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The Effect of Rambutan Leaves (*Nephelium lappaceum* Linn) Extract on Tail Bleeding Time in Mice Strain *Balb*-C

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ABSTRACT

Introduction: The most common post-odontectomy bleeding complications were bleeding cases on the first day with a prevalence of 96.6%. Complications of post-odontectomy bleeding if it lasts a long time and is not treated immediately can cause hypovolemic shock, fainting, and further can cause death. Rambutan leaves (*Nephelium lappaceum* Linn) have several active compounds of tannins, flavonoids, and saponins which have properties to help the hemostatic process by shortening bleeding time. **Aims:** To examine the effect of rambutan leaves extract (*Nephelium lappaceum* Linn) on tail bleeding time in balb-C strain mice. **Methods:** Twelve mice were randomly selected and divided into three groups accordingly; group K- (distilled water), group K+ (tranexamic acid 0.065 mg/g BW), and group P (rambutan leaves extract 260 mg/kg BW). After 60 minutes, the tails of mice were cut along 0.5 cm from the tip of the tail. Bleeding time was observed by turning on the stopwatch along with cutting the tail of the mice and turning it off after the bleeding stopped. **Results:** Rambutan leaves extract (*Nephelium lappaceum* Linn) intake at a dose of 260 mg/kg BW had a significant effect (p=0.021) on tail bleeding time, which was to shorten the bleeding time of cutting the tails of mice with Balb-C strain. **Conclusions:** Based on the results of the study, it can be concluded that rambutan leaves extract (*Nephelium lappaceum* Linn) has a significant effect on tail bleeding time, which can shorten the bleeding time of cutting the tail of mice with balb-C strain.

Keywords: post odontectomy bleeding complications, bleeding time, rambutan leaves extract

INTRODUCTION

The prevalence of post-extraction bleeding complications can be up to 31.5%.(1) The prevalence of post-extraction bleeding complications that often occurs is post-odontectomy. The most common post-odontectomy bleeding complication was bleeding on the first day with a prevalence of 96.6%.(2) Complications of post-odontectomy bleeding if it lasts a long time and not treated immediately will cause a lot of blood loss so that it can cause unwanted events such as hypovolemic shock, fainting, and further can cause death.(3) Therefore, post-odontectomy bleeding complications need to be controlled.

In systemic action, the hemostatic process can be assisted by pharmacological drug preparations, including epinephrine which acts as a vasoconstrictor and tranexamic acid as an antifibrinolytic. However, in some conditions and cases of patients at risk of bleeding complications, excessive use of hemostatic drugs such as epinephrine can affect the systemic circulation.(4) Continuous use of tranexamic acid can also cause adverse effects such as nausea, vomiting, diarrhea, dyspepsia, and headaches.(5)

The World Health Organization (WHO) recommends the use of traditional medicines from natural ingredients in treatment. The use of traditional medicine is generally considered safer than the use of modern medicine, where traditional medicine has relatively fewer side effects than modern medicine.(6) This study raised the potential of natural ingredients in rambutan leaves to help stop bleeding. Rambutan plant (*Nephelium lappaceum* Linn) has properties to help the hemostatic process. Rambutan leaves have several active compounds, including tannins, flavonoids, saponins, terpenoids, and total phenol content.(7) The content of tannins, saponins, and flavonoids can accelerate bleeding cessation. Based on the description described above, the researchers were

interested in studying the effect of rambutan leaves extract (*Nephelium lappaceum* Linn) on tail bleeding time in balb-C strain mice.[8]

METHODS

This type of research was a laboratory experimental research with the post test only control group design. This research was conducted after administering the ethical clearance of experimental animals and research procedures at the Health Research Ethics Commission (KEPK) Faculty of Dentistry, University of Jember (No: 109/PL17.8/PG/2021). The samples used were 12 male mices (mus musculus), aged 2-4 months, weight 20-40 grams, in healthy condition and without injuries. The tails of mice had criteria, including no wounds or scars, no inflammation, no defects, and healthy (active movement). The samples were divided into 3 groups, namely the negative control group (K-), namely the mice were given distilled water, the positive control (K+) was mice were given tranexamic acid 0.065 mg/gBW mice, and the treatment (P) was mice were given rambutan leaves extract 260 mg/kgBW mice.

The first step, identification of rambutan (*Nephelium lappaceum* Linn) was carried out at the Integrated Agricultural Development Unit, Jember State Polytechnic (No.1283/UN25.8/KEPK/DL/2021). The old rambutan leaves are picked from the tree at the same time and field. The old rambutan leaves have a dark green color, smooth leaves surface, and are taken under the young leaves ± 1 meter from the top of the rambutan branch. The old rambutan leaves were chosen because they contain the best active substances.[9]

The second step was making rambutan leaves extract by maceration method. 600 grams of fresh rambutan leaves were washed thoroughly with running water and then dried to remove water by airing in a room. The leaves were sliced into smaller pieces and then dried in an oven at 50°C for 12 hours. The dried rambutan leaves were mashed using a blender until they become 200 grams of simplicia. The simplicia was immersed in a glass jar with 96% ethanol solvent for 3x24 hours, while stirring. Mixing the marinade was done twice, namely in the morning and evening.[10] After 3 days, the macerate was filtered from the pulp using filter paper. Then evaporated using a rotary evaporator for 45 minutes at a temperature of 45-500 C. Then concentrated in a water bath at a temperature of 50°C to obtain a thick extract.[11] The dose of rambutan leaves extract used in this study was 260 mg/kg BW of mice given orally with distilled water as a solvent.

The next step was treatment according to the treatment of each group. The group of mice was treated orally with a gastric probe. After 60 minutes, the mice were put into a mouse tube with a size that fit the body of the mice. The tails of mice were examined with 70% alcohol for disinfection using a cotton swab then followed by cutting the tails of mice 0.5 cm long from the tip of the tail using sharp surgical scissors. Bleeding time was observed by turning on the stopwatch along with cutting the tail of the mice and turning it off after the bleeding stopped. The blood resulting from cutting the tail of mice can be dripped on absorbent paper. The results of the bleeding time of mice were recorded in each group. The results of calculations in each group were then analyzed using SPSS (statistical analysis program). The data from the research were carried out by the Shapiro-Wilk normality test to determine whether the data were normally distributed, then continued with the homogeneity test using the Levene test. In this study, the data were normally distributed and homogeneous, so it was continued with the One Way ANOVA parametric test and then continued with the LSD (Least Significance Different) test.

RESULTS

The results of the calculation of tail bleeding time in the group of mice that were given tranexamic acid at a dose of 0.065 mg/g BW (K+), the group of mice that were given aquadest (K-), and the group of mice given rambutan leaves extract at a dose of 260 mg/kg BW (P) in the mean form is presented in Table 1. Based on the data, the results of these calculations indicate that the average bleeding time could be written as K+ < P < K- (the bleeding time of the tails of the mice given tranexamic acid was shorter than the group of mice given rambutan leaves extract and the bleeding time of the tails of the mice given the tranexamic acid). Rambutan leaves extract is shorter than the group of mice given aquadest).

Tabel 1. Average bleeding time of mice strain balb-C in all groups

No	Group	n	Average bleeding time (minutes) ± SD	ANOVA
1.	K+	4	2.37 ± 0.43	0.002*
2.	P	4	3.8 ± 0.97	
3.	K-	4	5.5 ± 1.04	

Group K+: positive control group given tranexamic acid at a dose of 0.065 mg/gBW; Group P: the treatment group that was given rambutan leaves extract at a dose of 260 mg/kgBW; Group K-: The negative control group was given distilled water; N: number of samples; SD: standard deviation; ANOVA: analysis of variance; *There is a significant difference (P < 0.05).

The research data obtained were then tested for normality with the Shapiro-Wilk test, the results of the normality test showed a p-value of more than 0.05 (p>0.05), this indicates that the data is normally distributed, then continued with the Levene homogeneity test, and obtained homogeneous data because the p-value is more than 0.05 (p>0.05). The data from the calculation of bleeding time was then continued with the One Way ANOVA parametric test. Based on the results of the One Way ANOVA test, p-value of 0.000 (p <0.05) was obtained, this indicates a difference in the length of bleeding time in all study groups. The data was then continued with the LSD (Least Significance Different) test to determine the difference in the average bleeding time between the treatment groups (Table 2).

Tabel 2. LSD test results bleeding time in all groups

Group	K+	K-	p-value
		P value	
K+	-	0,001*	0,042*
K-	0,001*	-	0,021*
P	0,042*	0,021*	-

*There was a significant difference (p <0.05).

DISCUSSION

The results of the statistical analysis of the LSD test showed that the average bleeding time of the tails of mice in the group of mice given tranexamic acid at a dose of 0.065 mg/gBW and the group of mice given aquadest showed a significant difference (p=0.001). This shows the ability of tranexamic acid and aquadest in shortening tail bleeding time is not comparable. The bleeding time of the tails of the mice that were given tranexamic acid was shorter than the group of mice that were given aquadest. This proves that tranexamic acid is more effective.

Aquadest has neutral properties so that it does not have an effect on the process of stopping bleeding (hemostatic) in mice.[12] Meanwhile, in the group of mice given tranexamic acid, the bleeding time was shorter than the group of mice given distilled water because tranexamic acid functions as a hemostatic agent. Tranexamic acid is an antifibrinolytic agent that works by inhibiting the conversion of plasminogen to plasmin.[13] Plasmin is a fibrinolytic enzyme that can dissolve blood clots by slowly breaking down the fibrin network. In addition, plasmin plays a role in the destruction of fibrinogen, fibrin, and other clotting factors.[14] Tranexamic acid inhibits the breakdown of fibrin so that hemostatic can occur more effectively.[15]

The results of LSD test showed that the average bleeding time of the tails of mice in the group of mice given rambutan leaves extract was 260 mg/kg BW and the group of mice given aquadest showed a significant difference (p=0.021). This shows the ability of rambutan leaves extract and aquadest in shortening tail bleeding time is not comparable. The bleeding time of the tails of the mice that were given rambutan leaves extract was shorter than the group of mice that were given aquadest. This proves that rambutan leaves extract is more effective.

Tannins have the ability to help the hemostatic process. Several studies have been conducted to examine the effect of tannins as antifibrinolytic in the hemostatic process. Research conducted by Pereira at.al[16] tated that Brownea grandiceps flower extract containing tannins had the ability to inhibit plasmin activity. Plasmin is a proteolytic enzyme found in the blood. Plasmin in its inactive form is called plasminogen. Most of the plasminogen is bound to fibrin and some is free in the plasma. When the plasminogen is activated, there will be free plasmin and fibrin-bound plasmin. Activated plasmin bound to fibrin can dissolve fibrin deposits by slowly breaking down the fibrin network so that blood flow can be reopened.[17] Based on this, because tannins work by inhibiting plasmin activity, it can lead to increased blood clotting activity so that it can help the hemostatic process in stopping bleeding.

Saponins have the ability to help the hemostatic process. According to research by Ko et al[18], Korean red ginseng extract containing saponins has activity in inducing Plasminogen Activator Inhibitor-1 (PAI-1). PAI-1 is a linear glycoprotein produced by vascular endothelial cells, which functions as a major inhibitor of fibrinolytic activity. A high amount of PAI-1 causes a decrease in the ability of fibrinolysis which results in the accumulation of fibrin clots in the microcirculation.[19] PAI-1 works by inhibiting the formation of plasminogen.[20] Activated plasmin bound to fibrin can dissolve fibrin deposits by slowly breaking down the fibrin network so that blood flow can be reopened.[17] Based on this, because saponins work by inducing PAI-1 which can inhibit the formation of plasmin, it can lead to increased blood clotting activity so that it can help the hemostatic process in stopping bleeding.

Flavonoids work in helping to stop bleeding because they have a hemostatic effect. According to research by Zhou et al[21], a traditional Chinese plant, namely Celastrus orbiculatus fruit which contains flavonoids, has antifibrinolytic activity. The mechanism is related to prostacyclin modulation.[21] Flavonoids work in helping to stop bleeding by suppressing prostacyclin. In vivo, flavonoids have been shown to have the ability to inhibit the release of arachidonic acid. The inhibition of arachidonic acid release causes a lack of arachidonic substrate in the cyclooxygenase and lipoxygenase cycles which will then suppress the amount of prostacyclin.[12] Prostacyclin has a reciprocal relationship to PAI-1 production. Decreased prostacyclin levels can lead to increased PAI-1 levels.[22] A high amount of PAI-1 causes a decrease in the ability of fibrinolysis which results in the accumulation of fibrin clots in the microcirculation.[19] PAI-1 works by inhibiting the formation of plasminogen into plasmin.[20] Activated plasmin bound to fibrin can dissolve fibrin deposits by slowly breaking down the fibrin network so that blood flow can be reopened.[17] Based on this mechanism, flavonoids can help in stopping bleeding.

The results of the statistical analysis of the LSD test showed that the average bleeding time of the tails of mice in the group of mice given rambutan leaves extract was 260 mg/kg BW and the group of mice given tranexamic acid at a dose of 0.065 mg/g BW showed a significant difference (p=0.042). This shows that the rambutan leaves extract and tranexamic acid groups have the ability to shorten bleeding time which is not comparable. The bleeding time of the tails of mice given tranexamic acid was shorter than the group of mice given rambutan leaves extract. This proves that tranexamic acid extract is more effective.

Tranexamic acid was chosen as a positive control because tranexamic acid is a hemostatic agent that has been widely used to reduce blood loss in surgery and medical conditions associated with increased bleeding rates.[23] Tranexamic acid can reduce the need for blood transfusions, is used in the treatment of major trauma, and

prophylactically in surgery. In contrast to tranexamic acid whose effects have been clinically tested, extracts derived from plants such as rambutan leaves contain a substance called ballast. Ballast substances are impurity compounds contained in samples such as chlorophyll, fats, proteins, resins, waxes, and other non-polar compounds that can interfere with biological activity. So that the ballast substance can inhibit the work of the active substances found in rambutan leaves such as tannins, saponins, and flavonoids.[24]

CONCLUSION

Based on the results of the study, it can be concluded that the administration of rambutan leaves extract (*Nephelium lappaceum* Linn) has a significant effect on tail bleeding time, which can shorten the bleeding time of cutting the tail of mice with balb-C strain.

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