

## The Role of Healing Effect of Lip Balm Application *Cinnamomum burmannii* on lip wound based on Immunohistochemical Interleukin 6 (IL-6) Levels

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### Abstract

The oral mucosa goes through a complicated series of biological healing processes to regain equilibrium after being injured. To heal wounds, we need a material that can heal wounds quickly and well. Cinnamon is widely used for therapy because it has good anti-bacterial, anti-inflammatory, and antioxidant effects. The purpose of this study was to analyze the healing effect of the application of lip balm *Cinnamomum* on lip incisions based on the level of immunohistochemical interleukin-6.

This experimental study was conducted at the laboratory biomedical Faculty of Medicine, Universitas Islam Bandung. A 10 mm length and 4 mm depth incision lip mucous was surgically created in twenty-seven male rats with healthy, 200-300mg of body weight, male, 3-5 months. Animals were divided by simple random sampling were divided into three groups, group 1: the animal treated lip healing with distilled water (control). Group 2: the animal treat lip healing with placebo lip balm, and lastly, group 3: the animal treat lip healing with lip balm *Cinnamomum burmannii*. Each group performs observation on day-3, 7, and 14. To evaluate the effectiveness of treatment, a lip biopsy performs during each observation time. The immunoexpression IL-6 analysis was assessed by scoring distribution and intensity expression with a Leica microscope. There were significant differences in the level of immunoexpression Interleukin-6 in a group with treatment by lip balm *Cinnamomum burmannii*. By the time of observation, day 14 of group 2 have a greater level of IL-6 compared with the other groups.

Application lip balm *Cinnamomum burmannii* is effective in faster and good remodeling lip healing process by seen increasing level of IL-6.

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### Introduction

Timely wound healing is critical to maintaining the skin's function as a protective barrier. The shift from a pro-inflammatory to a reparative milieu must be strictly controlled. Interleukin (IL)-6 is an important regulator of inflammatory and reparative processes, in leukocyte differentiation, activation, and proliferation, endothelial cells, keratinocytes, as well as fibroblasts.<sup>1,2</sup> Pro- and anti-inflammatory cytokines were mostly produced by macrophages. Macrophages were also important makers of

healing elements. Pro- and anti-inflammatory cytokines were produced by neutrophils and lymphocytes. Healing-associated components were significantly produced by non-leukocytic cells.<sup>3</sup>

The oral mucosa goes through a complicated series of biological healing processes to regain equilibrium after being injured. While there are some broad parallels between the mouth cavity and cutaneous epithelium, there are also significant variations in the genomes and kinetics of wound healing. Clinical researchers are now looking at alternative medicines and prospective autotherapies to improve intraoral healing due to the paucity of effective treatments for oral mucosal lesions. The current comprehensive analysis examines endogenous variables that affect the caliber of tissue remodeling and covers the current gold standards for oral mucosal

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wound healing.<sup>4</sup> Lips are oral mucosal tissue. wound healing on the lips has its challenges. The body's epithelial tissues divide the fastest and have a built-in capacity for renewal and regeneration. This skill is critical for survival because epithelia serve as the final line of defense against the outside environment, shielding the underlying tissues. In homeostasis and after damage, tissue stem and progenitor cells are in charge of self-renewal and healing. When epithelial tissues are injured, they go through many stages of hemostasis, inflammation, proliferation, and remodeling, which frequently lead to fibrosis and scarring.<sup>5</sup>

*Cinnamomum burmannii* is an organic shrub that has long been used as a spice, preservative, and flavoring. *C. burmannii* essential oils, which mostly comprise mono- and sesquiterpenes such as borneol, linalool, and caryophyllene, have outstanding medicinal effects.<sup>6</sup> Chinese cinnamon from subcritical extraction consisted of (*E*)-cinnamaldehyde, eugenol, and coumarin, while its ethanol extract presented procyanidin trimer, (*E*)-cinnamaldehyde, and (*Z*)-cinnamaldehyde. In addition, methanol extract of Indonesian cinnamon from Kerinci, Sumatra, showed the presence of catechin, epicatechin, procyanidin B2, quercitrin, 3,4-dihydroxybenzaldehyde, protocatechuic acid, and cinnamic acid by ultrasonic extraction, Alcohols: Cyclohexane methanol. Aldehydes: Trans-cinnamaldehyde. Acetate: Trans-cinnamyl acetate; bornyl acetate; acetate; bornyl acetate. Terpinenes: (-)-Spathulenol; caryophyllene; D-borneol; eucalyptol; guaiol.

Cinnamon has a wide range of biological activities, such as antioxidant, anticoagulant, antidiabetic, anti-inflammatory, anti-tumor, anti-cancer, anti-microbial, anti-fungal, antiviral, and gastro-protective properties, treating dental problems, antipyretic, cytotoxic, gene expression, immunological response, hepatoprotective, immunomodulatory, neuroprotective, ultraviolet (UV) protective, sun protection, wound healing, and toxicological research, and lowering blood pressure, cholesterol, and lipids.<sup>7,8</sup>

This study aims to analyze the effect of natural lip balm *Cinnamomum burmannii* to treat lip healing by assessing the level of Immunoexpression Interleukin-6.

## Materials and methods

### Animal and Groups and treatment

All animal experiments were according to laboratory animal ethics ARRIVE guidelines 2.0 ethical clearance was obtained from the ethics committee Faculty of Medicine Unisba (001/KEPK-Unisba/II/2022), Indonesia. Twenty-seven adults male Wistar rats, weighing 200-300g. The animals were kept housed under standard animal laboratory conditions with free access to standard food and unlimited access to water in this situation, they were allowed to acclimate to the laboratory environment for 7 days. They were divided into three groups of treatments and three groups of observation time. The rats received anesthesia that was induced by an intramuscular injection of ketamine (50mg/kg BW). The lower lip of the animal was incision with a scalpel of 10mm in length and 4mm in depth. Each group received different materials of treatment. The first group is a normal control group that applied distillation water. The second group applied lip balm placebo (base), and the third group applied natural lip balm *C. burmannii*. The lip incision area applied daily treatment material to cover all the wound incisions. Lip incision areas were photographed on the intervention day and the last of each observation time, using a digital camera, and a ruler was used as a scale. At the end of the study, pictures of each day were analyzed by image raster software. On the day of each observation time, all animals were sacrificed and lip tissues of the incision area were collected. Each sample was cut and kept in formalin 10% to evaluate histological changes.

### Natural Lip balm *Cinnamomum burmannii*

Nature lip balm *C. Burmannii* was made based on a modification of previous research, 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, the addition of cinnamon leaf powder is as much as 18 grams. Once homogeneous, pour into a lip balm molds tube and measure the mixture's pH using a universal indicator. The mixture should then be left to sit at room temperature until it is perfectly solidified for 48 hrs to stabilize the lip balm.

### Immunoexpression IL-6 analysis

On days 3, 7, and 14, the animals were euthanized, and lower lip specimens were obtained. The material is then stained with IHC IL-6. The histoscore study was performed with a Leica light microscope.

Scoring=Distribution X Intensity

Intensity: 0=none, 1=<25%, 2=25-50%, 3=51-75%, 4=>75%. Distribution: 0=negative, 1=weak positive, 2=moderately positive, 3=strong positive, 4=very strong positive.

Positive: brown color stains inflammatory cell membranes and/or keratinocytes in the epidermis. Negative: brown color stains the cytoplasm or does not stain the membrane

### Statistical analysis

Comparison immunoexpression IL-6 from 9 experimental groups was analyzed using Kruskal Wallis correlation analysis (SPSS 26 software) and P < 0.05 showed statistical significance.

### 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 the application Cinnamomun burmannii lip balm compared to the placebo lip balm and control group by the level of Immunoexpression Interleukin-6.

Group	Distribution	Intensity	Score	P-Value
Day 3	2	1	2	0.030
	1	1	1	
	1	3	3	
Day 7	3	2	6	
	0	0	0	
	3	2	6	
Day 14	0	0	0	
	1	3	3	
	1	1	1	

**Table 1.** IL-6 immunoexpression of the control group.

IL-6 immunoexpression in the control group was highest on the 7th day of observation with an average score of 4 (Table 1). IL-6 immunoexpression in the group 1 (placebo lip balm) was highest on the 14th day of observation with an average score of 6 (Table 2). IL-6 immunoexpressions in the group 2 (lip balm Cinnamomun) was highest on the 14th day of observation with an average score of 12 (Table

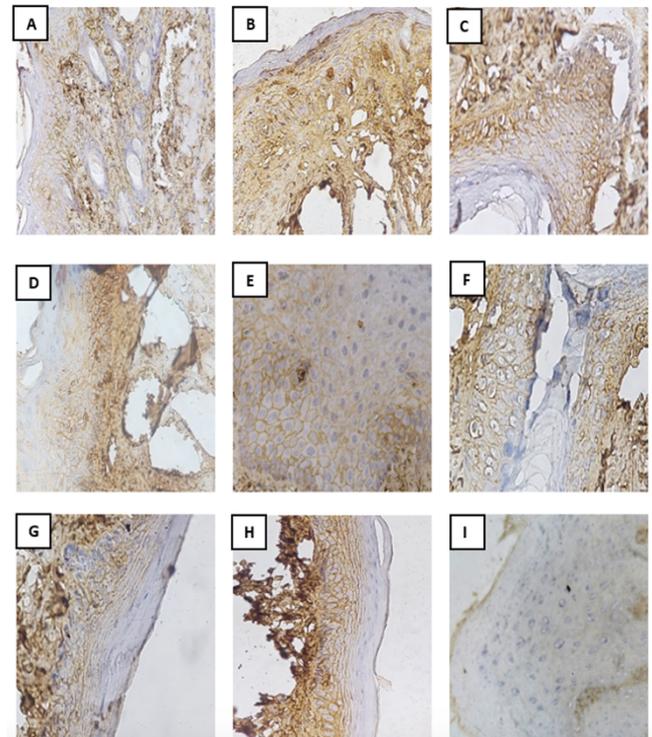
3). From the table, it can be seen that the highest value in group 2 with an observation time of day 14.

Group	Distribution	Intensity	Score	P-Value
Day 3	2	2	4	0.136
	3	2	6	
	2	2	4	
Day 7	3	2	6	
	3	2	6	
	3	1	3	
Day 14	4	1	4	
	2	3	6	
	3	3	9	

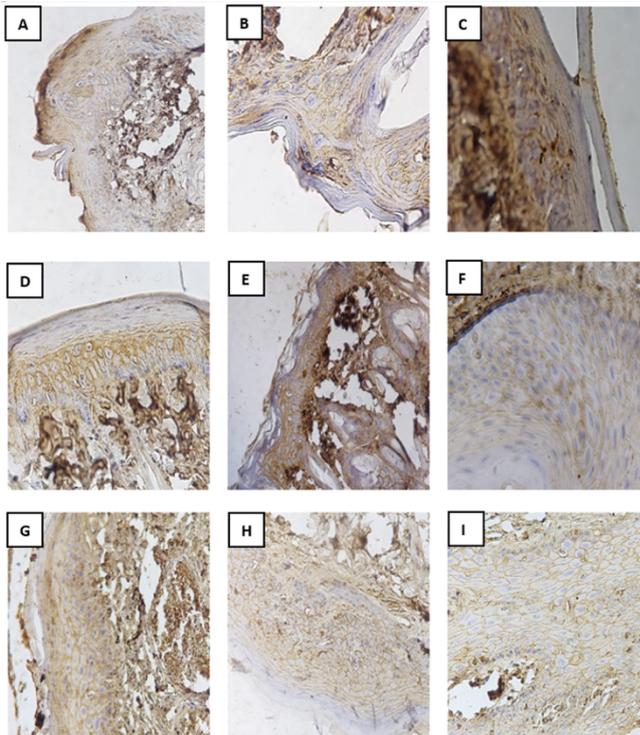
**Table 2.** IL-6 immunoexpression of group 1.

Group	Distribution	Intensity	Score	P-Value
Day 3	4	2	8	0.024
	4	2	8	
	3	2	6	
Day 7	3	2	6	
	4	2	8	
	4	2	8	
Day 14	4	3	12	
	4	3	12	
	4	3	12	

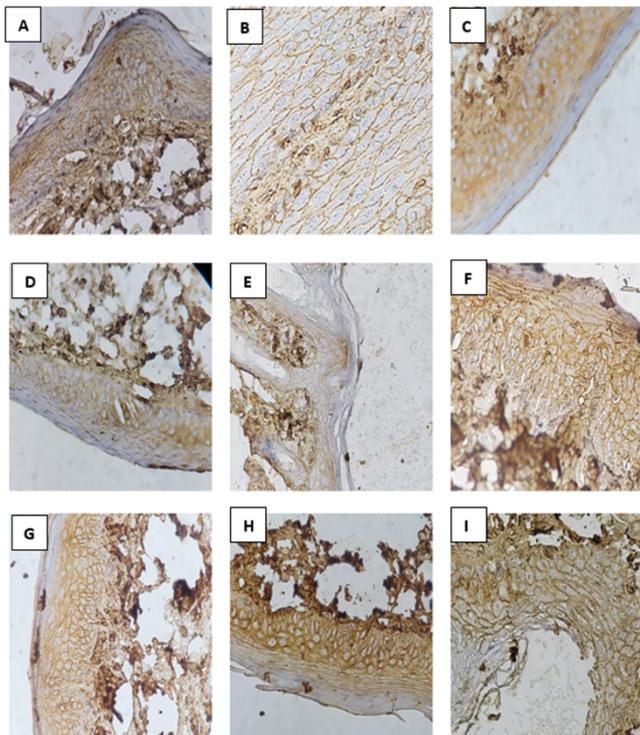
**Table 3.** IL-6 immunoexpression of group 2.



**Figure 1.** Immunoexpression of IL-6 on the 3rd day of observation. A, B, C control group. D, E, F treatment group 1. G, H, I treatment control group. 400x magnification.



**Figure 2.** Immunoeexpression of IL-6 on the 7th day of observation. A, B, C control group. D, E, F treatment group 1. G, H, I treatment group 2. 400x magnification.



**Figure 3.** Immunoeexpression of IL-6 on the 14th day of observation. A, B, C control group. D, E, F treatment group 1. G, H, I treatment group 2. 400x magnification.

IL-6-stained tissue sections (Figs 1, 2, and 3) of 27 preparations analyzed by pathologists. The highest distribution with spread up to >75% with staining intensity IL-6 on inflammatory cell membranes and/or keratinocytes in the epidermis had a strong intensity in group 2. On administration of cinnamon lip balm, IL-6 reactivity was detected in cell membranes and keratocytes. However, group 1 is mainly confined to cell membranes. There was a significant difference based on the time of observation, both the control group and the group that was given cinnamon lip balm. However, the highest value was in group 2.

### Discussion

In this study, giving cinnamon lip balm was effective in wound healing, by looking at the level of IL-6 immunoexpression with a score of 12. Distribution value >75% with strong intensity. The highest value was observed on the 14th day. In the control group, the level of IL-6 immunoexpression was highest on day 7, while in treatment group 1, namely the group given placebo lip balm, the level of IL-6 immunoexpression was highest on day 14. Wound healing is a complex and dynamic cascade of cellular and molecular interactions spanning four major phases: hemostasis, inflammation, proliferative, and remodeling. Therefore, several pharmacological activities such as anti-inflammatory, antioxidant, and antimicrobial activities can play an important role in the wound healing process. Interleukin-6 (IL-6) as a T cell product that appears to stimulate B cells will increase antibodies. IL-6 production appears to be a major regulator of inflammation and one of the few truly pleiomorphic cytokines, alongside IL-1 and tumor necrosis factor (TNF).<sup>9,10</sup>

Research on other natural ingredients was conducted by WY. Zhao stated that the administration of natural ingredients can inhibit inflammation by lowering IL-6 and can also increase angiogenesis by increasing VEGF, resulting in better wound healing. Wound management has now evolved in various ways and focuses on accelerating healing with the material applied. In the inflammatory phase, macrophages tend to be polarized to M1 and produce pro-inflammatory cytokines that will trigger tissue inflammation. Pro-inflammatory

cytokines produce TNF- $\alpha$ , IL-1, and IL-6 to stimulate the Toll-like receptor (TLR). TLRs are found on the surface of macrophages and stimulate pro-inflammatory cytokines and activate signaling pathways to activate NF- $\kappa$ B.<sup>11,12</sup> This study found that level of immunoeexpression Interleukin-6 increased in group 2, especially on day-14. This finding indicates that the healing process time by time began to improve. Cinnamon contains antimicrobial active chemicals such as cinnamaldehyde and eugenol, which is frequently employed as a preservative due to its antibacterial action. In vitro testing has shown that cinnamon extract is an efficient antimicrobial against *Staphylococcus aureus* and *Escherichia coli*.<sup>13</sup> *C. burmannii* contents (coumarins, polymers of proanthocyanidins A-type, and protonated heterodimer of flavan-3-ol group).<sup>14</sup> According to research by Helmy Abdou, et al. (2019), *C. burmannii* extract may have protective effects against the hepatic damage brought on by multi-walled carbon nanotubes (MWCNTs) by strengthening the antioxidant system and reducing the production of pro-inflammatory cytokines.<sup>15</sup>

In this study, giving cinnamon lip balm can accelerate healing, and the remodeling process is achieved faster, at the end of the third week the proliferation process increases, seen from the higher levels of IL-6. The main pro-inflammatory cytokine in the IL-6 family is interleukin (IL-6). The physiological regulation of immunological homeostasis, hematopoiesis, inflammation, development, and metabolism depends on reactions induced by each member of the IL-6 family. As a result, misregulation of these cytokine activities frequently causes chronic illness and malignancy; the clinical significance of this is demonstrated by the fact that medications that target the IL-6 pathway are effective in treating several autoimmune diseases.<sup>16,17</sup> An inflammatory cytokine with pleiotropic effects is interleukin-6 (IL-6). Chronic inflammatory conditions and multiple autoimmune diseases are linked to its dysregulation. IL-6 is a key target for the therapy of these complicated disorders due to its involvement in their pathogenesis.<sup>18</sup> Interleukin-6 (IL-6) is a cytokine that has a role in metabolism, brain cell maintenance, and both innate and acquired immunity. Numerous cell types, including myeloid cells, fibroblasts, endothelial cells, and lymphocytes, are capable of producing

IL-6. Toll-like receptors and IL-1 both have considerable stimulatory effects on IL-6 production. As a result, whether there is an infection or an inflammatory process, IL-6 levels in the body are elevated.<sup>19</sup>

Skin integrity needs to be quickly recovered following damage to sustain its functions. The extracellular matrix, resident skin cells, cytokines, chemokines, growth factors, and regulatory molecules all play a role in this process, as do peripheral blood mononuclear cells, chemokines, growth factors, and resident skin cells. The inflammatory phase, the proliferative phase, and the remodeling phase are the three consecutive and overlapping processes that make up the complex skin healing process. The inflammatory phase comprises hemostasis and cutaneous neurogenic inflammation, which begin immediately after damage and persist for about an hour. The rapid neutrophil recruitment to the damaged tissue during the first 24 hours was followed by its subsequent posterior reduction throughout the following week. Beginning the second day after the injury, inflammatory monocytes and macrophages gradually infiltrate the wound, increasing until they reach their maximum during the proliferative phase before beginning to decline over the next two weeks and taking over as the main mononuclear cell in the tissue repair process. Circulating lymphocytes go to the skin right after an injury, reaching a plateau by day 4. They remain there for another two weeks before starting to decline. The last stage begins two weeks after the damage and entails rebuilding the tissue that was initially created during the proliferation phase as well as the organization of a scar to restore the skin's integrity. This final phase can go on for months.<sup>20,21</sup>

A fundamental survival mechanism wound healing is usually taken for granted. There isn't a good categorization that adequately describes the wound healing process in the oral area, and there is very little information in the literature concerning disrupted wound healing. A series of intricate biological processes go into the healing of a wound. All tissues proceed in much the same way to finish the healing process with the least amount of scarring. In the warm oral fluid, which contains millions of microorganisms, the oral cavity is a wonderful habitat in which wound healing occurs.<sup>22,23</sup> For so, we need the greatest biocompatible substance for oral wound

healing.

The therapy used in this study was cinnamon. *Cinnamomum burmannii* has long been used as a medical therapy, one of which is to treat wounds. Wounds on the lips need healing materials that have a fast and good effect. Cinnamon contains flavonoid active compounds that have been tested for their properties as anti-inflammatory, antibacterial, and anti-oxidant. Due to its flavonoids and antioxidant characteristics, cinnamon has the potential to be utilized in cosmetics to prevent skin aging. Cinnamon (*C. burmannii*), a member of the Lauraceae family, was proposed because it is frequently used in traditional medicine and food flavoring and is known to be a rich source of polyphenols like flavonoids. The antioxidant activities and characteristics of cinnamon and its separated components, especially polyphenols, are thought to be responsible for their medicinally beneficial applications.<sup>7,24</sup>

Research conducted by Syahdiana Waty, et al. (2017) stated the antibacterial properties of *C. burmannii* as a mouth wash, there were alkaloids, flavonoids, saponins, and glycosides were detected in the extract. Nine bacterial species were identified as *Streptococcus mitis*, *S. sanguinis*, *S. salivarius*, *S. pluranimalium*, *S. pneumoniae*, *S. alactolyticus*, *Kocuria rosea*, *Kocuria kristinae*, and *Spingomonas paucimolis*. It showed that the extract of Cinnamon bark significantly inhibited *Streptococcus* growth, and it was effective as mouthwash.<sup>25</sup>

This study prepared this natural wound healing ingredient in the form of lipbalm. Lipbalm products are currently known as cosmetic products. Since ancient times, there has been a huge desire for cosmetics. The lips serve as grazing, suction, and speech-producing organs. It is made up of the orbicularis muscle, the skin, the superficial fascia, and the muscles that are inserted surrounding it. A continuous layer of dry, red mucous membrane covers the lips' outer edges. The coronary arteries that completely ring the buccal orifice close to the free border of the lips are found in the areolar tissue or submucous layer.<sup>26</sup> The emphasis on cosmetics made with natural ingredients has increased recently. The most popular cosmetic items for enhancing the beauty of the lips and giving cosmetics a spectacular touch are lip balm formulas. An all-natural method of preserving and enhancing lip health is lip balm. The basis of today's cosmetic

lips is the use of highly strong chemicals, which have a variety of adverse effects. Therefore, an attempt has been made in this work to research the natural components needed to create natural lip balms. It will be simpler to apply lip balm-packaged wound healing products to the lips, allowing for greater penetration into the wound and more effective healing.<sup>27</sup>

The limitation of this study is that different doses of cinnamon lip balm were not produced, necessitating future research to determine the ideal amount that can improve wound healing.

## Conclusions

Our finding indicates that the application of natural lip balm *Cinnamomum burmannii* is effective to accelerate lip wound healing by increasing the level of interleukin-6.

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## Declaration of Interest

The authors report no conflict of interest.

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