

Wound healing activity of Combination Propolis and Coconut Shell Liquid Smoke Ointment on wistar rat second degree Burns wound models

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ARTICLE INFO

Manuscript Received: 08 Dec, 2024

Revised: 25 Dec, 2024

Accepted: 27 Dec, 2024

Date of publication: 09 Jul, 2025

Volume: 15

Issue: 1

DOI: [10.56338/promotif.v15i1.6570](https://doi.org/10.56338/promotif.v15i1.6570)

KEYWORDS

Workplace nutrition interventions;
Employee health;
Dietary behavior;
Physical activity;
Mediterranean diet

ABSTRACT

Introduction: The delay in burns healing is a serious complication that can lead to disability and death. TGF- β 1 contributes significantly to the wound healing process. Chronic wounds that do not heal often indicate a loss of TGF- β 1 signalling. Propolis and coconut shell liquid smoke (CS-LS) are natural ingredients that contain antioxidant, anti-inflammatory, and analgesic compounds to accelerate wound healing. This study aimed to prove the effectiveness of using a topical combination of propolis and CS-LS in accelerating the healing process of degree II burns and their relationship with the expression of TGF- β 1 in serum. **Method:** A 14-day *in vivo* experimental investigation using Wistar rats was carried out with a total sample of 25 rats divided into 4 groups.; Group 1, treated with sulfadiazine ointment; Group 2, treated with propolis ointment; Group 3, treated with CS-LS ointment; Group 4, treated with the combination of propolis and CS-LS ointment, and 1 control group without treatment. Serum TGF- β 1 levels were determined by ELISA.

Result: Measurements of wound size were on days 0,3,7 and 14. This study obtained results the combination ointment group alone had significantly different TGF- β 1 levels from day 7 to day 14 and experienced a decrease in TGF- β 1 levels of 52.28 μ /mg. Based on wound size, there was a significant difference in all groups ($p < 0.05$), this was supported by the mean value of wound size which was smaller in the sulfadiazine ointment group and the combination (propolis and liquid smoke) ointment group.

Conclusion: The combination of propolis and coconut shell liquid smoke can be considered as an alternative in the treatment of second degree burns because of its ability to reduce TGF- β 1 and accelerate burn wound healing.

Keywords: wound healing, propolis, coconut shell liquid smoke, TGF- β 1.

Publisher: Fakultas Kesehatan Masyarakat Universitas Muhammadiyah Palu

INTRODUCTION

Burns on the skin and other tissues are due to heat, radiation, electricity, friction, or chemical contact. Burn trauma is the one of remain predominant problems in many countries. About 180,000 deaths occur each year from burns, and most of these deaths occur in developing countries [1–3]

Burn infection becomes a critical problem because it causes a delay in the maturation of the epidermis and leads to the formation of scar tissue. Infection is a common cause of morbidity and mortality in burn sufferers. This is due to the uncontrolled growth of bacteria.[4]. People with large area of wounds tend to cause difficulty in short-term wound closure and hinder long-term wound-healing process and scar development.[5] Burn treatment using a regimen of antimicrobial ointments, such as silver sulfadiazine, is used to reduce potential infection in minor injuries.

Nevertheless, such topical antimicrobials result in adverse effects and are slightly effective in wound-healing process. [6]

One of the mayor focuses of recent research on wound healing is the role of TGF- β and scar formation. [7] There are three isoforms of TGF- β (TGF- β 1, TGF- β 2, and TGF- β 3). These isoform appear to have overlapping functions and mediate their effects through intracellular SMAD pathways. TGF- β is involved in every phase in wound healing: inflammation, proliferative and remodelling[8]. Interestingly, chronic wounds that do not heal often indicate loss of TGF- β 1 signaling[9]. TGF-1 promotes endothelial progenitor cells, which enhances blood circulation to the wound area. TGF- β 1 is important in regulating the division of myofibroblasts, which are the focus of treatment for hypertrophic wounds and keloids. [10]

One of the herbal ingredients that is well-known to heal wounds is propolis. Propolis is one of the products produced by honeybees in the form of resin substances derived from plant buds and exudate, as well as beeswax mixtures. This natural ingredient has been known to the public since the first century to maintain health and treat various diseases, such as reducing swelling, reducing pain, and healing wounds.[11, 12] Propolis has shown beneficial effects in the wound healing process. It also reduces free radical activity (ROS) in the wound base which supports the repair process.[13] The well-established antimicrobial, antiviral, antifungal, anti-inflammatory, immunostimulatory properties of propolis make it as a potential candidate for remedy wound healing in animals.[14, 15] Some types of flavonoids present in propolis. [16, 17]

In addition to propolis, a natural ingredient that is also being widely studied as a wound medicine is coconut shell liquid smoke. Coconut (*Cocos nucifera*) is typically determined in tropical countries, including Indonesia, and is considered a fruit plant with many benefits, including its use as a traditional medicine from generation to generation. [18] Although its shell is often considered agricultural waste, it contains a variety of antioxidants. Coconut shell liquid smoke (CS-LS) is the result of coconut shells pyrolysis. The pyrolysis method involves a high temperature heating procedure that results in chemical degradation. This process results in liquid smoke as a result of the condensed wood smoke fumes. [19, 20] Since coconut shell liquid smoke is known to have antioxidant, anti-inflammatory, and antibacterial characteristics that may promote wound healing, it is being explored extensively as a topic for application to wounds.[21–23]

In this research, considering the benefit of propolis and coconut shell liquid smoke in wound healing, herbal ointment (composed of propolis and coconut shell) were investigated on second degree burn wound in rat.

METHOD

Research Type

This study is a true-experimental study to analyze the effect of a combination of propolis and liquid coconut shell smoke on TGF β levels in second-degree burns in Wistar rats.

Population and sample:

25 Wistar-albino male rats from our institution's animal lab—average weight: 300–350 g, average age: 3–4 months—were used in the study. The animals were fed a conventional meal and were kept separately in special cages. They were kept at a temperature of 23 ± 2 °C and a humidity level of 45%-55% on a 12 hour light-dark cycle.

Research Location

This study was carried out in the Hasanuddin University Faculty of Medicine's animal laboratory.

Topical Propolis and Coconut Shell Liquid Smoke

Propolis obtained from bees *Trigona Levicep* and Coconut Shell Liquid Smoke is obtained from Coconut shell in Jember, East Java. Topical Propolis and Coconut Shell Liquid Smoke is produced by CV. Indonesia Green Innovation.

Experimental Design

Animals were randomly divided into 5 groups of 5-member. The first group had no treatment as a negative control group (C). In the positive control group (P1), silver sulfadiazine ointment was used as the standard compound for wound healing. Test groups were treated with propolis ointment (P2), coconut shell liquid smoke ointment (P3), and combination of propolis and coconut shell liquid smoke ointment (P4). Animals in each treatment group had the

therapy ingredient applied to their wounds once daily. Animals' cage mattresses were changed every day to prevent wound infection.

Second Degree Burn Model

The chemical Jolen (Veet®), used in each treatment group, was used to eliminate hair from the treatment area. Rats were put to sleep in a particular box using ether inhalation till their level of awareness was reduced prior to burn injury. Povidone-iodine 1% was used to clean the region around the wound. According to the modified Guo method, burn wounds were produced by exposing subjects to an electrically heated piece of aluminum metal with a 2 cm diameter that had previously been heated to 100 °C for two seconds.

Tgf B Measurements

ELISA were performed on days 7 and 14 days following treatment. All blood samples were examined using the TGFβ elisa from BT LAB

Measurement Of Wound Area

By measuring the width of the wound and estimating and tracking the amount of time required for its full closure, wound healing was evaluated. From the time the wound was first created (day zero) until it had fully healed and closed, the wound surface area was measured on days 1, 3, 7, and 14.

Data Analysis

One way Anova test to determine the effect of treatment on TGF β concentrations and the Kruskal-Wallis test followed by the Mann-Whitney test to determine the effect of treatment on wound size. The normality test uses the Shapiro Wilk test. All data were examined using SPSS 23.0.

Ethical Approval

This Study was given acceptance by the local ethics commission under registration number 546/UN4.6.4.5.31/PP36/2022. Additionally, the work was completed in accordance with the ARRIVE reporting requirements for animal research.

RESULTS

The combination of Propolis and coconut shell liquid smoke could lower TGFβ level more significantly than the propolis group, coconut shell liquid smoke group, sulfadiazine group, and untreated group.

The independent t-test (table 1) showed the difference in TGF-β level in the proliferation phase during observation days. It was also found that only the combination group had significantly different TGF β level from day 7 to day 14 and showed a decrease in TGF β levels of 52.28 μ / mg. Therefore, it could be suggested that the topical combination of propolis and shell liquid smoke significantly decreased TGF β protein expression.

Table 1. Comparison of TGF β protein expression between observation groups

Days Post Wounding	Treatment Group; Mean±SD TGF β Level					p-value ^a
	K	P1	P2	P3	P4	
Day-7th	234,4±35,0	208,5±18,1	191,9±52,0	212,5±16,3	222,2±12,1	0,287
Day-14th	240,0±52,1	160,0±26,4	185,4±30,7	210,8±29,2	170,3±32,4	0,013
Difference	-5,57	48,50	6,50	1,75	52,28	0,328
p-value ^b	0,892	0,055	0,840	0,916	0,006	

^aOne-way anova test; ^bindependent t-test

The combination of propolis and coconut shell liquid smoke enhance wound closure on second degree burn wound model in rat.

Table 2. Comparison of wound sizes between groups

Day of observation	Observation group; Mean±SD wound size					p-value
	K	P1	P2	P3	P4	
Day 0	2,0±0,0	2,0±0,0	2,0±0,0	2,0±0,0	2,0±0,0	1,000 ^b
Day 3	1,98±0,02	1,62±0,03	1,93±0,08	1,85±0,06	1,61±0,38	0,003^b
Day 7	1,36±0,14	1,02±0,22	1,35±0,17	1,26±0,08	1,16±0,19	0,029^a
Day 14	0,30±0,10z	0,08±0,09	0,24±0,16	0,08±0,13	0,13±0,13	0,076 ^b
p-value ^c	0,002	0,002	0,002	0,002	0,002	

^auji one-way anova; ^buji kruskal-wallis; ^cuji friedman

Kruskal Wallis test (table 2) showed no difference in wound size on the day 0. Similarly, on the day 14 observation, there was no significant difference in wound size among observation groups. However, on the day 3, there was a significant difference in the entire group ($p < 0.05$). This was supported by the mean value of the wound size which was seen to be smaller in the sulfadiazine group and the combination group (propolis + CS-LS). To find out the groups are significantly different, then continued by the Mann-Whitney test, the results are presented below.

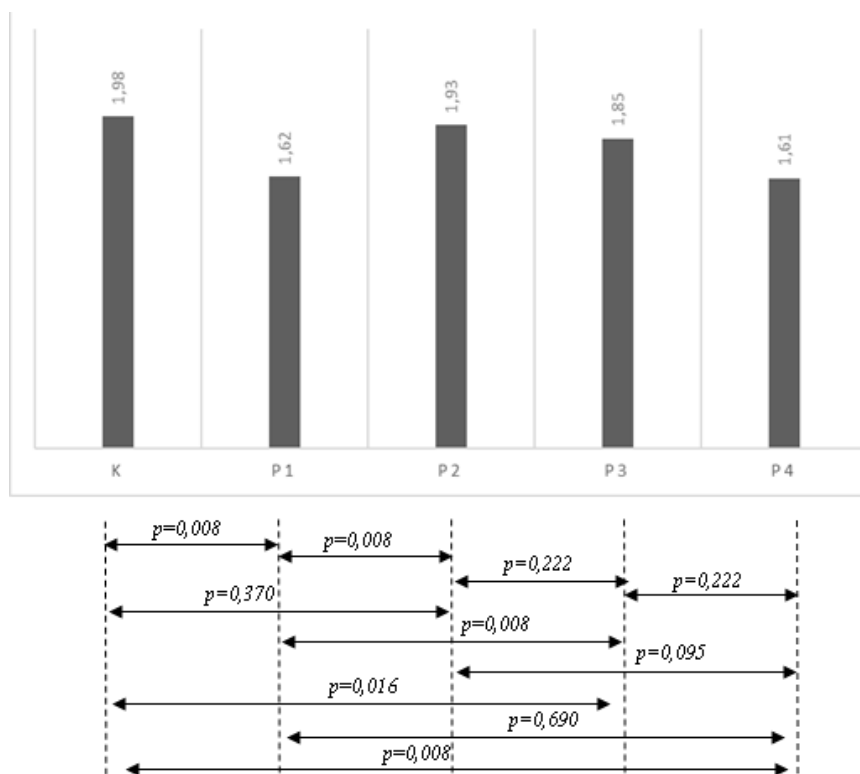


Figure 1 Result Mann-whitney Test of Wound Size On Day 3 Between Group

Figure 1 indicated significant differences in wound size in several groups, namely: 1) the control group and the sulfadiazine group with smaller wound size in the sulfadiazine group; 2) the control group and liquid smoke group with smaller wound size in the liquid smoke group; 3) control group and combination group (topical propolis and shell liquid smoke) with smaller wound size in the combination group; 4) sulfadiazine group and propolis group with smaller wound size in sulfadiazine group; 5) sulfadiazine group and liquid smoke group with smaller wound size in sulfadiazine group. This means that on day 3, sulfadiazine administration could accelerate wound healing better than the topical combination of propolis and shell liquid smoke, propolis group, liquid smoke group, and group without wound treatment in the Wistar rat degree II burn model.

The One-way ANOVA test showed significant differences in wound size across the observation groups on day 7 ($p < 0.05$) (Table 2). This was supported by a smaller wound size value on day 7 compared to the previous observation

day (day 0 and day 3). To find out which groups had different wound sizes on day 7, a post hoc Bonferroni test was conducted, as showed in Figure 2.

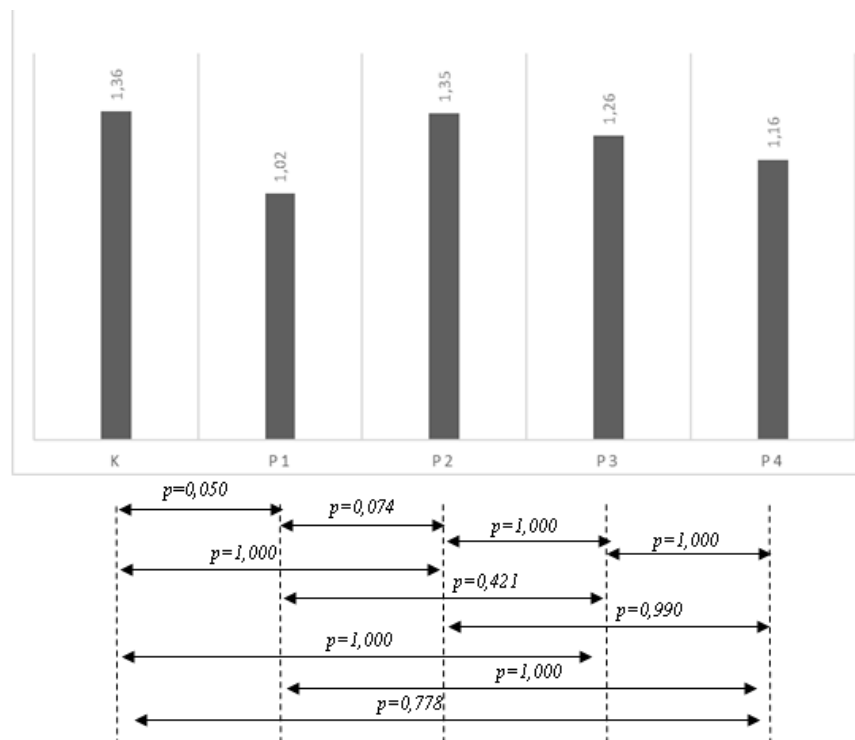


Figure 2. Result of the Mann-Whitney test of wound size on day 7 Between Group

Figure 2 illustrated that on day 7, there was a significant difference in wound size in several control and sulfadiazine groups, with a smaller wound size in the sulfadiazine group. This means that on day 7, sulfadiazine administration could accelerate wound healing better than the topical combination of propolis and shell liquid smoke, propolis group, liquid smoke group, and group without wound treatment in the Wistar rat degree II burn model.

Table depicted the result of the Friedman test to see the comparison of wound sizes in each observation day and obtained significantly different results on all observation days in each group. It was continued with the Wilcoxon test to see which groups were different. The results are presented in the chart below:

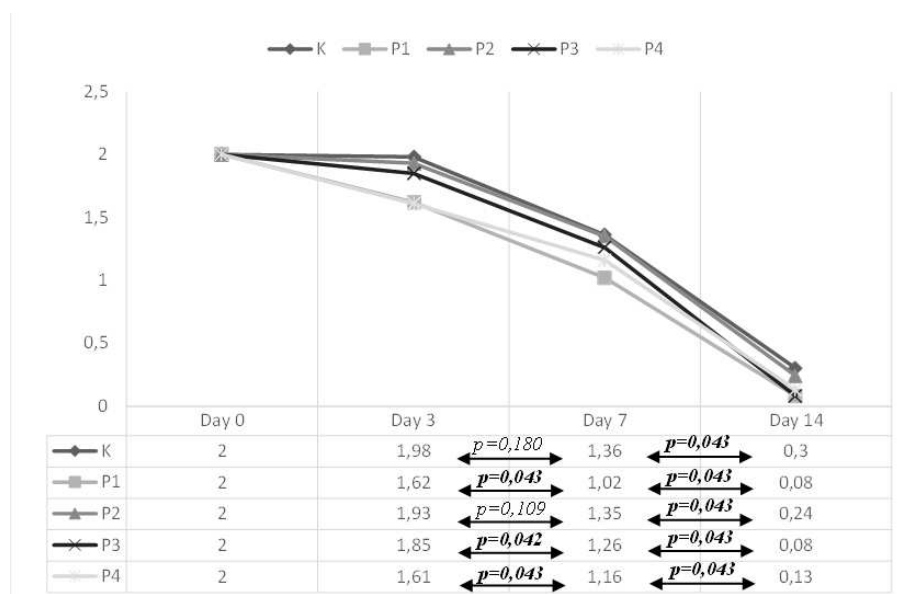


Figure 3. Result of the Wilcoxon test for Wound Size Between Observation Days

Figure 3 illustrated wound size at day 0, 3, 7 and 14 For the group given sulfadiazine, the group given liquid smoke and a topical combination of propolis + CSLS showed similar results on the wound size from day to day. The study finding was strengthened by wound image (figure 4) as follows:

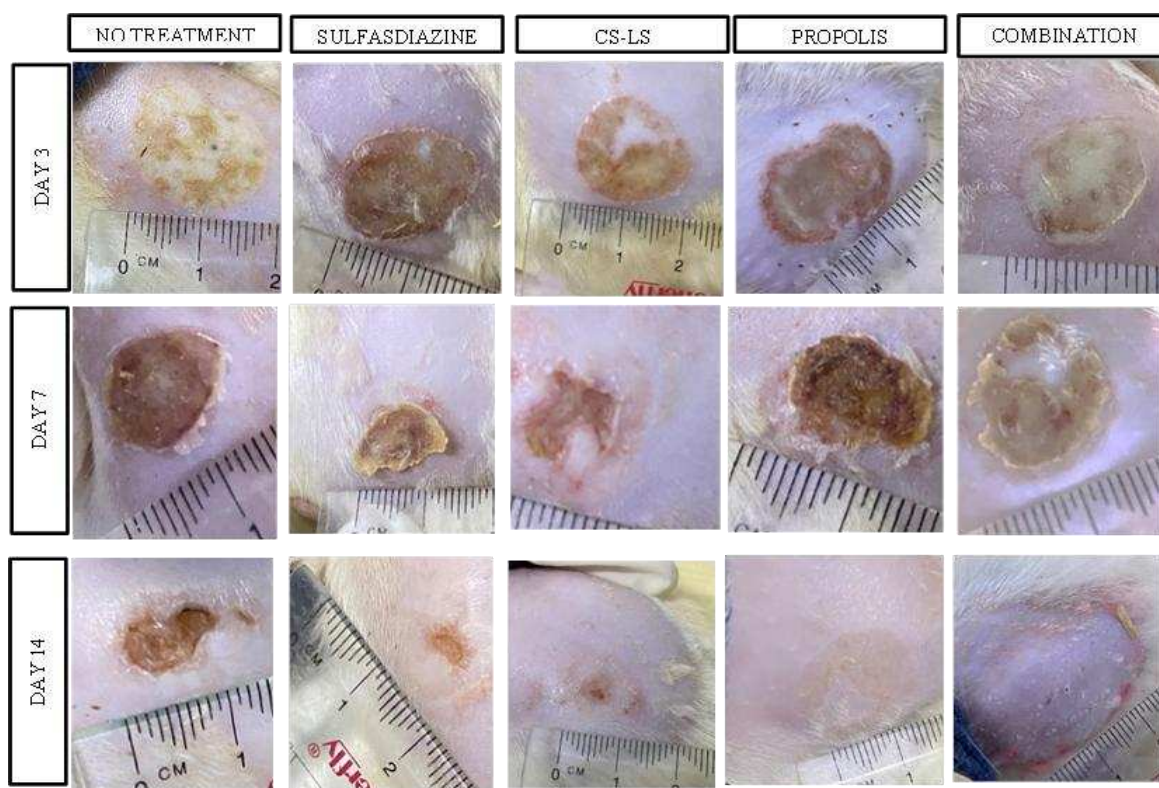


Figure 4. Wound Image On day 3,7 and 14 of observation days.

DISCUSSION

Wound healing is a natural physiological process to tissue injury. When the injury occurs, neutrophils and macrophages enter the injured tissue and produce ROS which can have beneficial and detrimental effects. High levels of ROS affect growth factor signaling thereby interfering with the remodeling of the Extracellular Matrix.[24, 25]

Among the many molecules known to affect wound healing, TGF- β , which is result in from platelets, macrophages, neutrophils, and fibroblasts affects all types of cells involved in all stages of wound healing. The intracellular signaling is initiated by TGF- β as the cytokine key, which binding to the type II threonine kinase serin receptor. Serin receptor plays a role in gene transcription, which contribute to wound-healing stages.[26, 27]

Transmembrane serine/threonine kinase receptors are involved in TGF- β signaling. The signal to the receptor causes a phosphorylation pass across different types of receptors, which makes it easier for receptors that control the Smad protein to bind intracellularly. Smads are cytoplasmic signaling molecules that have the ability to transmit TGF-1 signals from the cell membrane directly into the nucleus.[28] As a result, the TGF-1/Smad pathway contributes to and is significant in wound contraction. TGF-beta is also known to control cell differentiation, trigger the chemotaxis of inflammatory cells, and promote the buildup of extracellular matrix proteins. [29]. TGF is a polypeptide growth factor generated from human DNA that promotes healthy soft tissue repair mechanisms and corrects inadequate repair rates. These cells, which are necessary for the repair process; platelets, macrophages, endothelial cells, and fibroblasts, release this growth factor.[26, 27, 30, 31]

Due to its flavonoid content as well as a variety of vitamins, minerals, and enzymes, propolis has demonstrated positive effects in the wound healing process. [17] Caffeic acid phenethyl ester (CAPE) was also present in significant concentrations in the used propolis. Propolis-derived CAPE exhibits immunosuppressive properties, especially in T-cells, which are important in the development of a number of inflammatory disorders. Additionally, it decreases ROS activity in wounded tissue, aiding in the healing process. [32] By raising the amount of type I and type III collagen

in tissues, propolis also has a substantial impact on collagen metabolism. Collagen production increases and ROS levels decrease, which balances the extracellular matrix and produces granulation tissue.[2, 33, 34]

Due to the presence of cellulose, hemicellulose, and lignin in coconut shells, they can be pyrolyzed to produce liquid smoke. The phenolic chemicals found in CS-LS, such as 2-methoxyphenol (guaiacol), phenol, 4-ethyl-2-methoxyphenol (EMP), ascorbic acid, and flavonoids, are recognized to have anti-inflammatory properties that aid in wound healing.[20] By attaching to ROS and blocking nitric oxide (NO), phenols and guaiacol might reduce the expression of pro-inflammatory cytokines such tumor necrosis factor (TNF), interleukin 6 (IL-6), and interleukin 1 beta (IL-1). By blocking cyclooxygenase, they lessen prostaglandin E2 synthesis. [35] By boosting fibroblast and collagen proliferation, CS-LS promotes wound healing..[36] Free radicals that affect the production of pro-inflammatory and proangiogenic cytokines can be bound and inhibited by CS-LS. Therefore, it contributes to boosting VEGF production during the angiogenesis stage of wound healing.[37, 38]

Seeing the benefit of these two natural ingredients (propolis and coconut shell liquid smoke) in accelerating wound healing, we think that combining these two natural ingredients will have a better effect on wound healing. Both have been studied to have antimicrobial and inflammatory control effects that clear wounds from microbes, so that tissue regeneration becomes uninterrupted [17]. This condition strongly supports the optimal wound-healing process.

This is in line with our findings, where the combination of propolis and liquid smoke lowered TGF- β levels more significantly from day 7 to day 14. This indicates that the combination of propolis and liquid smoke can regulate the role of TGF- β in the wound-healing process. Shortly after the wound, the concentration of active TGF- β increased up to nine times (1 hour after the wound). The peak was on the third day. On day 7, TGF- β levels began to decline until they returned to the same concentration as normal skin. Unlike wounds that become hypertrophic scarring, it does not show an increase in TGF- β at the beginning of the wound. [39]

In this study, we also assessed the effect of the combination of these two natural ingredients on the acceleration of wound healing by measuring the wound size. Based on the results of data processing on wound measurements, it was seen that the wound size on day 3, 7, and 14 in the entire treatment group was smaller than the size of the wound in the control group where the wound size was the smallest, namely in the sulfadiazine group followed by a combination of Propolis and liquid smoke. This finding supports our hypothesis. Despite this, an assessment of the degree of epithelialization through microscopic examination is recommended for subsequent research. Wounds that do not have adjacent wound edges, wound contractions will make the wound shrink in size (secondary healing).

CONCLUSION

The results of this investigation show that the topical application of combination propolis and coconut shell liquid smoke enhances the wound repair process seen from wound size by decrease TGF β level on day 7 to day 14.

AUTHOR'S CONTRIBUTION STATEMENT

Conception and design of study: Aida Ayu Chandrawati, Marhaen Hardjo, Syahrijuita Kadir

Acquisition of data: Aida Ayu Chandrawati, Rosdiana Natsir, Sartini

Analysis and interpretation of data: Aida Ayu Chandrawati, Marhaen Hardjo, Syahrijuita Kadir

Statistic Analyze: Andi Alfian Zainuddin

Drafting the manuscript: Aida Ayu Chandrawati

Revising the manuscript critically for important intellectual content: Aida Ayu Chandrawati, Marhaen Hardjo, Syahrijuita Kadir, Rosdiana Natsir, Sartini, Andi Alfian Zainuddin

Approval of the final version of the manuscript to be published: Aida Ayu Chandrawati, Marhaen Hardjo, Syahrijuita Kadir, Rosdiana Natsir, Sartini, Andi Alfian Zainuddin

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

CV Indonesia Green Innovation had provided propolis products and coconut shell liquid smoke.

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