continues, causing more additional platelet withdrawals to form platelet plugs. This plug

is initially loose, but usually can successfully block the loss of blood if the wound is in a small blood vessel, but if the wound is large, a blood clotting mechanism is needed to stop bleeding (18).

The results of this study can be explained that the administration of 96% ethanol extract of papaya leaves at a dose of 2 gr / kg has an effect on blood cessation up to the same limit as the negative control group (normal). This is because the substances contained in papaya leaf extract are more optimal in stopping bleeding at that dose.

CONCLUSION

Giving papaya leaf extract (Carica papaya L.) at a dose of 0.5 g / kgBB, 1 g / kgBB and 2 g / kgBB can accelerate bleeding (bleeding time). Papaya leaf extract at a dose of 2g/kgBB gives the maximum effect in accelerating bleeding time (bleeding time).

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