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The Effect of Kepok Banana (*Musa paradisiaca*) Peel Extract on Macroscopic and Histopathological Features of Excision Wound Healing in Mice Skin

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ABSTRACT

Kepok banana peel extract is known to have a bioactive content that can accelerate wound healing. The present study sought to evaluate the effects of Kepok banana peel extract on the macroscopic and histopathological features of excision wound healing in mouse skin. A total of 24 BALB/c mice were divided into four treatment groups, with each group consisting of six mice. The mice were further divided into three subgroups based on observation days, including days 3, 6, and 9. Each mouse received two excision wounds. The four treatment groups included K1 (control), K2 (topical therapy using Kepok banana peel extract ointment 5%), K3 (topical therapy using Kepok banana peel extract ointment 10%), and K4 (topical therapy using Kepok banana peel extract ointment 15%). The Kepok banana peel extract was obtained using the maceration method, and the ointment Kepok was prepared as a cream with extract concentrations of 5%, 10%, and 15%, using bio cream as the base. Wound healing activity was evaluated across three phases, including inflammatory, proliferative, and remodeling. The parameters observed in the current study included macroscopic and histopathological characteristics of the wound. Macroscopic observations involved wound size, while histopathological analysis included quantification of inflammatory cells, fibrocytes, collagen density, and interleukin-6 expression. Therapy using Kepok banana peel extract ointment was administered for 9 days in the treatment groups. Macroscopic features of the wounds were observed daily, and skin samples from each group were collected on days 3, 6, and 9. The results demonstrated that the 5%, 10%, and 15% concentrations of Kepok banana peel extract formed wound healing areas on mouse skin on days 3, 6, and 9, and were able to reduce the number of inflammatory cells on days 3, 6 and 9 able to reduce IL-6 expression on days 3, 6 and 9, unable to increase fibrocytes on day 3, 6, and 9 and able to increased collagen density on days 6 and 9. The 15% concentration of Kepok banana peel extract applied for 9 days showed the greatest potential to accelerate wound-healing.

Keywords: Histopathological feature, Kepok banana peel, Macroscopic feature, Ointment, Skin, Wound healing

INTRODUCTION

The skin is the largest organ in the body that plays an important role in protecting body against external agents, maintaining body temperature and detecting sensory information from the outside environment (Wosgrau et al., 2015). Physical injuries resulting from surgical procedures, falls, burns, infectious diseases, or other pathological conditions cause damage or loss of skin structure and its function (Ahmad et al., 2021). The prevalence of skin wound in the population is relatively high, with this condition often being associated with various diseases and posing large socioeconomic burdens on patients and healthcare systems (Atzingen et al., 2013). Wounds can range from simple epithelial damage to the skin or extend deeper, reaching the subcutaneous tissue, potentially affecting underlying structures such as tendons, muscles, blood vessels, nerves, parenchymal organs, and bones (Velnar et al., 2009).

Wound healing in the skin is a multifaceted process characterized by interrelated and overlapping mechanisms, such as cell migration and proliferation, extracellular matrix synthesis, and the roles of growth factors and cytokines, which work together to promote tissue repair (Gushiken et al., 2021). A proper wound healing process is essential for the restoration of the anatomical and functional stability of impaired skin (Murthy et al., 2013). Various modern commercial topical drugs are commonly used to accelerate wound healing and reduce the risk of infection in the wound, but the use of these drugs has functional limitations and causes several side effects (D'abadia et al., 2022). Thus, alternative therapies are needed to accelerate wound healing, one of which is the use of traditional medicine that utilizes natural ingredients derived from plants (Maulidya et al., 2020).

Kepok banana peels, often regarded as bio-agricultural waste, are discarded in large quantities, contributing to environmental pollution. The decomposition of banana peels generates toxic byproducts, including methane, which has a global warming potential that is 21 times greater than that of carbon dioxide (CO2), thereby contributing to an increase

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in temperature and causing climate change (Syakri, 2019). The use of banana peels is expected to help overcome these environmental problems. Banana peels have been used as a traditional medicine across various countries, including several Asian countries, due to their rich bioactive content (Savitri et al., 2022). For instance, a study by Achmad et al. (2021) examined the effects of banana peel extract on the healing of incision wounds in the gingiva of mice. The findings demonstrated that banana peel extract could effectively accelerate the wound healing process by preventing bacterial infections and mitigating excessive inflammation. Banana peels are rich in flavonoids, saponins, and tannins, which are recognized for their potential to enhance the wound healing process (Syakri, 2019). The current study aimed to investigate the effects of Kepok banana peel extract on macroscopic and histopathological aspects of excision wound healing in mice.

MATERIALS AND METHODS

Ethical approval

This research has received ethics approval from the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (Number: 129/EC-FKH/Int/2023).

Study period

This research was conducted in January 2024 at the Department of Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, the Department of Histology, Faculty of Dentistry Medicine, Universitas Gadjah Mada, and the Pathology Laboratory of Prof. Dr. Sardjito Hospital, Yogyakarta, Indonesia.

Experimental designs

Twenty-four 2-month-old female BALB/c mice were randomly assigned to four treatment groups, with each group consisting of six mice further divided into three subgroups based on days, including day 3, day 6, and day 9. Each mouse received two excision wounds. The four treatment groups in the study included K1 (control), K2 (topical therapy using Kepok banana peel extract ointment 5%), K3 (topical therapy using Kepok banana peel extract ointment 10%), and K4 (topical therapy using Kepok banana peel extract ointment 15%). The mice were housed in individual cages and acclimatized for 7 days before treatment. The excision wounds were made using a 4 mm biopsy punch in the dorsal area with a wound diameter of 4 mm. The procedure was performed under general anesthesia, using a combination of ketamine (100 mg/kg body weight, intramuscular) and xylazine (10 mg/kg body weight, intramuscular; Plumb, 2008). The therapy using banana peel extract ointment was carried out for 9 days in the treated groups (K2, K3, and K4), and the observations of wound macroscopic morphology and wound diameter measurements were carried out on a daily basis. Necropsy and wound skin sampling of each group were carried out on days 3, 6, and 9. The collected wound skin samples underwent histopathological preparations by hematoxylin-eosin staining to observe the number of inflammatory cells and fibrocytes, Trichrome Masson staining to see collagen density, and immunohistochemical staining to examine the expression of Interleukin 6 (IL-6).

Wound-healing activity

Wound healing comprises three overlapping phases, including the inflammatory phase, the proliferative phase, and the remodeling/maturation phase (Ionita et al., 2022). The inflammatory phase was characterized by hemostasis and inflammation. The proliferative phase involved epithelialization, angiogenesis, and collagen deposition. In the remodeling phase, the wound undergoes contraction, reducing the visible scar tissue (Das, 2013).

Histological study

Sample collection was performed across four treatment groups, with each group consisting of six mice. Two wound skin samples were taken from each mouse, resulting in a total of 48 wound skin samples prepared for histopathological examination. The histopathological analysis in the present study included the quantification of inflammatory cell counts and fibrocyte numbers observed in the wound tissue using hematoxylin-eosin staining. Moreover, Masson's trichrome staining was employed to evaluate collagen density, and immunohistochemical staining was performed to analyze IL-6 expression in wound tissues.

Macroscopic analysis

The macroscopic analysis involved determining the excisional wound area in each mouse. The wound area was measured using a digital caliper to record the diameter, which was then used to calculate the wound area.

Statistical analysis

Data from macroscopic and histopathological analyses were statistically analyzed using IBM SPSS Statistics 26 (2019) software with α=0.05. One-way ANOVA was used for parametric data, while the Kruskal-Wallis test was employed for nonparametric data, with statistical significance set at $p < 0.05$. If significant differences were observed ($p < 0.05$) in the one-way ANOVA test, a follow-up analysis was conducted using Tukey's test. Similarly, if significant differences were found ($p < 0.05$) in the nonparametric Kruskal-Wallis test, a post hoc Kruskal-Wallis test was performed.

RESULTS AND DISCUSSION

Macroscopic features

Quantitative data from the macroscopic features for each treatment group were obtained from measuring the area of the wound using a digital caliper. Macroscopic observations of excisional wounds in mice treated with Kepok banana peel extract on days 3, 6, and 9 were shown in Figures 1,2, and 3. The results of the analysis of macroscopic feature data obtained from each group on days 3, 6, and 9 did not show any significant differences, as seen in Table 1. The lack of a significant difference in wound area among groups indicates that the wound healing process in the therapy groups (K2, K3, K4) proceeded in parallel with that of the normal healing group (K1/control).

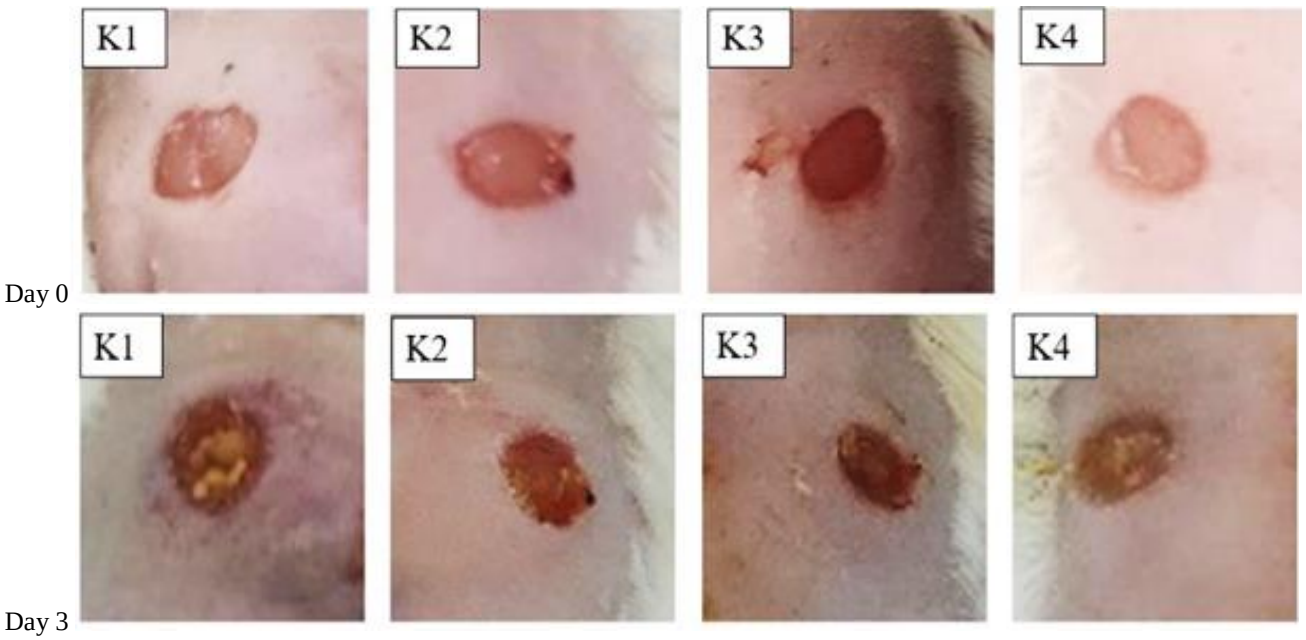

Figure 1. Macroscopic observations of excisional wounds in mice treated with Kepok banana peel extract on day 0 and day 3. **K1**: Control, **K2**: 5% Kepok banana peel extract ointment therapy, **K3**: 10% Kepok banana peel extract ointment therapy, **K4**: 15% Kepok banana peel extract ointment therapy.

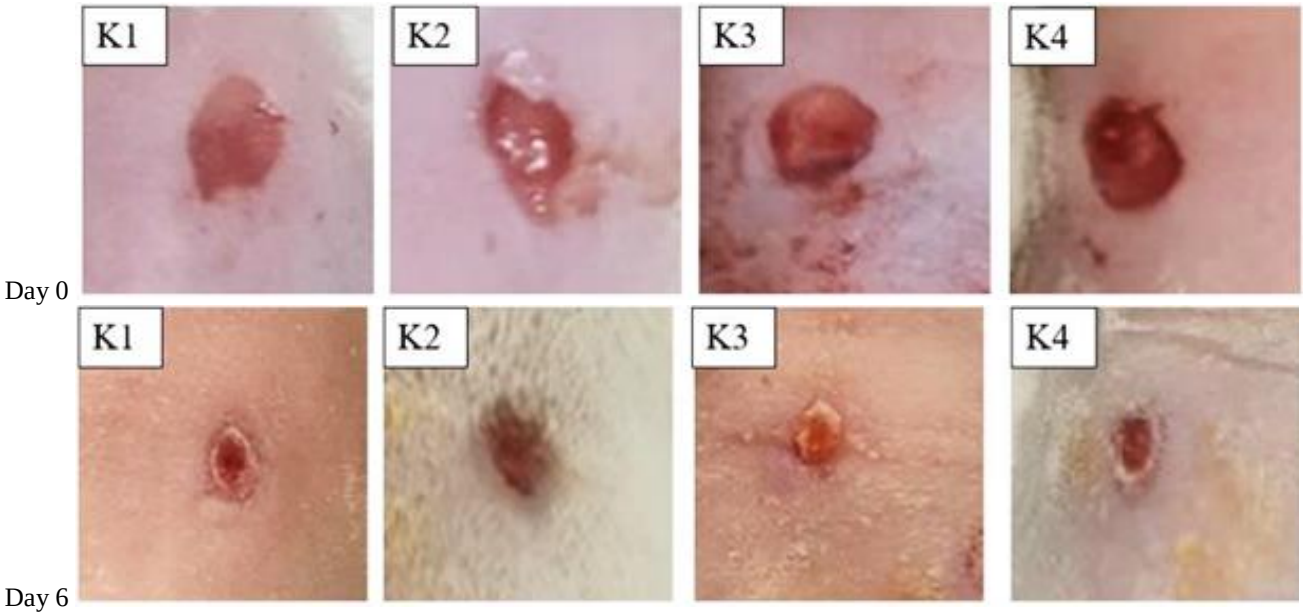

Figure 2. Macroscopic observations of excisional wounds in mice treated with Kepok banana peel extract on day 0 and day 6. **K1**: Control, **K2**: 5% Kepok banana peel extract ointment therapy, **K3**: 10% Kepok banana peel extract ointment therapy, **K4**: 15% Kepok banana peel extract ointment therapy.

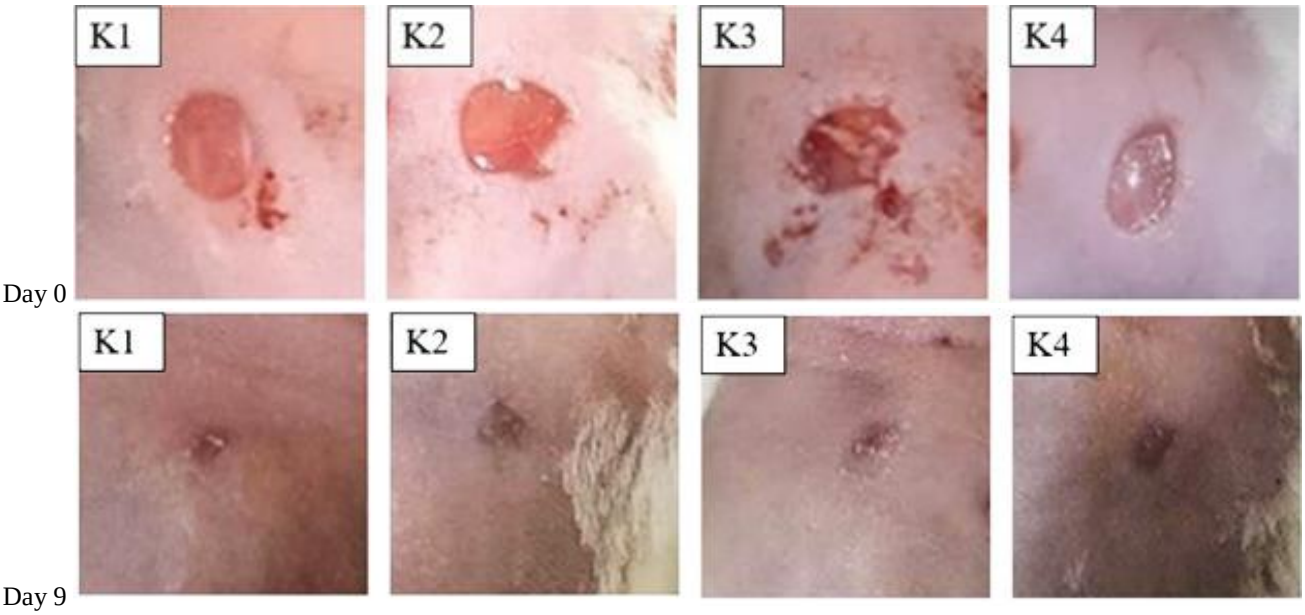

Figure 3. Macroscopic observations of excisional wounds in mice treated with Kepok banana peel extract on day 0 and day 9. **K1**: Control, **K2**: 5% Kepok banana peel extract ointment therapy, **K3**: 10% Kepok banana peel extract ointment therapy, **K4**: 15% Kepok banana peel extract ointment therapy.

Table 1. The results of excisional wound area measurements in Kepok banana (*Musa paradisiaca*) peel extract ointment groups on days 3, 6, and 9 after treatment

Groups \ Subgroups (Days)	Day 3	Day 6	Day 9
K1(0%)	12.52±2.84^{a}	2.86±2.05^{e}	0,00±0,00^{i}
K2(5%)	11.87±2.16^{a}	5.53±5.30^{e}	0,40±0,81^{i}
K3(10%)	10.71±1.84^{a}	3.88±2.78^{e}	0,05±0,10^{i}
K4(15%)	11.20±3.40^{a}	2.67±1.59^{e}	0,53±0,61^{i}
P value	0.783	0.603	0.471

Note: The absence of different notations indicates no significant difference between treatment groups within the same column ($p > 0.05$). K1: Control, K2: 5% concentration Kepok banana peel extract ointment therapy, K3: 10% concentration Kepok banana peel extract ointment therapy, K4: 15% concentration Kepok banana peel extract ointment therapy.

According to Rhea and Dunnwald (2020), the wound healing process in mice progresses through three phases included the inflammatory phase (days 0-5), the proliferative phase (days 3-14 days), and the remodeling/maturation phase (day 7-1 year). Therefore, the wound healing process that occurs on day 3 includes the inflammatory phase and the beginning of the proliferative phase. The inflammatory phase was characterized by the presence of hemostasis and inflammation. The proliferative phase was the phase of epithelialization, angiogenesis, and collagen deposition (Das, 2013). Kepok banana peel extract can heal wounds because it contains flavonoid compounds, saponins, and tannins (Syakri, 2019). While flavonoids can work as anti-inflammatory (Budiawan et al., 2023), tannins are known to have antibacterial activity to prevent infection in wounds, which can accelerate the contraction of fibrous tissue in the wound healing process (Budiawan et al., 2023). Fibrous contraction by fibroblasts triggers the formation of scar tissue, which will protect the formation of cells at the wound site (Budiawan et al., 2023). Saponins, as demonstrated in a study by Kim et al. (2011), inhibit inflammatory responses, promote epithelialization, and stimulate matrix synthesis, which was crucial for effective wound healing.

By day 6, the wound healing process advances into the proliferative phase, which was characterized by cell migration and proliferation, tissue synthesis granulation, and re-epithelialization. The granulated tissue was composed of a temporary extracellular matrix, macrophages, endothelial cells, and fibroblasts that provide strength to the skin. The re-epithelialization process plays a key role in closing the wound and restoring the skin's barrier function (York, 2022). Flavonoid compounds, tannins, and saponins contained in Kepok banana peel extract were compounds that can aid in healing wounds by helping in the proliferative phase by enhancing the vascularization during this phase (Meliawaty et al., 2021). Saponins can trigger the production of extracellular matrix and re-epithelialization, helping the wound healing process, especially in the proliferative phase (Kim et al., 2011). Moreover, tannins and saponins play a role in promoting fibroblast migration and proliferation (Marlinawati et al., 2023). Tannins also support the formation of new blood vessels by enhancing cell proliferation (Meliawaty et al., 2021).

On day 9, the wound healing process enters the proliferative and remodeling phases. The proliferative phase was marked by heightened migration and proliferation of keratinocytes, fibroblasts, endothelial cells, and leukocytes at the wound site. This phase also involved increased synthesis of extracellular matrix components, enhanced angiogenesis, and re-epithelialization, which together promote wound closure and restore the skin's barrier function. The remodeling phase was marked by the restructuring of the extracellular matrix, where collagen III was replaced with collagen I

(Gushiken et al., 2021). KepokSaponin compounds can stimulate matrix formation and re-epithelialization to accelerate wound closure (Kim et al., 2011). According to Meliawaty et al. (2021), saponins can help the formation of collagen I, which plays a role in stabilizing tissues formed in the *remodeling*/maturation phase.

Histopathological features

Inflammatory cell

Data on the number of inflammatory cells in each treatment group were obtained through microscopic observation at 400x magnification (Figure 4) in three fields of view. The average numbers of inflammatory cells in each treatment group for days 3, 6, and 9 are presented in Table 2. The statistical analysis conducted using the Kruskal-Wallis test revealed a significant difference among groups ($p < 0.05$) regarding the number of inflammatory cells on day 3, as indicated by the presence of different notations in the post-hoc Kruskal-Wallis test. Furthermore, the statistical analysis using the Kruskal-Wallis test for the number of inflammatory cells on day 6, along with the One-Way ANOVA for day 9, indicated significant differences among groups ($p < 0.05$), as evidenced by the different notations in the post hoc Kruskal-Wallis test (for day 6) and the subsequent One-Way ANOVA using Tukey's test (for day 9).

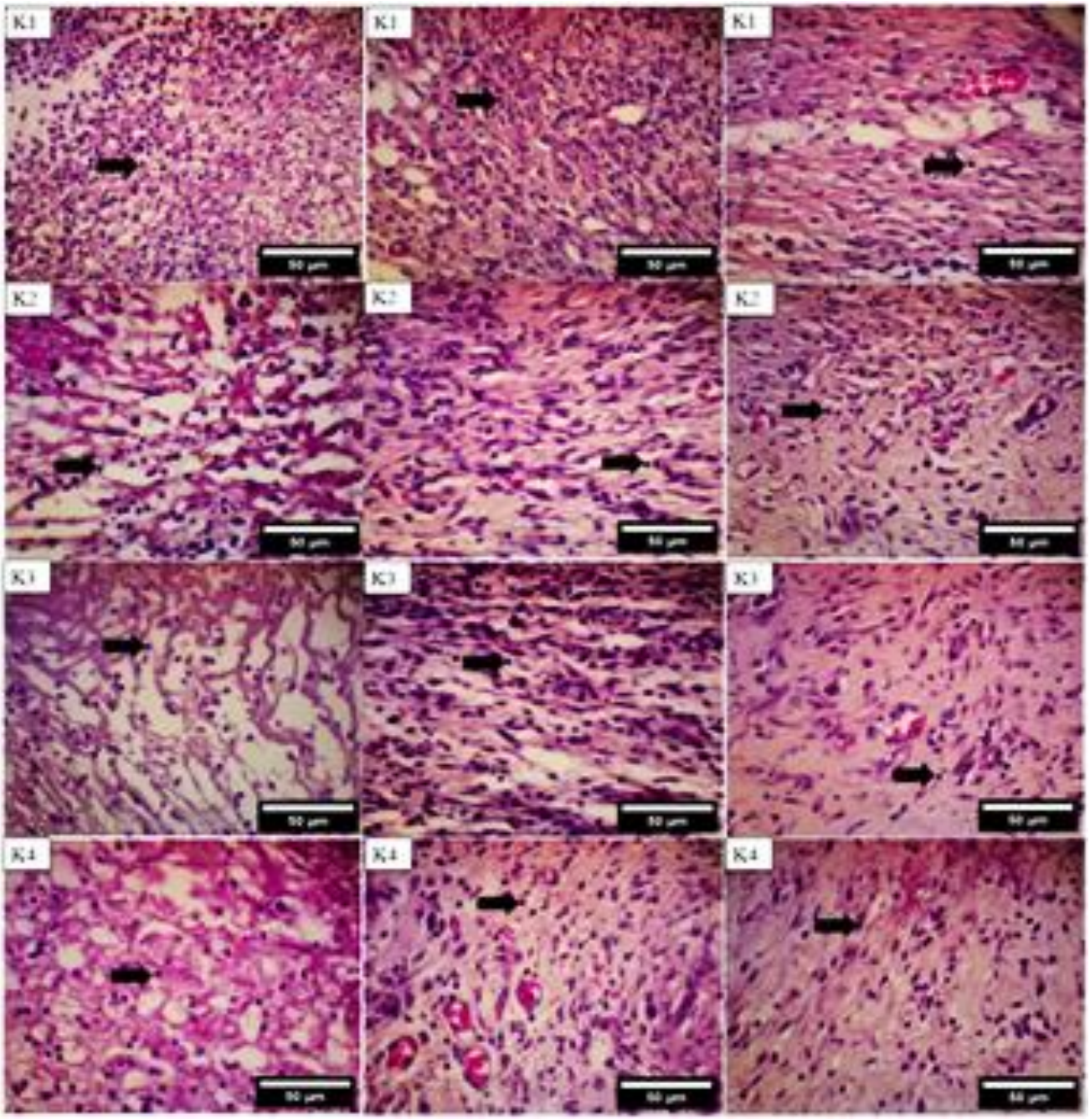

Figure 4. Histopathological characterization of inflammatory cells during wound healing in mice treated with Kepok banana peel extract (stained with hematoxylin-eosin, magnification 400x). Inflammatory cells are indicated by black arrows. **K1**: Control, **K2**: 5% concentration Kepok banana peel extract ointment therapy, **K3**: 10% concentration Kepok banana peel extract ointment therapy, **K4**: 15% concentration Kepok banana peel extract ointment therapy.

Table 2. Average number of inflammatory cells in Kepok banana (*Musa paradisiaca*) peel extract ointment groups on days 3, 6, and 9 after treatment

Groups \ Subgroups (Days)	Day 3	Day 6	Day 9
K1(0%)	251.08±108.46^{a}	159.92±22.95^{e}	115.83±12.56^{i}
K2(5%)	226.67±62.16^{a}	120.17±5.69^{e}	88.75±24.05^{ij}
K3(10%)	165.58±66.61^{ab}	119.75±9.34^{e}	69.58±23.22^{j}
K4(15%)	121±16.34^{b}	76.17±2.10^{f}	75.33±13.34^{j}
P value	0.036	0.023	0.022

Note: The presence of different notations indicates a significant difference among treatment groups within the same column ($p < 0.05$). K1: Control, K2: 5% concentration Kepok banana peel extract ointment therapy, K3: 10% concentration Kepok banana peel extract ointment therapy, K4: 15% concentration Kepok banana peel extract ointment therapy.

According to Geissler et al. (2022), inflammation was an integral part of the physiology of wound healing that aimed to clean the wound site from debris and pathogens. However, the excessive or prolonged inflammatory reaction can hinder the wound healing process and prevent complete recovery. The present study aligned with observations by Gunawan et al. (2019) that the infiltration of inflammatory cells during wound healing naturally decreases over time. The findings demonstrated that topical application of Kepok banana extract ointment on wounds can reduce the number of inflammatory cells, as indicated by the fewer inflammatory cells observed in the treatment groups (K2, K3, and K4) compared to the control group (K1). The large number of inflammatory products found indicates the level of inflammation experienced (Gunawan et al., 2019). An excessive and prolonged inflammatory response can lead to an increased injury in the tissues and worsen the healing process. Success in wound healing requires coordination between inflammation and resolution of inflammation (Eming et al., 2007).

The results of the current study reveal that the Kepok banana peel extract ointment reduced inflammatory cell counts on days 3, 6, and 9. This effect was attributed to the extract's flavonoid, tannins, and saponin content, which exhibit anti-inflammatory properties (Kim et al., 2011; Anandhi and Rajeskumar, 2023). Saponins function as anti-inflammatory by inhibiting inflammatory reactions in the early phases (Kim et al., 2011). Another anti-inflammatory mechanism that saponins have was the ability to inhibit the formation of exudate and inhibit the increase in vascular permeability (Fitriyani et al., 2011). Flavonoids can show anti-inflammatory activity by inhibiting the production of pro-inflammatory cytokines such as IL-6, IL1-1β, and TNF-α (Mayangsari et al., 2023). In addition to saponins and flavonoids, tannins also have anti-inflammatory activity but the mechanism of anti-inflammatory activity remains unclear (Fitriyani et al., 2011).

Interleukin 6

The data on interleukin 6 expression in each group on days 3, 6, and 9 of treatment with Kepok banana peel extract ointment was obtained by examining histopathological preparations of mouse skin using a microscope at 400x magnification (Figure 5). Observations were made in three different fields of view and analyzed using ImageJ 1.54g software. The average expression of interleukin 6 (IL-6) in each group on days 3, 6, and 9 of treatment with Kepok banana peel extract ointment is presented in Table 3. Statistical analysis conducted using SPSS software with one-way ANOVA revealed significant differences among groups ($p < 0.05$) concerning IL-6 expression on days 3, 6, and 9, as indicated by differing notations in the Tukey post-hoc test. The lowest IL-6 expression on day 3 was observed in group K4 (treatment with 15% banana peel extract ointment), with an average expression of 24.53 ± 1.57. On day 6, the lowest IL-6 expression was again observed in group K4, with an average expression of 15.60 ± 2.81. Finally, on day 9, group K3 (treatment with 10% banana peel extract ointment) exhibited the lowest IL-6 expression, averaging 9.49 ± 2.74.

Wound healing was a complex process involving the interaction of cellular cascades and biochemical actions that trigger structural improvements and the integrity function of injured tissues. This process involved many cell populations, extracellular matrices, and the action of dissolved mediators such as growth factors and cytokines (Ionita et al., 2022). One of the cytokines involved in the wound healing process was IL-6. Interleukin 6 was a multifunctional cytokine and was described as an inflammatory mediator (Nosenko et al., 2019). Interleukin 6 has a role in the wound healing process, but excessive expression of IL-6 can slow down the wound healing process because IL-6 provided a signal for leukocytes, thereby increasing the inflammatory process (Gulo et al., 2022). Although inflammation is an integral part of the physiological healing of wounds that aimed to clean the wound site from debris and pathogens, excessive or prolonged inflammatory reactions can impede the wound healing process, lead to incomplete recovery, and trigger chronic wounds (Geissler et al., 2022).

Based on the obtained results, Kepok banana peel extract therapy effectively reduced the expression of interleukin 6 on days 3, 6, and 9. This reduction can be attributed to the flavonoids and saponins present in Kepok banana peel extract, known to be able to inhibit or decrease pro-inflammatory cytokines, including interleukin 6. Khayri et al. (2022) report that flavonoids not only inhibit inflammation by targeting key signaling pathways, including Nuclear Factor-Kappa Beta (NF-κB), Mitogen-Activated Protein Kinase (MAPK), Extracellular Signal-Regulated Kinases (ERK), and the Akt pathway, but they also diminish the production of inflammatory cytokines. This reduction affects various cytokines, including Interleukin 6 (IL-6), Interleukin 8 (IL-8), Interleukin 1 Beta (IL-1β), Interleukin 17 (IL-17), Tumor Necrosis Factor Alpha (TNF-α), and Interferon gamma (IFN-γ). Yao et al. (2014) suggested that saponins exhibit anti-inflammatory effects by inhibiting the production of various pro-inflammatory cytokines, including Interleukin 6 (IL-6), Tumor Necrosis Factor Alpha (TNF-α), and Nitric Oxide (NO). This mechanism underscores the potential of saponins in

modulating inflammatory responses. In addition to flavonoids and saponins, banana peel extract also contains tannins, which possess anti-inflammatory properties (Fitriyani et al., 2011).

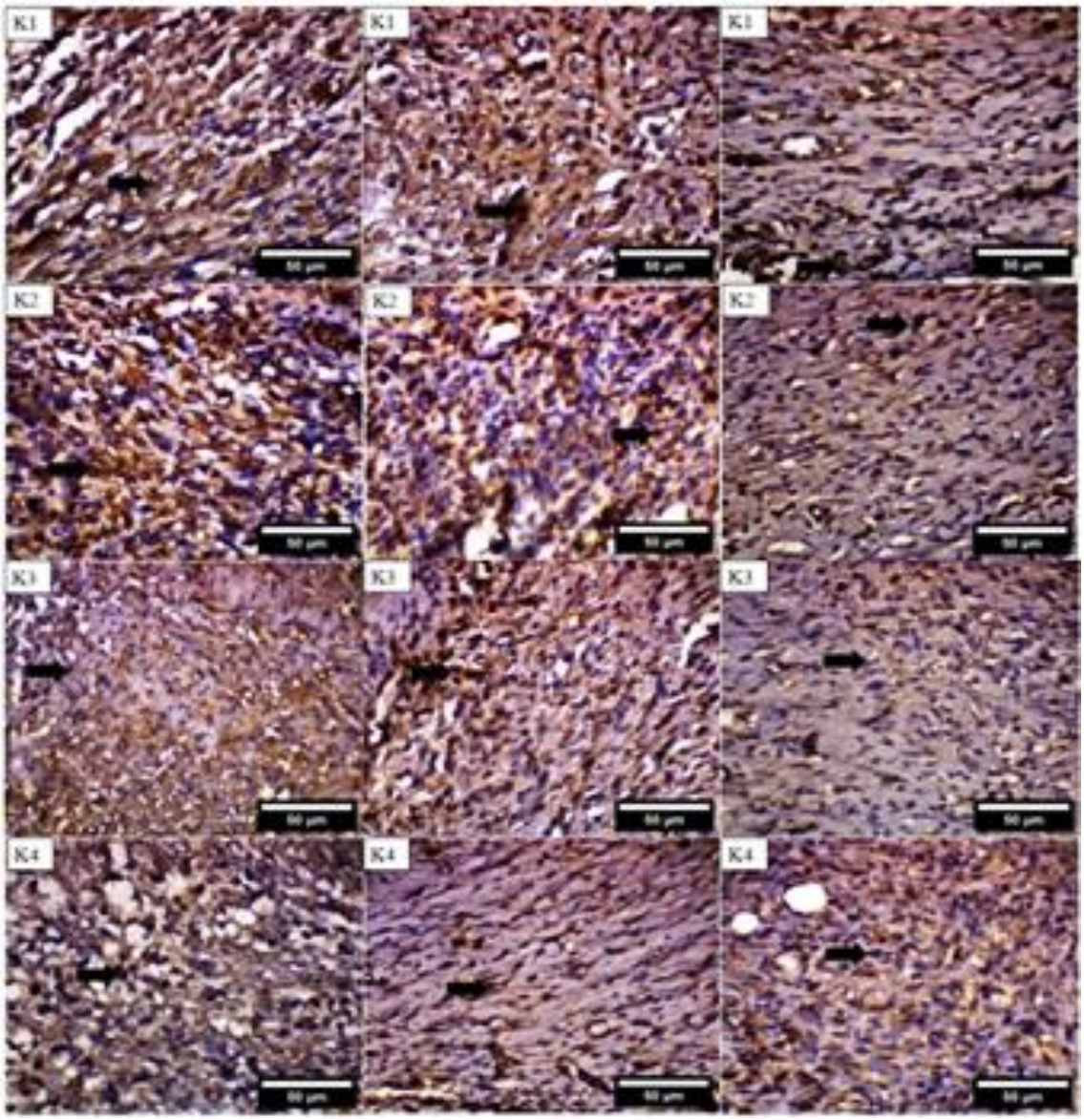

Figure 5. "Histopathological feature of Interleukin 6 (IL-6) expression during wound healing with Kepok banana peel extract (immunohistochemical staining, magnification 400x). The black arrows indicate IL-6 expression, which is stained brown in the immunohistochemical analysis. **K1**: Control, **K2**: Treatment with 5% Kepok banana peel extract ointment, **K3**: Treatment with 10% Kepok banana peel extract ointment, **K4**: Treatment with 15% Kepok banana peel extract ointment.

Table 3. Average number of Interleukin 6 (IL-6) expression in Kepok banana (*Musa paradisiaca*) peel extract ointment groups on days 3, 6, and 9 after treatment

Groups \ Subgroups (Days)	Day 3	Day 6	Day 9
K1(0%)	41.42±2.60^{a}	28.25±1.12^{e}	21.50±1.58^{i}
K2 (5%)	30.85±2.54^{b}	21.12±4.21^{f}	11.77±2.27^{j}
K3(10%)	29.05±2.73^{bc}	17.27±2.89^{f}	9.49±2.74^{j}
K4(15%)	24.53±1.57^{c}	15.60±2.81^{f}	12.81±3.84^{j}
P value	0.000	0.000	0.027

Note: The presence of different notations indicates a significant difference among treatment groups within the same column ($p < 0.05$). K1: Control, K2: 5% concentration Kepok banana peel extract ointment therapy, K3: 10% concentration Kepok banana peel extract ointment therapy, K4: 15% concentration Kepok banana peel extract ointment therapy.

Fibrocyte

Data on the number of fibrocytes in each group were obtained from observation using a microscope with 400x magnification in three fields of view (Figure 6). The average number of fibrocytes in each treatment group using Musa paradisiaca peel extract ointment on days 3, 6, and 9 is presented in Table 4. One-way ANOVA was used for statistical

analysis of fibroblast counts on days 3 and 9, while the Kruskal-Wallis test was employed for the counts on day 6. The results indicated no significant differences in fibrocyte numbers among groups on days 3, 6, and 9 after therapy with Kepok banana peel (Musa paradisiaca) extract ointment; therefore, no post-hoc tests were conducted.

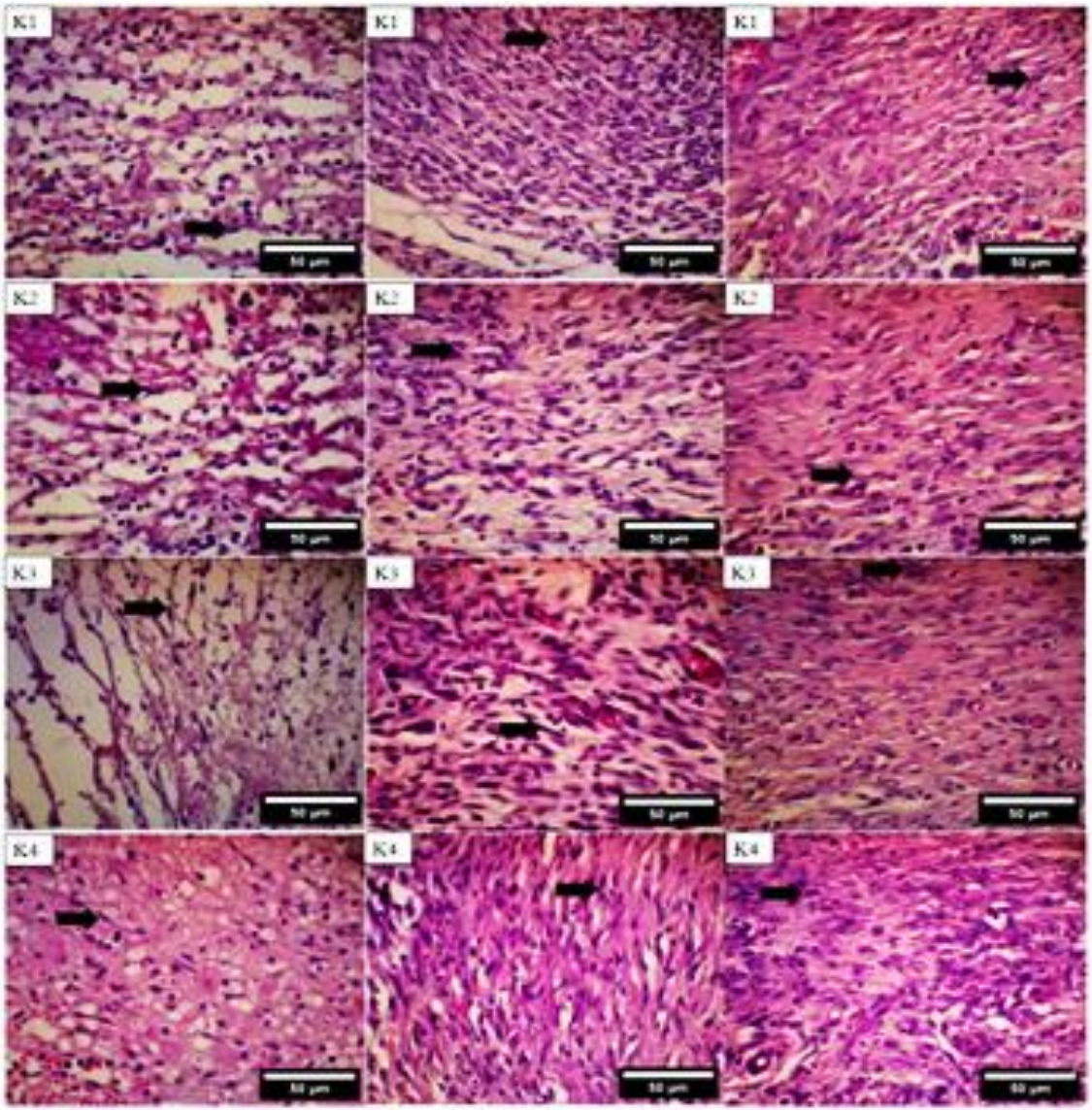

Figure 6. Histopathological examination of fibrocytes during the wound healing process with Kepok banana peel extract (hematoxylin-eosin staining, magnification 400x). Fibrocytes are indicated by black arrows. **K1**: Control, **K2**: Treatment with 5% Kepok banana peel extract ointment, **K3**: Treatment with 10% Kepok banana peel extract ointment, **K4**: Treatment with 15% Kepok banana peel extract ointment.

Table 4. The average number of fibrocyte cells in Kepok banana (*Musa paradisiaca*) peel extract ointment groups on days 3, 6, and 9 after treatment

Groups \ Subgroups (Days)	Day 3	Day 6	Day 9
K1(0%)	2.08±2.67^{a}	40.67±22.95^{e}	77.75±11.63^{i}
K2 (5%)	2.58±2.87^{a}	44.67±5.69^{e}	83.08±20.31^{i}
K3(10%)	2.67±1.65^{a}	67.17±9.34^{e}	99.17±2666^{i}
K4 (15%)	4.00±4.05^{a}	79.42±2.10^{e}	113±33.85^{i}
P value	0.817	0.431	0.219

Note: The absence of different notations indicates no significant difference between treatment groups within the same column ($p > 0.05$). K1: Control, K2: 5% concentration Kepok banana peel extract ointment therapy, K3: 10% concentration Kepok banana peel extract ointment therapy, K4: 15% concentration Kepok banana peel extract ointment therapy.

Fibrocytes were among the critical cells involved in the process of wound healing and tissue repair (Metz, 2003). These cells play an essential role in the wound healing process through several mechanisms, including their ability to function as antigen-presenting cells (APCs) during the inflammatory phase and their secretion of several proteins that trigger endothelial cell proliferation, migration, and angiogenesis (Xia and Ping, 2010). Fibrocytes play a significant role

in the proliferation phase of wound healing, participating in processes such as angiogenesis, collagen deposition, and wound contraction. Furthermore, they are also involved in tissue remodeling during the maturation phase of healing, contributing to the overall restoration of tissue integrity (de Oliveira and Wilson, 2020).

Kepok banana peel extract was known to contain flavonoids, saponins, and tannins, all of which can enhance wound healing through various mechanisms (Syakri, 2019). Flavonoids were recognized for their ability to enhance the production of growth factors essential for the wound healing process (Jaya et al., 2023). This ability can accelerate the transition from the inflammatory phase to the proliferative phase so that the wound healing process that occurs is faster, as compared to the physiological process (Jaya et al., 2023). Saponins have been shown to inhibit excessive inflammatory responses, promote re-epithelialization in wounds, and stimulate matrix synthesis during the wound healing process (Kim et al., 2011). Additionally, tannins possess both anti-inflammatory and antiseptic properties, which contribute to the acceleration of wound healing (Apriliana et al., 2021).

Collagen

Collagen density data for each group on days 3, 6, and 9 after treatment with Musa paradisiaca peel extract ointment was obtained through microscopic observation with 400x magnification (Figure 7) in three fields of view and processed with ImageJ software. Statistical analysis of the collagen density data was carried out with SPSS software. The Kruskal-Wallis test showed no significant difference in collagen density among groups on day 3. On day 6, one-way ANOVA revealed a significant difference between groups ($p < 0.05$) in collagen density, as indicated by distinct notations in the subsequent Tukey test. The highest collagen density was observed in group K4 (15% Kepok banana peel extract ointment).

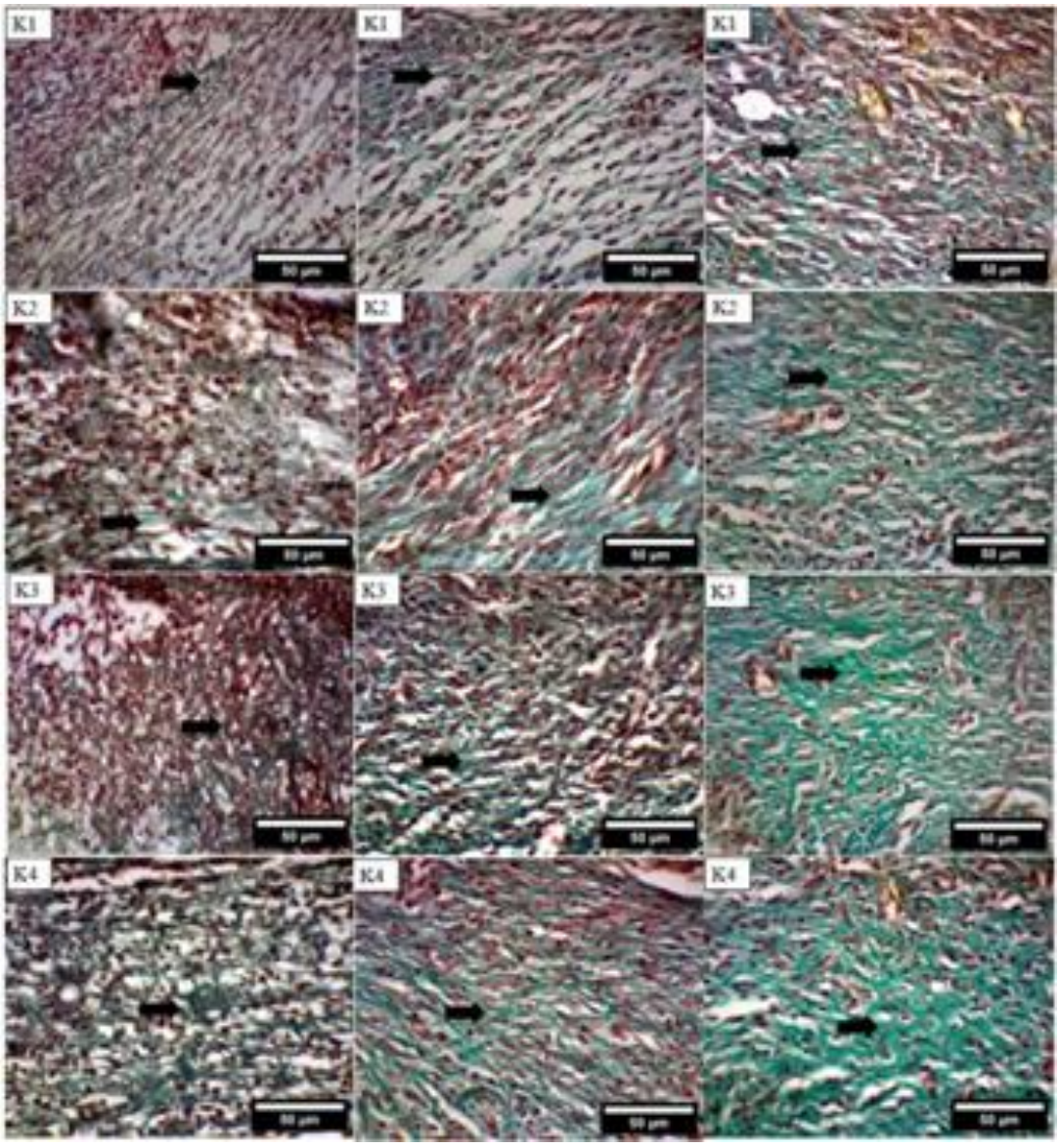

Figure 7. Histopathological feature of collagen fibers in wound healing with Kepok banana peel extract (*Masson's trichrome* staining, 400x magnification). The black arrows indicate collagen fibers stained green with *Masson's trichrome*. **K1**: Control, **K2**: 5% Kepok banana peel extract ointment therapy, **K3**: 10% Kepok banana peel extract ointment therapy, **K4**: 15% Kepok banana peel extract ointment therapy.

Table 5. Collagen density in Kepok banana (*Musa paradisiaca*) peel extract ointment groups on days 3, 6, and 9 after treatment

Groups \ Subgroups (Days)	Day 3	Day 6	Day 9
K1(0%)	19.02±8.72[a]	25.63±3.41[e]	29.55±4.77[i]
K2(5%)	22.60±1.52[a]	32.86±6.99[ef]	40.74±3.18[ij]
K3(10%)	25.40±3.11[a]	37.51±4.81[f]	44.40±6.97[j]
K4 (15%)	38.79±12.25[a]	43.36±5.19[f]	56.16±7.64[j]
P value	0.156	0.003	0.006

Note: The presence of different notations indicates a significant difference among treatment groups within the same column ($p < 0.05$). K1: Control, K2: 5% concentration Kepok banana peel extract ointment therapy, K3: 10% concentration Kepok banana peel extract ointment therapy, K4: 15% concentration Kepok banana peel extract ointment.

Additionally, statistical analysis using the Kruskal-Wallis test in SPSS demonstrated a significant difference between treatments ($p < 0.05$) in collagen density expression on day 9, as indicated by distinct notations in the Kruskal-Wallis post hoc test, with the highest collagen density found in group K4 (15% Kepok banana peel extract ointment).

Kepok banana peel extract was known to contain flavonoids, saponins, and tannins, which can accelerate wound healing (Syakri, 2019). These compounds interact with the growth factor receptors of fibroblasts, stimulating their activity and proliferation, thereby triggering collagen synthesis and accelerating granulation to accelerate wound healing. Flavonoids, saponins, and tannins can stimulate the proliferation and differentiation of fibroblasts into myofibroblasts. This stimulation leads to the synthesis of collagen and several other matrix proteins in large quantities (Marlinawati et al., 2023).

The inflammatory phase of wound healing was marked by hemostasis to stop bleeding and the activation and recruitment of immune cells. Collagen III was the first type of collagen produced during the wound healing process. Type III collagen was synthesized in the proliferative phase and was replaced with type I collagen in the remodeling/maturing phase. Fibroblasts were a major source of new collagen synthesis (Steiner et al., 2021).

By day 6, the wound healing process enters the proliferation phase (Das, 2013). This phase was characterized by the presence of fibroblast proliferation, collagen deposition, angiogenesis, formation of granulating tissue, and re-epithelialization (Steiner et al., 2021). The results of the present study demonstrate that Kepok banana peel extract therapy significantly increased collagen density on day 6 of the wound healing process. This increase in collagen density was attributed to the flavonoids, saponins, and tannins present in the banana peel extract. Flavonoid compounds inhibit Matrix Metalloproteinase (MMP), thereby increasing the amount of synthesis of collagen by fibroblasts for the formation of a new matrix and consequently, accelerating the wound healing process (Stipcevic et al., 2006). Saponins increase collagen production, thereby accelerating the wound healing process (Budiawan et al., 2023). Marlinawati et al. (2023) report that saponins can enhance fibronectin synthesis by fibroblasts and modify the expression of the Transforming Growth Factor Beta (TGF-β) receptor. Moreover, fibronectin is a versatile glycoprotein with binding sites for various macromolecules, including collagen, proteoglycans, and fibrin. Enhanced fibronectin synthesis promotes faster fibroblast migration, supporting the wound healing process by facilitating collagen production. Increased fibroblast migration to the wound site leads to higher collagen synthesis. Newly synthesized collagen combines with the existing collagen in the extracellular matrix, resulting in a denser matrix and promoting faster wound healing. Tannins can also enhance collagen synthesis by promoting the migration and proliferation of fibroblasts to the wound site (Rahati et al., 2020).

Based on the results obtained on day 9 of therapy with Musa paradisiaca peel extract ointment, the treatment with Kepok banana (Musa paradisiaca) peel extract was found to increase collagen density. This was evidenced by a higher average collagen density in the Kepok banana peel extract therapy group when compared to the control group. The higher collagen density in the therapy group with Kapok banana peel extract is due to the presence of flavonoids, tannins, and saponins. Flavonoid compounds inhibit MMP, thereby increasing enhancing collagen synthesis by fibroblasts and promoting the formation of a new extracellular matrix, which accelerates wound healing (Stipcevic et al., 2006). According to Deen et al. (2020), Falvonoids and tannins increased the viability and formation of collagen fibers, leading to an increase in the strength of the produced collagen fibers. Saponins enhanced collagen production during wound healing (Budiawan et al., 2023). They promote the migration and proliferation of fibroblasts to the wound site, which were the primary cells responsible for new collagen synthesis (Marlinawati et al., 2023). Additionally, saponins support the formation of type I collagen, a crucial element for stabilizing tissues during the remodeling phase (Meliawaty et al., 2021). Besides saponins, tannins also play a role by stimulating fibroblast migration and proliferation in the wound area, thereby boosting collagen synthesis and expediting the healing process (Rahati et al., 2023).

CONCLUSION

Topical therapy using Kepok banana peel extract ointments at purity percentages of 5, 10, and 15 formed wound healing areas on the skin of mice on days 3, 6, and 9. The treatment effectively reduced the number of inflammatory cells on days 3, 6, and 9, and decreased IL-6 expression on days 3, 6, and 9. While it did not increase fibrocyte levels on days 3, 6, and 9, it significantly enhanced collagen density on days 6 and 9. The 15% concentration of Kepok banana peel extract, applied for 9 days, exhibited the greatest potential in accelerating wound healing. Further research could utilize extracts from unripe Kepok banana peel, known to contain higher levels of bioactive tannins, and employ animal models with larger wound sizes to assess potential differences in wound closure rates among treatment groups.

DECLARATIONS

Authors' contributions

Sitarina Widyarini and Yuli Purwandari Kristianingrum supervised the study. Husnur Rukyat conceptualized, managed, and conducted data analysis and interpretation and drafted the manuscript. Husnur Rukyat and Dini Agusti Paramanandi performed all the experimental procedures. All authors read and approved the final edition of the manuscript.

Competing interests

The authors have not declared any conflicts of interest.

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Ethical considerations

The authors confirm that this manuscript is an original submission to this journal and has been screened for plagiarism.

Availability of data and materials

All original contributions from this study are provided within the article and supplementary materials. For further information, please reach out to the corresponding author.

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