APPLICATION OF RED DRAGON FRUIT EXTRACT GEL ON MUCOSAL WOUNDS HEALING OF WISTAR RAT

(APLIKASI GEL EKSTRAK BUAH NAGA MERAH TERHADAP PENYEMBUHAN LUKA MUKOSA RONGGA MULUT TIKUS WISTAR)

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ABSTRACT

Oral mucosa has some functions, like protecting the oral structure. But it is susceptible to trauma that causes a mucosal wound. One of the herbals used in wound healing is red dragon fruit. Red dragon fruit contains flavonoids, alkaloids, and tannin dan saponin that play a role in wound healing. This study aimed to determine the effect of red dragon fruit on the healing of oral mucosal wounds in Wistar rats. The research method used was a post-test-only control group design by making three groups: treatment, positive and negative control. Each group was divided into three small groups based on time: 3, 5, and 7 days, so there were nine small groups with four rats each. The wounds were made on the buccal mucosa using scalpel blade no.11, the length was 10 mm, and the depth was 1-2 mm. The research data were analyzed for normality with Shapiro-Wilk, homogeneity with Levene's test, two-way ANOVA,

and LSD Post Hoc test. The results were divided into three observation groups. Group-1 Epithelial cell thickness: The highest mean value of epithelial cell thickness was in the treatment group on days 3 and 5. Group-2 number of fibroblast cells: The highest mean value of fibroblast cells: treatment group (63.22) > control group (56.22) > negative group (47.44). Group-3 Angiogenesis: The highest mean angiogenesis value was in the treatment group on days 3 and 5. It was concluded that the application of red dragon fruit (*Hylocereus polyrhizus*) affected the healing of Wistar rats (*Rattus norvegicus*) buccal mucosal wounds.

Keywords: angiogenesis; red dragon fruit; wound healing

ABSTRAK

Mukoasa rongga mulut memiliki sejumlah fungsi, salah satunya adalah sebagai proteksi struktur mulut. Namun mukoasa tersebut rentan terhadapa trauma. Salah satu bahan herbal yang dapat digunakan pada penyembuhan luka adalah buah naga merah. Buah naga merah memiliki kandungan flavonoid yang berperan pada penyembuhan luka. Tujuan dari penelitian ini adalah mengetahui pengaruh aplikasi buah naga merah (Hylocereus polyrhizus) terhadap penyembuhan luka mukosa bukal rongga mulut tikus wistar (Rattus norvegicus). Metode penelitian menggunakan post test only control group design dengan membuat 3 kelompok yaitu: perlakuan, kontrol positif dan control negative. Masing-masing kelompok dibagi lagi menjadi 3 kelompok kecil berdasarkan waktu, yaitu: 3, 5 dan 7 hari, sehingga total ada 9 kelompok kecil dengan masing-masing 4 ekor tikus. Luka dibuta pada permukaan bukal, menggunakan scalpel blade nomer 11, dengan Panjang 10mm dan kedalaman 1-2mm. Data penelitian dianalisis uji normalitas dengan Saphiro-wilk, homogenitas dengan Levene's test, dilanjutkan uji two way anova dan LSD Post Hoc. Hasil penelitian dibagi dalam 3 grup pengamatan: Grup 1 Ketebalan sel epitel, Grup 2 Jumlah sel fibroblast, Grup 3 Angiogenesis. Hasil pengamatan grup-1: Nilai rerata ketebalan sel epitel tertinggi pada kelompok perlakuan hari

ke-3 dan ke-5. Hasil pengamatan grup-2: Nilai rerata jumlah sel fibroblast tertinggi berturut-turut sebagai berikut: kelompok perlakuan (63,22) > kelompok control (56,22) > kelompok negatif (47,44). Hasil pengamatan grup-3: Nilai rerata angiogenesis tertinggi pada kelompok perlakuan pada hari ke-3 dan ke-5. Disimpulkan bahwa aplikasi buah naga merah (Hylocereus polyrhizus) berpengaruh terhadap penyembuhan luka mukosa bukal rongga mulut tikus wistar (Rattus norvegicus).

Kata kunci: *angiogenesis; buah naga merah; penyembuhan luka*

INTRODUCTION

The oral mucosa is a tissue that lines the surface of the oral cavity, which functions to protect and defend against antigens. Periodontal or gingival surgical treatment is often traumatic. Trauma can appear as a wound where the body tissue is injured or damaged. This damage can be caused by many things, including sharp objects, chemicals, animal bites, electric shocks, and so on[1]. The prevalence of injuries due to trauma in the oral cavity is relatively high, which is around 3-24% in the population, and is more common in the elderly because of the thin mucosal structure. The cause of this trauma or injury is a diagnostic procedure, surgery, or during treatment.[2,3]

The formation of a wound in the tissue is always followed by a repair or healing process. A reaction of redness, heat, or pain accompanies healing. If the wound is not healed immediately, this wound has the potential to cause complications to discomfort in the mouth area. One form of complication that arises is an infection, which increases with age. The wound-healing process in the oral mucosa has similarities with the skin, consisting of hemostasis, inflammation, proliferation, and maturation.

The main goal of wound treatment is to restore the structure and function of skin tissue and minimize complications that arise. Povidone iodine is a commonly used antimicrobial agent and is relatively safe for minor acute wounds, but there is insufficient evidence to show its effectiveness in treating chronic wounds. There is a risk of complications of contact dermatitis.

Alternative medicine is now widely offered and stated to be good for wound

healing and have low side effects. Wound healing agents derived from medicinal plants or herbs are known to be able to fight infection and accelerate wound healing.[4] One type of plant used in wound healing is red dragon fruit, a variety of cactus. Dragon fruit contains flavonoid compounds (polyphenol compounds) such as phloretin-2-O-glucoside and myricetin-3 Ogalactopyranoside.[5] Phytochemical components such as flavonoid, alkaloid, tannin, and saponin in are antimicrobial, antioxidant, and cancer chemopreventive. The pharmacological activity of flavonoids is as an anti-inflammatory and analgesic. Flavonoids affect the inflammatory phase related to growth factors in releasing receptors for Platelet-derived Growth Factor (PDGF) and Transforming Growth Factor beta (TGF-β), which induces the proliferation and migration of fibroblasts to form an extracellular matrix to accelerate wound healing.[6] In this study, the effect of dragon fruit on wound healing is in 3 ways: an increase in epithelial cell thickness, the formation of fibroblast cells, and the occurrence of angiogenesis

METHOD

This research is true experimental with a post-test-only control group design because the population is homogeneous. The population in this study were Wistar rats (*Rattus norvegicus*) obtained from the Pharmacology laboratory of the Faculty of Medicine, Udayana University. Inclusion criteria: male and healthy Wistar rats, 2-3 months old, body weight 200-250 grams, healthy, characterized by active movements. Exclusion Criteria: rats lost 10% body weight after the adaptation period, and rats died during the adaptation period. Dropout criteria: sick rats during treatment, rats died before/during treatment. Thirty-six Wistar rats were divided into three groups, namely positive control (K+), negative control (K-), and treatment group (P); then, each group was divided into three times: 3, 5, and 7 days. The red dragon fruit was dried, oven at 40°C, macerated in ethanol 70% for 48 hours, filtrated, and then evaporated using a vacuum rotary evaporator at 50°C for 2 hours. Then 0,75g dried extract was mixed with 9.25g Na CMC 2% to make 7,5% extract gel. We used local intramuscular injection for the rat using 20mg/kg ketamine-xylazine before the incision. Wounds were made on the buccal mucosa using scalpel blade number 11, with 10mm length and 1-2mm depth. The gel was applied for 1 minute, two times a day, every 8 am and 4 pm, until seven days.

RESULT

Group 1 Epithelial Cell Thickness

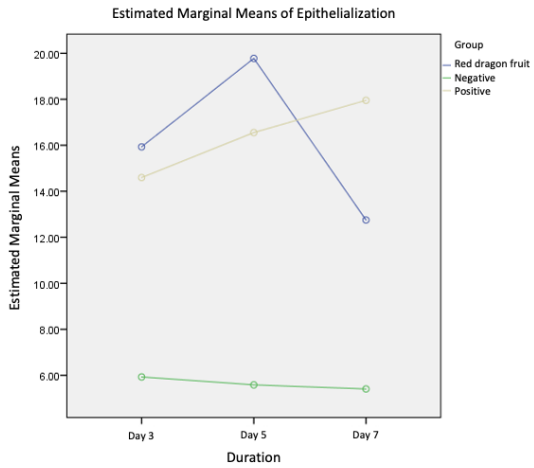

Figure 1. Graph of Epithelial Thickness on Days 3, 5, and 7 in Each Group.

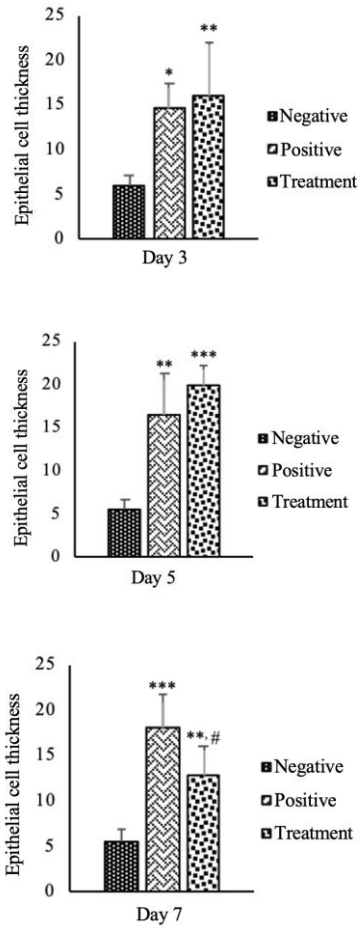

Figure 2. Graph of Average Epithelial Cell Thickness with 3, 5, and 7 Days of Administration (Thickness in m)

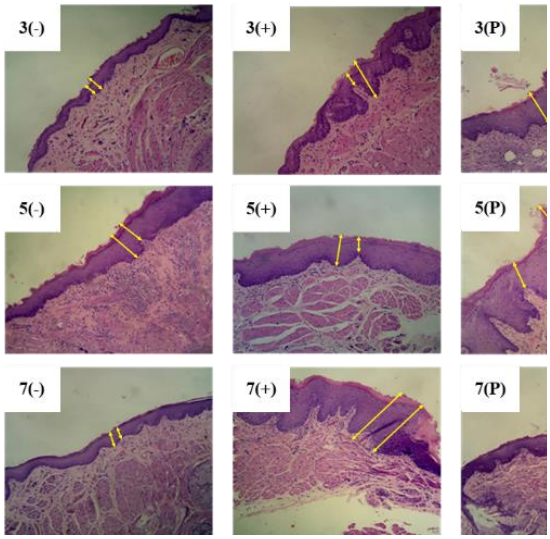

Figure 3. Epithelial Cell Thickness Histopathological Preparations with 3, 5, and 7 Days of Administration (100x Magnification) (Left Side is K(-), Middle is K(+), and Right Side is Treatment Group).

Group 2 Number of fibroblast cells

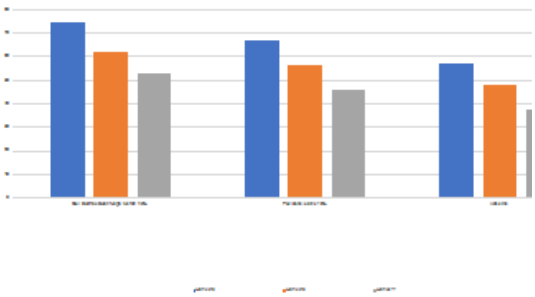

Figure 4. Graph of Average Number of Fibroblast Cells Seen from Each Treatment Group

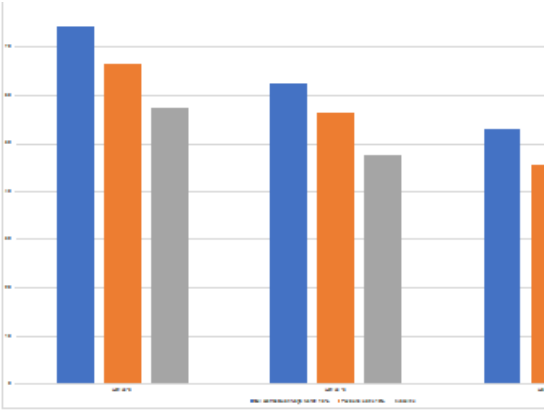

Figure 5. Graph of Average Number of Fibroblast Cells Seen from Each Day Group

The results of fibroblast cells observed on the 3rd day histopathological obtained the average fibroblast cell from the red dragon fruit extract gel (*Hylocereus polyrhizus*) treatment group 7.5% was 74.33, the povidone-iodine group was 66.66, and the vaseline group was 57.33.

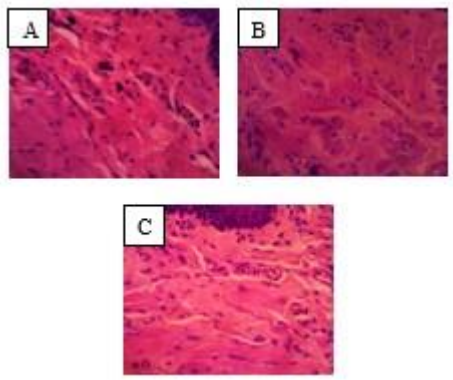

Figure 6. Microscopic Appearance of Fibroblasts in the Red Dragon Fruit (*Hylocereus polyrhizus*) (A), Povidone-Iodine (B), and Vaseline (C) Groups on Day 3 with HE Staining At 400x Magnification

The results of the observation of the number of fibroblast cells observed on day five histopathological obtained the average number of fibroblast cells in the red dragon fruit extract gel (*Hylocereus polyrhizus*) treatment group of 7.5% was 62.33, which was more significant than the group. Povidone iodine has an average number of fibroblasts at 56.33 and vaseline group with an average number of fibroblasts at 47.66.

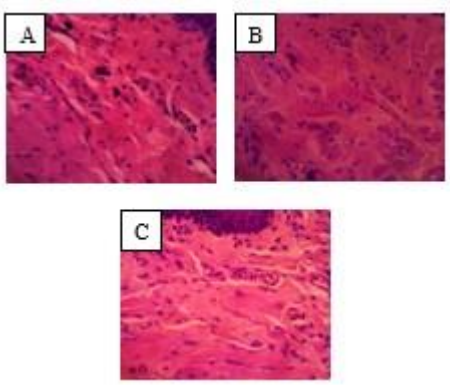

Figure 7. Microscopic Appearance Of Fibroblasts in Red Dragon Fruit (*Hylocereus polyrhizus*) (A), Povidone-Iodine (B), and Vaseline (C) Groups on Day 5 with HE Stains at 400x Magnification

The results observed on the 7th day histopathological obtained the average number of fibroblast cells in the red dragon fruit extract gel (*Hylocereus polyrhizus*) treatment group of 7.5%, and was 53, the average number of fibroblast cells in the povidone-iodine group was 45.66. The average number of fibroblasts in the vaseline group was 37.33.

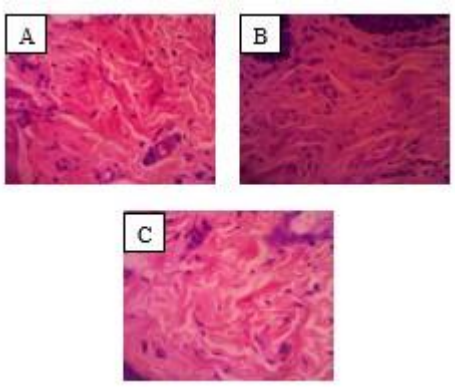

Figure 8. Microscopic Appearance of Fibroblasts in the Red Dragon Fruit (*Hylocereus polyrhizus*) (A), Povidone-Iodine (B), and Vaseline (C) Groups on Day 7 with HE Stains at 400x Magnification

Group 3 Angiogenesis

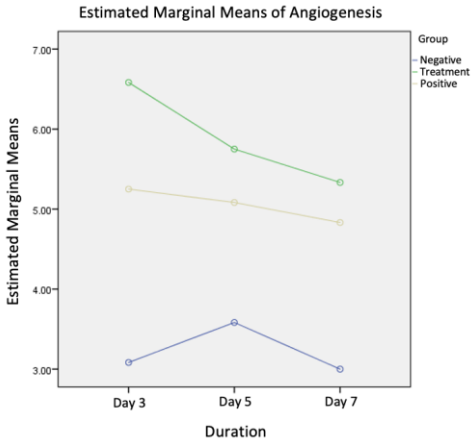

Figure 9. Graph of Total Angiogenesis between Duration (3, 5, and 7 Days) in Each Group

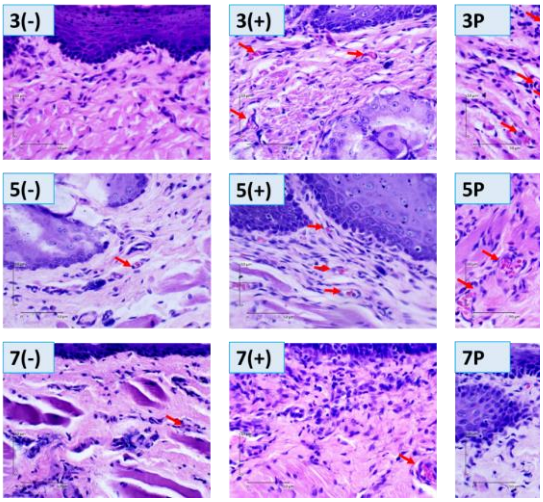

Figure 10. Histological Preparations of Total Angiogenesis with 3, 5, And 7 Days in Each Group (400x Magnification).
Description: Arrow Lines Indicate New Veins; (-)= Negative Group; (+)= Positive Group; P= Treatment Group

DISCUSSION

In this study, red dragon fruit extract concentration of 7.5% was used. The healing process of mucosal wounds can be seen from 3 aspects: epithelial cell thickness (group 1), increased number of fibroblast cells (group 2), and increased angiogenesis (group 3). The discussion will be explained according to the duration of the research day for each group.

On day 3, group 1, the treatment group had a mean epithelial thickness (15.93±5.94 m) higher than the positive or negative groups (5.927±1.21 m; 14.59±2.67 m), respectively. The treatment group also increased the average number of fibroblast cells (group 2) and angiogenesis (group 3).

It was caused by more fulfillment of the nutrients needed during the healing period. Chronic inflammatory cells such as lymphocytes and macrophages will reach maximum numbers, and tissue hypoxia will stimulate macrophages, followed by the release of growth factors to induce migration and proliferation of endothelial cells, which causes angiogenesis. The blood vessels formed will penetrate the wound fibrin matrix to create blood vessels.

On day 5 in group 1, the treatment group reached the highest point in the mean epithelial thickness (19.77±2.45 m) and statistically significant ($p<0.001$) when compared with the positive group with povidone-iodine (16.55±4.79 m) and the negative group with Vaseline (5.58±1.14 m). These results relate to the high flavonoids in red dragon fruit which accelerate wound healing by releasing pro-mitotic compounds. It included fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor-α (TGF-α), and epidermal growth factor (EGF). The formation of new capillary branches in the wound area supports faster epithelialization, so the red dragon, fruit extract treatment group reached the highest peak average thickness on day 5.

On the 5th day of observation in group 2, the number of fibroblasts in the treatment group decreased compared to the previous day's observation. It was because fibroblast cells begin to produce collagen, which will link the wound, affect the reepithelialization process, migrate and proliferate to form new connective tissue. In addition, fibroblasts also synthesize collagen, which affects the tensile strength at the wound healing site[7]. On the 3rd day after the injury, new collagen fibers have formed in the wound area, and then between the 5th and 20th day, there will be rapid collagen deposition followed by an increase in tissue strength.[8]

On day 5 in group 3, the treatment group saw an average decrease compared to day 3, but it was still higher than the other groups, namely the positive and negative groups. The cause of the reduction in the number of new blood vessels in the treatment group was that on day five, the angiogenesis process stopped after the number of new blood vessels needed was adequate, so it was already heading to the healing process.[9]

On the 7th day in group 1, the thickness of epithelial cells in the treatment group decreased. It was related to wound healing, where epithelial cells undergo maturation or remodeling as the final phase of wound healing. The stratified epithelial cells will structurally flatten, which leads to a decrease in the average thickness of the epithelium. In group 2, observing the number of fibroblast cells, the total number of fibroblasts was less from days 3 and 5.

As the wound-healing process progressed, proliferating fibroblasts and new blood vessels decreased.[9] Fibroblast cells have started to leave the granulation tissue and differentiate into myofibroblasts. Myofibroblasts are responsible for the wound contraction process that will form the strength of the scar tissue that is formed.[10] The Number of fibroblasts in the povidone-iodine group on all days was less than in the treatment group. It could be because povidone-iodine was more toxic to leukocytes and fibroblasts, resulting in the inhibition of neutrophil migration and a decrease in the number of monocytes so that the union of the wound edges could be disrupted.[18] In the 3rd group, the amount of angiogenesis in the treatment group decreased, but still higher than in the positive and negative groups. The decrease in the average number of new blood vessels on the 7th day occurred because the wound had closed and recovered, or the maturation (remodeling) stage had taken part. And on the 7th day, the wound had begun to heal, so the body began to stop forming new blood vessels. This decrease indicates that the angiogenesis formed on days 3 and 5 is considered sufficient to supply blood containing nutrients and other useful factors for healing so that the wound begins to close.[11, 12]

Red dragon fruit contains phytochemicals high in fiber, low in calories, and rich in antioxidants in flavonoids, tannins, alkaloids, steroids, saponins, Vitamin C, minerals, and chlorophyll. Red dragon fruit flesh and skin contain betalain, which has high antioxidant activity. Carotenoids, phenolics, and betalains are the most abundant compounds in tropical fruit. Polyphenols, as one of the phenolic compounds, have a total capacity for overall antioxidant activity. Phytochemical effects in the form of alkaloids, flavonoids (kaempferol and quercetin), tannins, and saponins have an essential role in wound regeneration.[12]

Flavonoid compounds in red dragon fruit extract also play an essential role in inducing the release of TGF-ß1, which causes the recruitment of inflammatory cells and induces macrophages to clean debris from dead epithelial tissue. Flavonoid compounds also cause an increase in the secretion of hydroxyproline which plays an essential role as an ingredient in collagen synthesis. Collagen in the wound healing process serves to provide strength and stabilization of other tissue structures to prevent worsening or interfere with the wound healing process.[12]

Alkaloid compounds in red dragon fruit acted as antimicrobials and antivirals. Activating the body's immune system is vital to prevent secondary infections that

inhibit wound healing. Tannin increases the proliferation and migration of fibroblasts to the wound area. as a wound closure in the acute phase and modulates the proliferation and differentiation of fibroblasts. It provides strength (tensile strength) through synthesizing collagen, extracellular matrix, and differentiation into myofibroblasts in the wound area and forming skeletal tissue. The skeletal tissue helped to mediate the migration of epithelial progenitor cells to the wound area, thereby helping accelerate reepithelialization.[12]

Saponin increases nutrient supply to the wound area through angiogenesis, thus helping the reepithelialization process. The formation of new blood vessel branches (angiogenesis) is related to the cytokine HIF-1α as a transcription factor in the shape of VEGF, which plays a role in inducing vascular endothelial differentiation to form new capillary branching. Research using asperosaponin VI showed increased activation of the HIF-1α/VEGF pathway, thereby inducing angiogenesis.[12]

CONCLUSION

Red dragon fruit *(Hylocereus polyhizuz)* application affected the healing process of the buccal mucosa of the oral cavity on Wistar rats (*Rattus norvegicus*) in terms of increased epithelial cell thickness, fibroblast cell count, and angiogenesis.

CONFLICT OF INTEREST

We declare no potential conflict of interest in the scientific articles we write.

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