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Topical virgin red palm oil improved wound healing by increasing fibroblasts, neovascularization and epithelialization in male Wistar rats (*Rattus norvegicus*)

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Background: The healing process of burns wound can be accelerated using natural compound and one example of alternative medicine is virgin red palm Oil (VRPO). However, scientific evidence that back this notion is still lacking. Therefore, this study aimed to assess the effect VRPO toward burn wound healing in male wistar rats (*Rattus norvegicus*).
Methods: An experimental randomized post-test only control group study was conducted using 28 male Wistar rats. Burn wound was created in all rats but only treatment group received VRPO while the control group was treated with placebo cream. Skin sample was obtained from each rat at day 4 and 11 for histological examination (fibroblasts density, neovascularization, and epithelialization).
Results: The results showed that VRPO improved fibroblast density, neovascularization, and epithelialization at day 4 compared to control group. However, at day 11, there was a decreased in fibroblast density and neovascularization while epithelialization continuously increased at double the rate of the control group.
Conclusion: Topical VRPO improved burn wound healing by increasing wound healing speed and epithelialization.

Keywords: Virgin Red Palm Oil (VRPO), fibroblasts, neovascularization, epithelialization

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INTRODUCTION

Management of burn wound focused on infection prevention and epithelial regeneration. There are three phases of wound healing, namely the inflammatory phase (2-3 days), proliferation or fibroplasia phase (3 days - 3 weeks) and remodeling phase (21 days - 1 year).[1] However, some natural products have been shown to enhance this process which potentially reduced medical burden to the patients.

One of such natural products is Virgin red palm oil (VRPO). VRPO has high level of carotenoids, lycopene, vitamin E, squalene, CoQ10, omega 9, omega 6, omega 3, and sterol which all can work simultaneously in enhancing wound healing. Carotenoids act as free radical scavenger while vitamin A and E support their anti-oxidant effect by modulating inflammation, improve fibroblast proliferation, increase collagen and hyaluronic synthesis and reduce MMP expression.[2,3] In addition, antioxidant also modulates the activity of neutrophils and macrophages in wound healing.[4] Vitamin E can also reduce the formation of scar tissue in chronic wounds.[5]

VRPO contains almost equal amounts of saturated and unsaturated fatty acids. Palmitic and oleic acids are most common fatty acid components in VRPO which increase skin moisture and increase the speed of normal wound healing process. The antibacterial properties of fatty acids prevent the colonization of pathogenic bacteria and secondary infections that can be dangerous at the time of injury.[6] Fatty acids also have immunomodulatory effects which accelerate wound closure, basement membrane formation, and epithelialization.[7] The immunomodulatory effect is achieved by downregulation of cyclooxygenase 2 (COX-2) and upregulation of type III collagen expression. In addition, they also modulate the level of inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10) and IL-17 mRNA (Cardoso *et al*., 2011; Alexander and Supp, 2014).[8,9]

Therefore, this study aimed to assess the effect of VRPO toward burn wound healing in male Wistar rats.

METHODS

This study was an experimental study using

randomized post-test only control group design. Subjects used in this study were 28 male Wistar rats, aged 3-3.5 month old and weighed 300-350 gram. Burns wound was made by shaving the back skin of the rat 2 x 2 cm (4 cm2 <10% of Total Body Surface Area) using iron plate that was heated in the boiling water (100°C). The plate was attached for 10 seconds until grade 2B burn was achieved.[9]

All rats were randomly divided into divided into four groups, namely two control groups which were treated with placebo cream and assessed at day 4 and 11 and two treatment groups which were treated with topical VRPO and evaluated at day 4 and 11. VRPO used in this study was Salmira® while vaseline was used as placebo.

At day 4 and 11 of treatment, the skin samples were collected at the burn site and examined histopathologically by using routine HE staining. The number of fibroblast cells, neovascularization, and epithelialization (thickness of the epidermal epithelium) was assessed under light microscope.

RESULTS

Histopathological examination showed that VRPO improved burn wound healing through three mechanisms. Regarding the fibroblast density, the treatment group had significantly higher fibroblast density at day 4 (treatment: 16.36 ± 1.06; control: 7.83 ±0.99; p< 0.01). However, fibroblast density was reversed at day 11. At this time, the mean fibroblast density in treatment group was 4.71 ± 0.39 while in control group, it was 6.89 ± 0.43 (p< 0.01) (Figure 1).

The effect of VRPO in neovascularization was also assessed and yielded an interesting result, similar to fibroblast. Initially, the average neovascularization in the treatment group at day 4 (P1(4)) was significantly higher than the control group (P1(4):18.78 ± 1.99 vs. P0(4): 2.43 ± 0.60; p<0.001). However, it was reversed at day 11 where the neovascularization was significantly higher in control group (P0(11)) compared to treatment group (P1(11)) (P0(11): 2.51 ± 0.32 vs. P1(11): 1.41 ± 0.38; p<0.001) (Figure 2).

Finally, the epidermal thickness was assessed to supplement the information obtained from fibroblast density and neovascularization. In contrary with fibroblast density and neovascularization, epithelial thickness was continuously increased both in controland treatment group. The difference could already be seen from day 4 (P0(4): 12.82 ± 3.15 µm vs. P1(4): 29.27 ± 9.47 µm; p<0.001) and continued to day 11 (P0(11): 25.12 ± 3.97 µm vs. P1(11): 66.83 ± 6.79 µm; p<0.001). At day 11, the epithelial thickness in treatment group was more than twice the thickness of control group and increased two times thicker compared to day 4 (Figure 3).

DISCUSSION

The results of this study indicated that VRPO has a positive effect in accelerating burn wound healing process. The finding in fibroblast density, neovascularization and epithelialization supported the hypothesis and can be explained using current understanding in wound healing process. In burn wound healing process, fibroblast begin to proliferate from day 3 after injury.[10] This process is accelerated by VRPO because it contains omega-3 or α-Linolenic Acid (ALA) that enhance fibroblasts migration to the injured areas.[11] In line with the formation of new connective tissue, some fibroblasts undergo phenotypic changes to actin-rich miofibroblast. Fibroblasts will stop producing collagen if type III collagen synthesis reaches its peak and granulation tissue is replaced by acellular scar tissue which leads to the decreased number of fibroblasts after day 7. Fibroblasts will then undergo apoptosis which explain why their number decreased at day 11.[12]

The Omega 6-containing in VRPO also enhance proinflammatory cytokines and growth factors production in a positive way to activate cell

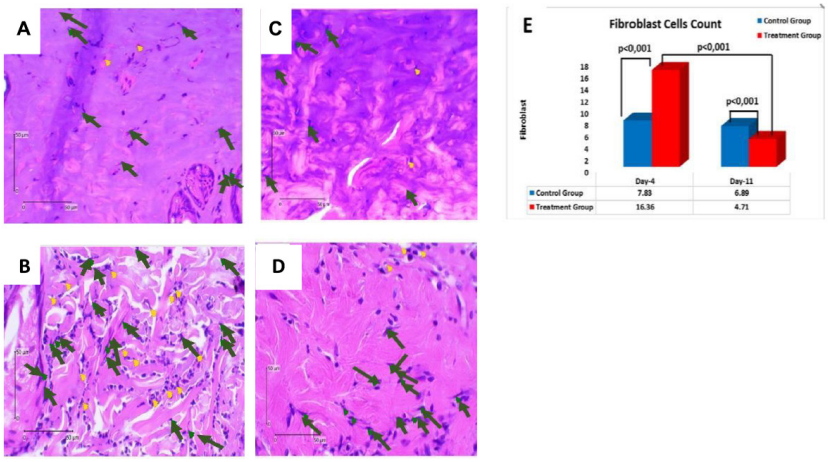

Figure 1. Histopathology assessment of dermal fibroblast. (A) Fibroblast density of the control group at day 4, (B) Fibroblast density of the treatment group at day 4, (C) Fibroblast density of the control group at day 11, (D) Fibroblast density of the treatment group at day 11, (E) Comparison and statistical analysis of fibroblast density between groups and evaluation times. (HE Staining, 400x magnification)

migration, proliferation, angiogenesis, and collagen deposition which ultimately accelerate wound closure. Topical applications of omega 9 also increase the levels of EGF and VEGF.[13] In addition, carotenoids inhibit MMP (Burgess, 2008) and increase TGF-β which consequently enhance NO levels.[3,14] Fatty acid content, especially Linoleic Acid (LA) and Oleic Acid (OA) in VRPO increase total cell migration through the wound during the repair process, facilitating the entry of growth factors into cells.[15,16]

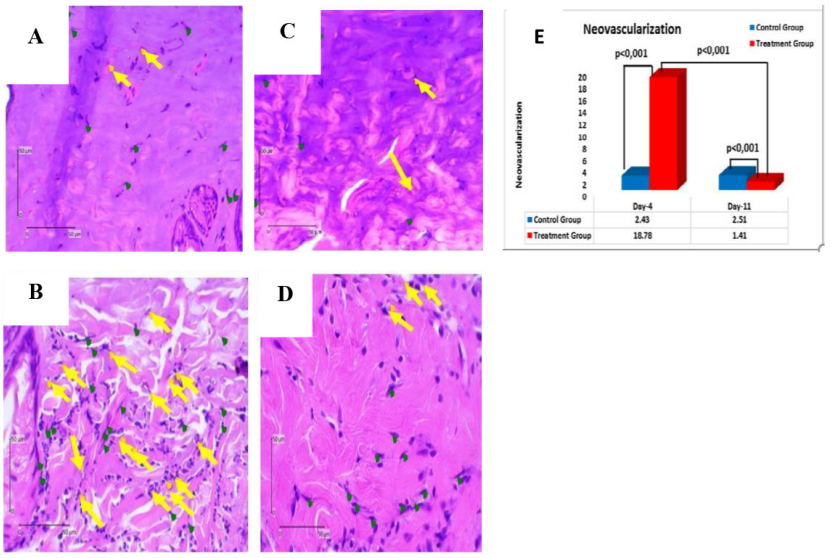

Figure 2. Histopathology of neovascularization. (A) Neovascularization of the control group at day 4, (B) Neovascularization of the treatment group at day 4, (C) Neovascularization of the control group at day 11th of examination, (D) Neovascularization of the treatment group at day 11, (E) Statistical analysis of neovascularization between the two periods and groups. (HE Staining, 400x magnification)

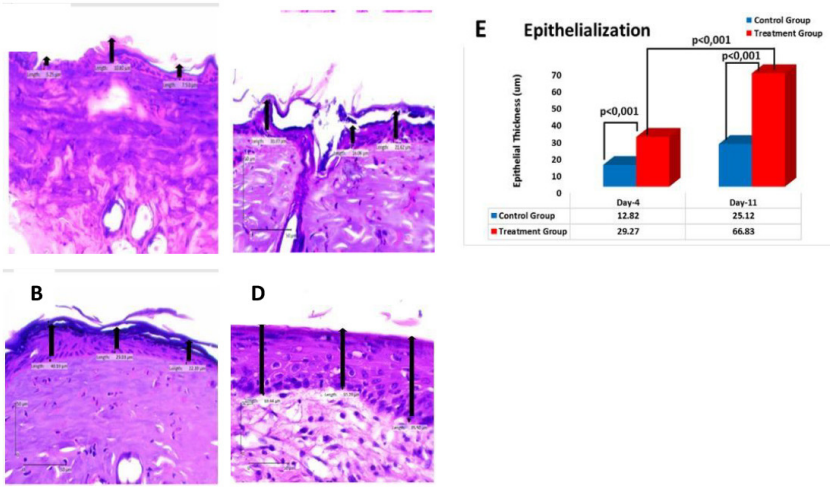

Figure 3. Histopathology of epithelialization. (A) Epithelial Thickness of the control group at day 4, (B) Epithelial Thickness of the treatment group at day 4**.** (C) Epithelial Thickness of the control group at day 11, (D) Epithelial Thickness of the treatment group at day 11, (E) Comparison and statistical analysis epithelial thickness between groups and evaluation times, (HE Staining, 400x magnification)

Regarding the re-epithelialization process, VRPO supports epithelialization due to its trienols which have potency to enhance proliferation and migration of epithelial cells.[17] These results are also supported by the Zampieri *et al* (2010), who stated that topical treatment of vitamin E enhanced skin moisture and epithelialization in all ages.[18]

In general, the results of this study indicate that topical administration of VRPO can accelerate the wound healing process. It is assumed that these effects are resulted from phytochemical content in VRPO which contains several important fatty acids or their derivatives. Some immunomodulators fatty acids such as α-Linolenic Acid (ALA) and Linoleic Acid (LA) modulate inflammatory reaction by balancing each other effect so there will be no excessive inflammatory reation.[13] Additionally, the oil itself acts as a barrier for microbial invasion which prevent infection during wound healing.[19] Furthermore, omega-3 or α-Linolenic Acid (ALA) in VRPO improve wound healing process by increasing fibroblasts migration to injured areas.[11]

On the other hand, tocopherol and carotenoids also prevent and protect injured or regenerated cells against oxidative damage from free radicals. Thus, these substances prevent excessive apoptosis or necrosis from injured cells. In addition, omega-6 or Linoleic Acid (LA) also accelerates wound closure especially after the 7th day of injury.[19]

CONCLUSION

The topical VRPO improved burn wound healing process in male Wistar mice by accelerating healing phases and improved epithelialization. Further study is needed to validate these findings by evaluating inflammatory cytokines and collagen density.

CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication

AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

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This study was self-funded without any contribution from third party.

ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Udayana University with approval letter number 417/KE-PH-Lit-2/VII/2019

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