Review Article

Efficacy of *Nigella sativa* in Wound Healing

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Skin rupture (open wound) intensifies bacterial colonization in underlying injured tissues. It initiates as a focal infection and might develop into an invasive and systemic infection. Multi-drug resistant bacteria have emerged as a global issue with their rising infections in humans and various animal species. The development of efficient alternative therapies could minimize antibiotic usage by countering antibiotic resistance. Alternative therapies can particularly be beneficial in treating mild infections. *Nigella sativa*, also known as black cumin or black seeds, is a medicinal herb native to the eastern Mediterranean, Northern Africa, Southwest Asia and the Indian subcontinent. The efficacy of *Nigella sativa* against Gram-positive and Gram-negative bacteria has been demonstrated in multiple studies. *Nigella sativa* is known to possess antioxidative, anti-inflammatory, wound healing and antibacterial properties. The antiseptic and antibacterial capability seems to facilitate the speedy recovery of wounds. This review summarizes recent studies related to *Nigella sativa* wound healing properties.

Key words: Wound, *Nigella sativa*, wound healing, antibacterial, anti-inflammatory, antioxidant properties

The skin shields the internal tissues from thermal, chemical and mechanical injuries. Bacterial colonization in the internal tissue increases after the skin rupture, which could develop into serious infection. Bacterial contamination and interaction inside the wounded tissues could hinder the healing process. Non-healed wounds are often characterized by pathological inflammation and suppurative discharge, which require broad-spectrum antibiotics to curb the microbial population[1]. However, multi-drug bacterial resistance has become a major public health-related global concern[2,3]. The international guidelines of antimicrobial usage in medicine consider *Nigella sativa* (*N. sativa*) and its active ingredient Thymoquinone (TQ) as an alternative therapy for treating skin lesions in animals and humans[4-7].

The presence of multi-drug-resistant bacteria is worldwide and their infections are increasing in humans and animals[8]. Therefore, the emergence of alternative therapies is necessary to reduce antibiotic applications for combating antibiotic resistance. Alternative therapies could especially help in treating mild infections. In this regard, *N. sativa* with antibacterial characteristics could serve as an effective alternative to traditional antibiotics[9].

Wounds are injuries that break the skin or other body tissues. Wounds can be only minor cuts or major injuries. Once a rupture in the skin occurs, our bodies immediately start the repair process to heal the damaged skin. Bacterial contamination causes wound infections. The outcome of wound healing is dictated by the interaction of the bacteria and the patient. The body sets a series of dynamic process, usually known as the "cascade of healing," in order to heal the damaged tissues[10]. These cascades of wound healing include highly organized cellular, humoral and molecular mechanisms[11]. They are classified into 3 overlapping cascades and synchronized in time and activity which include inflammation, proliferation and remodeling[12]. Abnormal wound healing occurs as a result of disruption of any one of these processes[13]. It is well established that sustained inflammation may lead to inadequate wound repair and subsequent fibrotic development[13]. At the cellular level to get complete wound repair, a perfect interaction of several cells, growth factors and cytokines are needed for healing of damaged skin[14]. T-lymphocytes migrate to the affected area and play a crucial role during the process. In addition

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macrophages, fibroblasts, platelets, neutrophils, monocytes, endothelial cells and keratinocytes[11] produce various growth hormones, cytokines and other survival or apoptosis-inducing agents which are of utmost importance in the wound healing process[15].

The active compounds in *N. sativa* extract are known to possess antioxidative, anti-inflammatory and antibacterial properties that facilitate the wound healing process[16,17]. Nevertheless, *N. sativa* extract has been revealed to induce chronic toxicity, which could be minimized by the addition of honey and other ingredients[18]. Therefore, this review summarizes the recent studies regarding the wound-healing properties of *N. sativa*, honey and their mixture.

MICROBIOLOGY OF CHRONIC WOUNDS

Bacterial contamination is the main problem that causes infections and may in many instances delays the process of repairing the damage. However, wound healing can take place in the presence of bacteria and some of the bacteria enhance the process. The interaction between the bacteria and the patient dictates the outcome of the wound healing. Physicians should recognize the stages of wound contamination, colonization and infection[19].

Contamination of wounds:

Chronic wounds usually get contaminated. This was done with the indigenous microflora and/or the surroundings.

Wound colonization:

A very common procedure for wound colonization involves the proliferating bacteria without a host response. Such bacteria are normal microflora of the skin like *Staphylococcus epidermidis*, *Pityrosporum* species, *Brevibacterium* species, *Corynebacterium* species and other coagulase-negative *Staphylococcus* and *Proprionibacterium acnes*[20,21].

Wound infection:

This results because of the invasion of proliferating bacteria not only the top outer layers of the wound, but also the deeper healthy viable tissues that are present on the periphery of the wound, leading to host response. Many pathogens are involved in this type of infection: Beta-hemolytic *Streptococcus* (*Streptococcus pyogenes*, *Streptococcus agalactiae*), *Proteus*, *Stenotrophomonas* (*Xanthomonas*), *Escherichia coli* (*E. coli*), *Klebsiella*, *Acinetobacter*, *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas*. The normal skin microbiota is predominating in early acute wound. This is followed by Beta-hemolytic *Streptococcus* and *S. aureus*. These are common organisms present in the ulcers in diabetic feet. Polymicrobial infection by facultative anaerobic Gram-negative bacterial rods like *Proteus*, *E. coli* and *Klebsiella* will be followed 4 w later. In the case of long-term chronic wounds infection, than aerobes more anaerobes will be found like *Stenotrophomonas* (*Xanthomonas*), *Acinetobacter* as well as *Pseudomonas*[20,21].

WOUND HEALING STAGES

Inflammation, proliferation and remodeling are the three crucial stages of wound healing. Blood and lymphatic fluids start outpouring from the wound immediately after the damage whereas the body's initial response is to stop the bleeding. The process known as blood clotting or hemostasis initiates within seconds to minutes post-injury. The body's emergency repair system is activated to restrict drainage for avoiding blood loss. Blood clotting stops bleeding and facilitates the healing of the affected skin area. During the process, arterial vasoconstriction occurs in the wounded endothelial lining and platelets start to aggregate in the damaged area. The platelet clumping and thrombosis initiation lead to the Adenosine 5' Diphosphate (ADP) release. The vasodilation soon follows the vasoconstriction allowing more thrombocytes and White Blood Cells (WBCs) to reach the injury site[22].

The initial inflammatory phase is a combination of chemotaxis and hemostasis. The inflammatory process is enhanced through the release of cytokines and mediators by the thrombocytes and WBCs. In addition, the synchronized secretion of multiple platelet-derived growth factors occurs to promote collagen degradation, re-epithelialization and growth of the new vessels. These growth factors enhance collagen synthesis by stimulating fibroblast division and multiplication[23,24].

Platelet activation causes the adhesion of inflammatory cells with the fibrin scaffold. Neutrophils carry out wound disinfection through the phagocytosis of bacteria and cellular debris[24].

The next step is the proliferative or granulation phase. During this phase, fibroblasts construct new glycosaminoglycans and collagen to stabilize the wound[25].

Then, the surrounding cells migrate towards the wound to begin the re-epithelialization. Initially, a

thin epithelial layer is made followed by a durable thicker layer to close the wound. It is followed by new vessels formation (angiogenesis), neovascularization and endothelial progenitor cells-based vessel formation (vasculogenesis)[26].

The laying of collagen fibers on the fibrin framework helps to mature the wound followed by enhanced wound contraction through continuous myofibroblasts and fibroblasts deposition[27]. Fig. 1 summarizes various wound healing stages.

N. sativa CHARACTERISTICS

N. sativa has been utilized since ancient times and received special attention in Islamic prophetic medicine. Prophet Muhammad PBUH mentioned that *N. sativa* could cure all types of diseases except death. The therapeutic efficacy of *N. sativa* has also been mentioned in religious books including the Bible[28]. There are multiple common names to describe *N. sativa* such as black cumin, black seed and "Habbatussauda". It is grown in various countries (Saudi Arabia, Pakistan, Turkey, India and Syria) on different continents (North Africa, Asia and Southern Europe). Black seed is comprised of numerous trigonal seeds[29].

TQ is the main ingredient of *N. sativa*. Both have been subjected to thorough *in vivo* and *in vitro* studies to assess their nephroprotective, anticancer, neuroprotective, antibacterial, anti-inflammatory and antioxidant properties[30,31].

N. sativa seeds have been used for centuries to cure various disorders. Several active compounds have been reported from *N. sativa* seeds including thymol, TQ and carvacrol (fig. 2). Multiple *in vivo* and *in vitro* pharmacological investigations have revealed the impacts of *N. sativa* seeds on different body organs[32]. The results indicated a broad range of pharmacological properties such as anti-inflammatory, antimicrobial, anticancer, anti-immune-boosting features[32]. *N. sativa* wound healing capability is supposed to mainly depend upon its antioxidant properties[16,33]. The animal models-based toxicity studies of *N. sativa* seeds and TQ have confirmed that they are safe to consume orally[34].

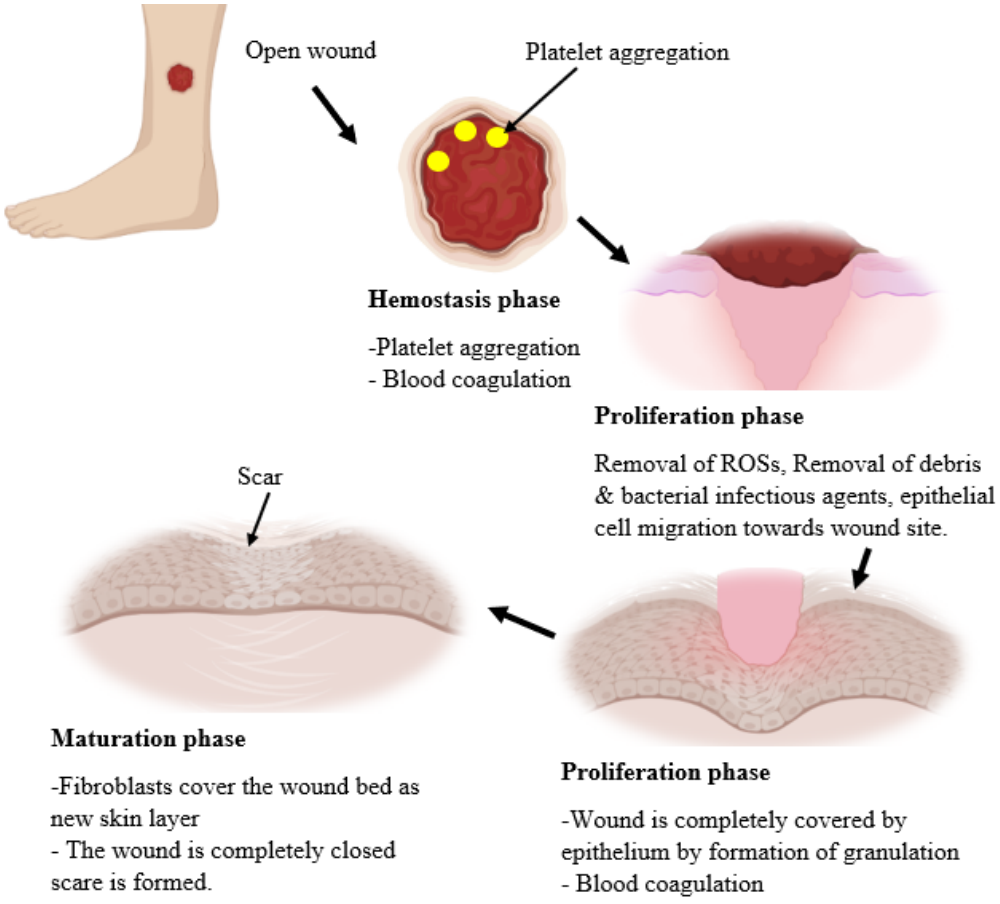

Fig. 1: Schematic illustration of the steps of wound healing and its characteristics

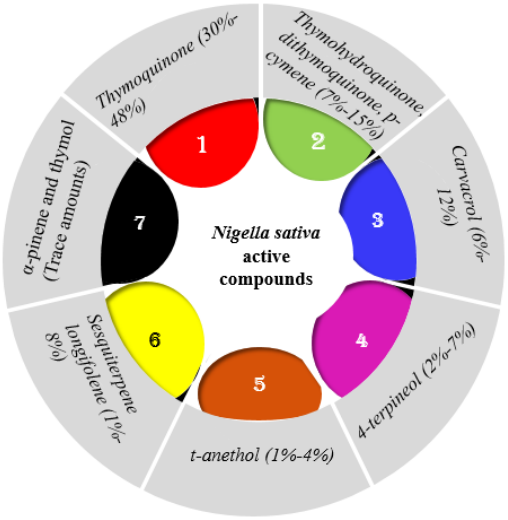

Fig. 2: Schematic diagram showing the most active compounds of *N. sativa* seeds

N. sativa is an annual, herbaceous and spontaneously growing trichomes-covered plant that could reach a height of 40-90 cm[18]. The shape of the *N. sativa* fruit is similar to a swollen capsule bearing 3-7 follicles and a large number of seeds are present in each follicle[35]. At ripening, the capsule opens and seeds fell outside to turn black. *N. sativa* medical applications started almost 2000 y ago in Asia and expanded to Africa and Europe[9]. *N. sativa* plant served as a spice to cure various ailments such as influenza, asthma, cough, hypertension, bronchitis, diabetes, headache, inflammation, eczema, dizziness and fever. *N. sativa* seeds have also been used to treat various diseases[36]. Moreover, the superficial rubbing of *N. sativa* seeds ether extract on the *staphylococcal* infected mice skin enhanced the healing process by reducing total and absolute differential WBCs counts, local infection, bacterial expansion, tissue impairment and inflammation[37]. Similarly, *N. sativa* aqueous extract has demonstrated a lower free radical scavenging activity and instigated fibroblast proliferation to enhance wound closure without affecting collagen synthesis[38].

Anti-inflammatory properties of *N. sativa*:

Medicinal plants are a valuable commodity. The applications of their extracts for healing skin wounds date back to ancient times[39]. This study elaborated that the hydroethanolic *N. sativa* extract (40 %) treatment was the best for healing wounds in diabetic rats within a short span. *N. sativa* ointment also exerted anti-inflammatory effects during the early healing period. Anti-inflammatory characteristics of *N. sativa* have also been established by Pop *et al.*[16].

Chen *et al.*[40] have revealed a common administration of nonsteroidal anti-inflammatory drugs after surgery to reduce inflammation, relieve pain and improve general conditions for enhancing the wound healing process. Therefore, medicinal plants like *N. sativa* with anti-inflammatory properties could help in accelerating the wound healing process[41].

N. sativa seeds and oil are known to effectively heal the wounds of farm animals[42]. *N. sativa* seed ether extract possesses anti-*staphylococcal* properties and could heal mice skin infections in addition to wound healing and tissue. It reduces WBC differential counts (absolute and total) to counter inflammation and local infection[37]. The application of *N. sativa* aqueous extract monolayer on gingival fibroblast reduces free radical scavenging and initiates the proliferation of gingival fibroblast to increase the wound healing potential without impacting collagen synthesis[38]. The mechanism involves the Transforming growth factor-beta (β) and increased basic fibroblast growth factor. The TQ administration (systemic or topical) has been reported to reduce oxidative stress and inflammation and enhance wound closure and healing process[43].

Anti-bacterial potential of *N. sativa*:

The efficacy of *N. sativa* against Gram-positive and Gram-negative bacteria has been demonstrated in multiple studies[44]. The antiseptic and antibacterial capability seems to facilitate the speedy recovery of wounds. Mahmoud *et al.* have revealed the absence of bacterial infection in rats treated with *N. sativa* extract[45].

During a study, *Staphylococcus aureus* growth was significantly stunted at an *N. sativa* extract concentration of 300 mg/ml in comparison to negative (distilled water) and positive (Azithromycin)

controls[35]. The active ingredients such as melanin and TQ are considered to mainly inhibit bacterial growth[34].

Different types of *N. sativa* crude extracts have been tested to assess their antibacterial potential against 6 Gram-positive and 16 Gram-negative isolates. Previously, the selected isolates had exhibited multiple kinds of antibiotic resistance, especially the Gram-negative isolates. *N. sativa* crude extract demonstrated promising antibacterial efficacy against some of the tested isolates. The water and alkaloid crude extracts of *N. sativa* were found to be most effective against these bacterial isolates. These extracts were observed to be more effective against Gram-negative bacteria as compared to Gram-positive bacteria[36]. Hannan *et al.* investigated the antibacterial potential of *N. sativa* against methicillin-resistant *Staphylococcus aureus* clinical isolates. The results revealed that all the methicillin-resistant *Staphylococcus aureus* strains were susceptible to *N. sativa* ethanolic extract at a concentration of 4 mg/disc whereas the Minimal Inhibitory Concentration (MIC) range was noted as 0.2-0.5 mg/ml.

According to Khksari *et al.*[46] the inhibition of wound infection (bacterial infections) accelerates the wound-healing process. Therefore, wound recovery is rapid in response to antibiotic administrations that help in controlling the infection[46]. Similarly, Salehi *et al.*[47] have reported that the antibacterial potential of *N. sativa* could ease the wound healing process.

N. sativa also contains thymol, which is considered a natural antibacterial ingredient and it can be used either individually or combined with other compounds. The antifungal, antibacterial and antioxidant properties of thymol are well-established[47]. Thymol presence in *N. sativa* in large quantities helps its hydroethanolic extract to rapidly counter skin and wound infections. Similarly, large thymol amounts in *Mentha piperita* essential oil could enhance the wound-healing process in rats[48]. During a study, the topical applications of *N. sativa* extract and mupirocin on neonates suffering from *Staphylococcal* pustular skin infections produced almost identical results. The applications of *N. sativa* extract against highly resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus*, have produced promising results in various investigations[49,50].

Acute and chronic lesions could be treated in various ways. Among these, antimicrobial therapy is also common as the treatment of bacterial-infected wounds becomes difficult. The removal of devitalized foreign matter from the extracellular matrix through surgery is also a complicated procedure. Several failures in recent wound treatment methods have been described whereas some techniques are quite expensive to follow.

Antioxidant potential of *N. sativa*:

N. sativa oil content ranges from 0.1 % to 1.5 % mainly containing TQ along with other ingredients[51]. Different *N. sativa* ingredients perform synergistically to exhibit antioxidant properties. Antioxidant characteristics present in it act as a potential cancer treatment tool. The reduced lipid peroxidation marker levels in the plasma enhance the antioxidant capability and facilitate the repairing of the cellular membrane's function and structure. Together, they provide a defensive environment to fight against cancer[51]. Ahmad *et al.*[35] have revealed that TQ prevented oxidative damage during *in vivo* and *in vitro* studies. The free radical scavenging by *N. sativa* could quicken the wound-healing activity[52]. Zareian *et al.*[53] have also elaborated that antioxidants based inflammation modulation helps in rapid wound healing. The antioxidant properties of *N. sativa* extract were found to be more potent than synthetic antioxidants (Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA))[54]. *N. sativa* seeds were also observed to exhibit gastroprotective activity in rats against indomethacin-induced ulcers and it reduced the ulcer index in comparison to the controls. The antioxidant activity plays a key role in the gastroprotective property as it increases the total hexose in gastric mucus secretion and gastric mucosa. *N. sativa* seeds could significantly decrease malondialdehyde protein levels, which is concurrent with the rise in mucus content, total thiol and total hexose as compared to the control group without influencing gastric acid secretion[55].

ROLE OF *N. sativa* IN WOUND HEALING

Wound healing is a complex process that should be overviewed from a histological perspective. Blood clotting is initiated immediately after the happening of the wound through thrombin production. Subsequent natural sub-processes that follow the blood clotting include inflammation, connective tissue cell proliferation and maturation[56].

During the first phase (a few hours after the wound incidence), inflammation cells (neutrophils or immunity-related multinuclear cells) move to the wound site for scavenging. The rise in neutrophil numbers enhances wound inflammation. Therefore,

the wound heals quickly at a smaller number of neutrophils. Later, halfway through the first phase, the number of immune system multinuclear cells decreases with the rise in mononuclear immune system cells. The inflammatory mononuclear cells are crucial for releasing different cytokines. These cytokines participate in the healing process through fibroblast retrieval and migration to the injury site. Moreover, during the final stages of the first phase, the vascular regeneration of epithelial tissue is initiated with a decrease in inflammation and infection-causing agents[57]. The histological investigations depicted a very small number of inflammatory neutrophils in the *N. sativa* extract-treated groups. Therefore, the inflammation in *N. sativa* extract-treated groups was lower as compared to diabetic phenytoin-treated and diabetic untreated groups. Similarly, rapid wound healing was observed in *N. sativa* extract-treated groups than in other groups. These findings are consistent with Joughi *et al.*[58] who reported anti-inflammatory activity and antibacterial ingredients of *Hypericum perforatum* flower extract. The combination of cotton oil and *Hypericum perforatum* ointment significantly decreased the population of inflammatory neutrophils and inflammation severity in the wounds. Likewise, the anti-inflammatory and antibacterial characteristics of *N. sativa* could reduce the inflammatory cell population and inflammation in wounds[52].

The decrease in infection-causing agents and inflammation during wound healing leads to angiogenesis to form the granulation tissue[59]. The tissue examinations have demonstrated significantly higher numbers of vascular epithelium in *N. sativa* extract-treated groups as compared to untreated groups. These findings confirm that topical *N. sativa* application could increase vascular regeneration or angiogenesis, which is similar to previous investigations[58].

The connective tissue cell proliferation or reproduction is the second phase in wound healing that begins on the 3rd d of wound incidence. This phase is characterized by angiogenesis, epithelialization, collagen deposition and formation of granulation tissue, which restore the skin tissue integrity. During this phase, immune cell numbers reduce at the wound site whereas fibroblast migration increases towards the wound[60].

A significant increase in the fibroblast migration towards the wound has been observed in tissues topically treated with *N. sativa* extract as compared to the phenytoin-treated and untreated tissues. Joughi *et al.*[58] have reported that the changes during the maturation phase could be linked to the antioxidant potential of the pharmaceutical agent. *N. sativa* antioxidant activity has been established, therefore, it could facilitate proliferation and rapid wound progress. Other plants with antioxidant potential might also exert similar impacts that could be established through further investigations.

Maturation is the third phase of wound healing, which is characterized by increased collagen synthesis and organization of collagen, alteration of granulation tissue into scar tissue, cell apoptosis and reduction in wound area. During the study, the collagen fibers were noted to be more organized with the increased diameter of the epidermis in the specimens treated with hydroethanolic extract ointment of *N. sativa* in comparison to other groups. Further microscopic examinations confirmed better collagen fibers organization and epidermis formation. These results confirmed previous reports about *N. sativa* properties and further elaborated that *N. sativa* extracts topical application could effectively mitigate the inflammation to enhance wound healing. However, *N. sativa* usage in diabetic patients requires in-depth clinical trials to assess its efficacy, safety and possible activity mechanisms in wound healing[56].

The scavenging of free radicals by *N. sativa* is considered as the main mechanism concerning wound healing and damaged skin repair. Topical *N. sativa* applications are also known to improve vascular regeneration or angiogenesis. Nourbar *et al.*[61] have reported a significant wound healing in diabetic rats in response to the topical application of *N. sativa* hydroethanolic extract in comparison to the controls. *N. sativa* and TQ participate in Type 2 Epithelial-Mesenchymal Transition (EMT) to boost wound healing, reduce inflammation and restrict organ fibrosis through EMT modulation[62].

Angiogenesis and enhanced fibroblast proliferation followed by collagen synthesis are the main features of *N. sativa* based wound healing[63]. Furthermore, *N. sativa* also alleviates bacterial infection and WBC numbers in the damaged tissue[17].

TQ is the main ingredient of *N. sativa*[64]. Bordoni *et al.*[33] have thoroughly investigated its four favourable properties (hepatoprotective, anticancer, antioxidant and anti-inflammatory) based on the 406 empirical evidence. It was further elaborated on the gastroprotective, antihistaminic, nephroprotective and antimicrobial properties of TQ[17]. Different

models have been developed to assess the effectiveness of wound healing therapies. Animal models are available at the pre-clinical level to study the co-morbidity at different skin depths. The wounds can be classified as full-thickness, partial-thickness and superficial based on the wound thickness. Superficial wounds (abrasion) are present at the epidermis level. Partial-thickness wounds (burns or lacerations) are present at the epidermis and dermis levels. The full-thickness wounds (gunshots or incisions) include all the skin layers and could expand to bones and muscles[17].

Several investigations have demonstrated enhanced *N. sativa*/TQ-based healing during all the phases of three wound types. *N. sativa* improves the healing process by restricting further bacterial infection and tissue damage, and by decreasing WBC count (absolute and total)[65]. Free radicals are known to obstruct wound healing activity. *N. sativa* oil could minimize the tissue protein carbonyl and malondialdehyde levels by promoting glutathione peroxidase, catalase and superoxide dismutase enzyme activity of wound healing[66]. The fatty acids (linoleic acid and oleic) of *N. sativa* help in maintaining the water barrier, which promotes wound healing through the selective transfer of components[67]. Fatty acids also activate neutrophil phagocytosis and release growth factors and cytokines to improve the wound condition[17]. Recently, nanotechnology has been employed to improve the *N. sativa* and TQ efficacy. Alexander *et al*.[68] conducted an *in vitro* study and reported that TQ-Loaded Nanostructured Lipid Carrier (TQ-NLC) presented better migration, proliferation and antioxidant efficiency than TQ alone. The impact was more prominent on 3 d Transfer, Inoculum 3×10^5 cells (3T3)-L1, which confirmed its antidiabetic and antioxidant potential. Turhan *et al*.[69] used a rat model to demonstrate the significantly higher efficiency of the Nano-silver and *N. sativa* oil combination in the wound healing process, which also notably reduced the scar formation in comparison to their individual treatments. During another study, the topical application of *N. sativa* essential oil on the wound of diabetic rats significantly increased oxidative stress and lipid peroxidation after streptozotocin administration[70].

N. sativa and honey are safe and cheaper natural resources that are being used for centuries to cure multiple human diseases[71]. Their combination could enhance the wound healing process by restricting the *N. sativa* seed-extract toxicity, especially when applied on a large surface area to cure chronic infections[18]. Allwayzy *et al*.[72] indicated an improved ear-wound healing process followed by significantly reduced wound area after 4 w in response to the application of black seed oil and honey mixture. The results confirmed the wound healing without any cell toxicity. Javadi *et al*.[73] employed a rat model to study the synergistic efficacy of *N. sativa* and honey mixture on wound healing. The mixture caused a significant reduction in the surface area of the wound as compared to the control group.

CONCLUSION

Impregnated tissue-engineered scaffolds of *N. sativa* showed promising results. Sharifi *et al*.[74] have suggested a nontoxic Poly (ε-Caprolactone (PCL))/ Poly (Lactic Acid (PLA)) novel hybrid scaffolds concentration in combination with *N. sativa* extract for a better wound healing process. These scaffolds are structured through double-nozzle electrospinning into the nanofibers to enhance cell proliferation, cell viability and biological efficiency. Several animal models based laboratory investigations have revealed promising synergistic efficacy of *N. sativa* and honey mixture for wound treatment. This is a novel approach that demands further clinical investigations to evaluate different types of *N. sativa* extracts and honey for wound treatment.

Conflict of interests:

The authors declared no conflict of interest.

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This article was originally published in a special issue, "Advanced Targeted Therapies in Biomedical and Pharmaceutical Sciences" Indian J Pharm Sci 2023:85(1) Spl Issue "173-181"