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ARTIKEL PENELITIAN

Effect of Topical Application of Black Seed Oil (*Nigella Sativa*) on Wound Healing in Diabetic Rat Ulcer Model

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Abstrak

Tujuan: Pengobatan tradisional terus dikembangkan sebagai alternatif untuk perawatan ulkus diabetikum dalam memenuhi beberapa keterbatasan pengobatan modern. Penelitian ini bertujuan untuk mengevaluasi penggunakan topikal minyak jintan hitam terhadap penyembuhan luka pada model tikul diabetes. **Metode:** 10 Tikus Percobaa diinduksi dengan streptozotocin dengan dosis 55mg/kg BB. Tikus diabetes dianastesi lalu luka dibuat luka dengan biopsy pusch dengan diameter 10 mm. Tikus dibagi menjadi 2 kelompok diantaranya kelompok tanpa perlakuan dan kelompok dengan perlakuan topikal 1 ml dengan black cumin oil 100% setiap hari selama 14 hari. Evaluasi penyembuhan luka dilakukan pada hari ke 7 dan 14. Analisis ekspresi gen menggunakan teknik molekuer dengan proses isolasi RNA menggunakanTriazol, Sintesi cDNA menggunakan kit sintesis dan diproses RT PCR. dan pehitungan menggunakan metode livak dan gambaran luka secara histopatologi menggunakan pewarnaan He. Analisis data menggunakan uji T. **Hasil:** Hasil penelitian menunjukan pada hari ke 7 tidak terjadi penurun ekspersigen TNF dengan $p>0,05$ sedangkan hari ke 14 menunjukan terjadinya penurunan ekspresigen TNF alfa dengan $p<0,001$. Gambaran histopatologi menunjukan perkembangan yang positif dengan memperlihatkan penyembuhan luka yang lebih baik dengan epitelisasi komplit, granulasi dengan sel radang yang lebih rendah dan fibroblast serta kolagen lebih tinggi dibandingkan kelompok kontrol. **Kesimpula:** Minyak jintan hitam dapat meningkatkan kecepatan penyembuhan luka pada tikus model ulkus diabetikum dengan menghambat ekspresigen TNF alfa.

Kata kunci: Black seed oil ; Penyembuhan luka ; TNF alfaa; Histopatologic

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Abstract

Objective : Traditional medicine is constantly being developed as an alternative to the treatment of diabetic ulcer in order to meet some of the limitations of modern medicine. This study aims to evaluate the use of topical black seed oil on wound healing in a diabetic rat model. **Method:**10 experimental rats were induced with streptozotocin at a dose of 55 mg/kg BW. Diabetic rats were anesthetized and then the wound was made with a biopsy push with a diameter of 10 mm. The rats were divided into 2 groups, namely the untreated group and the topical treatment group with 1 ml of 100% Black Seed Oil every day for 14 days. Wound healing evaluation was carried out on days 7 and 14. Gene expression analysis was performed using molecular techniques with RNA isolation process using Triazol, cDNA synthesis using synthesis kit and processed RT PCR. and calculation using the livak method and wound images histopathologically using He staining. Data analysis used T test. **Result:**The results showed that on day 7 there was no a decrease in TNF expression with p >0.05, while on day 14 it also showed a decrease in TNF alpha expression with p <0.001. Histopathological images showed positive development by showing better wound healing with complete epithelialization, granulation with lower inflammatory cells and higher fibroblasts and collagen compared to the control group. **Conclusion :**Black seed oil can increase the speed of wound healing in diabetic rat models by inhibiting TNF alpha expression.

Keywords: Black seed oil; Wouand healing; TNF alpha; Histophatology

INTRODUCTION

Diabetic wound healing is a process that involves cellular and biochemical responses both locally and systemically. It includes a coordinated, dynamic, and complex sequence of processes, including bleeding, coagulation, acute inflammatory response immediately after trauma, connective tissue and parenchymal cell regeneration, migration and proliferation, synthesis of extracellular matrix proteins, parenchymal and connective tissue regeneration, and collagen deposition.[1,2]The delay in diabetic wound healing is caused by complex factors such as decreased migration, proliferation, differentiation, apoptosis, keratinocyte, and fibroblast vascularization, and increased production of pro-inflammatory cytokines such as TNF-alpha.[3,1]

Hyperglycemia in diabetes can also damage the normal function of endothelial cells, which can further impair angiogenesis.[1,2]Wounds become chronically inflamed, unable to progress to the granulation stage due to an imbalance between ROS and oxidative stress, causing lipid peroxidation and further disruption of the function of fibroblasts and endothelial cells.[1,4] In normal conditions, cell metabolism produces eactive oxygen species (ROS) through antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase. Excess of these ROS can damage molecular structures such as proteins, lipids, and DNA.[5]

Diabetic wound care can be done locally by dressing and repeated debridement of necrotic tissue. However, the results of the treatment are not so significant with the occurrence of 14-12% amputation events, the treatment that is still being developed is to overcome the inflammatory response and the growth factors involved.[6]Traditional medicine is still being developed as an alternative for wound care to address some of the limitations of modern treatment. Limitations of modern treatment include high cost, antibiotic resistance. The availability of traditional-based treatment in wound management currently provides a balanced condition for accelerating the healing process as anti-inflammatory, accelerating growth factors and cell migration.[7]

Alternative ingredient that are often used for wound healing include black seed oil , which contains the main compound thymoquinone (TQ). TQ has various therapeutic effects, mainly associated with anti-inflammatory, antioxidant, anticancer, antibacterial, nephroprotective, and neuroprotective properties that have been revealed in various in vivo studies.[8,9] The positive effects of black seed oil in wound healing are mainly caused by the induction of angiogenesis, the increase of fibroblast proliferation, and collagen synthesis. [10] NS oil provides positive effects on the epithelialization and granulation tissue processes and reduces vascularity and inflammation during wound healing.[11]

Black seed oil has been reported to reduce tissue damage and bacterial infection.[12]

Research on black seed oil for the treatment of diabetic ulcers has not been widely conducted. Therefore, the researcher is interested in this study to evaluate the use of topical black seed oil in the wound healing process.

METHOD

This experimental study was conducted on 10 rats weighing 220-250g which were divided into a control group and a treatment group (100% Black seed oil) once a day for 14 days. All rats were kept in the Faculty of Pharmacy animal house under controlled temperature (23 ± 2°C) and 12-h dark/12-h light. Fasting blood glucose and random blood glucose tests were performed once a week for 4 weeks, taken from the tail vein blood. Rats were anesthetized using isoflurane, the wound was made with a biopsy pusch with a diameter of 10 mm. Skin sample collection in rats was performed on days 7 and 14. The rats were euthanized by the neck dislocation method. The initial step was to position the rat in the right-side recumbent position, then surgery was performed to isolate the skin by excision to the depth of the subcutaneous tissue. All experimental procedures consisting of animals complied with ethical guidelines and were approved by the relevant ethics committee no.42/UN.16.10.D.KEPK-FF/2023

TNF-α Gene Expression Analysis

RNA was isolated from tissue from all experimental groups using TRIzol® reagent (Thermo Fisher Scientific, CA, USA).Tissue was homogenized into the sample using a homogenizer using 1 mL TRIzol™ reagent per 50-100 mg tissue. Then 200 µl chloroform was added. Then centrifuge at 12,000 x g, 4 °C for 15 minutes. The upper or clear layer was transferred to a new sterile microfuge tube. 2x Isopropanol was added and incubated again for 10 minutes at room temperature. Centrifuge again for 10 minutes at 12,000 x g and 4 °C. The supernatant was then discarded and the pellet was washed with 350 µl 70% ethanol. Then, invert the tube and vortex gently. Centrifuge again for 5 minutes at 7500 x g at 4 °C. After the vacuum was finished, the pellet was resuspended in 25–40 µl RNase-free water (depending on the amount of pellet). RNA was then quantified and adjusted to a concentration of 1000 ng.[13]

cDNA Synthesis

cDNA synthesis was performed using a commercial kit from Thermo Fisher Scientific. The kit contains all the reagents needed for cDNA synthesis, including 5 µg total RNA, 1x RT buffer, 20 pmol oligodT, 4 mM dNTP, 10 mM DTT, 40 U SuperScript TMII RTase enzyme, and Nuclease Free Water. The reaction volume is 20 µl. cDNA synthesis was performed at 52 °C for 50 minutes following the working protocol as described in the manual kit.[13]

Realtime PCR

After RNA is converted into DNA, the next step is the process of DNA amplification using the RT-PCR technique. In the RT-PCR

process, gene primers that have been designed and optimized for temperature are used. Gene primers serve as a template for the DNA amplification process. The DNA amplification temperature is adjusted to the optimal temperature of the gene primer [14]

5'TGTGCCGCCGCTGTCTGCTTCACGCT-3'
5'GATGAGGAAAGACACCTGGCTGTAGA-3'

Measurement of TNF alpha expression level

The concentration of the gene in this study was measured using the relative quantification method according to the Livak method[15,14]

Histopathology

The skin tissue of experimental animals was fixed in 4% phosphate-buffered formalin and processed into paraffin blocks. Paraffin blocks were cut into thin sections with a thickness of 4mm and stained with Hematoxylin Eosin. Microscopic sections were observed under a light microscope using a CX 33 microscope. Photomicrographs were then taken with a 3.1MP Sony Exmor, Cmos, and Betaview program. Skin tissue was quantitatively measured for the thickness of the epidermis and dermis, as well as semiquantitatively for histological parameters, including edema, leukocytes, granulation, fibroblasts, collagen, and epithelialization, based on McMinn criteria. Epidermis thickness was measured at 40x magnification by drawing a straight line from the base of the epidermis to the upper boundary of the stratum granulosum under the stratum corneum at 10 different points and displayed as an average value in micrometers (µm). The thickness of the dermis was measured in the same way at 10 different points and displayed as an average value in micrometers (µm).[16]

Data Analysis

Data were analyzed using Graphpad Prism 9 with T-test with a 95% confidence interval. A p-value of less than 0.05 was considered significant**.**

RESULT AND DISCUSSION

Effect of Black Seed Oil on TNF-α Expression

No significant ($p>0.05$) decrease in TNF-α gene expression was observed in wound tissue compared to control (Figure 1). On day 14, a significant ($p<0.01$) decrease in TNF-α gene expression was observed compared to control (Figure 2).

Effect of Black Seed Oil on wound histology

The effect of Black Seed Oil on wound healing was analyzed using Hematoxylin staining. (Figure 3) shows that Black seed oil -treated wounds on day 7 showed better wound healing with complete epithelialization, granulation with lower inflammatory cells, and higher fibroblasts and collagen compared to the control group. The effect of Black Seed Oil on wound histology on day 14 (Figure 4) showed that the histological appearance of diabetic rat wounds treated with Black Seed Oil showed better wound healing with complete epithelialization, granulation with lower inflammatory cells,

and higher fibroblasts and collagen compared to the control group.

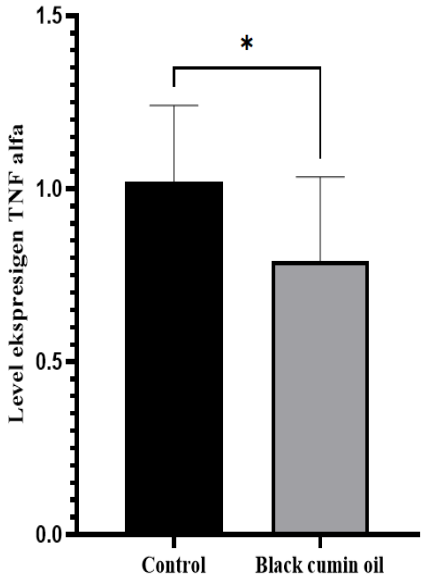


Figure 1. TNF-α gene expression after intervention on day 7. NS p>0.05

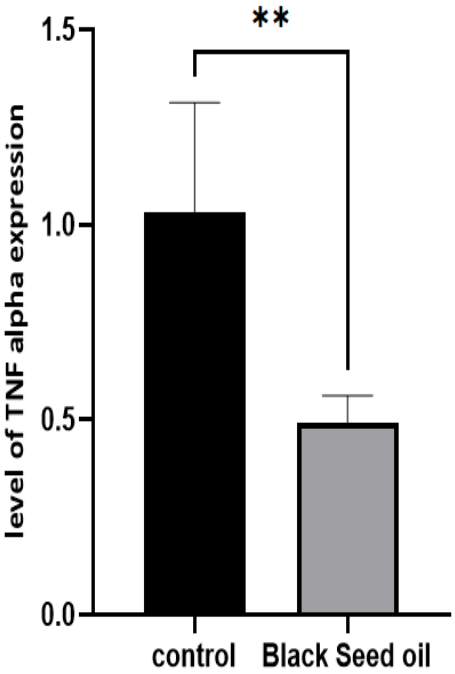
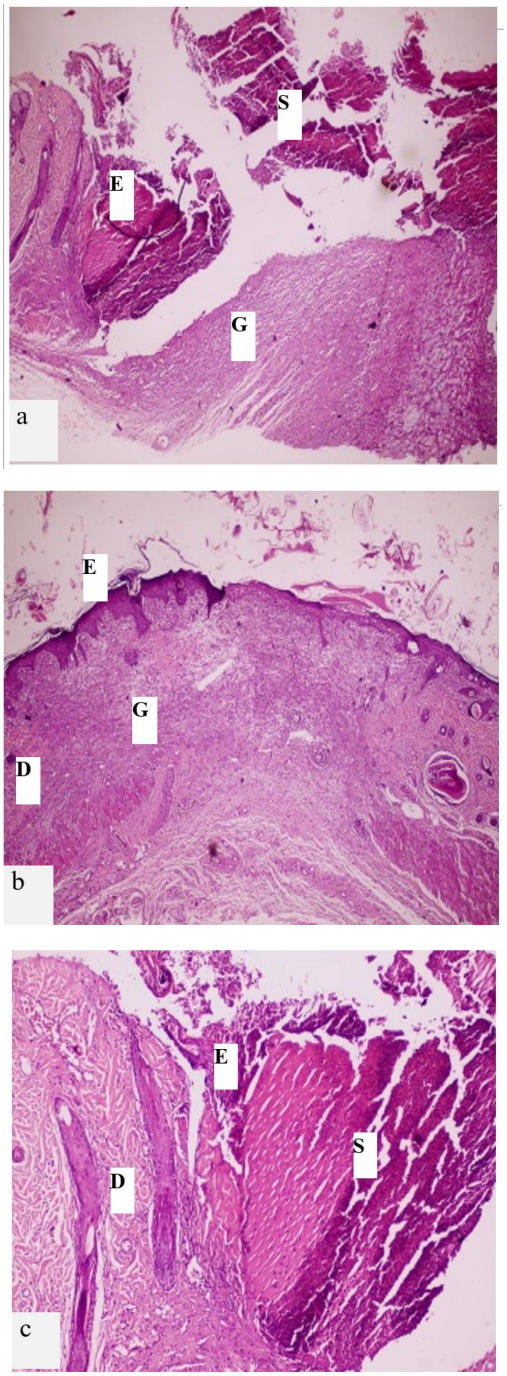
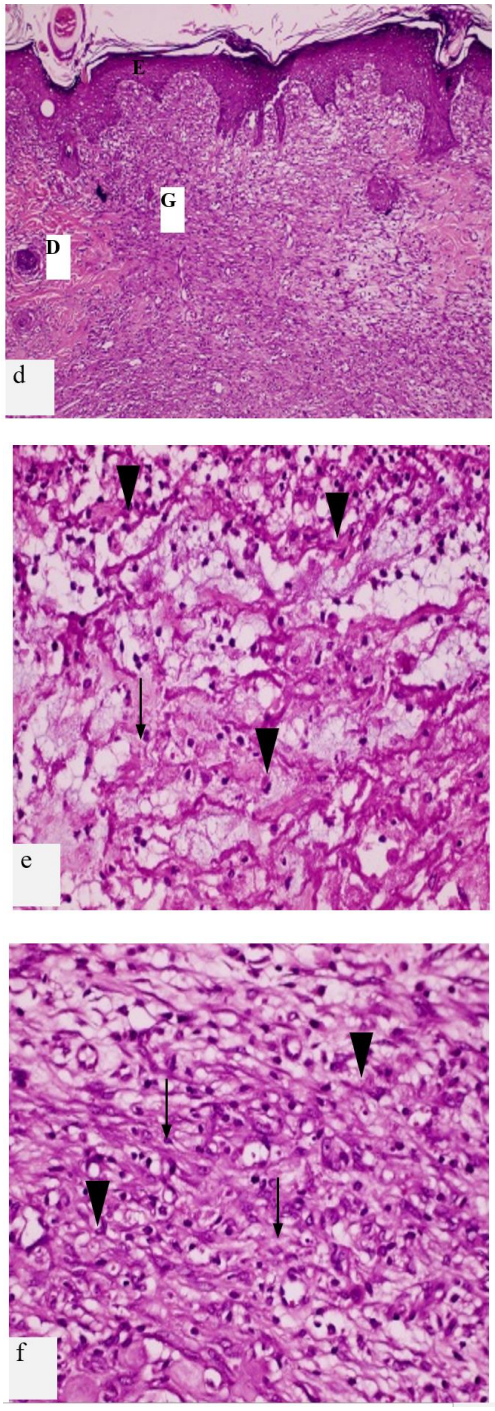


Figure 2. TNF-α gene expression after intervention on day 14. **p<0.001





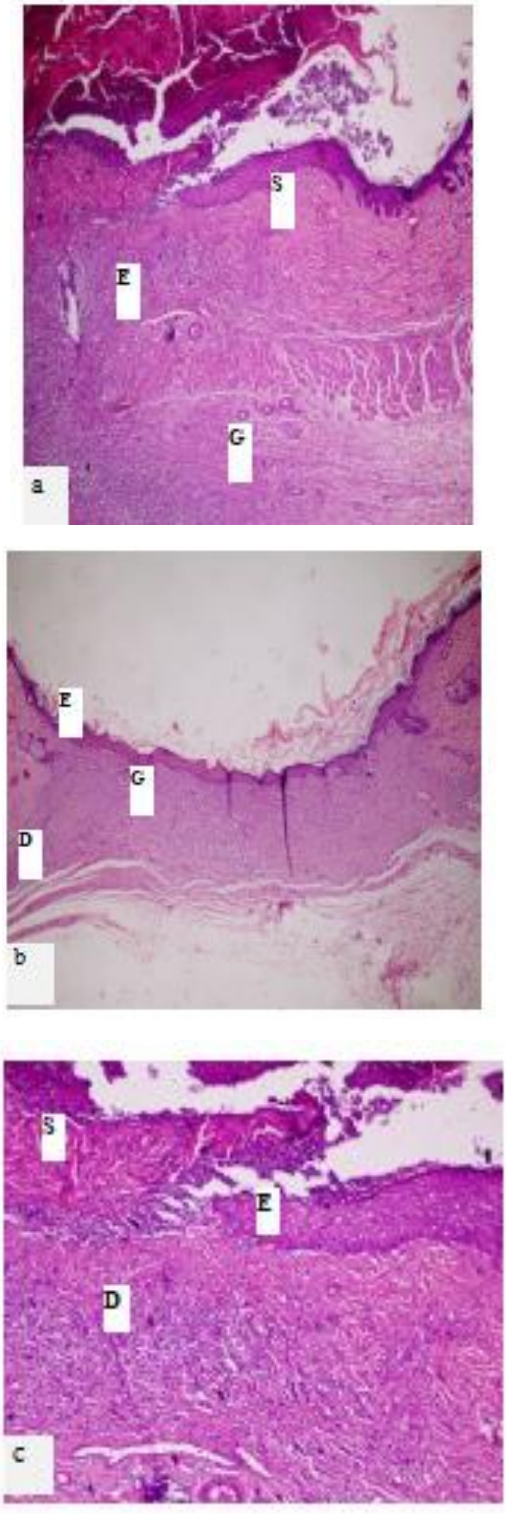


Figure 3. Histopathology on day 14. (a, c, e) diabetes control group while (b, d, f) group treated with Black seed oil . Showing the Epidermis (E) Showing the Dermis (D) showing Granuliation (G), showing Scrab (S).

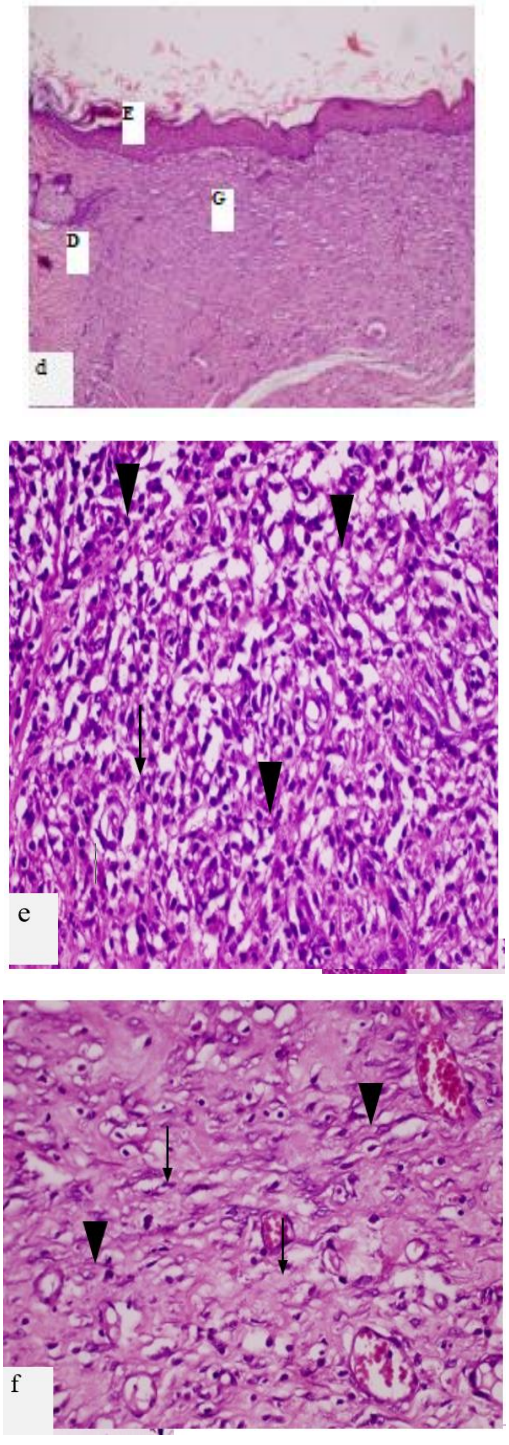


Figure 4. Histopathology on day 14. (a, c, e) diabetes control group while (b, d, f) group treated with Black seed oil . Showing the Epidermis (E) Showing the Dermis (D) showing Granuliation (G)

Discussion

Black Seed Oil has been studied to evaluate its effect on reducing pro-inflammatory cytokines such as TNF-α which are associated with chronic inflammatory conditions that occur in wound cases in diabetic ulcer model rats[17]. Javadi (2018)[18]. The smallest necrotic area was found in the treatment group with Nigella sativa oil. Other researchers have explained that Nigella sativa significantly reduced the wound size in the treatment group compared to the control group. [19] Other studies have also explained that Nigella sativa oil can reduce wound size by increasing collagen and vascularization, both of which are important in the wound healing process. [10]

The results of the TNF-α gene expression test using RT-PCR showed that Black Seed Oil can reduce TNF-α gene expression on day 14 with a P value of 0.001, which means that there is an effect of giving Black Seed Oil on wound healing. This study is also in line with the research conducted by Elghohari (2019)[20] Nigella sativa oil inhibits the inflammatory process, which can accelerate the wound healing process. [19]However, for diabetic wounds, the positive effects of NS have been reported to work at the base of the wound due to less inflammation and low infiltration of polymorphonuclear neutrophils in the group treated with NS compared to the control group[10]. NS improves wound healing in the inflammatory phase of diabetic ulcers. In the presence of diabetes, hyperglycemia causes a dysfunctional inflammatory

response due to the inflow of neutrophils that release cytotoxic enzymes and inflammatory mediators[21]. A previous study by the authors also showed that NSO gel reduced inflammation and increased reepithelialization and granulation tissue formation in diabetic rat wounds[11].

Other types of wounds in normal metabolic conditions have reported that NS accelerates the wound healing process in all phases, including the inflammatory phase, the proliferative phase, and the remodeling phase.[22]NS improves wound healing by reducing the total and absolute number of white blood cells and limiting tissue damage and bacterial spread.[12]NS oil has been found to reduce tissue malondialdehyde and protein carbonyl levels while preventing the inhibition of superoxide dismutase, glutathione peroxidase, and catalase enzymes, thus accelerating wound healing.[21]Furthermore, NS fatty acid components such as oleic acid and linoleic acid maintain the water barrier and enhance wound healing by selectively transferring nutrients in and out of the wound during the healing process.[11],Fatty acids activate neutrophil phagocytosis and release cytokines and growth factors, leading to improved wound healing[17],[23]. The assumption of the researchers in this study is that by shortening the inflammatory phase with a mechanism of inhibiting pro-inflammatory cytokines such as TNF alpha, the wound healing process will soon reach the proliferation and remodeling phase, thus the wound closure can occur perfectly.

CONCLUSION

Black Seed Oil treatment can reduce the expression of TNF-α gene which can accelerate wound healing in diabetic ulcer model mice. Histopathology findings on wound healing with Black Seed Oil showed a positive effect by accelerating the wound healing process.

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CONFLICT OF INTEREST (If Any)

None

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