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The combination of Virgin Coconut Oil (VCO) and *Nigella sativa* Oil (NSO) in wound healing: A histochemical study of diabetic wounds

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ABSTRACT: Wound healing presents a formidable challenge for individuals with Diabetes Mellitus (DM), mainly stemming from delayed epithelialization processes. Virgin Coconut Oil (VCO) and Nigella Sativa Oil (NSO) are natural agents commonly employed in DM wound care. Nonetheless, there has been limited histological exploration into their combined utilization. In this study, diabetic rats were divided into six groups: a positive control, individual VCO and NSO treatments, and a combination of VCO-NSO with volume ratios of 1:1, 2:1, and 1:2. These interventions were administered over 7 days, followed by the collection of tissue samples for histological examination. Statistical analysis was conducted using SPSS 18.0, where mean ± SD values underwent one-way analysis of variance (ANOVA). Post hoc comparisons between groups were performed using Dunnett's analysis. The findings of this investigation demonstrated that the application of VCO and NSO increased the thickness of both the epidermis and dermis compared to the untreated group. Notably, the VCO-NSO combination was more effective in reducing edema and decreasing macrophage and leukocyte counts than either the control group or the single VCO and NSO treatments. The synergistic effect of the VCO-NSO combination promoted better healing of DM-related wounds by attenuating inflammation and accelerating re-epithelialization. These compelling results suggest that combining VCO and NSO could be an invaluable approach for treating DM-associated wounds.

KEYWORDS: Dermis; epidermis; *Nigella sativa*; Virgin Coconut Oil; Wound healing.

1. INTRODUCTION

Delayed wound healing is a critical challenge in managing diabetic ulcers [1]. This delay primarily stems from hyperglycemia, which leads to heightened levels of free radicals, inflammatory cytokines, and thickening of the capillary basement membrane. These physiological changes, elucidated by Chang, contribute to the protracted healing process, often resulting in chronic wounds. Tragically, chronic wounds can progress to the point of necessitating amputation [2]. Moreover, diabetic ulcers significantly elevate the morbidity and mortality rates among individuals with diabetes. Diabetic patients with ulcers face a 2.5-fold higher risk of mortality compared to their counterparts without ulcers [3]. The healing process of diabetic ulcers involves several distinct phases, as identified [4]: the inflammation phase, re-epithelialization, granulation, and remodeling. A significant contributor to complications in diabetic ulcers is the prolonged inflammatory process, delayed re-epithelialization, and insufficient formation of new blood vessels [5].

During the proliferation phase, fibroblast and endothelial cells are crucial in covering the wound tissue, with endothelial cells playing an essential role in angiogenesis. The initiation of the inflammatory process is triggered by leukocyte migration [4]. However, there is a notable delay in leukocyte migration in the presence of hyperglycemia. This delay, as outlined by Yadav et al., subsequently exacerbates other stages of the wound healing process [6]. Various natural ingredients include Virgin Coconut Oil (VCO) [7], which is extracted from fresh coconut meat (*Cocos nucifera*) without using high heat or chemicals. VCO has been reported to have the potential for wound repair in diabetic patients [8]. Another study also reported that VCO could reduce *Streptococcus aureus* bacterial colonies in vitro, and can accelerate wound healing by activating Vascular

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Endothelial Growth Factor (VEGF) cytokine signaling [9]. Moreover, a reduction in the size of diabetic ulcers was observed in patients treated with VCO compared to those receiving 0.9% NaCl [10]. The promotion of wound re-epithelialization, characterized by decreased fibroblasts and preserved epidermal tissue, was observed with VCO application [11,12].

Nigella sativa Oil (NSO), also known as black cumin or habbatussauda oil, is extracted from the seeds of the *Nigella sativa* plant. NSO is renowned for its diverse therapeutic properties, including diuretic, anti-bleeding, antibacterial, antitumor, antimalarial, antidiabetic, and antihypertensive effects [13]. NSO also contains thymoquinone, a key component in wound care [14]. Thymoquinone's beneficial role lies in its ability to stimulate macrophage interleukin production, thereby inducing the release of chemotactic substances and growth factors crucial for wound healing. Moreover, it reduces the inflammatory response, damages bacterial cell lipid membranes, and suppresses IL-1β [13].

The potential combined effect of VCO and NSO in treating diabetic ulcers remains unexplored, despite their promise. This research aims to establish the effectiveness of VCO and NSO in healing diabetic ulcers.

2. RESULTS

A comparison of macroscopic wound morphology in the inflammatory phase between the study groups is shown. As shown in the Figure 1, the wounds in the treatment group shrink much faster than those in the positive control group, which received no treatment. Therefore, these results indicate that topical administration of VCO and NSO can promote wound healing in diabetic rats, as shown in Table 1. Comparison of wound diameter between treatment groups.

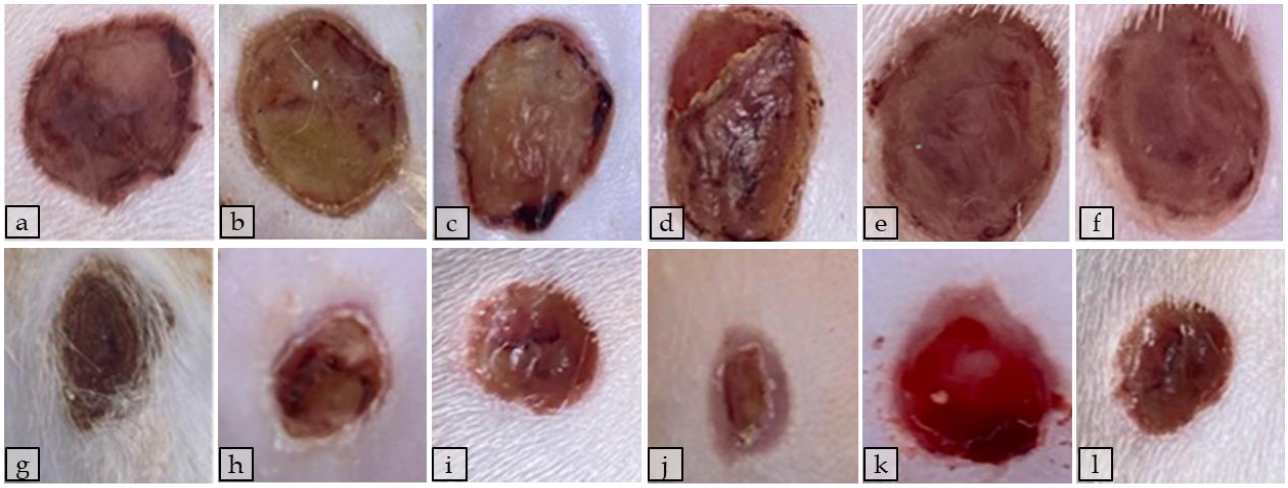

Figure 1. Wound tissue shape at various concentration ratios: Day 1: Diabetes control (a); treatment with VCO (b); treatment with NSO (c); treatment with the combination of VCO + NSO at 1:1 (d), 2:1 (e), 1:2 (f). Day 7: Diabetes control (g); treatment with VCO (h); treatment with NSO (i); treatment with a combination of VCO + NSO with a ratio of 1:1 (j), 2:1 (k), and 1:2 (l). The picture shown is the best picture (N = 5) in each group.

Table 1. Average Wound Diameter

No	Group	N	Pretest (mm)	Posttest (mm) and SD
1	Control	5	10	6.25±1.03
2	VCO	5	10	5.3±08
3	NSO	5	10	5.5±0.8
4	VCO-NSO 1:1	5	10	5.04±0.03
5	VCO-NSO 2:1	5	10	4.68±0.13
6	VCO-NSO 1:2	5	10	3.3±1.08

Description: N is the number of sample animals in one experimental group

The following are the research results from several analysis parameters carried out:

The wound diameter in the treatment group was smaller than in the control group. The smallest diameter was observed in the combination group compared to the single administration group. Wound size reduction was most significant with the combination product containing more VCO (2:1) or NSO (1:2).

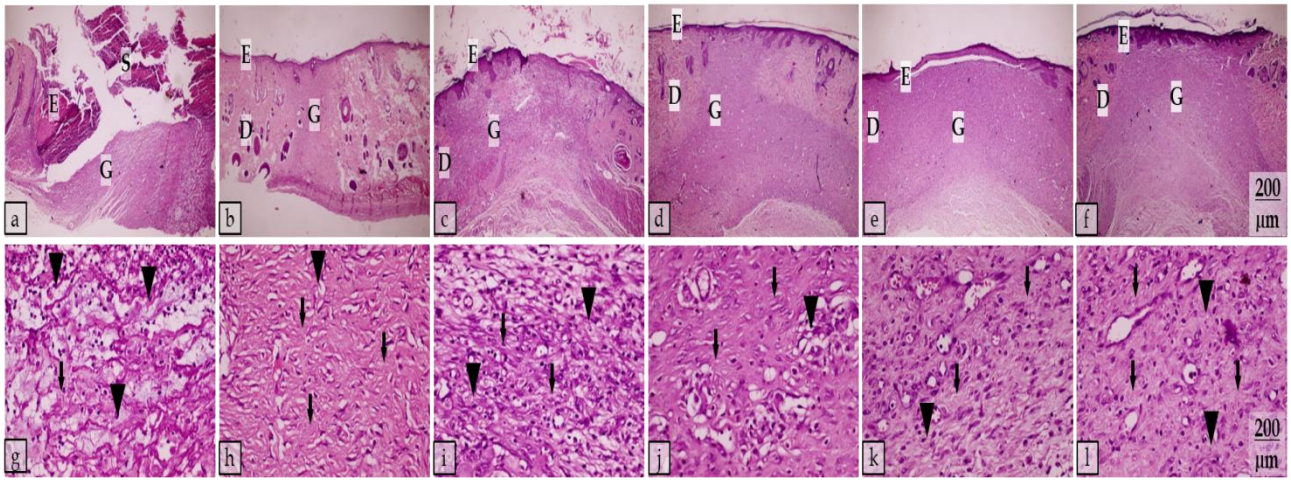

Figure 2. Histology of wound tissue on day 7: Diabetes control group (a, g); treatment with VCO (b, h); treatment with NSO (c, i); treatment with a combination of VCO + NSO with a ratio of 1:1 (d, j), 2:1 (e, k), and 1:2 (f, l). The picture used is the best picture (N = 5) in each group. This histological analysis is done through Hematoxylin and Eosin Staining.

The images show the epidermis (E) and dermis (D) layers, with post-wound granulation tissue (G) in the dermis containing collagen matrix (↓) along with inflammatory cells (▼). Control animals showed incompletely epithelialized, scab-covered wounds (S), characterized by granulation with loose connective tissue, a high presence of inflammatory cells, and low levels of fibroblasts and collagen. In contrast, treatment with VCO, NSO, or their combination showed improved wound healing, complete epithelialization, reduced inflammatory cells, and increased levels of fibroblasts and collagen. Notably, the VCO and NSO combination in a 2:1 ratio showed superior histological results compared to the other treatments.

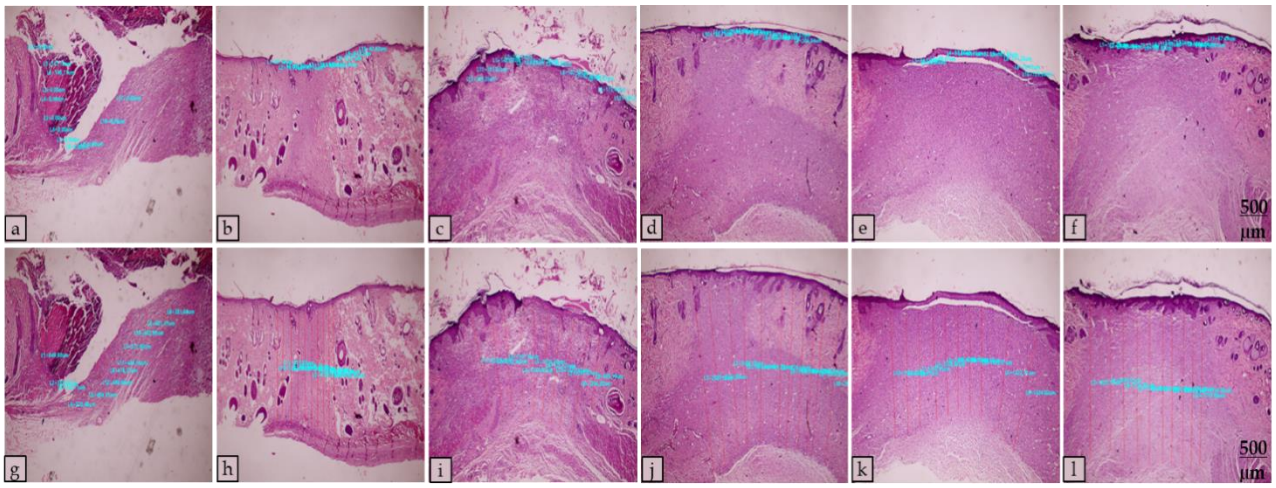

Figure 3. Microscopic measurement of epidermis and dermis thickness. Diabetic control group (a, g); treatment with VCO (b, h); treatment with NSO (c, I); treatment with VCO + black cumin combination, 1:1 (d, j); treatment with VCO + NSO combination, 2:1 (e, k); treatment with VCO + NSO combination, 1:2 (f, l). The image shown is the best image (N=5) in each group.

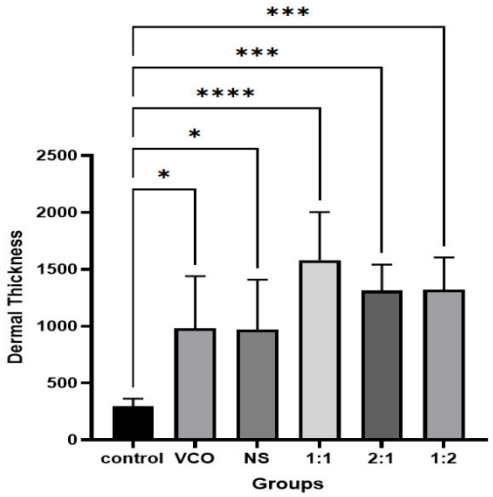

Figure 4. Shows the epidermal thickness after intervention on day 7 with significance levels of p < 0.05, p < 0.01, and **p < 0.001.

There is a difference in epidermal thickness between the groups. The thickness is the best in the VCO-NSO combination group, with the 1:1 VCO-NSO combination showing the best results. The thickness of the 2:1 VCO-NSO combination is comparable to the 1:2 combination.

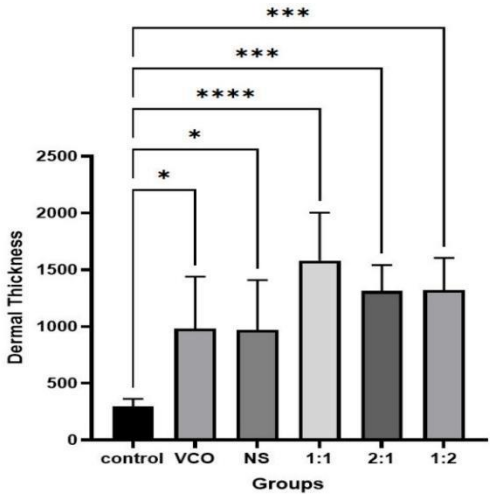

Figure 5. Shows the dermal thickness after intervention on day 7 with significance levels of p < 0.05, p < 0.01, and **p < 0.001.

There is a difference in dermis thickness between the groups. The thickness is the best in the VCO-NSO combination group, with the 1:1 VCO-NSO combination showing the best results. The thickness of the VCO-NSO 2:1 combination is comparable to the 1:2 combination. A brief explanation of the picture as a result.

Assessment of histological parameters of the skin on day 7 included epidermis thickness, dermis thickness, and histological scores based on McMinn criteria. The administration of VCO and black cumin, both individually and in combination, resulted in increased thickness of the epidermis and dermis. The mean thickness of the epidermis and dermis in combination group 1 was slightly higher than in the other groups. Histological parameters showed improvement in both single and combination treatments, based on the McMinn scoring system, compared to the control. The combination treatment generally yielded better histological parameters than the single treatment, with reduced edema, leukocytes, and macrophages, along with increased granulation density, fibroblasts, and collagen.

Table 2. Histological parameters

Group	Sample	Epidermis	Dermis	Edema	Leukocytes	Macrofag	Granules	Fibroblast	Collagen	Epithelial
Control	1	23.9	366.3	+++	+++	+++	+	++	+	+
	2	36.3	324.4	+++	+++	+++	++	+	++	+
	3	28.4	225.5	+++	+++	+++	+	++	+	++
	4	30.4	228.8	+++	+++	+++	+	+	+	+
	5	21.5	339.2	+++	+++	+++	+	++	+	+
VCO	1	75.5	1336.1	++	++	++	++	+++	+++	+++
	2	86.5	847.8	++	+++	++	++	+++	+++	+++
	3	125.5	242.1	++	+++	++	++	+++	+++	+++
	4	64.5	1331.7	+	++	+++	+++	++	+++	++
	5	83.5	1147.5	+	++	+++	++	++	++	++
Nigella Sativa Oil (NSO)	1	137.7	1217.8	+++	++	+++	+++	++	++	+++
	2	46.5	220.7	+++	+++	+++	++	++	++	++
	3	87.1	932.7	++	+++	+++	++	++	++	++
	4	122.7	1231.4	++	+++	+++	+++	++	++	+++
	5	54.3	1247.3	+++	+	++	++	++	+++	++
VCO-NSO 1:1	1	131.5	1975.9	+	+	+	+++	++	+++	+++
	2	144.4	1645.8	++	++	++	++	++	+++	+++
	3	139.5	1026.4	++	++	++	++	++	+++	+++
	4	125.6	1975.9	+	+	++	++	+	+++	+++
	5	142.9	1275.3	+	+	+	+++	+	+++	+++
VCO-NSO 2:1	1	78.6	1358.3	+	+	+	+++	++	+++	+++
	2	64.4	1158.5	+	+	+	++	++	+++	+++
	3	88.8	1348.3	+	+	+	++	+	+++	+++
	4	139.2	1054.4	++	+	++	++	+	+++	+++
	5	125.6	1648.5	++	++	++	+++	+	+++	+++
VCO-NSO 1:2	1	131.3	1754.1	+	+	+	+++	++	+++	+++
	2	89.2	1258.3	++	++	+	+++	+	+++	+++
	3	76.6	1428.3	+	+	+	++	+	+++	+++
	4	121.3	1034.4	++	++	+	+++	+	+++	+++
	5	125.3	1133.5	+	+	+	++	++	+++	+++

Description: + mild, ++ mild to moderate, +++ moderate, ++++ severe

3. DISCUSSION

Wound healing is a complex process comprising four phases: hemostasis, inflammation, proliferation, and remodeling [14]. Successful healing requires the precise coordination of various cell types, cytokines, and extracellular matrix molecules [4]. In diabetes mellitus (DM), this process is subject to various disruptions, including aberrations in fibroblast and keratinocyte cell function, vascular damage, and diminished leukocyte cell activity [15,16]. Numerous natural ingredients are found to be widely used in wound care, including VCO and NSO [17]. Both VCO and NSO offer a range of beneficial properties such as anti-inflammatory, antioxidant, and antibacterial effects. They also enhance interferon production, promote angiogenesis, and act as vasodilators [18]. These diverse effects are particularly valuable in wound repair, especially for diabetic ulcers [19]. As shown in Figure 1, the macroscopic assessment of wound diameter on day 7 did not reveal a significant difference between the control and treatment groups. The data presented in Table 1 confirm a statistically significant difference in wound diameter between the treated DM ulcer group and the untreated DM ulcer group (p-value <0.05). The wound diameter in the groups receiving VCO and NSO, whether individually or in combination, was smaller than that in the untreated DM ulcer group. Notably, the group treated with the VCO-NSO combination (1:2) demonstrated the smallest wound size among all the groups.

These findings align with the research of [30], which suggests that VCO is effective in wound healing and angiogenesis. VCO significantly promotes wound healing in diabetic rats by increasing collagen synthesis and re-epithelialization [31]. This observation can be attributed to the wound healing process being predominantly in the inflammatory phase, with epithelialization just commencing. Histological analysis of the wound tissue on day 7 showed an intact epidermis with developed dermal papillae in the VCO and NSO groups compared to the damaged epidermis in the control group (Figure 2). On day 7, the VCO and NSO groups showed high collagen content (3+ and 3+, respectively) (Table 2). Meanwhile, the control group contained fewer collagen and fibroblast cells (2+ and 1+, respectively) and more magrophage cells and leukocytes (3+). This study shows that VCO can alter the expression of several genes related to the inflammatory response in wound healing. Magrophages play an important role in providing effective immune function as part of innate immunity. These cells play an important role in immunomodulation by secreting cytokines that indicate an acute inflammatory response [20,21]. Table 2 data further indicates that leukocyte and macrophage levels were notably higher in the control, VCO, and NSO groups, which may explain the similar wound sizes observed. However, the VCO-NSO combination group displayed a noticeable difference in the macroscopic evaluation. This distinction could be attributed to the synergistic enhancement of the anti-inflammatory properties of VCO and NSO when combined [22]. In this combination group, leukocyte and macrophage levels were lower compared to the control group and the VCO and NSO single-treatment groups. The administration of VCO was more effective in reducing edema than NSO. There was no significant difference in edema reduction between the single NSO group and the positive control group, with a p-value of 0.009. However, the difference was highly significant when comparing the edema reduction between the VCO-NSO combination group and the positive control group, with a p-value of 0.000. The combination of VCO and NSO demonstrated comparable effectiveness in reducing edema to that of VCO alone. In terms of anti-inflammatory capabilities, both VCO and NSO, when administered individually, equally reduce the number of leukocytes and macrophages in the wound tissue. This is evidenced by a notable decrease in these cells. The anti-inflammatory efficacy is further enhanced when the two are combined (Table 2). The study also underscores the anti-inflammatory effects of both substances, showing that whether administered individually or combined, both VCO and NSO effectively reduce the number of leukocytes and macrophages in wound tissue. This suggests that the administration of a combination of VCO and NSO has the potential for a synergistic effect in alleviating inflammatory responses at the cellular level. In wound tissue proliferation, VCO and NSO exhibit comparable abilities to enhance granulation, fibroblast, collagen, and epithelial cell growth compared to the positive control group, with a p-value of less than 0.05. The proliferation abilities between the VCO and NSO groups do not show a significant difference, with a p-value greater than 0.05. This implies that both substances have similar capabilities in promoting the proliferation of wound tissue, as evidenced by increased granulation, fibroblast, collagen, and epithelial cell formation (Table 2).

Therefore, using a combination of VCO and NSO can be considered an effective strategy to stimulate wound tissue proliferation, with potential implications for improving the speed and quality of wound healing. NSO also possesses anti-inflammatory properties, and when combined with VCO in all comparisons, it enhances the anti-inflammatory effect beyond that of individual administration. Decreased levels of leukocytes and macrophages further support this enhanced effect observed in all combination groups. VCO and NSO are rich in antioxidants, which can expedite wound healing [23,24]. This finding aligns with reported results where VCO showed improved wound healing in an in vivo study. The VCO group showed significantly smaller wound size and a higher percentage of wound healing than the control group from day 8 onwards [18].

Additionally, the time required for wound closure in the VCO group (16 days) was notably shorter than in the control group (20 days). Previous research has established the individual benefits of Virgin Coconut Oil (VCO) and Nigella Sativa Oil (NSO) in facilitating wound repair by accelerating the epithelialization (proliferation) phase [25,26]. Additionally, the abundant antioxidant content in NSO can safeguard wounds against damage to the extracellular matrix, particularly under the high oxidative stress associated with DM ulcers [27]. This highlights the potent anti-inflammatory effect of VCO, as one hallmark of inflammation is the presence of tissue edema. Figure 2 (c, i, o) demonstrates complete epithelialization due to a single NSO administration.

Interestingly, the thickness of epidermal and dermal tissues is notably higher in untreated DM ulcers. This trend is similarly observed in the group subjected to single VCO administration (b, h, n). This suggests that VCO and NSO, whether administered individually or in various combinations, possess comparable abilities to promote epithelialization in epidermal and dermal cells. However, the VCO-NSO combination group (1:1) significantly differed from the other treatment groups, demonstrating the highest average

thickness of both the epidermis and dermis (Figures 4 and 5). In essence, the VCO-NSO (1:1) combination yielded superior results in enhancing the epithelialization of epidermal and dermal tissues. Notably, the epidermis appeared thicker, and the granulation tissue appeared denser in the VCO-NSO combination group, providing increased strength to the newly formed tissue, as evident in Figure 2. These findings align with the research of [28], which indicated that ozonated Nigella sativa oil accelerated wound healing by reducing the duration of epithelialization.

Histopathological scoring data further revealed that the control group exhibited high inflammation at the wound site, coupled with increased vascular permeability [29]. While new fibroblast proliferation occurred, there was no development of epithelialization in this group. In contrast, the NSO group displayed lower inflammation than the control group. Moreover, fibroblast activity and epithelialization were observed in the NSO group, suggesting its positive influence on wound healing. The study also revealed that higher levels of fibroblast cells were present in the DM ulcer group when VCO and NSO were administered individually. However, the number of fibroblast cells decreased when VCO and NSO were combined.

Interestingly, when evaluating collagen levels, it was observed that the VCO-NSO combination treatment group exhibited an increase in collagen content compared to the untreated group. This finding is noteworthy as it suggests that the VCO-NSO combination can augment collagen production, a crucial factor in wound healing, without an increase in fibroblast cells. Combining VCO and NSO may synergize collagen synthesis, significantly enhancing wound healing [30]. Collagen is a vital protein that contributes significantly to the extracellular matrix formation [25,31]. The reduced collagen levels observed in the untreated DM ulcer group are associated with prolonged wound healing, as evident in Figure 1. The untreated group exhibited larger wound sizes than the treated group.

This highlights the potent anti-inflammatory effect of VCO, as one hallmark of inflammation is the presence of tissue edema. NSO also possesses anti-inflammatory properties, and when combined with VCO in all comparisons, it enhances the anti-inflammatory effect beyond that of individual administration. Decreased levels of leukocytes and macrophages further support this enhanced effect observed in all combination groups. VCO and NSO are rich in antioxidants, which can expedite wound healing [23,24].

Additionally, VCO can reduce the number of circulating leukocytes, mononuclear cells, and polynuclear cells, which play a role in immunomodulation by releasing proinflammatory cytokines. These cytokines are responsible for inflammatory responses, including edema, fever, and tissue damage [32]. In line with this, the study provides insights into the anti-inflammatory effect of VCO on skin inflammation, demonstrating its ability to suppress pro-inflammatory cytokine stimulation in Human Monocytic Leukemia (THP-1) cells. This suppression was observed at both the protein and gene expression levels [25]. NSO inhibits leukotriene synthesis and histamine release and acts as an antioxidant. In addition, it provides crucial immunomodulatory effects by enhancing T-cell responses [33,34].

As shown in Figure 4, there is a significant difference in the epidermal thickness between the VCO-NSO 1:1 combination group and the control group, with a p-value less than 0.0001. This result indicates that administering the VCO-NSO 1:1 combination has a notable impact on the epidermal thickness in the wound tissue compared to the control group. Furthermore, compared to the other treatment groups, the findings suggest that administering the VCO-NSO 1:1 combination yields the most significant improvement in epidermal thickness in the wound tissue. This discovery indicates that the VCO-NSO 1:1 proportion significantly benefits the epidermal regeneration process more than the other formulations tested. The statistical interpretation, noting a very low p-value (less than 0.0001), signifies that the difference in epidermal thickness between the VCO-NSO 1:1 combination group and the control group is statistically significant and reliable, and not merely coincidental. The outermost layer of the skin, known as the epidermis, assumes a crucial role in wound healing. Although it is not directly engaged in forming scar tissue or facilitating the cohesion of skin layers, the epidermis performs various functions that support and impact the healing of wounds [35].

While not directly participating in scar tissue formation, the proliferation of epidermal cells contributes to restoring the skin's structural integrity. These cells multiply to cover the wound site and establish a new protective layer. The process of keratin formation, occurring in the outermost layer of the epidermis, known as the stratum corneum, provides additional mechanical strength and resilience to the healing skin [36]. Keratin, being a protein, adds rigidity and resistance to the epidermal layer. While the epidermis primarily functions to protect the skin against potential infections and assist in structural restoration during the healing process, the critical roles in scar tissue formation and the cohesion of skin layers are performed by the underlying layer, the dermis. Collaborative efforts between the epidermis and dermis are indispensable for

ensuring effective wound healing and overall skin recuperation. The combination of VCO-NSO 1:1 is also highly effective in promoting dermal thickness.

Figure 5 shows that the VCO-NSO 1:1 combination significantly differs from the control group, with a p-value of less than 0.0001. While using VCO and NSO individually also yields positive outcomes in dermal thickness, the combined 1:1 formulation demonstrates superior results with a p-value of less than 0.01. This observation suggests that the synergistic effect of combining VCO-NSO 1:1 enhances their efficacy in promoting dermal thickness. The significant difference, as indicated by the very low p-value, emphasizes the substantial impact of the VCO-NSO 1:1 combination on dermal thickness compared to both the control group and the single applications of VCO and NSO. The dermis, located beneath the epidermis and above the hypodermis, plays a crucial role in wound healing. This layer contributes significantly to the healing process by housing various structures and components. The dermis plays multiple roles in wound healing through its structural and functional aspects. It contains collagen and elastin fibers that provide strength, elasticity, and structural support to the skin [30,37]. During wound healing, collagen is re-organized to form solid and elastic scar tissue. The dermis also houses immune cells such as macrophages, which play a role in clearing the wound area of bacteria, dead cells, and other debris [38]. The inflammatory process in the early stages of wound healing involves the immune system's response to protect the body from infection.

In summary, the findings underscore the remarkable effectiveness of the VCO-NSO 1:1 combination in enhancing dermal thickness, surpassing the outcomes achieved by a single application of VCO or NSO. This highlights the potential benefits of combining these substances in wound care interventions, suggesting a promising avenue for further research and clinical application in optimizing dermal healing processes. The study results strongly support the effectiveness of the VCO-NSO 1:1 combination in enhancing epidermal thickness in wound tissue, indicating significant implications for developing more efficient wound care strategies. This study suggests the need for further research to refine the combination based on the concentration of the active ingredients that play the most significant role in wound healing. The objective is to identify the optimal formulation to treat DM ulcers effectively.

4. CONCLUSION

VCO and NSO have demonstrated their potential to enhance wound healing in DM ulcers by promoting wound tissue epithelialization and mitigating the inflammatory process. Moreover, combining VCO and NSO yields superior results compared to single administrations.

5. MATERIALS AND METHODS

5.1 Materials and Tools

In this study, various tools and materials were employed. The tools included glassware, Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC-MS), microtome, water bath, incubator, microscope, glass objects, centrifuge, vortex, sterile microtubes, glucometer, biopsy punch, animal cages, and synthesis kit from Thermo Fisher Scientific. The materials used consisted of distilled water, methanol, ethanol, n-Hexane, isoflurane, streptozotocin, formalin, xylol I, II, and III solutions, paraffin, Hematoxylin and Eosin staining, Trizol reagent, chloroform, and mouse skin tissue. Additionally, VCO and NSO were obtained from herbal shops, with VCO sourced from the Golden Khallazz brand and NSO from the Habba Syifa brand. Samples were stored at room temperature throughout the research.

5.2 Test Animals

This study used Wistar male white rats as experimental subjects, with an age range of 3 months and body weight between 200-250 grams. The Wistars were housed in stainless steel cages under controlled environmental conditions, with the room temperature maintained between 25-28°C. The Wistar cages were equipped with wood shavings as bedding, which were replaced weekly to maintain cleanliness. 5 Wistars occupied each cage, and there was no mixing of Wistars from different experimental groups to prevent cross-contamination and ensure the validity of the study results. Lighting was provided for 12 hours a day, from 09.00-21.00, and the Wistars were provided with adequate food (BioFeed pellets produced by Karunia Kasih Abadi, Klaten, Indonesia) and clean drinking water.

Routine checks were conducted daily to monitor the clinical and behavioral status of the Wistars, ensuring no clinical or behavioral changes occurred during the study. No Wistar showed signs of death, decreased appetite/growth, or behavioral changes during the study. The Wistars were grouped into 6 groups, each consisting of 5 Wistars, and the duration of treatment was 7 days. The groups were as follows: 1) Wistar

DM+ulcer; 2) Wistar DM+ulcer+VCO; 3) Wistar DM+ulcer+NSO; 4) Wistar DM+ulcer+VCO+NSO (1:1 ratio); 5) Wistar DM+ulcer+VCO+NSO (2:1 ratio); and 6) Wistar DM+ulcer+VCO+NSO (1:2 ratio).

5.3 Diabetes Induction

To induce type 1 diabetes in the rats, we administered a single injection of 50 mg/kg body weight of streptozotocin, dissolved in 50 mM citrate buffer with a pH of 4.5, resulting in a final concentration of 40 mg/ml. We initially assessed baseline blood glucose levels before the streptozotocin injection and repeated the measurements 7 days after the injection to confirm the successful induction of diabetes. Blood glucose levels were determined using phenol 4-aminoantipyrine glucose oxidase (GOD-PAP), obtained from Diagnostic Systems International. The diagnosis of type 1 diabetes was established if the blood glucose levels exceeded 200 mg/dl (11.1 mmol/liter) 7 days after the streptozotocin injection. The average blood glucose level in diabetic rats, measured before the infliction of wounds, was 245 mg/dl.

5.4 Wounding

The diabetic rats were subjected to anesthesia by administering 0.2 mL of 2% lidocaine dissolved in 1 mL of 0.9% NaCl via subcutaneous injection. Following anesthesia, the dorsal area where the hair had been shaved was prepared by applying 70% alcohol. Subsequently, a 10 mm diameter and 0.3 mm depth injury, reaching the dermal layer, was inflicted using a biopsy punch.

5.5 Data Collection

Histology preparations were initiated by fixing the skin tissue samples in a 10% formalin solution for 18-24 hours. After this fixation process, the tissue samples were immersed in distilled water for 1 hour to remove the fixation solution. The subsequent step involved dehydration, achieved through a sequence of graded ethanol solutions with concentrations of 70%, 80%, and 90%. Following dehydration, the clearing process was undertaken. The skin samples were immersed in xylol I, II, and III solutions for 20 minutes each, allowing them to become transparent. Subsequently, the skin samples were placed in liquid paraffin I, II, and III within a paraffin incubator set at a temperature of 58-60°C. This was followed by embedding; the paraffin blocks, once solidified, were thinly sliced to a thickness of 5 micrometers using a microtome. These sections of NSO were then immersed in a water bath set at 40°C. The prepared sections were carefully lifted and positioned on glass slides, subsequently dried overnight in an incubator maintained at a temperature of 60°C. To facilitate visualization, Hematoxylin-Eosin staining was carried out. The HE-stained slides were examined using a bright-field microscope at 40x magnification to perform a semi-quantitative assessment of angiogenesis, ulceration, re-epithelization, inflammatory infiltration, hemorrhage, and congestion. These factors were graded on a scale of 0 (not present), 1 (mild), 2 (mild to moderate), 3 (moderate) and 4 (severe). The analysis of leukocyte infiltration, granulation tissue formation, fibroblast presence, collagen deposition, and epithelialization was conducted by staining with Gomori trichrome and assessed under a bright-field microscope, applying the same scoring criteria as used for the HE-stained slides [39,40]. Microscopic preparations were examined using a CX 33 light microscope. Photomicrographs were captured using a 3.1MP Sony Exmor camera equipped with a CMOS sensor and the Betaview program. Quantitative measurements of skin tissue included epidermal and dermal thickness. Additionally, semi-quantitative histologic parameters such as edema, leukocyte infiltration, granulation tissue formation, fibroblast presence, collagen deposition, and epithelialization were assessed based on the McMinn criteria.

5.6 Statistical Analysis

Statistical analysis was conducted using SPSS 21.0, where mean ± SD values underwent one-way analysis of variance (ANOVA). Post hoc comparisons between groups were performed using Dunnett's analysis.

Human and animal rights: This research has obtained ethical approval through an ethical assessment conducted by the Ethical Commission of the Faculty of Pharmacy, Andalas University, with reference number 42/UN.16.10.D.KEPK-FF/2023.

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REFERENCES

[1] Pouget C, Dunyach-Remy C, Pantel A, Schuldiner S, Sotto A, Lavigne JP. Biofilms in diabetic foot ulcers: Significance and clinical relevance. Microorganism. 2020;8(10):1580. https://dx.doi.org/10.3390/microorganisms8101580 .

[2] Jalilian M, Sarbarzeh PA, Oubari S. Factors related to severity of diabetic foot ulcer: a systematic review. Diabetes Metab Syndr Obes Targets Ther. 2020;13:1835. https://dx.doi.org/10.2147/DMSO.S256243.

[3] Monteiro-Soares M, Boyko EJ, Jeffcoate W, Mills JL, Russell D, Morbach S, Game F. Diabetic foot ulcer classifications: a critical review. 2020 ;36 Suppl 1:e3272. https://dx.doi.org/10.1002/dmrr.3272.

[4] Forsythe RO, Hinchliffe RJ. Assessment of foot perfusion in patients with a diabetic foot ulcer. Diabetes Metab Res Rev. 2016;32:232–238. https://dx.doi.org/10.1002/dmrr.2756.

[5] Kuhlmann M, Wigger-Alberti W, Mackensen YV, Ebbinghaus M, Williams R, Krause-Kyora F, Wolber R. Wound healing characteristics of a novel wound healing ointment in an abrasive wound model: A randomised, intra-individual clinical investigation. Wound Med. 2019;24(1):24–32. https://dx.doi.org/10.1016/j.wndm.2019.02.002.

[6] Nur SA, Anggraini SS, Dafriani P, Morika HD. Reduction of Staphylococcus aureus bacteria collaboration in diabetic ulcers by giving virgin coconut oil. Int J Community Med Public Heal. 2020;7(12):4733. https://dx.doi.org/10.18203/2394-6040.ijcmph20205142.

[7] Hekmatpou D, Mehrabi F, Rahzani K, Aminiyan A. The effect of aloe vera clinical trials on prevention and healing of skin wound: A systematic review. Iran J Med Sci. 2019;44(1):1. https://dx.doi.org/10.30476/ijms.2019.40612.

[8] Rafsanjani MS, Naeini AT, Meimandi-parizi A, Nowzari F, Wani MM, Iraji A. Wound healing effect of Carum carvi L. on the incised skin wound in male rats: Histopathology, total protein and biomechanical evaluations. Vet Med Sci. 2022;8:2726–2737. https://dx.doi.org/10.1002/vms3.961.

[9] Marsius SH, Jansen S. The activity of combination of hydrolyzed Virgin Coconut oil and chitosan toward wound healing parameters on NIH 3T3 cells using ın vitro methods. Asian J Pharm Res Dev. 2019;7(3):14–19. https://dx.doi.org/10.22270/ajprd.v7i3.534.

[10] Dafriani P, Nur SA, Morika HD, Marlinda R. Virgin Coconut Oil (VCO) Accelerated Wound Healing Process in Diabetes mellitus (DM) Patients With Diabetic Ulcer in dr. Rasidin Hospital, Padang, Indonesia. J Aisyah J Ilmu Kesehat. 2020;5(2):221–224. https://dx.doi.org/10.30604/jika.v5i2.375.

[11] Nagase S, Matsue M, Mori Y, Honda-Ogawa M, Sugitani K, Sumitomo N, Okamoto S. Comparison of the antimicrobial spectrum and mechanisms of organic virgin coconut oil and lauric acid against bacteria. J Wellness Heal Care. 2017;41(1):87–95. https://dx.doi.org/10.1177/0963689719881366.

[12] Suryani S, Sariani S, Earnestly F, Marganof M. A comparative study of Virgin Coconut Oil, Coconut oil and palm oil in terms of their active ıngredients. Processes. 2020;8:1–11. https://dx.doi.org/10.3390/pr8040402.

[13] Han MC, Durmuş AS, Sağliyan A, Günay C, Özkaraca M, Kandemir FM, Çomakli S, Firat Öztopalan D. Effects of Nigella sativa and Hypericum perforatum on wound healing. Turkish J Vet Anim Sci. 2017;41(1):99–105. https://dx.doi.org/10.3906/vet-1603-26.

[14] Chang M, Nguyen TT. Strategy for treatment of infected diabetic foot ulcers. Acc Chem Res. 2021;54(5):1080–1093. https://dx.doi.org/10.1021/acs.accounts.0c00864.

[15] Vitse J, Bekara F, Byun S, Herlin C, Teot L. A double-blind, placebo-controlled randomized evaluation of the effect of low-level laser therapy on venous leg ulcers. Int J Low Extrem Wounds. 2017;16(1):29–35. https://dx.doi.org/10.1177/1534734617690948.

[16] Hu B, Gao M, Boakye-Yiadom KO, Ho W, Yu W, Xu X, Zhang XQ. An intrinsically bioactive hydrogel with on-demand drug release behaviors for diabetic wound healing. Bioact Mater. 2021;6(12):4592–4606. https://dx.doi.org/10.1016/j.bioactmat.2021.04.040.

[17] Kmail A, Said O, Saad B. How Thymoquinone from Nigella sativa Accelerates Wound Healing through Multiple Mechanisms and Targets. Curr Issues Mol Biol. 2023;45(11):9039–9059. https://dx.doi.org/10.3390/cimb45110567.

[18] Varma SR, Sivaprakasam TO, Arumugam I, Dilip N, Raghuraman M, Pavan KB, Paramesh R. In vitro anti-inflammatory and skin protective properties of Virgin coconut oil. J Tradit Complement Med. 2019;9(1):5–14. https://dx.doi.org/10.1016/j.jtcme.2017.06.012.

[19] Widianingrum DC, Noviandi CT, Salasia SIO. Antibacterial and immunomodulator activities of virgin coconut oil (VCO) against Staphylococcus aureus. Heliyon. 2019;5(10):e02612 https://dx.doi.org/10.1016/j.heliyon.2019.e02612.

[20] Varma SR, Sivaprakasam TO, Arumugam I, Dilip N, Raghuraman M, Pavan KB, Ra M, Paramesh R. In vitro anti-in fl ammatory and skin protective properties of Virgin coconut oil. J Tradit Complement Med. 2019;9(1):5–14. https://dx.doi.org/https://doi.org/10.1016/j.jtcme.2017.06.012.

[21] Mohammadi A, Blesso CN, Barreto GE, Banach M, Majeed M, Sahebkar A. Macrophage plasticity, polarization and function in response to curcumin, a diet-derived polyphenol, as an immunomodulatory agent. J Nutr Biochem. 2019;66:1–16. https://dx.doi.org/10.1016/j.jnutbio.2018.12.005.
[22] Randhawa MA, Alenazy AK. An active principle of Nigella sativa L., thymoquinone, showing significant antimicrobial activity against anaerobic bacteria. J Intercult Ethnopharmacol. 2016;6(1):97-101. https://dx.doi.org/10.5455/jice.20161018021238.
[23] Syarif F, Susilaningsih N, Bakhtiar Y. The Effectivity of Hydrolysed VCO for 2nd Degree Burn Injury on Wistar Rats: Based on VEGF expression and collagen thickness. J Clin Med. 2021;8(3):273–280. https://dx.doi.org/10.36408/mhjcm.v8i3.561.
[24] Rohman A, Irnawati, Erwanto Y, Lukitaningsih E, Rafi M, Fadzilah NA, Zakaria Z. Virgin coconut oil: extraction, physicochemical properties, biological activities and its authentication analysis. Food Rev Int. 2021;37(1):46–66. https://dx.doi.org/10.1080/87559129.2019.1687515.
[25] Yuniati R, Subchan P, Riawan W, Khrisna MB, Restiwijaya M, Dyan Kusumaningrum NS, Nur M. Topical ozonated virgin coconut oil improves wound healing and increases HSP90α, VEGF-A, EGF, bFGF, and CD34 in diabetic ulcer mouse model of wound healing. F1000Research. 2021;9:580. https://dx.doi.org/10.12688/f1000research.22525.3.
[26] Sallehuddin N, Nordin A, Bt Hj Idrus R, Fauzi MB. Nigella sativa and its active compound, thymoquinone, accelerate wound healing in an in vivo animal model: a comprehensive review. Int J Environ Res Public Health. 2020;17(11):4160. https://dx.doi.org/10.3390/ijerph17114160.
[27] Manju S, Malaikozhundan B, Vijayakumar S. Antibacterial, antibiofilm and cytotoxic effects of Nigella sativa essential oil coated gold nanoparticles. Microb Pathog. 2016;91:129–135. https://dx.doi.org/10.1016/j.micpath.2015.11.021.
[28] Canpolat I, Eröksüz Y, RT. Effects on the wound healing process using Ozonated oils (sesame, Nigella sativa, Hypericum perforatum) in rats. Turk J Vet Res. 2021;5(1):25–33. https://dx.doi.org/10.1016/j.jtumed.2018.02.008.
[29] Elgohary HM, Al Jaouni SK, Selim SA. Effect of ultrasound-enhanced Nigella sativa seeds oil on wound healing: An animal model. J Taibah Univ Med Sci. 2018;13(5):438–443. https://dx.doi.org/10.1016/j.jtumed.2018.02.008.
[30] Mutlu HS, Erdoğan A, Tapul L. Autologously transplanted dermal fibroblasts improved diabetic wound in rat model. Acta Histochem. 2020;122(5):151552. https://dx.doi.org/10.1016/j.acthis.2020.151552.
[31] Rasouli M, Rahimi A, Soleimani M. The interplay between extracellular matrix and progenitor/stem cells during wound healing: Opportunities and future directions. Acta Histochem. 2021;123(7):151785. https://dx.doi.org/10.1016/j.acthis.2021.151785.
[32] Liu C, Lu Y, Du P, Yang F, Guo P, Tang X, Lu G. Mesenchymal stem cells pretreated with proinflammatory cytokines accelerate skin wound healing by promoting macrophages migration and M2 polarization. Regen Ther. 2022;21:192–200. https://dx.doi.org/10.1016/j.reth.2022.06.009.
[33] Majdalawieh AF, Fayyad MW. Immunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: A comprehensive review. Int Immunopharmacol. 2015;28(1):295–304. https://dx.doi.org/10.1016/j.intimp.2015.06.023.
[34] Shaterzadeh-yazdi H, Noorbakhsh M foad, Hayati F, Samarghandian S, Farkhondeh T. Immunomodulatory and anti-inflammatory effects of thymoquinone. Cardiovasc Haematol Disord - Drug Targets. 2018;18(1):52–60. https://dx.doi.org/10.2174/1871529X18666180212114816.
[35] Li Y, Zhang J, Yue J, Gou X, Wu X. Epidermal stem cells in skin wound healing. Adv Wound Care. 2017;6(9):297–307. https://dx.doi.org/10.1155/2020/9148310.
[36] Yang R, Liu F, Wang J, Chen X, Xie J, Xiong K. Epidermal stem cells in wound healing and their clinical applications. Stem Cell Res Ther. 2019;10(1):1–14. https://dx.doi.org/10.1186/s13287-019-1312-z.
[37] Lim CH, Sun Q, Ratti K, Lee SH, Zheng Y, Takeo M, Ito M. Hedgehog stimulates hair follicle neogenesis by creating inductive dermis during murine skin wound healing. Nat Commun. 2018;9(1):4903. https://dx.doi.org/10.1038/s41467-018-07142-9.
[38] Rippa AL, Kalabusheva EP, Vorotelyak EA. Regeneration of dermis: Scarring and cells involved. Cells. 2019;8(6):607. https://dx.doi.org/10.3390/cells8060607.
[39] Dias FGG, de Freitas Pereira L, Furtado RA, Magalhães GM, Miguel MP, Dias LGG, Tavares DC. Topical formulations containing Copaifera duckei Dwyer oleoresin improve cutaneous wound healing. Avicenna J Phytomedicine. 2021;11(2):120. https://dx.doi.org/10.22038/ajp.2020.
[40] Satria D, Nazliniwaty OAH, Rahma L, Rahmah A, Nasution FAR, Nur M. The effects of the combination of ethanol binahong leaf (Anredera cordifolia) and mobe leaf (Artocarpus lakoocha) extract gel on fibroblast and osteocyte proliferation in wound healing post tooth extraction socket on wistar rats (Rattus norvegicus). Farmacia. 2022;70(5):925–31. https://dx.doi.org/10.31925/farmacia.2022.5.19.