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Efficacy of Avocado Oil Cream in Wound Repair: Macroscopic Analysis, Inflammatory Cells Count, and Collagen Density

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ABSTRACT

The wound healing process goes through a series of complex stages that are mutually continuous, namely inflammation, proliferation, and maturation. Following wound formation and the release of proinflammatory cytokines and growth factors, inflammation occurs immediately after the hemostasis phase. Proliferation occurs when products processed by the growth factors are present. The final stage, maturation, is portrayed by the plan of the extracellular network. This study utilized oil from avocado fruit (*Persea americana*), which contains linoleic and oleic acid content that supports skin tissue repair. The study aimed to explore the effects of using avocado oil cream on the area of wound healing, the number of inflammatory cells, and the collagen density. Twenty-four female mice, aged eight weeks, were used as experimental animals by making excision wounds using a 4 mm biopsy punch on the dorsal skin on the left and right sides. The mice were divided into four groups based on the percentage of avocado oil in the topical cream included Group K (control, topical cream without avocado oil), Group P1 (5% topical avocado oil cream), Group P2 (10% topical avocado oil cream), and Group P3 (15% topical avocado oil cream). Macroscopic examination of the wounds was conducted daily on days 3, 6, and 9 after topical cream treatment using a digital caliper. A total of 48 skin tissue samples were collected from days 3, 6, and 9 after cream application, which were then processed for histopathology evaluations using hematoxylin-eosin staining and Masson's Trichrome staining. Hematoxylin-eosin staining was used to count the inflammatory cells, and Masson's Trichrome staining was employed to assess collagen density. The results revealed that avocado oil had a great impact on wound closure after 9 days of 15% avocado oil cream treatment, reducing the inflammatory cells after 3-6 days of 10% avocado oil cream therapy, and increasing collagen density after 9 days of 15% avocado oil cream application, as compared to the control, non-avocado oil cream group. Avocado oil can help close wounds, reduced the number of inflammatory cells, and increased collagen density when used in topical pharmaceutical formulations. Avocado oil cream may, therefore, be considered a viable option for wound repair treatment.

Keywords: Avocado oil, Collagen, Inflammatory cell, Wound repair

INTRODUCTION

The skin, as the largest organ in the animal's body, plays an essential role as the first line of defence against external threats. Damage to the skin, such as wounds, results in a loss of anatomical and functional integrity (Poljsak et al., 2019). Closing wounds and repairing damaged tissues are critical for restoring skin function to its original state, with an emphasis on minimizing healing time and side effects (Landen et al., 2016). In recent decades, research related to wound healing has continued to be carried out both from the molecular systematics of wound healing and the choice of therapies that can be used to obtain the best healing conditions. The treatments using natural ingredients to repair damaged skin tissue have recently gained popularity. In this line, phytomedicine is a field of science that studies using plants as ingredients for therapy or preventing damage to the body (Sichani et al., 2021). In Indonesia, a country rich in natural biodiversity and resources, research on herbal therapies has grown significantly, providing opportunities to explore alternative wound healing treatments using locally available natural resources.

Avocado (*Persea americana*), a fruit native to the Americas, is rich in vitamin B12, calcium, iron, magnesium, protein, and fibres. Avocado also acts as an anti-aging agent, prevents stroke, has analgesic and anti-inflammatory effects, is anti-diabetic and anti-ulcer, helps wound healing, and contains antioxidants (Ranade and Thiagarajan, 2015; Gupta et al., 2018). The study conducted by de Oliveira et al. (2013) was on utilizing avocado oil for wound healing therapy in mice. Avocado oil has been used in treating psoriasis, wrinkles, and stretch marks (Ranade and Thiagarajan, 2015). Moreover, avocado oil is rich in oleic acid and linoleic acid, found also in olives and olive oil, and helpful in skin repair (de Oliveira et al., 2013; Li et al., 2013).

The wound healing process involves a series of complex and continuous stages involved inflammation, proliferation, and maturation. Inflammation begins immediately after the hemostasis phase following the formation of

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the wound and the release of proinflammatory cytokines and growth factors. Proliferation occurs with the presence of products processed by the growth factors. The final stage of maturation is characterized by the arrangement of the extracellular matrix and scar tissue (Velnar et al., 2009). The overall goal of these wound-healing stages is to achieve normal skin conditions regarding anatomical and physiological functions (Landen et al., 2016). The present study aimed to determine the effects of topical avocado oil cream therapy on the area of wound healing, the number of inflammatory cells, and the collagen density.

MATERIALS AND METHODS

Ethical approval

The Research Ethics Commissions of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia, approved this research under document number 128/EC-FKH/Int./2023.

Study period and location

This research was conducted from November 2023 to February 2024 at the Department of Pathology, Faculty of Veterinary Medicine, Faculty of Dentistry, and Faculty of Medicine, Universitas Gadjah Mada, Indonesia.

Cream preparation

The cream formulation consisted of a mixture of avocado oil and bio-cream. The concentrations of avocado oil cream used in this study were 0%, 5%, 10% and 15%. The avocado oil cream was an oil-in-water (o/w) type of cream in which water was used as a solvent that can dissolve the dissolved components formulated in the cream. Water which was free from toxins, pollutants, and microbes was used in making the cream. The emulsion formed by the water helps to stabilize the oil-in-water mixture, classifying it as an oil-in-water emulsion (Rai et al., 2019).

Animal preparation

The study involved 24 eight-week-old female Deutschland Denken Yoken (DDY) mice, with an average body weight of 30 grams. The mice were divided into four treatment groups with three different time points, with two mice per group. Each mouse had two excisional wounds, resulting in four wounds in each group. The animals were purchased from the Integrated Laboratory for Research and Testing at Universitas Gadjah Mada, Indonesia. The mice were kept individually, in 12h light/dark cycle, and at constant temperature (25^0C). They were given food and water *ad libitum* throughout the experiment.

Wound model and treatments

The laboratory animals were wounded by making excision wounds using a 4 mm biopsy punch on the dorsal skin on the left and right parts (Lin et al., 2003; Masson-Meyers et al., 2020). The excisional wounds were suitable for histological assessment (de Oliveira et al., 2013). The 24 mice were divided into Groups K, P1, P2 and P3. Groups P1, P2, and P3 were treated topically with avocado oil cream at 5%, 10%, and 15% respectively, once daily on the wound sites (Bessera et al., 2020). The control group (Group K) received a topical application of a 0% avocado oil cream. A digital caliper measured the wound area on days 3, 6, and 9 after treatment. After the final measurements, the mice were euthanized by cervical dislocation and necropsied (Liu et al., 2022).

Histological study

The 48 wound tissues were collected and fixed in 10% formalin for 24 hours, and subsequently dehydrated in ethanol, cleared in xylol, embedded in paraffin wax, and sectioned by a microtome (Bessera et al., 2020). The tissue sections (5 µm thickness) were placed on slides and stained with hematoxylin-eosin for inflammatory cell observation and Masson's Trichrome for collagen density assessment. A binocular light microscope (Olympus CX-23, Japan) with 400x magnification was used to observe the number of inflammatory cells and collagen density, with three images captured per tissue. Observation of the number of inflammatory cells in histopathological slides was conducted using a microscope at 400x magnification across 3 fields of view, followed by counting using Image Raster software. Collagen density analysis was performed through microscopic observation (400x) of histopathological slides stained with Masson's Trichrome. Three fields of view were used to obtain the average collagen density using ImageJ software.

Macroscopic study

Measurement of wound areas and wound healing areas were conducted daily from the first day to the ninth day of topical administration by a digital caliper. The wound area was calculated using the Formula 1.

$$A = \pi . rx . ry, \qquad \text{(Formula 1)}$$

where "A" represented the area (mm^2) of the wound, "rx" was the x-axis, and "ry" was the y-axis of the wound.

The wounds were measured and the data were collected on days 3, 6 and 9.

Statistical analysis

The data for the wound areas was analysed statistically using SPPS (ver. 27, 2020). Inflammatory cell counts were performed in the dermis area from three different fields of view using Image Raster 3 software. The collagen density was determined using ImageJ software. The average number of inflammatory cells and collagen density from each group were analysed using the One-way ANOVA test or Kruskall-Wallis's test (SPSS, version 27, 2020, Statistic), with $p < 0.05$ as considered statistically significant.

RESULTS

Wound area

The wound area was measured using a caliper at three time points included 3,6, and 9 days after treatment with cream in Groups K, P1, P2, and P3. In general, the average area of wound healing showed a decrease in wound size after three days of treatment (Figure 1). Topical therapy after six days revealed a declining trend in wound size (Figure 2). Moreover, after nine days of topical medication, the wounds were the most closed in Groups P1, P2, and P3 (Figure 3). Topical treatment with 15% avocado oil cream (Group P3) showed a significantly better reduction in wound size compared to the group without avocado oil (Group K) after three days of therapy ($p < 0.05$). The wound area after 6 days of treatment showed no difference between the groups ($p > 0.05$). However, after nine days of topical application, treatments with 10% (Group P2) and 15% (Group P3) avocado oil cream resulted in significantly better wound healing ($p < 0.05$) compared to the group without avocado oil therapy (Table 1).

Inflammation cells

Inflammatory cells were counted in three different areas of the dermis layer in histopathological images (Figure 4) for all groups. Overall, the average number of inflammatory cells after 3, 6, and 9 days of therapy in the treatment groups declined, as compared to Group K (Table 2), indicating an improved condition. Moreover, significant differences ($p < 0.05$) were observed after treatment with avocado oil cream. After three days, Groups P2 and P3 had significantly fewer inflammatory cells than Group K ($p < 0.05$). Similar results were found for the 6-day medication, where Groups P2 and P3 showed a significantly better reduction in inflammatory cells ($p < 0.05$) compared to the group with 0% avocado oil. Meanwhile, there were no differences ($p < 0.05$) between groups after nine days of therapy.

Collagen density

The density of collagen was assessed from three different regions in Trichrome Masson's histopathological images (Figure 5). Briefly, after three days of therapy, collagen density in Groups P1, P2, and P3 showed an increasing trend compared to Group K, although there were no significant differences between the groups ($p > 0.05$). On days 6 and 9, collagen density increased significantly in the groups treated with avocado oil cream ($p < 0.05$). Six days after treatment, Group P3 had significantly higher collagen density ($p < 0.05$) than Group K. Meanwhile, by the ninth day of treatment, Group P2 showed significantly greater collagen density ($p < 0.05$) compared to Group K (Table 3).

Table 1. Means number of wound sizes at 3, 6, and 9 days of treatment (mm^2) with avocado oil cream

Group (Avocado oil cream concentration) \ Day of treatment	3	6	9
K (0%)	11.61 ± 1.46^{a}	4.71 ± 1.29^{e}	2.55 ± 0.32^{i}
P1 (5%)	10.97 ± 2.00^{ab}	4.49 ± 1.22^{e}	1.99 ± 0.41^{ij}
P2 (10%)	8.41 ± 1.78^{ab}	5.56 ± 1.98^{e}	1.41 ± 0.50^{jk}
P3 (15%)	8.06 ± 0.81^{b}	4.26 ± 2.56^{e}	0.95 ± 0.20^{kl}
P value	0.016	0.339	0.009

K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: cream with 15% avocado oil. ^{f} to ^{e} and ^{jk} to ^{i}: Mean within a column with different lowercase superscripts differ significantly ($p < 0.05$).

Table 2. Means number of inflammatory cell measurements (cell) at 3, 6, and 9 days of treatment with avocado oil cream

Group (Avocado oil cream concentration) \ Day of treatment	3	6	9
K (0%)	401.33 ± 50.19^{a}	258.75 ± 14.98^{e}	119.92 ± 13.85^{i}
P1 (5%)	284.83 ± 37.76^{ab}	191.17 ± 12.68^{ef}	103.83 ± 9.02^{i}
P2 (10%)	168.08 ± 6.19^{b}	155.08 ± 15.65^{f}	104.58 ± 11.14^{i}
P3 (15%)	169.50 ± 41.42^{b}	152.08 ± 22.83^{f}	89.75 ± 13.12^{i}
P value	0 < 0.001	0.003	0.4

K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: cream with 15% avocado oil. ^{f} to ^{e} and ^{jk} to ^{i}: Mean within a column with different lowercase superscripts differ significantly ($p < 0.05$).

Table 3. Means number of collagen density measurements (%) at 3, 6, and 9 days of treatment with avocado oil cream

Group (Avocado oil cream concentration) \ Day of treatment	3	6	9
K (0%)	16.97 ± 1.26^{a}	23.06 ± 4.35^{e}	44.13 ± 2.91^{i}
P1 (5%)	17.67 ± 3.75^{a}	25.83 ± 3.56^{ef}	46.27 ± 3.05^{ij}
P2 (10%)	18.59 ± 5.30^{a}	29.84 ± 3.38^{ef}	51.78 ± 1.06^{jk}
P3 (15%)	19.11 ± 1.33^{a}	31.55 ± 2.66^{f}	49.23 ± 3.28^{ij}
P value	0.81	0.02	0.09

K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: Cream with 15% avocado oil. ^{f} to ^{e} and ^{jk} to ^{i}: Mean within a column with different lowercase superscripts differ significantly ($p < 0.05$).

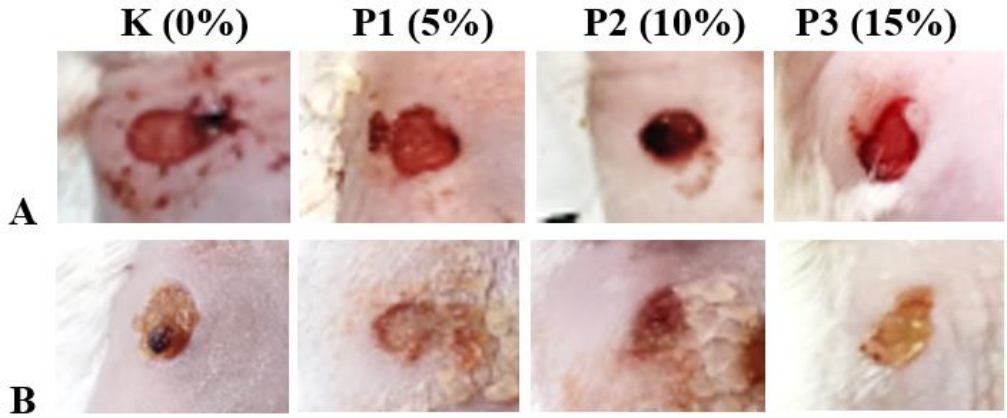

Figure 1. Wound healing after three days of avocado oil cream treatment in mice. K: Cream with no avocado oil; P1: cream with 5% avocado oil; P2: Cream with 10% avocado oil; P3: Cream with 15% avocado oil; **A**: Day 0, **B**: Day 3

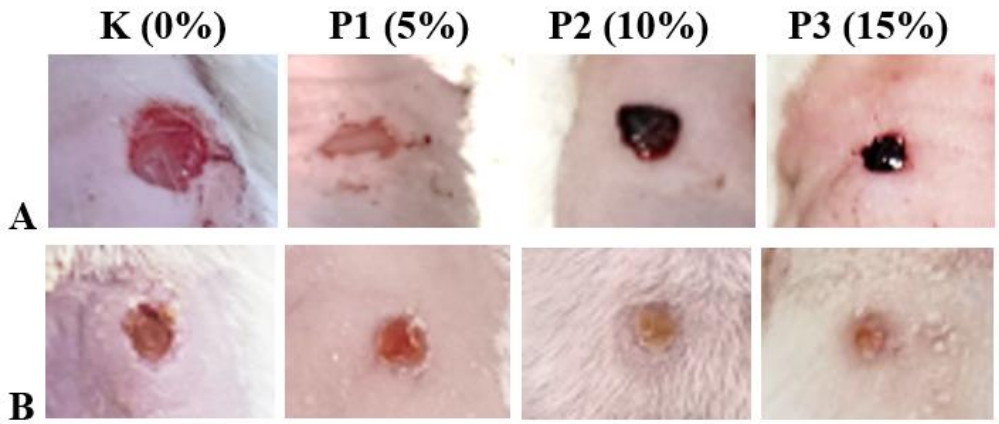

Figure 2. Wound healing after six days of treatment with avocado oil cream in mice. K: Cream with no avocado oil; P1: cream with 5% avocado oil; P2: Cream with 10% avocado oil; P3: Cream with 15% avocado oil; **A**: Day 0, **B**: Day 6

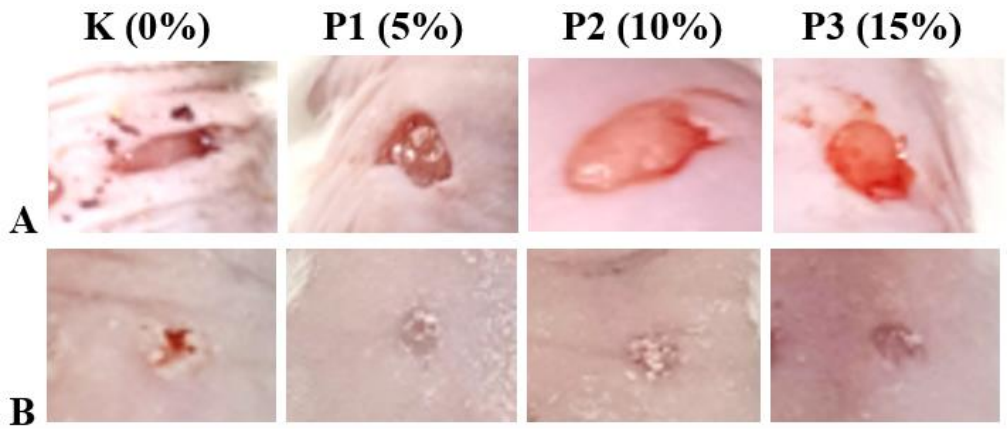

Figure 3. Wound healing after nine days of treatment with avocado oil cream in mice. K: Cream with no avocado oil; P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: Cream with 15% avocado oil; **A**: Day 0, **B**: Day 9.

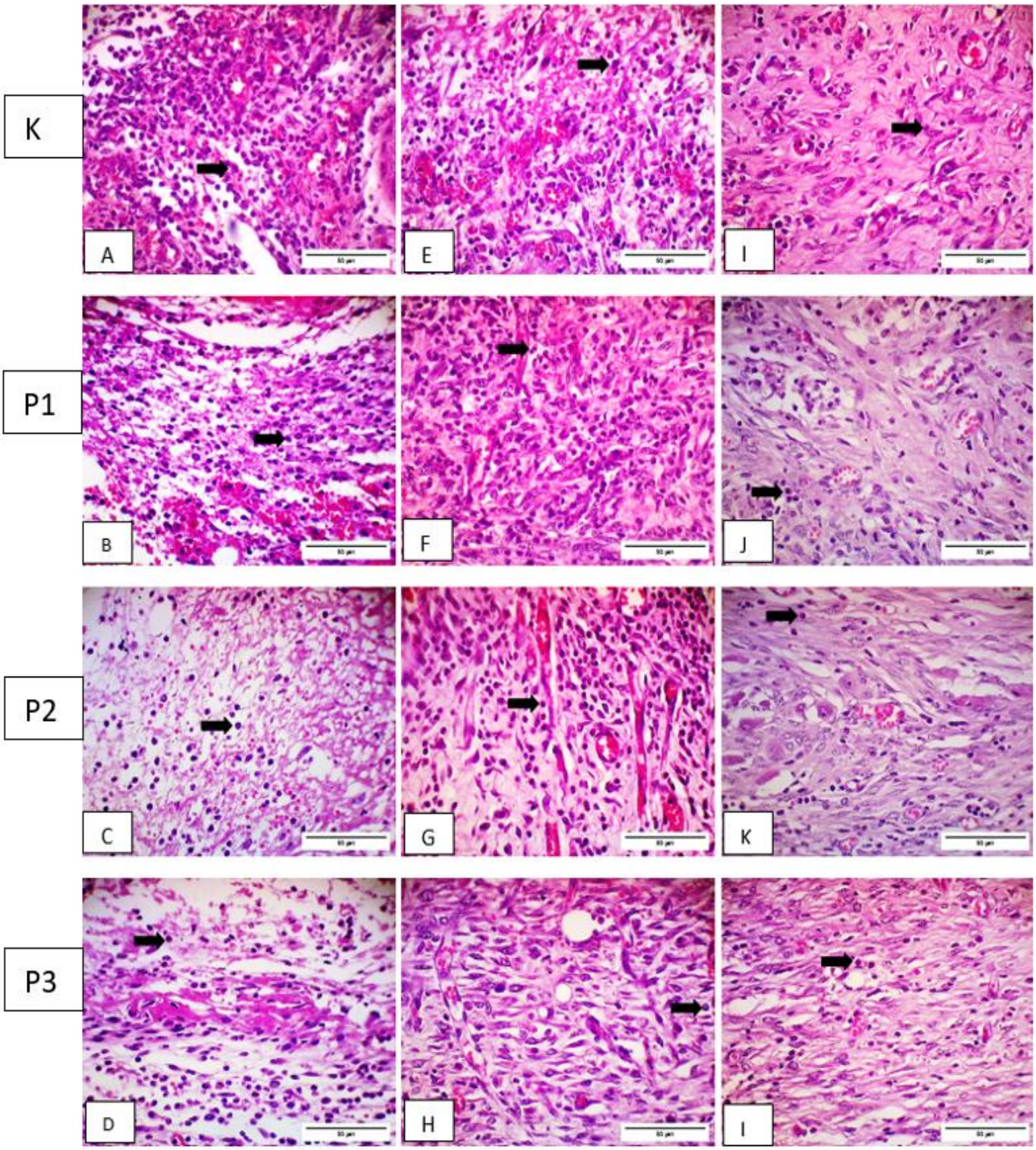

Figure 4. Inflammatory cell in wound healing with avocado oil cream in mice on days 3, 6 and 9. Day 3 (**A-D**), day 6 (**E-H**), day 9 (**I-L**). K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: Cream with 15% avocado oil, black arrow: Inflammatory cell, magnification 400x.

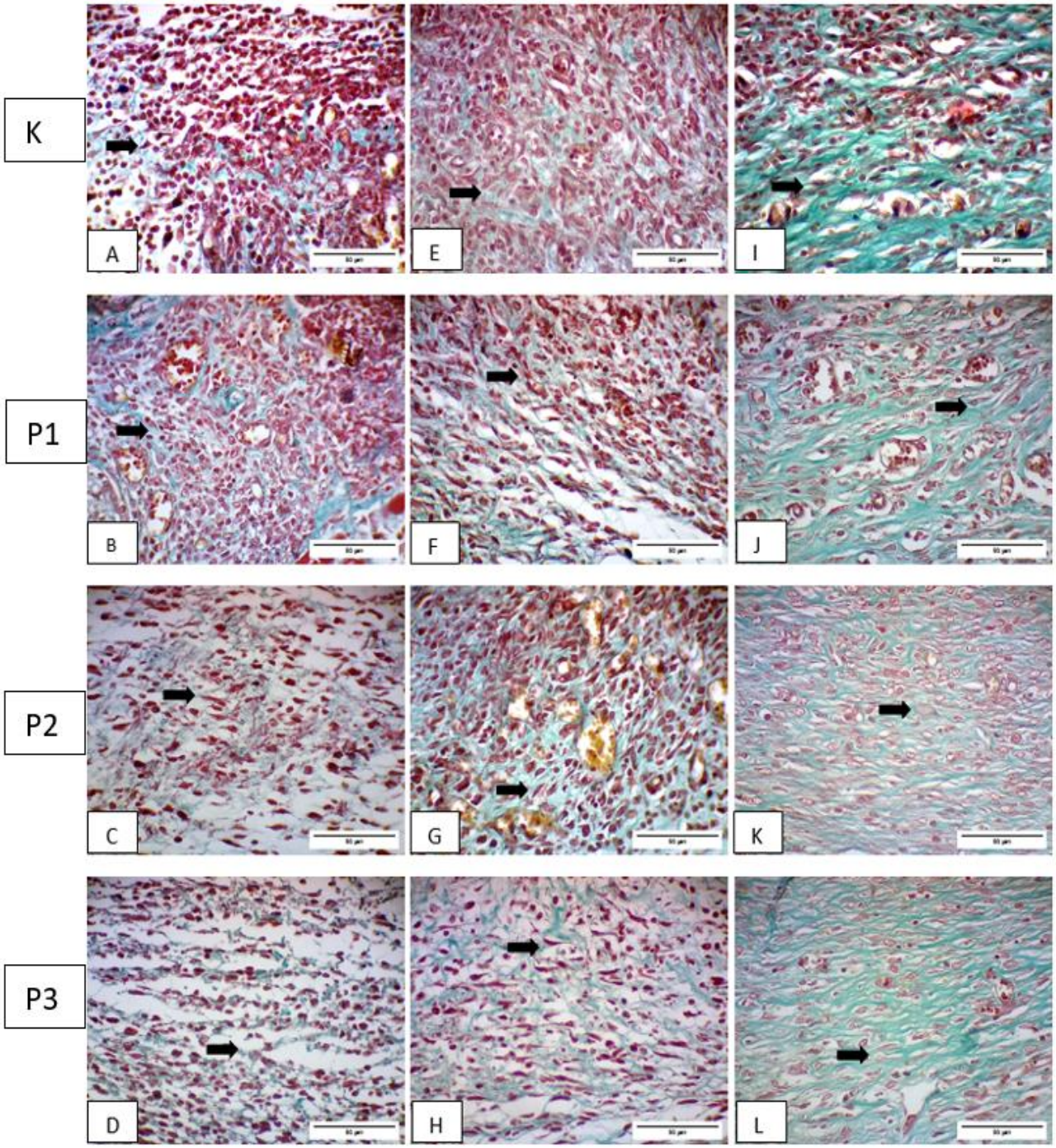

Figure 5. Collagen density (Trichrome Masson) in wound healing with avocado oil cream in mice on days 3, 6, and 9. Day 3 (**A-D**), day 6 (**E-H**), day 9 (**I-L**). K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: Cream with 15% avocado oil. Black arrow: Collagen, 400x, 2024.

DISCUSSION

The present study provided evidence that avocado oil cream significantly affects wound healing. The wound healing process included the three stages of inflammation, proliferation, and maturation. The inflammation stage occured from the time of wound formation until three days later (Kaifa et al., 2021). The proliferation stage typically begins on the fourth day of wound healing (Wahyuni et al., 2024). The maturation stage was the final stage of wound healing, characterized by wound closure and the restoration of tissue strength to 20–80% of its original state (Wahyuni et al., 2024). The wound area in Groups P1, P2, and P3 throughout the 9 days yielded data that corroborate the macroscopic image of a closed wound with a wound size of 1.99 + 0.41 mm^2, 1.41 $\pm$ 0,50 mm^2 and 0.95 $\pm$ 0.20 mm^2. These results suggested that avocado oil had the potential to be an alternative wound therapy. According to research by de Oliveira et al. (2013), avocado oil possesses anti-inflammatory properties. The oil was rich in essential fatty acids, such as linoleic acid and oleic acid, which act as anti-inflammatory agents. These two acids prevent the inflammatory process from going beyond the standard wound healing time so that chronic wound conditions do not occur. The mechanism inhibited

the multiplication of inflammatory cells (Lin et al., 2018; Pegoraro et al., 2021). Linoleic acid enhances wound closure by shortening the bleeding time and stabilizing fibrin and fibroblast migration. Another role of linoleic acid was to accelerate the inflammatory phase allowing the proliferation phase to commence sooner, thereby shortening the wound healing period (Silva et al., 2018). According to Pegoraro et al. (2021), oleic acid can inhibit the occurrence of oedema, showing its anti-inflammatory effect. Moreover, topical administration of linoleic and oleic acid in the wound healing process in rats had resulted in significant closure in the wound area (Ishak et al., 2019).

The findings of the current study reveal that avocado oil cream reduced the number of inflammatory cells in the process of wound healing. At 3 - 6 days post-treatment, both 10% and 15% avocado oil creams significantly reduced the number of inflammatory cells compared to the group without avocado oil ($p < 0.05$). However, after 9 days of treatment, there were no significant differences ($p > 0.05$) between the groups. Oedema and infiltration of inflammatory cells were the main changes in wound healing in the dermis layer (Widyarini et al., 2023). The proliferation stage can begin when the number of inflammatory cells decreases (Lin et al., 2003; Velnar et al., 2009). When the inflammatory response was working well and the number of inflammatory cells was higher, as observed on days 3 and 6 in comparison with day 9, the wound is healing well (Wosgrau et al., 2015). According to the study by Pegoraro et al. (2021), topical oleic acid therapy has anti-inflammatory effects; therefore, it can be an alternative to cutaneous inflammation therapy. Linoleic and oleic acids were two types of compounds that were involved in the inflammatory process. The wound can receive a significant influx of neutrophils from both acids. Linoleic corrosive effect influences aggravation through neutrophil movement (Silva et al., 2018; Guidoni et al., 2019). Neutrophils control the beginning of wound healing through cell migration to the inflammatory area, phagocytosis of cellular debris and microorganisms, and the release of pro-inflammatory cytokines (Pereira et al., 2008).

Collagen was one of the main components in the wound healing process. The results of this study indicated a significant difference ($p < 0.05$) in collagen density after 6 days of treatment with 15% avocado oil cream compared to the control group (Group K). Furthermore, after 9 days of therapy with avocado oil cream, a significant increased ($p < 0.05$) in collagen density was observed with 10% avocado oil cream compared to the group without avocado oil. Collagen was a connective tissue that provides a structural framework for tissue regeneration (Al-Henhena et al., 2011). Fibroblasts were cells responsible for synthesizing collagen. Progressive collagen synthesis will support the formation of excellent and optimal connective tissue (Sembiring et al., 2021). Collagen production in wound healing will increase because it repairs damaged or lost tissue (Gunawan et al., 2019). Collagen induced platelet activation and aggregation, resulting in the deposition of fibrin clots at the wound site. The inflammatory phase encourages the proliferation of fibroblasts that synthesize collagen and extracellular matrix. Collagen produced a robust inflammatory response, thus paving the way for faster wound healing (Mathew-Steiner et al., 2021). The initial process of the proliferation stage was assisted by fibroblasts that will produce collagen. Collagen will connect the tissues in the wound to help restore the strength of the skin tissue and accelerate wound closure (Sentat and Permatasari, 2015). During the maturation stage, young collagen will develop into mature collagen. Young collagen undergoes degradation or restructuring to become more prominent, organized, and bundle-shaped, eventually forming dermal tissue (Bodas and Shinde, 2021).

Previous research by de Oliveira et al. (2013) and Lin et al. (2018) demonstrated that topical application of avocado oil on injured rat skin significantly increased collagen levels. Oleic and linoleic acids were active ingredients in avocado oil that support wound healing. Oleic acid has been shown to restore and enhance collagen levels in both acute and chronic wounds (Ventura et al., 2021). Linoleic acid played a vital role in the expression of fibrinolytic system components that regulate collagen production (Ferreira et al., 2011). The release of cytokines was enhanced by linoleic acid, which also promotes the growth and differentiation of fibroblasts, keratinocytes, and endothelial cells. These processes accelerated the synthesis of collagen, which in turn aids in tissue healing. The composition of these two acids promotes the production of new collagen to replace damaged collagen, which aids in the healing process of wounds (Ishak et al., 2019). The notable findings of this study included the efficacy of 15% avocado oil cream in achieving significant wound closure after 9 days of treatment, 10% avocado oil cream in effectively reducing inflammatory cells within 3-6 days, and 15% avocado oil cream in significantly increasing collagen density after 9 days of application.

CONCLUSION

The application of avocado oil has demonstrated a great impact on wound closure after 9 days of treatment with 15% avocado oil cream, reducing the inflammatory cells after 3-6 days of 10% avocado oil cream therapy, and increasing collagen density at 15% of avocado oil application after 9 days. Future studies should focus on extending treatment duration, exploring higher concentrations of avocado oil, and assessing specific cytokine expressions to provide more detailed insights into its wound-healing mechanisms.

DECLARATIONS

Authors' contributions

Dini Agusti Paramanandi conceptualized, managed, and conducted data analysis and interpretation. Dini Agusti Paramanandi and Husnur Rukyat performed all the experimental procedures. Yuli Purwandari Kristianingrum

supervised the study. Sitarina Widyarini conceptualized and supervised the study. All authors read and approved the final manuscript.

Competing interests

The authors have not declared any conflict of interest.

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Ethical consideration

The authors confirm that the manuscript has been checked for plagiarism and submitted to this journal originally.

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material. For inquiries, please contact the corresponding author.

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