MEDISAINS
Jurnal Ilmiah Ilmu - Ilmu Kesehatan

Original Article

In vivo study: effect of black cumin seeds extract (*Nigella sativa* Linn.) on wound healing

Azafanisa Agilta Velovexia [1], Nasrudin Andi Mappaware [2]✉, Andi Dhedie Prasatia Sam [2], Ida Royani [2], Nur Fadhillah Khalid [2]

[1] *Medical Education Study Program, Faculty of Medicine, Universitas Muslim Indonesia, Makassar, South Sulawesi, Indonesia*
[2] *Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Muslim Indonesia, Makassar, South Sulawesi, Indonesia*
[2] *Department of Orthopedics, Faculty of Medicine, Universitas Muslim Indonesia, Makassar, South Sulawesi, Indonesia*
[2] *Department of Nutrition Science, Faculty of Medicine, Universitas Muslim Indonesia, Makassar, South Sulawesi, Indonesia*
[2] *Department of Pharmacology, Faculty of Medicine, Universitas Muslim Indonesia, Makassar, South Sulawesi, Indonesia*

ARTICLE INFORMATION

Received: December 27, 2024
Revised: February 7, 2024
Accepted: February 14, 2024

KEYWORDS

Nigella sativa; Wound Healing; Inflammation; Leukocytes

CORRESPONDENCE

Phone: +62 822-9216-8162

E-mail: nasrudin.nasrudin@umi.ac.id

A B S T R A C T

Background: Wound healing is a complex biological process involving inflammatory, proliferative, and remodelling phases. The increasing interest in natural therapies has led to the exploration of black cumin (*Nigella sativa*) for its immunomodulatory, anti-inflammatory, and antioxidant properties. While previous studies have examined *Nigella sativa* effects on acute wounds, limited research has assessed its impact on leukocyte levels and its efficacy compared to conventional treatments.

Purpose: This study evaluates the effects of *Nigella sativa* seed extract on incisional wound healing and leukocyte levels in Rattus norvegicus, determining its potential role in accelerating tissue regeneration and modulating inflammation.

Methods: A quasi-experimental study with a post-test-only non-equivalent control group design was conducted using 24 male Wistar rats, divided into four groups: negative control (distilled water), positive control (povidone-iodine), and two treatment groups receiving *Nigella sativa* extract once or twice daily. Wound area reduction was measured on days 7 and 14, and leukocyte levels on day 7. Statistical analysis included Kruskal-Wallis, One-way ANOVA, and post hoc tests ($p < 0.05$).

Results: *Nigella sativa*-treated groups exhibited significantly faster wound closure than controls. By day 14, complete closure was observed in both treatment groups, while residual wounds remained in controls. Leukocyte levels were significantly higher in the treatment groups than in the negative control ($p = 0.001$), indicating an enhanced immune response. No significant difference was found between once- and twice-daily treatments ($p = 0.166$ for wound area, $p = 0.996$ for leukocytes).

Conclusion: *Nigella sativa* extract accelerates wound healing by promoting wound contraction and modulating immune responses. The findings support its potential as a natural alternative for wound management, with effects comparable to povidone-iodine.

INTRODUCTION

Wounds are defined as damage or loss of epithelial integrity of the skin caused by trauma, extreme temperatures, chemical exposure, explosions, electric shock, or animal attacks.[1] The wound healing process occurs in three distinct and sequential phases: inflammation, proliferation, and remodelling.[2] During the inflammatory phase, the immune system responds by activating white blood cells, or leukocytes, which are crucial in defending the body against infections. An increase in leukocyte counts is commonly associated with infection and inflammation, highlighting their importance in the wound healing process.[3,4]

Over time, wound care management has significantly evolved, with herbal-based treatments increasingly recognized as viable alternatives to conventional chemical antibiotics. Many herbal plants have been utilized as topical medicines to enhance wound healing, offering a more natural approach to wound management.[5] Among them, black cumin (*Nigella sativa*) has garnered significant

https://doi.org/10.30595/medisains.v23i1.25269

attention due to its extensive medicinal properties. This herbaceous plant from the Ranunculaceae family is often called a "miracle herb" for its diverse therapeutic benefits.[6,7]

Traditionally, *Nigella sativa* has been used to treat ailments such as asthma, fatigue, and wounds.[7,8] Its essential oil contains bioactive compounds such as thymoquinone, thymol, nigericin, thymohydroquinone, carvacrol, nigericin, and alpha-heder in,[9] which exhibit various pharmacological properties, including immunomodulatory, antioxidant, antibacterial, anti-inflammatory, and tissue regenerative effects, making *Nigella sativa* a promising candidate for wound healing applications.[10–13] Several previous studies have investigated the effects of *Nigella sativa* on acute wound healing. However, many of these studies have not thoroughly examined variations in dosage or specific application methods. Furthermore, most research has only compared untreated wounds with those treated with *Nigella sativa*, without including a positive control group such as conventional antiseptics for a more rigorous comparison.[14–16]

Additionally, while prior research has assessed the impact of *Nigella sativa* on leukocyte levels, no study has specifically explored its effects on leukocyte responses in conjunction with incisional wound healing.[14–16] Understanding this interaction is crucial, as leukocytes play a pivotal role in orchestrating the inflammatory response, influencing the rate and quality of wound healing. Addressing these research gaps is essential for developing safer and more effective natural therapies for wound management and immune regulation.

This study aims to evaluate the effects of *Nigella sativa* seed extract on the incisional wound healing process and changes in leukocyte levels in Rattus norvegicus. By investigating these effects, this study seeks to provide deeper insights into the potential of *Nigella sativa* seed extract in accelerating tissue regeneration and its role in modulating immune responses during the inflammatory phase of wound healing.

METHOD

Study Design

This quasi-experimental study uses a post-test-only non-equivalent control group design. The experiment was conducted to evaluate the effect of *Nigella sativa* Linn. extract on wound healing in an in vivo model.[17]

Study Site

The study was conducted from June to July 2024 at the laboratories of the Faculty of Medicine and the Faculty of Pharmacy, Universitas Muslim Indonesia.

Materials

TThis study utilized a variety of biological samples, treatment substances, and laboratory equipment essential for the experimental procedures. The biological sample used was Wistar strain white rats (Rattus norvegicus), which served as the experimental subjects. The primary treatment substance was *Nigella sativa* extract, which was tested for its effects on wound healing. Additional reagents included povidone-iodine for antiseptic purposes, distilled water (Aquadest) as a solvent, and lidocaine as a local anesthetic.

Various laboratory materials were employed to ensure proper handling and application of treatments, including gloves, face masks, alcohol swabs, cotton buds, 1cc syringes, standard rat feed, and capillary pipettes. The experimental procedures required precise documentation and measurement tools such as a digital camera, digital weighing scale, ruler, and marker. To maintain a controlled experimental environment, rat cages, food and water containers, and wool gloves were used for safe animal handling. Additionally, essential surgical and laboratory instruments included a scalpel with surgical blades, a hair clipper for preparing the wound site, and ethylenediamine tetraacetic acid (EDTA) tubes for sample collection. The selection of materials and equipment was based on their relevance to ensuring the accuracy and reproducibility of the study, adhering to standard laboratory protocols in experimental wound healing research.

Plant Extraction Process

Nigella sativa Linn was extracted using the maceration method with 90% ethanol as the solvent. A total of 2 kg of finely ground *Nigella sativa* seeds was soaked in 3000 mL of 90% ethanol and continuously stirred using a mixer for 2–3 hours to ensure uniform extraction. The mixture was then left to stand for 24 hours for complete maceration.

Following the maceration process, the solution was filtered to separate the solid residues from the ethanol extract. The filtrate was subsequently concentrated using a rotary evaporator to remove the solvent, yielding the final ethanolic extract of *Nigella sativa* seeds. This concentrated extract was then stored under appropriate conditions until further use in the study.[18]

In Vivo Procedure

Animal Preparation

A total of 24 Wistar strain white rats were used in this study and randomly divided into four treatment groups. Group I (Negative Control), Group II (Positive Control), Group III (Treatment I) and Group IV (Treatment II). Before the experiment, the rats underwent an acclimatization period of one week, during which they were provided with standard feed and maintained under natural room temperature and humidity conditions to ensure adaptation to the experimental environment.[19]

Wound Induction Procedure

Prior to wound induction, each rat was anesthetized with an intramuscular injection of 0.08 mL lidocaine to minimize pain and ensure immobilization during the procedure. The dorsal fur of the rats was shaved using a hair clipper,

creating a 3 cm diameter hairless area, followed by disinfection with 70% alcohol. A standardized incisional wound (2 cm long) was created on the dorsal region using a sterile No.11 scalpel. Each rat received 0.5 µL of the assigned treatment per application, applied topically to the wound site.

Experimental Procedure

The assigned treatments were administered topically after wound induction according to the respective experimental groups. Group I (Negative Control) received distilled water (Aquadest), while Group II (Positive Control) was treated with povidone-iodine. In the treatment groups, Group III (Treatment I) received *Nigella sativa* extract once daily, whereas Group IV (Treatment II) received the extract twice daily. Each application consisted of 0.5 µL of the respective treatment, ensuring uniform dosage across all subjects.

The interventions were carried out under controlled conditions, with treatments applied directly to the wound site at consistent intervals. On day seven, wound measurements were performed, and blood samples were collected to analyze leukocyte levels. This provided insight into the inflammatory response and potential immunomodulatory effects of *Nigella sativa* extract. On day fourteen, a second wound measurement was conducted to evaluate the progression of wound healing and closure.

All procedures followed standardized laboratory protocols to ensure the accuracy and reproducibility of the experimental outcomes. The rats were monitored daily for any signs of distress, infection, or complications to maintain ethical research standards and ensure the reliability of the results.

Variables and Measurement

This study evaluated wound healing based on two primary indicators: wound area reduction and leukocyte levels. The wound area was measured at two-time points, day 7 and day 14, using a digital calliper to assess the rate of wound contraction. The wound size was recorded in square centimetres (cm). A significant reduction in wound area over time indicated better healing progression.

In addition to wound size, leukocyte levels were analyzed to evaluate the inflammatory response and potential immunomodulatory effects of *Nigella sativa* extract. Blood samples were collected on day 7, and leukocyte counts were measured to determine differences in immune response among the treatment groups. A decrease in leukocyte levels was considered an indicator of reduced inflammation and improved wound healing.

Statistical Analysis

The data obtained in this study were analyzed using One-way ANOVA for normally distributed data (leukocyte levels) and Kruskal-Wallis analysis for non-normally distributed data (wound area) to assess differences in wound area reduction and leukocyte levels between the control and treatment groups after intervention. The Mann-Whitney and Tukey post hoc tests were conducted to determine significant differences between specific groups in wound healing outcomes.[20]

Ethical Consideration

This research has been approved by the research ethics committee of the Medical Faculty, University of Brawijaya, No.395/A.1/KEP-UMI/VIII/2024.

RESULTS

Wound Area Reduction

The wound healing process was evaluated by measuring the wound area on day 1 (baseline), day 7, and day 14. The wound size at day 1 was 2.00 ± 0.00 cm² across all groups, confirming uniform baseline wound induction (Table 1). By day 7, differences in wound contraction were observed among the groups. The negative control group had a mean wound area of 1.45 ± 0.14 cm², while the positive control group showed a slightly smaller wound size of 1.35 ± 0.19 cm². In contrast, the treatment groups receiving *Nigella sativa* extract exhibited more pronounced wound contraction. The once-daily treatment group (Treatment I) had a mean wound size of 0.84 ± 0.45 cm². In contrast, the twice-daily treatment group (Treatment II) demonstrated the most significant reduction, with a mean wound area of 0.39 ± 0.52 cm².

By day 14, complete wound closure (0.00 ± 0.00 cm²) was observed in both *Nigella sativa* treatment groups, while residual wound areas remained in the control groups. The negative control group retained a wound size of 0.22 ± 0.19 cm², whereas the positive control group showed a mean wound area of 0.05 ± 0.13 cm². Statistical analysis revealed a significant difference in wound area reduction between the groups ($p = 0.001$), indicating that the intervention substantially affected wound healing.

Further pairwise comparisons using the Whitney test demonstrated no significant difference between the negative and positive control ($p = 0.297$). However, significant differences were found between the negative control and both treatment groups. The once-daily *Nigella sativa* extract treatment (Treatment I) showed a significantly smaller wound area compared to the negative control ($p = 0.004$), and the twice-daily treatment (Treatment II) also showed a significant difference ($p = 0.006$).

Similarly, significant differences were observed between the positive control group and both treatment groups, with Treatment I showing a significantly smaller difference. The wound area compared to the positive control ($p = 0.008$) and Treatment II also exhibited a significant difference ($p = 0.016$). In contrast, no significant difference was found between the two treatment groups (Treatment I vs. Treatment II) ($p = 0.166$), suggesting that increasing the frequency of *Nigella sativa* extract application beyond once daily did not provide a statistically significant additional benefit.

Table 1. Mean Differences in Wound Area Between Groups (n=6 per group, cm)

Groups	Baseline	Day-7	Day-14	p-value *
Negative control	2.0±0	1.45±0.14	0.22±0.19	0.001
Positive control	2.0±0	1.35±0.19	0.05±0.13	
Treatment I	2.0±0	0.84±0.45	0±0	
Treatment II	2.0±0	0.39±0.52	0±0	

***** Kruskal-Wallis Test

Table 2. Comparison of Wound Area Differences Between Groups

Groups	Mean rank	p-value*
Negative control vs. Positive control	7.58 vs 5.42	0.297
Negative control vs. Treatment I	9.5 vs 3.5	0.004
Negative control vs. Treatment II	9.33 vs 3.67	0.006
Positive control vs. Treatment I	9.25 vs 3.75	0.008
Positive control vs. Treatment II	9.0 vs 4.0	0.016
Treatment I vs. Treatment II	7.92 vs 5.08	0.166

***** Mann Whitney Test

These findings suggest that *Nigella sativa* extract significantly accelerated wound healing compared to both control groups. However, the lack of significant difference between once-daily and twice-daily applications of the extract indicates that a single daily application may be sufficient to achieve optimal wound healing effects.

Leukocyte Count Analysis

The leukocyte count was measured on day 7 to assess the inflammatory response and potential immunomodulatory effects of *Nigella sativa* extract on wound healing. The results showed variations in leukocyte levels across the different groups, with higher leukocyte counts observed in the treatment groups receiving *Nigella sativa* extract (Table 3).

The negative control group had the lowest leukocyte count, with an average of 6.367×10³/µL, followed by the positive control group, which showed a slightly higher leukocyte count of 7.725×10³/µL. The treatment groups exhibited even higher leukocyte levels, with Treatment I having an average leukocyte count of 8.808 × 10³/µL and Treatment II showing the highest leukocyte count of 8.933 × 10³/µL. Statistical analysis revealed a significant difference in leukocyte levels among the groups (p = 0.001), indicating that the intervention substantially affected the immune response.

Further post hoc analysis (Tukey) provided insights into specific group differences (Table 4). There was no significant difference between the negative and positive control (p = 0.129), suggesting that Povidone iodine did not elicit a markedly different leukocyte response compared to untreated wounds. However, significant differences were observed between the negative control and treatment groups. Treatment I showed significantly higher leukocyte levels (p = 0.003), and Treatment II showed a significant increase in leukocyte levels (p = 0.002).

When comparing the positive control group to the *Nigella sativa* treatment groups, no significant differences were observed (p = 0.283 for Treatment I, p = 0.201 for Treatment II), indicating that *Nigella sativa* extract elicited a comparable immune response to povidone-iodine. Additionally, no significant difference was found between Treatment I and Treatment II (p = 0.996), suggesting that increasing the *Nigella sativa* application beyond once daily did not significantly enhance leukocyte activation.

These findings suggest that *Nigella sativa* extract induces a higher leukocyte response than untreated wounds and is comparable to povidone-iodine in modulating immune activation during the inflammatory phase of wound healing. However, increasing the application frequency did not significantly increase the immune response, indicating that a once-daily application may be sufficient for optimal immune stimulation.

Table 3. Differences in Leukocyte Levels Between Groups (n=6 per group, µl)

Groups	Leukocyte count x10³/µl	p-value*
Negative control	6.367	0.001
Positive control	7.725	
Treatment I	8.808	
Treatment II	8.933	

***** One-way ANOVA Test

Table 4. Comparison of Leukocyte Level Differences Between Groups

Groups	Mean diff	p-value*
Negative control vs. Positive control	-1.358	0.129
Negative control vs. Treatment I	-2.441	0.003
Negative control vs. Treatment II	-2.566	0.002
Positive control vs. Treatment I	-1.083	0.283
Positive control vs. Treatment II	-1.208	0.201
Treatment I vs. Treatment II	-0.125	0.996

***** Tukey Post Hoc Test.

DISCUSSION

The results of this study demonstrated that *Nigella sativa* extract significantly accelerates wound healing, as evidenced by a greater reduction in wound area in the treatment groups compared to the control groups. By day 14, complete wound closure was observed in the groups receiving *Nigella sativa* extract, whereas residual wound

areas remained in the control groups. Furthermore, the leukocyte count analysis revealed that the treatment groups had significantly higher leukocyte levels than the negative control group, indicating a possible immunomodulatory effect of *Nigella sativa* extract in wound healing. These findings align with previous research that highlights the role of *Nigella sativa* in enhancing wound recovery through its anti-inflammatory, antioxidant, and immunostimulatory properties.[7–13]

The acceleration of wound healing in the *Nigella sativa* treatment groups can be attributed to its bioactive compounds, which influence various phases of the wound healing process. Thymoquinone, one of the major active components, plays a crucial role in the inflammatory phase by inhibiting cyclooxygenase (COX) activity, thereby reducing prostaglandin production.[21] This mechanism prevents prolonged vasodilation and helps control excessive inflammation, allowing a smoother transition to the proliferative phase. Additionally, saponins in *Nigella sativa* stimulate the production of vascular endothelial growth factor (VEGF), which promotes angiogenesis and matrix formation, thereby facilitating granulation tissue development.[13]

During the proliferation phase, flavonoids contribute to epithelialization by triggering the reconstruction of the damaged epidermal layer.[22] These compounds enhance extracellular matrix (ECM) production, which supports keratinocyte migration from the basal membrane to the wound surface.[23] This process is essential for effective tissue regeneration. In the remodelling phase, zinc plays a vital role in fibroblast proliferation and collagen synthesis, while fatty acids support new cell formation, contributing to the maturation of healed tissue.[24] The combination of these bioactive compounds allows for faster and more efficient wound closure, as observed in this study.

This study's findings are consistent with previous research demonstrating that *Nigella sativa* extract accelerates wound closure by promoting the inflammatory phase and expediting the transition to the proliferation phase.[25] Other studies have confirmed that *Nigella sativa* enhances angiogenesis and ECM production, facilitating improved skin regeneration.[6,26] Research on diabetic wound models has shown that topical application of *Nigella sativa* improves granulation tissue formation and re-epithelialization.[13,27,28] The hydroethanolic extract of *Nigella sativa* has been particularly noted for its ability to reduce wound size and inflammation, further supporting its efficacy as a wound-healing agent.[15]

Furthermore, *Nigella sativa* antimicrobial properties contribute to its effectiveness in wound healing. Studies have demonstrated its ability to inhibit the growth of methicillin-resistant Staphylococcus aureus (MRSA), thereby reducing infection risks and enhancing the wound-healing process.[29] The presence of VEGF and platelet-derived growth factor (PDGF) in *Nigella sativa* extract further supports angiogenesis and tissue regeneration, reinforcing its potential as a therapeutic agent for wound management.[30]

The leukocyte count analysis in this study indicated a significant increase in leukocyte levels in the treatment groups compared to the negative control group ($p = 0.001$). This suggests that *Nigella sativa* extract may stimulate immune responses, aiding wound healing. The increased leukocyte levels can be attributed to ascorbic acid and thymoquinone, which are known to enhance immune function.[7] These compounds activate T-helper cells, increase phagocytic activity, and stimulate macrophage-driven leukocyte production to defend against infection and foreign substances.[29]

Additionally, thymoquinone has been shown to modulate pro-inflammatory cytokines, reducing excessive inflammation while maintaining an effective immune response. Previous studies have confirmed that *Nigella sativa* is an immunostimulant, increasing total leukocyte counts and enhancing specific and non-specific immune responses.[21] The findings from this study align with research indicating that *Nigella sativa* extract improves immune activity, thereby supporting the wound healing process.[26]

Although a significant difference in wound healing and leukocyte levels was observed between the negative control group and the treatment groups, no significant difference was found between the once-daily and twice-daily administration of *Nigella sativa* extract ($p = 0.166$ for wound area reduction and $p = 0.996$ for leukocyte levels). This suggests that while *Nigella sativa* extract effectively accelerates wound healing, increasing the frequency of application beyond once daily may not necessarily provide additional benefits. Previous studies have reported a dose-dependent relationship between *Nigella sativa* extract and immune response, with higher doses leading to increased leukocyte counts.[31,32] However, the stimulatory effects may plateau beyond a certain threshold, as observed in this study.

CONCLUSIONS AND RECOMMENDATION

The findings of this study confirm that *Nigella sativa* extract enhances wound healing by accelerating wound contraction and modulating immune responses. Thymoquinone, saponins, flavonoids, zinc, and fatty acids contribute to faster wound closure by promoting the inflammatory, proliferative, and remodelling phases of healing. Additionally, its immunomodulatory properties stimulate leukocyte production, aiding in infection defence and tissue repair. The absence of significant differences between once-daily and twice-daily applications suggests that a single daily administration is sufficient for optimal therapeutic effects. Given its antimicrobial, anti-inflammatory, and immunostimulatory properties, *Nigella sativa* extract holds promise as a natural wound-healing agent. It may serve as an alternative or adjunct therapy in clinical wound management. Future studies should explore

the long-term effects of *Nigella sativa* extract, evaluate its efficacy in different wound types, and further investigate potential synergistic effects with other natural compounds to optimize its therapeutic application in wound care.

REFERENCES

1. Maftei A, Holman AC, Merlici IA. Using fake news as means of cyber-bullying: The link with compulsive internet use and online moral disengagement. *Comput Human Behav*. 2022;127:107032. https://doi.org/10.1016/j.chb.2021.107032
2. Kangal MKO, Regan JP. Wound Healing. In: StatPearls Publishing; 2025. https://www.ncbi.nlm.nih.gov/books/NBK535406/
3. Grubbs H, Manna B. Wound Physiology. In: StatPearls Publishing; 2025. https://www.ncbi.nlm.nih.gov/books/NBK518964/
4. Malech HL, Deleo FR, Quinn MT. The role of neutrophils in the immune system: an overview. *Methods Mol Biol*. 2014;1124:3-10. doi:10.1007/978-1-62703-845-4_1
5. Vaillant AAJ, Sabir S, Jan A. Physiology, Immune Response. In: StatPearls Publishing; 2025. https://www.ncbi.nlm.nih.gov/books/NBK539801/
6. Moses RL, Prescott TAK, Mas-Claret E, Steadman R, Moseley R, Sloan AJ. Evidence for Natural Products as Alternative Wound-Healing Therapies. *Biomolecules*. 2023;13(3). doi:10.3390/biom13030444
7. Hannan MA, Rahman MA, Sohag AAM, et al. Black Cumin (*Nigella sativa* L.): A Comprehensive Review on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. *Nutrients*. 2021;13(6). doi:10.3390/nu13061784
8. Ahmad A, Husain A, Mujeeb M, et al. A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pac J Trop Biomed*. 2013;3(5):337-352. doi:10.1016/S2221-1691(13)60075-1
9. Rahman M, Yang DK, Kim GB, Lee SJ, Kim SJ. *Nigella sativa* seed extract attenuates the fatigue induced by exhaustive swimming in rats. *Biomed reports*. 2017;6(4):468-474. doi:10.3892/br.2017.866
10. Eid AM, Elmarzugi NA, Abu Ayyash LM, Sawafta MN, Daana HI. A Review on the Cosmeceutical and External Applications of *Nigella sativa*. *J Trop Med*. 2017;2017:7092514. doi:10.1155/2017/7092514
11. Abbas M, Gururani MA, Ali A, et al. Antimicrobial Properties and Therapeutic Potential of Bioactive Compounds in *Nigella sativa*: A Review. *Molecules*. 2024;29(20). doi:10.3390/molecules29204914
12. Pop RM, Sabin O, Suciu Șoimița, et al. *Nigella sativa*'s Anti-Inflammatory and Antioxidative Effects in Experimental Inflammation. *Antioxidants (Basel, Switzerland)*. 2020;9(10). doi:10.3390/antiox9100921
13. Ciesielska-Figlon K, Wojciechowicz K, Wardowska A, Lisowska KA. The Immunomodulatory Effect of *Nigella sativa*. *Antioxidants (Basel, Switzerland)*. 2023;12(7). doi:10.3390/antiox12071340
14. Sallehuddin N, Nordin A, Idrus RBH, 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. doi:10.3390/ijerph17114160
15. Susilowati R, Rohmanningrum UM. Effective Combination of *Nigella sativa* and Trigonella Foenum-Graecum Seed Extract on Wound Healing in Diabetic Mice. *J Biodjati*. 2023;8(1):106-116. doi:10.15575/biodjati.v8i1.19968
16. Nourbar E, Mirazi N, Yari S, Rafieian-Kopaei M, Nasri H. Effect of Hydroethanolic Extract of *Nigella sativa* L. On Skin Wound Healing Process in Diabetic Male Rats. *Int J Prev Med*. 2019;10(1):18. doi:10.4103/ijpvm.ijpvm_276_18
17. Yaman I, Durmuş AS, Çeribaşı S, Yaman ME. Effects of *Nigella sativa* and Silver Sulfadiazine on Burn Wound Healing in Rats. *Vet Med (Praha)*. 2010;55(12):619-624. doi:10.17221/2948-vetmed
18. Miller CJ, Smith SN, Pugatch M. Experimental and quasi-experimental designs in implementation research. *Psychiatry Res*. 2020;283:112452. doi:10.1016/j.psychres.2019.06.027
19. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *J Pharm Bioallied Sci*. 2020;12(1):1-10. doi:10.4103/jpbs.JPBS_175_19
20. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. *Lab Anim*. 2018;52(2):135-141. doi:10.1177/0023677217724823
21. Kim J, Kim DH, Kwak SG. Comprehensive guidelines for appropriate statistical analysis methods in research. *Korean J Anesthesiol*. 2024;77(5):503-517. doi:10.4097/kja.24016
22. Liu Y, Huang L, Kim MY, Cho JY. The Role of Thymoquinone in Inflammatory Response in Chronic Diseases. *Int J Mol Sci*. 2022;23(18). doi:10.3390/ijms231810246
23. Qin W, Xu L. Pathways linking relative deprivation to blood pressure control: the mediating role of depression and medication adherence among Chinese middle-aged and older hypertensive patients. *BMC Geriatr*. 2023;23(1). doi:10.1186/s12877-023-03769-6
24. Deng X, Gould M, Ali MA. A review of current advancements for wound healing: Biomaterial applications and medical devices. *J Biomed Mater Res B Appl Biomater*. 2022;110(11):2542-2573. doi:10.1002/jbm.b.35086
25. Lin PH, Sermersheim M, Li H, et al. Zinc in Wound Healing Modulation. *Nutrients*. 2017;10(1). doi:10.3390/nu10010016
26. Nourbar E, Mirazi N, Yari S, et al. Effect of Hydroethanolic Extract of *Nigella sativa* L. on Skin Wound Healing Process in Diabetic Male Rats. *Int J Prev Med*. 2019;10:18. doi:10.4103/ijpvm.IJPVM_276_18
27. 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. doi:10.3390/cimb45110567
28. Herman A, Herman AP. Herbal Products and Their Active Constituents for Diabetic Wound Healing-

Preclinical and Clinical Studies: A Systematic Review. *Pharmaceutics*. 2023;15(1). doi:10.3390/pharmaceutics15010281

29. Apaydin YB, Gedikli S. Wound Healing Effects of *Nigella sativa* L. Essential Oil in Streptozotocin Induced in Diabetic Rats. *GSC Biol Pharm Sci*. 2019;7(3):30-39. doi:10.30574/gscbps.2019.7.3.0095
30. Elmowalid GA, Ahmad AAM, El-Hamid MIA, et al. *Nigella sativa* Extract Potentially Inhibited Methicillin Resistant Staphylococcus Aureus Induced Infection in Rabbits: Potential Immunomodulatory and Growth Promoting Properties. *Animals*. 2022;12(19):2635. doi:10.3390/ani12192635
31. Palanisamy CP, Alugoju P, Jayaraman S, Poompradub S. *Nigella sativa* L. Seed Extracts Promote Wound Healing Progress by Activating VEGF and PDGF Signaling Pathways: An in Vitro and in Silico Study. *F1000research*. 2023;12:436. doi:10.12688/f1000research.132106.1
32. Salem A, Bamosa A, Alam M, et al. Effect of *Nigella sativa* on general health and immune system in young healthy volunteers; a randomized, placebo-controlled, double-blinded clinical trial. *F1000Research*. 2021;10:1199. doi:10.12688/f1000research.73524.2
33. Wei J, Wang B, Chen Y, et al. The Immunomodulatory Effects of Active Ingredients From *Nigella sativa* in RAW264.7 Cells Through NF-κB/MAPK Signaling Pathways. *Front Nutr*. 2022;9:899797. doi:10.3389/fnut.2022.899797