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Original Article

The Effectiveness of Macrophage Hydrolyzed VCO Cream in Healing Second Degree Burns in Wistar Rats

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Abstract

Background : Burns are a global public health issue, with many studies on topical medications that are effective in second-degree burns. Hydrolyzed VCO (hVCO) contains lauric acid, polyferol and alpha tocopherol which are beneficial in wound healing. This study was conducted to determine the effectiveness of hVCO cream macrophage formation for second degree burn wound healing in Wistar rats.
Methods : A parallel group study was conducted on thirty wistar rats randomly divided into six groups induced second degree burns. Basic cream was applied to two control groups on the 6th and 12th day, 70% hVCO was applied to two groups on the 6th and 12th day, and 100% hVCO was applied to two groups on the 6th and 12th day. Histopathological examination of macrophage formation was conducted in each treatment.
Results : The groups with hydrolyzed VCO cream on day 12 (p=0.089) had higher macrophage formation than the same hVCO group on day 6 (p=0.354). The macrophage count of hVCO in each group showed an increase.
Conclusion : 100% hVCO is effective in accelerating second degree burn wound healing in terms of macrophage count.

Keywords : Burns, hVCO, Macrophage count

INTRODUCTION

Wounds are tissue damage or loss caused by trauma from sharp or blunt objects, changes in temperature, chemical substances, electric shock explosions, or animal bites.[1] While burns are a type of trauma with high morbidity and mortality, a global public health issue, especially in low-middle income countries. World Health Organization (WHO) estimates 300,000 deaths every year worldwide due to burns, with mortality rate in 11.6 deaths per 100,000 population per year in Southeast Asia.[2,3] In Indonesia, as a tropical country with uncontrolled use of fire, it is reported that burn injuries caused about 195.000 deaths annually and ranked as 6th most unintentional injuries.[15] Health problems due to burns effect patient's physical ability to carry out daily activities, which affects psychological and socio-economic aspects.[4] Treatment for burns varies based on the depth and classification of the burn (degree 1 as epidermal burn, degree II as superficial dermal burn, degree II as mid dermal burn, and degree III as full thickness burn.[5–7] Compared with other degrees, burn wounds II (superficial and mid dermal) is most often found where there is loss of dermis layer and peripheral nerve fibers causing severe pain in patient. Treatment is expected to fasten wound healing process and restore physiological function of the skin.[6,8]

Second degree burn wound is often treated with topical medication, namely Silversulfadiazine cream. Side effects of this cream such as kidney toxicity, leukopenia, antibiotic resistance, allergic reactions, and delayed wound healing will happen if it is used for a long time.[9] Due to these adverse side effects, many studies have been carried out to find more effective and efficient active ingredients for topical medicines from natural ingredients such as Virgin Coconut Oil (VCO). VCO was made from *Cocos nucifera*. High content of phytosterols as unsaturated fatty acids makes VCO widely used in cosmetics sector as antiinflammatory agent. Topical hydrolyzed VCO is also known to have antibacterial function.[10,11] C. nucifera as the main ingredient for hydrolyzed VCO is very easy to find in tropical countries like Indonesia. The process is easy to carry out and does not require large costs. Therefore, researchers wanted to assess the effectiveness of hydrolyzed VCO cream in certain doses, especially on parameters examination of the number of macrophages in the healing phase of second degree burns in Wistar rats. This study aims to prove that hydrolyzed VCO (hVCO) can be an alternative topical medication for second degree burns with macrophages as parameter, which is known to act as a predominant and continuous source of cytokines production in burn injury.

METHODS

Research Design

This is an experimental research with randomized posttest only with parallel group design. Burn was inducted in the back of rats in 3x5cm area with stainless rod attached for 15 seconds. Basic cream was applied to two control groups on the 6th and 12th day, 70% hVCO was introduced to two groups on the 6th and 12th day, 100% hVCO was introduced to two groups on the 6th and 12th day. Histopathological examination of macrophage formation was conducted in each treatment.

Research Sample

Rattus novergicus rats aged 8–10 weeks, with body weight around 100–150 grams and male sex were selected with the inclusion criteria of rats. Mice were kept in stainless steel cages with a 12-hour light cycle. Rat food was given ad libitum.

Time and location of Research

Research and data collection were carried out for 3 months. This research was carried out in five places, namely PT. Victoria Care Indonesia for making VCO product, STIFAR Semarang Laboratory as a place for making VCO cream, LPPT FK UGM as a place for treatment of experimental animals, Anatomical Pathology section Faculty of Medicine, Sebelas Maret State University, Solo and Anatomical Pathology section Faculty of Medicine, Sultan Agung University, Semarang for histopathological examination.

Research Variable

The independent variable of this research are 70% hVCO and 100% hVCO and the dependent variable is macrophage count in pathology anatomy examination.

Research Implementation

36 male *Ratus norvegicus* rats that met the inclusion and exclusion criteria were adapted for 7 days, randomized, and inducted to second degree burn wound. Group X1 was given hVCO 70% cream for six days, group X2 was given hVCO 100% cream for six days, group X3 was given basic cream for six days, then the tissue was removed and examined. Group X4 was given hVCO 70% cream for twelve days, group X5 was given hVCO 100% cream for twelve days, group X6 was given basic cream for twelve days, then the tissue was removed and examinated.

Hydrolized VCO was made from 50 grams of oil with 70% and 100% NaOH ethanol. Cream formula was made in oil-in-water emulsion. Macrophage count was measured in immunohistochemistry examination using binocular microscope with 400x magnification and hematoxylin eosin staining.

Data Analysis

Data obtained from research observations are in the form of macrophage count. The data was tested for normality using one-way Anova test. Data analysis will be continued with Post-Hoc Test to determine differences between groups. The Kruskal Wallis Non-Parametric Test will be carried out if the data is not normally distributed. The test was then followed by Mann Whitney Test to test the mean difference between each treatment group. The *p value* of significant differences was <0.05 with 95% confidence interval. Data analysis was carried out with SPSS 25 for Windows software.

RESULTS

Descriptive Analysis

In this study, normality test was carried out by Shapiro Wilk with the results shows on Table 1.

Macrophage Count

Normality and homogeneity tests show normal data with homogeneous distribution indicated by the acquisition of a *p value* >0.05 in all groups. One Way ANOVA test showed *p value* was <0.05 indicating significant differences in all groups. The test then continued with uji Post Hoc Games Howell test to analysis each difference. However, Howell's Post Hoc Games showed that the data was not normally distributed, hence to identify further differences in macrophages count, the test continued using the Kruskal-Wallis test (Table 2).

From the results of Kruskal-Wallis difference test, it was found that there was significant differences (*p value* <0.001). To determine differences in macrophages between treatment groups, the Mann-Whitney Post Hoc test was used. The results showed significant differences in X3 group versus versus X2, X6, X4 dan X5 group; significant differences in X1 group versus versus X2, X4 dan X5 group; significant differences in X2 group versus versus X6, X4 dan X5 group; significant differences in X6 group versus versus X4 dan X5 group.

DISCUSSION

In burn injuries, macrophages play the role of phagocytosis, cleaning necrotic tissue, pathogenic microorganisms and foreign objects. These functions lead to enhancement in macrophages levels. Monocytes as leukocyte cells are recruited from the circulatory system to the site of infection or injury. Monocytes secrete proinflammatory and angiogenic factors, also differentiate into macrophages to clean extracellular debris that enters the wound within 48–96 hours after

TABLE 1
Descriptive and Shapiro-Wilk Test Results

Group	Mean ± SD	Median (Min–Max)	$p^{£}$
X3	3.73 ± 1.12	3.90 (1.80 – 4.80)	0.271*
X1	3.77 ± 3.33	3.30 (0.00 – 9.80)	0.354*
X2	11.17 ± 4.42	10.60 (6.00 – 19.40)	0.089*
X6	1.47 ± 1.24	1.80 (0.00 – 4.00)	0.296*
X4	0.07 ± 0.16	0.00 (0.00 – 0.00)	0.000*
X5	0.00	0.00	–

TABLE 2
Macrophage Kruskal-Wallis Test Results

Group	Mean ± SD	*p*
X3	3.73 ± 1.12	<0.001*
X1	3.77 ± 3.33	
X2	11.17 ± 4.42	
X6	1.47 ± 1.24	
X4	0.07 ± 0.16	
X5	0.00	

Note: * Significant ($p < 0.05$)

TABLE 3
Post Hoc Mann-Whitney Test Results

Group I	Group II	*p*
X3	X1	0.521
	X2	0.004*
	X6	0.016*
	X4	0.003*
	X5	0.002*
X1	X2	0.010*
	X6	0.146
	X6	0.013*
	X5	0.007*
X2	X6	0.004*
	X4	0.003*
	X5	0.002*
X6	X4	0.049*
	X5	0.022*
X4	X5	0.317

Note: * Significant ($p < 0.05$)

injury. Macrophages can also secrete various cytokines and chemokines to stimulate cell proliferation and collagen deposition, promoting vascularization and granulation. There are two patterns of macrophage activation, namely classical macrophage activation (M1) which acts as immune cells and inflammatory cells in the early phase of wound healing. Activation of alternative macrophages (M2) as repair cells and dominates in the later stages of wound healing.[12]

Administration of VCO was able to show changes in macrophage function, with various increasingly high doses. In this study, macrophages increase in second degree burns on days 6 and 12 tended to be higher in wound care with 70% hydrolyzed VCO cream compared to 100% hydrolyzed VCO cream and controls. This result is due to the content of single chain fatty acids in VCO which keeps the wound tissue at optimal humidity for the formation of new cells. Single chain fatty acids also play a role in preventing bacterial invasion. On the contrary, previous research which analyzed the number of neutrophils after 70% hydrolyzed VCO cream usage found that the number of neutrophils was not too high on day 6 and decreased further on day 12 compared to 100% hydrolyzed VCO cream. The increase in neutrophils in the inflammatory phase stimulates the expression of FGF and VEGF. FGF and VEGF will activate the invasion of fibroblasts produced by macrophages. Fibroblasts proliferate and produce matrix proteins such as fibronectin, hyaluronic acid, collagen and proteoglycans. Fibroblasts also form an extracellular matrix that helps keratinocyte cell migration. Overall it may be said that administration of VCO has strong anti-inflammatory properties as well as other physiologic processes.[12]

Other study showed that angiogenesis in the 70% hydrolyzed VCO cream group had the highest number of new blood vessels on day 12 compared to the 100% hydrolyzed VCO cream and control group. Angiogenesis as a part of skin components plays an important role in new tissue formed process which connected with wound healing process. Angiogenesis stimulated by vascular endothelial growth factor (VEGF), a pro-inflammatory cytokine produced by endothelial damage and also by neutrophils. Angiogenesis, collagen deposits, and granulation tissue formation are important processes that occur in this phase.[13,14]

CONCLUSION

The results of this study concluded that administration of hydrolyzed VCO cream was more effective than administration of basic cream in healing second degree burns, in terms of the function and number of macrophages in Wistar rats. The administration of hydrolyzed VCO cream was 70% and 100% more effective in healing second degree burns, on days 6 and 12 of observation, compared to administration of basic cream.

Ethical Approval
This research has received approval from the Health Research Ethics Committee, Faculty of Medicine, Diponegoro University with Ethical Clearance.

Conflicts of Interest
The authors declare that there was no conflict of interest.

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Author of Contributions
FS, N were involved in planning and supervised the work, FS performed the measurements, processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. FS performed the xyz calculations and statistical analysis. FS, N aided in interpreting the results and worked on the manuscript. All authors discussed the results and commented on the manuscript.

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