Medica Hospitalia
Journal of Clinical Medicine
Med Hosp 2021; vol 8 (3) : 273–280

Original Article

The Effectivity of Hydrolysed VCO for 2nd Degree Burn Injury on Wistar Rats : Based on VEGF Expression and Collagen Thickness

Fahmi Syarif[1], Neni Susilaningsih[2], Yuriz Bakhtiar[3]

[1]Department of Surgery, Faculty of Medicine, Diponegoro University
[2]Histology Section of the Medical Faculty of Diponegoro University
[3]Section of Neurosurgery, Department of Surgery, Faculty of Medicine, Diponegoro University

p-ISSN: 2301-4369 e-ISSN: 2685-7898
https://doi.org/10.36408/mhjcm.v8i3.561

Accepted: April 30th, 2021
Approved: August 11th, 2021

Author Affiliation:
Department of Surgery,
Faculty of Medicine,
Diponegoro University

Author Correspondence:
Fahmi Syarif
Prof. H. Soedarto, S.H. Street, Tembalang,
Semarang, Central Java50725,
Indonesia

Email Address:
dr.fahmisyarif@gmail.com

Abstract

Latar belakang : Burn wounds remain a serious problem in several countries. Silver sulfadiazine cream is commonly used as a burn therapy. Long–time use, however, might cause negative side effects. Several alternatives with better safety profile, including virgin coconut oil (VCO), are opted. VCO contains lauric acid, polyphenol, and alpha-tocopherol and can serve as an alternative as it increases Vascular Endothelial Growth Factor (VEGF) expression and collagen thickness in the burn wound healing phase. This study was conducted to evaluate the effectivity of hydrolysed VCO cream on vascular endothelial growth factor (VEGF) expression and collagen thickness for healing 2nd degree burn injury in wistar rats

Methods : This is arandomised controlled trial with post-tests only involving 36 male Wistar rats induced to a second–degree burn in 6 paralled groups.Rats in the groups I & IV were treated with 70% hydrolysed VCO, groups II & V were treated with 100% hydrolysed VCO, and groups III & VI as control groups were treated with base cream. The effects of hydrolysed VCO and base cream on second–degree burn healing indicated by VEGF expression and collagen thickness were measured between 6 and 12 days of treatment.

Results : The highest VEGF expression was achieved in the group treated with 70% hydrolysed VCO after 12 days (23.52 ± 14.72). Both control groups terminated after 6 dan 12 days (6.67 ± 7.32 and 2.90 ± 0.90 respectively) showed the lowest VEGF expression. The application of hydrolysed 70% and 100%VCO cream did not significantly increase collagen thickness on day 6 (6.38 ± 3.29, 9.87 ± 3.95) and 12 (15.63 ± 3.00, 17.85± 4.94) compared to control.

Conclusion : 70% and 100% hydrolyzed VCO is more effective in treating second–degree burns than base cream, determined from VEGF and collagen thickness.

Keywords : hydrolysed VCO, Second-degree burns, VEGF, Thickening of Collagen, Healing of burns in rats

INTRODUCTION

Wound can be defined as the loss or damage of some tissue caused by trauma to sharp or blunt objects, temperature changes, chemicals, electric shocks, or animal bites.[1] Thermal, chemical, and electrical trauma are the causes of burns.[2,3] With high morbidity and mortality rate, burns remain a concern as they may cause a long–term disability, affecting patient's psychology and economy.[4]

Burn management depends on wound classification.[5] Difference in wound depth and width requires different care.[6] Burns can be classified based on wound depth as the first–degree burn damages epidermal layer, the second–degree burn affects some parts of dermal layer, and the third–degree burn or full-thickness burn wound affects the entire dermis layer.[7,8] The second–degree burn wound involves the loss of the dermis layer including peripheral nerves which can be very painful.[7] Immediate care is needed to prevent further tissue damage.[9]

Wound healing process occurs in three consecutive phases involving inflammatory, proliferation and remodeling phases.[10] Vascular Endothelial Growth Factor (VEGF) is a significant factor in the inflammation phase that takes place immediately after an injury.[11] VEGF stimulates angiogenesis reducing tissue hypoxia and metabolism deficiency.[11] Collagen also played an important role in wound healing.[12] Hemostasis that occurs in the first phase of wound healing relies on collagen ashemostatic agent.[12] Thrombocytes adheres to collagen, causing collagen to swell and release substances that induce hemostasis.[12] Remodeling phase also relies on collagen.[11,13]

Topical Silver sulfadiazine is a commonly used drug for a second–degree burn.[14] It can prevent and treat infection, but long–term use is not recommended as side effects involving nephrotoxicity, leukopenia, antibiotic resistance, allergic reaction, and delayed wound healing may occur.[14] Several studies found alternatives with better safety profiles such as VCO for management of burn wounds.[14]

Coconut oil, known as Virgin coconut oil (VCO) is a product of the plant *Cocos nucifera*, commonly found in South East Asia, and islands around the Indian and Pacific Ocean.[15,16] It is deemed safe for oral consumption or external use.[16] VCO contains lauric acid, polyphenol, and alpha–tocopherol.[16] VCO exhibits anti-oxidant, anti-thrombotic, hypolipidemic, anti-bacterial, anti-dermatophyte, anti-viral, and immunostimulant abilities.[16] Hydrolysis in VCO helps to break down triglyceride chain into monoglyceride and produces free fatty acid.[17] The main fatty acid in hydrolyzed VCO is lauric acid and monolaurin.[18] These fatty acids and monoglyceride components can suppress virus and bacteria growth by destroying virus structure and bacteria cell wall, thus promoting wound healing.[19]

In the present study, the effectiveness of hydrolyzed VCO on healing second–degree burn will be analyzed based on VGEF expression and collagen thickness.

METHODS

The study was conducted on November 2019 included 36 Wistar male rats aged 8–10 weeks and weighed 100 ± 50 grams each. Rats were housed in individually ventilated cages with 12/12–hour light/dark cycles, food and water *ad libitum*. A second–degree burn using a 50 g stainless–steel rod (10 mm diameters) was induced to all rats. The rats were randomized and divided into six equal groups, each containing 6 rats. Each of the group was given different treatment. Group I & IV rats were treated with 70% hydrolysed VCO, group II & V rats were treated with 100% hydrolysed VCO, group III & VI rats serve as control group treated with base cream. VCO was given once every day with 1–2 mm thickness on the second-degree burn wound area after cleaning the wound with normal saline and sterile gauze pad. VCO was obtained from PT. Victoria Care Indonesia. To obtain hydrolysed VCO, 50 grams of VCO was mixed with 70% or 100% ethanol NaOH to hydrolysed the VCO.

The group I–III were terminated after 6 days of treatment while group IV–VI were terminated after 12 days of treatment. Samples were harvested, then immunohistochemistry staining was done to measure VEGF levels using Allerd score. Collagen thickness was observed using Sirius and Picro Sirius red stain kit (Leica) measured based on fraction area percentage using imageJ application by principal investigator.

Statistical analysis was done using SPSS 25.0 software. Data normality was assessed using Shapiro-Wilk test. Normally distributed data were analyzed using One Way Anova Test, continued with Post–hoc test. Abnormally distributed data was analysed using Kruskal Wallis test, continued with Mann–Whitney test. Statistical significance was set as $p<0.05\%$ with 95% of confidence interval.

This study has been reviewed and approved by Ethical Committee of Faculty of Medicine Diponegoro University. Experiment was conducted in Animal Laboratory of LPPT UGM. Immunohistochemistry staining was done in Anatomical Pathology Lab of Universitas Negeri Sebelas Maret Solo. Sirius red staining and histopathology examination were done in Anatomical Pathology Lab of UNISSULA Semarang.

RESULT

Consort Diagram showed on Figure 1.

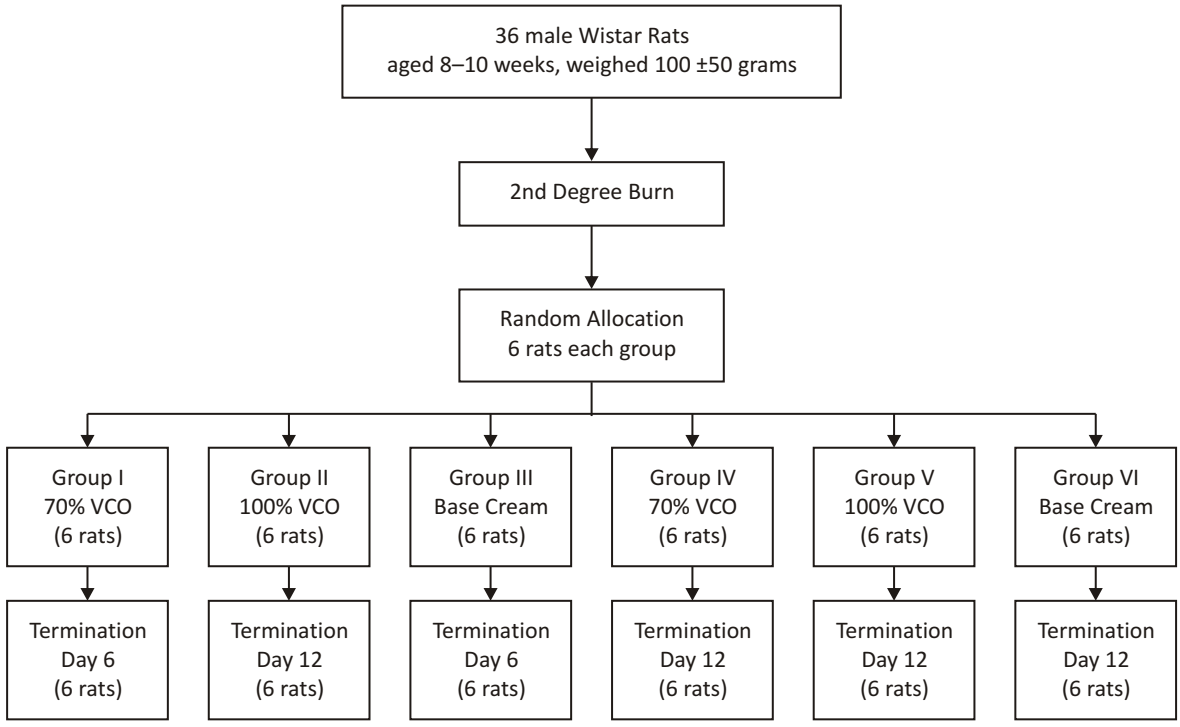

Figure 1. Consort Diagram

VEGF Expression

Through immunohistochemistry staining, expression of VEGF was measured. The highest VEGF expression was achieved in group treated with 70% hydrolysed VCO and terminated after 12 days (23.52 ± 14.72), while both control groups treated with base cream and terminated after 6 dan 12 days (6.67 ± 7.32; 2.90 ± 0.90) showed lowest VEGF expression. (Table 1)

As shown in Table 2, no significant difference were found between group 1,2, and 3, (*p*-value >0,05), while group 4, 5, and 6 shown significant difference (*p*-value <0,05). The ANOVA test in group IV, V, and VI followed by a T–independent test, showing the increase in VEGF levels in second–degree burns tended to be higher groups that received 70% and 100% hydrolysed VCO cream compared to groups that received base cream on day 12. As shown in table 3, there is no significant differences on VEGF expression between group receiving 70% hydrolysed VCO and 100% hydrolysed VCO (*p*-value =0,488).

Based on Fig 2, there was a significant difference in the VEGF level between groups administered hydrolysed VCO cream on day 6 and 12. The groups with the hydrolysed VCO cream on day 12 showed higher VEGF levels than those on-day 6.

Collagen thickness in wound tissue was stained with Sirius red staining. The area fraction was measured with the ImageJ application (fig 3). The application of hydrolysed VCO cream (70% and 100%) on the 6th (group I and II) and 12th days (group IV and V) increased collagen

TABLE 1
VEGF Expression

Group	Mean ± SD	*p*-value
I 70% VCO 6th day	10,80 ± 9,05	0,205*
II 100% VCO 6th day	12,30 ± 8,40	0,508*
III placebo 6th day	6,67 ± 7,32	0,040
IV 70% VCO 12th day	23,52 ± 14,72	0,607*
V 100% VCO 12th day	18,28 ± 10,10	0,832*
VI Placebo 12th day	2,90 ± 0,90	0,072*

TABLE 2
Kruskal Wallis in VGEF Expression

Group	Mean ± SD	*p*
I	2,75 ± 0,61	0,368
II	2,67 ± 5,16	
III	1,50 ± 0,63	

*Significant (*p* <0,05)

TABLE 3
One Way ANOVA test in VEGF Expression

Group	Mean ± SD	*p*	*Levene Test*
IV	23,52 ± 14,72	0,009*	0,006
V	18,28 ± 10,10		
VI	2,90 ± 0,90		

*Significant (p <0,05); **Homogen (p >0,05)

TABLE 4
VEGF expression differences between two groups

Group	*p*
I – II	0,773§
I – III	0,150‡
II – III	0,337‡
IV – V	0,488§
IV – VI	0,019§*
V – VI	0,013§*
I – IV	0,102§
II – V	0,291§
III – VI	0,873‡

*Significant (p <0,05); §*Independent t*; ‡*Mann Whitney*

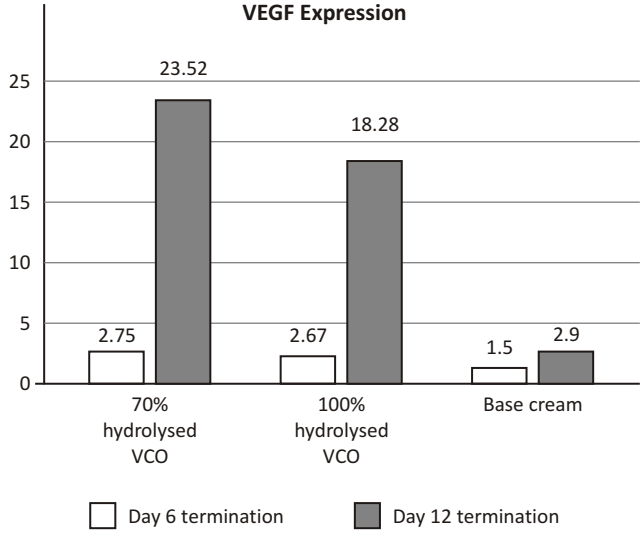

Figure 2. VGEF Expression Chart

thickness higher than base cream. However, no significant difference was found between group I, II and III (p > 0.05), and group IV, V and VI (p>0.05).

The application of hydrolysed VCO cream on the second degree burn wounds increases collagen thickness in each group, however, this increase is not statistically significant.

DISCUSSION

In this study, hydrolysed VCO is more effective comparedto base creamin healing second–degree wound based on VEGF expression and collagen thickness. The increase in VEGF levels occurs due to compounds in hydrolysed VCO that played an important role in wound healing, such as tocopherols, phytosterol or polyphenols, and single–chain fatty acids.[15] Polyphenols increase levels of ROS which induces VEGF expression.[20] VEGF itself is the main stimulants for angiogenesis and vasculogenesis.[11] VEGF also potentiates microvascular hyperpermeability, which can precede or co–exist with angiogenesis.[10]

In this study, the VEGF expression on day 6 was higher in the group of 70% hydrolysed VCO cream compared withthe two other groups. Similar findings also occur on day 12 of treatment. This could be due to the content of single–chain fatty acids in VCO and the application of hydrolysed VCO cream to condition the wound tissue at optimal humidity for the formation of new cells.[21] Aside from aiding the wound healing process, single–chain fatty acids also play a role in preventing bacterial invasion.[22]

Fibroblasts proliferate and produce matrix proteins such as fibronectin, hyaluronic acid, collagen, and proteoglycans.[23] Fibroblasts also form an extracellular matrix that assists migration of keratinocyte cells.[23] New blood vessels, macrophages together with fibroblasts will compose a granulation tissue that is bound by a matrix protein.[24] This phase lasts on the 4th or 5th day until the 21st day of wound healing.[25] Angiogenesis in group treated with 70% hydrolysed VCO cream showed the highest number of new blood vessels on day 12 compared to group treated with 100% hydrolysed VCO cream and control group. Angiogenesis, collagen deposits, and granulation tissue formation are important processes that occur in this phase.[9,22,23] Research conducted by Ibrahim (2017) regarding VCO and VEGF explained similar findings where VCO played a role in wound healing and angiogenesis, bimolecularly mediated by the regulation of the VEGF entry route.[21]

Collagen also plays a very important role in the wound healing process.[27] The protein substance in collagen helps increasing the surface tension of wounds.[9] Collagen also has an important role as a hemostatic agent, as platelets adhere to collagen, causing the release of substances needed to initiate the hemostasis process.[10] Collagen–platelet interactions depend on the degree of polymerization of collagen maturation and its positive effect on collagen molecule.[11,17,25]

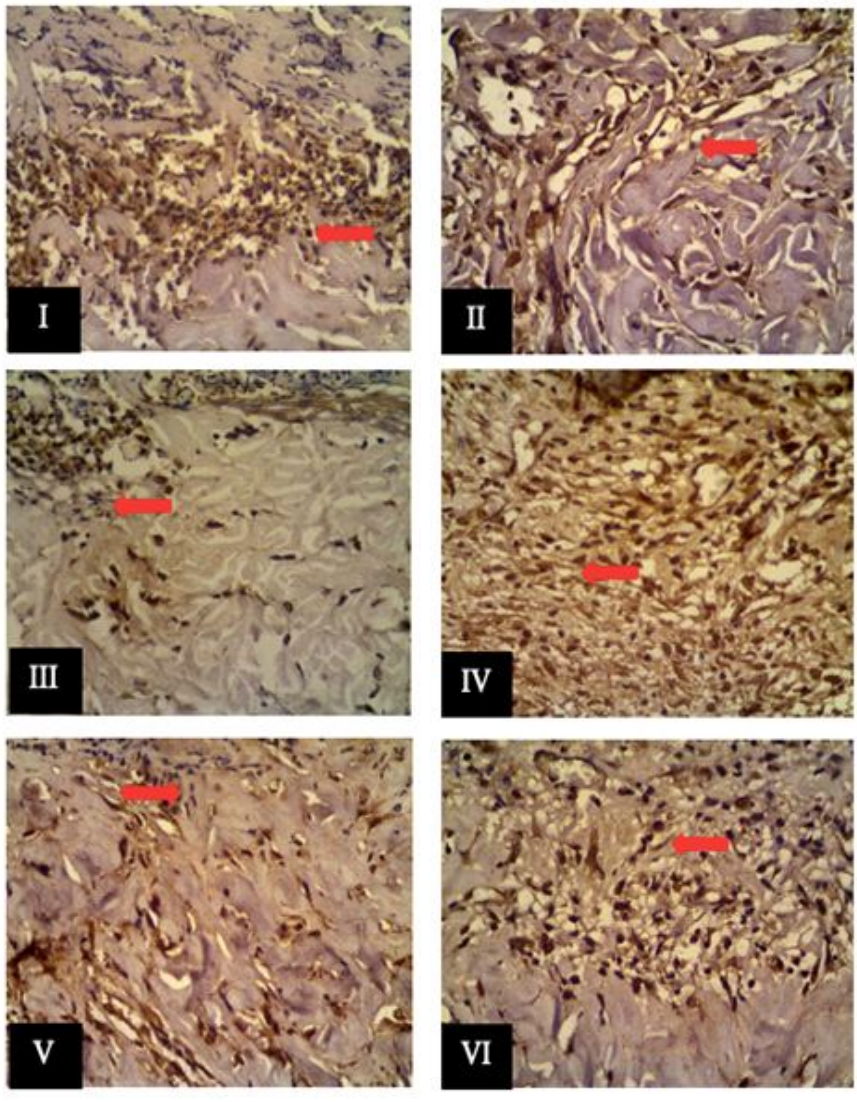

Figure 3. VEGF distribution in wound tissue histology preparations with 400x magnification in six groups. Histological preparations are made by immunohistochemical staining.

The exact mechanism of collagen interactions remains unclear,but previous studies show the first stage of wound healing process, namely the collagen and platelet interactions, is hemostasis, followed byvasoconstriction and vasodilation.12During vasodilation, the non-traumatic area becomes more permeable.17 A flow of hormones, plasma proteins,electrolytes, antibodies, fluids, and PMN leukocytes occurs.27 At the site of trauma, rapid accumulation of PMN leukocytes and macrophages takes place.27 Collagen has chemotaxis properties against monocytes.27 Monocytes such as macrophages function in phagocytosis of bacteria in the wound area and clean debris.27 Macrophages will attract fibroblasts to the wound site and collagen synthesis begins.27The delay in wound failure is due to the decreased number of macrophages.28

TABLE 5
Kruskall Wallis and One-Way Anova Testin Collagen Fraction

Group	Mean ± SD	*p*	*Levene Test*
I	6,38 ± 3,29	0,066¥	0,261**
II	9,87 ± 3,95		
II	5,43 ± 1,94		
IV	15,63 ± 3,00	0,778£	
V	17,85 ± 4,94		
VI	15,02 ± 2,32		

*Significant (p <0,05); **Homogen (p>0,05); ¥One Way ANOVA; £Kruskal Wallis

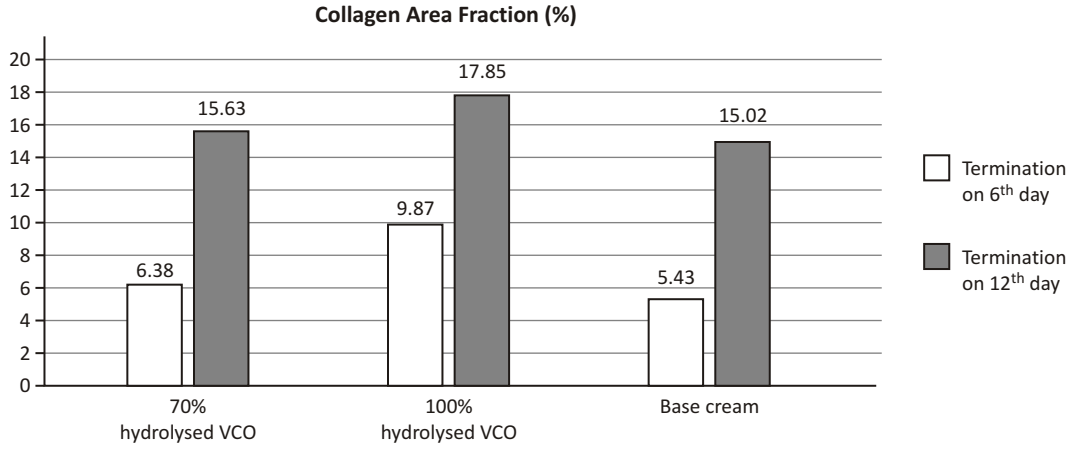

Figure 4. Collagen Area Fraction Graph

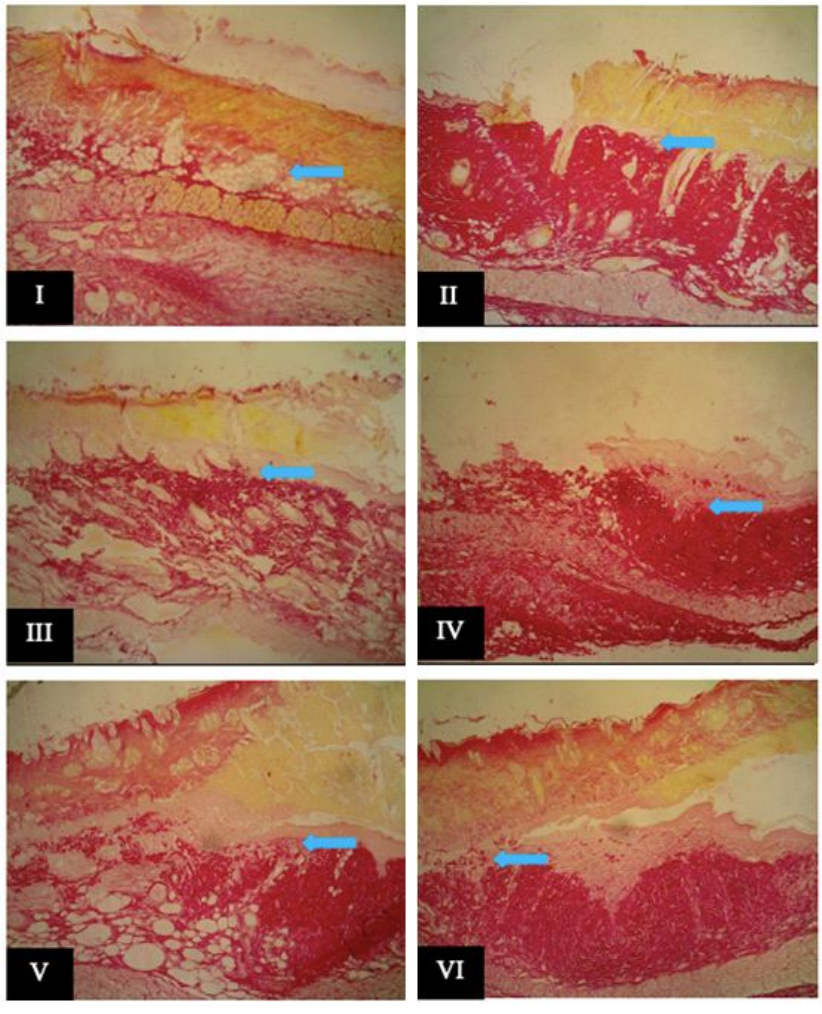

Figure 5. Collagen distribution on histological wound tissue preparations in each group. Histological preparations was made with sirius red staining.

Fibroblasts are the most abundant components in granulation tissue.[25] Collagen synthesis and deposits are important events that occur in the proliferation phase and the process of wound healing in general.[21] In our study, the number of fibroblast on group treated with 70% hydrolysed VCO cream was higher compared to group

with 100% hydrolysed VCO cream and control group after 6 days of treatment. On 12th day of treatment, group with 70% hydrolysed VCO cream has a higher amount of fibroblast compared to the control group. In line with the previous study of Silalahi J, *et al*, (2015) which stated 70% hydrolysed VCO has the fastest time (± 12 days) of burn wound diameter reduction compared to bioplacenton.[18] Hydrolysed VCO is a monoglyceride, that acts as a potent antibacterial and suppresses the inflammatory process and accelerates the remodeling process.[18] As a monoglyceride, VCO can also form free fatty acids, inducing the expression of VEGF and FGF which will later trigger fibrin activation to form collagen.[29]

Collagen is secreted into the extracellular space in the form of procollagen.[29] This form further divides in the terminal segment and is called tropocollagen.[30] As it combines with other tropocollagen molecules, collagen filaments is formed. These filaments then recombine to form fibrils, which then combine to form collagen fibers.[30] Forms of filaments, fibrils, and fibers occur in the glycosaminoglycan matrix, hyaluronidase acid, chondroitin sulfate, dermatan sulfate, and heparin sulfate produced by fibroblasts.[30] Collagen synthesis starts on the 3rd day after injury and takes place quickly around 2–4 weeks.31 Collagen synthesis is controlled by the collagenase enzyme and other factors that affect collagen and then new collagen will be formed.[32] Previous study conducted by Soliman AM , *et al* (2018) proved VCO was better than silver sulfadiazine cream (SSD) in healing diabetic wounds by stimulating re-epithelialization and collagen synthesis better.[33]

Collagen remodeling process in the maturation phase is influenced by the time of collagen synthesis and the degradation of collagen.[2] Collagenase and metalloproteinase in the wound remove excess collagen as the synthesis of new collagen continues.[12] Gradually the fibronectin disappear, hyaluronidase acid and glycosaminoglycans are replaced by proteoglycans.[3] Collagen type III is replaced by type I collagen and water will be absorbed from the scar tissue.[12] As the collagen fibers seal together, collagen cross–linking occurs, reducing the thickness of the scar tissue and increasing wound strength.[31]

The 100% hydrolysed VCO cream, however, was no longer more effective compared to base cream on increasing the amount of VEGF expression and collagen thickness on the 6th day of treatment. This might be due to damage of some active substances produced after chemical hydrolysis.[34] As the study was only conducted on the 6th and 12th day, the peak value of VEGF levels in the wound healing phase and the final phase of wound healing is not known. Collagen thickness assessment was only done on day 6 as the end of the inflammatory phase and at day 12 where the proliferation phase of wound healing has not been completed. Therefore, the thickness of collagen at the beginning of the inflammatory phase and the end of the proliferation phase is unknown.

CONCLUSION

In conclusion, the 70% and 100% hydrolyzed VCO are more effective in treating second–degree burn wounds compared to base cream, indicated by VEGF and collagen thickness. The application of 70% hydrolysed VCO after 12 days significantly increased VEGF expression but did not increase collagen thickness significantly. As this study was done in animal model, study on human should be conducted. Observation after all wound healing phases completed could help to describe the effect of VCO in wound healing better.

REFERENCES

1. Sjamsuhidajat R, JongWD: Buku ajar ilmu bedah Samsuhidajat-De Jong. 4th Edition. Jakarta: EGC. 2014.
2. Shehan H, Peter D: ABC of burns :Pathophysiology and types of burns. BMJ328(7453):1427–9, 2004.
3. Garcia-Espinoza, Aguilar-Aragon, Ortiz-Villalobos, Garcia-Manzano RA and Antonio BA: Burns: Definition, Classification, Pathophysiology and Initial Approach. Los Angeles : Gen Med. 5 : 5, 2017.
4. Departement of Information Evidence and Research. WHO methods and data sources for global burden of disease estimates 2000–2016. Geneva; 2018.
5. Tiwari V. K. (2012). Burn wound: How it differs from other wounds?. Indian journal of plastic surgery : official publication of the Association of Plastic Surgeons of India, 45(2), 364–373. https://doi.org/10.4103/0970-0358.101319
6. Garcia-Espinoza, Aguilar-Aragon, Ortiz-Villalobos, Garcia-Manzano RA and Antonio BA: Burns: Definition, Classification, Pathophysiology and Initial Approach. Los Angeles : Gen Med. 5 : 5, 2017.
7. Papini R: Management of burn injuries of various depths. BMJ329(7458):158–60, 2004.
8. The Australian and New Zealand Burn Association Limited: Emergency Management of Severe Burns. Indonesia Kolegium Ilmu Bedah Indonesia. 2013.
9. Rowan MP, Cancio LC, Elster EA, Burmeister DM, Rose LF, Natesan S, *et al*: Burn wound healing and treatment: review and advancements. Crit Care 19:243,2015.
10. Qing C: The molecular biology in wound healing and non-healing wound. Chin J Traumatol20:189–193, 2017.
11. Clauss M: Molecular biology of the VEGF and the VEGF receptor family. Semin ThrombHemos26:561–569, 2000.
12. Ruszczak Z: Effect of collagen matrices on dermal wound healing. Adv. Drug Deliv. Rev 55(12):1595–1611, 2003.
13. Munire K, Ozgok K, John-Paul R: Wound Healing.United States StatPearls Publishing. 2019.
14. Mousavi SA, Mousavi SJ, Zamani A, Nourani SZ, Abbasi A, Nasiri E, *et al*: Comparison of Burn Treatment with Nano Silver-Aloe Vera Combination and Silver Sulfadiazine in Animal Models 24:3, 2019.
15. Manisha D, Shyamapada M: Coconut (Cocos nucifera L. : Arecaceae) : In health promotion and disease prevention. . Asian Pac J Trop Med4(3):241–7, 2011.
16. Lima EBC, Sousa CNS, Meneses LN, Ximenes NC, A. SJM, Vasconcelos GS, et al: Cocos nucifera (L.) (Arecaceae) : A phytochemical and pharmacological review. Braz J Med Biol Res48(11): 953–64, 2015.

17. Christina B, Wilma B, Donald B, Curtis K, James MJ, Ronald S, *et al*: Final Report on the Safety Assessment of Cocos nucifera (Coconut) Oil and Related Ingredients. Int J Toxicol 30:5S–16S, 2011.
18. Silalahi J, Surbakti C: Burn wound healing activity of hydrolysed virgin coconut oil. Int J Pharm Tech Res 8(1):67–73, 2015.
19. Yade P, Silalahi J, Putra EDL: Antibacterial activity of hydrolysed virgin coconut oil. Asian J Pharm and Clin Res 7:90–4, 2014.
20. Nguyen VTA, Le TD, Phan HN, Tran LB: Antibacterial activity of free fatty acids from hydrolysed virgin coconut oil using lipase from candida rugosa. J Lipids 2017: 1–7, 2017.
21. Ibrahim AH, Li H, Al-Rawi SS, Majid ASA, Al-Habib OA, Xia X, *et al*: Angiogenic and wound healing potency of fermented virgin coconut oil: in vitro and in vivo studies. Am J Transl Res 9:4936–44, 2017.
22. Sun, Y., &O'Riordan, M. X. (2013). Regulation of bacterial pathogenesis by intestinal short-chain Fatty acids. Advances in applied microbiology, 85, 93–118. https://doi.org/10.1016/B978-0-12-407672-3.00003-4
23. Cooper, R. L., Segal, R. A., Diegelmann, R. F., & Reynolds, A. M. (2015). Modeling the effects of systemic mediators on the inflammatory phase of wound healing. Journal of theoretical biology, 367, 86–99. https://doi.org/10.1016/j.jtbi.2014.11.008
24. Xue, M., & Jackson, C. J. (2015). Extracellular Matrix Reorganization During Wound Healing and Its Impact on Abnormal Scarring. Advances in wound care, 4(3), 119–136. https://doi.org/10.1089/wound.2013.0485
25. Tracy, L. E., Minasian, R. A., & Caterson, E. J. (2016). Extracellular Matrix and Dermal Fibroblast Function in the Healing Wound. Advances in wound care, 5(3), 119–136. https://doi.org/10.1089/wound.2014.0561
26. Schultz GS, Chin GA, Moldawer L, *et al*. Principles of Wound Healing. In: Fitridge R, Thompson M, editors. Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists [Internet]. Adelaide (AU): University of Adelaide Press; 2011. 23.
27. Thiruvoth FM, Mohapatra DP, Kumar D, Chittoria SRK, Nandhagopal V. Current concepts in the physiology of adult wound healing. PlastAesthet Res 2015;2:250–256. http://dx.doi.org/10.4103/2347-9264.158851
28. Gonzalez AC CT, Andrade ZA, Medrado AR: Wound healing - A literature review. An Bras Dermatol91(5):614–20, 2016.
29. Tsirogianni AK, Moutsopoulos NM, Moutsopoulos HM: Wound healing : Immunological aspects. Injury, Int J Care Injured37S:S5–S12, 2006.
30. Malhotra, V., Erlmann, P., & Nogueira, C. (2015). Procollagen export from the endoplasmic reticulum. Biochemical Society transactions, 43(1), 104–107. https://doi.org/10.1042/BST20140286
31. Thiruvoth FM, Mohapatra DP, Kumar D, Chittoria SRK, Nandhagopal V: Current concepts in the physiology of adult wound healing. PlastAesthet Res 2:250–6, 2015.
32. Prasetyono TOH: General concept of wound healing, revisited. Med J Indones18(3):208–16, 2009.
33. Davison KE, Marshall WS, Garcia GE: Sources of collagen for Biomaterials in skin wound healing. Bioengineering (Basel) 6 (3), 2019.
34. Soliman AM, Lin TS, Ghafar NA, Das S: Virgin coconut oil and diabetic wound healing: histopathological and biochemical analysis. Eur J Anat 22:135–44, 2018.