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(RESEARCH ARTICLE)



## Wound healing effects of *Nigella sativa* L. essential oil in streptozotocin induced in diabetic rats

Apaydin Yildirim Betül <sup>1,\*</sup> and Gedikli Semin <sup>2</sup><sup>1</sup> Ataturk University Veterinary Faculty, Department of Biochemistry, Erzurum, Turkey.<sup>2</sup> Ataturk University Veterinary Faculty, Department of Histology-Embryology, Erzurum, Turkey.

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### Abstract

*Nigella sativa* has been widely used in traditional Turkish medicine for several treatments, specially wounds and diabetes disorders. However, the effects of this plant essential oil on wound healing have not yet been clearly explained. Thus study is required to develop new and effective treatment methods to deal with this subject. This present study was focused on utilization of *Nigella sativa* L. essential oil (NSE) on topical agent for diabetic wound treatment. A total of 72, Sprague–Dawley male rats were used in the present study. The rats were divided into nine groups (n=8). Streptozotocin (STZ) was given at single dose of 60 mg/kg/i.p. The animals showing diabetes (Blood glucose level >250 mg/dL) will be selected for wound groups. Wounds were created by punch (5 mm on dorsal region of each rat). On the 3th, 10th and 14th post-wounding day, the rats were sacrificed and dissected wound tissues. The results of this study showed that lipid peroxidation and oxidative stress significantly increased after STZ application. MDA and GSH levels, GPx, SOD and CAT activities were measured in plasma and wound tissues of the diabetic and treatment groups. While GSH, GPx, SOD and CAT levels in the plasma and wound tissues of the rats were decreased while MDA level was increased compared to the control group in the diabetes group. GSH, GPx, SOD and CAT levels were increased in the *Nigella sativa* L. essential oil treatments compared to the diabetes group while MDA level was decreased. All changes in biochemical parameters were directly proportionaled with histopathological changes of the wound tissues. NSE can be a play role of reducing of the lipid peroxidation, oxidative stress and associated complications and plays a beneficial role in the treatment of diabetic wound.

**Keywords:** Diabetes mellitus; Essential oil; *Nigella sativa* L.; Rat; Oxidative stress

### 1. Introduction

The prevalence of diabetes and other chronic diseases is increasing in many countries with the increase of the aging population. As a result, the complications are very common and very difficult to recover diabetic wounds cause serious problems for public health. Chronic wound healing is a more complex process than normal wound healing in diabetes mellitus, and it may negatively affect the wound healing process.

Interest in the use of plant extracts and other agents on wound healing in traditional and complementary medicine is increasing. These agents generally promote the establishment of a favorable environment for wound healing by joining the wound healing process [1]. Some plant extracts are used as natural and safe antioxidants and play a serious act in blocking oxidative stress caused by free radicals. In recent years, this agents have attracted the attention of scientists [2, 3].

\* Corresponding author

E-mail address: [betul\\_apaydin@hotmail.com](mailto:betul_apaydin@hotmail.com)

Treatment of diabetic wounds requires a professional specializations in an approach. Antibiotic dressings, topical antibiotics, debridement and antiseptic agents are used to examination infections and support the wound healing process [4, 5]. Some wounds do not respond to these treatments and all of practices are deficient, making the wound worse [6]. For these reasons, it is necessary to develop a suitable treatment method for the treatment of diabetic wounds.

In diabetic states, a prolonged wound healing process, prolonged proliferation of numerous cell types and a large number of reactive oxygen species that through damage protein constitutions of extracellular matrix components, proliferation of proteolytic enzymes and inflammation cytokines, and oxidative stress are extremely prolonged [7].

*Nigella sativa L.* is a good food source with essential compounds. Seeds of various civilizations and cultures are used as herbal medicine to protect and treat diseases [8]. Concentrated in its essential oil, the cyber, which is concentrated in its oil, has health-enhancing and antioxidant activity thanks to its active ingredients such as 4-terpineol thymocinone, thymol, carvarol and t-anethole [9]. Thanks to their essential components such as Thymoquinone, they are effective as free radical scavengers in lipid peroxidation [10]. In this study, the effects on wound healing in diabetes wounds of *Nigella sativa L.* seed essential oil was investigated in rats because it is thought to be an essential source for the use of *Nigella sativa L.* essential oil as a topical agent in wound treatment.

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## 2. Material and methods

### 2.1. Reagents

All chemicals were purchased from Sigma Aldrich (St. Louis, USA). All of solution were prepared in freshly distilled water.

### 2.2. Materials

*Nigella sativa L.* seed materials which purchased from spice shop in Erzurum and dried under shade and protected it from sunlight. We roasted all of seed material and powdered in a blender coarsely before preperation of essential oil.

### 2.3. Preperation of *Nigella sativa L.* essential oil

*Nigella sativa L.* essential oil (NSE) were recovered by the method Usanmaz Bozhoyuk and Kordali [11]. This samples (500 g) were subjected to hydrodistillation (seed material in boiling water) using a Clevenger type apparatus for 4 hours. Hydrodistillation of *Nigella sativa L.* seed yielded 0.6% (w/w) of essential oil. The yields were based on dry materials of seed samples.

### 2.4. Animals and experimental procedure

A total of 72 Sprague–Dawley 200–250 g male rats were used in the present study. The experiments were conducted according to the ethical norms approved by the Ethic Committee of Experimental Animal Teaching and Researcher Center (No: 06.10.2017 36643897-000-ATA-131). Sprague–Dawley rats were purchased from the Medical and Experimental Application and Research Center (Erzurum, Turkey). Throughout the animal experiments were processed following the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were kept in standard laboratory conditions under natural light and dark cycle and were fed with standard food for one week, in order to adapt to the laboratory conditions. 18 h before from the experiments, they were fasted overnight, but allowed free access to water. Eight animals were used for each group of study. Rats were divided into the three main groups such as Control, diabetic (STZ-DM) and diabetic+ *Nigella sativa L.* essential oil (STZ-DM+NSE) groups. Each of these main groups was separated into three subgroups for 3<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> day post-operative follow ups.

### 2.5. Induction of diabetes

Diabetes was induced in rats by administration of a single (60 mg/kg b.w.) intraperitoneal injection of STZ (Sigma). STZ was dissolved in citrate buffer (0.1 M, pH 4.5). Seven days after the injection, the blood glucose levels were measured from tail vein. Each animal with a blood glucose level above 250 mg/dL was considered to be diabetic. Blood glucose concentrations were determined using a Glucometer-elite commercial test (Bayer), based on the glucose oxidase method. In all experiments, rats were fasted for 18 h prior to STZ injection.

## 2.6. Wound induction protocol

All of the rats were anaesthetized with intraperitoneal injection of thiopental sodium (55 mg/kg). The dorsal area was completely shaved using an electrical shaver and sterilized with 70% ethanol. In addition, 2% lidocaine was applied as a topical anaesthesia. Two 5 mm excision wounds were created on the upper back of each animal with a dermal punch. The wounding day was accepted as day 0. Control and STZ-DM groups were not applied anything and daily 10 µL of the NSE was topically applied to duplicate wounds on STZ-DM+NSE groups rats. On the 3<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> post-wounding day, the rats were sacrificed by decapitation and dissected wound tissues. Wound samples were taken all of rats for biochemical (-80 °C) and histopathological examinations (%10 formaldehyde).

## 2.7. Measurement of wounds area and wound contraction

The wound contraction was measured by transparent graph papers on days 3<sup>rd</sup>, 10<sup>th</sup>, and 14<sup>th</sup>, as macroscopically, the wound contraction diameter and the excision wound was calculated according to following equation:

$$\text{Wound Contraction Percentage (\%)} = (\text{A Day 0} - \text{A Day X}) / \text{A Day 0} \times 100$$

A Day X= Diameter of wound day 3<sup>th</sup>, day 10<sup>th</sup> and day 14<sup>th</sup>

A = Diameter of wound.

## 2.8. Biochemical analysis

All animals were sacrificed on 20th day by under anesthesia, and dissected wound tissues for biochemical analysis. Wound tissues were homogenized in 0.05M pH 7.4 Tris buffered saline, centrifuged at 12000rpm for 15 min at 4°C and taken supernatants. The total protein amount was measured according to the method of Lowry et al. 1951 [12], MDA [13, 14], GSH [15, 16] levels; GPx [17], SOD [18] and CAT [19] activities were measured in wound and plasma of the rats.

## 2.9. Histopathological analysis

One of the wound tissues of rats was removed and fixed in 10% buffered neutral formalin solution for 72 hours. Tissue samples were embedded in paraffin after xylene and ascendent ethanol series. The paraffin blocks were cut 5-µm thick using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany). The sections of all groups were stained with Mallory's triple stain modified by Crossman. The stained specimens were examined under a light microscope (Nikon eclipse i50, Tokyo, Japan) and photo images were taken for histopathological evaluation. For histopathologic evaluation, each specimen was examined in regards of inflammation, granulation tissue amount, fibroblast maturation, collagen deposition, re-epithelialization, neovascularization.

## 2.10. Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) followed using SPSS software package, version 22.0 p values <0.05 were considered as significant. The results were presented as mean ± standard error (SEM) for 8 rats in each group.

## 3. Results

### 3.1. Biochemical findings

In this wound model, plasma and wound tissues MDA, GSH levels, GPx, SOD and CAT activity were determined and presented in Table1 and Table 2.

When MDA levels were compared in plasma and wound tissues in the 3<sup>th</sup> day, 10<sup>th</sup> and 14<sup>th</sup> days, it was found that MDA levels in STZ-DM group increased compared to control and STZ-DM+NSE group and this increase was statistically significant (P <0.001). In the STZ-DM+NSE group, there was a decrease in MDA level compared to diabetes group and this decrease was statistically significant (p <0.001).

While GSH levels, GPx, SOD and CAT activities in plasma was decreased compared to other groups in diabetes group (STZ-DM), it was increased in treatment group (STZ-DM+NSE) compared to diabetes and control group. This increase was found to be statistically significant in the comparison of measured values on the same day (p <0.001). There was no significant difference in the control group (p <0.01) between the 3<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> days compared to the control group.

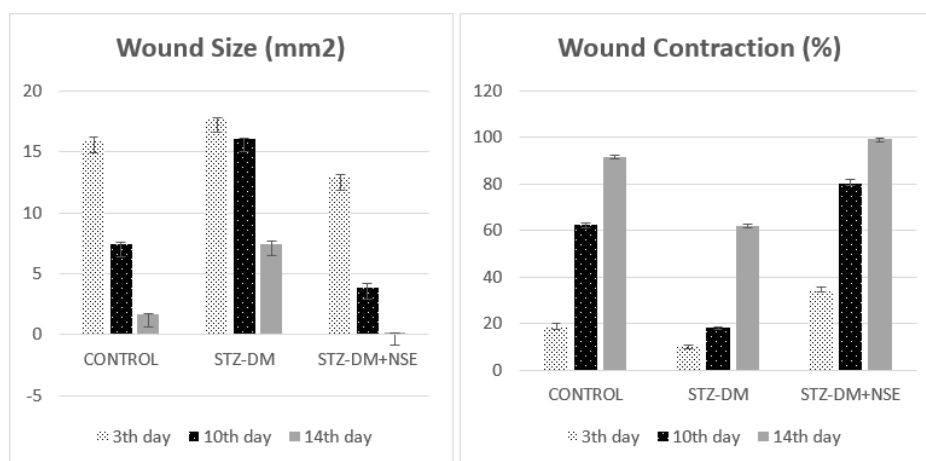
Table 1 shows that the topical application of NSE led to a decrease in the MDA level on the 3<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> day (P < 0.001) and an increase in the GSH levels, GPx, SOD and CAT activities were found in the STZ-DM+NSE group when compared with the other two groups (P < 0.001).

**Table 1** The effects of *Nigella sativa* L. essential oil on plasma biochemical parameters (ANOVA)

	Day	Control Group	STZ-DM Group	STZ-DM+NSE Group	P
MDA mmol/L	3	17.58±0.05 <sup>cA</sup>	26.28±0.40 <sup>aC</sup>	19.70±0.37 <sup>bA</sup>	***
	10	13.60±0.10 <sup>cB</sup>	30.07±0.10 <sup>aB</sup>	16.25±0.44 <sup>bB</sup>	***
	14	12.32±0.43 <sup>bC</sup>	31.35±0.19 <sup>aA</sup>	9.38±0.43 <sup>cC</sup>	***
P		***	**	***	
GSH mmol/L	3	2.06±0.01 <sup>aB</sup>	1.82±0.03 <sup>bA</sup>	2.12±0.06 <sup>aB</sup>	***
	10	2.07±0.00 <sup>aB</sup>	1.58±0.05 <sup>bB</sup>	2.22±0.06 <sup>aB</sup>	***
	14	2.31±0.00 <sup>bA</sup>	1.42±0.01 <sup>cC</sup>	2.57±0.03 <sup>aA</sup>	***
P		***	***	***	
GPx U/mL protein	3	0.23±0.00 <sup>aB</sup>	0.20±0.01 <sup>bA</sup>	0.22±0.00 <sup>aB</sup>	***
	10	0.24±0.00 <sup>aAB</sup>	0.19±0.00 <sup>bAB</sup>	0.24±0.00 <sup>aB</sup>	***
	14	0.25±0.00 <sup>aA</sup>	0.17±0.00 <sup>bC</sup>	0.26±0.01 <sup>aA</sup>	***
P		*	**	***	
SOD U/mL	3	10.47±0.01 <sup>bC</sup>	10.08±0.02 <sup>cA</sup>	14.10±0.17 <sup>aB</sup>	***
	10	14.66±0.05 <sup>aA</sup>	10.93±0.23 <sup>bB</sup>	14.67±0.42 <sup>aB</sup>	***
	14	12.66±0.17 <sup>bB</sup>	9.54±0.12 <sup>cC</sup>	16.97±0.25 <sup>aA</sup>	***
P		***	***	***	
CAT kU/L	3	175.15±0.79 <sup>aB</sup>	140.55±0.84 <sup>bA</sup>	177.95±0.80 <sup>aC</sup>	***
	10	179.291±0.40 <sup>bA</sup>	138.62±0.70 <sup>cAB</sup>	193.18±1.86 <sup>aB</sup>	***
	14	180.92±0.49 <sup>bA</sup>	136.47±0.41 <sup>cB</sup>	217.05±0.99 <sup>aA</sup>	***
P		***	**	***	

\*\*\*P<0.001 and a,b,c: Values with different superscripts within one row differ significantly.\* P<0.05, \*\*P<0.01, \*\*\*P<0.00 and A,B,C: Values with different superscripts within one column differ significantly.

Measurement of wounds area and wound contraction were determined and presented in Figure 1.



**Figure 1** Effect of the *Nigella sativa* L. essential oil on wound size and wound contraction (%) during days 3<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> in diabetic rat

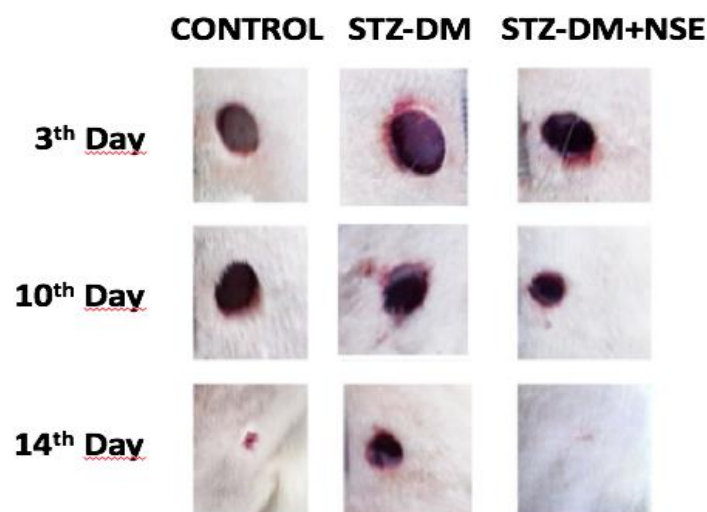
**Table 2** The effects of *Nigella sativa* L. essential oil on wound tissues biochemical parameters (ANOVA)

	Day	Control Group	STZ-DM Group	STZ-DM+NSE Group	P
MDA nmol/g tissue	3	25.67±0.42 <sup>bA</sup>	34.30±0.33 <sup>aC</sup>	15.87±0.76 <sup>aC</sup>	***
	10	18.59±0.19 <sup>bB</sup>	37.01±0.52 <sup>aB</sup>	14.21±0.24 <sup>aC</sup>	***
	14	17.07±0.22 <sup>bC</sup>	48.05±0.31 <sup>aA</sup>	9.62±0.11 <sup>bC</sup>	***
P		***	***	***	
GSH mmol/g tissue	3	0.67±0.01 <sup>a</sup>	0.44±0.01 <sup>bA</sup>	0.69±0.01 <sup>aC</sup>	***
	10	0.70±0.01 <sup>b</sup>	0.41±0.00 <sup>bB</sup>	0.84±0.01 <sup>aB</sup>	***
	14	0.73±0.03 <sup>b</sup>	0.39±0.01 <sup>cC</sup>	0.94±0.02 <sup>aA</sup>	***
P		NS	***	***	
GPX U/g protein	3	26.47±0.04 <sup>bB</sup>	26.08±0.00 <sup>cA</sup>	26.60±0.03 <sup>aC</sup>	***
	10	26.75±0.05 <sup>aA</sup>	26.03±0.01 <sup>bB</sup>	26.79±0.04 <sup>aB</sup>	***
	14	26.82±0.03 <sup>bA</sup>	26.01±0.00 <sup>cC</sup>	27.06±0.03 <sup>aA</sup>	***
P		***	***	***	
SOD EU/g protein	3	10.42±0.30 <sup>bB</sup>	8.91±0.21 <sup>cA</sup>	15.08±0.49 <sup>aC</sup>	***
	10	10.91±0.26 <sup>bAB</sup>	8.45±0.07 <sup>cAB</sup>	17.35±0.26 <sup>aB</sup>	***
	14	11.82±0.30 <sup>bA</sup>	8.14±0.11 <sup>bC</sup>	18.70±0.26 <sup>aA</sup>	***
P		**	**	***	
CAT U/g tissue	3	169.54±0.40 <sup>bC</sup>	134.97±0.31 <sup>cA</sup>	185.11±1.68 <sup>aC</sup>	***
	10	171.02±0.24 <sup>bB</sup>	131.73±0.29 <sup>cB</sup>	201.48±1.21 <sup>aB</sup>	***
	14	177.14±0.34 <sup>bA</sup>	122.66±0.68 <sup>cC</sup>	242.29±0.73 <sup>aA</sup>	***
P		***	***	***	

\*\*\*P<0.001 and a,b,c: Values with different superscripts within one row differ significantly. \*\*P<0.01, \*\*\*P<0.00, NS: Non-significant (P>0.05) and A, B, C: Values with different superscripts within one column differ significantly.

### 3.2. Histopathologic results

Wounds photographs of a representative rat from all group were taken on days 3<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> (Figure 2)



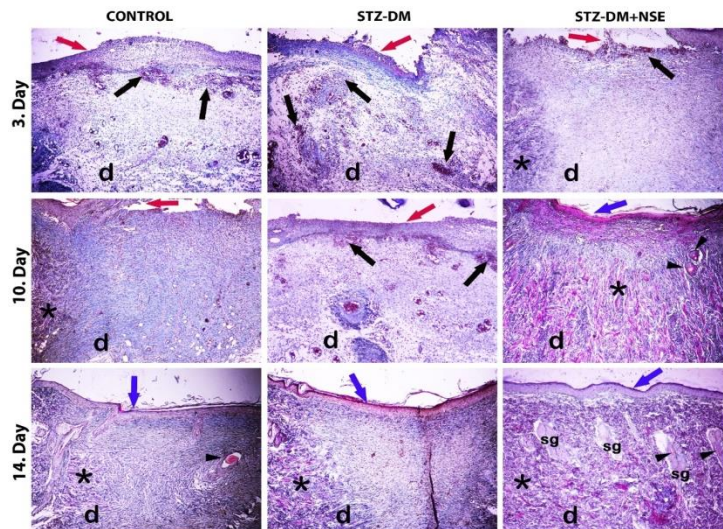
**Figure 2** Effect of *Nigella sativa* L. essential oil representative photographs of macroscopic image of excision wound healing on day 3<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> in diabetic rats

Effect of *Nigella sativa L.* essential oil representative photomicrograph of rat wound tissue at days 3<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> after wounding (Figure 3).

The sections were evaluated histopathologically for the development of inflammation, granulation tissue, fibroblast formation, collagen deposition, re-epithelium and vessel formation. Accordingly, in the STZ-DM group on the 3<sup>th</sup> day, there were more inflammatory sites, hemorrhagic areas and more granulation tissue compared to control and STZ-DM+NES groups. There was quite irregular collagen accumulation in the dermis. The level of improvement in the other two groups was better than the STZ-DM group. Epithelial formation was not observed in three groups on the 3<sup>th</sup> day.

There was more healing in all groups in the 10<sup>th</sup> day than in the 3<sup>th</sup> day. In the STZ-DM+NES group, it was observed that epithelial tissue and keratin structure were formed compared to control and STZ-DM groups. In addition, a more regular dermis structure, collagen and fibroblast development was observed. The areas of inflammation were almost lost. The recovery rate in the control group was higher than in the STZ-DM group.

On the 14<sup>th</sup> day of the experiment, STZ-DM+NES group was the group with the highest level of epidermal and dermal regeneration compared to the other two groups. In this group, the wound area was completely closed, epithelial tissue and keratin were completely formed. There was also a more regular dermis structure. In the dermis of STZ-DM+NES group, more intense sebaceous gland and hair follicle formation could be seen. Epithelial and keratin formation was observed in the control and STZ-DM groups, but the healing of the wound area was less in the STZ-DM group than in the control group (Figure 2).



**Figure 3** Photomicrograph of rat skin at days 3<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> after wounding

Red arrows regenerated epidermis, blue arrows: epitel and keratin structure, black arrows: inflammation and hemorrhage areas, d: dermis, asterisk: collagen deposition, black arrow head: hair follicle, sg: sebaceous gland. Crossman's Modified Mallory Triple Staining. Bar: 100 µm.

#### 4. Discussion

Wound healing, a normal complex process initiated after injury, is divided into four phases, including haemostasis, inflammation, proliferation, and tissue remodeling. The process requires coordinated interactions among various cell types, biochemical mediators, and extracellular matrix molecules within a specific time. Unlike acute wounds, diabetic wound show many molecular abnormalities in healing cascade involving defects in fibroblast and keratinocyte functions [20, 21], angiogenesis impairment [22] and loss of phagocytic activity [23].

There are many herbal products that have antioxidant activity used under the name of dressing materials for therapeutic purposes in topical wound healing promotion. The seeds of *Nigella sativa L.* (black seed, black cumin), *Nigella damascene* and *Nigella arvensis* are used in folk medicine and as a spice. The black seed is cultivated commonly in Konya, Burdur, Isparta and Afyon regions in Turkey. *Nigella sativa* consists of essential oil (0.38-0.49%), protein (20-30%), fixed fat (30-40%), melantin, saponin, tannin and nigellin. *Nigella sativa L.* has many beneficial effects such as wound healing, antimicrobial, antibacterial, antihelminthic, antitumor, anti-inflammatory, antihistaminic, vasodilator, bronchodilator, regulates allergic reactions, reduces sugar and cholesterol, stimulating bone marrow, increases interferon production, decrease anxiety [24-28].

Bruits and Bucar [26] found that *Nigella sativa* L. oil had especially antioxidants and free radical sweeping features but not prooxidant. *Nigella sativa* L. has been widely used to healing wound [29-33]. Sarkhail et. al, 2011 [34] investigated the burn healing efficiency of Black seeds (*Nigella sativa* L.) The results of the study suggest that burn wound healing potential of seeds may be due to anti-inflammatory, antioxidant and antimicrobial activities of main compounds oil [34].

In a study by Yaman *et al.* [31], using *N. sativa* in a rat model of burn wound injury was associated with improved wound healing with no macroscopic or microscopic signs of infection. In another experimental study by Paheerathan *et al.*, revealed *Nigella sativa* L. seed powder shows mark significant accelerating wound healing activity in incised wound model on wistar albino rats [35].

A study found that thymoquinone as one of the *N. sativa*-active compounds could prevent oxidative damage. *N. sativa* is thought to speed up wound healing through scavenging free radicals [36]. According to Zareian *et al.* study, modulating inflammation and using antioxidants contribute to speeding up wound healing [37]. *N. sativa* extract has antioxidant property that is even more potent than the antioxidant properties of synthetic antioxidants [26]. Accordingly, it can be argued that one of the reasons for speeding up wound healing is antimicrobial and antiseptic effects. None of *N. sativa* extract-treated rats acquired infection in this study, which is consistent with Aydin *et al.* studies [36].

Using human gingival fibroblast as a monolayer, aqueous extract of *N. sativa* exhibited low free radical scavenging activity and induced gingival fibroblast proliferation with accelerated wound closure activity despite its non-significant effect on collagen synthesis [32].

Sari *et al.*, 2013, [38] showed that *Nigella sativa* L. oil gel reduced inflammation and improved reepithelialization and granulation tissue formation in the wounds of diabetic rats. Another study of Sari *et al.* 2018, [39] showed that wound reepithelialization was also better with the Aloa vera gel than with the *Nigella sativa* L. oil gel [39]. Yaman *et al.*, [31] Many study showed that *Nigella sativa* L. oil could promote the healing of oral ulcers and burn wounds [31, 39, 40]. *N. sativa* seed essential oil was also associated with significant reduction of wound surface area compared to the control and STZ-DM groups. These findings are consistent with the results of the present study.

This study revealed that diabetic wounds displayed impairment in re-epithelialization, cell migration and proliferation, as well as granulation tissue formation, leading to deteriorated wound healing process. These abnormal patterns were clearly improved by NSE treatment. During proliferation phase, fibroblasts are stimulated to migrate and proliferate into the wound for production of the matrix proteins, hyaluronan, fibronectin, proteoglycans, as well as collagen fibre. Previous studies demonstrated that fibroblasts isolated from diabetic wounds had lower migratory activity and mitogenic responses, compared with non-diabetic wounds [20, 41]. Due to high glucose levels, migration and proliferation of keratinocytes were impaired, resulting in inadequate re-epithelialization [21].

Additionally in our study, wound closure rates were assessed daily throughout the study and it was found that this rate was significantly higher ( $p < 0.001$ ) in the STZ-DM+NSE group compared to the control and STZ-DM groups. After 3<sup>th</sup>, 10<sup>th</sup> day and 14<sup>th</sup> day in wound treatment, macroscopical findings demonstrated that the granulation tissue was more smooth and a live and histological parameters showed that wound healing developed healthier and vascularization reduced more markedly in experimental groups compared to control and STZ-DM.

The induction of experimental diabetes in the rats using chemicals which selectively destroy pancreatic  $\beta$ - cells is very convenient and simple to use as streptozotocin (STZ) that acts as diabetogenic agent mediated by reactive oxygen species [40].

Under diabetic condition, inflammation and oxidative stress have been extremely prolonged with a sustained expression of inflammatory cytokines, proteolytic enzymes, and large amounts of reactive oxygen species that directly damage protein structures of extracellular matrix components and modify the function of several cell types, leading to an impaired wound healing process [7].

The production of free radicals is an integral part of body metabolism but imbalance results is oxidative stress. The excessive lipid peroxidation results in destruction of cellular membranes that could leads to cell death and degenerative disorders [42]. Previously, scientists elaborated that the *N. sativa* essential oil tends to normalize the level of lipid peroxidase, lactate dehydrogenase, glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) enzymes [43].

Some other researchers outlined the importance of *N. sativa* L. as antioxidant that could be useful in pathological conditions related to free radicals. However, the combination and in vivo role of oxidative stress and antioxidants is still a matter of conjecture. The antioxidant potential seems inversely linked with oxidative damage, however, the interactions between antioxidant potential of *N. sativa* in oxidative stress conditions needs research interventions conducted comprehensively. In this study, we investigated to the role of *Nigella sativa* L. fixed and essential oils in improving the antioxidant status and attempted to explore their mechanisms and molecular targets. For the purpose, we measured the oxidative damage due to oxidative stress and determined the antioxidant potential of the body through measuring MDA, GSH levels; GPx, SOD and CAT activities total in plasma and wound tissues.

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## 5. Conclusion

These results demonstrate that the *Nigella sativa* L. essential oil may improve acute cutaneous wound repair, mainly via shortening the duration of epitalization. Topical application of *Nigella sativa* L. essential oil may be considered as an alternative therapeutic modality to enhance cutaneous wound healing. *Nigella sativa* L. essential oil might have a role in reducing of the oxidative stress, lipid peroxidation and associated complications and plays a utility role in the treatment of diabetic wound. Therefore we believe that *Nigella sativa* L. essential oil can be used as an alternative agent to existing treatments in the future wound healing due to its antioxidant, antimicrobial and anti-inflammatory effects.

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## Compliance with ethical standards

### *Acknowledgments*

We appreciate the expert technical help of Prof. Dr. Saban Kordali.

### *Disclosure of conflict of interest*

Betul Apaydin Yildirim and Semin Gedikli declare that they have no conflicts to declare.

### *Statement of ethical approval*

The experiments were conducted according to the ethical norms approved by the Ethic Committee of Experimental Animal Teaching and Researcher Center (No: 06.10.2017 36643897-000-ATA-131).

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