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THE EFFECTIVENESS OF GEL ADMINISTRATION OF KALIMBAJO STEM BARK EXTRACT (LENNEA COROMANDELICA) ON INCREASING COLLAGEN DENSITY IN HEALING PROCESS OF FEMALE MICE (MUS MUSCULUS) INCISION WOUNDS

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Abstract

The wound healing process can cause wounds in the perineum. One of the plants known to have this potential as a traditional medicine is kalimbajo (Lennea coromandelica). The purpose of the study is to analyze the effectiveness of administering kalimbajo stem bark extract gel (Lannea Coromandelica (Houtt.) Merr) on increasing collagen density and the formation of epithelial tissue in the healing process of incision wounds in female Mus musculus. Methodology: True experimental using a quantitative method with a Post Test Only Control Group was used as a research design. The sample was divided into 5 groups. Kruskall-Wallis was used as data analysis. Results: The results showed that Kalimbajo bark extract gel with a concentration of 14% was more effective in healing incision wounds for 8.33 compared to a concentration of 6%, 10%. The best concentration for epithelial cell formation in the wound healing process was 14% more effective compared to the 6%, 10%, negative control, and positive control concentrations in epithelial cell formation in the wound healing process. Conclusion: It can be concluded that kalimbajo stem bark extract with a concentration of 14% is the best concentration to help the healing process of incision wounds, increase collagen density, and growth of epithelial cells.

Keywords: Gel Extract, Collagen Density, Wound Healing, Sectio Cessarea (SC), Kalimbajo (Lennea Coromandelica).

1. INTRODUCTION

According to the World Health Organization (WHO) in 2020, there were 2.7 million cases of perineal rupture in pregnant women, expected to reach 6.3 million in 2050. In Asia, perineal rupture in society accounts for 50% of the incidence of perineal rupture in the world (Sudianti et al., 2023). The incidence of perineal rupture in Indonesia increased from the previous year or by 60% in 2013 to 67.2% in 2014 (Indah Dewi Sari et al., 2022).

One of the factors that affect the occurrence of perineal wounds is the technique of assisted labor (Prawitasari et al., 2019), while in the indications of episiotomy measures such as fetal emergency, fetal presentation, the use of vacuum extraction and forceps where the degree of injury can reach degree III. Wound care in the perineum needs attention because it can cause dysfunction of the female genital

organs, such as bleeding, the entry of germs that can cause infection, and prolapse of the genital organs (Triyanti et al., 2019).

In the history of medicine, various types of plants have been found that were used by ancient communities as the main source in the treatment of diseases (Muhammad Tahir & Winda, 2023). Traditional wound care is currently more in demand by the public because it is not only easy to show, but also has relatively few side effects. One of the plants that can be used as a wound medicine is Javanese Bark (Lannea coromandelica (Hout) Merr). Javanese wood or in the Bone community, especially in Kajuara District, known as "Kalimbajo", is a plant that is often found in the yards of houses and roadsides (Nurihardiyanti et al., 2020).

Based on phytochemical studies, the bark of the Kalimbajo plant (Lannea coromandelica (Hout) Merr) has been reported to contain steroid compounds, terpenoids, saponins, tannins, and flavonoids. Flavonoid compounds can play a role in wound healing by stopping bleeding through vasoconstriction mechanisms in blood vessels, antidote free radicals, and anti-inflammatory, while tannin compounds can act as astringents in wounds, and saponin compounds increase the speed of epitalization and help in the formation of collagen which acts as a wound healer (Manik et al., 2019).

In Indonesia, research conducted by (Arum et al., 2022) showed that transdermal patch preparations of ethanol extract from Javanese bark have effectiveness in healing cut wounds in male white rats. The use of extracts, simplicia, or parts of a plant used as medicine has maximum efficacy if provided with a gel-like formula. Therefore, with this gel formula, it is hoped that it can be used as a practical and effective medicine by the community, both wound healing drugs in obstetrics, namely perinuem wounds and SC wounds, as well as wounds caused by sharp object scratches and others. The difference between this study and the previous study was about the concentration. In the previous research, the form of patches was provided with concentrations of 3%, 6%, and 9%, while in this study, the form of a gel was provided with a concentration of 6%, 10% and 14%. From the above description, it is the basis for researchers to conduct in vivo research on the effectiveness of administering kalimbajo stem bark extract gel (Lannea coromandelica (Houtt.) Merr) on increasing collagen density in the healing process of female musculus incision wounds.

2. EXPERIMENTAL SECTION

2.1 Tools and Materials

The tools used during this research were: Animal cages, cell phone cameras, sterile handscon, bent, jars, aluminium foil, surgical boards and trays, callipers, frequent paper, scissors, anatomical tweezers, scalpel, sterile masks. The materials used in this study were: kalimbajo stem bark, female mice, povidone iodine solution 10%, alcohol 70%, cotton, mouse feed, gallon water, cotton buds and formalin.

2.2 Method

This study was a pure research (true experimental) with a *Post Test only Control Group* design. The manufacture of kalimbajo bark extract gel was carried out in the laboratory of Pharmaceutical Biology, Almarisha Madani University, Makassar. The population in this study were female mice selected using the Simple Random Sampling method. The sample was divided into 5 groups, namely the positive control group with the administration of povidone iodine, the negative control group with the

administration of the gel without EKBK, the treatment group 1 with the administration of 6% concentrated EKBK gel, the treatment group 2 with the administration of 10% concentrated EKBK gel and the treatment group 3 with the administration of 14% concentrated EKBK gel. Data analysis was carried out using the Kruskall-Wallis test.

2.3 Procedure

30 female mice weighing 20-35 grams were adapted and kept in groups for 6 days in experimental animal cages made of plastic. The test animals were divided into 5 treatment groups, namely the P1 group as the 6% EKBK group (administration of kalimbajo bark extract gel at a concentration of 6%), the P2 group as the EKBK group 10% (administration of kalimbajo bark extract gel at a concentration of 10%), the P3 group as the EKBK treatment group 14% (administration of kalimbajo bark extract gel at a concentration of 14%), the group (K-) as a negative control (the administration of gel without kalimbajo bark extract), and group (K+) as a positive control (with the administration of povidone iodized). Each group consisted of 6 mice, the treatment was carried out for 14 days.

3. RESULT AND DISCUSSION

The flavonoid compounds plays a role in wound healing in the inflammatory phase through the vasoconstriction mechanism in blood vessels when the wound occurs. In addition, flavonoids also work in activating T cells, deferrating, and multiplying (Rachmanita et al., 2019). In the proliferation phase, the formation of collagen in repairing and healing wounds is characterized by an increase in the number of fibroblasts. Fibroblasts have an important role in the wound healing process, where fibroblasts are responsible for producing protein structure products that are used in the tissue reconstruction process, so that after the occurrence of the wound fibroblasts move actively and move to the wound gap, the collagen synthesized by the fibroblasts is also increasing, the more the number of fibroblasts is affected by the number of macrophages (Chen et al., 2019).

The other compounds found in kalimbajo bark extract is saponins. This saponin compound can activate TGF-β signaling by increasing the ability of TGF-β receptors when binding to TGF-β so that the more TGF-β will be activated, the more fibroblasts will move to the wound gap, so that after moving to the wound gap, these fibroblasts will later be induced again by TGF-β to multiply (Chen et al., 2019). The content of kalimbajo bark extract gel applied to animal wound areas tries to stimulate the synthesis of growth factors, including fibroblast growth factor (FGF) so that it increases the activity of fibroblast cells to produce collagen and form connective tissue so that the wound heals faster (Sutiyo, 2020).

Based on table 1, the average wound healing based on Nagaoka's score showed clearly that the EKBK group of 14% has an average faster healing time of 8.33 days, a healing score of 2.33, followed by the EKBK group of 10% with an average healing time of 11.33 days, a healing score of 2. Then followed the EKBK group of 6% with an average healing time of 11.67 days, a healing score of 2. Moreover, the next is followed by the negative control group with an average healing time of 12.67 days and the group with an average of the longest cure among all groups was the positive control group with a healing time of 13 days. Since there were no infections and allergic reactions in all groups, each group got a score of 3. The results of macroscopic observation of wound healing clinically showed a value of $p= 0.030$ ($p<0.05$), so it was

concluded that there was a difference in wound healing time between groups. Furthermore, a follow-up test was carried out for Kruskall-Wallis, a significant value was found in the EKBK treatment group of 14%. These results proved that the administration of EKBK gel at a concentration of 14% provides progressive wound healing faster than EKBK gel concentration of 10% and 6%, as well as the control group. This research is in line with previous research that used rats with transdermal patches of 96% ethanol extract of Javanese bark can accelerate the wound healing process with a concentration of 9% % (Arum et al., 2022).

Table 1: Macroscopic Average Wound Healing based on Nagaoka Score

Group	Healing Time (Days)	Healing Score	Local Infection	Allergic Reaction
EKBK 6%	11.67	2	3	3
EKBK 10%	11,33	2	3	3
EKBK 14%	8,33	2,33	3	3
NEGATIVE CONTROL	12,67	2	3	3
POSITIVE CONTROL	13	2	3	3

Applying the EKBK gel to cut wounds affected the wound healing process which began with the inflammatory phase. The inflammatory phase began from the occurrence of the wound until the 5th day. In this phase, wound healing began as soon as tissue and blood vessel damage occured that caused platelets to come out, platelets formed blood clots; therefore, that bleeding stopped. This inflammatory process was characterized by the activity of neutrophils and macrophages. Neutrophils and macrophages produced free radicals called Reactive Oxigen Species (ROS) which were part of the immune system and function to clean wounds from bacteria. Not only that, ROS could also be a signal to modulate several signaling pathways that regulated blood coagulation, thrombosis, migration, proliferation, fibrosis and angiogenesis of each phase of wound healing. Molecules on ROS appeared if the hydrogen peroxide was low. However, it was different if it was in high levels, ROS would damage the tissue severely, so that the presence of ROS in the body for a long time would inhibit the wound healing process (Chen et al., 2019).

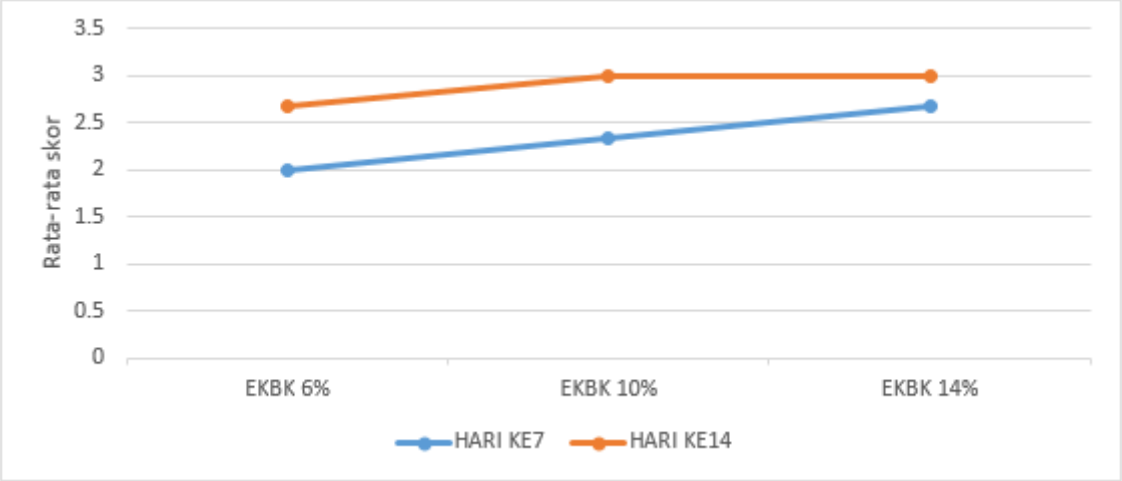

Figure 1: Average collagen density score in the EKBK group of wound healing degree on hispathology test

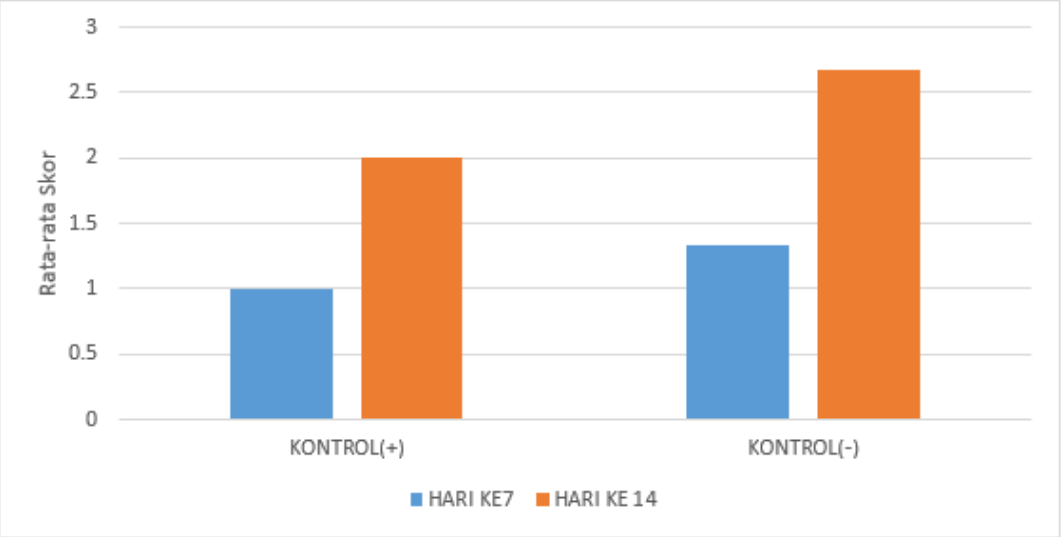

Figure 2: Mean collagen density score in the wound healing degree control group on the histopathology test

Based on figure 1 and 2, the average collagen density score for the treatment group and control group increased from day 7 to day 14 of each group. For the EKBK group of 6% there was an increase in the average collagen score on day 7 from score 2 to 2.67 on day 14. Meanwhile, for the EKBK group of 10% there was an increase in the average collagen score on day 7 from 2.33 to 3 on day 14, as well as in the EKBK group of 14%. It showed that there was an increase in the average collagen score on day 7 from 2.67 to 3 on day 14. Meanwhile, in figure 2, the positive control group also increased the average collagen score on day 7 from 1 to 1.33 on day 14, while the negative control group increased the average collagen score from 2 on day 7 to 2.67 on day 14.

Table 2: Percentage of collagen density score

GROUP	COLLAGEN DENSITY PERCENTAGE GROUP					
	DAY 7			DAY 14		
COLLAGEN SCORE	**1**	**2**	**3**	**1**	**2**	**3**
EKBK 6%	0	100	0	0	66,67	33,33
EKBK 10%	0	66,67	33,33	0	0	100
EKBK 14%	0	33,33	66,67	0	0	100
POSITIVE CONTROL	100	0	0	0	100	0
NEGATIVE CONTROL	66,67	33,33	0	0	66,67	33,33
P Value	**0,031***			**0,061**		

** = p<0.05 is significant (Kruskall-Wallis Test)*

Based on table 2 above, the percentage of collagen density on the 7th day was obtained by the EKBK group of 6%, there was a score of 1 and a score of 3 with a percentage of 0%, a score of 2 with a percentage of 100%. In the EKBK 10% group, there was a score of 1 with a percentage of 0%, a score of 2 with a percentage of 66.67% and a score of 3 with a percentage of 33.33%. In the 14% group, there was a score of 1 with a percentage of 0%, a score of 2 with a percentage of 33.33% and a score of 3 with a percentage of 66.67%. The positive control group obtained a score of 1 with a percentage of 100%, a score of 2 and a score of 3 with a percentage of 0%. The negative control group obtained a score of 1 with a percentage of 66.67%

and a score of 2 of 33.33%. Meanwhile, on the 14th day, the collagen density score in the EKBK group was 6% of 0% for a score of 1, 66.67% for a score of 2 and 33.33% for a score of 3. The EKBK group was 10% for score 1 and score 2 is 0%, for score 3 was 100%. Meanwhile, the EKBK group was 14% for score 1 and score 2 is 0%, for score 3 was 100%. The positive control group had a score of 1 and a score of 3 with a percentage of 0%, a score of 2 with a percentage of 100%, while the negative control group obtained a score of 1 with a percentage of 66.67% and a score of 2 of 33.33%.

Based on the results of the scoring above, a statistical test using Kruskall-Wallis was then carried out to see the significant value of the increase in collagen density between the groups. The results of the Kruskall-Wallis test on day 7 showed a value of p=0.031 (p<0.05) meaning that there was a difference in the increase in collagen density between groups. While on the 14th day, it showed a value of p= 0.061 (p>0.05) meaning that there was no difference in the increase in collagen density between groups.

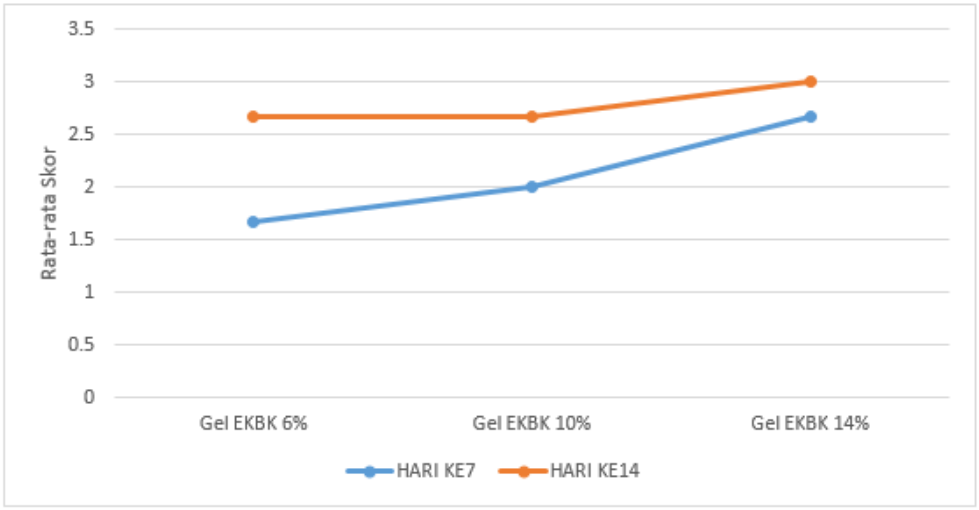

Figure 3: Average score of epithelial cell formation in the EKBK group degree of wound healing in hispathology test

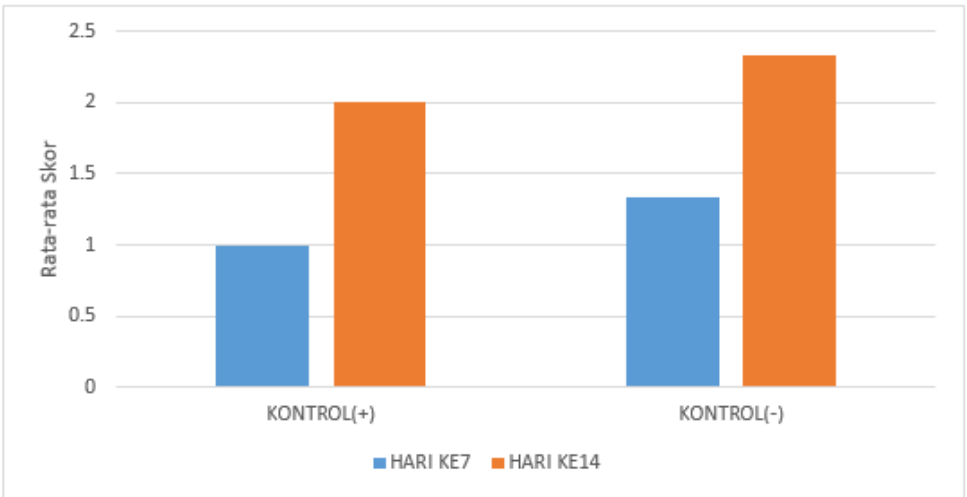

Figure 4: Average epithelial cell formation score in the control group for incision wound healing in hispathology test

From the figure 3 above, it showed that the average score of epithelial cell formation for the treatment group and control group increased from day 7 to day 14 in each group. In the EKBK group of 6%, there was an increase in the average epithelial cell score on day 7 from 1.67 to 2.67 on the 14th day, for the EKBK group of 10% there was an increase in the average epithelial cell score from 2 on day 7 to 2.67 on day 14, as well as in the EKBK group of 14% that there was an increase in the average epithelial cell score from 2.67 on day 7 to 3 on day 14. Meanwhile, in figure 4, the positive control group, the average epithelial cell score also increased on day 7 from 1 to 1.33 on day 14 while the negative control group also increased the average epithelial cell score from 2 on day 7 to 2.33 on day 14.

Table 3: Epithelial Cell formation score percentage

GROUP	EPITHELIAL CELL FORMATION PERCENTAGE					
	DAY 7			DAY 14		
COLLAGEN SCORE	1	2	3	1	2	3
EKBK 6%	33,33	66,67	0	0	66,67	33,33
EKBK 10%	0	100	0	0	33,33	66,67
EKBK 14%	0	33,33	66,67	0	0	100
Positive Control	100	0	0	0	100	0
Negative Control	66,67	33,33	0	0	66,67	33,33
P-value	0,044*			0,165		

* = p<0.05 is significant (Kruskall-Wallis Test)

Based on table 3, the percentage of epithelial cell formation score on the 7th day was obtained by the EKBK group of 6%. There was a score of 1 with a percentage of 33.33%, a score of 2 with a percentage of 66.67% and a score of 3 with a percentage of 0%. In the 10% EKBK group, there was a score of 1 and a score of 3 with a percentage of 0%, a score of 2 with a percentage of 100%. In the 14% group, there was a score of 1 with a percentage of 0%, a score of 2 with a percentage of 33.33% and a score of 3 with a percentage of 66.67%. The positive control group obtained a score of 1 with a percentage of 100%, a score of 2 and a score of 3 with a percentage of 0%, while the negative control group obtained a score of 1 with a percentage of 66.67% and a score of 2 of 33.33%. Meanwhile, on the 14th day, the epithelial cell score in the EKBK group was 6% of 0% for score 1, 66.67% for score 2 and 33.33% for score 3. The EKBK group of 10% was 0% for score 1, score 2 was 33.33%, for score 3 was 66.67%. The EKBK group was 14% at 0% for score 1 and score 2, for score 3 was 100%. The positive control group had a score of 2 with a percentage of 100% and scores 1 and 3 with a percentage of 0%. While the negative control group had a score of 2 with a percentage of 66.67% and a score of 33.33%.

Based on the results of the scoring above, a statistical test using Kruskall-Wallis was then carried out to see the significant value of the formation of epithelial cells between groups. The results of the Kruskall-Wallis statistical test on day 7 showed a value of p=0.044 meaning that there was a difference in the formation of epithelial cells between groups. Meanwhile, on the 14th day, the value of p = 0.165 was shown, meaning that there was no difference in the formation of epithelial cells between groups. It can be seen that the administration of EKBK gel at a concentration of 14% showed the effectiveness of the application of EKBK gel and it increased collagen density and the formation of epithelial tissue in the wound healing process compared to the concentrations of 6% and 10% and the control group, because the kalimbajo bark extract at a concentration of 14% contained more active substances, especially

the content of falvonoids which played a role in increasing collagen survival, preventing cell damage, preventing wound infections, and accelerating the wound healing process. In addition, the alkaloid compounds could stimulate cell proliferation agents for collagen production and saponin compounds which played a role in increasing tensile stress and increasing collagen formation and better epitalization.

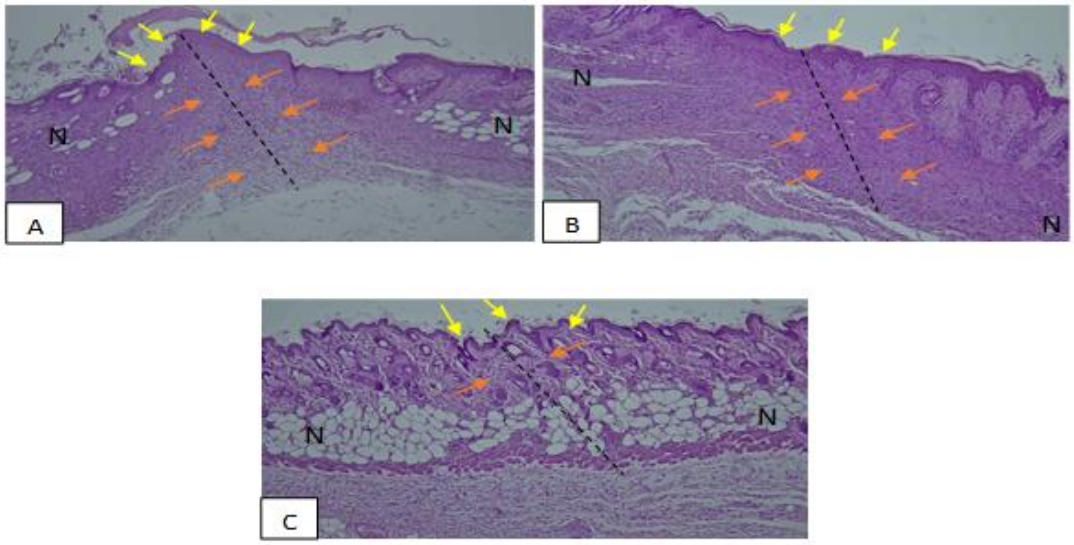

Figure 5: Histopathological picture of 100x magnification (→) Collagen, (→) epithelial cells (---) cut wounds

In Figure 4, based on the results of histopathology tests using a microscope with a magnification of 100x, the assessment of tissue samples in each group was adjusted to the scoring level that had been determined by the anatomical pathologist. In figure A, it can be seen that around the area of the arrow the density of collagen formed was still thin and the color was still pale compared to the surrounding area, while the formation of epithelial cells looked imperfectly fused and did not have the same thickness as the surrounding area; therefore, it was given a score of 1. The picture B showed around the area of the arrow of collagen density which was formed at medium density and the color was the same as the surrounding area, while the formation of epithelial cells was almost completely fused and did not have the same thickness as the surrounding; therefore, it was given a score of 2. The picture C showed around the arrow area of the collagen density formed that was dense and the same as the surrounding cells as well as the formation of epithelial cells formed have fused perfectly and have the same thickness as the cells around it; therefore, it was given a score of 3.

Therefore, the results of this study can be implicated topically to treat wounds on the surface of the skin. Kalimbajo stem bark extract gel can be used for wounds on the skin including burns and incisions. In the field of obstetrics, the use of kalimbajo stem bark extract gel can be used as a postpartum wound medicine such as perineal rupture wounds and SC scar wounds. The use of kalimbajo stem bark extract gel has additional advantages not only accelerating wound healing, but also easier to apply and it does not have a toxic effect.

4. CONCLUSION

Kalimbajo bark extract gel with a concentration of 14% is more effective compared to the 6% and 10% concentrations in the healing process of mice cuts. Kalimbajo stem bark extract gel with a concentration of 14% is more effective compared to the concentration of 6%, 10%, negative control and positive control in increasing collagen density in the wound healing process. Kalimbajo bark extract gel with a concentration of 14% is more effective compared to the concentration of 6%, 10%, negative control and positive control in the formation of epithelial cells in the wound healing process.

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