

Examined Study Of The Impact Of Ethanol Extract From Sirih China (*Peperomia pellucida* L.) Ointment On The Histological Appearance Of The Epidermis And The Duration Of Burn Healing In Mice (*Mus musculus*)

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ABSTRAK

Luka bakar adalah kondisi di mana terjadi kerusakan pada lapisan epidermis hingga lapisan dermis. Struktur kulit mengalami kerusakan dari epitelium hingga lapisan basal dermis. Anda dapat menggunakan lada Cina herbal (*Peperomia pellucida* L.) untuk perawatan luka bakar. Studi ini bertujuan untuk mengevaluasi dampak pemberian salep daun sirih Cina, dengan variasi konsentrasi dan formula, terhadap durasi penyembuhan luka bakar dan diameter penutupan luka. Metode Ada 30 tikus *Mus musculus* dalam penelitian ini. Membagi mereka menjadi lima kelompok: kelompok reguler tanpa luka bakar, kelompok kontrol positif yang menerima gentamisin, kelompok perlakuan 1 yang menerima salep ekstrak etanol konsentrasi rendah 5% daun sirih Cina (EECB), kelompok perlakuan 2 yang menerima salep EECB konsentrasi rendah 10%, dan kelompok perlakuan 3 yang menerima salep EECB konsentrasi tinggi 15%. Hasil menunjukkan aktivitas penutupan luka dengan mengukur diameter luka bakar yang diobati dengan salep lada Cina konsentrasi 15% pada hari ke-18 dan konsentrasi 15% menutup luka bakar. Perbedaan histopatologis terlihat pada kelompok K3, di mana kepadatan kolagen mendapat skor +3 ($\pm 0,06$). Kami menemukan korelasi positif yang sangat lemah (0,000) antara histopatologi (kolagen) dan diameter luka serta korelasi negatif yang kuat (-0,0527) antara parameter histopatologi (kolagen) dan durasi penyembuhan luka bakar. Kesimpulan. Penelitian menyimpulkan bahwa efektivitas pemberian salep daun sirih Cina dengan konsentrasi 15% diperoleh.

Kata kunci : Luka Bakar, *Peperomia pellucida*, Lama Penyembuhan, Histologi, Diameter luka

ABSTRACT

Burns are a condition where there is damage to the epidermis layer down to the dermis layer. The skin structure sustains damage from the epithelium to the basal layer of the dermis. You can use the herbal Chinese pepper (*Peperomia pellucida* L.) for burn treatment. This study aims to evaluate the impact of administering Chinese betel leaf ointment, with variations in concentration and formula, on the duration of burn wound healing and the diameter of wound closure. Methods There were 30 *Mus musculus* mice in this study. Divided them into five groups: a regular group without burns, a positive control group that received gentamicin, treatment group 1 that received a low concentration 5% ethanol extract of Chinese betel leaf (EECB) ointment, a treatment group 2 that received a low concentration 10% EECB ointment, and a treatment group 3 that received a high concentration 15% EECB ointment. Results demonstrate wound closure activity by measuring the diameter of burn wounds treated with a 15% concentration of Chinese pepper ointment on the 18th day and a 15% concentration closed the burn wound. Histopathological differences were seen in the K3 group, where collagen density scored +3 (± 0.06). We found a very weak positive correlation (0.000) between histopathology (collagen) and wound diameter and a strong negative correlation (-0.0527) between the histopathological parameter (collagen) and the duration of burn wound healing. Conclusion. The research concludes that the effectiveness of administering Chinese betel leaf ointment with a concentration of 15% was obtained.

Keyword: Wound burn, *Peperomia pellucida*, Healing time, Diameter burn

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I. INTRODUCTION

1. Background

Burns, the most common form of skin injury, are typically caused by heat, chemicals, radiation, and electricity. Burn patients can experience various complications, including trauma, infection, electrolyte imbalance, burn-related disabilities, and scars. Many factors influence the healing of burn wounds, including the depth of the burn, the victim's age, the anatomical position of the wound area, infection, and the source or cause of the burn (Esmat & Ahmad, 2018). According to the World Health Organization (2018), burns generally cause health problems that lead to 180,000 deaths annually, categorizing their prevalence as high worldwide. Mortality in Southeast Asia reaches 11.6% each year. The depth and extent of the burned skin, the victim's health status, their age, and inadequate treatment all influence the morbidity and mortality rates. Based on the 2018 Riskesdas data in Indonesia, burns rank second (1.3%) in the proportion of injury types overall (2.6%), which includes fractures, sprains, wounds (abrasions and lacerations), amputations, eye injuries, concussions, and internal organ injuries (Darotin et al., 2023). We divide the process of burn wound healing into three main phases: the inflammatory phase, the proliferative phase, and the maturation phase. In this phase, which is the process of tissue maturation, inflammation begins to subside, forming new pale and thin tissue. Histopathological studies on burn models in mice (*Mus musculus*) show these changes in the epidermis and other skin layers in response to burn trauma. Burn wound healing involves regenerating epidermal cells and reorganizing skin structure to restore skin function and integrity. (Mutiarawati et al., 2022). The theory by Matthew et al. (2015) explains that the first treatment for a first-degree burn at home is to remove the victim from the burn's source and then run clean water (not ice water) over the burned area for 15-30 minutes. Keep the area clean and apply a cold compress (not ice) until the pain subsides. You can administer antibiotics or antiseptics through creams, ointments, or solutions to prevent infection and speed up burn healing. Additionally, anti-inflammatories can be administered to reduce the inflammation caused by burns (Hiamawan, 2022). People use plants as herbal medicine because they cause minimal side effects. One such method involves using the Chinese pepper plant (*Peperomia pellucida L.*) as an antiseptic to expedite burn healing. In her research, Mutiarawati (2022) found that applying Chinese pepper extract ointment on rats with burns resulted in rapid collagen granulation growth in the skin tissue within approximately 4 days. The wound's quality of collagen granulation tissue formation characterizes the wound healing process. The thicker the formed collagen granulation tissue, the shorter the wound healing process. We suspect active compounds like saponins, tannins, flavonoids, phenols, and essential oils are responsible for increased collagen granulation tissue.

2. Statement problem

1. The formulation of Chinese pepper leaf ointment (*Peperomia pellucida L.*) for burn wound coverage based on wound diameter?
2. The formulation of Chinese pepper leaf ointment (*Peperomia pellucida L.*) on wound closure based on healing duration?
3. Does the formulation of Chinese pepper leaf ointment (*Peperomia pellucida L.*) show differences in the histology of the epidermis of burn wounds between groups?
4. What is the relationship between burn wound closure and histology?

3. Purposes of research

This study proves the effect of *Peperomia pellucida* ointment on the duration of burn wound healing in burn wound model rats.

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4. Advantages of Research

Increasing researchers' knowledge and supporting literature on the numerous benefits of Chinese pepperleaf (*Peperomia pellucida L.*) which is beneficial for long-term research on the wound healing acceleration process.

II. METHODS

The research design uses a quasi-experimental approach and has obtained approval from the animal ethics committee with No. 0329/KEPH-FMIPA/20204 and the animal ethics committee of the FMIPA University of Sumatera Utara. The research was conducted at the Pharmacology and animal Laboratory, Faculty of Medicine, Universitas Sumatera Utara, and Department of Pharmacy, Faculty of Health, Universitas Haji Sumatera Utara.

Extraction of *Perperromia pellucidai* (PP)

The maceration method was used with 70% ethanol solvent. 500 g of simplicia powder is placed into the maceration vessel, with a ratio of 1:10. pour 3,750 parts of ethanol solvent, cover it, and let it sit for 5 days protected from sunlight while frequently stirring the solvent. After 5 days, the mixture is filtered with filter paper, and the residue is soaked again with 1250 ml of 70% ethanol for 2 days and then filtered again. The obtained macerate was evaporated using a rotary evaporator at a temperature of 50°C, a speed of 70 rpm, and a pressure of 0.7 to remove the ethanol solvent from the extract.

III. RESULTS AND DISCUSSION

Obtained a thick extract of Chinese betel leaf weighing 57.20 grams with a yield of 11.44%. The thickened extract is placed in a well-sealed container and stored in the refrigerator to prevent mold from developing on the extract.

Table 1. Average Results of Burn Wound Closure Diameter

Groups	Burn wounds (mm) (Mean±SD)	Min-Max	p Value (>0.05)	p Value (significant) (<0.05)
G1	-	-	-	0.017*
G2	10.02±0.82	9.18-10.84	0.608	
G3	7.16±2.17	4.99-9.33	0.181	
G4	6.40 ±2.12	4.28-8.52	0.984	
G5	7.65±0.93	6.72-8.59	0.269	

Abb: G1: Negative control: Control group (healthy); G2: Positive Control: Gentamicin; G3: Treatment 1: PP Ointment (5%); G4: Treatment 2: PP Ointment (10%); G5: Treatment 3: PP ointment (15%); *Anova test: (<0.05)

Based on Table 4.1, the smallest wound diameter was found in the G5, with an average diameter of 6.40±2.12. This result is better than gentamicin, which has an average wound diameter of 7.65±0.93. Then, the second-best result was followed by G4. Whereas G5 is not better than any group because the average diameter of its wounds is larger than G3, G4, or G5. The smallest minimum and maximum values were obtained in G5, with a minimum value of 4.28 and a maximum of 8.51. This result is smaller when compared to other groups. A significance test was conducted using the Anova test for the wound diameter results, and a significance value of 0.017 was obtained (significant). Based on the graph above for group 1 with a 5% concentration of Chinese betel leaf ointment, there was no significant decrease on the first day. On the 2nd to the 5th day, there was an increase in wound diameter. From day 6 to day 18, there was a significant decrease, with a healing percentage of 20%. the same study was conducted by Sianipar et al. (2024) on burn wound healing using a 5% ethanol extract gel of Chinese betel leaf, which showed a healing

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percentage of 24.4% and an average wound healing diameter of 9.15 ± 8.63 over 15 days, as shown in Figure 1.

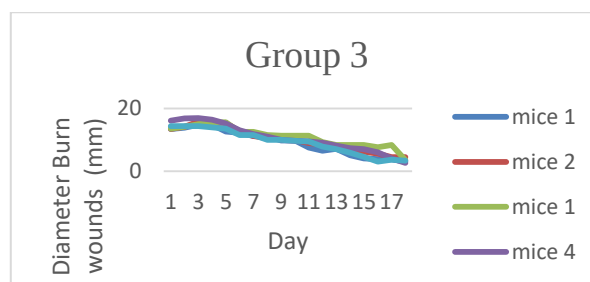


Figure 1. Diameter of burn wounds during observation (**Group 1**)

Group 2 in Figure 2, with a 10% concentration of Chinese pepper ointment, showed a decrease in wound diameter on the 3rd day. On the 16th day, a significant decrease in wound diameter was observed in mouse number 3, followed by mouse number 4 on the 18th day, with the wound diameter reaching zero. This indicates that the formation of fibres and collagen in the skin epidermis has been perfectly completed, with a healing percentage of 60%.

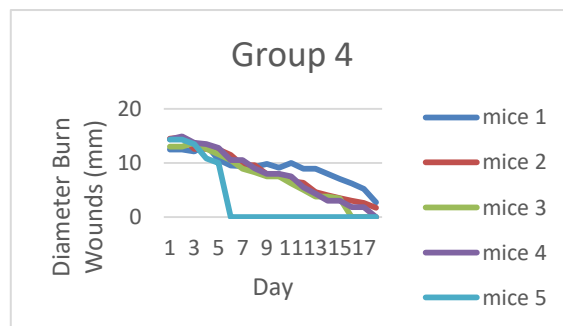


Figure 2. Diameter of burn wounds during observation (**Group 2**)

Figure 3 shows Group 3, with a 15% concentration of Chinese betel leaf ointment, showed a reduction in wound diameter on the 6th day. On the 14th day, the wound diameter reduction reached zero, observed in mouse number 4, followed by mouse number 3, 2, and 1, with a healing percentage of 98%.

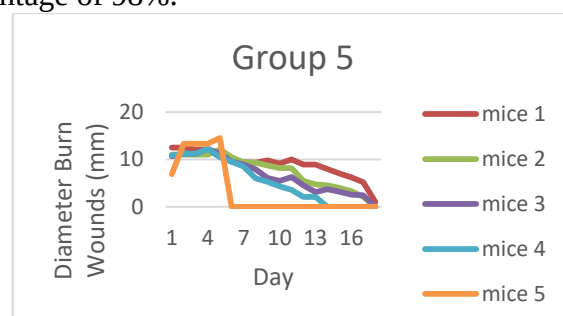


Figure 3. Diameter of burn wounds during observation (**Group 3**)

Figure 4 shows that the gentamicin group, which serves as the positive control, showed a decrease in wound diameter starting from days 5 and 6, and by days 16 to 18, the wound diameter reached zero. This differs from the 15% concentration of Chinese pepper leaf ointment.

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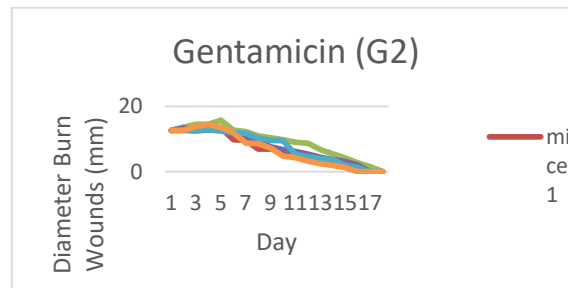


Figure 4. Diameter of burn wounds during observation (**Group 4**)

Average Collagen Density in the Histology of Mice Given Treatment

Collagen density is interpreted semi-quantitatively by observing it. According to Novriansyah (2008), the histopathological scoring parameters for collagen density are based on the calculation of 1 field of view at a magnification of 100 times.

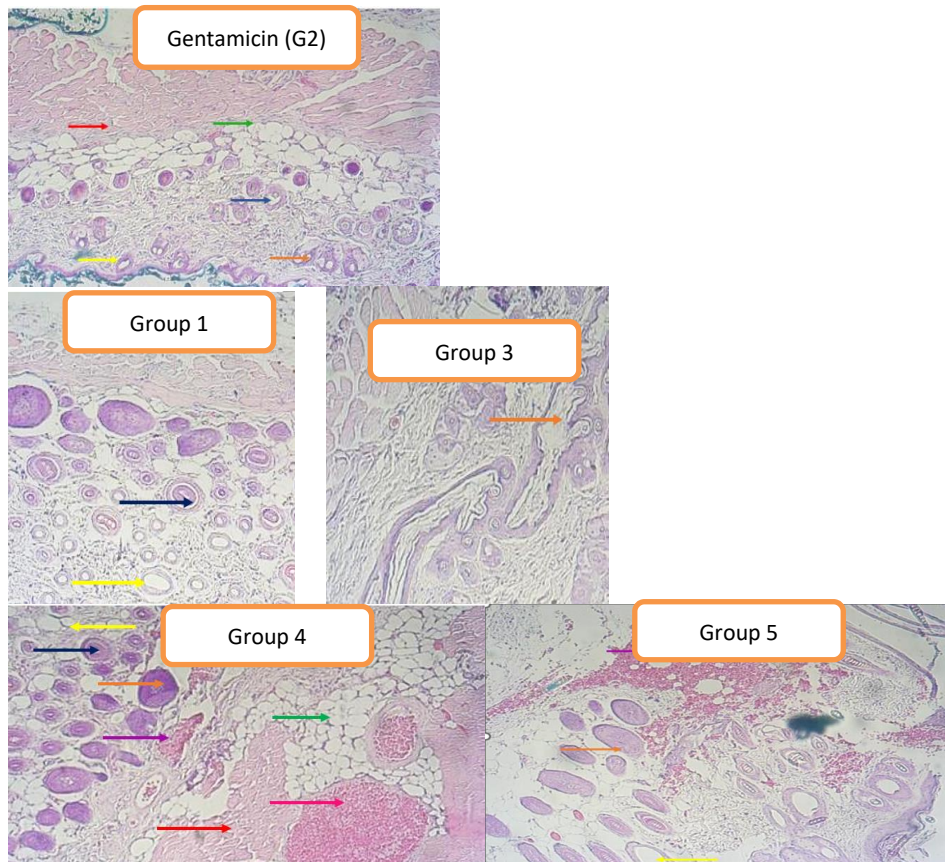
Table 2. Average Collagen Density in the Histology of Mice Given Treatment

Group	Collagen (Mean SD)	Interpretation
G1	3.09±0.08	The density of collagen fibres in the wound area is dense; the skin structure appears normal, starting from the epidermis to the dermis. Hypodermis and muscle layer
G2	2.32±0.08	The density of collagen fibres in the wound area is moderate. Epithelialization has been observed in some layers, with bleeding and mild mononuclear inflammatory cells. there are fibroblasts present
G3	1.63±0.12	The density of collagen fibres in the wound area is low. In some areas of the skin, the epidermal layer was not found, bleeding and oedema were observed, and severe infiltration of inflammatory cells was observed. In the dermis layer, light fibrosis formation is observed around the inflammation.
G4	2.49±0.12	The density of collagen fibres in the wound area is moderate. Perfect epithelialization has been observed in the epidermal layer, with mild infiltration of mononuclear inflammatory cells and moderate positioning of fibroblast cells.
G5	2.90±0.06	The density of collagen fibres in the wound area is approaching close. Perfect epithelialization has been observed in the dermis layer, with light infiltration of mononuclear inflammatory cells and light infiltration of fibroblast cells, and some hair follicles are already visible.

Abb: G1: Negative control: Control group (healthy); G2: Positive Control: Gentamicin; G3: Treatment 1: PP Ointment (5%); G4: Treatment 2: PP Ointment (10%); G5: Treatment 3: PP ointment (15%);

Based on Table 2, the skin burns in mice that were given a burn for 5 seconds with an iron plate appeared pale white, and there was oedema on the first day. The burn was then treated with Chinese betel leaf extract ointment at concentrations of 5%, 10%, 15%, and gentamicin ointment (positive control) for 18 days. On day 5, the wound appeared to dry, and scabs formed on the surface of the burned skin. On day 10, the scabs in the G5, G4, and gentamicin treatments began to peel off partially, except for G3. On day 18, it was observed that groups G5, G4, and gentamicin had developed new skin tissue, and there were no scabs visible on the surface of the wounded skin, except for G3, which still had scabs on the surface of the wounded skin.

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Histological (→) Epidermis, (→) Vacuole, (→) Collagen, (→) Epithelial Cells, (→) Fibroblasts, (→) Mononuclear Inflammatory Cells, (→) Hemorrhage.

Collagen has an important role in the wound-healing process. Collagen has capabilities that include hemostasis, interaction with platelets, interaction with fibronectin, increased fluid exudation, enhancement of cellular components, growth factors, and promotion of fibroplasia processes and sometimes epidermal proliferation.

IV. CONCLUSION

The average wound closure diameter on the first day for each group was obtained: G3: 10.02; G4: 7.16; G5: 6.40, and Gentamicin: 7.65, with a wound diameter of 0 on the last day in the K3 group. Applying Chinese betel leaf ointment with a concentration of 15% showed faster burn wound closure compared to Chinese betel leaf ointment with 10% and 5% concentrations on the 18th day. Histological differences in the skin's epidermis were observed among the groups given Chinese betel leaf ointment. G5 (15%) Chinese betel leaf ointment showed more explicit epidermis formation and epithelialization formation than the 5% and 10% concentrations. A very weak (0.000) but positive relationship was obtained between the histopathological parameter (collagen) and the diameter of burn wound closure, and a strong (-0.537) but negative relationship was obtained between the histopathological parameter (collagen) and the duration of burn wound healing.

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Accepted Date	Revised Date	Decided Date	Accepted to Publish
11 Desember 2024	26 Desember 2024	07 Januari 2025	Ya