

The Effectiveness of Kenikir Leaf Extract (*Cosmos Caudatus Kunth*) on Bleeding Time in Mice (*Mus Musculus*) Tails

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ABSTRACT

Background: Continuous bleeding will inhibit the formation of blood clots and cause the wound healing process to be hampered, thereby endangering the patient's life. Kenikir leaves contain flavonoid compounds, tannins, and saponins which have the potential as natural hemostatic agents in shortening bleeding time.

Aim: To analyze the effectiveness of kenikir leaf extract (*Cosmos caudatus kunth*) on bleeding time in the tails of mice (*Mus musculus*).

Material and Methods: This type of research is a laboratory experiment with a post test only control group design method. The samples used were 12 mice and divided into 3 groups, namely negative control (K-), positive control (K+), and treatment (P). All samples were adapted for 7 days, then fasted for 4 hours, then given each ingredient according to the group, then waited for 60 minutes and cut the mice tails along 0.5 cm from the tail end. Blood is dropped on filter paper every 30 seconds until the bleeding stops, the results of the calculation of bleeding time were analyzed using the Anova and LSD analysis.

Results: The average bleeding time in the K-, K+, and P groups was 7.25 minutes, 1.87 minutes and 3.37 minutes. Anova analysis results obtained a significance value ($p < 0.05$), which means that there are differences in all groups. LSD analysis showed that there were significant differences between K- and K+ groups and K- and P groups, whereas in the K+ and P groups no significant differences.

Conclusion: Kenikir leaf extract can shorten the bleeding time in mice tails.

KEYWORDS: *cosmos caudatus kunth*, bleeding time, flavonoids, tannin, saponins.

ARTICLE DETAILS

Published On:
26 January 2023

Available on:
<https://ijmscr.org/>

INTRODUCTION

Prolonged bleeding is one of the common complications after tooth extraction. The frequency of bleeding after tooth extraction can reach 31.5%.¹ Bleeding that occurs continuously and excessively will inhibit the formation of blood clots, causing the wound healing process to be hampered and can become a place for pathogenic bacteria to enter which can lead to an infection.² The risk of bleeding will increase if the patient has various systemic conditions, including hypertension, diabetes mellitus, taking anticoagulants and failure of postoperative instructions.³

Both local and systemic measures should be applied to stop bleeding. Systemic action can be done by administering hemostatic drugs orally or by injection, some of which are

epinephrine as a vasoconstrictor, or tranexamic acid.⁴ The use of hemostatic drugs can cause side effects if used in the long term, so a natural substitute is needed that has hemostatic properties but is still safe or has minimal side effects.⁵

One of the natural substitute that have the potential as hemostatic drugs is kenikir leaves. The content of substances in kenikir leaves shows various active compounds including flavonoids, saponins, terpenoids, alkaloids, tannins and essential oils.⁶ Based on the research of Sari *et al.* (2019) regarding 15% kenikir leaf extract which was applied topically to the back wounds of mice stated that kenikir leaf extract has the potential as a wound healing drug, due to flavonoid compounds and tannins that help in wound healing activity.⁷ According to Chiaretti⁸ that flavonoids are one of

the most effective compounds to treat bleeding. Therefore, kenikir leaves have potential as a natural hemostatic drug because of their high antioxidant content, namely flavonoids, tannins, and saponins that can play a role in shortening bleeding time.

MATERIAL AND METHODS

This type of research is a laboratory experiment with a post test only control group design method. This research has been carried out with ethical clearance at the Health Research Ethics Commission, Faculty of Dentistry, University of Jember with letter number 1282/UN25.8/KEPK/DL/2021.

The samples used were 12 male mice (*mus musculus*), aged 2-4 months, weight 20-40 grams, in healthy condition and without injuries. The samples is divided into 3 groups, namely the negative control group (K-) or the mice were given aquadest, the positive control (K+) or mice were given tranexamic acid 0.065 mg/g, and the treatment (P) or mice were given kenikir leaf extract 2.8 mg/g.

The first step is to extract the leaves of kenikir (*cosmos caudatus kunth*), previously the kenikir leaves have been identified at the UPT for Integrated Agricultural Development of the Politeknik Negeri Jember with letter number 110/PL17.8/PG/2021. The kenikir leaves were washed and then dried in a cabinet dryer at a temperature of 50°C ±12 hours, then mashed using a blender and then sieved. The fine powder of kenikir leaves was put into a glass jar and added 400 ml of 96% ethanol solvent for 3x24 hours, stirring occasionally. The maceration results were filtered with filter

paper and concentrated with a vacuum rotary evaporator water bath at a temperature of 60° C to obtain a thick kenikir leaf extract.

The next stage was treatment, where all samples were adapted for 7 days, then fasted for 4 hours, then each ingredient was given according to the group, then waited for 60 minutes and cut the tails of mice 0.5 cm long from the tip of the mice's tails.⁹ Blood is dripped on filter paper every 30 seconds until the bleeding stops.¹⁰ The filter paper used was divided into 16 squares. Bleeding time is calculated by multiplying the number of boxes filled with blood by 30 seconds. The results of calculations in each group were then analyzed using SPSS. The data from the research were carried out by the Shapiro-Wilk normality test, 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 Anova parametric test and then continued with LSD (Least Significance Different) analysis.

RESULTS

From the comparison results of the average length of bleeding time for each group, the negative control group (K-) showed the longest bleeding time compared to the K+ and P groups. The positive control group (K+) showed the shortest bleeding time compared to the K- and P groups, while the treatment group (P) showed a shorter bleeding time when compared to the K- group but longer than K+, so that when sorted according to the longest bleeding time it was K-> P > K+.

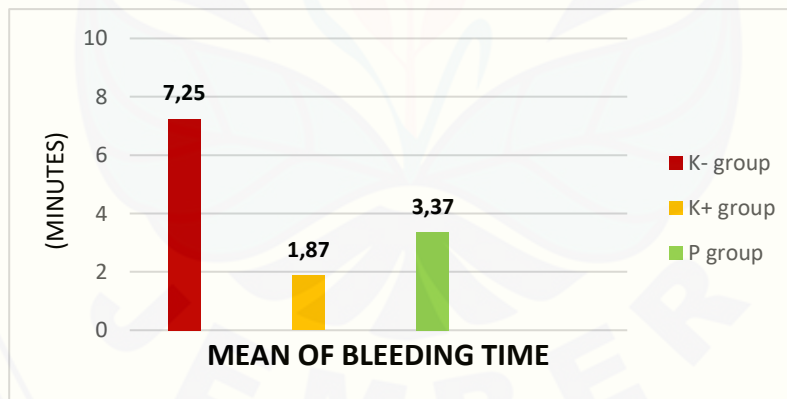


Figure 1. Average length of bleeding time in the Negative Control (K-), Positive Control (K+), and Treatment (P) groups.

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 Anova parametric analysis. Based on the results with Anova, a significance value of 0.000 or ($p < 0.05$) was obtained, this indicates a difference in the length of bleeding time in all study groups. These data were then followed by LSD analysis to determine the difference in the mean bleeding time between groups (**Table 1**).

Table 1. The results of LSD analysis conducted between groups in the study based on the length of bleeding time

| Groups | Groups | Sig. |
|--|--------|-------|
| K- | K+ | .000* |
| | P | .001* |
| K+ | K- | .000* |
| | P | .069 |
| P | K- | .001* |
| | K+ | .069 |
| *: There is a significantly difference . | | |

DISCUSSION

LSD analysis show that between the K- and K+ groups, there was a significantly difference, likewise, the K- and P groups also showed a significantly difference. The K- group or negative control in the form of aquadest did not give any physiological or hemostatic effect on bleeding time in mice because it acted as a negative control which was determined to produce negative results.

The shortened bleeding time in P group when compared to K- group is thought to be caused by the presence of active compounds in kenikir leaves that can shorten bleeding time, namely, flavonoids, tannins, and saponins, so that the LSD analysis results show a significantly difference between P and K- groups which means that kenikir leaf extract in P group has an effect on bleeding time in the form of shortening bleeding time in mice.

The mechanism of flavonoids in shortening bleeding is a vasoconstriction mechanism.¹⁴ Flavonoids can maintain vascular permeability and increase capillary vascular resistance, so that blood vessels will experience vasoconstriction which will stop bleeding. Flavonoids also act on the microvascular endothelium to reduce hyper permeability and inflammation.¹¹ Flavonoid compounds in 100 g of kenikir leaf powder are 1089.79 mg.¹² Tannin compounds are astringent which have the ability to form a complex with macromolecules, especially proteins.¹³ Tannins also precipitate blood proteins, thereby inducing the synthesis of thromboxane A2 to increase platelet aggregation, and accelerate the formation of a temporary platelet plug in injured blood vessels.¹⁴ Based on the statement from the research of Galang et al. (2015) that tannins are one of the astringent materials that can precipitate thrombin and play an important role in the hemostatic mechanism at the stage of blood clotting. The deposited thrombin will convert fibrinogen into fibrin threads at the site of blood loss which then forms a collection of fibrin threads that can stop bleeding.^{18,19} The active compounds of saponins also have the ability to be antiseptic by forming a persistent foam which has a high level of toxicity against microorganisms present in the wound area, so that the wound does not experience severe infection.¹⁵

LSD analysis between P and K+ groups showed no significantly difference, but based on the data from the

calculation of the average length of bleeding time, the average value of the K+ group was slightly shorter than P group, this could be due to the effect of weaker pharmacological use of traditional medicine can be caused by the complex ballast or banar compounds commonly found in plants so they interfere with natural ingredients in producing biological activity,¹⁶ but statistically there is no significantly difference, This proves that P group or mice given kenikir leaf extract had a hemostatic effect on mice even though the average value of bleeding time in P group was slightly above K+ group. Therefore, kenikir leaves have potential as an alternative to natural hemostatic drugs, but more in-depth research is still needed.

Various conditions that the researcher cannot control can affect bleeding time. The ability of mice to respond to platelet thrombus formation to stop bleeding after an injury, platelet reactivity, the possibility of plasma enzymatic reactions in mice, physical stress in experimental animals, and flavonoid compounds, tannins, and saponins that must be tested again can affect bleeding time.¹⁷

CONCLUSION

Kenikir leaf extract can shorten the bleeding time in mice tails.

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