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Coconut Shell Liquid Smoke Promotes Burn Wound Healing

Vita Murniati Tarawan, MD, PhD[1], Kemala Isnainiasih Mantilidewi, MD, PhD[1], Ika Miqra Dhini, MD, MHS[1], Putri Teesa Radhiyanti, MD, MHS[1], and Endang Sutedja, MD, PhD[1,2]

Abstract

The aim of this study was to evaluate burn wound healing activity of coconut shell liquid smoke (CS-LS) in a burn wound animal model. Burn wound–induced mice were treated with CS-LS (CS-LS group), povidone iodine 10% (povidone group), or NaCl 0.9% (NaCl group). Application of CS-LS promoted wound contraction compared to that of the povidone and NaCl groups ($P < .05$). This study showed a positive correlation between the number of fibroblasts and wound contraction. The number of fibroblasts was highest in the CS-LS group, compared to that of the povidone and NaCl groups ($P < .05$). In conclusion, CS-LS promotes burn wound healing by one possible mechanism, by increasing the number of fibroblasts. The results indicate that further experimental trials are needed to develop CS-LS as an alternative topical drug for burn wound healing.

Keywords

burn wound, coconut shell liquid smoke, fibroblast, wound contraction

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Burn wound has become a major trauma problem worldwide due to its complications.[1] Complications can be prevented by proper early management to prevent pain, discomfort, and minimal scar formation.[2,3] Burn wound can be classified based on its grade according to depth and severity. Grade 1 (superficial) is limited to the epidermal layer, wherein a white plaque and minor pain occur at the site of injury and repair takes place spontaneously and the burn wound resolves within 3 to 4 days without any complication. Grade 2 (partial thickness) involves all epidermis and the upper part (papillary) of the dermis and may also involve the deep (reticular) dermis. This type of wound clinically can be seen by erythema and the formation of a superficial blister. Grade 2 involves a longer healing process of about 1 month, with minimal cicatrix. Grade 3 burns occur with loss of epidermis until the subcutaneous tissue. Grade 4 burns involve a deeper layer of muscle, tendon, and ligament tissue.[4] Of all the burn cases most presents as grades 2 and 3 burn injuries.[5]

The healing process involves 3 phases, inflammation, proliferation, and remodeling. Phase 1, the inflammation process, is the process in which injured tissues release cytokines and growth factor that attract neutrophils and monocytes within the first 24, or 36 to 72 hours after the wound, respectively. Once in the tissue the monocytes are activated and transformed into macrophages. The second phase of proliferation involves a key role of fibroblasts, the principal residents of dermal layer, to proliferate and then secrete extracellular matrix components such as collagen, thereby providing strength for the wound's contraction. Last, the remodeling process involves the balance between synthesis and degradation of extracellular matrix to organize the newly formed extracellular matrix, resulting in a normal strength and elasticity of the skin.[6]

The most common antiseptic used for various wounds is povidone iodine 10%.[7] However, many side effects may occur such as skin irritation, and for the same reason, people may look at alternative topical agents for wound healing. Many cell metabolisms involve oxidative reaction and result in the

[1] Universitas Padjadjaran, Bandung, Indonesia
[2] Dr. Hasan Sadikin Hospital, Bandung, Indonesia

Corresponding Author:
Kemala Isnainiasih Mantilidewi, MD, PhD, Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Padjadjaran, Jalan Eijkman No. 38, Bandung 40161, Indonesia.
Email: kmantilidewi@gmail.com

production of reactive oxygen species. In the healing process, a high rate of cell metabolism is needed to repair the injury and therefore reactive oxygen species production will be increased.[8] Based on this consideration, there is an increasing focus on the use of antioxidants to protect from deleterious effects of reactive oxygen species in causing further tissue injury, and thereby promoting the healing process.[3,9] The source of antioxidant can be from many resources including traditional medicine.[6,10]

Coconuts (*Cocos nucifera*) are found in tropical countries and are considered as important fruit crops, including their use as traditional medicines. Even though the shells often are considered as agro wastes, they contain the highest antioxidant properties.[10] Pyrolysis method, a chemical change brought about at 400°C, will result in liquid smoke, a solution due to the condensation of vapor of wood smoke.[11] Coconut shell pyrolysis will result in a product called coconut shell liquid smoke (CS-LS).

CS-LS is increasingly studied for its application in various conditions due to its chemical components, such as phenols, due to their antioxidant, anti-inflammatory, and antimicrobial properties.[10] Other alternative topical agents derived from *Argemone mexicana* L, *Aloe vera* L, and *Nerium indicum* Mill contain antioxidant polyphenols that promote wound healing.[12] Despite the reported medicinal importance and activities of CS-LS, the effect of CS-LS in promoting wound healing is still unclear. In this article, we have studied the effect of CS-LS as an alternative topical agent for burn wound.

Materials and Methods

Materials

Herbals. The herbals used in this study were all commercially available as liquid smoke and were purchased from small industry CV Wulung Prima, supervised by the Faculty of Agricultural Technology, Bogor Agricultural University (Bogor, Indonesia). The herbals that were used in the current project were disposed. Any future research planned to be done requires freshly prepared herbals to maintain the quality of the herbals in every experiment.

Experimental Animals. Male mice ages 8 to 10 weeks, weighing 20 to 30 g, were kept in cages with free access to food and water.

Chemicals. The following chemicals were acquired: povidone iodine 10% (Betadine; Kimia Farma Tbk, Jakarta, Indonesia), penthotal (Bratachem, Jakarta, Indonesia), and chloroform (Bratachem).

Methods

Burn Wound Induction. Burn wound induction was performed following a Mason-Walker burn model as guideline, with several modification.[4] On burn induction day (day 1) mice were anesthetized using penthotal 4 mg/kg BB IP before burn wound induction. A thin metal-half cylinder was heated for 60 seconds above an open flame and then pressed softly on a clean-shaved dorsal area for 10 seconds to produce a grade 2 burn area of 1 cm diameter. Immediately after burn wound induction, mice were randomized and divided into 3 groups, each consisting of 12 mice. They received topical application of 1 of the following 3 treatments: CS-LS (CS-LS group), normal saline 0.9% (NaCl group) as control, and 10% povidone iodine (povidone group) as another control because of its common use as an antiseptic during burn wound treatment.[7] The 2 times daily application was continued for 25 days with the wound left open.

Assessment of Wound Healing. Macroscopic evaluation of wound healing was done by measuring wound contraction. The measurement was done at 1, 5, 10, and 25 days after burn induction. The wound area is measured by imprinting the area on a transparent paper, and then the diameter of wound is measured using a ruler.

Wound area was then calculated using the following formula: $\pi \times r^2$, where $\pi = 3.14$ and r is half of the wound diameter. By taking into account the initial size of the wound (day 1) as 100%, the percentage of wound closure (wound contraction) was calculated using the following formula[2]:

$$\frac{(\text{Initial wound size [Area on day 1]}) - \text{Area on day 5, 10, or 25}}{\text{Area on day 1}} \times 100\%$$

Histological Analysis. Microscopic evaluation was done following measurement of wound contraction on days 1, 5, 10, and 25. Three mice from each group were anesthetized, sacrificed, and skin tissue of the burn wound (approximately 0.5 cm × 0.5 cm × 0.5 cm size) was taken surgically. Mouse skin tissues were fixed with 4% paraformaldehyde in phosphate-buffered saline for 3 hours at room temperature, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Images were obtained with a light microscope. Three fields of view at 200× and 400× magnification were randomly taken from each mouse sample to count the number of fibroblasts manually.

Statistical Analysis

The percentage of wound area and the number of fibroblasts for each group are presented as mean ± standard deviation and were analyzed by analysis of variance followed by Bonferroni's test. Correlation test was done by Pearson correlation test. All the statistical analyses were performed with SPSS software version 16.0 for Windows, with a P value of <.05 considered as statistically significant.

Results

Acceleration of Wound Healing by CS-LS Through Promotion of Wound Contraction

Observation of wound healing was done by calculating wound contraction. The observation was done by taking into account the initial size of the wound (day 1) as 100%, and then wound contraction was calculated on days 5, 10, and 25 to evaluate the inflammation, proliferation, and remodeling phases, respectively. Our results showed that on day 5 there was a significant difference of wound contraction in the CS-LS group when compared to that in the NaCl and povidone groups ($P < .001$). On day 10 the percentage of wound contraction was significantly different in the CS-LS group when compared to that in the povidone group ($P < .001$). Last, on day 25 the difference of percentage of wound contraction was statistically significant in the CS-LS group when compared to that in the NaCl group ($P < .05$; Figure 1). These data indicate that the CS-LS group

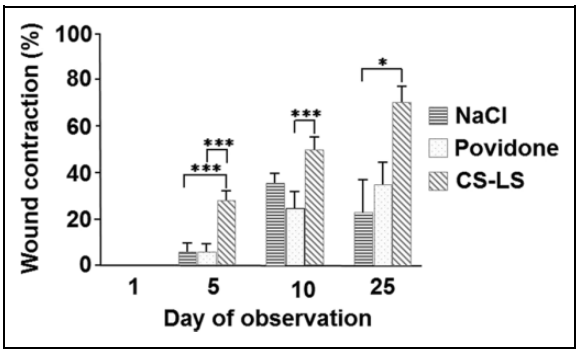

Figure 1. Importance of coconut shell liquid smoke (CS-LS) in the wound contraction. Measurement of wound contraction was done at 1, 5, 10, and 25 days after burn induction. *$P < .05$, ***$P < .001$ (one-way ANOVA and Bonferroni's test).

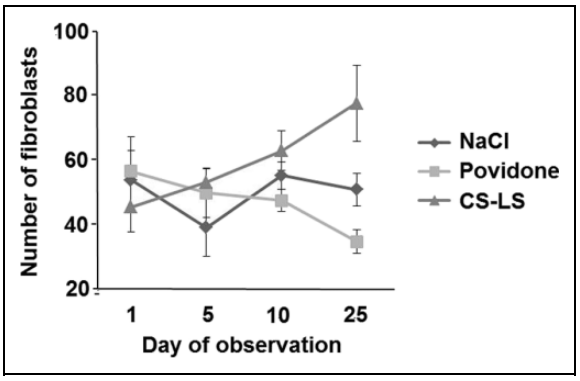

Figure 2. Effect of coconut shell liquid smoke (CS-LS) in the number of fibroblasts. After measuring wound contraction on days 1, 5, 10, and 25 of observation, 3 mice from each group were sacrificed and skin tissues were obtained for paraffin block and then processed for histological analysis using hematoxylin-eosin staining. Data are means ± SE from 3 mice, with 3 fields of view being examined for each mice.

shows faster wound contraction when compared to both the povidone and NaCl groups.

Application of CS-LS Increases the Number of Fibroblasts

Following measurement of wound contraction on days 1, 5, 10, and 25 in all mice, 3 mice from each group were sacrificed and skin tissues were obtained for paraffin block and was processed for histological analysis using hematoxylin-eosin staining. Microscopic images were taken using a light microscope and the number of fibroblasts was counted manually and the mean number of fibroblasts presented (Figure 2). We observed that although the number of fibroblast showed a similar increasing trend for both CS-LS and NaCl groups, by one way analysis the significant difference in the number of fibroblasts between day 1 and day 25 occurred only in the CS-LS group and not in the NaCl group. In contrast, the trend showed a decrease in number as wound healing progressed in the povidone group (Table 1).

Table 1. Correlation Between Number of Fibroblasts and Time of Observation.

Treatment	Pearson Correlation Coefficient	*P*
NaCl	.066	.840
Povidone	−.595	.041
CS-LS	.720	.008

Abbreviations: NaCl, sodium chloride; CS-LS, coconut shell liquid smoke.

Table 2. Correlation Between Wound Contraction and Number of Fibroblasts.

Treatment	Pearson Correlation Coefficient	*P*
NaCl	.590	.047
Povidone	.593	.005
CS-LS	.506	.004

Abbreviations: NaCl, sodium chloride; CS-LS, coconut shell liquid smoke.

Treatment of CS-LS Promotes Wound Contraction Through Increasing the Number of Fibroblasts

When we tried to show the correlation of wound contraction with fibroblasts, all treatment groups showed that wound contraction was directly correlated with the number of fibroblasts (Table 2).

Discussion

There are several goals during management of burn wounds: reduction of pain, prevention of infection, dehydration and wound conversion, and last rapid healing to minimize scar formation.[1,6] We compared the use of CS-LS, a traditional medicine as an alternative topical agent for burn wound, to those commonly used in society, normal saline and povidone iodine.

The wound healing process undergoes 3 processes, inflammation, proliferation, and remodeling.[13] The 3 processes can overlap. Our results first showed that CS-LS accelerates wound healing through the promotion of wound closure (Figure 1). Overall, the CS-LS group showed steady acceleration of wound contraction compared to both NaCl and povidone groups, yet statistically the significant difference among the groups varies with time of healing.

The effect on wound contraction among the treatment groups is most significant on day 5, of which obvious wound contraction was seen in the CS-LS group while it was limited in the NaCl and povidone groups. However, on day 10, the percentage of wound contraction was significantly different in the CS-LS group when compare to that in the povidone group. Last, on day 25, the difference of percentage of wound contraction was statistically significant in the CS-LS group when compared to that in the NaCl group. Only CS-LS application showed a steady increased rate of wound contraction in a time-dependent manner, indicating a normal rate of wound healing

process compared to that of the application of normal saline or povidone iodine.

Components CS-LS that may participate in the wound healing process are flavonoids, phenols, and tannins, which act as antioxidants, antimicrobials, and anti-inflammation agents.[10,12,14] Flavonoids are known to mediate the wound healing process by increasing collagen and protein, as well as decreasing lipid peroxidation in granulation tissue. These outcomes indicate a free radical scavenging activity.[14] Besides flavonoids, phenols in CS-LS also have antioxidant effects.[12] Phenolic compounds protect against increased oxidative stress that can prevent normal wound contraction.[10] With antioxidants, inhibition of prostaglandin, cyclooxygenase, and lipoxygenase can activate proline hydroxylase to reduce tissue injury.[15]

A previous study indicated that flavonoids, besides their antioxidant properties, also possess antimicrobial activity that helps in wound contraction and faster epithelization.[16] Experimental study using an excision wound model has also shown promising results. In this study, topical application of an Indian traditional medicine consisting of several herbals with antimicrobial activity on excision wounds revealed faster reduction in wound area as compared to the application of either modern topical medicine or untreated controls.[17] Therefore, the effect of CS-LS in enhancing wound contraction almost to full contraction may be mediated through antioxidant and antimicrobial properties. These findings suggest that further investigation is needed to study the role of individual fractionate compounds of CS-LS in the promotion of wound contraction in burn wounds.

Another parameter of wound healing observed in this study is the function of fibroblasts in mediating wound healing. When we compared individual treatments, although the number of fibroblasts showed a similar increasing trend for both CS-LS and NaCl groups, the significant difference in the number of fibroblasts between day 1 and day 25 was only seen in the CS-LS group and not in the NaCl group. In contrast, a decreasing trend was observed in the povidone group (Figure 2 and Table 1).

The second phase of the healing process involves a key player, fibroblasts. Attracted by secreted fibroblast growth factor from neutrophils, fibroblasts proliferate and synthesize collagen. The extracellular matrix produced will strengthen the edges of the wound and causes wound contraction. In between the 2 edges called granulation tissue is the site of fibroblasts. During wound healing fibroblasts in conjunction with other cells consume oxygen that dissolve with superoxide, the molecules important to fight infection, and further produce more growth factors. Therefore, fibroblast migration and proliferation in the wound site is critical for the wound healing process.[6]

We propose that a possible mechanism of CS-LS in promoting burn wound healing is by increasing the number of fibroblasts. Our results show the direct correlation between wound contraction and fibroblasts (Table 2). Several constituents of CS-LS that may promote fibroblasts proliferation are tannins and phytosterols. One study found that these compounds promote the wound healing process by increasing capillary formation as well as fibroblast proliferation. These result in enhancement of the rate of epithelization.[18] Phenols with its antioxidant activity reduce lipid peroxidation that stimulates fibroblasts activity for the synthesis of fibronectin, thus promoting wound healing.[8] Evaluation of different active compounds of CS-LS in fibroblast proliferation remains to be determined in future studies. In the wound healing process, fibroblasts transform into myofibroblasts functions to secrete collagen to ensure the tensile strength of the wound.[6] Therefore, studies on the effect of CS-LS on myofibroblast formation should become an important issue.

Conclusions

Our data support the beneficial use of CS-LS compared to normal saline and povidone as a topical agent in mice with burn wound. Further research studies such as evaluation of individual fractionate compounds in CS-LS in wound healing and clinical trials must be done to develop CS-LS as an alternative topical drug to promote burn wound healing.

Authors' Note

This study performed animal experimental methods and has followed guideline principles (3Rs) on the care and use of laboratory animals described by Russel and Burch in 1959.

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Author Contributions

VMT participated in the design of the research, supervised the experiment, and provided mentorship support. KIM supervised the experiment, drafted the article, and provided mentorship support. IMD participated in the design of the research and conducted the experiment. PTR supervised the experiment. ES participated in the design of the research, supervised the experiment, and provided mentorship support. All authors read and approved the final version of the article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

This study was approved by the Ethical Committee of Faculty of Medicine Universitas Padjadjaran and was conducted in accordance with the internationally accepted principles for laboratory animal use and care (No. 459/UN6.C2.1.2/KEPK/PN/2014).

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