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Effects of Lavender Oil on Wound Healing in an Experimental Diabetes Model in Rats: A Randomized Controlled Trial

Deneysel Diyabet Modeli Oluşturulmuş Ratlarda Lavanta Yağının Yara İyileşmesine Etkisi: Randomize Kontrollü Bir Çalışma

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ABSTRACT

Objective: Lavender oil has antimicrobial, anti-inflammatory, analgesic properties as well as beneficial activities on wound healing. This study aims to determine the effect of lavender oil on wound healing in an experimental diabetes model in rats.

Methods: This randomized controlled experiment included three diabetic and three non-diabetic groups of 42 male Wistar albino rats. A 12-mm-diameter, full-thickness wound was created on the backs of the rats. Lavender oil, Madecassol, and 0.9% sodium chloride [normal saline (NS)] were applied as wound dressings. During macroscopic evaluation of wound healing, wound-healing percentage was calculated using the Walker formula, and wound area was determined using the ImageJ image analysis program. For microscopic evaluation, the tissue samples were taken from the rats on days 1, 7, and 14. Hematoxylin-Eosin staining findings and the distributions of vascular endothelial growth factor-A (VEGFA), collagen-I, and collagen-III were determined.

Results: In all groups, the highest wound-healing percentage and the lowest wound-area measurements were observed in those treated with lavender oil. Lavender oil increased inflammatory cell infiltration and angiogenesis, and accelerated granulation tissue formation and re-epithelialization. The VEGFA and collagen-III levels on day 7, and the collagen-I levels on day 14, were highest in those treated with lavender oil. Although rats treated with lavender oil differed significantly from those treated with NS in wound healing, there was no difference between rats treated with Madecassol and those treated with lavender oil.

ÖZ

Amaç: Lavanta yağı, antimikrobiyal, antiinflamatuar, analjezik özelliklerinin yanı sıra yara iyileşmesi üzerinde de yararlı etkilere sahiptir. Bu çalışmanın amacı deneysel diyabet modeli oluşturulmuş ratlarda lavanta yağının yara iyileşmesi üzerine etkisini belirlemektir.

Yöntemler: Bu randomize kontrollü deneysel çalışmada, 42 erkek Wistar albino sıçandan oluşan üç diyabetik ve üç diyabetik olmayan grup yer almaktadır Sıçanların sırtlarına 12 mm çapında, tam kalınlıkta yara oluşturuldu. Lavanta yağı, Madecassol ve %0,9 sodyum klorür [serum fizyolojik (SF)] yaralara uygulandı. Yara iyileşmesinin makroskobik değerlendirilmesinde; Walker formülüyle yara iyileşme yüzdesi ve ImageJ görüntü analizi programıyla yara alanları hesaplandı. Mikroskobik değerlendirmede; 1., 7., 14. günlerde sıçanlardan doku örnekleri alındı. Hematoksilen-Eozin boyama bulguları ve vascular endothelial growth factor-A (VEGFA), kollajen-I, kollajen-III dağılımları belirlendi.

Bulgular: Tüm gruplarda en yüksek yara iyileşme yüzdesi ve Image J ölçümlerinde en düşük yara alanının, lavanta yağıyla pansumanı yapılan gruplarda olduğu saptandı. Lavanta yağının; inflamatuar hücre infiltrasyonunu ve anjiyogenezisi arttırdığı, granülasyon doku oluşumunu ve reepitelizasyonu hızlandırdığı belirlendi. 7. günde VEGFA, kollajen-III, 14.günde ise kollajen-I düzeyinin en yüksek lavanta yağı uygulanan gruplarda olduğu saptandı. Yara iyileşmesi açısından lavanta yağı-SF grupları arasında anlamlı farklılık saptanırken, madecassol-lavanta arasında fark olmadığı belirlenmiştir.

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ABSTRACT

Conclusions: On both macroscopic and microscopic examination, dressing with lavender oil was effective in promoting wound healing in all groups.

Keywords: Diabetic foot, lavender oil, rats, wound care, nursing

ÖZ

Sonuç: Lavanta yağıyla pansumanın tüm gruplarda makroskopik ve mikroskobik olarak yara iyileşmesinde etkili olduğu belirlendi.

Anahtar Sözcükler: Diyabetik ayak, lavanta yağı, sıçanlar, yara bakımı, hemşirelik

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by major abnormalities in carbohydrate, fat, and protein metabolism, resulting from the hormone insulin (1,2). DM is a progressive disease, and uncontrolled DM can lead to acute and chronic complications (3). One of these complications is the diabetic foot, which can develop in association with neuropathy, peripheral arterial disease, infection, and immune system disorders (4,5). Although the negative effects of diabetes on wound healing cannot be fully explained, hyperglycemia is thought to affect this process due to factors such as impaired collagen synthesis; decreased fibroblast proliferation and reduced growth factor production; increased apoptosis of cells within scar tissue; impaired angiogenesis; defective granulation tissue formation; and increased risk of infection due to decreased chemotaxis and phagocytosis (3,6,7). To prevent pathogen proliferation and to enable debridement of necrotic tissue, treatments currently used for wound healing include application of antimicrobial agents. Although applied simultaneously, the effects of both treatments are not permanent (6). Topical application of compounds containing antioxidants will be beneficial, especially for wound healing and protection of tissues against oxidative damage. Furthermore, certain plant species can be used to treat both acute and chronic wounds (8). Lavender is one of these medicinal plants, and its oil is known to have antimicrobial, anti-inflammatory, and analgesic properties, as well as beneficial biological activities in wound healing. Studies have concluded that the chemical components in lavender oil, such as α-borneol, α-terpinene, terpinen-4-ol, α-terpineol, linalyl acetate, and linalool, are effective in wound healing (9–11). A review of the evidence on the effects of lavender oil on wound healing found that wounds treated with lavender oil exhibited an increased healing rate, elevated collagen expression, and enhanced activity of proteins involved in tissue remodeling (12). Additionally, lavender oil has the potential to accelerate the formation of granulation tissue, remodel the tissue through collagen replacement, and support wound healing by promoting early wound contractions mediated by transforming growth factor beta (TGF-β) (13). This study aimed to assess the effect of lavender oil on the healing of diabetic wounds. Teamwork is essential to the healing of diabetic wounds. Among the team members, nurses play a central role as the primary providers of care (14). Currently, the discipline of nursing is producing new high-quality evidence on care through rigorous research. In this context, the use of experimental animals in nursing constitutes research that strengthens evidence-based nursing knowledge, advances the development of nursing practices, and fosters innovation in nursing (15). Because wound care is one of the primary responsibilities of nurses, it is considered a basic research topic. For this reason, it is essential to conduct various studies in nursing science, including animal (preclinical) and clinical research. This study aimed to evaluate the effects of lavender oil on wound healing in an experimental rat model of diabetes.

MATERIALS AND METHODS

Animal Experimentation and Study Groups

In this study, Wistar albino male rats aged 4–5 months, with an average weight of 250–350 g, were used. These rats were obtained from the Kahramanmaraş Sütçü İmam University, Faculty of Medicine, Experimental Research Laboratory. During the experiment, the rats were housed in rooms with a 12-h light/dark cycle, at a room temperature of 22°C±2°C and a humidity level of 45%–50%, and were provided with tap water and a standard portion of pellet food. With the alpha level (type I error) set at 0.05 and the power set at 0.80 in the statistical power analysis, the required sample size was 48. The effect size was interpreted using Cohen's criteria (17). The study consisted of three diabetic and three non-diabetic groups. The rats were randomly assigned to the groups using the Research Randomizer software (https://www.randomizer.org/) (16). Due to anesthesia complications, six rats died: some during wound dehiscence and others after tissue removal on the seventh day. The study included 42 rats.

Non-DM-normal saline (NS), (n = 7): The dressing was applied daily with 0.9% sodium chloride (NaCl) solution.

Non-DM-lavender oil (n = 7): The dressing was applied with 0.5 mL of lavender oil daily.

Non-DM-madecassol, (n = 7): For the positive control, the dressing was treated with 0.5 g of Madecassol ointment daily.

DM-NS, n = 7: The dressing was applied daily using 0.9% NaCl solution.

DM-lavender oil, (n = 7): The dressing was applied with 0.5ml lavender oil every day

DM-madecassol (n = 7): The dressing was applied using 0.5 g of Madecassol ointment daily.

Development of Experimental Diabetes Model and Incisional Wound Model

To develop the diabetes model, streptozotocin (Sigma-Aldrich, Germany) was administered intraperitoneally to rats at a dose of 60 mg/kg after 18 h of food deprivation. Rats with blood glucose levels > 250 mg/dL, measured from the tail vein 48 h after induction, were considered diabetic (18). The rats were weighed sequentially, and, after determining the appropriate doses from their weights, they were induced. Plasma glucose levels were measured from the tail vein 24 hours after induction, and rats with levels of 250 mg/dL or higher were considered diabetic. No action was taken in the non-DM groups. To develop the wound model, rats were anesthetized intraperitoneally with xylazine (10 mg/kg) and ketamine (50 mg/kg). The rats' dorsal hair was shaved without damaging the skin, which was then cleaned with a povidone-iodine solution.

Three full-thickness skin-defect wounds, each 12 mm in diameter and containing the panniculus carnosus muscle, were created by punch biopsy; the dorsal midline wounds were separated by at least 15 mm. To avoid impairing the evaluation of wound healing when tissue was taken on days 1, 7, and 14, three wounds were opened in each rat. The day the wounds were created was considered day 0, and wound care was provided for 14 days.

Wound Care Materials and Dressing Application

Lavender oil (Lavandula angustifolia L., natural), with a density of 0.879 g/mL at 25℃, was obtained from Sigma-Aldrich (Germany). This natural lavender oil, whose stability and quantification tests were performed by the company, was applied locally to the wounds. Prior to application of lavender oil to the wounds, potential irritant effects were evaluated, and no allergic or irritant reactions were detected. As a positive control and consistent with the literature, Madecassol pomade (Bayer, Germany) was used as a reference drug (19,20). Madecassol is an ointment derived from the extract of Centella asiatica, a medicinal plant that accelerates connective tissue and prevents scars formation (21). The NS solution provided by Eczacıbaşı/Baxter (Türkiye) was used as a negative control. The rats were dressed at the same time each day for 14 days. In the DM and non-DM groups, 0.5 mL lavender oil and 0.5 g Madecassol pomade were applied topically to the wounds with a sterile sponge, and the NS was applied dropwise to the wounds. The wounds were left open after the application. At the end of the study, the rats were euthanized by an overdose of anesthetic.

Data Collection Method

Research data were obtained from day 0 (wound creation) to day 14 (study completion). In this study, wound healing data were collected macroscopically and microscopically. For macroscopic examination, after the rats' wounds were identified, wound measurements were obtained using a digital caliper on days 3, 5, 7, 11, and the percentage of healing was calculated using the Walker formula given below.

Walker Formula

% wound area = (Wound area on day X/Wound area on day 0) × 100.

Wound area closure percentage on day X = 100%−% unclosed wound area.

Additionally, the wound was photographed on days 3, 5, 7, 11, and 14 using a digital camera, and the wound area was measured using the ImageJ image analysis program. ImageJ is a Java-based image-processing program available free of charge for public use at https://imagej.nih.gov/ij/download.html. The calculations and results of the program are internationally recognized and may be used in scientific studies (22–24).

For the microscopic examination, one-fourth of the tissue samples were taken from the wounds of the rats in all groups on days 1,7 and 14. Some tissue samples taken for light microscopic examination were stained with Hematoxylin–eosin (H&E), while other sections were stained for vascular endothelial growth factor A (VEGFA) (Abcam, England), collagen-I (Abcam, England), and collagen-III (Abcam, England) by the indirect immunoperoxidase method to determine their distribution.

H&E Staining

The tissues taken from the wound sites were subjected to routine tissue processing after 48 h of fixation in a 10% neutral-buffered formalin solution. The tissues were blocked, sectioned at 5 µm, and stained with H&E for examination by light microscopy. Parameters used to evaluate wound healing on histopathological examination were scored using the method of Galeano et al. (25,26).

Indirect Immunoperoxidase Staining

Tissue sections 5-µm thick taken from the wound areas were placed on polylysine-coated slides and incubated at 60 °C for 1 h for immunohistochemical staining. Then two changes of xylene clearing, 30 min each, were performed. They were further rehydrated through a graded alcohol series and then held in distilled water for 10 min. To inhibit endogenous tissue peroxidase, the sections incubated in Dakopen solution for 15 min at room temperature were treated with 3% H_2O_2 for 5 min. The sections were washed three times for 5 min each with phosphate-buffered saline and then treated with a blocking solution for 10 min. After the blocking solution was removed from the tissue, the sections were incubated overnight with primary antibodies against VEGFA, collagen-I, and collagen-III. The following day, the sections were washed three times with phosphate-buffered saline and then stained with secondary antibodies against VEGFA, collagen-I, and collagen-III for 30 min each. To detect the visibility of the immunohistochemical reaction, the sections that were washed three times with phosphate-buffered saline for 5 min each wash were also stained with 3-Amino-9-ethylcarbazolefor 5min. After the background staining was completed with Mayer's hematoxylin, the sections were washed with distilled water for 10 min and covered with a mounting medium. After indirect immunohistochemistry, the samples are evaluated by the histology technician and scored as following; no findings of immunoreactivities "0", partial/slight "1", complete but immature or mild "2", complete and mature/moderately rated"3", and severe "4" (25,26).

Ethical Dimensions of the Research

Consistent with the Declaration of Helsinki, developed by the World Medical Association and with the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees (dated: 07.06.2006, number: 26220), the researcher received the Certification of Experimental Animal Usage following training of the personnel involved in the use of animals for experimental research. Prior to conducting the research, approval was obtained from Kahramanmaraş Sütçü İmam University Experimental Animals Ethics Committee on June 6, 2018 (session number: 2018/08; decision number: 05). Informed consent was not applicable, as this study did not involve human participants and was conducted using experimental animals.

Statistical Analysis

The data obtained from the study were entered into SPSS version 22.0 (SPSS Inc, Chicago, IL, USA) for statistical analyses. Data were analyzed with parametric tests when assumptions of normality were met, and with non-parametric tests when those assumptions were not met. Student's t-test and the Mann-Whitney U test were used to examine differences between the two groups, depending on the results of the normality test. For comparisons among multiple groups,

the Kruskal–Wallis test was used when the data were not normally distributed; post hoc tests were used for pairwise comparisons. In the statistical analysis, the level of significance was set at $p < 0.05$.

RESULTS

Macroscopic Results

During macroscopic examination on days 3, 5, 7, 11, and 14, the healing percentage was calculated using the Walker formula, and the wound area was measured with the ImageJ image-analysis program. By day 14, among the groups, rats whose wounds were dressed with lavender oil had the highest wound-healing percentage. A significant difference between the NS and Lavender groups was observed in the diabetic group on days 5, 7, 11, and 14, and in the non-DM group on days 5, 11, and 14 ($p < 0.05$). Similar wound-healing percentages were observed in the control groups treated with Madecassol and Lavender. On all days, as measured using the ImageJ program, the lowest wound area was observed in the lavender oil group, and the highest average wound area was observed in the groups dressed with NS. Significant differences were observed between the DM and non-DM groups on day 7, between the NS and Lavender groups on days 11 and 14, and between the Lavender group and the other groups. ImageJ measurements showed significant differences between the NS and Lavender groups on day 7 within both the DM and non-DM groups, and between the Lavender group and the other groups on days 11 and 14 (Figure 1; $p < 0.05$). There were no complications or infections in the wounds (Figure 2).

Microscopic Results

Microscopic examination for the study was performed by the histology technician. The parameters for each tissue on H&E staining, including inflammatory cell infiltration, increase in granulation tissue, angiogenesis, and levels of re-epithelialization, were considered. On the first day, the highest level of inflammatory cell infiltration was measured in the DM and non-DM groups treated with lavender oil. Formation of granulation tissue, angiogenesis, and re-epithelialization were not observed on day 1 in any group(s). For Granulation tissue formation on day 14, the highest mean score was observed in the DM-Madecassol, DM-Lavender, and non-DM-Lavender groups. The highest mean angiogenesis score was observed in the groups treated with lavender oil on days 7 and 14. The highest mean level of re-epithelialization was observed in the groups dressed with lavender oil on day 7, and in the DM-Madecassol, DM-Lavender, and non-DM-Lavender groups on day 14 (Table 1, Figure 3). VEGFA, collagen-I, and collagen-III levels were evaluated in tissues stained with indirect immunoperoxidase. On days 7 and 14, the highest mean VEGFA level was observed in the groups dressed with lavender oil. When collagen-I levels were evaluated, the highest mean across all days was observed in the DM-Lavender and non-DM-Lavender groups. The highest mean collagen-III level was observed in the groups dressed with lavender oil on day 7. The lowest mean score was observed in the NS groups on day 14 (Table 2; Figures 4–6).

DISCUSSION

Previous studies evaluated the effects of lavender oil on wound healing, but the effects on diabetic wounds were not examined (3,10,12,13,27,28). Studies have shown that lavender oil stimulates wound contraction and skin regeneration by increasing activities of antioxidant enzymes; accelerates the development of granulation tissue, collagen replacement, and wound closure; and may support wound repair by promoting early wound contraction via TGF-β (10,12,13). Given these characteristics, lavender oil is thought to accelerate wound healing. In this study, rats treated with lavender oil had the highest wound-healing percentages and the lowest wound-area measurements at 14 days compared with other groups (Figure 1).

Reduction of inflammatory cells in diabetic individuals increases susceptibility to wound infection, and prolongation of the inflammatory phase further delays wound healing (3,6). Mori et al. (13) reported that topical application of lavender oil induced inflammation in wound lesions. In a study by Koca Kutlu et al. (27) on wound healing in rats, the authors found no signs of local infection in groups treated with lavender oil. However, to our knowledge, no study in the available literature has examined the effects of lavender oil on diabetic wounds. In this study, lavender oil showed the highest level of inflammatory cell infiltration on day 1 across all groups, whereas on day 7 the highest level was observed in groups dressed with Madecassol. Nevertheless, there was no significant difference between the groups on day 7. On day 14, the lowest mean scores were measured in the DM-Lavender and non-DM-Madecassol groups. These findings show that lavender oil produces results consistent with the positive control and with the literature, and that it affects inflammatory cell infiltration.

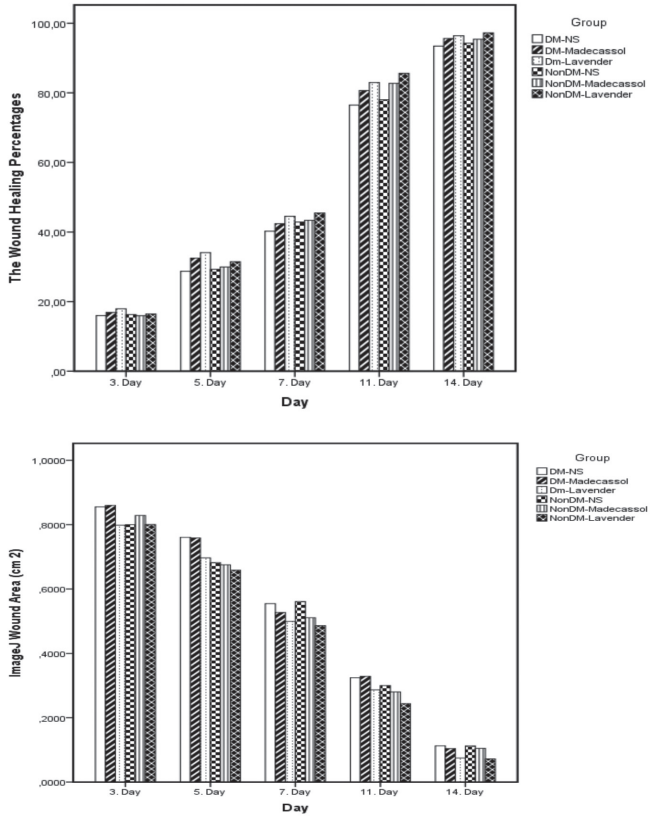

Figure 1. Walker formula, The Wound Healing Percentages and ImageJ Wound Area (cm^2)

Table 1. Hematoxylin-eosin staining results.

	Group	1 day	7 day	14 day
İnflammatory cell infiltration	DM-NS (n = 7)	0.86 ± 0.69	1.71 ± 0.76	1.86 ± 0.69d
	DM-Madecassol (n = 7)	0.86 ± 0.69	2.43 ± 0.98	1.57 ± 0.53d
	DM-lavender (n = 7)	1.14 ± 0.69	1.86 ± 0.69	0.43 ± 0.53
	Non-DM-NS (n = 7)	1.57 ± 0.53	2.71 ± 1.11	1.71 ± 0.49
	Non-DM-Madecassol (n = 7)	2.29 ± 1.11[a]	2.86 ± 0.69	0.29 ± 0.49[a,c]
	Non-DM-lavender (n = 7)	2.43 ± 0.98[a]	2.71 ± 0.76	0.43 ± 0.53[a,c]
Granulation tissue increase	DM-NS (n = 7)	0.0 ± 0.0	1.57 ± 0.98	1.57 ± 0.53
	DM-Madecassol (n = 7)	0.0 ± 0.0	3.00 ± 0.82[b]	2.86 ± 0.90[b]
	DM-lavender (n = 7)	0.0 ± 0.0	3.00 ± 0.82[b]	2.86 ± 0.90[b]
	Non-DM-NS (n = 7)	0.0 ± 0.0	2.57 ± 1.13	2.71 ± 1.11[a]
	Non-DM-Madecassol (n = 7)	0.0 ± 0.0	3.71 ± 0.49[a]	2.86 ± 0.69[a]
	Non-DM-lavender (n = 7)	0.0 ± 0.0	3.71 ± 0.49[a]	3.14 ± 0.69[a]
Angiogenesis	DM-NS (n = 7)	0.0 ± 0.0	0.71 ± 0.49[d]	0.71 ± 0.49
	DM-Madecassol (n = 7)	0.0 ± 0.0	0.86 ± 0.69[d]	2.29 ± 1.11[b]
	DM-lavender (n = 7)	0.0 ± 0.0	1.57 ± 0.53	2.43 ± 0.98[b]
	Non-DM-NS (n = 7)	0.0 ± 0.0	1.57 ± 0.53[a]	2.14 ± 0.69[a]
	Non-DM-Madecassol (n = 7)	0.0 ± 0.0	1.57 ± 0.53[a]	3.29 ± 0.76[a,c]
	Non-DM-lavender (n = 7)	0.0 ± 0.0	1.71 ± 0.49[a]	3.29 ± 0.95[a,c]
Re-epithelialization	DM-NS (n = 7)	0.0 ± 0.0	0.14 ± 0.38	1.57 ± 0.79
	DM-Madecassol (n = 7)	0.0 ± 0.0	0.86 ± 0.69	3.29 ± 0.76[b]
	DM-lavender (n = 7)	0.0 ± 0.0	1.29 ± 0.76[b]	3.29 ± 0.76[b]
	Non-DM-NS (n = 7)	0.0 ± 0.0	0.86 ± 0.69[a]	2.43 ± 0.53
	Non-DM-Madecassol (n = 7)	0.0 ± 0.0	0.86 ± 0.69[a]	3.43 ± 0.53[a,c]
	Non-DM-lavender (n = 7)	0.0 ± 0.0	1.43 ± 0.53[a]	3.71 ± 0.49[a,c]

[a]: The difference between the DM-NS group and the non-DM group on the specified days was $p < 0.05$.
[b]: The difference between the DM-NS group and the other diabetic groups on the specified days was $p < 0.05$.
[c]: The difference between the non-DM-NS group and the other non-DM groups on the specified days was $p < 0.05$.
[d]: The difference between the DM-Lavender and diabetic groups on the specified days was $p < 0.05$.

Granulation tissue begins to form during the proliferation phase of wound healing, approximately 4 days after the lesion, providing the basis for the remodeling and maturation phase (29,30). In this study, because granulation tissue development began during the proliferative phase, no granulation tissue was observed on the first day. High blood glucose levels impair fibroblast formation and collagen synthesis, causing impaired development of granulation tissue. Therefore, in diabetic individuals, wound healing is impairedand the wound closure time is prolonged (3,6,31). Lavender oil accelerates the development of granulation tissue in the early stages and promotes tissue remodeling through collagen replacement (13). In dogs with wounds dressed with lavender oil, the proliferative phase had an earlier onset, and the development of granulation tissue and collagen deposition were increased (28). In this study, on day 7, when development had increased, the highest mean granulation tissue was measured in the lavender oil and Madecassol groups. A significant difference was observed between the DM-NS group and the other DM groups on days 7 and 14 (Table 1; $p < 0.05$). The fact that lavender oil gave similar results to Madecassol led to positive results in our study. Consistent with these results, lavender oil appears to accelerate the development of granulation tissue.

Angiogenesis is a coordinated process that occurs during the proliferation phase (29,32). In this study, angiogenesis did not occur on day 1. The angiogenesis process started to occur on day 7 and reached its peak level on day 14. Hyperglycemia in individuals with diabetes impairs angiogenesis and delays wound healing. This situation plays an important role in the development of the diabetic foot (31,33,34) . An *in vivo* study demonstrated that high glucose concentrations impair angiogenesis by causing loss of endothelial integrity, endothelial cell detachment, and increased sensitivity of endothelial cells (35). Angiogenesis was decreased in diabetic groups compared with the non-DM groups. A significant difference between the DM-NS and non-DM groups was observed on days 7 and 14 ($p < 0.05$). The angiogenesis phase is crucial to wound healing. Therefore, it is necessary to prevent diabetes-induced impairment of angiogenesis, which contributes to diabetic skin ulcers, particularly diabetic foot ulcers. In this study, in the DM and non-DM groups, the highest level of angiogenesis was evaluated in the groups dressed

with lavender oil on days 7 and 14 (Table 1). This suggests that lavender oil is effective at promoting angiogenesis.

Re-epithelialization, which occurs in the proliferative phase of wound healing, begins 24h after the injury with migration of keratinocytes to the wound site (32,36). Re-epithelialization was not observed in all groups on day 1 of the study. Moreover, re-epithelialization was lower in diabetic rats than in non-diabetic rats, and a significant difference was observed between the DM-NS group and the non-DM groups on days 7 and 14 (Table 1; $p < 0.05$). The findings of this study indicate that diabetes delays re-epithelialization by inhibiting keratinocyte migration on the extracellular matrix, thereby delaying re-epithelialization during the proliferative phase (31,37). The re-epithelialization process is stimulated by growth factors such as epidermal growth factor (EGF), TGF-β, and fibroblast growth factor; this stimulation is important for rapid closure of the wound area (33). In a study of wound healing in rats, lavender oil accelerated re-epithelialization and wound closure by increasing EGF secretion (27). In this study, re-epithelialization levels were high in the lavender

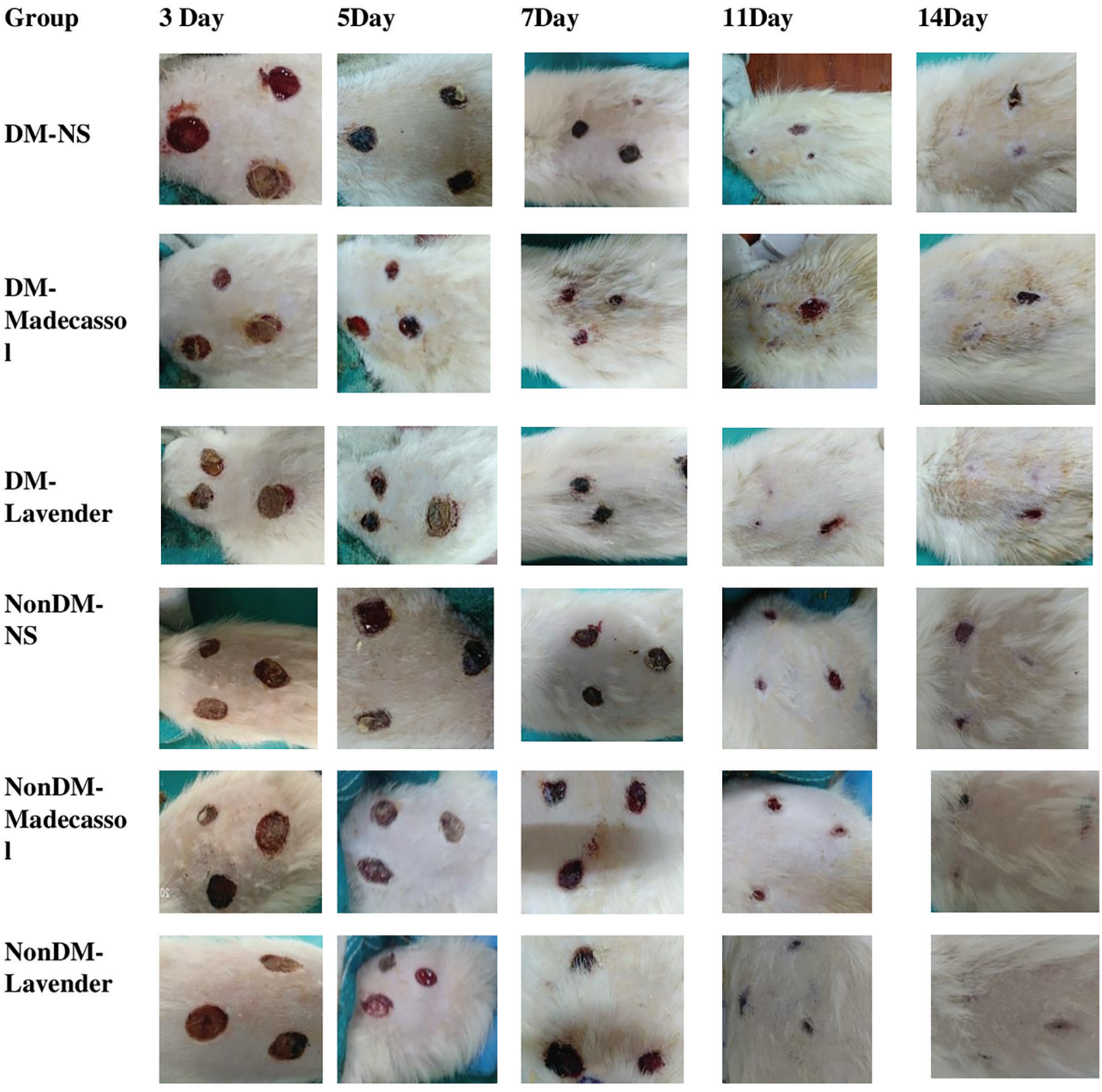

Figure 2. Examples of the wound healing process in groups based on time.

Table 2. Immunohistochemistry VEGFA, collagen-I, and collagen-III levels in groups based on days.

	Group	1 day	7 day	14 day
VEGFA	DM-NS (n = 7)	0.0 ± 0.0	0.14 ± 0.38	0.14 ± 0.38
	DM-Madecassol (n = 7)	0.14 ± 0.38	2.29 ± 1.11[b]	2.00 ± 0.82[b]
	DM-lavender (n = 7)	0.29 ± 0.49	2.43 ± 0.98[b]	2.43 ± 0.53[b]
	Non-DM-NS (n = 7)	0.14 ± 0.38	3.57 ± 0.53[a]	2.71 ± 0.76[a]
	Non-DM-Madecassol (n = 7)	0.29 ± 0.49	3.71 ± 0.49[a]	3.00 ± 0.82[a]
	Non-DM-lavender (n = 7)	0.0 ± 0.0	4.00 ± 0.0[a]	3.14 ± 0.90[a]
Collagen-I	DM-NS (n =7)	0.14 ± 0.38	0.43 ± 0.53	1.00 ± 0.58
	DM-Madecassol (n = 7)	0.71 ± 0.76	1.57 ± 0.53[b]	2.29 ± 0.49[b]
	DM-lavender (n = 7)	0.86 ± 0.69	1.71 ± 0.76[b]	2.71 ± 0.49[b]
	Non-DM-NS (n = 7)	1.14 ± 0.38[a]	1.43 ± 0.53[a]	2.14 ± 0.69
	Non-DM-Madecassol (n = 7)	1.14 ± 0.69[a]	1.71 ± 0.76[a]	2.86 ± 0.90[a]
	Non-DM-lavender (n = 7)	1.43 ± 0.53[a]	1.86 ± 0.69[a]	3.71 ± 0.49[a]
Collagen-III	DM-NS (n = 7)	0.29 ± 0.49	0.71 ± 0.76	0.43 ± 0.53
	DM-Madecassol (n = 7)	0.29 ± 0.49	2.57 ± 0.79[b]	1.71 ± 0.76[b]
	DM-lavender (n = 7)	0.43 ± 0.79	3.00 ± 0.58[b]	1.57 ± 0.53[b]
	Non-DM-NS (n = 7)	0.43 ± 0.53	2.14 ± 0.69	1.43 ± 0.53[a]
	Non-DM-Madecassol (n = 7)	1.00 ± 0.82	3.00 ± 0.82[a]	1.71 ± 0.76[a]
	Non-DM-lavender (n = 7)	1.14 ± 0.69	3.71 ± 0.49[a]	1.86 ± 0.69[a]

[a]: The difference between the DM-NS group and the non-DM group on the specified days was $p < 0.05$.
[b]: The difference between the DM-NS group and the other diabetic groups on the specified days was $p < 0.05$.
[c]: The difference between the non-DM-NS group and the other non-DM groups on the specified days was $p < 0.05$.
[d]: The difference between the DM-Lavender and diabetic groups on the specified days was $p < 0.05$.

groups on days 7 and 14. By day 14 of the study, a significant difference was found between the NS group and the lavender and Madecassol groups in both the DM and non-DM groups. No difference was observed between the positive-control groups for lavender oil and Madecassol. This shows that lavender oil yields results consistent with the positive control and accelerates wound healing by promoting re-epithelialization.

VEGF is an important angiogenic growth factor that regulates vascular permeability in the wound-healing process; it also contributes to the revascularization of the wound site, development of new granulation tissue, and epithelialization (38,39). Errors in VEGFA release cause delays in wound healing (40–42). In this study, the VEGFA levels were evaluated in tissues obtained from the wound area. Studies have shown that the synthesis of various growth factors, including VEGF, is decreased in wounds of streptozotocin-induced diabetic mice (43). Reduced expression of VEGF in non-obese diabetic mice decreased angiogenesis, whereas increased VEGF expression could increase it (42). In this study, average VEGFA levels were lower in the diabetic groups than in the non-DM groups, and a significant difference in VEGFA levels between the DM-NS and non-DM groups was observed on days 7 and 14. Previous studies have reported that an increase in VEGF levels in the wound area can promote healing in diabetic wounds by improving angiogenesis and increasing perfusion. Many different factors also contribute to wound healing through multiple activities, including collagen deposition and re-epithelialization (44,45). In this study, the highest VEGFA levels in the DM and non-DM groups were observed in rats treated with lavender oil. Additionally, a significant difference was observed between the DM-NS group and the other diabetic groups on days 7 and 14 (Table 2; $p < 0.05$). Dressing with lavender oil increased VEGFA levels and showed a positive effect on wound healing.

İn the early stages of wound healing, collagen-III is first to occur, as the scar formation progresses, collagen-I increases as it is better regulated against mechanical stress in the remodeling phase (46-48). In the results of this study, collagen-III levels were found to be higher on day 7 than on other days in all groups, whereas the collagen-I levels were found to be higher on day 14. In addition, the mean scores of collagen-I and collagen-III were found to be lower in the diabetic groups. The results of this study indicate that diabetes affects collagen synthesis. In the study of Mori et al. (13) on wound healing in rats, it has been stated that, compared with the control groups, the number of fibroblasts synthesizing collagen increased in wounds treated with lavender oil. Furthermore, 4 days after injury, the collagen-III and collagen-I level, significantly increased in wounds treated with lavender oil compared with those treated with a control solution. In this study, the collagen levels in the DM and non-DM groups were found to be higher in those dressed with lavender oil on all days. Although dressing with lavender oil in diabetic rats increased the collagen-I and III levels, since there is no study on the use of lavender oil in diabetic wound healing in the literature, a comparison could not be made. It was determined that there was a significant difference between the NS and lavender

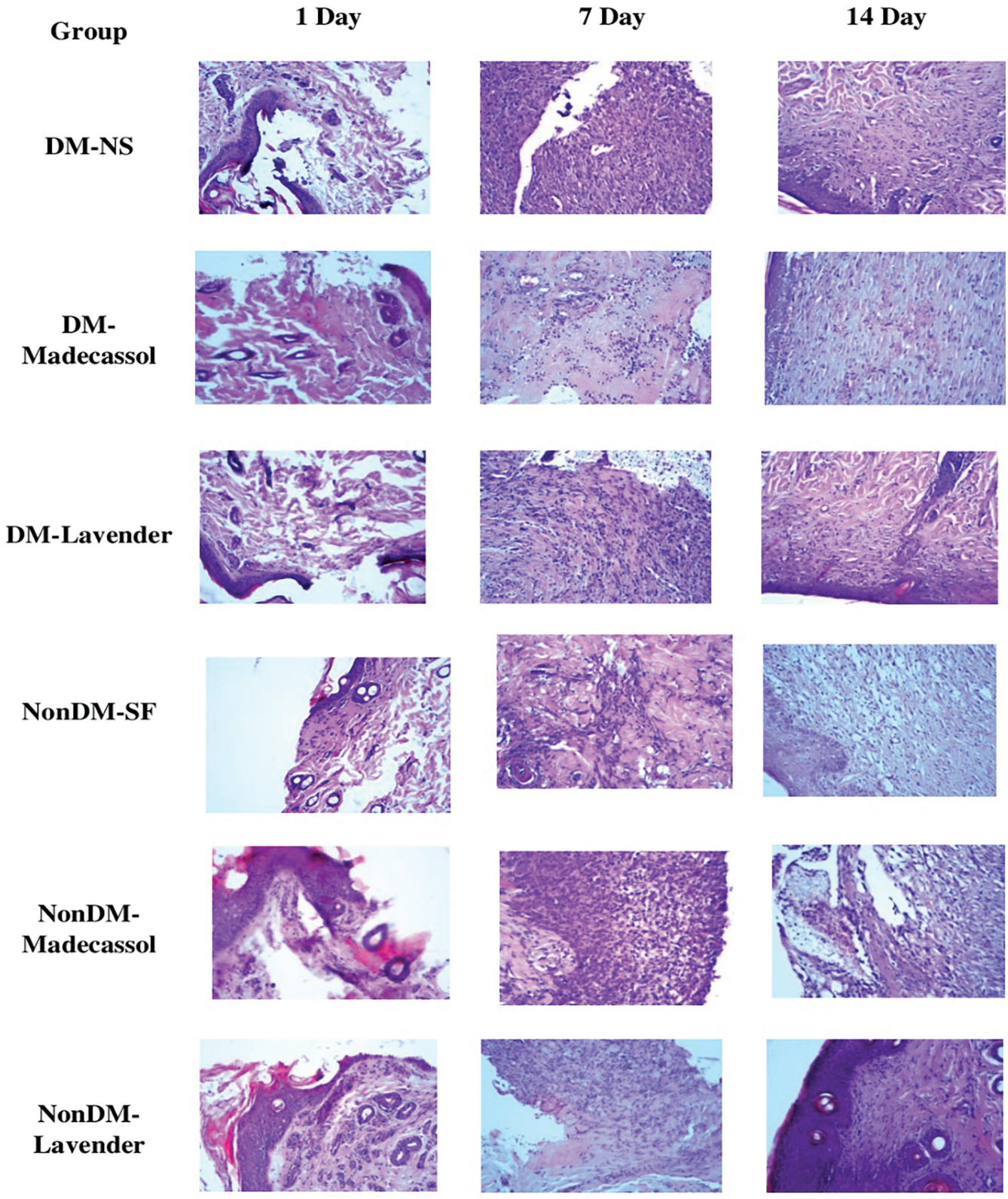

Figure 3. Examples of the Hematoxylin–Eosin staining process in groups based on time.

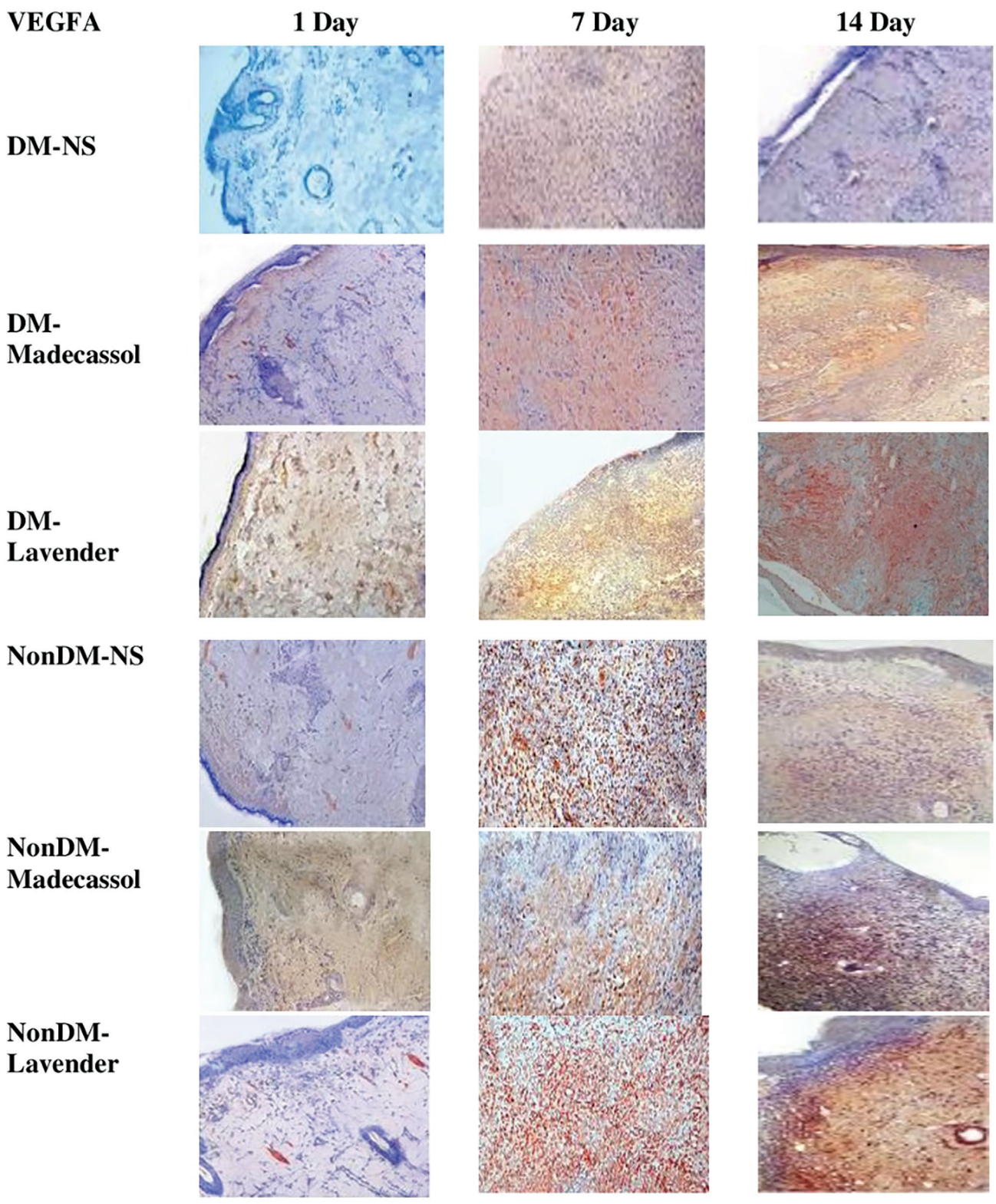

Figure 4. VEGFA immunohistochemistry staining samples in groups based on time.

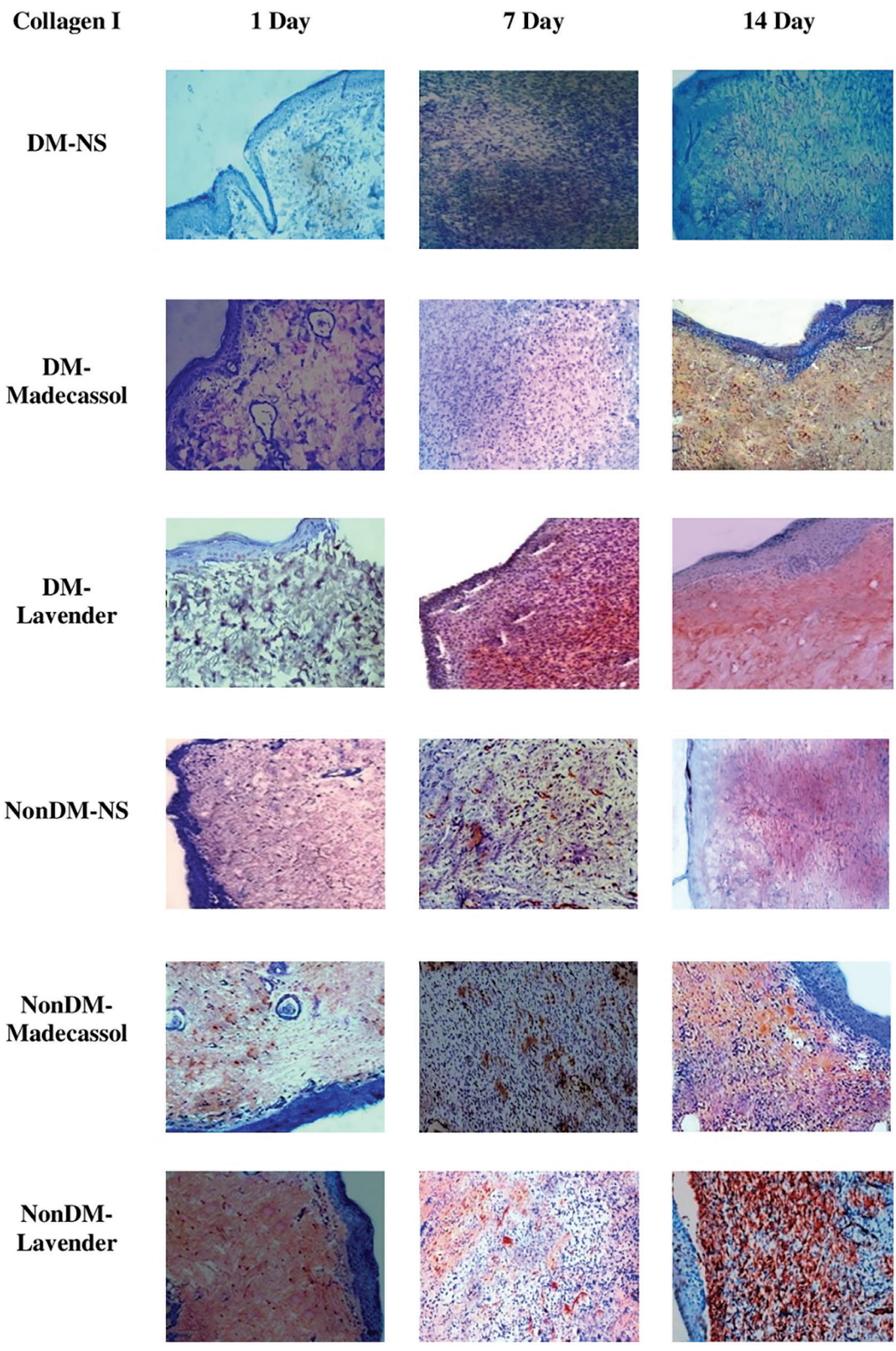

Figure 5. Collagen-I immunohistochemistry staining samples in groups based on time.

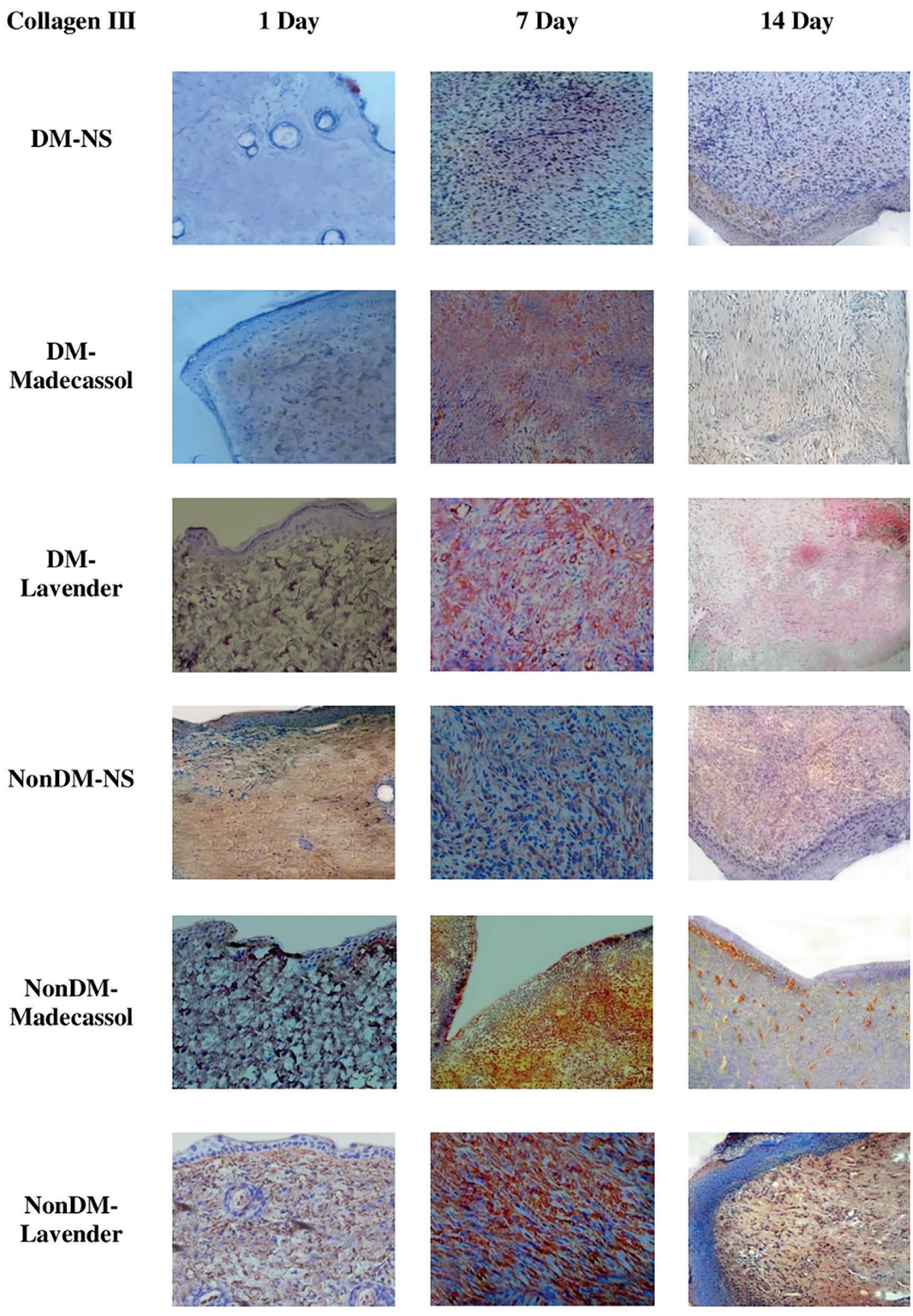

Figure 6. Collagen-III immunohistochemistry staining samples in groups based on time.
DM: Diabetes mellitus, NS: Normal saline

groups. However, no significant difference was found between the Madecassol and lavender groups. This indicates that lavender oil gives results that are consistent with those of the positive control and that it is effective on collagen synthesis.

Study Limitations

The present study is situated within the preclinical stage of research. While the findings obtained from experimental animal models provide significant insights and predictive value regarding the efficacy of lavender oil in wound healing, they do not directly represent clinical outcomes in humans. To establish the clinical utility of these findings for human subjects, further investigations based on these results must be designed as clinical trials. Additionally, as this research was conducted on acute wounds created via full-thickness skin defects in diabetic rats, the results are representative of diabetic acute wounds specifically. Consequently, the scope of the study findings is limited to the context of acute wound healing.

CONCLUSION

Lavender oil dressing was found to be effective in wound healing, both macroscopically and microscopically, in all groups. The results of this study suggest that the positive effects of lavender oil on wound healing may increase the likelihood of using it as a novel dressing material in combination with conventional treatment. It's recommended to plan randomized, controlled clinical trials to evaluate the application of lavender oil in the treatment of diabetic wounds and to identify the active constituent(s) responsible for its healing effect.

The results:

• Wounds dressed with lavender oil healed faster than those dressed with NS or Madecassol.

• On day 14, groups dressed with lavender oil showed the highest wound-healing percentage and the smallest wound area.

• Lavender oil produced results consistent with those of the positive control, Madecassol.

Ethics

Ethics Committee Approval: Consistent with the Declaration of Helsinki, developed by the World Medical Association and with the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees (dated: 07.06.2006, number: 26220), the researcher received the Certification of Experimental Animal Usage following training of the personnel involved in the use of animals for experimental research. Prior to conducting the research, approval was obtained from Kahramanmaraş Sütçü İmam University Experimental Animals Ethics Committee on June 6, 2018 (session number: 2018/08; decision number: 05).

Informed Consent: Informed consent was not applicable, as this study did not involve human participants and was conducted using experimental animals.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.G., A.Y., Concept: M.G., Ö.O., A.Y., A.Y., Design: M.G., Ö.O., A.Y., A.Y., Data Collection or Processing: M.G., A.Y., A.Y., Analysis or Interpretation: M.G., Ö.O., A.Y., A.Y., Literature Search: M.G., Ö.O., A.Y., A.Y., Writing: M.G., Ö.O.

Conflict of Interest: No conflict of interest was declared by the authors.

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