Effectiveness Test of Wound Healing based Virgin Coconut Oil toward Commercial Products on Rabbits

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***Abstract*—The skin is permanently exposed to the external environment and is very vulnerable to the appearance of various types of lesions such as burns, boils, and other injuries. Wound healing is a complex biological event and is influenced by extrinsic and intrinsic factors which can cause complications. Virgin Coconut Oil (VCO) is rich in the role of vitamin E in the mechanism of cell retention in cell organs, significantly increasing the activity of antioxidant enzymes, fibroblast proliferation, and neovascularization. This study aims to determine the effectiveness of the VCO in experimental cuts. Subjects (rabbits suffering from cut wounds by treating VCO commercial products topically) were divided into four groups (i.e., negative control, positive control treatment with betadine, treatment with artificial VCO, treatment with commercial VCO. Then the wound diameter was measured on days 0, 3, 7, 15 and21. On the last day, treated rabbits were anesthetized, and wound tissue was taken for histological examination. The results showed that the VCO treatment had potentially developed into wound care products. The VCO administration group affected the epithelial cell thickness by about 641.5 µm lower than other groups, and also have an average number of epithelial cells more than other groups.**

Keywords—Skin, VCO, Wound Healing, Virgin Coconut Oil

I. INTRODUCTION

The skin is a part of the body that acts as an intermediary between internal and external organs as well as being protective from outside disturbances such as solar heat, fire, hot water, pressure, scratches, and other disorders [1]. The skin also acts as a body bolt sensor related to touch, pressure, temperature, pain, and spontaneous warnings if there is a sudden disturbance. Besides, the skin has a role in the exchange of fluids, salts, gases, and heat [2].

The skin is permanently exposed to the external environment; the skin is very vulnerable to the appearance of various types of lesions, such as burns, ulcers, and cuts [1]. About 6 million patients come to the emergency department for laceration treatment each year [3]. Open wounds often need to be treated for several days, weeks, or even months until the wound is closed. Most wounds heal without complications [4].

Wound healing is a complex biological event and is influenced by extrinsic and intrinsic factors, which can cause complications. Injuries caused by heat or fire or other causes can be fatal if not followed by serious treatment [5]. Local factors such as oxygen and infections and systemic in the form of age, sex hormones, stress, drugs, smoking and carbohydrates, protein, and amino acids play an important role in influencing the wound healing process [7].

Wound healing requires a complex sequence of biological processes, which are generally divided into three phases, including invasion of inflammatory cells, the proliferation of tissue repair cells, and tissue renovation. The extent of wound healing depends on many factors, including the size of the wound, the blood supply to the area, the presence of foreign bodies, microorganisms, age, patient health, patient nutritional status, drug use, and systemic disease variations [8]. The wound healing process is given in Fig. 1.

Besides, many wound care products have the potential to remove wounds without damaging healthy tissue, reducing infection, and increasing the rate of wound healing [9]. Traditional therapies have been used for centuries, especially by populations living in rural areas in developing countries. Usually, this therapy involves the use of compounds derived from herbs and animals, living organisms, silver, and traditional sanitary napkins. On the other hand, modern therapy consists of the use of grafts, new bandages, biotechnology skin substitutes, and cell/growth factor therapy [10].

Iranians have long used plant medicine to treat many diseases. In Iran for example, since thousands of years and has existed in hundreds of books stating that traditional medicine in the form of alternative medicine is very good for health. For thousands of years also tropical countries have been using coconut from the Cocos nucifera tree as part of health food and maintained as a livelihood. Coconut oil is a vegetable oil extracted from old coconut seeds from old heads [12].

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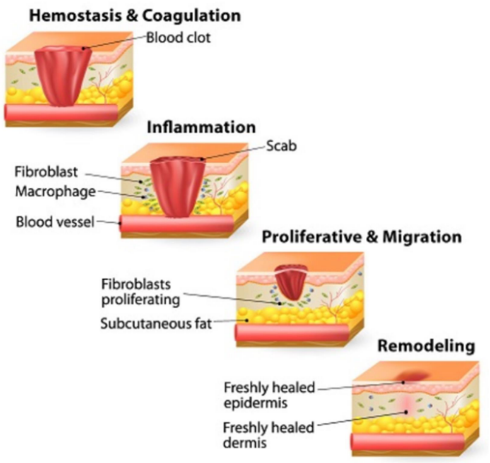

Fig. 1 Wound Healing Process [21].

Virgin Coconut Oil (VCO) is the result of pure fresh coconut meat extraction at low temperatures without involving chemicals. Physical methods for producing VCO include pressing, washing with water, settling, filtering, and centrifugation processes. However, the natural way to produce VCO is through the fermentation process, which occurs through microorganisms that occur naturally. The most important qualifications of VCO must be free from water, to avoid rancidity, high levels of antioxidants, vitamins, and lauric acid [13].

The VCO is rich in the role of vitamin E in the mechanism of cell retention in cell organs, significantly increasing the activity of antioxidant enzymes, fibroblast proliferation, and neovascularization. Coconut oil is stored to be applied to the skin even on injured people with a minimum risk of allergies. Previously research said that wounds treated with VCO healed faster, increased collagen tissue, increased fibroblast proliferation, and neovascularization of wounds [14, 15].

As explained earlier that VCO has been widely used as an ingredient of traditional medicine for wound healing. The results of other studies also explain that VCO has an antimicrobial character to wound infection in this case VCO has the ability to increase epitalization [16-19]. In this research, the treatments with VCO was used and compared with betadine treatment for the fastest wound healing.

II. METHODOLOGY

The tools used in this study were grated coconut, tools/hydraulics for coconut milk, ruler 10 mm and vernier caliper (sketmat), Blender machine, light microscope, object-glass, deck glass, container, filter paper, large funnel, small funnel, spatula, plastic bottles, measuring cups, water hose, small dipper, large filter, small filter, cloth napkin, scalpel, razor, gloves, mouth mask, iron plate 2 x 2 cm. The ingredients used in this study were fresh old coconut, 20 healty rabbit (Oryctolagus cuniculus) male 1 kg, lidocaine cream 9%, Fatty acid methyl esters (FAME), and Betadine.

In vivo examination, the 20 rabbit test was divided into 4 groups (5 rabbits dor each group) which are Group (negative control) was a group of rabbits without treatment,Group II (positive control) was rabbits with topical treatment of 10% betadine ointment, Group III (treatment group I) was rabbits with topical VCO treatment. Group IV (treatment group II) was rabbits with topical VCO commercial product treatments. Before the experiment, rabbits test were adjusted for one week in the room with temperature (22-25 ° C) and 12 hours light/ dark cycle. In the adjusted period, the rabbits test were only feed with commonly food and the drinking water ad libitum. The experiment was completed with ethical cleareance from the health and science commission, University of Sumatera Utara, Indonesia.

The skin of the rabbits were shaved on the spot area (the vertebral column area with a distance of 2.5 cm from both sides of the ear). The shaved skin area was anesthetized with 9% lidocaine for 5 minutes until it completely numb. After the incision has formed, the VCO and 10% betadine ointment were immediately applied. Whereas in the negative control group, the wound treatment only uses aquadest. The wound treatment must be carefully applied. Each of them was cleaned first using aquadest before the treatment. The wound medicine was given twice a day. The steps to manage the wound are prepared gauze and arranged rabbits to facilitate the action. The wound is covered with gauze that has been moistened by VCO and commercial VCO products in groups III and IV, respectively, as thick as 0.5-1 mm until it is considered capable of covering the entire wound surface. As for the positive control group, the wound was smeared using a 10% betadine ointment 1-2 mm thick to cover the entire wound surface. Then the wound is closed with sterile gauze. In the negative control group, the wound was covered with three layers of sterile gauze moistened with aquadest, then placed over the incision, then covered with five layers of sterile gauze. Bandages are replaced when conditions are full. Hand-wound cuts with VCO, commercial VCO products, 10% betadine ointment, and sterile aquades control group were carried out for ± 21 days. Observations were made on days of 3, 7, 11, 15, and 21, respectively.

Forth, skin wound samples of each rabbits for each group were taken for histopathological studies with small excision after 21 days of treatment. Samples were fixed in 10% formalin buffer, processed, blocked with paraffin, then cut to 5 µm, and stained with hematoxylin and eosin. The slides are then read under a light microscope to see collagen tissue, epithelialization, and neovascularization.

In the last step, the recorded data were analyzed using Statistical Package for Social Science. The normality of the data was evaluated using Saphiro-Wilk test (normal if $p > 0.05$). The normal data was then tested with One Way Anova for data distribution and the data variance of each group was tested using the Post hoc Bonferroni method. If it does not meet the experiment requirements then a Kruskal-Wallis non-parametric test is performed.

III. RESULTS AND DISCUSSIONS

The observations of the measurement data on the average length of the wound (back of the rabbits) in each group for each measurement day can be seen in Table I. The smallest of wound diameter on day 21 was in the treatment group K1 and K2 given commercial VCO and artificial VCO.

Furthermore, the largest diameter on the 21st day is in the group without treatment. VCO provides a faster recovery effect compared to Bioplasenton. Statistical tests showed that a significant difference (P <0.05) was obtained from the negative control group against the positive control group that was treated with bioplacentons. The fastest wound closure occurred on the 21st day, namely in the K1 and K2 treatments with an average cut length of 0.1 cm, while the wound closure, which has the most massive average length of the wound, is on the K-treatment 0 8 cm. Based on the Table I shows that the administration of VCO has a faster wound closure affect both commercial VCO and artificial VCO and faster than the group given betadine and in the group without treatment the incision wound length occurred. Based on statistics, the results of K- have a significant difference p <0.05 to K +, K1, and K2. Table I clearly shows the process of reducing wound length for each group (K-, K +, K1, K2) on each measurement day by 46.67%, 86.67%, 93.33%, and 93.33%, respectively. Overall, the healing process was faster in the K1 and K2 groups than others. On day 7, the K1 group was the fastest at around 50%.

A layer of epithelium will cover the wound that begins with mitosis of the epidermal basal cell and was followed by epithelial displacement down the wound edge. The epithelium moves as a sheet until it is in contact with other epithelial cells. The reepithelialization process starts a few hours after the wound and complete reepithelialization usually occurs 24-48 hours on the wound with the edges close to one another, but requires more time on a wide wound. The wound surface restores epithelial integrity and epithelialization originates from the basement membrane. Epithelial cells of the wound begin to show increased mitotic activity and migrate the connective tissue that is still alive. Fig. 2 is visual observasion on the wound healing of each group rabbits for measurement days of 0, 7, and 21, respectively.

TABLE I. LENGTH OF WOUND AND EPITHELIAL CELL THICKNESS

Group	Day -					Thickness (µm)	Cell Number
	0	3	7	15	21		
K-	1,5	1,48	1,35	1,2	0,8	1.050,4	4,4
K+	1,5	1,30	0,90	0,6	0,2	689.1	6,7
K1	1,5	1,28	0,75	0,5	0,1	645.6	83,
K2	1,5	1,25	0,80	0,4	0,1	641.5	8,5

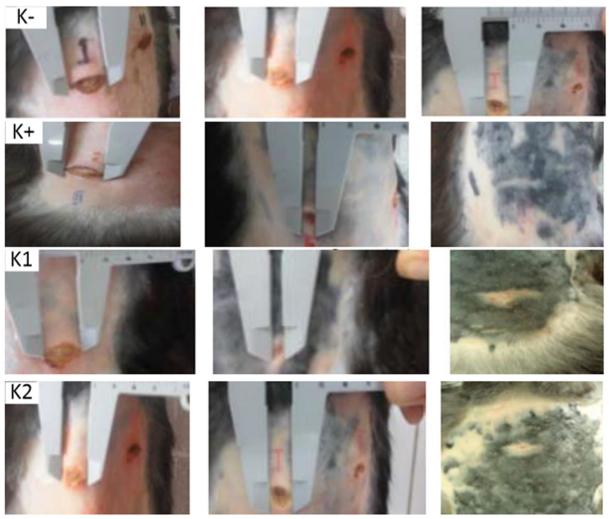

Fig.2. Observation of the Wound Healing.

The highest average value of epithelial thickness produced by the negative control group without treatment was about 1,050.4, while the lowest was in the K1 about 641.5 that treated with commercial VCO. Based on statistics, it was known that the negative control group had a significant difference (p <0.05) compared to the others group.

The data on the average number of epithelial lesions in the K-negative control, K + positive control, K1 commercial VCO administration group, K1 artificial VCO administration group can be seen in Table I and visually shown in Fig. 3. The highest average number of epithelial cells produced by the commercial VCO administration group was 8.5, while the lowest was in the negative control group without treatment 4.4. Based on statistics, it was known that the negative control group had a significant difference (p <0.05) compared to the others group.

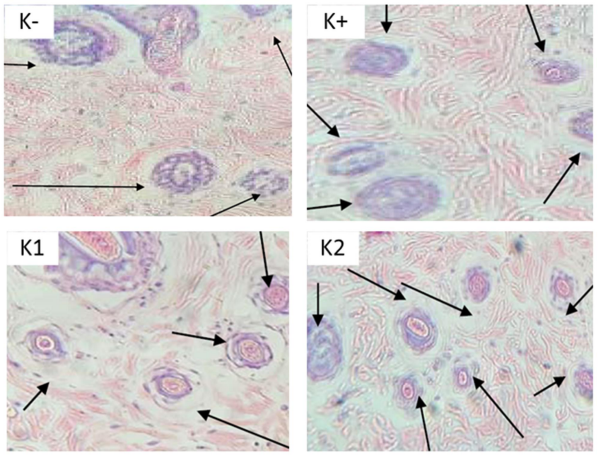

Fig.3. Observation of Number Epithelial Cell.

According to previously research [20], states that the potential of the antioxidant content in oil flavonoids may be one of the causes of increased wound healing. Flavonoids are antioxidants that are stronger several times compared with vitamin C. Previous studies on VCO found tannins. Tannins are known to form a protective layer on the wound so that the wound can be protected from microorganisms that have

the potential to cause infection. The potential of the tannin content also may be one of the causes of the increase in the speed of wound healing because it can act as an antibacterial. Histologically, the wound healing process is achieved when the tannin content in plants can increase cell proliferation and prevent oxidation. According to previously research [21], indicate that flavonoids function as inhibitors or prevent the growth of microorganisms by damaging cell walls in bacteria. Saponins has a function as an antiseptic that can kill bacteria and can prevent the bacteria from growing back so that no infection occurs in the wound. Wound healing was a complex process because of the various bio-cellular and biochemical activities that occur on an ongoing basis. The combination of vascular response, cellular activity, and chemical formation as a mediating substance in the wound area were interrelated components in the wound healing process. The process of wound healing was highly effected by the role of migration and proliferation of fibroblasts in the injury area. The proliferation of fibroblasts at the wound healing stage indicates a rapid healing process. The primary process of fibroblast growth will occur on the 14th day until the 20th-day post-injury, and after that, there will continue to be improved until the skin structure returns to normal [22, 23]. The reepithelialization process will re-produce an intact epidermal layer to cover the wound so that it can be protected from the outside environment. The reepithelialization process consists of several phases, namely the first phase of migration, the second phase of proliferation, and the third phase of keratinocyte differentiation. Keratinocyte migration and proliferation is influenced by several factors, namely Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), Transforming Growth Factor-β (TGF-β), Transforming Growth Factor (TGF-), Insulin-like growth factor (EGF) IGF-1), and Hepatocyte Growth Factor (HGF) [24]. During periods of intense vascular and cellular reaction, the epithelium rapidly generates to restore its protective function [25].

IV. Conclusion

Based on the experiement results, it can be concluded that the provision of VCO is potentially developed into wound treatment products. Both commercial and artificial VCO treatment groups had a greater wound closure effect that was proven on the day 21 about 93,33% which the wound covered than the bioplacenton group. The VCO treatment group had a lower 645.6 µm effect on epithelial cell thickness compared to other groups, and also had an average number of epithelial cells more than the other groups.

Acknowledgment

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