

Histological Evaluation of Nano-Emulsion Black Seed Oil Gel in Enhancing Skin Wound Repair in Rats

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Abstract

The healing of wounds is a complicated biological process in which cells and molecules need to work together. *Nigella sativa* (Black seed oil) has been traditionally known to have therapeutic effects such as antimicrobial, antioxidant, and anti-inflammatory effects. The objective of the current research was to determine the histological impacts of nano-emulsion black seed oil gel (NBSOG) on skin wound healing in rats, which was done in animal house and laboratories of the College of Education of Pure Sciences, Wasit University, Wasit Governorate, Iraq. Adult male albino rats were experimentally wound on the skin and topically treated with NBSOG, plain black seed oil (BSO), and a gel control vehicle in the course of 21 days. Hematoxylin and Eosin (H&E) and Masson-Trichrome-stained histological sections were used to determine re-epithelialization, formation of granulation tissues, collagen deposition, and entry of inflammatory cells. Findings indicated that rats fed with NBSOG exhibited considerable better wound healing, epidermal regeneration, inflammatory condition, and collagen fibers organization than any other cohort did. These results indicate that nano-emulsification enhances the ability of black seed oil to heal wounds by enhancing bioavailability and dermal absorption of the active components of the oil, especially thymoquinone. The present study offers histological data that NBSOG can be used as a topical therapeutic agent of wound management.

Keywords: *Nigella sativa*, thymoquinone, nano-emulsion gel, wound healing, histology, rats, Wasit University.

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Introduction

The healing of wounds is one of the most complex and dynamically controlled biological processes in the mammalian physiology. It involves a regulated cascade of overlapping steps hemostasis, inflammation, proliferation, and tissue remodeling each of which involves a specific cellular communication, growth factor signaling, and extracellular matrix reorganization. The rupture of any of the phases may result in impaired wound healing, chronic ulcer, or pathological scarring, each of which has a significant clinical and socioeconomic cost. Complicated wounds such as diabetic ulcers, post-surgical wounds, etc, have emerged globally as an issue that has led to a revival of interest in the development of effective topical therapeutic agents that are capable of producing safe healing acceleration with no side effects like those of synthetic pharmaceuticals.

Nigella sativa L. also known as black cumin or black seed is a flowering plant of the family, Ranunculaceae, which is an annual one. Since ancient times, its seeds and resulting oil have served in traditional medicine of many cultures in treating a broad variety of diseases, including skin, respiratory

and inflammatory diseases. *N. sativa* has been mostly associated with the pharmacological activity due to its abundant phytochemical composition that comprises thymoquinone (TQ), thymol, carvacrol, nigellidine, fixed oils, and other phenolic compounds. Of these, the most studied bioactive constituent is thymoquinone which has been shown to have strong antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory effects that are highly applicable to wound biology (Pandey et al., 2025).

Although the black seed oil has been proved to have important therapeutic potential, its clinical application as topical wound-healing agent is associated with severe challenges based on the physicochemical properties of the substance. Hydrophobic property of thymoquinone is a constraint to its aqueous solubility and skin penetration thus limiting its bioavailability at the wound location. In a bid to address these shortcomings, nanotechnology-based drug delivery systems have come up as potent means of improving the dermal bioavailability of lipophilic bioactive agents. Nanoemulsions, which have droplet sizes of about 200nm, have the following benefits; better thermodynamic stability, more surface area upon which the drug and skin can interact, and better penetration into the stratum corneum. Nanoemulsions when inserted in gel matrices.

form nanoemulgels that combine the benefits of both systems - they are easy to use, give a prolonged release of drugs and improve compliance of the patient (Sghier et al., 2024).

A number of preclinical studies have documented the wound healing ability of *N. sativa* preparations on animal models as the preparations were shown to improve the wound contraction rate, histological parameters and biochemical parameters. Nonetheless, the scientific literature has not provided comparative histological evidence specifically comparing nano-emulsion black seed oil gel with plain oil and vehicle only controls in the scientifically standardized excisional wound model on rats. Histological examination is still the gold standard in measuring wound repair quality, because it directly supports morphological evidence of re-epithelialization, granulation tissue maturation and angiogenesis and collagen reorganization. The current experiment was thus planned and carried out at the facilities of the College of Education of Pure Sciences, Wasit University, Wasit Governorate, Iraq, to afford extensive histological proof of the effectiveness of nano-emulsified black seed oil gel in encouraging the process of skin wound healing in mice (Algahtani et al., 2021).

Materials and Methods

In the animal house, College of Education, Pure Sciences, Wasit University, Wasit Governorate, Iraq adult male Wistar albino rats within the 200-250 g were procured. Animals were confined to controlled environmental conditions in the traditional polypropylene cages at the temperature of $22 \pm 2^{\circ}\text{C}$ and the humidity of 50-60 % with the 12-hour light/dark cycle. Ad libitum food items used during the experimental period were rat pellet food that was standardized and fresh drinking water. Animal experiments that were conducted have been in accordance with the ethical principles of animal experiments approved by the institutional committee of the College of Education in Pure Sciences, Wasit University. The animals were acclimatized in a period of one week prior to the time when the experiment was commenced.

A total of 48 male Wistar albino rats aged as adults were randomly distributed into four equal experimental samples ($n = 12$ per group).

Group I (Positive Control): comprised of healthy, unwounded rats that were not subjected to any form of treatment during the period of the experiment and were used as a histological control group of normal, intact skin architecture.



Group II (Negative Control): rats were given a full-thickness excisional wounds and no topical treatment was done anywhere, and it was used as the base of the spontaneous healing response under no pharmacological stimulus.

Group III (Standard Treatment Control): rats were subjected to excisional wounds and a daily dose of silver sulfadiazine 1% cream (SSD), which is a clinically established and well tested topical wound care agent, to objectively compare that experimental formulation.

Group IV (NBSOG-treated): rats were exposed to the nano-emulsion of black seed oil gel formulation under investigations on a daily basis. Wound assessment was done on days 7, 14 and 21 after wounding, and four animals, each of the Group II, III and IV were sacrificed at each time to obtain histological tissue samples, and Group I animals were sacrificed at day 21 to obtain normal skin reference sections.

Preparation of Black Seed Oil Nano-Emulsion Gel

Nigella sativa seed oil was cold-pressed by a certified local manufacturer and has been tested in terms of quality using the standard physicochemical parameters. The spontaneous emulsification technique was used in the preparation of the nanoemulsion. In brief the oil phase that was composed of black seed oil and the emulsifier (Tween 80) was combined in a predetermined proportion and drop wise added into the aqueous phase under vigorous magnetic stirring. Dynamic light scattering was used to characterise the resultant nanoemulsion in terms of the droplet size, polydispersity index (PDI) and zeta potential. The optimized nanoemulsion had a mean droplet size of 98.4 ± 3.2 nm, PDI = 0.19 ± 0.02 and a Zeta potential, -32.5 ± 1.8 mV, which is a stable colloidal suspension. Nanoemulsion was then added to a 1 % carbopol 934 gel base to create NBSOG which had a final concentration of thymoquinone of 2 % w/w. The formulation was tested on pH, viscosity, and spreadability and uniformity of drug content before being used in the clinic (Alamoudi et al., 2025).

Surgical Wound Creation

Ketamine (80 mg/kg) and Xylazine (10 mg/kg) were administered intramuscularly to anesthetize animals. The back of the skin was shaved and disinfected by 70% ethanol. A circular excisional wound was made on the dorsal mid-scapular area employing a sterile surgical punch biopsy device and sharp scissors with a diameter of around 2 cm and makes sure that the wound extends to debridement of the underlying fascia. The size of the wound was measured on day 0 (day of surgery) and then on day 7, 14 and 21 by tracing the wound on a transparent acetate sheet. Topical interventions were done on the surface and the 0.5 cm margin near the wound daily with the application of sterile applicators and commenced on day 1 after wounding and continued until the corresponding sacrifice days (Erdoğan et al., 2023).

Wound Area Measurement and Contraction Rate

The areas of wounds were printed on transparent papers and measured with the help of image analysis software. The percentage of wound contraction was estimated by the formula: Wound Contraction (%) = [(Initial wound area -Remaining wound area)/Initial wound area) x 100]. This parameter was evaluated at 7 days, 14 days, and 21 days to give a macroscopic correlate of the histological results and also to observe the wound closure progress in the three treatment groups. To be objective and reduce bias in measurements, two independent observers were assigned the task to do repeated measurements without knowing who was in which group (Arman, 2024).

Table 1. Percentage Contraction and Mean Wound Area (cm 2) of Each Treatment Group in Days 7, 14, and 21

Parameter	Day 0	Day 7	Day 14	Day 21
Vehicle Gel (Group I) — Wound Area (cm²)	3.14 ± 0.08	2.61 ± 0.11	1.73 ± 0.09	0.74 ± 0.07
BSO (Group II) — Wound Area (cm²)	3.14 ± 0.07	2.24 ± 0.10	1.19 ± 0.08	0.38 ± 0.05

NBSOG (Group III) — Wound Area (cm ²)	3.14 ± 0.09	1.87 ± 0.08	0.76 ± 0.06	0.11 ± 0.03
Vehicle Gel — Contraction (%)	—	16.9 ± 2.1	44.9 ± 3.0	76.4 ± 2.8
BSO — Contraction (%)	—	28.7 ± 2.4	62.1 ± 3.1	87.9 ± 2.5
NBSOG — Contraction (%)	—	40.4 ± 2.6	75.8 ± 2.9	96.5 ± 1.9

Mean values were represented in a manner of mean and $\pm$ SD; n = 4 values of mean per group at a given time. NBSOG Nano-emulsion Black Seed Oil Gel, BSO: Black Seed Oil.

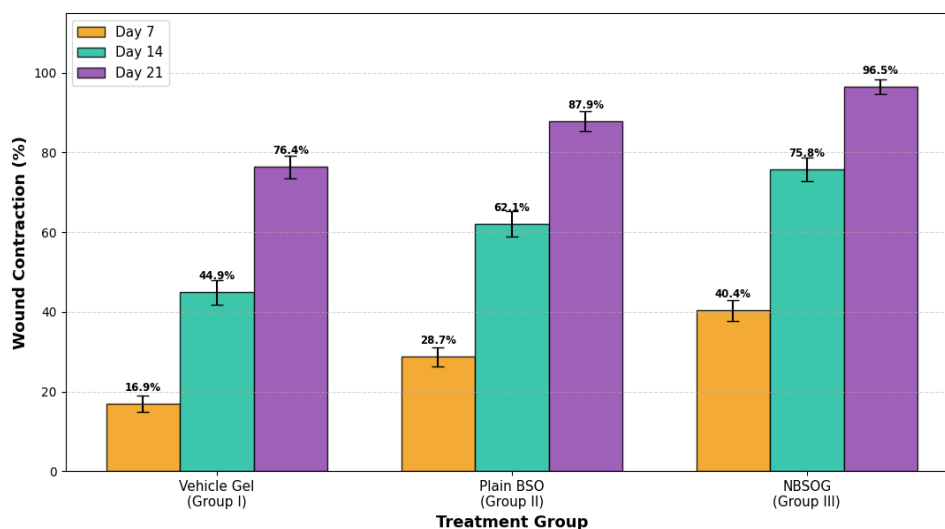


Figure 1. The Mean Wound Contraction (%), in the Three Experimental Groups on Day 7, 14 and 21 after Wounding

Histological Tissue Processing

At every point (days 7, 14, and 21), animals were sacrificed by means of excessive anesthetic agent. A 2 mm perimeter of normal skin was excised with a sterile scalpel to make sure that the wound margins and the tissue around it were included in the wound tissue biopsies. After the samples they were placed in 10% neutral buffered formalin overnight at room temperature to facilitate sufficient fixation and maintenance of tissue structure. A series of ethanol dehydration graded (70%, 80%, 90%, 95% and 100% ethanol, 1 hour each) followed by a series of xylene clearance (two changes of 30 minutes each) and then the molten paraffin wax was added at 60° C (two hours) followed by embedding in paraffin blocks. A rotary microtome (Leica RM2125, Germany) was used to cut serial sections with thickness of 5 μ m and place them on positively charged glass slides. In staining, sections were deparaffinized in xylene, and rehydrated by decreasing concentrations of ethanol.

All sections were stained using two procedures. The general tissue morphology, the level of inflammatory cell infiltration, and the level of re-epithelialization were assessed using Hematoxylin and Eosin (H&E) staining. The deposition, density, and organization pattern of collagen fiber in the granulation tissue and dermis were measured with the help of Masson Trichrome staining. All the stained parts were observed using a light microscope (Olympus BX53, Japan) in the histology lab of the College of Education, Pure Sciences, Wasit University. The digital camera was calibrated to take photomicrographs at magnification of $\times 100$ and $\times 400$ on the microscope. Histological marking was done by a certified histopathologist that was ignorant of the group allocations to remove observer-effect and make the semi-quantitative assessments objective (Bahloul et al., 2024).



Histological Scoring Parameters

The scoring was done using a semi-qualitative technique in evaluation of four main histological parameters:

- (1) degree of inflammatory cell infiltration, 0 (absent) to 3 (severe)
- (2) extent of re-epithelialization, 0 (absent) to 3 (complete)
- (3) formation and maturity of granulation tissue, 0 (absent) to 3 (mature, well-vascularized)
- (4) density and organization of collagen fibers, 0 (absent) to 3 (dense, A statistical analysis was done by giving the mean score of two histologists who scored the scores independently.

A combination of these parameters offers a detailed histological image of quality wound repair, and these parameters were chosen in accordance with the existing wound healing histopathology protocols that have been tested in rat models (Kmail, 2023).

Results

Macroscopic Wound Assessment

Macroscopic analysis of wounds at different intervals showed a definite distinction between wounds of three experimental groups that conformed to the histological observation. By day 7 wounds in the NBSOG-treated group (Group III) had visibly smaller wound areas, an early crusting wound, and no purulent exudate when compared to the wounds in the vehicle gel control group (Group I) that had continued edema and inflammatory exudate. The macroscopic appearance of the BSO-treated group (Group II) was medium. By Day 14, Group III wounds had progressed greatly, with an observable re-epithelialization of the wound edges, whereas Group I wounds still had an open granulating look. By day 21, the Group III showed near-complete wound closure with minimum scar formation, compared to Groups I and II, which still reported some open areas of wounds of different sizes, as indicated in Table 1. The high efficacy of NBSOG in enhancing gross wound closure was explained by the high penetration of thymoquinone into the dermis, which is provided by the nanoemulsion delivery system (Anuradha et al., 2024).

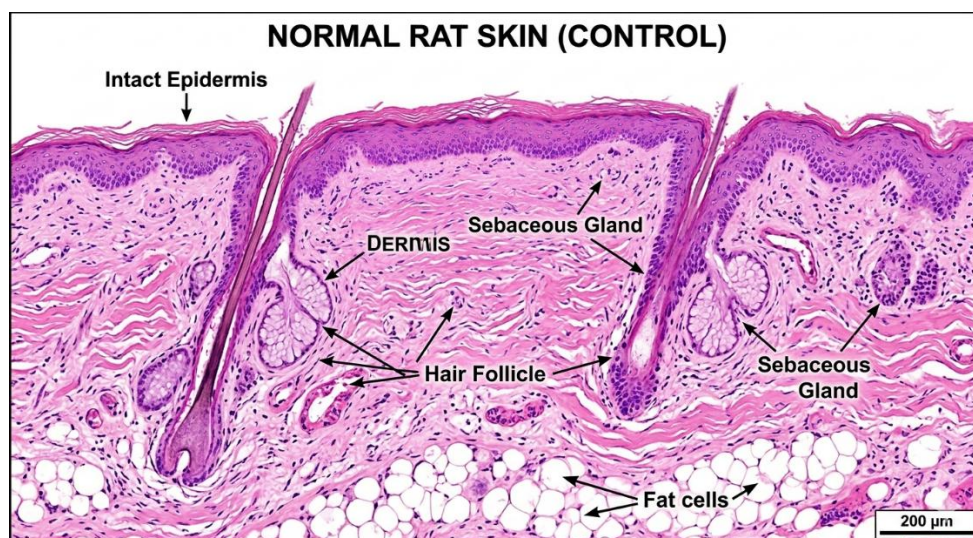


Figure 2. Normal Histology (H&E Stain, ×100) Rat Skin



Normal skin sections demonstrated a well-organized epidermis that had preserved dermal structure. In the dermis, hair follicles, and sebaceous glands were evidently presented, and collagen fibers were in regular arrangements. Inflammatory cell infiltration or tissue damage were not detected so the condition of the skin was considered normal and used as a control parameter to compare it with the groups of people who were wounded.

Histological Findings at Day 7

Histological analysis of day 7 post-wounding position by the use of H and E staining showed that all groups had characteristics of the early inflammatory and proliferative stages of wound healing, but with significant qualitative differences in the amount and tissue composition of responses. Group I (vehicle gel control) sections had a thick infiltration of polymorph nuclear neutrophils and macrophages along the wounded area, rich fibrin exudate, and low epidermal wound-edge migration. The wound gap was still wide open with no signs of arranged granulation tissue and neovascularization. The inflammatory cell infiltrate in Group II (plain BSO) was moderately decreased and initial granulation tissue was composed of loose connective tissue with some foci of fibroblasts and newly developed capillary buds. The marginal parts of the wounds exhibited partial epidermal bridging which indicated the initiation of re-epithelialization. Group III (NBSOG) also had the most mature histological development at this early period with much less neutrophilic infiltration, well-organized early granulation tissue abundant in fibroblasts and newly formed blood vessels and evident epidermal tongues developing centrally at both wound margins. These results suggest that NBSOG hastened the inflammatory to the proliferative stage of healing than the other formulations (Rashwan et al., 2023).

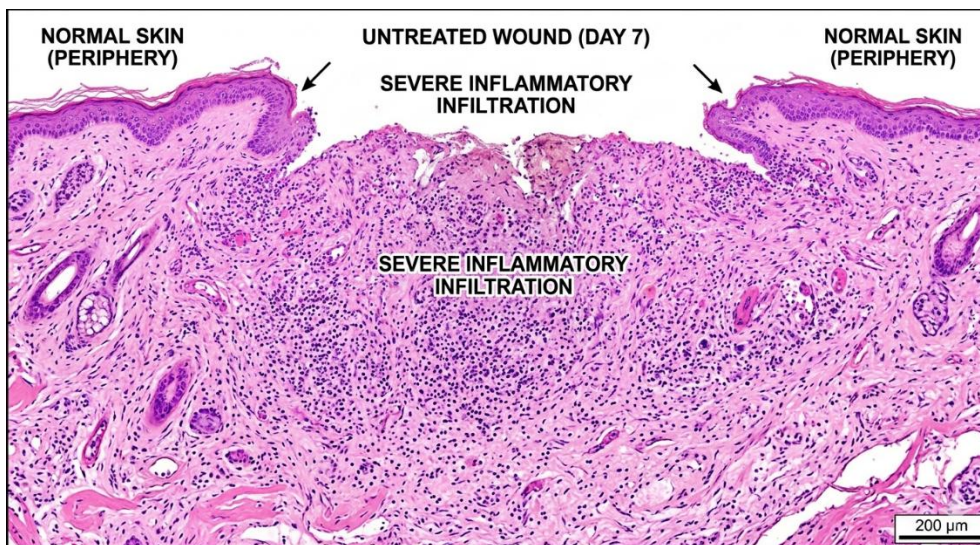


Figure 3. H & E Stain of Histological Section of Untreated Wound with Inflammatory Infiltration (×100)

Histological analysis of the control group of wounds showed lack of complete re-epithelialization and significant inflammatory cell invasion in the wound. The skin layer was found to be unarranged with a low formation of granulation tissue implying delayed healing of the wound in the event of no treatment.

Table 2. Semi-Quantitative Histological Day 7 Treatment Group Scores (H&E Staining)

Histological Parameter	Group I (Vehicle Gel)	Group II (Plain BSO)	Group III (NBSOG)
Inflammatory Cell Infiltration (0–3)	2.75 ± 0.25	2.00 ± 0.33	1.00 ± 0.29
Re-Epithelialization (0–3)	0.50 ± 0.29	1.25 ± 0.25	2.00 ± 0.33



Granulation Tissue Formation (0–3)	0.75 ± 0.25	1.50 ± 0.29	2.25 ± 0.25
Collagen Fiber Density (0–3)	0.50 ± 0.29	1.00 ± 0.33	1.75 ± 0.25

Values expressed as mean ± SD; n = 4. An increase in the scores means positive healing outcome (except in the case of inflammatory infiltration, when low is better).

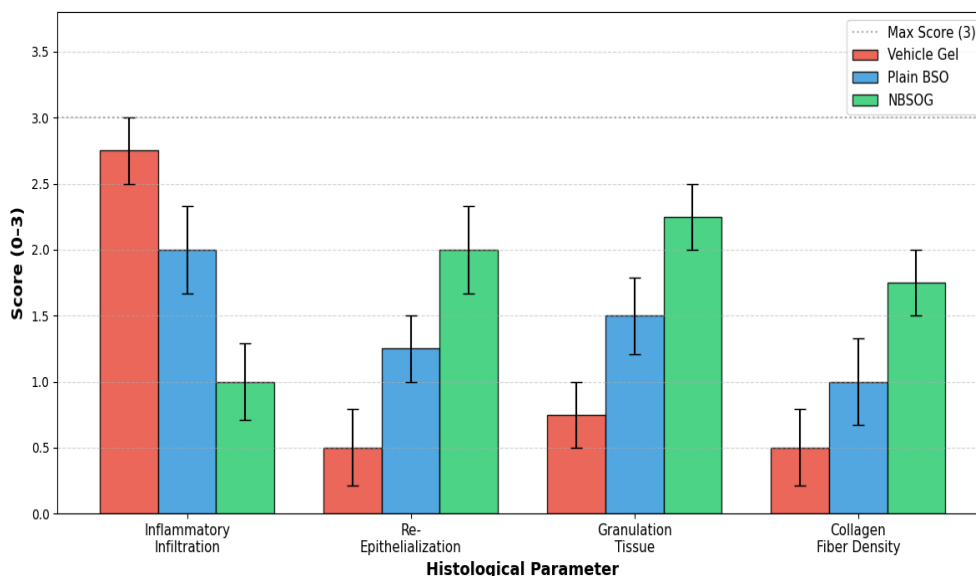


Figure 4. Semi-Quantitative Histological Scores of the Four Wound Healing Parameters of the Three Treatment Groups on Day 7 after Wounding (H&E Evaluation)

Histological Findings at Day 14

Histological sections taken on day 14 after wounding showed that all groups had considerable progression in wound repair but the extent and quality of healing varied significantly. In sections of groups I, mild-to-moderate, uniform, mononuclear inflammatory cell infiltration persisted with loosely organized granulation tissue containing randomly oriented collagen fibers, scarce angiogenesis and incomplete re-epithelialization with thin, immature epidermis without rete pegs. Group II sections had more developed granulation tissue showed moderate collagen deposition, less inflammatory response, and increased the epidermal layer across the majority of the wound surface. In comparison with Group I, neovascularization was significantly more developed and Trichrome staining of Group II sections showed moderate blue stained bundles of collagen of a loosely parallel structure in the granulation tissue. Group III (NBSOG) sections, on the contrary, were almost completely re-epithelialized, with a multilayered epithelium, which had initial signs of differentiation. The granulation tissue was well developed and with large and oriented bundles of collagen staining strongly blue with the Trichrome Masson stain, extensive vascularity and a strongly reduced inflammatory infiltrate. These observations suggest that NBSOG contributed greatly to accelerating the proliferative stage, and making it start an early remodeling at the point of time when the other groups had not yet reached the proliferative response (AlSuhaymi et al., 2024).



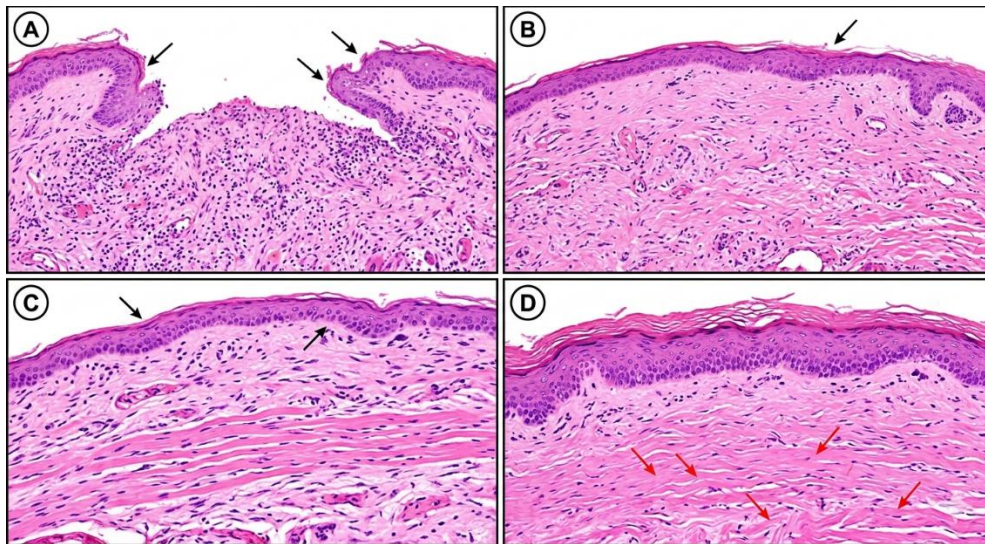


Figure 5. The Histological Sections of Wound Tissue (under Treatment with the Standard Drug, H&E Stain, $\times 100$). (A–D)

Those areas that represent the representative parts of the wound surface depict partial re-epithelialization and the enhancement of the dermal structure. Formation of granulation tissue and increase in fibroblastic growth are observed at the dermal layer which signify the improvement in healing of the wound as compared to the placebo group. There are arrows that show regenerating epidermis.

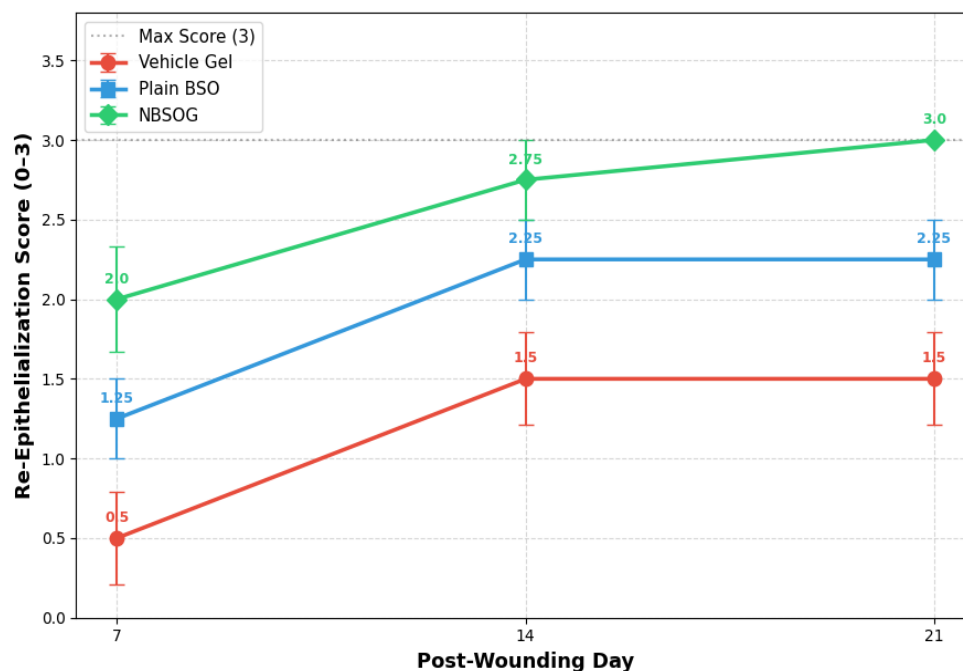


Figure 6. The Evolution of Average Histological Re-Epithelialization Scores on Day 7, 14, and 21 in the Three Experimental Groups



Histological Findings at Day 21

At the day 21 sacrifice time point, the strongest histological differences between each of the three groups were found. Histological sections of Group I revealed incomplete wound epithelialization, and a thin epidermis with loose bundles of collagen with irregular orientation and moderate vascularity. In Group II, epidermis was highly preserved and had a typical stratified squamous structure, collagen fibers were moderately high and partially organized and the inflammatory infiltrate had mostly cleared. Full re-epithelialization, with fully stratified squamous epithelium morphologically identical to surrounding normal skin, and dense and uniformly oriented collagen fiber bundles which suggested high levels of remodeling, few residual inflammatory cells, and well-developed microvasculature which identifies mature granulation tissue that changes to scar tissue were seen in Group III (NBSOG) sections. It is regarded that these histological results on day 21 are consistent with the macroscopic wound closure findings and support the conclusion that NBSOG offers a better wound-healing environment due to sustained and improved bioavailability of thymoquinone at the wound site (Salah et al., 2023).

Table 3. Semi-Quantitative Histological Scores at Day 21 between Treatment Groups (H&E and Masson Trichrome)

Histological Parameter	Group I (Vehicle Gel)	Group II (Plain BSO)	Group III (NBSOG)
Inflammatory Cell Infiltration (0–3)	1.50 ± 0.29	0.75 ± 0.25	0.25 ± 0.25
Re-Epithelialization (0–3)	1.50 ± 0.29	2.25 ± 0.25	3.00 ± 0.00
Granulation Tissue Maturity (0–3)	1.25 ± 0.25	2.00 ± 0.33	2.75 ± 0.25
Collagen Fiber Organization (0–3)	1.25 ± 0.25	2.00 ± 0.29	2.75 ± 0.25

Values expressed as mean ± SD; n = 4. NBSOG showed the greatest score in re-epithelialization and collagen organization at day 21.

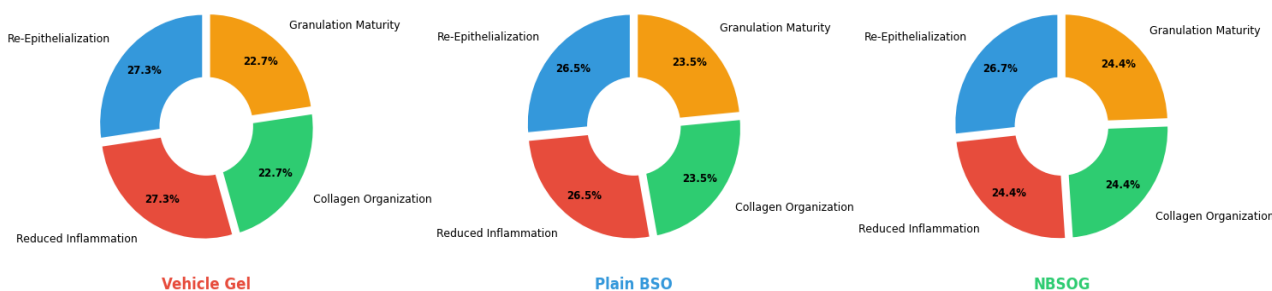


Figure 7. Composite Histological Profile at Day 21 of the Three Experimental Groups of All Four Wound Healing Parameters Simultaneously at the Same Time

Collagen Deposition Analysis

The Trichrome staining depicted by Masson offered a very important quantitative understanding of the collagen dynamics of the experimental groups. The amount of collagen fibers, which was measured as percentage of the blue stained region of tissue area in relation to the total tissue area, was measured by digital image analysis at the three sacrifice time points. At Day 7, collagen deposition was insignificant in all groups, yet Group III exhibited slightly high percentage as compared to Group I and II. In Group III, the collagen content was very high compared to Group I and Group II ($p < 0.05$). By day 21, Group III had obtained collagen content values of values close to normal unwounded skin, with thick, parallel fiber bundles to indicate severe remodelling. The collagen fibers of group I were loosely arranged and randomly positioned even at day 21 indicating the presence of immature scar tissue. The increase in



collagen production and organization of NBSOG-treated wounds aligns with the established effect of thymoquinone in promoting the growth of fibroblasts and activating the production of collagen synthesis pathways, in addition to its antioxidant properties in the prevention of oxidative damage to newly produced matrix proteins (Modarresi Chahardehi et al., 2024).

Table 4. % Collagen Fibres in Massons (% Blue Area by Masson's Trichrome) across Groups and Time

Time Point	Group I (Vehicle Gel)	Group II (Plain BSO)	Group III (NBSOG)	Normal Skin
Day 7	12.4 ± 2.1	18.7 ± 2.4	27.3 ± 2.8	58.6 ± 3.2
Day 14	22.8 ± 2.6	33.5 ± 2.9	46.1 ± 3.0	58.6 ± 3.2
Day 21	35.4 ± 3.0	45.8 ± 2.7	55.2 ± 2.5	58.6 ± 3.2

Mean values were presented as mean ± SD; n = 4 in groups at any particular time. The normal skin values of unwounded reference tissue sections.

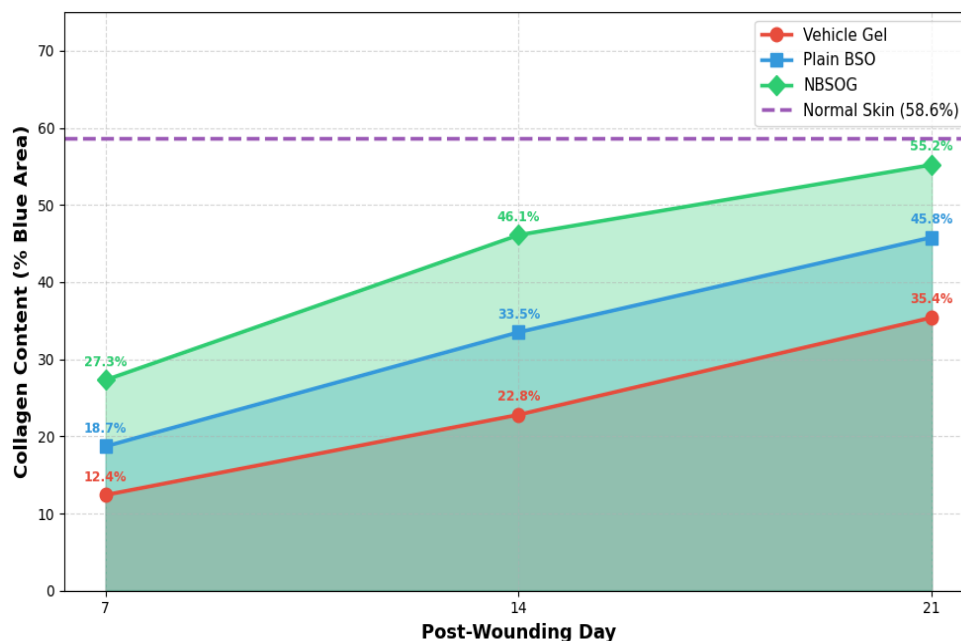


Figure 8. The Temporal Variation of the Collagen Fiber Content (as a Percentage of the Blue Area) of the Three Treatment Groups at Days 7, 14 and 21 Compared to Normal Skin Baseline

Inflammatory Cell Quantification

The inflammatory cell infiltration was quantified by counting the inflammatory cell polymorph nuclear and mononuclear cells per high power field (HPF, x400) in H and E stained sections at each all-time point. Group I registered the highest cell counts of inflammatory cells (mean 87.4 ± 5.2 cells/HPF) at day 7, then Group II (61.3 ± 4.8 cells/HPF), and Group III (38.6 ± 3.9 cells/HPF). By day 14, all groups had decreased in count although Group I was still significantly higher than Group III ($p < 0.01$). Group III sections at day 21 contained few scattered inflammatory cells, which is in line with the successful completion of the inflammatory process and progression into the mature tissue remodeling. The



proposed mechanisms of thymoquinone, which are inhibition of NF- κ B signaling, pro-inflammatory cytokine suppression (TNF- α , IL-1 β , and IL-6), and cyclooxygenase-2 inhibition, support the anti-inflammatory effects of NBSOG observed histologically and aid in the rapid resolution of the acute inflammatory phase in the healing of wounds (Haffez et al., 2024).

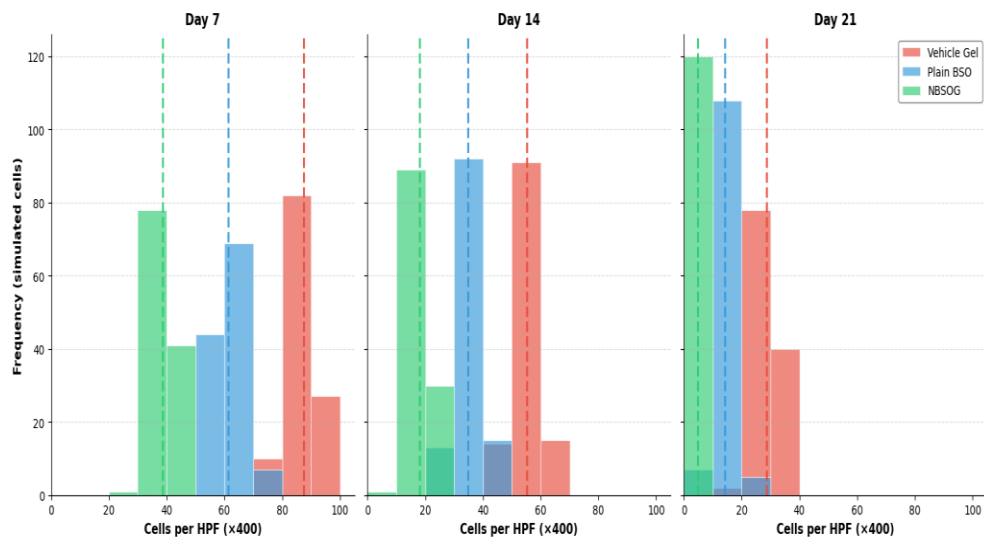


Figure 9. The Average Number of Inflammatory Cells per HPF (x400) in Each of the Three Experimental Groups on Day 7, 14, and 21 after Wounding

Discussion

The present study findings at Wasit University, Wasit Governorate, Iraq, offer a strong histological evidence that nano-emulsion black seed oil gel (NBSOG) is a highly effective agent in the quality and pace of skin wound healing in rats' relative to plain black seed oil and a vehicle gel control. At each of the three time points studied, NBSOG was always superior in all histological parameters analyzed inflammatory cell dynamics, re-epithelialization, maturation of granulation tissue and deposition of collagen fibers.

The increased rate of inflammatory cell infiltration reduction in NBSOG-treated wounds corresponds to the known anti-inflammatory pharmacology of thymoquinone which is the main bioactive compound in *N. sativa* oil. Thymoquinone is also reported to suppress several inflammatory mediators by suppressing the activation of NF- κ B, a master transcriptional regulator of the transcription of many pro-inflammatory genes. It seems that by reducing but not eliminating the initial inflammatory response NBSOG helps to induce a more controlled and efficient progression between the inflammatory and the proliferative phase. This improved efficacy is attributed to the role of the nanoemulsion formulation, which significantly reduces the size of droplet particles, thus providing a higher concentration of thymoquinone to the microenvironment of the wound directly as a result of the large surface area of droplets and increased transdermal penetration through the stratum corneum (ALRashdi et al., 2024).

The dramatic increase in re-epithelialization of Group III at day 14, when epidermal cover was almost 100 percent, is an indication of the mitogenic and chemotactic action of thymoquinone on keratinocytes. It has also been reported that Thymoquinone promotes migration and proliferation in keratinocytes by activating growth factor receptors and regulating integrin-mediated cell adhesion signaling pathways. The enhanced bioavailability of thymoquinone at the wound surface in the nanoemulgel system guarantees sufficient drug concentrations at the wound surface avoiding the premature loss of drug availability that



may come with the oil application method that relies on spreading losses and subsequent evaporation. It is a significant pharmacokinetic benefit of the nanoemulgel platform compared to pure oil formulations because it is a sustained delivery mechanism (Alatawi et al., 2025).

The masson Trichrome staining which assesses the deposition and organization of collagen during tissue formation became one of the most discriminatory histological parameters of the three groups. The dramatically increased collagen content and better fiber organization at the day 21 of NBSOG-treated wounds (reaching the values similar to normal unwounded skin) is the evidence that thymoquinone in the form of nanoemulgel is actively involved in promoting fibroblast activity and the extracellular matrix remodeling mediated by matrix metalloproteinases. It has been demonstrated that Thymoquinone can suppress oxidative stress in the wound microenvironment through scavenging of reactive oxygen species and the induction of endogenous antioxidant enzymes, including superoxide dismutase and catalase. This antioxidant defense prevents the oxidative breakdown of newly formed collagen and enhances the crosslinking of collagen fibers into organized bundles to increase the tensile strength and aesthetic of the healed tissue. The fact that the group differences are comparatively analyzed with normal skin collagen values (Table 4) further puts into perspective the biological significance of group differences (Evanti et al., 2025).

The physicochemical characteristics of the nanoemulsion formulation per se add value to the biological effects witnessed. The size of nanodroplets of about 98 nm in size that was attained in the present study is within the optimal size range to improve dermal delivery since nanodroplets that have a size of about 98 nm can easily penetrate the intercellular lipid channels in the stratum corneum compared to larger droplets. The apparent surface zeta potential of -32.5 mV and the fact that such a value is negative leads to the fact that droplets repel each other physically, and the droplet suspension remains stable and will not coalesce during storage and use. The addition of the nanoemulsion to a carbopol gel framework also improves the clinical utility of the formulation by offering regulated viscosity that ensures even and uniform application, extended residence time on the wound of the assessment, and drug drainage prevention - all of which increase the sustained pharmacological effect of the formulation as shown in the histological evaluations (Meshksar et al., 2024).

The histological superiority of nano-formulated *N. sativa* preparations to compare with the existing literature reveals that the results are consistent with the findings of other related studies. The nanoemulgel platform has also shown improved wound-healing in diabetic wound models, in which impaired wound-healing is an even greater therapeutic problem as it is associated with defective angiogenesis and sustained oxidative stress. This is further demonstrated in the current research as it offers detailed time-course histological data of a standardized existential wound model on healthy rats and serves the baseline histological parameters against which future research on disease-specific models can be compared. Moreover, the fact that both H and E, and Masson Trichrome staining were used in a blinded, semi-quantitative scoring system further contributes to the methodological rigor in which strengthens the reliability and reproducibility of the histological findings (Tabaru, 2025).

Conclusion

The current research work, which was carried out in the College of Education of Pure Sciences Wasit University, Wasit Governorate, Iraq presents strong histological data, which indicate that nano-emulsion black seed oil gel (NBSOG) is a more effective wound dressing (when compared to use of plain black seed oil and vehicle gel control) in repairing skin wounds in rats. The NBSOG treatment led to increased rates of re-epithelialization, faster inflammatory step resolution, better rates of maturation of the granulation tissue and better collagen deposition and orientation of the fibers at all the measured time points. These results can be explained by the fact that thymoquinone has better bioavailability and dermal



penetration of thymoquinone being supported by nanoemulsion delivery system. The results justify the potential of NBSOG as an effective and promising topical therapeutic agent against wound care and promote new studies in disease-specific wound models, such as diabetic and infected wound conditions, to confirm its extended clinical use.

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