Effectiveness of natural lip balm cinnamon (Cinnamomum burmannii) in accelerating the incision wound healing process in *Rattus norvegicus*

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ABSTRACT

Introduction: Cinnamomum burmannii is commonly used in the treatment and is thought to be an anti-inflammatory medicinal herb that may hasten recovery. Lip wound healing is a critical need for aesthetic performance. The purpose of this study is to evaluate the efficacy of natural lip balm *C. Burmannii* has been shown to speed up the healing of incision wounds in rats (*Rattus norvegicus*). Methods: This research was carried out in the laboratory biomedical Faculty of Medicine, Universitas Islam Bandung. In twenty-seven male rats weighing 200-300 g, a lip mucous incision of 10 mm length and 4 mm depth was surgically created. Simple random sampling was used to divide the animals into three treatment groups based on wound treatment after lip incision (The control group applied distillation water, the first group applied a lip balm placebo/base, and the second group applied natural lip balm *C. burmannii* and then divided into three observation times (days 3rd, 7th, and 14th). A lip biopsy was performed during each observation time to determine the success of the therapy. The macroscopic examination was carried out by counting the number of lip wounds that were closed around, while the microscopic examination was carried out by counting epithelialization with a Leica microscope and image raster software. Results: The length of the lip wound has greatly decreased in the *C. Burmannii* group with a p-value of 0.048 (p<0.05, and epithelialization was comparable across the board. Conclusion: The use of *Cinnamomum burmannii* natural lip balm is effective in accelerating lip wound healing by bringing the lip wound closer together. Effectiveness of Natural lip balm Cinnamon (Cinnamomum burmannii) in accelerating the incision wound healing process in *Rattus norvegicus*.

Keywords: lip balm; *cinnamomum burmannii*; wound; epithelialization; healing process

p-ISSN: 1979-0201; e-ISSN: 2549-6212; Available from: [http://jurnal.unpad.ac.id/pjd/article/view/9593](http://jurnal.unpad.ac.id/pjd/article/view/9593)  
DOI: 10.24198/pjd.vol34no3.9593  
Submission: May 28, 2022; Accepted: Nov 31, 2022; Published online: Nov 31, 2022

INTRODUCTION

Wound healing is an essential survival mechanism that is sometimes disregarded. The research on interrupted wound healing is limited, and there is no standard categorization for wound healing in the oral region. Wound healing is made up of a series of intricate biological processes. To complete the

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healing process with the fewest possible scars, all tissues follow an almost identical pattern.

The outcome of wound healing in the oral cavity might differ from that of a clinically healed wound. Wound healing occurs in the mouth cavity, which is a unique environment hosting millions of microorganisms. In the oral cavity, lesion healing can range from a clinically healed wound with minimal scarring and histologically normal connective tissue beneath epithelial cells to severe trismus induced by fibrosis. Oral wound healing is influenced by a variety of local and general variables, and a better knowledge of these aspects can aid in addressing difficulties that contribute to poor oral wound healing.1

There are slight differences in wound healing in the skin and oral mucosal areas. Oral and cutaneous wound closure and inflammatory responses can be stimulated by human saliva. As a result, saliva might be a promising new treatment option for open skin wounds.2 Wound healing is a multi-step mechanism that improves the structure and function of injured tissues. Multiple growth factors and cytokines produced at the wound site regulate the process precisely. Any modifications that impede the healing process might accelerate tissue injury and prolong the repair process. Infections, underlying disorders, and drugs can all lead to poor wound healing.3 Bioactive chemicals present in foods and medicinal plants are appealing molecules for the creation of novel medications to treat various degrees of diseases, including those connected with inflammatory processes, which are frequently linked to oxidative stress.4

Natural compounds having anti-inflammatory, antioxidant, antibacterial, and pro-collagen synthesis capabilities have been studied extensively as wound healing agents. In developing countries, traditional medicine is used by 80% of the population to manage fundamental medical concerns. As a result, research has lately been done to identify novel effective, and safe therapeutic compounds derived from natural sources for the management of a range of ailments. For many years, indigenous plants have been employed for this purpose by many tribes all over the world.5 Cinnamon (Cinnamomum spp., Lauraceae family) refers to a group of about 250 evergreen trees native to Asia, China, and Australia. Burmannii Cinnamon (also called Korintje, Java, or Indonesian cinnamon). Cinnamon extracts and essential oils have been obtained from several sections of the plant, including the leaves, bark, fruits, root bark, flowers, and buds. There are more than 80 compounds known, and their compositions change owing to a variety of variables.

Cinnamomum spp. Main Constituents and Antibacterial Properties Against Oral Pathogens. The primary ingredient of cinnamon EO and extracts was reported to be trans-Cinnamaldehyde (t-Cinnamaldehyde or (E)-Cinnamaldehyde). Cinnamon’s organoleptic and antibacterial characteristics are mostly due to this molecule. Although it is generally associated with clove, eugenol is another potent component found in cinnamon essential oil. Its powerful antibacterial qualities may have a key impact on dental health because it is one of the most prevalent components in cinnamon EO and extracts. Linalool, B-Caryophyllene.6 Cinnamon is widely used in medicine and is proven as an antihyperlipidemic, immunomodulatory, antihypercholesterolemic, and analgesic.7,8,9,10

Lip balm is a type of wax that is applied to the lips as a moisturizer to keep them from drying out and cracking. Lip moisturizers are widely used in situations when protection is required, such as when the air humidity is low or the temperature is too low to prevent water evaporation and mucosal epithelial cells on lips. Because of the weak protective function, the lips are particularly vulnerable to external impacts, such as excessive heat, which can cause the lips to become dry, chapped, and dull-colored Lip salve is a cosmetic preparation manufactured with the same foundation as lipstick, although there are significant variations between the lipstick and a lip moisturizer that are mostly functional.

Lip balm creation employing the most natural materials possible and formulation assessment These natural ingredients and safer alternatives to synthetic excipients were used to effectively construct a lip balm formulation.11,12 Natural materials are now strongly suggested for medical therapy since they are safer and less expensive. Cinnamon has previously been used as a medicine to reduce urea levels, repair chronic inflammation, antidepressant, and treat liver damage.13,14,15 Cinnamon is used as a treatment because it contains active chemicals. Cinnamaldehyde and
cannabinoids were important chemicals with anti-inflammatory and antibacterial effects. Other research has not employed cinnamon as a wound healing material in the face area, which demands not just rapid but also aesthetically good healing materials. Therefore, we conducted this study to analyze the effectiveness of natural lip balm C. Burmannii to accelerate the lip wound healing process in rats (Rattus norvegicus).

**METHODS**

**Animal and groups and treatment**

27 adults male Wistar rats, weighing between 200 and 300g. The animals were kept in regular animal laboratory housing with free access to standard food and unlimited access to water in this environment, and they were given a week to get acclimated to the environment. Animals were divided into three treatment groups based on the method used to treat lip wounds (the control group was given distilled water, group 1 was given placebo lip balm, and group 2 was given C. burmannii); and finally, each treatment group was divided into three observation time groups (Day 3, 7, and 14). Ketamine (50 mg/kg BW) was injected intramuscularly to induce anesthesia in the rats. A scalpel that measured 10mm in length and 4mm in depth was used to make an incision on the animal’s lower lip. The lower lip of the animal was incised with a scalpel of 10mm in length and 4mm in depth. Each group received different materials for treatment. The control group used distilled water, the first group used a placebo (basic) lip balm, and group 2 used C. burmannii natural lip balm. All of the wound incisions were to be covered by the daily therapy material given to the lip incision area. Using a digital camera and a ruler as a scale, the lip incision sites were photographed on the intervention day and at the end of each observation period. At the end of the research, image raster software examined the images from each day.

All animals were sacrificed the day of each observation period, and the lip tissues around the incision were collected. Each sample was cut into pieces and preserved in 10% formalin to assess histological changes.

**Natural lip balm cinnamomum burmannii**

Cinnamomum plants were obtained from the Surakarta plantation which had been tested for plant determination at the ITB School of Life Science and Technology with registration number 1282/IT1.C11.2/TA.00/2022. Nature lip balm C. Burmannii was made with the composition of 45g olive oil, 24g candelilla wax, and 45g cupuaçu butter, then put in a water bath at 100 °C to form a homogeneous mixture called the base. Furthermore, up to 18 grams of cinnamon leaf powder are added. Once homogenous, pour into a lip balm molds tube and use a universal indicator to determine the pH of the liquid. To stabilize the lip balm, let the liquid at room temperature for 48 hours until completely solidified.

**Clinical evaluation**

Ulcer length was measured with a ruler and photo analyses by image raster software. All measurements were performed by the same operator. Ulcer length was evaluated on day 3, day 7, and day 14 by camera handphone with specification 50MP, 10x optical zoom (Samsung S22 ultra).

**Histological analysis**

The animals were put to sacrifice on days 3, 7, and 14 so that lower lip samples could be taken for histological analysis. The specimens were rapidly fixed in 10% neutral buffered formalin for at least 24 hours. Using the standard method, the fixed specimens were embedded in paraffin. The prepared paraffin blocks were divided into pieces that were 5 m thick. These sections were stained with hematoxylin and eosin. Using a Leica microscope and image raster software, the microscopic study was evaluated by counting epithelialization and the macroscopic analysis by counting lip wounds closer. At 100 and 400 times magnification, tissue images were taken. In addition to that, pathologists do analysis in five different fields of view.

**Statistical analysis**

The relationship between the ulcer length and epithelialization variation from 9 experimental groups was analyzed using Kruskal Wallis
correlation analysis (SPSS 26 software) and p<0.05 showed statistical significance. The Faculty of Medicine Unisba’s ethics committee granted approval for all animal research in accordance with the Laboratory Animal Ethics ARRIVE guidelines 2.0 (001/KEPK-Unisba/II/2022).

RESULTS

In this study, the healing process of the rat’s lip in each group was evaluated, and the results showed a considerable accelerating activity related to Cinnamomum burmannii lip balm compared to the placebo lip balm and control group. The lip healing area was significantly reduced in natural lip balm C. Burmannii treated (P<0.05) (Table 1).

There was a significant lip wound closer (p<0.05) in a group with natural lip balm C. Burmannii application. The length of the ulcer decreased by observation time among a group (Figure 1). There was no significant change in epithelial length in all groups. Significant changes

| Group | Observation Day | Length (cm) | P value |
|-------|----------------|-------------|---------|
| C     | Day-3          | 0.9         | 0.9     |
|       | Day-7          | 0.9         | 0.9     |
|       | Day-14         | 0.9         | 0.9     |
| G1    | Day-3          | 1           | 0.9     |
|       | Day-7          | 0.9         | 0.8     |
|       | Day-14         | 0.8         | 0.8     |
| G2    | Day-3          | 0.7         | 0.6     |
|       | Day-7          | 0.7         | 0.6     |
|       | Day-14         | 0.5         | 0.4     |

Figure 1. Lip wound closer of experimental animal based on groups and observation day (the 3rd, 7th, and 14th day)
were seen on the third and seventh days of observation. Table 2 shows the results of measuring the thickness of epithelialization. Based on the treatment group, the average thickness in group 2 was better than the control group and placebo, but not statistically significant. Based on the time

| Control | Mice 1 | Mice 2 | Mice 3 |
|---------|--------|--------|--------|
| Day-3   | [A]    | [B]    | [C]    |
| Day-7   | [D]    | [E]    | [F]    |
| Day-14  | [G]    | [H]    | [I]    |

Figure 2. Epithelialization in the control group. Lower lip mucous were stained by Hematoxylin Eosin: A) B) C). Control group in day 3rd observation time; D) E) F). Control group in day 7th observation time; G) H) I). Control group in day 14th observation time.

| Group 2 | Mice 1 | Mice 2 | Mice 3 |
|---------|--------|--------|--------|
| Day-3   | [A]    | [B]    | [C]    |
| Day-7   | [D]    | [E]    | [F]    |
| Day-14  | [G]    | [H]    | [I]    |

Figure 3. Epithelialization in treatment group (group 1). Lower lip mucous were stained by Hematoxylin Eosin: A) B) C). Group 1 in day 3rd observation time; D) E) F). Group 1 in day 7th observation time; G) H) I). Group 1 in day 14th observation time.
of observation on the 3rd and 7th day, there were significant differences between the groups.

**DISCUSSION**

This present study showed natural lip balm *C. Burmannii* significantly accelerates lip wound healing by decreasing the length of lip wounds. Direct apposition of the epithelium to underlying collagen-dense connective tissue. Despite substantial structural and physiological similarities, skin and mouth mucosa recover in radically different ways in response to damage. The oral mucosa has a proclivity for quick restoration of barrier function with minimal underlying fibrosis, whereas skin heals slowly and scars accumulate. Matricellular proteins, which modulate cell activity, have been found to play important roles in cutaneous healing, although their involvement in oral mucosa repair is unclear. Wounds in the oral mucosa have been proven to heal substantially quicker and with less scar formation than skin wounds. This finding is accompanied by a decreased inflammatory response, which can be attributable to a decrease in neutrophil recruitment, and pro-inflammatory cytokines. The

| Observation Day | Group of treatment | C  | G1  | G2  | P value |
|----------------|--------------------|----|-----|-----|---------|
| Day-3          |                    | 0.04| 0.06| 0.07| 0.027   |
| Day-7          |                    | 0.06| 0.08| 0.11| 0.3     |
| Day-14         |                    | 0.06| 0.07| 0.23| 0.038   |
| Day-14         |                    | 0.09| 0.14| 0.15| 0.038   |
| Day-14         |                    | 0.05| 0.14| 0.25| 0.148   |
| Day-14         |                    | 0.13| 0.19| 0.13| 0.397   |
| P value        |                    | 0.807| 0.663| 0.397|

Figure 4. Epithelialization in treatment group (group 2). Lower lip mucous were stained by Hematoxylin Eosin: A) B) C). Group 2 in day 3th observation time; D) E) F). Group 2 in day 7th observation time; G) H) I). Group 2 in day 14th observation time.
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Pathology of inflammation is initiated by complex processes triggered by wound lip incision. The ulcer can directly activate macrophages, which trigger the production of inflammatory mediators. The pharmacological reduction of herbal medicine inflammatory mediators is regarded as one of the necessary conditions to alleviate various disorders caused by macrophage activation. C. Burmannii is used as an anti-inflammatory drug. Cinnamaldehyde, the principal constituent of leaf C. Burmannii.

Cinnamaldehyde has been demonstrated to exhibit antibacterial activities. However, many of the molecular pathways that contribute to lip healing being quicker than skin healing are still unknown. In recent years, several research has revealed that the extracellular matrix (ECM) composition is an important element in soft tissue healing. Matricellular proteins, in particular, have received a lot of attention recently due to their role in direct cell behavior modification by periostin as a possible treatment to promote soft tissue repair or decrease fibrosis.17,18

In the current investigation, we discovered that cinnamaldehyde significantly enhanced the epithelialization of lip incisions in rats while lowering pro-inflammatory markers. Wound healing necessitates a well-coordinated combination of numerous biological and molecular activities. Angiogenesis is a vital phase in wound healing because it regenerates blood vessels and allows for the oxygen supply required to encourage repair and vessel development. Angiogenesis is a series of processes that results in the development of new blood vessels from the pre-existing vascular network and is critical in wound healing.

For treatment, traditional medicinal herbs and pure chemicals extracted from plants have been employed. C. Burmannii contains the active chemicals cinnamaldehyde and coumarin, which serve as angiogenesis promoters. The possible method involves increasing VEGF secretion and activating both the PI3K/AKT and MAPK signaling pathways.19,20,21

These findings are similar to those of Khadeeja M Ali et al, who show that administration inhibited proinflammation production, enhanced anti-inflammatory markers IL-1B and TNF-α, and oxidative stress minimized the increase in the level in experimental animal models.22 Clinical studies performed by several researchers have demonstrated the application of medicinal plants to accelerate the healing process with anti-inflammatory effects by their bioactivity abilities to promote wound healing and prevent infection without grave side effects.23,24,25,26 According to another study, cinnamaldehyde effectively inhibited ox-LDL-induced vascular smooth muscle cells (VSMCs) proliferation, migration, and inflammatory cytokine overproduction, as well as foam cell formation in VSMCs and macrophages.27 It has been demonstrated in several research with other animals that epithelialization increases. Amin Daemi et al. in 2019 used topical Cinnamomum verum increased wound healing and may be used to treat diabetes patients’ wounds.28 On the other hand, the present study showed, the length of epithelialization was not significantly different among a group. It should be noted that this research used an animal model. However, there are inherent limitations in any animal model of human illness; in comparison to human lip inflammation, the experimental inflammation’s duration of the disease is brief and follows an acute phase.

CONCLUSION

Our finding indicates that the application of the natural lip balm Cinnamomum burmannii is effective to accelerate lip wound healing by faster lip wound closer.

ACKNOWLEDGEMENT

This study was funded by the Faculty of Medicine Universitas Islam Bandung and many thanks to the laboratory biomedical and the staff for their assistance in animal handling. Moreover, the authors would like to extend gratitude to the department of pathology anatomy for histological analyses.

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