Effects of Nigella sativa and Hypericum perforatum on wound healing

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Abstract: In this study, it was aimed to determine the influence of applying Nigella sativa (NS) and Hypericum perforatum (HP) on wound healing. A total of 42 female albino 2-month-old Wistar rats were randomly separated into 3 main groups. Each of these main groups was separated into two subgroups for 7- and 14-day postoperative follow-ups. A total of 84 excisional skin wounds, 5 mm in diameter, were created on the backs of the rats. Placebo cream (control group), 50% NS oil cream, and 50% HP oil cream were applied topically for the first, second, and third group, respectively. The wounds were checked daily. Seven rats from each group were euthanized on the 7th and 14th days after the operation. Skin samples taken from wound areas were examined histologically and biochemically. The measurements revealed that the wounds healed faster in the study groups than in the control group (P < 0.001). It was found that NS had more antioxidant properties than the other treatments, and HP increased wound healing via its positive effects on epithelialization and granulation. The clinical, biochemical, and histological findings showed that NS and HP increased the healing of skin wounds.

Key words: Nigella sativa, Hypericum perforatum, wound healing, rat

1. Introduction
Skin wounds have long healing processes, and therefore they decrease the life quality of patients. The healing of wounds in a short time without any infections or scars is the desired outcome. In recent years, there have been many studies conducted on alternative treatment methods for wound healing (1–4). The studies related to wound healing aim to achieve wound healing without scar formation as well as accelerating the wound healing (4). However, studies are still ongoing to achieve optimal wound healing.

From ancient times, herbal drugs have been used to treat wounds, and in many cultures their uses continue in traditional medicine. Historically, all medicinal preparations were derived from plants. Today, a significant number of drugs are developed from plants that are active against a number of diseases (5).

Nigella sativa (NS) extract and its oil have antineoplastic, antibacterial, antifungal, anthelmintic, antiinflammatory, and antioxidant effects, and they stimulate the immune system. These effects stem from the active compounds in the contents of NS. There are many active compounds in NS such as thymoquinone, dithymoquinone, thymol, carvacrol (volatile acids), nigellicine, nigellimine-N-oxide, nigellidine (alkaloids), and α-hedrin (triterpene) (6–8). It has been suggested that thymoquinone acts as an antioxidant and prevents membrane lipid peroxidation in tissues (9).

Hypericum perforatum (HP) is used in various ways in wound healing. It is reported in the literature to have antispasmodic, sedative, antiseptic, and antidepressive effects as well as wound-healing effects (10,11). In addition, it is known to be effective against bacteria, viruses, inflammation, and pain (11,12).

Today, traditional medicine is commonly used in wound healing. The aim of this study was to compare the effects of NS and HP in the form of an oil cream in concentration of 50% on wound healing in terms of clinical, biochemical, and histological aspects.

2. Materials and methods
2.1. Chemicals
NS, HP oil cream, and placebo cream were used. To prepare NS and HP oil creams, NS oil (Hel-Kim, 50 mL, Mersin, Turkey) and HP oil (Aksu Vital, 20 mL, Istanbul, Turkey) were separately mixed with equal amount of placebo cream.
2.2. Animals
Forty-two 2-month-old female Wistar albino rats weighing 220–250 g were used and the study was conducted in the Fırat University Experimental Research Center. Standard rat food was given to the rats, and they were left free in the cages. The approval for the study was received from the Local Ethics Committee of Fırat University, Turkey (Protocol No: 2015/09). The rats were anesthetized by administering 6 mg/kg xylazine hydrochloride (Rompun, 23.32 mg/mL, Bayer) and 85 mg/kg ketamine hydrochloride (Ketalar, 50 mg/mL, Parke-Davis) intramuscularly.

2.3. Treatment protocol
Two open skin wounds, 5 mm in diameter, were formed on both sides of the median line of the backs of the rats by punch biopsy (Figure 1). The same procedure was repeated for all the rats, and a total of 84 excisional skin wounds were created. Each of the rats had two wounds in total. The rats were randomly separated into 3 main groups, each comprising 14 rats. The placebo cream, 50% NS oil cream, and 50% HP oil cream were used topically for the first group (control group, n = 14), second group (n = 14), and third group (n = 14), respectively. The main groups were separated into two subgroups, each having seven rats to be examined on the 7th and 14th days. The applications were performed twice a day during the follow-up period. After the operation, seven rats from each group were euthanatized on the 7th and 14th days. Skin samples were taken from the rats for histological and biochemical examinations.

![Figure 1](image.png)

**Figure 1.** Effect of topical application of 50% NS and 50% HP on wound healing. Control group: The group that received no treatment.
2.4. Examination of the wound and wound area measurements
The wounds were checked on a daily basis. Statistical comparisons of wound measurements were carried out on the 7th and 14th days postoperatively. The borders of the wounds were drawn on transparent paper and recorded. These drawings were transferred to millimetric paper to measure the wound areas. The day on which wound contraction started, the rate of the contraction of the wound, the day on which epithelialization was first observed, and the last wound size were evaluated in these examinations.

2.5. Biochemical analysis
The malondialdehyde (MDA) levels in the skin homogenate were measured using the thiobarbituric acid reaction in accordance with the method described by Placer et al. (13) and were expressed as nmol/g of tissue. The skin catalase (CAT) activity was measured in accordance with the method described by Aebi (14) and expressed as kat/g of protein. The protein concentration was also measured in the supernatant according to the method described by Lowry et al. (15). The glutathione (GSH) level of the skin was measured in accordance with the method described by Sedlak and Lindsay (16) and expressed as nmol/g of skin tissue. The glutathione peroxidase (GPx) activity in skin tissue was defined in accordance with the method described by Matkovics et al. (17) and expressed as U/g of protein. The production of superoxide radicals produced by xanthine and xanthine oxidase, following the reaction of nitro blue tetrazolium and the formation of formazan dye, was used to measure superoxide dismutase (SOD) activity (18).

2.6. Histological examination
Necropsies of the rats were performed after euthanasia, and the skin samples were transferred to a 10% buffered formalin solution. The samples were processed with routine follow-up processes and immersed in paraffin blocks. The sections (5 µm) taken from the blocks were evaluated by staining with hematoxylin and eosin under a light microscope in terms of epithelialization, collagen accumulation and granulation tissue, angiogenesis, and inflammatory cell infiltration.

2.7. Statistical analysis
SPSS 22.0 was used for the statistical analysis. Nonparametric Kruskal–Wallis H test analyses were used to identify differences between the groups. The nonparametric Mann–Whitney U test was used to calculate statistical differences between the 7th and 14th days within each group. The values were presented as mean ± standard error of mean (SEM). P < 0.05 was considered significant.

3. Results
3.1. Clinical results
Mortality was not seen in the rats during the study. Rats did not damage the wounds of other rats during the follow-up period. A lesser degree of inflammation signs was found in the NS and HP groups compared to the control group. A significant difference was not observed in terms of wound colors among the groups during the postoperative period. Less exudate was observed in the wounds treated with NS or HP cream. The measurements of the wound areas revealed that the wound healing was better in the NS and HP groups when compared to the control group (Figure 1).

The results of the wound measurements are given in Table 1, and the wound areas and the contraction rates are given in Table 2. It was observed that the wound areas were significantly smaller in the NS and HP groups as compared to the control group on days 7 and 14 (Table 1, P < 0.001). Wound healing increased significantly in all groups between the 7th and 14th days (Table 1, P < 0.01). According to the results (Table 2), the percentages of wound contraction were greater in the NS and HP groups than in the control group. The largest wound areas (%) were observed in the rats of control group.

3.2. Biochemical results
Table 3 shows that the topical applications of NS and HP led to a decrease in the MDA level on the 7th day (P < 0.001) and an increase in the GSH (P < 0.01), CAT (P < 0.001), GPx (P < 0.001), and SOD (P < 0.01) levels. A significant decrease in the MDA level and an increase in the GPx and SOD levels (P < 0.001) were found in the NS group when compared with the other two groups on the 14th day (P < 0.001). The GSH and CAT levels were lower

Table 1. Influence of the topical application on the wound size changes (mm²).

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>NS</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19.625</td>
<td>19.625</td>
<td>19.625</td>
</tr>
<tr>
<td>7</td>
<td>7.75 ± 0.42ab</td>
<td>1.08 ± 0.10ab</td>
<td>1.58 ± 0.15ab</td>
</tr>
<tr>
<td>14</td>
<td>3.66 ± 0.16ab</td>
<td>0.06 ± 0.02ab</td>
<td>0.25 ± 0.06ab</td>
</tr>
</tbody>
</table>

The values are given as mean ± SEM.
Control: The group that received no treatment. NS: Topical application of 50% NS oil cream. HP: Topical application of 50% HP oil cream.
ab, Values in the same row with different letters are significantly different (P < 0.001).
A,B, Values in the same column with different letters are significantly different (P < 0.01).
SEM: Standard error of the mean.
in the NS group than in the other two groups on the 14th day (P < 0.001). The biochemical data showed significant changes between the 7th and 14th days within each group (Table 3).

### 3.3. Histological results

The levels of epithelialization, granulation tissue, collagen accumulation, and angiogenesis on the seventh day were higher in the rats in the HP group compared to the control and NS groups. No differences were noted between the groups in terms of inflammatory cell infiltration (Figure 2).

The levels of granulation tissue and collagen accumulation were also higher in the rats in the HP group than in the control and NS groups on the 14th day. However, no differences were observed between the groups in terms of epithelialization, angiogenesis, and inflammatory cell infiltration. In addition, the severe edema observed in the granulation tissue of the control group was less in the HP group (Figure 2).

### 4. Discussion

Wound healing is a biological process including inflammation, cell migration, collagen deposition, angiogenesis, and reepithelialization (3,4). There are many studies conducted on herbal treatment methods to speed
up the wound-healing process. The herbal products that will be used for this purpose must not be toxic or have side effects (1–4).

Herbal drugs have been used as therapeutics since ancient times (5). Many therapeutic effects of NS extracts, including antiinflammatory, antioxidant, antibacterial, anthelmintic, antidiabetic, and antitumor effects, have been reported (6–9). HP has also been reported to have antispasmodic, sedative, antiseptic, and antidepressants effects on wound healing (10–12). In this study, it was aimed to comparatively investigate the effects of NS and HP on wound healing in a wound model. This study also aimed to develop a fast, efficient, reliable, easy, and cheap alternative treatment method without complications through clinical, biochemical, and histological evaluations.

Figure 2. Seventh day: Control group: immature granulation tissue and hemorrhagic areas (*); NS group: immature granulation tissue and hemorrhagic areas (*); HP group: increased granulation tissue (*), neovascularization (arrow head), and epithelialization (arrow) [(hematoxylin and eosin (H&E)]. 14th day: Control group: granulation tissue in severe edema (*); NS group: granulation tissue (*); HP group: mature granulation tissue (H&E).
Different test animals such as the rabbit (1) and rat (19–21) have been used in various studies. The rat model used in this study is simple and reproducible. The wound healing model provides an in vivo approach for investigating the healing of wounds in domestic animals.

It has been reported that there are different methods to create wound models (1,19). In this study, punch biopsy was used to create round full-thickness wounds. The use of such a simple technique saved time in creating the wound model.

Studies have shown that infection is the primary cause of mortality in patients with wide wounds. For this reason, many investigators have tried to design suitable treatment procedures to decrease the risk of infection of wounds and shorten the duration of treatment in patients with wounds (22,23). In this study, no mortality was seen in the animals. It was observed that a lesser degree of inflammation signs was found in the treatment groups than in the control group. In addition, less exudate was determined in the NS and HP groups compared with the control group.

Wound contraction is another key property of wound healing that contributes to minimization of infection and promotion of rapid wound closure (20). In this study NS and HP caused significantly accelerated wound contraction as compared to the control group on days 7 and 14 (P < 0.001). According to the wound area and the contraction rates, wound healing was found to be better in the treatment groups (Tables 1 and 2) (P < 0.001). The results obtained in the study were consistent with previous findings (10,24).

Oral treatment with NS seeds ensured 80% protection against oxidative stress and carcinogenesis. However, a combination of honey and NS seeds ensured a 100% protection. The serum MDA and NO levels significantly decreased in both cases compared with the active controls (25). Intraperitoneally administered thymoquinone, which is an active component of NS, was found to be efficient in decreasing the oxidative stress in an aorta ischemia and reperfusion model (26). Hosseinzadeh et al. (6) reported that thymoquinone and NS had inhibitory effects against the lipid peroxidation process in cerebral ischemia and reperfusion injury in rats. It was also found that the HP extract had flavonoids and phenolic acids, and exerted antioxidant and antiinflammatory effects in animal models (10–12).

On the 7th and 14th days of the study, the MDA, GSH, CAT, GPx, and SOD levels were examined, revealing antioxidant effects of HP and NS. NS had the highest antioxidant influence (Table 3).

The deposition of collagen, which is observed in the proliferative stage of the healing process, indicates wound healing (27). NS was reported to have antimicrobial, antioxidant, antiinflammatory, and immunomodulatory properties, and the NS used in a burn model sped up the healing process in a histological manner (7). In the present study, the granulation tissue and collagen deposition in the wound area constantly increased in the HP group on the 7th and 14th days after the injury compared with the other groups. These findings were consistent with those of the previous studies (21,27).

The amentoflavone, hyperforin, and hypericin in HP render antiinflammatory, antibacterial, and antiviral properties to the plant (28). HP increased fibroblast migration, collagen deposition, and revascularization; sped up tissue regeneration; and ensured better epithelialization (10,29). In the present study, the angiogenesis and epithelialization in the rats in the HP group on the 7th day and the granulation tissue and collagen accumulation on the 7th and 14th days were higher compared with the other groups, as also shown by previous studies (10,21,29).

It was also reported that HP increased wound healing significantly in second-degree experimental thermal burns compared with sulfadiazine treatment if used four times a day (24). Mukherjee et al. (21) demonstrated that HP increased wound healing when administered orally. Another study (30) found that HP helped in healing cesarean wounds.

The biochemical data obtained in this study showed that the antioxidant effect of NS was greater than that of HP. NS was found to speed up wound healing, probably owing to its antioxidant property, and HP contributed more to the wound healing in histological examinations.

It can be concluded that NS exerts a wound-healing effect through its antioxidant property while HP enhances wound healing via epithelialization and granulation-encouraging effects. The topical application of NS and HP in the form of an oil cream at 50% concentration increased open wound healing in a reliable manner.

In addition, it is thought that the data obtained in this study may be a source of reference in wound healing or treatment.

References
