

Administration of Cocor Bebek Leaves Gel (*Kalanchoe Pinnata*) 20% Increased Neovaskularization, Number of Fibroblast Cells and Epitelization in the Wound Healing Process of Male Wistar Rats (*Rattus Novergicus*)

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Abstract: *Background:* Wounds are damage or deviation from normal anatomical and functional structures. Physiological changes that occur in the tissue regeneration process after injury are neovascularization, increased number of fibroblast cells and the occurrence of epithelialization. Cocor bebek leaves extract contains flavonoids, tannins, saponins and phenolic compounds that can accelerate the wound healing process. The purpose of this study was to prove that the gel of cocor bebek (*Kalanchoe Pinnata*) leaves extract could increase neovascularization, the number of fibroblasts, and epithelialization in the wound healing process of the skin of male Wistar strain mice (*Rattus Norvegicus*). *Methods:* This study used a randomized posttest only control group design using 28 male Wistar strain rats (*Rattus Norvegicus*) aged 4-6 months, 150-200 grams weight and healthy, divided into 4 groups, each group of 7 heads. After being injured, group P0 (4) was given base gel 2x / day for 4 days, group P1 (4) was given cocorebek leaves extract gel 2x / day for 4 days, group P0 (11) was given base gel 2x / day for 11 days, group P1 (11) was given cocor bebek leaves extract gel 2x / day for 11 days. All rats were given oral amoxilin 10 mg 3 times / day for 3 days. On days 4 and 11, skin samples were taken from the wound site for histopathological examination to see neovascularization, fibroblast cells, and epithelialization with Haematoxylin Eosin staining. *Result:* On day 4, the results showed that the mean number of neovascularization in the treatment group (4.60 ± 3.37) was significantly higher than the control group (1.44 ± 1.55) ($p < 0.05$). There was a significant difference in the mean number of fibroblasts in the treatment group (45.71 ± 9.95) which was higher than the control group (27.51 ± 12.50). The mean epithelial length of the control group (85.71 ± 28.35) and the treatment group (175.71 ± 46.66) ($p < 0.05$) were significantly different. On day 11, the results showed that the mean number of neovascularization in the treatment group (3.01 ± 0.95) was significantly higher than the control group (1.76 ± 0.23) ($p < 0.05$). There was a significant difference in the mean number of fibroblasts in the treatment group (68.09 ± 7.87) which was higher than the control group (29.84 ± 10.11). The mean epithelial length of the control group (195.57 ± 61.61) and the treatment group (296.86 ± 56.14) ($p > 0.05$) was significantly different. *Conclusion:* From the above results it can be concluded that giving 20% cocor duck leaves extract gel in the wound healing process of male Wistar rats increased the number of fibroblast cells, neovascularization and reepithelialization.

Keywords: Cocor bebek leaves, neovascularization, fibroblasts, epithelization, wound healing

1. Introduction

In the development of time, technology and science also continue to develop, in which medical science is included. Various kinds of efforts were made to maintain and maintain human health. In various developing countries such as Indonesia, the level of public awareness of the importance of health is still low, this is due to inadequate economic and educational factors, so that knowledge about health is not well understood. As well as knowledge about the aging process, there are still many misconceptions about the aging process.¹

The wound is the loss or destruction of some body tissue. Wounds can also be defined as physical damage that occurs as a result of the opening or breakage of the skin which causes an imbalance of normal skin function and anatomy.² As we get older, a decrease in the function of organs in the body will also occur, which can cause disease. If the person has a wound, the ability to heal the wound will also decrease.³ The wound healing process slows down at all

phases as a person ages.³ Because one of the effects of aging is the inhibition of the wound healing process, treatments that speed up the wound healing process are one of the steps of Anti-Aging Medicine. So that in this study, the relationship with Anti-Aging Medicine is that the process of slowing wound healing due to aging can be prevented and inhibited by giving cocor bebek leaves extract gel (*Kalanchoe Pinnata*).

2. Methods

This study used a randomized posttest only control group design using 28 male Wistar strain rats (*Rattus Norvegicus*) aged 4-6 months, 150-200 grams weight and healthy, divided into 4 groups, each group of 7 heads. After being injured, group P0 (4) was given base gel 2x / day for 4 days, group P1 (4) was given cocorebek leaves extract gel 2x / day for 4 days, group P0 (11) was given base gel 2x / day for 11 days, group P1 (11) was given cocor bebek leaves extract gel 2x / day for 11 days. All rats were given oral amoxilin 10 mg 3 times / day for 3 days. On days 4 and 11, skin

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3. Results

On day 4, the results showed that the mean number of neovascularization in the treatment group (4.60 ± 3.37) was significantly higher than the control group (1.44 ± 1.55) ($p < 0.05$). There was a significant difference in the mean number of fibroblasts in the treatment group (45.71 ± 9.95) which was higher than the control group (27.51 ± 12.50). The mean epithelial length of the control group (85.71 ± 28.35) and the treatment group (175.71 ± 46.66) ($p < 0.05$) were significantly different. On day 11, the results showed that the mean number of neovascularization in the treatment group (3.01 ± 0.95) was significantly higher than the control group (1.76 ± 0.23) ($p < 0.05$). There was a significant difference in the mean number of fibroblasts in the treatment group (68.09 ± 7.87) which was higher than the control group (29.84 ± 10.11). The mean epithelial length of the control group (195.57 ± 61.61) and the treatment group (296.86 ± 56.14) ($p > 0.05$) was significantly different.

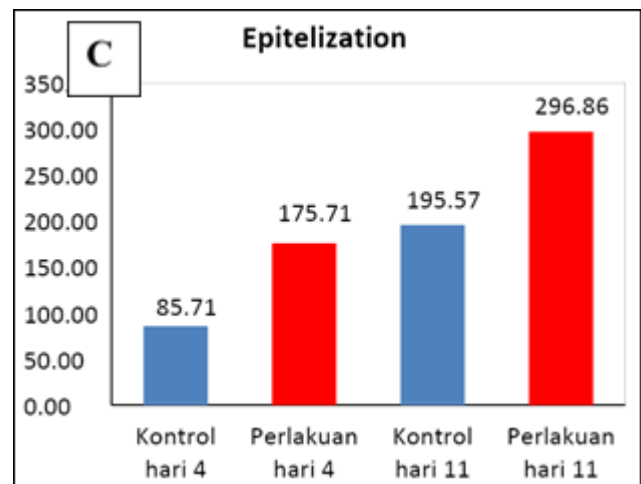
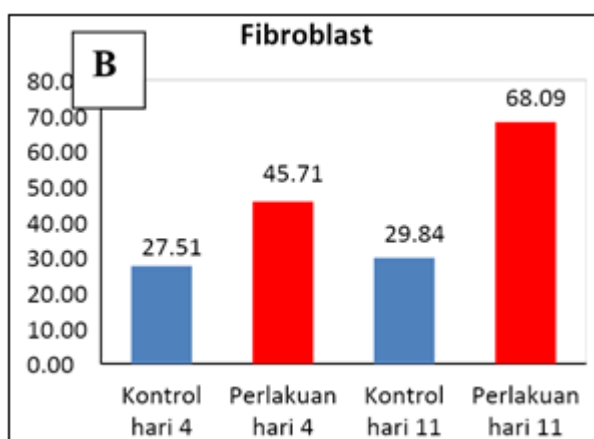
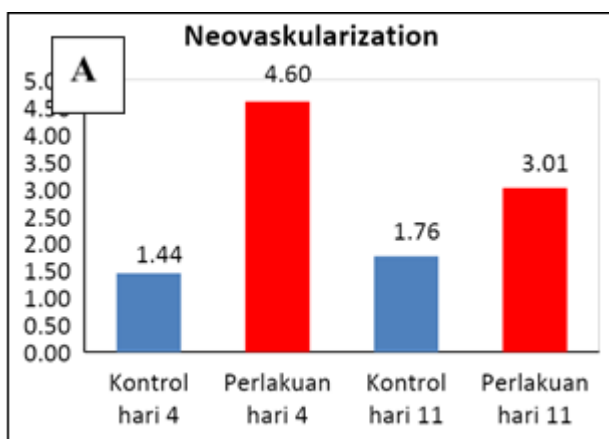


Figure 1: (A) Comparison of Neovaskularization, (B) Comparison of Fibroblast and (C) Comparison of Epitelization

4. Discussion

Cocor Bebek Leaves Extract Gel Effect on Neovascularization

The results of this study showed that the mean neovascularization in the control group on the 4th day after being given oral amoxicillin for 3 days and given basic gel every day for 4 days was 1.44 ± 1.55 / LP while in the treatment group on the 4th day after being given oral amoxicillin for 3 days and 20% cocor bebek leaves extract gel every day for 4 days increased to 4.60 ± 3.37 / LP. In the control group on day 11 the mean of neovascularization increased to 1.76 ± 0.23 / LP and in the 11th day treatment group neovascularization decreased slightly to 3.01 ± 0.95 / LP.

The results of the 4th day treatment group had the highest mean neovascularization compared to the other three groups with p value < 0.001 . This shows that giving cocor bebek leaves extract gel for 4 days can increase neovascularization in the wound healing process of mice with a significant difference ($p < 0.05$). Meanwhile, the 11th day treatment group had the lowest mean neovascularization compared to the other three groups. This shows that on the 11th day the rats had wound healing.

When compared with the physiology of wound healing, the results of this study are consistent with the maximum number of neovascularization rates occurring on the 4th day, physiologically the peak occurs on the 3-5th day of injury and will begin to decline on 7-8 days after the injury.⁴ It can also be observed that there is a significant difference between the control and treatment groups, where the treatment group has a higher mean of neovascularization. And on the 11th day according to the physiology of wound healing, where the wound had healed and entered the remodeling stage so that in the control and treatment groups the mean neovascularization decreased. In the 11th day treatment group, the lowest mean of neovascularization was obtained and it was significantly different when compared to the control group.

Cocor Bebek Leaves Extract Gel Effect on the Number of Fibroblast Cells

The results of this study showed that the mean number of fibroblast cells in the control group on day 4 was 27.51 ± 12.50 cells / LP, while in the treatment group on day 4 it increased to 45.71 ± 9.95 cells / LP. On the 11th day of the control group the mean number of fibroblasts still seemed to slightly increase to 29.84 ± 10.11 cells / LP and in the 11th day treatment group the mean number of fibroblasts increased to 68.09 ± 7.87 cells / LP. In the 11th day treatment group, it is expected that the number of fibroblasts will peak and will gradually decrease until the 21st day. In the results of this study, both the control group and the treatment group showed that the number of fibroblasts was still increasing. This is related to the process of wound healing in the proliferation phase, where fibroblasts will continue to increase on day 3 and reach their peak on day 14 and gradually decrease until day 21.⁷

This shows that giving cocor bebek leaves extract gel can increase the number of fibroblasts in the wound healing process of mice with a significant difference ($p < 0.05$). And this shows that on the 11th day in both the control group and the treatment group the wound healing process was still ongoing.

Giving 20% cocor bebek leaves extract gel to rats wounds could increase wound healing activity. The increase in wound healing activity is due to the fact that cocor bebek leaves extract contains flavonoids, tannins and saponins, which are cardiac glycosides. The flavonoid content in cocor bebek leaves is a good reducing compound, thought to have anti-inflammatory and anti-bacterial properties seen from its mechanism of action by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane.⁵

In a previous study using 15g of Indian variety cocor bebek leaves extract, using petroleum jelly and mupirocin as negative and positive controls in injured albino rats (Sprague dawley), on day 11 showed a significant increase in wound healing by percentage. The increase in the treatment group was 86.3%, while in the negative control group it was 68% and the positive control group was 85.5%. It was explained that the histological analysis of tissue samples in the control group showed that collagen fibers were still sparse and scattered, whereas in the treatment group the collagen fibers were denser and more deposited.⁶

Cocor Bebek Leaves Extract Gel Effect on Epithelialization

The results of this study showed that the mean of epithelialization in the control group on day 4 was 85.71 ± 28.35 μm , while in the treatment group on day 4 was 175.71 ± 46.66 μm . In the control group on the 11th day of epithelialization increased to 195.57 ± 61.61 μm and in the 11th day treatment group the mean epithelialization also increased to 296.86 ± 56.14 μm .

The treatment group had a higher mean of epithelialization than the control group with p value < 0.001 . This shows that

giving cocor bebek leaves extract gel can increase epithelialization in the wound healing process of mice with a significant difference ($p < 0.05$). The 11th day treatment group had the highest mean of epithelialization compared to the other three groups.

This shows that the epithelialization process goes better by administering cocor bebek leaves extract gel to wound healing in mice. The increase in the mean of epithelialization on day 4 and day 11 in the group given cocor bebek leaves extract gel indicated a better and faster wound healing process. This is due to the flavonoid content in cocor bebek leaves extract. Flavonoids have been shown to increase epithelial cell migration and proliferation as well as increase myofibroblast synthesis, migration and activity.

The saponins and tannins in cocor bebek leaves extract also play a role in the wound healing process. The mechanism of action of saponins in wound healing is to stimulate the formation of type 1 collagen which plays an important role in the wound closure process and increases tissue epithelialization.⁸

Cocor Bebek Leaves Extract Gel in Anti-Aging Medicine

The results of this study indicate that giving cocor bebek leaves extract gel can improve neovascularisation and epithelialization so that it can significantly accelerate the wound healing process in rats' skin wounds. This is good news, considering that the wound healing process is slowing down as you age. In fact, giving compounds rich in antioxidants can accelerate and even improve wound conditions.

Aging is defined as a process of decline in organ function that occurs over time. One of them is the symptom of aging, which causes the effectiveness of the healing process in wounds to decrease, thereby disrupting the course of the wound healing process that is experienced.⁹

Thus, giving cocor bebek leaves extract gel can be the first step in anti aging medicine in accelerating wound healing in someone who has experienced aging, which of course still requires further research so that it can actually be used in humans. The faster wound healing process in the aging process will certainly improve the quality of life of the sufferer, reduce the risk of scarring, chronic wounds and tissue death.

Cocor bebek leaves extract gel (*Kalanchoe Pinnata*) contains flavonoids, tannins, saponins which in general can increase neovascularization, the number of fibroblast cells and increase epithelialization, so in general it can accelerate the wound healing process.

Flavonoid content of cocor bebek leaves extract as much as 2523.15 mg / 100g, with a total phenol of 1391.12 mg / 100g GAE, tannin content of 1519.80 mg / 100g TAE, antioxidant capacity 279.32 mg / L GAEAC with 50 Inhibition Capacity % 2130,7346 ppm, saponin content (+), alkaloid content (-). When compared with other extracts that have been studied, namely binahong leaves extract 40% with a flavonoid content of 11.23 mg / kg, an antioxidant capacity of 3.68 mmol / 100mg, alkaloids 85.583 ppm can

heal wounds quickly but until day 21, the process healing is not normal up to 100%.¹⁰ Previously carried out research on wound healing by giving acacia leaves extract with a flavonoid content of 5868.62 mg / 100g, an antioxidant capacity of 171401.44 mg / L GAEAC, 50% Inhibitor Capacity of 15.51 mg / dl can accelerate the wound healing process, it's just that The number of fibroblasts and neovascular cells produced was lower than that of cocor bebek leaves extract.

5. Conclusion

In conclusion, the results of this study indicated that the administration of cocor bebek leaves gel (*Kalanchoe Pinnata*) 20% increased neovascularization, number of fibroblast cells and epithelization in the wound healing process of male wistar rats (*Rattus Novergicus*).

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