https://www.jurnal-umbuton.ac.id/index.php/Pencerah
https://doi.org/10.35326/pencerah.v8i4.3007

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SANG PENCERAH
Jurnal Ilmiah Universitas Muhammadiyah Buton

E-ISSN: 2655-2906, P-ISSN: 2460-5697 Volume 9, No 2, Year 2023

Comparison of Propolis Extracts and Bioplacenton at Epidermal Re-epithelialization Process in Burn Wound of Mice (*Mus musculus*)

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Article Info

Received 06 *January 2023*

Approved 10 *April* 2023

Published 04 *May* 2023

Keywords:
Burn, Epidermis, Propolis Extract, Re-Epithelialization

Abstrak

Propolis extract is thought to stimulate the process of re-epithelialization of the epidermis. This research was an experimental study with a post-test only control group design. This study used experimental animal, male mice (Mus musculus) Swiss webster strain. Mice were grouped randomly into a control group and a treatment group. Each mouse was given treatment in the form of burn on the back. The control group was the group that was not given propolis or bioplacenton extract. The treatment group was given 5%, 10%, and bioplacenton propolis extract, respectively. Skin histopathological tissue collection was carried out on day 1, day 4, and day 21 to observe the process of re-epithelialization of the epidermis. The results showed that the means of epithelial thickness in the control group and the 5% and 10% propolis extract treatment were significantly thicker on the 21st day of observation when compared to the control group. The administration of bioplacenton showed an increase in the re-epithelialization process of the epidermis on the 4th day. The administration of 10% propolis extract was better in increasing the mean epithelial thickness on the 4th day than the group given 5% propolis extract and bioplacenton.

1. Introduction

Burn is a global public health problem due to its high mortality and morbidity. Burn mortality is over 95% in low- and middle-income countries. Death is not the

only consequence of burn. Many burn sufferers end up experiencing disability (morbidity), so that it often causes stigma and community rejection. (Smolle et al., 2017)

The problem experienced by burn patients, in addition to complications, is the long process of healing burn. The wound healing process can be divided into three phases, the inflammatory, proliferative, and maturation phases. The inflammatory phase occurs for 3-4 days. In this phase, vascular changes and cellular proliferation occur. The wound area undergoes platelet aggregation and releases serotonin, and epithelialization begins to take place. Second, the proliferative phase that lasts from the end of the inflammatory phase until day 21, where there are fibroblast proliferation, angiogenesis, and the process of epithelialization. Third, the maturation phase, which occurs from day 21 up to 2 years, in which the collagen maturation process occurs, then cellular and vascular activities decrease. The final form of this phase is the forming of a pale, thin, limp scar tissue without pain or itching. (Tottoli et al., 2020)

Drugs that are efficacious for treating wounds that have been widely known so far are silver sulfadiazine, bacitrain, and mafenide acetate, those which act as anti-microbial agents. Hydrocolloids and Hydrogels are widely used as absorptive dressings and have also been shown to accelerate the wound healing process. (Yastı et al., 2015) One of the widely used dosage forms for treating skin burn in Indonesia is Bioplacenton. Bioplacenton is a gel that can be used for wound healing. Bioplacenton contains 10% placenta extract and 0.5% neomycin sulphate. Placental extract works to trigger the formation of new tissue and neomycin sulphate prevents infection in the wound area.(Hendy & Lister, 2019)

Propolis extract has been known by the Indonesian people as a traditional medicine which contains flavonoids in the wound healing process. Research on the effect of propolis on burns has not been widely reported. Flavonoids have many benefits, including as anti-inflammatory, antimicrobial, and antioxidant. The antioxidant effect of propolis extract is shown by the content contained in flavonoids, namely caffeic acid phenethyl ester (CAPE), which is a high level antioxidant. CAPE inhibits excessive oxidative reactions of inflammatory processes and cell metabolism in wound. CAPE inhibits the release of arachidonic acid from cell membranes and inhibits the activity of COX-1 and COX-2, so that inflammatory mediators are not formed. (Oryan et al., 2018). This shows that propolis has the potential to heal burn. In this research, we compare the effect propolis and bioplacentone on burn wound healing.Propolis extract has potention to accelerate the healing of burn and the process of re-epithelialization of the epidermis. In addition, it contains caffeoylquinic acid and cinnamon acid, both of which also have antimicrobial effects. (Coelho et al., 2017) Therefore, the authors are interested in conducting research on the comparison of the use of propolis extract with bioplacenton in the process of re-epithelialization of the epidermis in burn mice (Mus musculus).

2. Methods

The design of this research was experimental study with post-test only control group design. This study used mice as experimental animals. Calculation of the minimum number of mice required referred to the Federer formula. The mice used in this study were male Swiss webster strain mice with a body weight of 20-40

grams and an age of 8-12 weeks. A total of 48 mice were used in this study which were divided into 4 groups. The control group was not given propolis or bioplacenton extract, group 1 was given 5% propolis extract, group 2 was given 10% propolis extract and group 3 was given bioplacenton ointment. The handling and treatment methods for experimental animals applied in this study had been approved by University Health Research Ethics Committee of Bengkulu University with protocol number 23/UN30.14.9/LT/2019.

The mice were from the Animal Laboratory of the Bandung Institute of Technology and kept in separate cages at the Life Sciences Learning Resource, Ruyani, Bengkulu Province. Before entering the treatment stage, all experimental animals were acclimatized for 7 days. During the experiment, the room temperature ranged from 23˚C to 27˚C. The foods and drinks were provided ad libitum. After the acclimatization process, the mice were burned. Burns were created by first shaving about 2 cm of hair around the back area to be burned. The skins were disinfected with 70% alcohol. Burns on the back of mice were made using a metal plate measuring 20 mm. After that, the mice would be given propolis or bioplacenton topically evenly on every part of the skin that was burned.

The propolis extract used in this study was pure propolis made in ointment preparations for topical use. The ointment base was adeps lanae and vaseline. The ointment formula used was a basic standard formula.(Rojczyk et al., 2020) Then, skin tissue samples were taken on day 1, day 4 and day 21. The skin samples were then stored in a small tube containing 10% formalin solution. Preparation of skin histopathological tissue was carried out at the Anatomical Pathology Laboratory of the Muhamad Yunus Regional General Hospital, Bengkulu Province. Histopathological preparations were made by staining with hematoxylin-eosin. The preparations were then read and analyzed at the Research Laboratory of the Faculty of Medicine and Health Sciences, Bengkulu University with an Olympus CX-22 binocular microscope. The images obtained would then be analyzed using Image-J software.

3. Findings and Discussions

3.1 Findings

Microscopic view of the epithelial thickness from the skin epidermis

The histopathological description of epithelial thickness of the epidermis tissue (Figure 1) showed that there was an increase in the epithelial cells thickness in the control and treatment groups on the 1st, 4th, and 21st days. An increase of epithelial thickness of epidermis tissue was also seen in the treatment group given 5%, 10% propolis or bioplacenton.

Epidermal Thickness Measurement as an Indicator of Skin Re-epithelialization Process

The results of the measurement of epithelial thickness showed that there was an increase in the epithelial thickness of the skin tissue after burn (Figure 2). In the control group, there were thickening process, namely on day 1 with a mean ± SD (5.45 + 1.69 µm), on day 4 with a mean ± SD (12.20 + 7.17 µm), and day 21 with a mean ± SD (28.44 + 3.52 µm). The same process happened in the treatment group 1 which was given 5% topical propolis extract. On the 1st day, the epithelial thickness of the post-burn skin tissue was obtained with a mean ± SD (4.83 + 0.12

µm) followed by an increase in epithelial thickness on the 4th day (10.87 ± 2.22 µm) and getting thicker on the 21st day (37.79 ± 1.78 µm).

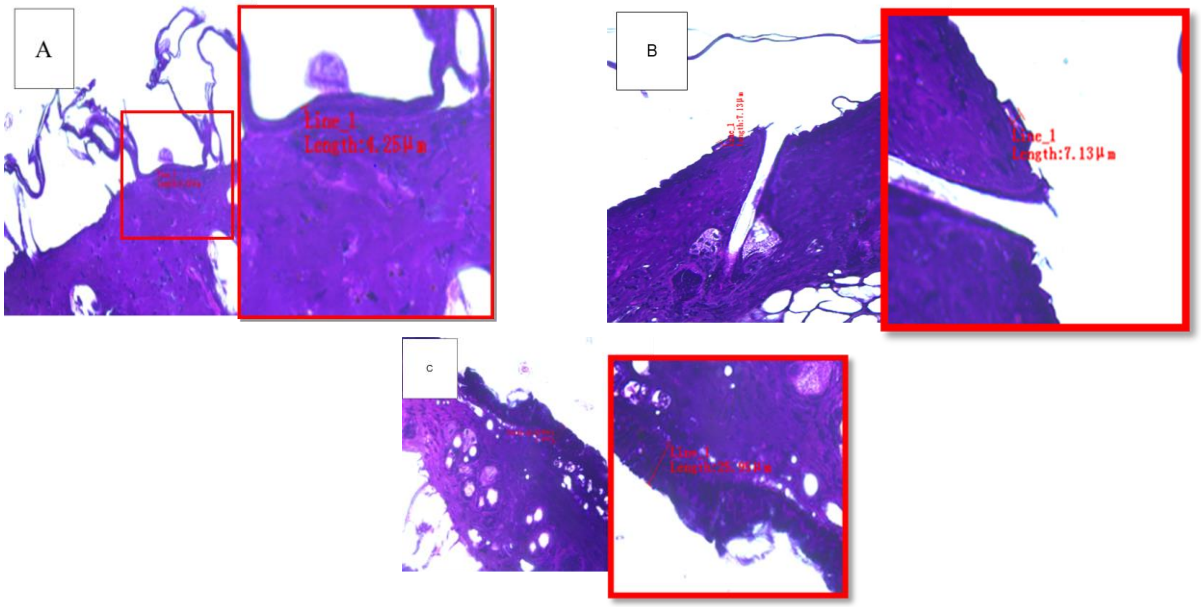

Figure 1. 1 A) Histopathological appearance of the control group on day 1; 1B) Skin Histopathological Overview of the Control Group on day 4; 1C Skin Histopathological Overview of the Control Group on day 21; Magnification 400 x (Binocular CX-22).

In treatment group 2 given 10% topical propolis extract on the 1st day, the epithelial thickness of post-burn skin tissue was obtained with a mean ± SD (5.25 ± 2.01 µm) followed by an increase in epithelial thickness on the 4th day (17.65 ± 7.55 µm) and getting thicker on day 21 (62.16 ± 0.36 µm). In treatment group 3 given bioplacenton on the 1st day, the epithelial thickness of the post-burn skin tissue was obtained with a mean ± SD (6.42 ± 1.93 µm) followed by an increase in epithelial thickness on the 4th day (14.99 ± 0.98 µm) and getting thicker on the 21st day.

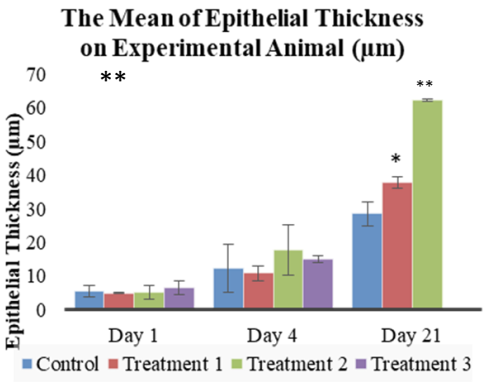

Figure 2. The mean epithelial thickness in experimental animals. Control: mice that were given burns only; Treatment 1: Burns were given 5% propolis extract; Treatment 2: Burns were given 10% propolis extract; Treatment 3: Burns given bioplacenton; *: p 0.05; ** : p 0.001

In treatment group 3 (bioplacenton), namely on the 21st day, it was obtained the value of an epithelial thickness of 30.62 µm, which was almost the same as the thickness of epithelial tissue in the control group and the 5% propolis extract group. The 10% propolis extract group was the group with the best re-epithelialization compared to other treatments in this study.The results of the post hoc LSD test to see the differences in each group showed that there was no significant difference in the thickness of epithelial cells on day 1 among the control group (P0), the 5% propolis extract treatment group (p: 0.724), 10% propolis extract (p:0.911) and bioplacenton (p:0.586). This shows that the treatment with propolis extract (55 and 10%) and bioplacenton has not been able to increase the thickness of epithelial cells on day 1.

On the 4th day, there was no significant difference in the thickness of epithelial cells in the control group (P0) when compared to the treatment group with 5% propolis extract (p: 0.815), 10% propolis extract (p: 0.366), and bioplacenton (p: 0.630). This shows that the treatment with propolis (5% and 10%) and bioplacenton has not been able to increase the thickness of epithelial cells on the 4th day. On the 21st day, there was a significant difference in the thickness of epithelial cells in the control group (P0) when compared to the 5% (p: 0.027) and 10% (p: 0.001) propolis extract treatment groups. This shows that treatment with propolis extract (5% and 10%) could increase the thickness of epithelial cells on day 21.

3.2 Discussions

Mice (Mus musculus) are usually selected as test animals because of their small size, relatively short life span and easy availability. The test animals used must come from traceable sources, of the same age, and acclimatized to the conditions of the testing laboratory first. The determination of the test should be carried out on both sexes of the test animal whenever possible. However, this research only utilized one gender, namely male, in order to avoid hormonal bias factor. The selection of experimental animals was due to the fact that mice some crucial characteristics, namely living in the laboratory, easy to handle, timid, photophobic, tended to gather with each other, having a tendency to hide, and more active at night. Their normal body temperature is 37.4˚C, then normal respiration rate is 163 per minute. (Pallas et al., 2020) During the adaptation period, the condition of the experimental animals used was quite good with adequate food and drink throughout the study period (Vadell et al., 2014). An increase of epithelial thickness of epidermis tissue shown in the treatment group given 5%, 10% propolis or bioplacenton. The results of this study were supported by other studies that examined the effect of topical administration of propolis extract on the process of re-epithelialization of the epidermis in burnt mice. (Suriawanto et al., 2021) The study showed that the re-epithelialization process took place on days 1 to 21. Epidermal matriks like metalloproteinases also support re-epithelialization during the skin healing. (Michopoulou & Rousselle, 2015) The data obtained showed that on the 1st and 4th day, there was not statistically significant among these groups, but microscopically it was seen that re-epithelialization occurred continuously from day 1 to 21. (Pradipta, 2010; Suriawanto et al., 2021). In this study, microscopic images on day 4 showed that the propolis extract group was 10% thicker than the control group and the treatment group. This shows that the administration of 10% propolis extract was

better in increasing epithelial thickness on day 4 when compared to the control, 5% propolis extract, and bioplacenton groups.

The treatment with propolis extract (55 and 10%) and bioplacenton has not been able to increase the thickness of epithelial cells on day 1. This is because on the first day the wound healing process was still in the inflammatory phase. The inflammatory phase is the body's reaction to the wound that occurs immediately after the injury, up to 3-4 days. (Turabelidze & Dipietro, 2013) In this phase, two physiological activities occur, namely hemostasis (blood clotting) and a cellular inflammatory phase. During the process of hemostasis, the broken blood vessel in the wound that causes bleeding will try to stop it by vasoconstriction, constriction of the severed blood vessel end (retraction), and a hemostatic reaction. This phase has not undergone the epithelialization process to reshape the wound surface.(Tottoli et al., 2020; Yulita, 2018)

On the 4th day, there was no significant difference in the thickness of epithelial cells in the control group (P0) when compared to the treatment group with 5% propolis extract (p: 0.815), 10% propolis extract (p: 0.366), and bioplacenton (p: 0.630). This shows that the treatment with propolis (5% and 10%) and bioplacenton has not been able to increase the thickness of epithelial cells on the 4th day. There was no significant difference because on day 4, the second stage of inflammatory process was still ongoing, in which the second stage was the cellular inflammation phase. In this case, there is a migration process of polymorphonuclear leukocytes and macrophages out of the capillaries and into the damaged area as a reaction to chemotactic agents stimulated by injury. (Mulder et al., 2021) Furthermore, leukocytes secrete hydrolytic enzymes that help in digesting bacteria and wound debris. While macrophages will engulf microorganisms and cell debris through the process of phagocytosis. In addition, macrophages will also secrete angiogenesis growth factor (AGF) which stimulates the growth of new epithelial cells and blood vessels and attracts fibroblasts, so that re-epithelialization has not occurred. (Pastar et al., 2014)

Based on the results of observations and data obtained in this study, it appeared that the process of re-epithelialization or epidermis tissue formation had occurred since day 1. The addition of epithelial formation continued on the 4th and 21st days, although the significant difference among the administration of 5% topical propolis extract, 10% topical propolis extract, and the control group was only seen on the 21st day of observation. Due to the observation of histopathological tissue, it appeared that the administration of 10% topical propolis with a frequency of 2 times in a day could increase the thickness of re-epithelialization in burnt mice compared to the control group, as seen from the size of the thicker epidermal layer. This illustrates that the administration of propolis played a role in helping the re-epithelialization process in the post-burn epidermis, especially in the proliferative phase (day 4 to day 21).

This is probably due to the high flavonoid content in propolis which had a major role in the wound healing process. Flavonoids can function as antioxidants, antimicrobials, and anti-inflammatory. In the event of injury or tissue damage, inflammatory mediators are released by neutrophils, macrophages, endothelial cells, and cells that have important vasomotor effects. Inflammatory mediators will affect platelet function and even act as cytotoxic free radicals. Propolis flavonoids are rich in vitamins and act as antioxidants and it is mentioned that propolis

antioxidants are better than vitamin E. (Oryan et al., 2018). The other natural compound like aloe vera, chitosan and virgin coconut oil also have potential in supporting re-epithelialization process in wound healing (Ashouri et al., 2019; Silalahi & Surbakti, 2015)

In addition, the content of quercetin and caffeic acid phenethyl ester (CAPE) in propolis causes an anti-inflammatory effect by inhibiting the cyclooxygenase and lipooxygenase pathways, so that arachidonic acid released by damaged cells does not turn into inflammatory mediators, such as thromboxane, prostaglandins, and leukotrienes. (Oryan et al., 2018) Based on the data obtained in the study, several supporting studies, and the various ingredients contained in propolis indicate that topical administration of propolis to burns in mice can increase the epithelial thickness of epidermis tissue.

4. Conclusion

Administration of 5% propolis extract on burns increased epithelial thickness starting on day 1, day 4 to day 21. The administration of 10% propolis extract on burns increased epithelial thickness from day 1 to day 21. Giving bioplacenton on day 4 histopathologically showed an increase in the process of epidermal re-epithelialization. Based on microscopic observations, administration of 10% propolis extract on day 4 was better in increasing epithelial thickness when compared to administration of 5% propolis extract, bioplacenton, and control.

5. Acknowledgement

Thank you for M. Yunus Hospital Bengkullu, Bhayangkara Hospital Bengkulu and Research Laboratory Faculty of Medicine and Health Sciences Bengkulu University that have support this research.

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