narra j

Original Article

Effect of hemp seed oil on accelerating wound healing: Evaluation of wound size reduction, epithelialization, granulation tissue formation, and vascularization in murine models

Safrizal Rahman[1,2*], Mohammad A. Rivai[3], Dasrul Dasrul[4] and Onarisa Ayu[1,2]

[1]Division of Orthopedics and Traumatology, Department of Surgery, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [2]Division of Orthopedics and Traumatology, Department of Surgery, Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia; [3]Department of Surgery, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [4]Laboratory of Animal Reproduction, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

*Corresponding author: safrizalrahman@usk.ac.id

Abstract

Essential oils have gained interest in wound management, with prior studies exploring combinations of hemp seed oil (*Cannabis sativa*) and other oils. However, single-oil strategies may offer simpler formulation, reducing the risk of interactions while preserving therapeutic benefits. The aim of this study was to explore the effect of hemp seed oil on accelerating wound healing, focusing on wound size reduction, epithelialization, granulation tissue formation, and vascularization in murine models. An in vivo with a post-test-only control group was conducted using 36 male *Mus musculus* mice (3–4 months, 150–250 grams) which were divided into three groups: negative control (NC), positive control (PC, treated with chloramphenicol ointment twice daily), and treatment group (TG, treated with hemp seed oil 400,000 mg/mL twice daily). Mice were euthanized on day 3, 7, 14, and 21 for wound healing assessment, including macroscopic evaluation (visual observation, wound size, and wound healing rate) and microscopic evaluation (epithelialization, granulation tissue formation, and vascularization). The present study found that the TG group demonstrated smaller wound sizes on day 14 (p<0.001) and day 21 (p<0.001). This group also enhanced wound healing rates observed on day 14 (p<0.001) and day 21 (p=0.001) compared to PC and NC groups. Epithelialization was significantly higher in the TG group compared to PC and NC groups on day 14 (p=0.007), while granulation tissue formation showed significant improvement on day 3 (p=0.045), day 14 (p=0.028), and day 21 (p=0.003). Additionally, TG group showed significantly greater new blood vessel formation on day 21 (p=0.001) compared to the PC and NC groups. In conclusion, hemp seed oil demonstrated significant potential in accelerating wound healing processes suggesting a superior effect compared to chloramphenicol ointment. Therefore, hemp seed oil may serve as a promising natural and cost-effective adjunct for wound management.

Keywords: Essential oil, epithelialization, hemp seed oil, granulation tissue, wound healing

Introduction

Wound healing is a complex biological process affecting millions of individuals globally, particularly patients with chronic wounds, who face significant physical, psychological, and social

https://narraj.org/

Received: December 5, 2024 - Accepted: March 27, 2025 - Published online: March 28, 2025

burdens, including pain, reduced mobility, depression, and anxiety [1-4]. Chronic wounds impose a significant burden on quality of life and present a challenge to healthcare systems [1,2]. While standard treatments, including dressings, antibiotics, and surgical interventions, remain the cornerstone of wound management, there is increasing interest in adjunctive therapies to enhance wound healing outcomes [5-7]. Emerging and cost-effective approaches, such as advanced wound dressings, skin substitutes, exogenous growth factor-based therapies, and systemic treatments, are essential for improving patient care and ensuring healthcare sustainability [8].

In recent years, the application of essential oils of natural compounds that is derived from plants has attracted considerable interest in wound management [9-16]. Hemp seed oil (*Cannabis sativa*) has gained attention for its distinctive properties due to its rich composition of bioactive compounds, including polyunsaturated fatty acids, terpenoids, and cannabinoids, which may offer anti-inflammatory, antimicrobial, and skin-regenerative benefits [17-19]. Phytol from hemp seed oil has been shown to exhibit anti-inflammatory activity by reprogramming human monocyte-macrophages towards anti-inflammatory phenotypes, thereby reducing inflammatory responses [15]. Hemp seed oil also contains polyunsaturated fatty acid triglycerides, particularly linoleic and linolenic acids, which contribute to its antioxidant properties [16]. Additionally, hemp seed oil contains tetrahydrocannabinol and cannabidiol, which provide anti-inflammatory, analgesic, antioxidant, and antimicrobial properties that support wound healing by reducing inflammation, preventing infections, and promoting tissue regeneration [20]. Furthermore, hemp seed oil has been demonstrated to reduce oxidative stress in *Drosophila melanogaster* larvae, potentially aiding in the prevention and treatment of conditions induced by reactive oxygen species [16].

A previous study has focused on combining hemp seed oil with other oils, hypothesizing synergistic effects from the diverse bioactive compounds [21]. Although such combinations may provide broader benefits, identifying the specific contributions of individual components becomes more challenging. A single-oil strategy might simplify formulation and reduce the risk of potential interactions among various compounds. The aim of this study was to explore the effect of hemp seed oil on accelerating wound healing, focusing on wound size reduction, epithelialization, granulation tissue formation, and vascularization in murine models. Murine models are particularly advantageous due to genetic similarities to humans, well-characterized immune responses, and availability of standardized procedures, providing reliable and reproducible results for investigating wound healing mechanisms.

Methods

Study design and setting

An in vivo study with a post-test-only control group was conducted at Laboratory of Animal Research and Laboratory of Histology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. The present study was conducted in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [22], although blinding was not implemented for researchers or veterinarian. A total of 36 male *Mus musculus* mice, aged 3–4 months and weighing 150–250 grams, were employed. Mice were assigned to three experimental groups: negative control group (NC), which included mice with created wounds treated with only physiological 0.9% normal saline (NaCl); positive control group (PC), which included mice with created wounds treated topically with chloramphenicol ointment twice daily; and treatment group (TG), which included mice with created wounds treated topically with hemp seed oil twice daily. Hemp seed oil used in the present study was Hemp Oil Extract 400,000 mg/mL (Shenzhen Mingji Technology, Guangdong, China), a pure hemp seed extract. Chloramphenicol was chosen as the positive control due to its well-established antimicrobial properties and common use in wound healing models. The wounds were created as 2 cm × 0.2 cm elliptical wounds on the dorsal skin, extending to the subcutaneous layer. Three mice from each group were euthanized on days 3, 7, 14, and 21. Wound healing was assessed macroscopically through visual observation, wound size, and wound healing rate, while microscopic evaluation employed modified Nagaoka criteria, assessing epithelialization, granulation tissue formation, and vascularization [23].

Sample size and sampling method

Sample size calculation was based on Federer formula, determined by number of experimental groups and number of experimental attempts, resulting in a minimum of 8 mice per group. To account for a potential dropout rate of 10%, a correction was applied using formula n=n/(1–f). Following this adjustment, the minimum sample size required was calculated to be 10 mice per group, resulting in a total of 30 mice. However, the present study included 12 mice per group to account for the euthanasia of 3 mice at each time point (days 3, 7, 14, and 21). Microsoft Excel v.2021 (Microsoft, Redmond, Washington, US) was used for sample randomization into each group. Neither the researchers nor the veterinarian was blinded.

Eligibility criteria

Mice were included based on following criteria: (1) healthy; (2) male; (3) aged 3–4 months; and (4) weighing between 150–250 grams. Conversely, exclusion criteria were as follows: (1) female; (2) presence of structural or functional abnormalities; and (3) ongoing infection. Drop-out criteria were: (1) deceased mice; (2) signs of infection at the wound site (e.g., swelling, redness, or pus); (3) severe lethargy; and (4) consistently refusing to eat for an extended period. Infected mice were excluded and treated accordingly, and mice exhibiting severe lethargy or consistently refusing to eat were monitored and excluded if no improvement occurred despite supportive care.

Animal preparation

Mice were housed in specially designed cages (30 × 20 × 20 cm), made of plywood and wire mesh, in well-shaded, tranquil rooms with a controlled environment. Each murine was placed in a separate cage. Mice were provided the same quantity and type of food, and a tray beneath the cages collected urine and feces, cleaned daily. Ventilated cages were maintained at a temperature of 25–27°C and humidity of 50–60%. A 12:12 light-dark cycle was maintained, with lights on at 5:30 AM. Mice were fed 10 grams of formulated pellet feed twice a day, receiving a total of 20 grams of pellets per day. Clean water was provided ad libitum through specialized bottles. Acclimatization period lasted for 7 days. Mice experiencing a weight loss of more than 10% during the acclimatization period were excluded.

Wound model creation

Wounds were created under anesthesia induced by intraperitoneal administration of ketamine 5 mg/kg body weight (BW) and xylazine 0.08 mg/kg BW in lower abdominal quadrant. Fur on the dorsal region of the mice was trimmed, shaved, and cleaned with 70% alcohol. The incision was performed using a surgical scalpel #10 blade, creating a 2 cm × 0.2 cm elliptical wound on the dorsal skin, extending to the subcutaneous layer. To ensure consistent incision length, a ruler was used for measurement, while the uniform depth of the wound was confirmed by marking the scalpel to indicate the maximum depth during the incision process.

Intervention

Mice were assigned to three experimental groups: NC, which included mice with created wounds treated with only physiological 0.9% NaCl; PC, which included mice with created wounds treated topically with chloramphenicol ointment twice daily; and TG, which included mice with created wounds treated topically with hemp seed oil 400,000 mg/mL twice daily. A volume of 0.5 mL of 0.9% NaCl was applied directly to the wound using a pipette. Chloramphenicol ointment was applied topically to the wound twice daily, with approximately 0.5 grams dispensed using a spatula and evenly spread over the wound using a sterile cotton swab. Hemp seed oil was applied topically to the wound using a micropipette (0.5 mL per application) and evenly distributed over the wound area with the pipette tip. Hemp seed oil used in the present study was Hemp Oil Extract 400,000 mg/mL (Shenzhen Mingji Technology Co, Guangdong, China), a pure hemp seed extract. Wound healing observations for each group were conducted daily for 21 days. On days 3, 7, 14 and 21, three mice from each group were anesthetized using a ketamine-xylazine combination (75–100 mg/kg BW + 5–10 mg/kg BW, respectively) via intramuscular injection in hind limb muscle, with anesthesia lasting 10–30 minutes. After anesthesia, cervical dislocation was performed to terminate the mice. Dorsal region, from which skin samples were harvested, was cleaned and then excised to a thickness of approximately 3 mm, extending 1–1.5 cm^2 into the

subcutaneous layer. Collected skin samples were then fixed in 10% neutral-buffered formalin. Carcasses of the mice were subsequently incinerated.

Histopathological sample preparation

Samples underwent a series of preparation steps, including Masson Trichrome staining of the skin tissue. The first step in the histological preparation process was fixation, where tissue sections approximately 2 cm in size were placed on glass slides and immersed in formalin solution for 24 hours followed by standard histopathology sample preparation steps. The prepared slides were then stained with Masson Trichrome, following a series of steps involving xylene, alcohol, absolute alcohol, running water, and finishing with Canada balsam to ensure the sections adhered firmly to the glass slide and cover slip. Finally, the slides were examined under an electric microscope (Nikon Instruments, Tokyo, Japan) at 20 µm and 30 µm scales, and photographs were taken. Quantitative histopathological scoring was performed on one tissue section, divided into four quadrants (upper right, lower right, upper left, and lower left). Microscopic observations were made using a microruler on the ocular lens of the microscope at a 1:1,000 scale, with a magnification of 400×.

Histopathological measurement

Microscopic evaluation of wound healing was performed by a pathologist who assessed epithelialization, granulation tissue formation, and vascularization at 40× magnification, followed by further examination at 100× to 400× magnifications under an electric microscope (Nikon Instruments, Tokyo, Japan). Tissue slides were observed under a light microscope at 400× magnification, with five different fields of view examined in a zigzag manner. Observations were conducted using a blinded method, and the results from the five fields of view were averaged.

Study variables

In the present study, dependent variables included macroscopic wound healing evaluation (visual observation, wound size, and wound healing rate) and microscopic wound healing evaluations (epithelialization, granulation tissue formation, and vascularization, measured using the modified Nagaoka criteria) [23]. Visual observation documented three parameters: wound color, scab formation, and new skin formation over 21 days. These parameters were assessed daily through direct visual inspection. Wound color indicated the healing stage, transitioning from red (R) to brown (B), pink (P), and finally white (W). Scab formation was recorded as present or absent, marking the stages of wound drying and scab detachment. New skin formation was documented as present or absent, representing the emergence of epithelialized skin over the wound. Wound size, defined as the wound's surface area, was measured on days 1, 3, 7, 14, and 21 using a caliper. The longest length and widest perpendicular width were multiplied to approximate the area, recorded in cm^2. The wound healing rate was calculated as the percentage reduction in wound size over time, using the formula: ((initial area) - (area at day x)) / initial area ×100%. Measurements were taken on days 1, 3, 7, 14, and 21, with results presented as percentages.

Microscopic assessment of wound healing in the present study involved the preparation of histological tissue slides of the wound area using Masson Trichrome staining, followed by examination under an electric microscope (Nikon Instruments, Tokyo, Japan), at 400× magnification. For epithelialization, a score of 1 indicates minimal formation, a score of 2 reflects moderate formation, and a score of 3 represents complete regeneration. Granulation tissue formation is scored from 1 to 4, with a score of 1 indicating a thin granulation tissue layer, 2 for a medium layer, 3 for a thick layer, and 4 for a very thick layer. Vascularization is assessed by counting blood vessels, with a score of 1 for 1–2 blood vessels per field of view, 2 for 3–4 blood vessels, 3 for 5–6 blood vessels, and 4 for more than 7 blood vessels per field. The results for epithelialization, granulation tissue formation, and vascularization were averaged across five fields of view, with the data reported as unitless values. A higher histological score indicates better wound healing, while a lower score reflects poorer healing. The final maximum score is 11 and the minimum score is 3.34.

Statistical analysis

SPSS version 25.0 software (IBM, New York, US) was employed for data analysis, with $p<0.05$ considered as statistically significance. Continuous data were presented as mean and standard deviation (for normally distributed data) and median (minimum-maximum) for non-normally distributed data and categorical data were presented as frequency and percentages. Shapiro-Wilk test and Levene homogeneity test were utilized to assess data normality. For normally distributed data, a one-way analysis of variance (ANOVA) test was conducted, while Kruskal-Wallis tests was used for non-normally distributed data. Duncan's post hoc tests were conducted to identify specific differences between group means when an analysis of variance test showed significant results. Two-way ANOVA with repeated measures was employed to assess how the time factor (days 3, 7, 14, and 21) interacts with the group (NC, PC, and TG).

Results

Macroscopic evaluation: Effect of hemp seed oil on visual observation, wound size, and wound healing rate in wound models using mice

Visual observation

Based on visual observations of wound healing, a color change of the wound was noted, the transition from brown to pink, and eventually to white, varied between groups. The color change from red to brown occurred on day 1 across all experimental groups (**Table 1**). In the NC group, the color changed from brown to pink on day 8, followed by a transition to white, starting from day 14 until day 21. In the PC group, the color change from brown to pink occurred on day 7, and the wound turned white from day 14 until day 21. In the TG group, the color change from brown to pink occurred more quickly, on day 7, with the wound turning white from day 11 until day 21 (**Table 1**).

In the NC group, scab formation began on day 4, with new skin forming on day 17. In the PC and TG groups, scab formation began on days 2 and 3, respectively (**Table 1**). New skin formation occurred on average on day 15 in the PC group and on day 13 in the TG group, indicating a faster healing process in the TG group. Visual observations also indicated that wounds, initially moist, began to dry as soon as scabs formed on the wound surface. The detachment of the scab coincided with the drying of the wound, signaling the growth of new cells in the skin, which facilitated the quicker detachment of the scab and the closure of the wound edges, leading to new skin formation. The speed of scab formation and new skin development reflects the rate of wound healing (**Table 1**). These visual observation findings demonstrated that hemp oil treatment resulted in faster wound healing compared to the chloramphenicol ointment and the normal saline-treated group.

Table 1. Visual observations of wound color, scab formation, and new skin development across experimental groups from day 1 to day 21

Group	Parameters	Day																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NC	Wound color	R	B	B	B	B	B	B	P	P	P	P	P	P	W	W	W	W	W	W	W	W
	Scab formation	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-
	New skin formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	✓	✓
PC	Wound color	R	B	B	B	B	B	P	P	P	P	P	P	P	W	W	W	W	W	W	W	W
	Scab formation	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-	-
	New skin formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	✓	✓	✓	✓
TG	Wound color	R	B	B	B	B	B	P	P	P	P	W	W	W	W	W	W	W	W	W	W	W
	Scab formation	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-
	New skin formation	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓

NC: negative control group; PC: positive control group; TG: treatment group; R: red; B: brown; P: pink; W: white.

Wound size

Wound size decreased over time in all experimental groups, with varying rates of reduction (**Figure 1**). The greatest reduction in wound size by day 21 was observed in the TG group, with a

decrease of 0.10±0.03 cm², followed by the PC group at 0.22±0.08 cm² (**Table 2**). The smallest reduction in wound size was observed in the NC group at 0.75±0.13 cm². In the NC group, the reduction in wound size was slower compared to the PC and TG groups. By day 7, the TG group demonstrated a more rapid wound size reduction than the PC and NC groups. However, on days 14 and 21, the reduction in wound size in the PC and TG groups was similar and occurred more quickly than in the NC group (**Table 2**).

One-way ANOVA results indicated that the wound size on days 1, 3, and 7 did not show significant differences between the experimental groups, with *p*-values of 0.179, 0.070, and 0.236, respectively (**Table 2**). However, the wound size showed significant differences on days 14 ($p<0.001$) and day 21 ($p<0.001$). These findings suggested that hemp seed oil treatment did not significantly affect wound size during the early stages of healing (days 1, 3, and 7), but had a significant effect on wound size during the later stages of healing (days 14 and 21).

Duncan's post-hoc test results indicated that on day 14, NC group differed significantly from both the PC and TG groups, but no significant difference was found between the PC and TG groups (**Table 2**). Although hemp oil resulted in a smaller wound size, it did not show a statistically significant difference compared to the PC. On day 21, the same pattern was observed, with the TG group having a smaller wound size than the NC and PC groups (**Table 2**). These results confirmed that hemp seed oil treatment accelerated the reduction in wound size during the healing process from day 14 wound healing process compared to chloramphenicol ointment treatment. The results of the two-way ANOVA with repeated measures indicated significant main effects for both the time factor and the experimental group factor, as well as a significant interaction between these factors (**Table 3**). The highest F value was found for the time factor, indicating that time was the primary factor influencing wound size reduction. The group factor and the time × group interaction were also significant, suggesting that both the type of treatment and the combination of time with treatment play crucial roles in the wound healing process. The coefficient of determination (R^2) of 0.994 indicated that the model effectively explains the variability in wound size (**Table 3**). Therefore, both time and treatment group significantly affected wound size reduction, with a significant interaction between these factors.

Table 2. One-way ANOVA and duncan's post hoc test results for wound size reduction among experimental groups on days 1, 3, 7, 14, and 21

Time	Wound size (cm²), mean±SD			*p*-value
	Negative control (NC)	Positive control (PC)	Treatment group (TG)	
Day 1	4.27±0.12	4.34±0.12	4.13±0.12	0.179
Day 3	3.93±0.12	3.87±0.12	3.61±0.19	0.070
Day 7	3.12±0.27	2.89±0.17	2.83±0.10	0.236
Day 14	1.91±0.16[a]	1.13±0.12[b]	0.74±0.05[b]	<0.001*
Day 21	0.75±0.13[a]	0.22±0.08[b]	0.10±0.03[b]	<0.001*

[a-b] Analyzed using Duncan's post hoc test; Means with different superscripts were significantly different from other experimental groups within the same time point, while those with the same superscript indicated no significant difference at $p=0.05$
*Statistically significant at $p<0.05$

Table 3. Two-way ANOVA with repeated measures for wound size reduction, showing the effects of time, group, and the time × group interaction

Source	Degree of freedom	Mean-squared	F	*p*-value	R^2
Time	4	24.921	1300.090	<0.001	0.994
Group	2	1.012	52.775	<0.001	
Time × group	8	0.154	8.042	<0.001	
Error	30	0.019			

Wound healing rate

The wound healing rate data indicated a progressive increase in the percentage of wound healing over time across all experimental groups. The highest healing rate was observed in the TG group, with a healing rate of 23.32±12.29% on day 3, 54.19±3.38% on day 7, 92.96±1.34% on day 14, and 99.94±0.03% on day 21 (**Table 4**). This was followed by the PC group, with healing rates of 20.54±4.52% on day 3, 56.02±2.94% on day 7,97.00±0.26% on day 14, and 99.73±0.18% on day 21. The lowest healing percentage was observed in the NC group, with healing rates of

15.06±4.99% on day 3, 46.59±6.57% on day 7, 79.92±2.25% on day 14, and 96.86±0.94% on day 21 (**Table 4**).

One-way ANOVA results indicated that the percentage of wound healing on days 3 and 7 did not differed significantly across the experimental groups, with *p*-values of 0.488 and 0.233, respectively (**Table 4**). However, significant differences were observed on days 14 ($p<0.001$) and day 21 ($p=0.001$), respectively. These results suggested that hemp seed oil treatment did not significantly affect the wound healing rate on days 3 and 7, but had a significant effect on wound healing rate on days 14 and 21.

Figure 1. Wound size was measured on days 1, 3, 7, 14, and 21 using a caliper, indicating that hemp seed oil treatment accelerated wound healing compared to chloramphenicol ointment and normal saline.

Duncan's post-hoc analysis of the data showed that the average wound healing rate on days 14 and 21 in the TG group was significantly higher than in both the NC and PC groups (**Table 4**). On day 14, a significant difference was observed, with the TG group showing the highest effectiveness, followed by the PC group, and the NC group being the least effective. On day 21, the TG group showed the most effective outcome, with no significant difference between the PC and NC groups (**Table 4**). These results indicated that hemp seed oil treatment was more effective than chloramphenicol ointment in accelerating wound healing in the day 14 and day 21 of wound healing.

The results of the two-way ANOVA with repeated measures showed significant main effects for both the time factor and the group factor (**Table 5**). The time factor had the highest F-value, indicating that time was the primary factor influencing wound healing rate. The group factor also showed a significant effect, suggesting that the type of treatment played a role in the wound healing rate. However, the interaction between time and group was not significant, indicating that the effect of the group remained consistent across the time points. The R^2 value of 0.991 indicated that the model explains 99.1% of the variability in wound healing rate (**Table 5**).

Therefore, the findings indicated that both time and treatment group significantly influence wound healing rate, with time being the dominant factor and the treatment effect consistent across time points.

Table 4. One-way ANOVA and duncan's post-hoc test results for wound healing rate among experimental groups on days 3, 7, 14, and 21

Time	Wound healing rate (%), mean±SD			*p*-value
	Negative control (NC)	Positive control (PC)	Treatment group (TG)	
Day 3	15.06±4.99	20.54±4.52	23.32±12.29	0.488
Day 7	46.59±6.57	54.19±3.38	56.02±2.94	0.233
Day 14	79.92±2.25[a]	92.96±1.34[b]	97.00±0.26[c]	<0.001*
Day 21	96.86±0.94[a]	99.73±0.18[a]	99.94±0.03[b]	0.001*

[a-c] Analyzed using Duncan's post hoc test; means with different superscripts were significantly different from other experimental groups within the same time point, while those with the same superscript indicated no significant difference at $p=0.05$
*Statistically significant at $p<0.05$

Table 5. Two-way ANOVA with repeated measures for wound healing rate, showing the effects of time, group, and the time × group interaction

Source	Degree of freedom	Mean-squared	F	*p*-value	R^2
Time	4	16617.234	814.257	<0.001	0.991
Group	2	214.014	10.487	<0.001	
Time × group	8	36.786	1.803	0.116	
Error	30	20.408			

Microscopic evaluation: Effect of hemp seed oil on epithelialization, granulation tissue formation, and vascularization in wound models using mice

Epithelialization

On day 3, NC group had the highest epithelialization (1.87±0.31), while TG group had the lowest (1.20±0.35) (**Table 6**). By day 7, epithelialization had increased in all experimental groups, with PC group showing the highest value (2.13±0.23). On day 14, TG group exhibited greater epithelialization (2.67±0.23) compared to PC group (1.87±0.31) and NC group (2.87±0.23). By day 21, epithelialization levels were comparable across groups, with TG group slightly higher (2.13±0.12) (**Table 6**).

Based on the one-way ANOVA results, the epithelialization on days 3, 7, and 21 did not show significant differences across the experimental groups, with *p*-values of 0.108, 0.680, and 0.098, respectively (**Table 6**). However, significant differences were observed on day 14 ($p=0.007$). These results indicated that hemp seed oil treatment did not significantly affect the epithelialization on days 3, 7, and 21, but had a significant effect on day 14 of wound healing.

Duncan's post-hoc test results revealed that the average epithelialization score on day 7 in the TG group was significantly higher compared to the NC and PC groups (**Table 6**). The PC group also showed a significantly higher epithelialization score compared to the NC group. These findings demonstrated that hemp seed oil treatment can accelerate epithelialization in the wound healing process on day 14.

The results from the two-way ANOVA with repeated measures showed that the time factor had a significant effect on epithelialization, indicating that changed over time influenced the epithelialization (**Table 7**). The group factor, however, was not significant, suggesting that the type of treatment did not significantly affect the epithelialization. The interaction between time and group was significant, indicating that the effect of time on epithelialization was influenced by the treatment group. The R^2 value of 0.813 indicated that the model explained 81.3% of the variability in epithelialization (**Table 7**). Therefore, the findings suggested that time significantly influences epithelialization, while the treatment group alone did not have a significant effect. However, the interaction between time and treatment indicated that the impact of time on wound healing varied depending on the treatment group.

The histopathological evaluation of epithelialization revealed that minimal epithelialization occurred on day 3, indicating the early stages of epithelialization (**Figure 2**). As the healing process progressed, a notable increase in epithelialization thickness was observed on days 7, 14,

and 21. Both the TG and the PC group showed significantly greater epithelialization thickness compared to the NC group, which exhibited moderate epithelialization progression. These findings suggested that the hemp seed oil treatment and chloramphenicol both enhanced the epithelialization process, leading to thicker epithelial layers over time. In contrast, the NC group demonstrated slower progression, with a less pronounced increase in epithelial thickness.

Table 6. One-way ANOVA and Duncan's post hoc test results for epithelialization among experimental groups on days 3, 7, 14, and 21

Time	Epithelialization, mean±SD			*p*-value
	Negative control (NC)	Positive control (PC)	Treatment group (TG)	
Day 3	1.87±0.31	1.47±0.31	1.20±0.35	0.108
Day 7	1.93±0.23	2.13±0.23	2.00±0.35	0.680
Day 14	2.87±0.23[a]	1.87±0.31[a]	2.67±0.23[b]	0.007*
Day 21	1.93±0.94	2.00±0.00	2.13±0.12	0.098

[a-b] Analyzed using duncan's post hoc test; means with different superscripts were significantly different from other experimental groups within the same time point, while those with the same superscript indicated no significant difference at $p=0.05$
*Statistically significant at $p<0.05$

Table 7. Two-way ANOVA with repeated measures for epithelialization, showing the effects of time, group, and the time × group interaction

Source	Degree of freedom	Mean-squared	F	*p*-value	R^2
Time	3	1.373	21.678	<0.001	0.813
Group	2	0.021	0.333	0.720	
Time × group	6	0.406	6.415	<0.001	
Error	30	0.063			

Granulation tissue formation

On day 3, PC group showed the highest granulation formation (1.93±0.42), exceeding NC group (1.60±0.20) and TG group (1.13±0.23) (**Table 8**). By day 7, granulation increased in all experimental groups, with TG group achieving the highest value (2.20±0.20). On day 14, TG group showed significantly greater granulation (3.07±0.31) compared to NC group (2.07±0.12) and PC group (2.20±0.53). By day 21, PC group and TG group displayed similar granulation (2.33±0.23 and 2.33±0.31, respectively), both higher than NC group (1.40±0.00) (**Table 8**).

Based on the one-way ANOVA results, the granulation tissue formation on days 3, 14, and 21 showed significant differences across the experimental groups, with *p*-values of 0.045, 0.028, and 0.003, respectively (**Table 8**). However, the granulation tissue formation on day 7 did not show significant differences ($p=0.187$). The findings showed that hemp seed oil treatment significantly affect granulation tissue formation during wound healing in mice on days 3, 14, and 21, but had no significant effect on day 7.

Duncan's post-hoc test results indicated that on day 3, the average granulation tissue formation in the PC group was significantly higher compared to the NC and TG groups, with no significant difference observed between the NC and TG groups (**Table 8**). On day 14, the TG group showed significantly higher granulation tissue formation compared to the PC and NC groups; however, no significant difference was observed between the PC and NC groups. On day 21, both the TG and PC groups showed significantly higher granulation tissue formation compared to the NC group; however, there was no significant difference between the TG and PC groups (**Table 8**). These findings demonstrated that hemp seed oil treatment was more effective than chloramphenicol in accelerating granulation tissue formation on day 14 during the wound-healing process; however, the effect was similar on day 21.

The results from the two-way ANOVA with repeated measures revealed significant main effects for both the time factor and the group factor, suggesting that both time and treatment type significantly affect granulation tissue formation (**Table 9**). Additionally, the interaction between time and group was also significant, indicating that the effect of time on granulation tissue formation differed across treatment groups. The R^2 value of 0.812 indicated that the model explained 81.2% of the variability in granulation tissue formation (**Table 9**). Therefore, the

findings suggested that both time and treatment type significantly affected granulation tissue formation, with the effect of time varying across treatment groups.

Table 8. One-way ANOVA and Duncan's post hoc test results for granulation tissue formation among experimental groups on days 3, 7, 14, and 21

Time	Granulation tissue formation, mean±SD			*p*-value
	Negative control (NC)	Positive control (PC)	Treatment group (TG)	
Day 3	1.60±0.20[a]	1.93±0.42[b]	1.13±0.23[a]	0.045*
Day 7	1.80±0.35	2.00±0.00	2.20±0.20	0.187
Day 14	2.07±0.12[a]	2.20±0.53[a]	3.07±0.31[b]	0.028*
Day 21	1.40±0.00[a]	2.33±0.23[b]	2.33±0.31[b]	0.003*

[a-b] Analyzed using Duncan's post hoc test; Means with different superscripts were significantly different from other experimental groups within the same time point, while those with the same superscript indicated no significant difference at $p=0.05$
*Statistically significant at $p<0.05$

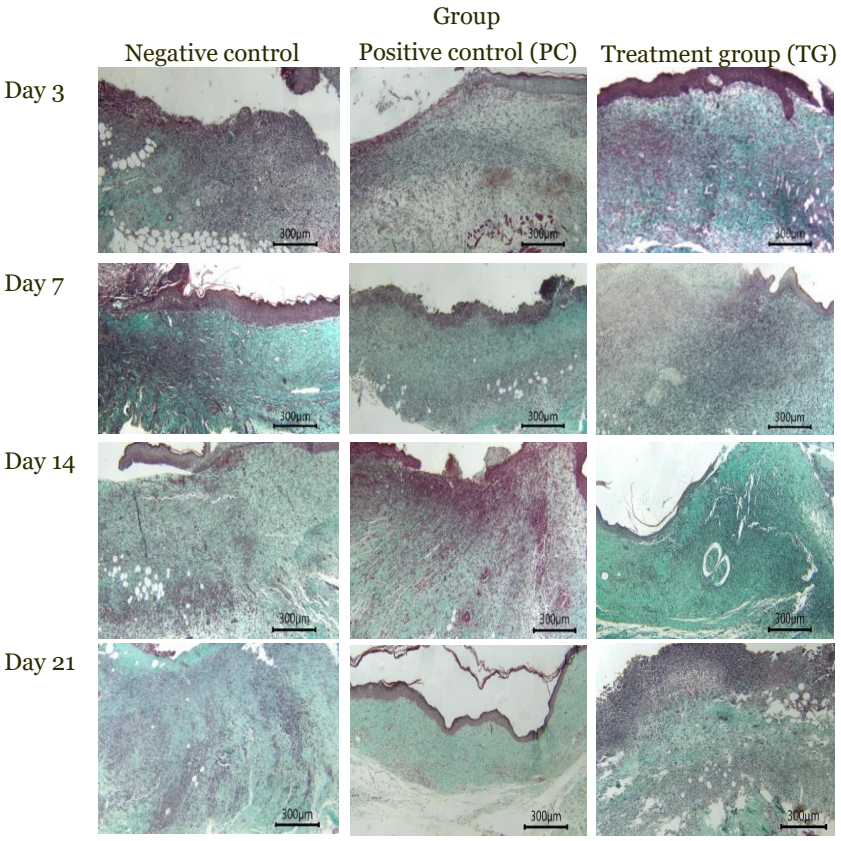

Figure 2. Histopathological evaluation of epithelialization at 400× magnification revealed minimal epithelialization on day 3, with progressive increases observed on days 7, 14, and 21. The treatment group (TG) and positive control (PC) group demonstrated greater epithelialization thickness, while the negative control (NC) group showed only moderate progression.

Table 9. Two-way ANOVA with repeated measures for granulation tissue formation, showing the effects of time, group, and the time × group interaction

Source	Degree of freedom	Mean-squared	F	*p*-value	R^2
Time	3	0.822	10.273	<0.001	0.812
Group	2	0.764	9.556	0.001	
Time × group	6	0.714	8.926	<0.001	
Error	30	0.080			

The histopathological evaluation of granulation tissue formation showed a clear progression in tissue development across the study period (**Figure 3**). On day 3, granulation tissue was absent in all groups, indicating that the early stages of granulation tissue formation had not yet initiated. By day 7, thin, scattered granulation fibers began to form in all groups, suggesting the

onset of the granulation phase of healing. However, the fibers remained sparse and disorganized, reflecting the early stage of granulation tissue development. On day 14, a noticeable increase in the quantity of granulation fibers was observed, with the TG demonstrating a denser deposition of fibers compared to the PC and NC groups. Although the fibers in all groups were still disorganized at this point, the TG group exhibited more pronounced granulation tissue formation, which indicated a more effective wound healing response (**Figure 3**).

By day 21, granulation tissue had filled the wound gaps in all groups, becoming denser and more mature (**Figure 3**). The TG group showed the most advanced stage of granulation tissue formation, with dense and well-organized fibers, suggesting accelerated healing. All groups exhibited more mature Type I granulation tissue on day 14 compared to day 7, but the TG group stood out with significantly enhanced granulation formation, particularly when compared to the NC and PC groups. These findings suggested that hemp seed oil treatment facilitated more efficient and accelerated granulation tissue formation, promoting better wound healing compared to the other groups (**Figure 3**).

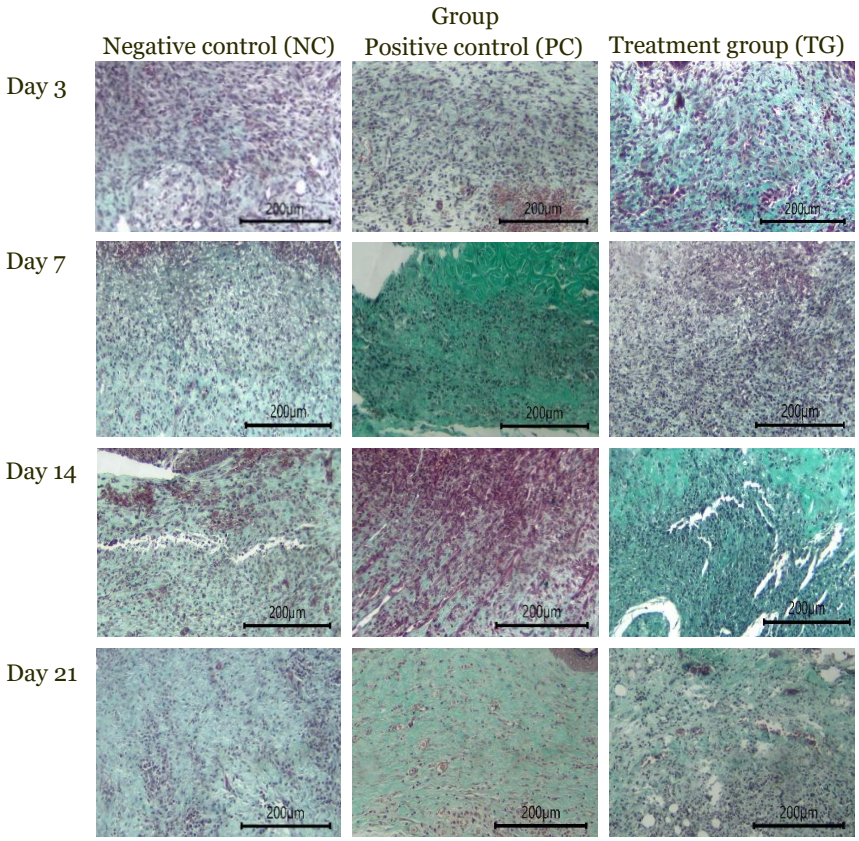

Figure 3. Histopathological evaluation of granulation tissue formation under 400× magnification revealed, on day 3, granulation was absent in all groups. By day 7, thin, scattered granulation fibers were observed. On day 14, granulation fibers increased, with the treatment group (TG) showing denser deposition than the positive control (PC) and negative control (NC) groups, though the fibers remained disorganized. By day 21, granulation tissue filled the wound gaps and became denser. All groups exhibited more mature Type I granulation on day 14 compared to day 7, with the TG group showing notably enhanced formation compared to the NC and PC groups.

Vascularization

On day 3, vascularization was lowest in TG group (1.07±0.12) and highest in PC group (1.40±0.20) (**Table 10**). By day 7, vascularization increased in TG group (1.60±0.00), slightly exceeding PC group (1.33±0.12) and NC group (1.73±0.42). On day 14, TG group showed the highest vascularization (2.60±0.20), followed by PC group (2.07±0.46) and NC group (1.93±0.23). By day 21, TG group maintained the highest vascularization (1.93±0.12), exceeding PC group (1.47±1.29) and NC group (1.40±0.00) (**Table 10**).

Based on the one-way ANOVA results, the scores for vascularization in the wound tissue on days 3, 7, and 14 did not show significant differences across the experimental groups, with *p*-values of 0.072, 0.216, and 0.0929, respectively (**Table 10**). However, on day 21, there was a

significant difference (p=0.001). These results suggested that hemp seed oil treatment did not significantly affect new blood vessel formation on days 3, 7, and 14, but had a significant effect on new blood vessel formation on day 21 of the wound healing process.

According to Duncan's post-hoc test results, the average score for vascularization on day 21 was significantly higher in the TG group compared to both the NC and PC groups (**Table 10**). The PC group also showed a significantly higher score compared to the NC group. These findings demonstrated that hemp seed oil treatment accelerates the formation of vascularization on day 21 during the wound healing process in mice.

Table 10. One-way ANOVA and Duncan's post-hoc test results for vascularization among experimental groups on days 3, 7, 14, and 21

Time	Vascularization, mean±SD			p-value
	Negative control (NC)	Positive control (PC)	Treatment group (TG)	
Day 3	1.33±0.12	1.40±0.20	1.07±0.12	0.072
Day 7	1.73±0.42	1.33±0.12	1.60±0.00	0.216
Day 14	1.93±0.23	2.07±0.46	2.60±0.20	0.092
Day 21	1.40±0.00a	1.47±1.29b	1.93±0.12c	0.001*

[a-c]Analyzed using Duncan's post hoc test; Means with different superscripts were significantly different from other experimental groups within the same time point, while those with the same superscript indicated no significant difference, at p=0.05;
*Statistically significant at p<0.05

The results from the two-way ANOVA with repeated measures showed that the time factor has a significant effect on vascularization, indicating that vascularization changed over time (**Table 11**). However, the group factor and the interaction between time and group were not significant, suggesting that the type of treatment and the combination of time and treatment did not significantly influence vascularization. The R^2 value of 0.567 indicated that the model explained 56.7% of the variability in vascularization (**Table 11**). Therefore, the findings suggested that vascularization was significantly influenced by time, but the treatment type and the interaction between time and treatment did not have a significant impact. The model explained 56.7% of the variability in vascularization.

Table 11. Two-way ANOVA with repeated measures for vascularization, showing the effects of time, group, and the time × group interaction

Source	Degree of freedom	Mean-squared	F	p-value	R^2
Time	3	1.382	7.449	0.001	0.567
Group	2	0.191	1.030	0.372	
Time × group	6	0.218	1.174	0.353	
Error	30	0.186			

The histopathological evaluation of vascularization showed a progressive increase in the formation of new blood vessels across all experimental groups over the study period (**Figure 4**). On day 3, the initial stages of vascularization were evident in all groups, although the number of newly formed blood vessels remained limited at this early stage of wound healing. By day 7, a modest increase in vascularization was observed in all groups, indicating the ongoing development of new blood vessels. However, the TG showed a more pronounced increase in blood vessel formation compared to both the NC and PC groups, suggesting that the treatment had a stimulatory effect on vascularization (**Figure 4**).

On day 14, vascularization was more pronounced, with further formation of blood vessels. The TG group continued to exhibit a higher number of new blood vessels compared to the NC group, indicating that the hemp seed oil treatment further accelerated the process of vascularization (**Figure 4**). The PC group also showed improved vascularization, though it did not surpass the TG group. By day 21, vascularization reached its peak in all groups, with the TG group showing the most substantial vascular network. This enhanced vascularization in the TG group suggested that the treatment effectively promoted the formation of new blood vessels (**Figure 4**). Thus, the findings indicated that hemp seed oil treatment significantly enhanced

vascularization compared to the NC group, with the most notable improvement observed over the entire study period.

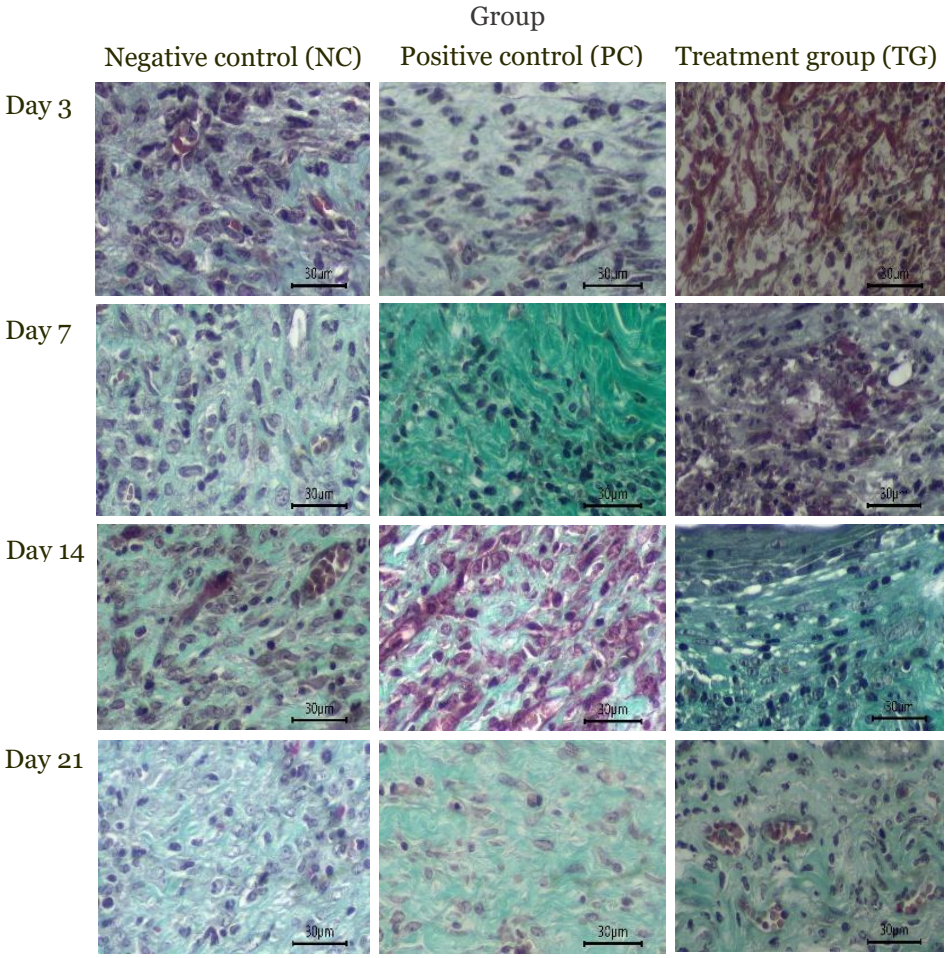

Figure 4. Histopathological evaluation of vascularization under 400× magnification revealed a gradual increase in new blood vessel formation on days 3, 7, 14, and 21 across all experimental groups. The treatment group (TG) group exhibited a higher number of new blood vessels compared to the NC group, indicating enhanced vascularization.

Discussion

Wound healing is a complex biological process that occurs in distinct yet overlapping stages: hemostasis, inflammation, proliferation, and remodeling [24,25]. During hemostasis, clot formation halts bleeding and creates a fibrin matrix as a scaffold for cell migration [26]. In the inflammatory phase, immune cells, such as neutrophils and macrophages, clear debris and pathogens while releasing cytokines to signal tissue repair [4]. The proliferation stage involves fibroblast activity, angiogenesis, and collagen deposition, facilitating granulation tissue formation [27]. Finally, in the remodeling phase, collagen is reorganized, and the wound achieves tensile strength [28]. Effective wound management requires therapies that address each of these stages to ensure optimal healing [29].

In the early stages of wound healing (days 1, 3, and 7), hemp seed oil treatment in the present study did not significantly differ from chloramphenicol ointment in reducing wound size. Hemp seed oil demonstrated superior effectiveness in accelerating wound size reduction compared to chloramphenicol ointment during days 14 and 21, indicating its potential as a supportive therapy for prolonged wound healing phases. While both treatments improved epithelialization, the significant effect observed on day 14 in the present study suggested that hemp seed oil may provide particular benefits during this critical stage of wound healing, potentially accelerating the transition to tissue remodeling. The findings of the present study aligned with the established wound healing process, where epithelialization typically began on days 3 to 4 post-injury,

accompanied by neovascularization and fibroblast accumulation [30]. Keratinocytes migrated to regenerate the basal membrane, and once restored, proliferation peaks around day 4 [4]. The epithelial layer thickens, forming a new cornified layer, and keratinocytes reattach to the basal lamina [4]. By day 7, fibroblasts begin secreting type III collagen and transition into myofibroblasts, preparing the wound for the subsequent proliferative and remodeling phases [25]. The significant increase in epithelial thickness on day 14 in the hemp seed oil group was consistent with the peak proliferative activity of keratinocytes and the formation of a new cornified epithelial layer, suggesting that hemp seed oil may enhanced keratinocyte proliferation and differentiation.

During the proliferative phase, keratinocytes migrate to close the wound gap, angiogenesis reforms blood vessels, and fibroblasts replace the early fibrin clot with granulation tissue [4,25]. In the present study, hemp seed oil treatment significantly accelerated granulation tissue formation during wound healing, particularly on day 14, where it outperformed chloramphenicol. However, its effect on day 21 was comparable to chloramphenicol. By day 14, hemp seed oil treatment demonstrated denser fiber deposition and more pronounced granulation tissue formation compared to the chloramphenicol and control groups, although the fibers remained somewhat disorganized. By day 21, granulation tissue in the hemp seed oil treatment exhibited the most advanced and well-organized structure, indicating accelerated and more efficient healing compared to the chloramphenicol and control groups.

Furthermore, in the present study, hemp seed oil treatment significantly increased vascularization on day 21, with no effect observed on days 3, 7, or 14. On day 14, vascularization was more evident in histopathological evaluation, and the hemp seed oil treatment continued to outperform the negative control group. By day 21, vascularization peaked, with the hemp seed oil treatment demonstrating the most substantial vascular network compared to chloramphenicol and negative control groups. Vascularization plays a critical role in wound healing, as insufficient new blood vessel formation can result in ischemia and tissue loss [30]. Vascular formation begins from day 1 to day 3; as healing advances, vascularization intensifies, peaking around days 7 and 14. During this period, the number of new blood vessels increases significantly, reflecting the wound's increased demand for nutrients and oxygen [30]. This peak in vascularization also marks the initiation of vascular remodeling, where immature blood vessels mature and organize into a more stable and functional network [30]. The process of vascular remodeling was crucial for transitioning from the initial stages of wound healing, which involve inflammation and cell proliferation, to the later stages, such as tissue maturation and scar formation [4]. This enhanced vascularization in the hemp seed oil-treated group aligned with the typical progression of vascularization observed in wound healing, where blood vessel formation begins on days 1–3, peaks around days 7 and 14, and transitions into vascular remodeling. By day 21, when the vascular network was fully established and remodeling occurs, hemp seed oil treatment offered a significant improvement compared to both the chloramphenicol and negative control groups in promoting angiogenesis.

Hemp seed oil contains a variety of bioactive components, each with the potential to accelerate specific stages of wound healing [31]. Polyunsaturated fatty acids, such as omega-3 and omega-6, can modulate inflammation by regulating pro- and anti-inflammatory cytokine production, aiding the inflammatory phase [31]. Terpenoids and flavonoids in hemp oil possess antioxidant properties, which reduce oxidative stress and support tissue repair during proliferation [32]. Additionally, cannabinoids such as cannabidiol exhibit anti-inflammatory and antimicrobial effects, crucial for infection control and balanced immune responses [17]. The combined actions of these compounds suggested that hemp seed oil may enhance wound healing through multiple pathways, addressing both the inflammatory and proliferative stages effectively.

The findings of the present study highlighted the efficacy of hemp oil in accelerating wound healing processes, particularly wound size reduction, epithelialization, granulation tissue formation, and vascularization, with results indicating superior effect compared to chloramphenicol ointment. These outcomes aligned with previous research exploring the synergistic potential of combined essential oils in wound healing, such as a novel formulation comprising sesame, hemp, wild pistachio, and walnut oils, which demonstrated significant improvements in wound contraction and epithelialization time in animal models of burn wounds

[21]. While combined formulations leverage the diverse bioactive compounds of multiple oils, the specific contributions of individual components remained unclear. Hemp seed oil, as a single-agent treatment, simplifies formulations and avoids potential interactions among bioactive compounds, yet still exhibits robust therapeutic potential. In the present study, hemp seed oil demonstrated significant acceleration of wound size reduction, epithelialization, granulation tissue formation, and vascularization, outcomes comparable to those observed with more complex oil combinations [21].

Clinically, the present study suggested that hemp oil holds promise as a natural and potentially cost-effective adjunct to current wound management strategies in humans. Existing studies have highlighted the safety profile of topical cannabinoid products, with minimal irritation or sensitization observed in patch tests on healthy participants [19,33,34]. However, rare cases of allergic contact dermatitis with a follicular pattern following repeated applications have been reported [35]. Furthermore, in vitro findings indicated that cannabinoid products exhibit tolerability comparable to historically non-irritating substances [19].

Despite these encouraging outcomes, further investigation is required to establish the efficacy and safety of hemp oil in human wound management [36]. The use of a murine model (*Mus musculus*) in the present study may not completely replicate human wound healing processes, limiting the direct applicability of the findings to human clinical settings. Furthermore, the present study period was limited to 21 days, which may not fully capture the long-term effects of hemp seed oil on wound healing. Moreover, the use of only one dose of hemp seed oil restricts the ability to assess dose-dependent effects. Typically, three doses (minimum, standard, and maximum) are used to determine the optimal therapeutic dose. The single dose used may represent a suboptimal level, potentially explaining the lack of significant results in certain variables. Future studies should incorporate multiple doses to better understand the dose-response relationship and identify the most effective dose for wound healing. Rigorous clinical trials are essential to determine its potential role as an alternative or complementary therapy for enhancing wound healing outcomes in clinical settings.

Conclusion

Hemp seed oil demonstrated significant potential in accelerating wound healing processes, particularly in promoting wound size reduction, epithelialization, granulation tissue formation, and vascularization, indicating a superior effect compared to chloramphenicol ointment. Hemp seed oil may be considered a promising adjunct or alternative treatment for wound management, particularly for patients seeking natural and cost-effective options. Nevertheless, further research is required to assess its effectiveness and safety in managing wounds in humans.

Ethics approval

Protocol of the present study was reviewed and approved by Ethical Committee for Animal Research, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia (Approval number: 304/KEPH/VII/2024).

Acknowledgments

The authors would like to thank the staff of Laboratory of Animal Research and Laboratory of Histology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools and methodologies in the following capacities of which AI-based language models ChatGPT was employed in the language refinement (improving grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

How to cite

Rahman S, Rivai MA, Dasrul D, Ayu O. Effect of hemp seed oil on accelerating wound healing: Evaluation of wound size reduction, epithelialization, granulation tissue formation, and vascularization in murine models. Narra J 2025; 5 (1): e1878 - http://doi.org/10.52225/narra.v5i1.1878.

References

1. Betts A, Yeowell G, Odeyemi I, *et al.* Systematic review of patient perspectives and quality of life outcomes associated with use of topical interventions for treatment of chronic wounds. Value Health 2018;21:S242.
2. Fearns N, Heller-Murphy S, Kelly J, *et al.* Placing the patient at the centre of chronic wound care: A qualitative evidence synthesis. J Tissue Viability 2017;26(4):254-259.
3. Kodange C. Screening for depression in patients with chronic wounds. Adv Skin Wound Care 2021;34(9):502-503.
4. Wilkinson HN, Hardman MJ. Wound healing: Cellular mechanisms and pathological outcomes. Open Biol 2020;10(9):200223.
5. Mirhaj M, Labbaf S, Tavakoli M, *et al.* An overview on the recent advances in the treatment of infected wounds: Antibacterial wound dressings. Macromol Biosci 2022;22(7):e2200014.
6. Howell RS, Gorenstein S, Castellano M, *et al.* Wound care center of excellence: Guide to operative technique for chronic wounds. J Am Coll Surg 2018;226(2):e7-e17.
7. Symulewicz M, Korol F, Edyko P, *et al.* New technologies in the surgical treatment of wounds. J Edu Health Sport 2023;46(1):22-34.
8. Oliveira A, Simões S, Ascenso A, *et al.* Therapeutic advances in wound healing. J Dermatolog Treat 2022;33(1):2-22.
9. Mohammed HA, Qureshi KA, Ali HM, *et al.* Bio-evaluation of the wound healing activity of *Artemisia judaica* L. as part of the plant's use in traditional medicine; phytochemical, antioxidant, anti-inflammatory, and antibiofilm properties of the plant's essential oils. Antioxidants 2022;11(2):332.
10. García-Salinas S, Evangelopoulos M, Gámez-Herrera E, *et al.* Electrospun anti-inflammatory patch loaded with essential oils for wound healing. Int J Pharm 2020;577:119067.
11. Jaramillo V, Díaz E, Muñoz LN, *et al.* Enhancing wound healing: A novel topical emulsion combining cw49 peptide and lavender essential oil for accelerated regeneration and antibacterial protection. Pharmaceutics 2023;15(6):1739.
12. Avola R, Granata G, Geraci C, *et al.* Oregano (*Origanum vulgare L.*) essential oil provides anti-inflammatory activity and facilitates wound healing in a human keratinocytes cell model. Food Chem Toxicol 2020;144:111586.
13. Pérez-Recalde M, Ruiz Arias IE, Hermida ÉB. Could essential oils enhance biopolymers performance for wound healing? A systematic review. Phytomedicine 2018;38:57-65.
14. Costa MF, Durço AO, Rabelo TK, *et al.* Effects of carvacrol, thymol and essential oils containing such monoterpenes on wound healing: A systematic review. J Pharm Pharmacol 2019;71(2):141-155.
15. Claro-Cala CM, Grao-Cruces E, Toscano R, *et al.* Acyclic diterpene phytol from hemp seed oil (*Cannabis sativa L.*) exerts anti-inflammatory activity on primary human monocytes-macrophages. Foods 2022;11(15):2366.
16. Vitorović J, Joković N, Radulović N, *et al.* Antioxidant activity of hemp (*Cannabis sativa L.*) seed oil in *drosophila melanogaster* larvae under non-stress and H2O2-induced oxidative stress conditions. Antioxidants 2021;10(6):830.
17. Kongkadee K, Wisuitiprot W, Ingkaninan K, *et al.* Anti-inflammation and gingival wound healing activities of *Cannabis sativa L.* subsp. sativa (hemp) extract and cannabidiol: An in vitro study. Arch Oral Biol 2022;140:105464.

18. Mikulcová V, Kašpárková V, Humpolíček P, *et al.* Formulation, characterization and properties of hemp seed oil and its emulsions. Molecules 2017;22(5):700.
19. Maghfour J, Rietcheck H, Szeto MD, *et al.* Tolerability profile of topical cannabidiol and palmitoylethanolamide: A compilation of single-centre randomized evaluator-blinded clinical and *in vitro* studies in normal skin. Clin Exp Dermatol 2021;46(8):1518-1529.
20. Citti C, Linciano P, Panseri S, *et al.* Cannabinoid profiling of hemp seed oil by liquid chromatography coupled to high-resolution mass spectrometry. Front Plant Sci 2019;10:120.
21. Mehrabani M, Seyyedkazemi SM, Nematollahi MH, *et al.* Accelerated burn wound closure in mice with a new formula based on traditional medicine. Iran Red Crescent Med J 2016;18(11):e26613.
22. Percie du Sert N, Hurst V, Ahluwalia A, *et al.* The arrive guidelines 2.0: Updated guidelines for reporting animal research. BMC Vet Res 2020;16(1):242.
23. Nagaoka T, Kaburagi Y, Hamaguchi Y, *et al.* Delayed wound healing in the absence of intercellular adhesion molecule-1 or L-selectin expression. Am J Pathol 2000;157(1):237-247.
24. Singer AJ. Healing mechanisms in cutaneous wounds: Tipping the balance. Tissue Eng Part B Rev 2022;28(5):1151-1167.
25. Almadani YH, Vorstenbosch J, Davison PG, *et al.* Wound healing: A comprehensive review. Semin Plast Surg 2021;35(03):141-144.
26. Cañedo-Dorantes L, Cañedo-Ayala M. Skin acute wound healing: A comprehensive review. Int J Inflam 2019;2019:1-15.
27. Visha M, Karunagaran M. A review on wound healing. Int J Clinicopathol Correlation 2019;3(2):50.
28. Gonzalez AC de O, Costa TF, Andrade Z de A, *et al.* Wound healing-A literature review. An Bras Dermatol 2016;91(5):614-620.
29. Bosanquet DC, Harding KG. Wound healing: Potential therapeutic options. Br J Dermatol 2022;187(2):149-158.
30. Rodrigues M, Kosaric N, Bonham CA, *et al.* Wound healing: A cellular perspective. Physiol Rev 2019;99(1):665-706.
31. Ishihara T, Yoshida M, Arita M. Omega-3 fatty acid-derived mediators that control inflammation and tissue homeostasis. Int Immunol 2019;31(9):559-567.
32. Mnekin L, Ripoll L. Topical use of *Cannabis sativa L*. Biochemicals. Cosmetics 2021;8(3):85.
33. Weigelt MA, Sivamani R, Lev-Tov H. The therapeutic potential of cannabinoids for integumentary wound management. Exp Dermatol 2021;30(2):201-211.
34. Kibret BG, Patel S, Niezgoda J, *et al.* Evidence-based potential therapeutic applications of cannabinoids in wound management. Adv Skin Wound Care 2022;35(8):447-453.
35. Clark E, Nilsson U, Samaran Q, *et al.* Allergic contact dermatitis from *Cannabis sativa* (hemp) seed oil. Contact Dermatitis 2022;87(3):292-293.
36. Patel PM, Lio PA. Safety and sourcing of topical cannabinoids: Many questions, few answers. J Clin Aesthet Dermatol 2021;14(8):49-51.