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Effect of Combined *Centella asiatica* Extract and Standard Therapy on Incision Wound Healing Rate in BALB/c Mice (*Mus musculus*)

Ala'raaf Nada Sofnidar, Renni Yuniati*, Buwono Puruhito
Universitas Diponegoro, Semarang, Indonesia
Email: renniyuniati6@gmail.com

Abstract

Wounds represent a disruption of biological tissue integrity affecting organs, skin, and mucosal membranes. Several conventional wound healing modalities remain suboptimal, necessitating the exploration of more effective therapeutic approaches. *Centella asiatica* (gotu kola) is a traditional medicinal plant widely recognized for its wound healing, anti-inflammatory, antimicrobial, and antioxidant properties, primarily attributed to its triterpenoid content, particularly asiaticoside. This study aimed to determine the effect of combining *Centella asiatica* extract cream with standard therapies on the rate of incision wound healing in BALB/c mice. A true experimental study with a post-test only control group design was conducted using male BALB/c mice (*Mus musculus*), randomly divided into four groups (n = 6): K1 (NaCl 0.9%), K2 (gentamicin 0.1%), P1 (NaCl 0.9% + 16% *Centella asiatica* extract cream), and P2 (gentamicin 0.1% + 16% *Centella asiatica* extract cream). Incision wounds (1 cm in length, 0.2 cm in depth) were created on the dorsal region. Wound length was measured on days 1, 3, 7, 14, and 21. Data were analyzed using the Kruskal–Wallis test, Mann–Whitney U test, one-way ANOVA, and post-hoc LSD test. Significant differences in wound length reduction were observed among the four groups on day 1 (p = 0.001) and day 7 (p = 0.014). On day 3, one-way ANOVA revealed significant intergroup differences (p = 0.003). The P2 group consistently demonstrated the fastest wound length reduction throughout the observation period. By day 14, no significant differences were found (p = 0.368), and by day 21, complete wound closure was achieved in all groups.

Keywords: *Centella* asiatica; gentamicin; incision wound; wound healing; BALB/c mice.

INTRODUCTION

Wounds are defined as damage to the integrity of biological tissues, including organs, skin, and mucosal membranes, and can be described as physical injuries that result in the disruption of normal skin surface continuity (Herman & Bordoni, 2022). The Ministry of Health of the Republic of Indonesia reported a national injury prevalence of 8.2%, with the highest prevalence in South Sulawesi Province (12.8%) and the lowest in Jambi Province (4.5%), underscoring the significant burden of wound-related conditions in the country (Risma et al., 2018). Based on the mechanism of occurrence, wounds are classified into several types, one of which is an incision wound caused by sharp objects such as surgical instruments (Aminuddin et al., 2020). If not managed promptly and appropriately, incision wounds can lead to serious complications, including infection, hemorrhage, dehiscence, and impaired organ function (Febriana, 2019; Oktaviani et al., 2019).

Optimal wound healing is characterized by rapid tissue recovery without adverse effects (Ozay et al., 2019). Numerous local and systemic factors influence the wound healing process, including wound depth, nutritional status, and metabolic abnormalities (Labib & RW, 2022). While conventional wound care agents such as sodium chloride (NaCl 0.9%) are widely used in clinical settings for wound irrigation, NaCl 0.9% is not an antiseptic and lacks bactericidal properties (Wibowo & Comariyati, 2017). Gentamicin, an aminoglycoside antibiotic, is commonly employed as a topical agent for the prevention and treatment of wound infections (Chaves, 2022). However, the search for more effective and accessible wound healing agents continues, particularly those derived from natural sources (Criollo-Mendoza et al., 2023).

Traditional medicinal plants have long been recognized for their therapeutic potential in wound management. Among these, *Centella asiatica* (Linn.) Urban, commonly known as gotu kola or *pegagan* in Indonesian, has been extensively used in herbal and alternative medicine formulations for treating a wide range of conditions, including gastrointestinal diseases, gastric ulcers, eczema, and wounds (Jhansi & Manjula, 2019). The wound healing properties of *Centella asiatica* are primarily attributed to its triterpenoid compounds, with asiaticoside being the most pharmacologically significant constituent. Asiaticoside plays a critical role in accelerating wound healing and reducing wound fibrosis through the stimulation of collagen synthesis and the promotion of angiogenesis (Zuniarto & Fauzia, 2017). Furthermore, *Centella asiatica* exhibits notable antimicrobial and antioxidant activities that contribute to its wound healing efficacy (Azis et al., 2017).

The urgency of this research stems from several converging factors. First, the high prevalence of wounds in Indonesia (8.2% national prevalence) and the potential for serious complications when healing is delayed necessitate the exploration of more effective therapeutic options. Second, conventional therapies such as NaCl 0.9% provide wound irrigation but lack direct therapeutic activity, while gentamicin, though effective against bacteria, does not directly stimulate the cellular processes of wound healing, such as collagen synthesis and angiogenesis. Third, the increasing concern over antimicrobial resistance highlights the need for adjunctive therapies that can reduce reliance on antibiotics while maintaining or improving healing outcomes. Fourth, *Centella asiatica* is widely available, affordable, and culturally accepted in Indonesian traditional medicine, making it a promising candidate for integration into standard wound care protocols, provided its additive benefits can be demonstrated.

The novelty of this research lies in three interconnected contributions. First, this study provides the first systematic evaluation of the combination of 16% *Centella asiatica* ethanol extract cream with two different standard therapies (NaCl 0.9% and gentamicin 0.1%) in a controlled animal model, enabling assessment of additive versus synergistic effects. Second, this research employs a true experimental design with a post-test only control group approach across four groups (negative control, positive control, and two treatment groups), allowing direct comparison of combination therapy against each standard therapy individually. Third, this study measures wound healing progression at multiple time points (days 1, 3, 7, 14, and 21), capturing both early-phase acceleration and complete wound closure, providing a comprehensive temporal profile of the combination effect. This represents an original contribution to wound healing research, bridging the gap between traditional herbal medicine knowledge and evidence-based combination therapy protocols.

Despite the growing body of evidence supporting the wound healing properties of *Centella asiatica*, limited studies have investigated the combined effect of *Centella asiatica* extract with standard wound care therapies, particularly NaCl 0.9% and gentamicin 0.1%, on incision wound healing. Therefore, this study aimed to evaluate the effect of combining 16% *Centella asiatica* ethanol extract cream with standard therapies on the rate of incision wound healing in BALB/c mice (*Mus musculus*).

METHOD

Study Design and Ethical Approval

This study employed a true experimental design with a post-test only control group approach. The research was conducted at the Experimental Animal Laboratory, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia, over a period of approximately 1.5 months. Ethical clearance was obtained from the Health Research Ethics Committee (KEPK) of the Faculty of Medicine, Universitas Diponegoro / Dr. Kariadi Hospital, Semarang, prior to the commencement of the study.

Experimental Animals

Twenty-four male BALB/c mice (*Mus musculus*) were obtained from a certified supplier in Semarang and selected through simple random sampling. The inclusion criteria were male sex, age of 8-12 weeks, body weight of 20-30 grams, healthy appearance, and active behavior. The exclusion criteria included visible anatomical abnormalities and signs of illness during the acclimatization period. The mice were acclimatized to laboratory conditions for one week prior to the experiment, housed in standard cages (6 mice per cage), and provided with standard feed three times daily and water *ad libitum*.

Preparation of *Centella* asiatica Extract Cream

The 16% *Centella asiatica* ethanol extract cream was formulated using the composition presented in Table 1.

Table 1. Composition of *Centella asiatica* extract cream formulation

No.	Component	Concentration (%)	Function
1	*Centella asiatica* herbal extract	1.5	Active ingredient
2	Stearic acid	12	Emulsifier and solvent
3	Cetyl alcohol	2	Emulsifier
4	Triethanolamine (TEA)	2	Binder
5	Glycerin	10	Humectant
6	Methylparaben	0.1	Preservative
7	Propylparaben	0.08	Preservative
8	Aquadest	*Ad* 100	Vehicle

The water phase (aquadest, methylparaben, TEA) and oil phase (stearic acid, cetyl alcohol, glycerin, propylparaben) were heated separately to 70°C using a water bath. Both phases were then combined in a mortar and stirred until a homogeneous cream mass was formed. The *Centella asiatica* herbal extract (1.5%) was subsequently incorporated into the cream base. Gentamicin 0.1% cream used in this study was a commercially available product manufactured by Kimia Farma (Indonesia).

Experimental Procedure

The mice were anesthetized subcutaneously with 0.2 mL of 2% lidocaine. The dorsal area was shaved and disinfected with 70% alcohol. A sterile scalpel was used to create an incision wound of approximately 1 cm in length and 0.2 cm in depth on the dorsal region of each mouse.

Following wound creation, the 24 mice were randomly allocated into four groups (n = 6 per group): K1 (negative control) received daily application of NaCl 0.9%; K2 (positive control) received daily application of gentamicin 0.1% cream; P1 (treatment group 1) received daily application of NaCl 0.9% followed by 16% *Centella asiatica* extract cream; and P2 (treatment group 2) received daily application of a combination of gentamicin 0.1% cream and 16% *Centella asiatica* extract cream. All treatments were applied once daily using sterile gloves throughout the 21-day observation period.

Outcome Measurement

Wound length (cm) was measured macroscopically using a calibrated ruler and a magnifying loop on days 1, 3, 7, 14, and 21 post-wounding. Photographic documentation was obtained at each observation time point to record the wound healing progression.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics software. The Shapiro-Wilk test was employed to assess data normality. For normally distributed data, One-Way ANOVA followed by the Least Significant Difference (LSD) post-hoc test was used to compare differences among groups. For non-normally distributed data, the Kruskal-Wallis test was applied, followed by the Mann-Whitney U test for pairwise comparisons. Statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

All 24 mice survived the entire 21-day observation period without any dropouts, mortality, or signs of severe illness. The progression of wound healing was evaluated based on the reduction in wound length across the four treatment groups.

Wound Length Across Observation Days

The mean wound length measurements for each group at each observation time point are presented in Table 2.

Table 2. Mean wound length (cm) across treatment groups and observation days

Observation Day	K1 (NaCl 0.9%)	K2 (Gentamicin 0.1%)	P1 (NaCl + ***C. asiatica***)	P2 (Gentamicin + ***C. asiatica***)	p- value
Day 1	0.85 ± 0.10	0.80 ± 0.06	0.75 ± 0.05	0.55 ± 0.05	0.001[a]
Day 3	0.70 ± 0.09	0.58 ± 0.12	0.53 ± 0.10	0.40 ± 0.15	0.003[b]
Day 7	0.37 ± 0.05	0.28 ± 0.16	0.23 ± 0.10	0.13 ± 0.08	0.014[a]
Day 14	0.17 ± 0.14	0.18 ± 0.17	0.20 ± 0.06	0.08 ± 0.08	0.368[b]
Day 21	0.00	0.00	0.00	0.00	1.000[a]

Values are expressed as mean ± SD. [a]Kruskal-Wallis test; [b]One-Way ANOVA.

On day 1, the Shapiro-Wilk test indicated non-normal data distribution for groups P1 and P2 ($p = 0.004$). The Kruskal-Wallis test revealed a significant difference among the four groups ($p = 0.001$). On day 3, data were normally distributed across all groups, and One-Way ANOVA demonstrated a significant difference ($p = 0.003$). On day 7, the Kruskal-Wallis test again showed significant intergroup differences ($p = 0.014$). By day 14, ANOVA indicated no significant difference among the groups ($p = 0.368$), and by day 21, all wounds had achieved complete closure.

Pairwise Comparisons

The results of pairwise comparisons between treatment groups are presented in Table 3.

Table 3. Pairwise comparison p-values between treatment groups

Comparison	Day 1	Day 3	Day 7
K1 vs K2	0.342	0.104	0.368
K1 vs P1	0.075	0.025*	0.028*
K1 vs P2	0.003*	<0.001*	0.003*
K2 vs P1	0.171	0.474	0.360
K2 vs P2	0.003*	0.015*	0.070
P1 vs P2	0.003*	0.066	0.102

*Statistically significant ($p < 0.05$). Day 1 and Day 7: Mann-Whitney U test; Day 3: LSD post-hoc test. Days 14 and 21 were not included as no significant overall differences were detected.

The pairwise analysis demonstrated that on day 1, the P2 group showed significantly shorter wound lengths compared to K1 ($p = 0.003$), K2 ($p = 0.003$), and P1 ($p = 0.003$). On day 3, K1 showed significantly longer wounds compared to P1 ($p = 0.025$) and P2 ($p < 0.001$), while K2 also differed significantly from P2 ($p = 0.015$). On day 7, K1 exhibited significantly longer wounds compared to P1 ($p = 0.028$) and P2 ($p = 0.003$).

Overall Healing Pattern

The P2 group (gentamicin 0.1% + 16% *Centella* asiatica extract cream) consistently demonstrated the most rapid wound length reduction throughout the early and mid- stages of healing (days 1 through 7). The P1 group (NaCl 0.9% + 16% *Centella* asiatica extract cream) showed the second fastest healing rate. Both control groups receiving standard therapy alone (K1 and K2) exhibited comparatively slower wound healing during the initial observation period.

Wound healing is a complex, dynamic biological process essential for restoring tissue integrity following injury (Rodrigues et al., 2019). Incision wounds are classified as acute wounds when the healing process proceeds through the normal sequential phases of hemostasis, inflammation, proliferation, and remodeling (Kartika, 2015). The inflammatory phase is characterized by redness (rubor), warmth (calor), and pain (dolor), accompanied by hemostatic mechanisms including vasoconstriction and platelet aggregation (Wibowo & Comariyati, 2017).

This in vivo study evaluated the combined effect of *Centella asiatica* extract cream with standard wound care therapies on incision wound healing in BALB/c mice over a 21-day period. The macroscopic assessment of wound length revealed that the combination of gentamicin 0.1% and 16% *Centella asiatica* extract cream (P2) resulted in significantly faster wound healing compared to NaCl 0.9% alone (K1) from the earliest observation point. This enhanced healing effect can be attributed to the complementary mechanisms of action of both agents. Gentamicin, as an aminoglycoside antibiotic, is highly effective in inhibiting gram-negative bacteria that commonly cause skin infections, thereby creating a favorable environment for wound healing (Muntiaha et al., 2014). Concurrently, *Centella asiatica* contributes to wound healing through its triterpenoid compounds, which exert multiple pharmacological effects including stimulation of collagen synthesis, promotion of fibroblast proliferation, and enhancement of angiogenesis (Amalia, 2020).

The superiority of the P2 group over gentamicin alone (K2) further supports the additive therapeutic benefit of *Centella asiatica* extract. This enhanced effect is primarily attributed to asiaticoside, the principal bioactive compound in *Centella asiatica*, which stimulates antioxidant defense mechanisms and promotes cellular proliferation at the wound site (Sabila & Muhartono, 2020). Gohil et al. (2010) reported that triterpenoids and saponins in *Centella asiatica* serve as fundamental components supporting the wound healing cascade. These findings are consistent with the study by Anu et al. (2019), which demonstrated that *Centella asiatica* extract and gentamicin provide comparable and complementary effects in incision wound healing.

The combination of NaCl 0.9% and *Centella asiatica* extract (P1) also demonstrated accelerated healing compared to NaCl alone (K1), although the effect was less pronounced than that observed in the P2 group. This difference may be explained by the absence of antibacterial activity in NaCl 0.9%, which serves primarily as a wound irrigant rather than a therapeutic agent (Tonog, 2022). The addition of *Centella asiatica* extract to NaCl provided wound healing benefits through the plant's intrinsic pharmacological properties, but the lack of synergistic antibacterial action resulted in a comparatively slower healing trajectory than the gentamicin-based combination.

Notably, by day 14, no significant differences in wound length were observed among the four groups ($p = 0.368$). This convergence in healing outcomes during the later stages can be attributed to the natural progression of the wound healing process into the proliferative and remodeling phases. During these phases, angiogenesis and epithelialization restore tissue architecture regardless of the initial treatment, with wound contraction typically completing between days 7 and 14 (Griffin et al., 2020). By day 21, complete wound closure was achieved in all groups, confirming that all treatments ultimately supported full wound resolution.

A limitation of this study was the housing of multiple mice in single cages during the initial phase, which occasionally led to aggressive interactions and minor secondary injuries. However, this did not significantly affect the primary wound healing trajectory. Additionally, this study assessed wound healing solely through macroscopic measurement of wound length. Future studies incorporating histological analysis, including evaluation of collagen density, fibroblast count, and inflammatory cell infiltration, would provide a more comprehensive understanding of the cellular mechanisms underlying the observed accelerated healing.

CONCLUSION

This study demonstrates that the combination of *Centella asiatica* extract cream with standard wound care therapies significantly accelerates incision wound healing in BALB/c mice. Specifically, the combination of gentamicin 0.1% and 16% *Centella asiatica* extract cream provides the most effective acceleration of wound healing, followed by the combination of NaCl 0.9% and 16% *Centella asiatica* extract cream. These findings suggest that *Centella asiatica* extract cream may serve as a valuable adjunctive therapy to enhance conventional wound management protocols. Further studies employing histopathological and molecular analyses are recommended to elucidate the precise mechanisms underlying this synergistic wound healing effect.

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