The Effectiveness of Sialang Honey in Burn Healing Through the Expression of Vascular Endothelial Growth Factor (VEGF) in Rattus norvegicus

Firdath Rubenzani[1], Syamsul Rizal[1], Mirnasari Amirsyah[1], Budiman[2], Khairan Khairan[3]

[1]Department of Plastic Reconstructive and Aesthetic Surgery, Dr. ZainoelAbidin General Hospital, University of Syiah Kuala, Banda Aceh, Indonesia
[2]Department of Plastic Reconstructive and Aesthetic Surgery, Gatot Subroto Army Hospital, Jakarta, Indonesia
[3]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Syiah Kuala, Banda Aceh, Indonesia

*Email correspondence : firdathrubenzani@gmail.com

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Abstract

Full-thickness burns require rapid revascularization to prevent tissue necrosis and support the healing process. Sialang honey, a wild multifloral honey from Indonesia, has traditionally been used for wound care, but its specific mechanism in modulating angiogenesis remains poorly understood. This study aimed to evaluate the effectiveness of Sialang honey on the expression of Vascular Endothelial Growth Factor (VEGF) in a mouse model with full-thickness burns. A true experimental study with a post-test only control group design was conducted using 27 male Wistar rats (Rattus norvegicus). Full-thickness burns were induced and treated daily for 14 days. Rats were randomly divided into three groups: Negative Control (0.9% NaCl), Treatment (Topical Sialang Honey), and Positive Control (1% Silver Sulfadiazine). Skin tissue was collected on days 5, 7, and 14. VEGF expression was detected using immunohistochemical (IHC) staining and measured via H-Score. Data were analyzed using One-Way ANOVA and Post-Hoc LSD Test ($p<0.05$). Treatment with Sialang Honey significantly increased VEGF expression compared to the negative control group. Peak expression occurred on day 7 (mean difference vs. control: $p=0.000$), indicating strong angiogenesis during the proliferation phase. Notably, VEGF levels in the honey group decreased significantly on day 14 ($p<0.05$), mimicking the physiological transition to the remodeling phase. The honey group showed a superior angiogenic response compared to Silver Sulfadiazine on day 7. Sialang honey effectively accelerated burn wound healing by modulating VEGF expression promoting an initial surge in angiogenesis followed by a physiological decline. These findings support the potential of Sialang honey as an effective topical agent for burn management.

Keywords: Sialang Honey, Vascular Endothelial Growth Factor (VEGF), Angiogenesis.

INTRODUCTION

Burns healing takes time because of complex tissue trauma that disrupts physiological homeostasis and skin integrity (Jeschke et al., 2020). Deep and full-thickness burns are the longest to heal because the burns have damaged the dermis and its vascular network (Burgess et al., 2022; Cretu et al., 2025). The natural healing process is often delayed, increasing the risk of infection, prolonged inflammation, and the formation of hypertrophic scars (Jeschke et al., 2020; Żwierełło et al., 2023). It is not just the burns that take time to heal, but the scars become a concern, too. First, to heal the wound, therapeutic strategies that accelerate wound healing mechanisms, particularly during the proliferation phase, are critical for optimal recovery.

The wound healing process is highly dependent on angiogenesis, which is the formation of new capillary blood vessels from existing blood vessels, which is very important for delivering oxygen and nutrients to regenerating tissue (Wilkinson & Hardman, 2020; Żwierełło et al., 2023). Vascular Endothelial Growth Factor (VEGF) is the main angiogenic factor that regulates this process (Z. Liu et al., 2023; Wang et al., 2024). Produced by macrophages, fibroblasts, and keratinocytes during the proliferation phase, VEGF expression typically begins around day 5, peaks between days 12 and 14, and facilitates the formation of granulation tissue (Shams et al., 2022; Wilkinson & Hardman, 2020). Insufficient VEGF expression is often correlated with delayed healing and chronic wounds, making the upregulation of this growth factor a primary therapeutic target.

Silver sulfadiazine is widely used for antimicrobial control, but these treatments can sometimes cause cellular toxicity or inhibit the re-epithelialization process (Schaefer, 2017; Wasiak & Cleland, 2015). Therefore, it is good to use natural products with bioactive properties. The natural product with bioactive properties that we propose in this study is honey. Honey can promote autolytic debridement, maintain a moist wound environment, and provide broad-spectrum antimicrobial activity because of its hydrogen peroxide content and high osmolality (Halim & Dwimartutie, 2020).

Sialang honey, a wild multifloral honey produced by Apis dorsata bees in the Riau region of Indonesia, is one type of honey that is proven beneficial to heal wound, including burns (Asmara & Nurlia, 2020). Why is Sialang honey very special? unlike typical monofloral honey, Sialang honey contains a different phytochemical profile, in that it has high concentrations of phenolic acids and flavonoids (Rodica et al., 2021). Previous studies suggest that these bioactive compounds possess strong antioxidant and anti-inflammatory properties, which may exceed those found in standard cultivated honey (Asmara & Nurlia, 2020; Ritonga & Daulay, 2019). It is estimated that the antioxidant capacity of Sialang honey creates an optimal microenvironment that stimulates macrophage activity and fibroblast proliferation, potentially increasing VEGF expression and accelerating angiogenesis.

Sialang honey has antimicrobial benefits, especially to heal wounds, and many studies have proven this. However, we found limited literature on the molecular mechanism of Sialang honey in modulating angiogenic factors such as VEGF in burns. To test the effect of Sialang honey on burn wounds, we used rats as the models. Rattus norvegicus (Norwegian rat) is an ideal model for this study due to its physiological and anatomical similarities to the wound healing process in humans (Hedrich, 2020). Therefore, this study aims to evaluate the effects

of topical application of Sialang honey on VEGF expression and wound healing acceleration in a rat model with burn wounds.

METHODS

Study Design and Ethical Approval This study is a laboratory experimental study with a Post-Test Only Control Group Design using a Complete Randomized Design (CRD). All procedures for handling experimental animals are in accordance with the 3R principles (Replacement, Reduction, Refinement). Test Animals The study sample consisted of 27 male white rats (Rattus norvegicus) of the Wistar strain, with the following inclusion criteria: age 2-3 months, weight 200-250 grams, and in healthy and active condition. Prior to treatment, the rats were acclimated for 7 days in standard laboratory cages with a 12-hour light-dark cycle, temperature of 25±2°C, and fed standard feed and drinking water ad libitum. Burn Model Creation and Grouping The rats were anesthetized using [specify type of anesthesia, e.g., intramuscular Ketamine-Xylazine]. The fur on the back area was shaved, then a third-degree (full-thickness) burn wound was created by applying a 2 cm diameter hot metal plate that had been heated to 100°C for 5-10 seconds to the skin on the back of the mouse.

The experimental animals were randomly divided (simple random sampling) into 3 treatment groups (n=9 animals per group): Group P0 (Negative Control): The wound was covered with sterile gauze moistened with 0.9% physiological NaCl solution. Group P1 (Treatment): The wound was coated with 0.5 mL of pure Sialang honey (100%). Group P2 (Positive Control): The wound was coated with 0.5 grams of 1% Silver Sulfadiazine cream.

Wound treatment was performed once a day for 14 consecutive days. Before applying the test material, the wound was gently cleaned with physiological NaCl solution to remove dirt or previous treatment residues.

Three mice from each group were euthanized on days 5, 7, and 14. Skin tissue from the wound area was biopsied and fixed in 10% Neutral Buffered Formalin (NBF) solution for paraffin block preparation. The tissue was sectioned at a thickness of 4-5 microns for two types of staining: Hematoxylin Eosin (H&E) staining, used for general histopathological examination, viewing tissue structure, and descriptively assessing the wound healing phase. Immunohistochemistry (IHC) staining: Performed specifically to detect VEGF expression. The preparations were incubated using anti-VEGF primary antibodies, followed by secondary antibodies and DAB (Diaminobenzidine) chromogen. Positive VEGF expression was indicated by the formation of brown deposits in the cytoplasm of endothelial cells and inflammatory cells.

VEGF expression data were calculated based on histochemical scores (H-Scores) or the percentage of positive cells in 5 fields of view. Data normality was tested using the Shapiro-Wilk test. Since the data were normally distributed ($p > 0.05$), analysis was continued with a One-Way ANOVA test to examine differences between groups, and a Post-Hoc LSD test to determine the significance between pairs of groups. Data analysis was performed using statistical software with a 95% confidence level ($\alpha = 0.05$).

RESULT

Microscopic examination of Rattus norvegicus tissue revealed the following results :

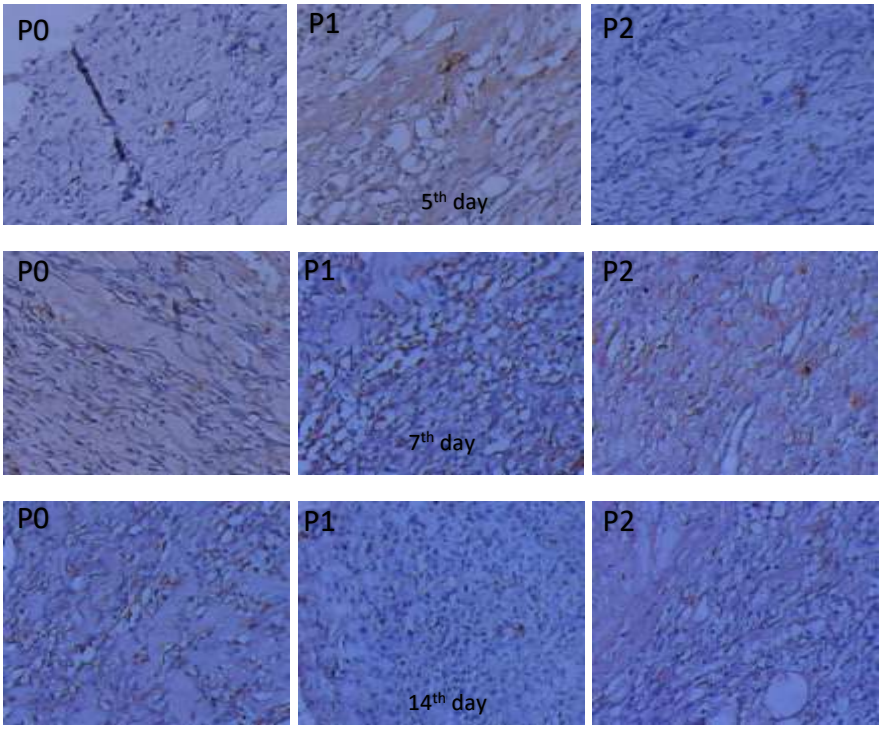

Figure 1. Immunohistochemical (IHC) staining of VEGF expression in skin tissue of *Rattus norvegicus*

Immunohistochemical (IHC) analysis showed that VEGF expression was concentrated in the cytoplasm of inflammatory cells and endothelial cells, marked by brown deposits. The intensity of VEGF expression varied significantly throughout the day of observation. Meanwhile, the cytoplasm was not colored by HE colour or VEGF antibody staining. From the results of microscopic examination, it was found that on 5^{th} day VEGF started to appear, and on 7^{th} day VEGF high increased which was marked by the brownish colour of Immunohistochemical (IHC) staining in microscopic examination. Then on 14^{th} day the brownish colour started to decrease with the cell composition starting to solid.

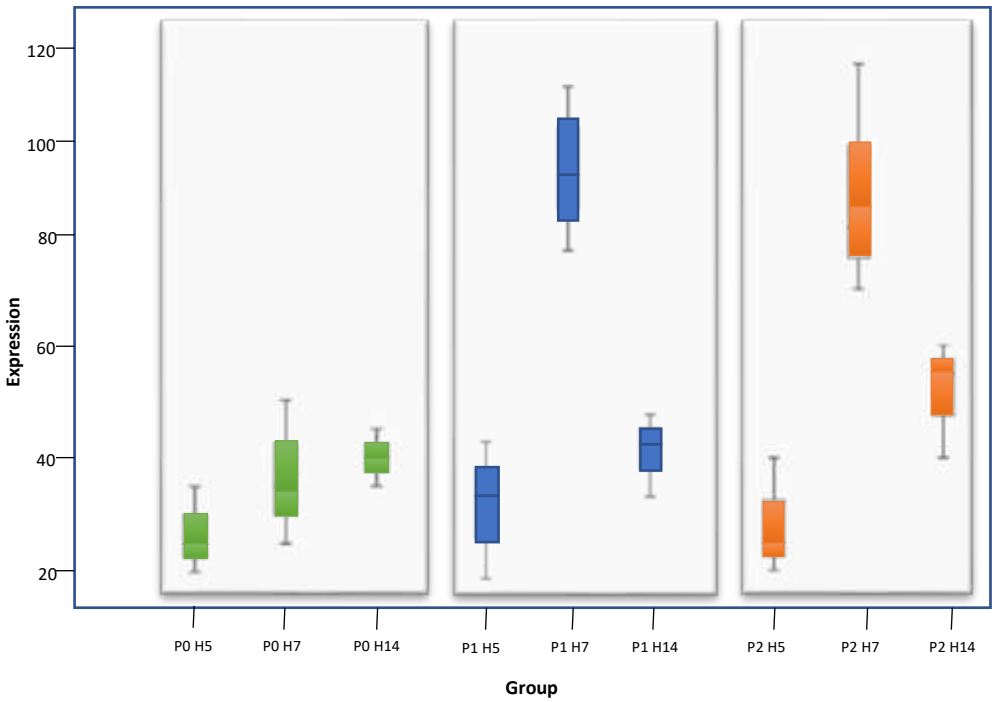

Figure 2. Comparison of VEGF expression between groups

The table is calculated using the Hystochemical Score (H-Score) by combining the colouring intensity and the percentage of coloured cells at every intensity and the SPSS test is performed using the One-Way ANOVA test. The results of the one-way ANOVA test were obtained with p = 0.00 (<0.05), it mean full thickness burn patients wound care with Sialang Honey has a strong effectiveness to accelerate epithelialization through on VEGF expression. Based on the table, it is found that the results of burn treatment using Sialang Honey have the

Table 1. *Post-Hoc Result*

		Different	CI 95%		*p*
			Minimum	Maximum	
P0 H5 vs	P0 H7	-10,00	-30,83	10,83	0,327
	P0 H14	-13,33	-34,16	7,50	0,195
	P1 H5	-6,67	-27,50	14,16	0,510
	P1 H7	-68,33	-89,16	-47,50	0,000*
	P1 H14	-16,667	-37,50	4,16	0,110
	P2 H5	-1,67	-22,50	19,16	0,868
	P2 H7	-60,33	-81,16	-39,50	0,000*
	P2 H14	-25,00	-45,83	-4,17	0,021*
P0 H7 vs	P0 H14	-3,33	-24,16	17,50	0,741
	P1 H5	3,33	-17,50	24,16	0,741
	P1 H7	-58,33	-79,16	-37,50	0,000*
	P1 H14	-6,67	-27,50	14,16	0,510
	P2 H5	8,33	-12,50	29,16	0,412
	P2 H7	-50,33	-71,16	-29,50	0,000*
	P2 H14	-15,00	-35,83	5,83	0,148
P0 H14 vs	P1 H5	6,67	-14,16	27,50	0,510
	P1 H7	-55,00	-75,83	-34,17	0,000*

		Different	CI 95%		*p*
			Minimum	Maximum	
	P1 H14	-3,33	-24,16	17,50	0,741
	P2 H5	11,67	-9,16	32,50	0,255
	P2 H7	-47,00	-67,83	-26,17	0,000*
	P2 H14	-11,67	-32,50	9,16	0,255
P1 H5 vs	P1 H7	-61,67	-82,50	-40,84	0,000*
	P1 H14	-10,00	-30,83	10,83	0,327
	P2 H5	5,00	-15,83	25,83	0,620
	P2 H7	-53,67	-74,50	-32,84	0,000*
	P2 H14	-18,33	-39,16	2,50	0,081
P1 H7 vs	P1 H14	51,67	30,84	72,50	0,000*
	P2 H5	66,67	45,84	87,50	0,000*
	P2 H7	8,00	-12,83	28,83	0,430
	P2 H14	43,33	22,50	64,16	0,000*
P1 H14 vs	P2 H5	15,00	-5,83	35,83	0,148
	P2 H7	-43,67	-64,50	-22,84	0,000*
	P2 H14	-8,33	-29,16	12,50	0,412
P2 H5 vs	P2 H7	-58,67	-79,50	-37,84	0,000*
	P2 H14	-23,33	-44,16	-2,50	0,030*
P2 H7 vs	P2 H14	35,33	14,50	56,16	0,002*

The Sialang Honey group (P1) showed the highest VEGF expression on Day 7 compared to all other groups. The mean difference between P1 and the Negative Control group (P0) on Day 7 was highly significant ($p = 0.000$), indicating strong angiogenesis induction. On Day 7, Sialang Honey (P1) also showed significantly higher VEGF expression compared to the Silver Sulfadiazine group (P2) ($p < 0.05$), indicating superior potential in stimulating the early phase of angiogenesis. In the Sialang Honey group, VEGF levels decreased significantly from Day 7 to Day 14 ($p = 0.000$). This decrease is very important, as it signals the transition from the proliferation phase to the remodeling phase, preventing excessive granulation tissue formation.

DISCUSSION

The results of this study indicate that topical application of Sialang Honey effectively modulates VEGF expression in thick-thickness burns. The most important finding is a significant increase in VEGF on Day 7 in the honey-treated group. Previous study reported that VEGF will increase from the 3rd day to the 6th day, with an average increase on the 5th day depend on the cell's response to the effects of cell hypoxia. Production expression of VEGF proves that the epithelialization process was starting (Sari et al., 2022). VEGF has a peak appearance between the 7th and 9th day and will decrease in number after the amount of MMP (Matrix metalloproteinase) begins to balance with the amount of VEGF, it could happen between on 12th and 14th day (Brem et al., 2009). Microscopic examination also proves that

Silver Sulfadiazine and Sialang Honey have an angiogenesis process as evidenced by the expression of VEGF. Research by Reine Z et al. said that Sialang Honey and pure forest honey have a high flavonoid acids compared to farmed honey. Flavonoid acids consist of quercetin, apigenin, genistein and kaempferol has function to activate anti-hypoxia factor (HIF -1) which will provoke the release of erythropoietin receptors. The effect of erythropoietin receptor attachment to the edge of the cytoplasm will induce the process of angiogenesis through the expression of VEGF (Zhafirah et al., 2023).

The highest level of VEGF expression, which means the angiogenesis process has the highest level from the 3 groups. Research by Luh Gede et al, proved that VEGF expression will start to appear between the 3rd and 5th day, and will enter the peak phase on the 7th and 9th day and begin to decrease, less on the 14th day. This is because of the lack of Plasmacytoid Dendritic Cells (pDC), which will decrease on the 10th day, so that VEGF will also decrease. In full-thickness burns, VEGF has effects on vascular hyperpermeability and monocyte aggregation. Full-thickness burns cause damage to the epidermis and dermis because of the inflammatory reaction. When the inflammatory process enters the final phase, HIF-1 starts to release, which activates erythropoietin receptors to initiate VEGF release in response to the start of the proliferation phase. The effect of VEGF initiated in Full Thickness burns is the occurrence of many new vascularizations and will be followed by the formation of granulation tissue. The granulation tissue will temporarily become a barrier to replace the dermis and epidermis until the epithelialization process is complete. Full-thickness burns often prolong the proliferation phase marked by hypergranulation because VEGF does not decrease on the 12th – 14th day due to the major burn process, and the epithelialization reaction will be disturbed (Abdulazeem et al., 2022).

According to Ayyasi et al., forest honey has the highest flavonoid acid content compared to other honeys, where one of the substances contained in this flavonoid acid is quercetin and H_2O_2. Quercetin can stimulate the release and activation of VEGF in the process of wound healing of full-thickness and deep dermal burns. H_2O_2 can confirm the stimulation of VEGF after the reaction between exudate and glucose oxidation in honey. Silver Sulfadiazine as a positive control has a strong effect on the angiogenesis process because it has a pro-fibroblast effect that stimulates fibroblasts, maintains moisture, and as an anti-bacterial in the wound area, so it is often widely used as a wound treatment in burn wounds (Almas et al., 2020; X. Liu et al., 2023).

From the findings above, we can conclude that this study provides scientific evidence supporting the use of Sialang Honey as an effective topical dressing for full-thickness burns. We can see that there is a significant increase in VEGF expression on Day 7 indicates that Sialang Honey effectively accelerates the proliferation phase by stimulating rapid angiogenesis, which is essential for nutrient delivery and tissue survival. The physiological decrease in VEGF on Day 14 in the honey-treated group indicates a normal healing process that transitions well into the remodeling phase, and this can reduce the risk of pathological scar formation (e.g., keloids) often associated with prolonged high VEGF levels. After carrying out this study, we could say that Sialang Honey offers a promising, cost-effective, and accessible alternative to standard treatments such as silver sulfadiazine, particularly in resource-limited areas where modern wound dressings may be scarce.

This study has several limitations. First, as an *in vivo* animal study using *Rattus norvegicus*, these findings may not be directly applicable to human clinical outcomes due to physiological differences in skin structure and healing mechanisms (loose skin in rats versus tight skin in humans). Second, this study focused only on VEGF as a single marker of angiogenesis; it did not evaluate other inflammatory cytokines (e.g., IL-6, TNF-α) or collagen maturation (Type I/III ratio), which could provide a more comprehensive understanding of the healing process. Third, the observation period was limited to 14 days, making it impossible to evaluate the long-term quality of scars and their tensile strength. Future studies involving human clinical trials and longer observation periods are recommended to validate these findings.

CONCLUSION

Burn wounds takes long time to heal, but natural product like honey can not only heal the wound, but also reduce the scar formation. After giving treatments to rats using Sialang honey, we found that Sialang Honey is effective for treatment in full thickness burns through the process of releasing VEGF antibodies, which causes the angiogenesis process better than use of NaCl and Silver Sulfadiazine. The wound care with NaCl, Sialang Honey, Silver Sulfadiazine had the highest VEGF expression on 7th day and decreased on 14th day. This process occurred in all treatments (NaCl, Sialang Honey, Silver Sulfadiazine), but the use of Sialang Honey had the highest VEGF expression compared to the others.

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