J Prima Med Sains (2023) 5(2):126-129
https://doi.org/10.34012/jpms.v5i2.4402

ORIGINAL ARTICLE

Anti-inflammatory potential of lemongrass leaves in wound healing

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Abstract

The inflammatory stage is a normal phase of wound healing; however, inflammation can become chronic and lasts for months or years. This is a post-test with control group design study, divided with 20 male white rats into 4 groups: control group (K), treatment group 1 (P1), treatment group 2 (P2), and treatment group 3 (P3). Lemongrass leaf extract gel, simplicia leaves, stems, and roots of citronella were extracted by maceration using a 1:5 ratio of simplicia to solvent. Gel was applied twice a day (morning and evening) to each group, adhering the formulation. Macroscopic observations were made to assess the wound condition and incision length. The length of the incision wound was measured using a ruler every two days for a span of fourteen days. As the sample size was less than 50, a normality test was performed using the Shapiro-Wilk test, followed by a One-way ANOVA and a Post Hoc test. The study found that wound healing was slowest in the control group but faster in the groups treated with 10% and 15% lemongrass leaf extract. Secondary metabolites at a concentration of 15% had a positive impact on wound healing, whereas at lower concentrations, they only inhibited microorganisms, making them less effective for wound healing.

Keywords: gel extract, lemongrass leaf, inflammation, wound incision

Introduction

Inflammation is a response of the immune system to irritation. Irritants can originate from pathogens or foreign objects, such as accidental contamination with dirt on the fingers. Inflammation does not solely commence when a bacterial infection occurs, resulting in pus formation or an ineffective healing process; rather, it begins when the body strives to combat potentially harmful irritation.[1–3] When inflammation occurs within the body, various types of immune cells may be involved, releasing inflammatory mediators such as bradykinin and histamine. These substances dilate small blood vessels in the tissue and increase blood flow to the injured area. This process causes redness and a warm sensation in the inflamed area.[4,5] Although swelling occurs in the inflammation process, it subsides as fluids released by cells are transported out of the tissue.[6] Injured tissue is repaired, lost tissue is replaced, and the epithelial layer is restored. Keratinocytes, fibroblasts, vascular endothelial cells, and immune cells all play crucial roles in supporting inflammation, cell migration, and angiogenesis.[7]

The wound-healing mechanism in the body involves a complex interaction between inflammatory mediators and cells. Wounds can heal with secondary intention without intervention, or wound edges can be stitched, stapled, or otherwise treated to facilitate primary intention healing.[8,9] While the inflammatory stage is a common phase in the wound-healing process, it can escalate into chronic inflammation. Chronic inflammation, also known as long-term inflammation, can persist for several months to several years. Generally, the extent and effects of chronic inflammation vary depending on the cause of the injury and the ability of the body to repair and cope with damage.[10] Chronic wounds can lead to further complications

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for patients, such as disability, sepsis, loss of function, or amputation. In other situations, the healing process may become overly active, forming excessive scar tissue and resulting in complications such as hypertrophic scars and keloids. Hypertrophic scars and keloids may be associated with itching, burning, or pressure pain.[9]

Aromatic plants and medicinal herbs continue to be the primary choice of alternative and traditional treatments in developing countries. These plants are often referred to as herbal medicines. Many herbal therapies are currently used in medicine and serve as anti-inflammatory, antifungal, and analgesic agents. The use of medicinal plants is frequently embraced by communities with limited or no access to medical assistance.[11,12] Lemongrass (*Cymbopogon citratus*) belongs to the grass family, Poaceae. It is a prominent medicinal and aromatic plant cultivated in tropical and subtropical regions of Asia, South America, and Africa. The essential oil of lemongrass leaves is extracted by steam distillation from dried or fresh plant material.13 Steam distillation yields essential oil along with hydrosol or aromatic water, often used to prevent inflammation and microbial infections.[13] Previous studies have indicated the presence of bioactive compounds in lemongrass, such as flavonoids, phenolic acids, and tannins, which play a role in various phases of wound healing.[14–16] This study aims to analyze the effectiveness of lemongrass leaf extract in preventing the inflammatory process in cut wounds.

Method

This study employed a post-test with a control group design and was conducted from March 2023 to May 2023 at the Riwandi Pet Shop and Animal House in Medan City. Lemongrass leaves were obtained from Lumbanpoki Village, Samosir Island, North Sumatra, and identified at the Medanese Herbarium (MEDA), Plant Taxonomy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. Twenty male white rats were divided into four groups: control group (K), treatment group 1 (P1), treatment group 2 (P2), and treatment group 3 (P3). The instruments used in this research included rat cages, gloves, digital scales, probes, markers, blenders, pipettes, 3 ml syringes, feed containers, rotary evaporators, mixers, freezers, freeze dryers, and masks. Healthy white rats without physical defects, distilled water, 80% ethanol, 1% HCL, label paper, rat feed and drink, and lemongrass extract were used.

Before administering the treatments, all Wistar strain white rats underwent a seven-day acclimatization process at the Animal House, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan. The rats were given time to adapt to their new environment, as well as their food, and beverages. Rat feed and drink were provided according to standard requirements. In the preparation of lemongrass leaf extract, gel, lemongrass leaf, stem, and root s*implicia* were extracted using the maceration method with a simplisia-to-solvent ratio of 1:5. Maceration was performed with 90% ethanol for 24 h with shaking at 130 rpm. The solvent and *simplicia* were separated using filter paper. The residue was macerated again with ethanol, yielding two extractions. The filtrates from the two macerations were combined and concentrated using a rotary evaporator at 50°C. The lemongrass extract was concentrated by evaporation in a water bath using a porcelain dish, and the percentage yield of the extract was calculated. Subsequently, the lemongrass extract was dissolved in part of the ethanol and stirred until a gel base was formed. Methyl paraben, dissolved in propylene glycol, was added and stirred until homogeneous. The lemongrass extract was then added to the gel base, stirred until homogeneous, and distilled water was added. The prepared lemongrass extract gel formulation was stored in a sealed container.

Table 1. Preparatory formula of lemongrass leaf extract gel

Ingredients	Formula		
	F1	F2	F3
Lemongrass leaf ethanol extract	5%	10%	15%
HPMC (hidroksipropil Metilselulosa)	1 gr	1 gr	1 gr
Propilenglikol	15 gr	15 gr	15 gr
Methyl paraben	0,1 gr	0,1 gr	0,1 gr
TEA (Trietanolamin)	2 drops	2 drops	2 drops
Distilled Water (Aquadest)	100	100	100

The treatment was initiated with topical anesthesia using lidocaine solution on the back. The back of the mouse was disinfected with 10% povidone-iodine. The fur around the wound (back) was shaved and treated with 70% alcohol. Subsequently, the mice were injured by making a 1 cm incision at a depth of 0.2 cm or until the subcutaneous layer on the back using a sterile scalpel. The wound was irrigated with distilled water until the bleeding stopped. Each group received the following treatments: a) Group 1 (K): Incised wounds on mice without any treatment; b) Group 2 or Treatment Group-1 (P1): Incised wounds on mice

treated with 5% lemongrass leaf extract gel topically; c) Group 3 or Treatment Group-2 (P2): Incised wounds on mice treated with 10% lemongrass leaf extract gel topically; and d) Group 4 or Treatment Group-3 (P3): Incised wounds on mice treated topically with 15% lemongrass leaf extract gel. The lemongrass leaf extract gel was applied twice daily (morning and evening) at the same time for each group, according to the formulation. The wound conditions and length of the incised wounds were observed macroscopically. Measurement of the incised wound length using a ruler was conducted every 2 days for 14 days.

Mouse body weight data from each group were analyzed using SPSS version 26. Because the sample size was less than 50, the statistical tests used included normality testing with the Shapiro-Wilk test, followed by One-way ANOVA and Post Hoc tests.

Results

Observations of wound healing were conducted every 2 days for 14 days for four treatment groups: those treated with 5% lemongrass leaf extract gel (P1), 10% lemongrass leaf extract gel (P2), 15% lemongrass leaf extract gel (P3), and the control group (K). Total wound healing occurred on the 14th day in the treatment group P3. However, in treatment groups P1, P2, and K, total wound healing did not occurred by the 14th day, although in the P2 group, nearly complete healing was observed at 0.01 cm on the 14th day. Based on the average length of wounds in each group, it can also be concluded that wound healing in

group P3 occurred more rapidly, followed by group P2. The slowest wound healing occurred in the control group (K) (see Table 2).

Table 2. Average wound length (cm)

Day	K	P1	P2	P3
2	0.97	0.89	0.79	0.73
4	0.85	0.74	0.63	0.56
6	0.77	0.63	0.51	0.44
8	0.64	0.43	0.36	0.18
10	0.50	0.22	0.26	0.10
12	0.29	0.18	0.08	0.02
14	0.08	0.04	0.01	0.00
Average	0.59	0.45	0.38	0.29

The Shapiro-Wilk test results indicate that the data is normally distributed. The wound length from day 1 to day 14 shows significant values ($p>0.05$) in all groups (K=0.679; P1= 0.171; P2=0.421; P3=0.377). The healing process of incised wounds in each group (K, P1, P2, and P3) observed after 14 days of treatment was tested for homogeneity using the One-way Anova test. The results indicate that The variance of the research data for the control group (K), treatment group 1 (P1), treatment group 2 (P2), and treatment group 3 (P3) was homogeneous or came from a population with the same variance of 0.560 ($p>0.05$). Further testing with Bonferroni Post Hoc Test showed differences in the average wound healing length in white Wistar rats (Rattus norvegicus), marked with "*".

Discussion

The wound-healing process consists of inflammation, proliferation, and maturation phases. The average wound healing phase from the first day to the 4th day in the group treated with 10% lemongrass leaf extract gel (P2) and 15% lemongrass leaf extract gel (P3) showed an inflammation phase, indicating a faster reduction in wound length compared to the 5% lemongrass leaf extract gel (P1) and the control group (K), which experienced inflammation until the 6th day. This occurred due to the presence of bioactive compounds in lemongrass, such as flavonoids that can stop bleeding in wounds and act as anti-inflammatory agents influencing the production of inflammatory cells in the inflammation phase of wound healing. Tannins, acting as astringents, can reduce the permeability of the mucosa, strengthening mucosal binding and preventing irritation. Tannins also indirectly affect mucosal and bacterial wall permeability, causing bacteria to shrink and die. The phenolic acid content in lemongrass plays a role in preventing cell damage due to free radicals, thus preventing inflammation and swelling.

Table 3. Bonferroni post hoc test results

(I) Group	(J) Group	Mean diff. (I-J)	p
Control	Treatment 1	.12400*	0.000
	Treatment 2	.18800*	0.000
	Treatment 3	.26200*	0.000
Treatment 1	Control	-.12400*	0.000
	Treatment 2	.06400*	0.001
	Treatment 3	.13800*	0.000
Treatment 2	Control	-.18800*	0.000
	Treatment 1	-.06400*	0.001
	Treatment 3	.07400*	0.000
Treatment 3	Control	-.26200*	0.000
	Treatment 1	-.13800*	0.000
	Treatment 2	-.07400*	0.000

*) The mean difference is significant at the 0.05 level

The study found that the group treated with 15% lemongrass leaf extract gel was more effective in healing incised wounds in white mice compared to the groups treated with 5% and 10% lemongrass leaf extract gel. This is because, at a concentration of 15%, secondary metabolites in lemongrass extract already have an effect on wounds.

However, at lower concentrations, they only inhibit microorganisms, making them less effective in wound healing.

This is in line with the opinion of Purbowati et al[17] stating that if antibacterials are used at low concentrations, they only inhibit (bacteriostatic), but at high concentrations, they become bactericidal. The results also align with research conducted by Hairi [14] indicating that lemongrass extract at 100% and 50% concentrations can accelerate the healing of labial mucosal wounds in mice, as observed from the wound length. The higher the concentration of the extract, the faster the wound healing, possibly due to a higher concentration of active substances in the extract. However, there was no significant difference between lemongrass extract concentrations of 100%, 50%, and 25%.

Conclusion

In conclusion, the study found that the slowest wound healing occurred in the control group, while the groups treated with 10% and 15% lemongrass leaf extract showed faster healing rates. The secondary metabolites at a concentration of 15% had an effect on the wound, whereas at lower concentrations, they only inhibited microorganisms, making them less effective in wound healing.

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