Effects of *Nigella sativa* and silver sulfadiazine on burn wound healing in rats

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ABSTRACT: This experiment was conducted in order to compare the effects of *Nigella sativa* (NS) and silver sulfadiazine (SSD) cream on healing of burn wounds in rats. Fifty four adult, male Wistar-albino rats were divided into three groups of equal numbers. A burn was generated on the backs of all the rats. The burned areas in the first, second and third groups were covered with daily cold cream (control), SSD cream and NS cream (50% NS oil + 50% cold cream), respectively. Four, nine, and 14 days later, the rats were sacrificed and the burned skin tissue samples were collected for histopathological examinations. Histopatological evaluations on the 4^{th}, 9^{th} and 14^{th} days showed burn healing to be better in the NS and SSD groups with respect to the control group. Wound healing was significantly different among the groups at 4^{th}, 9^{th} and 14^{th} days ($P < 0.001$). In conclusion, application of NS and SSD cream are effective in healing burn related skin wounds in the rat model.

Keywords: *Nigella sativa;* silver sulfadiazine; burn; rat

Silver sulfadiazine (SSD) is the topical agent of choice in severe burns and is used almost universally today in preference to compounds such as silver nitrate and mafenide acetate. SSD cream, while being effective, causes some systemic complications including neutropenia, erythema multiforme, crystalluria and methemoglobinemia (Gracia, 2001; Gregory et al., 2002; Hosnuter et al., 2004).

Nigella sativa (NS), an annual herbaceous plant of the Ranunculaceae, has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of various diseases (Phillips, 1992). NS contains 36–38% fixed oil, proteins, alkaloids, saponins and 0.4–2.5% essential oil (Lautenbacher, 1997). The main compounds are thymoquinone (30–48%), *p*-cymene (7–15%), carvacrol (6–12%), 4-terpineol (2–7%), *t*-anethole (1–4%) and the sesquiterpene longifolene (1–8%) (Burits and Bucar, 2000). Several pharmacological effects, e.g., antibacterial, antiparasitic and anti-inflammatory effects have been attributed to NS or its active compounds which include thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellimine-*N*-oxide, nigellidine and alpha-hedrin (Randhawa and Al-Ghamdi, 2002). Thymoquinone is reported to prevent oxidative injury in various *in vitro* and *in vivo* studies in rats (Daba and Abdel-Rahman, 1998; Mansour et al., 2001; Yaman and Balikci, 2010). Thymoquinone has been suggested to act as an antioxidant and was reported to prevent membrane lipid peroxidation in tissues (Mansour et al., 2002). These effects seem to be related to inhibition of eicosanoid generation, namely thromboxane B2 and leucotrienes B4 (by inhibiting cyclooxygenase and 5-lipooxygenase, respectively), and membrane lipid peroxidation (Hosseinzadeh et al., 2007). Varol (2008) suggested application of topical NS oil to accelerate wound healing.

The purpose of this study was to compare the effects of NS cream dressing and SSD on clinical and histological healing rates of skin burn wounds in a rat model.

MATERIAL AND METHODS

All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were

approved by the Committee on Animal Research at University of Firat, Elazig, Turkey.

Chemicals. NS oil (Copyright © 2005 Origo Gıda Kimya Tarim Urn. San. Ve Tic. Ltd. Sti. – Gaziantep, Turkey; the company produces NS oil by cold pressing fresh seeds without the use of chemicals), SSD cream (Silverdin, Deva, Silver sulfadiazine, 10 mg/g) and cold cream (Botafarma, 12.5% spermacetin + 12% white wax + 56% liquid paraffin + 0.5% borate of soda + 19% distilled water) were used in this study.

Animals. Fifty four adult, male Wistar-albino rats weighing approximately 200–250 g and aged six to eight months were used in this experimental study. Rats were supplied by the Experimental Animal Unit of the University of Firat in Elazig, Turkey. Within the same unit, the rats were housed in plastic cages in a temperature-controlled room with a 12-h light/dark cycle, and were fed *ad libitum* with rodent chow. The rats had free access to water throughout the experiment.

Anesthesia. The rats were anesthetized with single intramuscular injections of 6 mg/kg xylazine hydrochloride (Rompun, Bayer, 23.32 mg/ml) and 85 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, 50 mg/ml).

Thermal injury. The rats were anesthetized, their backs were shaved and prepared with 10% antiseptic povidone-iodine solution (Kim-Pa, Poviiodeks, 10% povidone-iodine) and burn areas of 1 cm diameter were created.

Skin burns were created as described by Durmus et al. (2009), Han et al. (2005) and Hosnuter et al. (2004). Animals were subjected to full-thickness second-degree skin burns with 1 cm surface area diameter by brass probes. The brass probe was immersed in boiling (100 °C) water until thermal equilibrium was reached. The probe was then placed on the back of the rats for 20 s without applying pressure. All animals were immediately resuscitated with lactated Ringer's solution (2 ml/100 g body weight) applied intraperitoneally.

Animal experiments. Rats were randomly divided into three equal groups of eighteen animals each:

1. Control group: Immediately after burning, burn areas were covered with cold cream twice a day for 14 days.
2. SSD group: Immediately after burning, burn areas were covered with SSD cream twice a day for 14 days.
3. NS group: Immediately after burning, burn areas were covered with 50% NS oil + 50% cold cream twice a day for 14 days.

The wounds were clinically observed in all groups every day. Four, nine, and 14 days later, the rats were sacrificed after being anesthesized.

Histopathological examination. Burned skin tissue samples were collected after sacrificing the rats for histopathological examination purposes. These tissue samples were fixed in 10% neutral-buffered formalin solution, embedded in paraffin wax, cut into 5 μm-thick sections and stained with hematoxylene-eosin and Masson's trichrome stain for examination by light microscopy.

Statistical analysis. The thickness of granulation tissue was examined at the centre of each wound and recorded. The data were expressed as means ± standard errors (SEM). Differences between group means and between days four, nine, and 14 were estimated using a one-way analysis of variance (ANOVA) and a Duncan test was performed for multiple comparisons using the SPSS 12.0 for Windows. Results were considered as statistically significant at $P < 0.001$.

RESULTS

No mortality was observed in the animals during the study.

Wounds in the control group displayed a greater degree of inflammation on the basis of the three clinical signs of the inflammatory process: heat, redness, and swelling which appeared to be lessened in wounds treated with NS and SSD.

Histopathological evaluations on days four, nine, and 14 showed the burn healing to be better in the NS and SSD groups with respect to the control group (Figure 1).

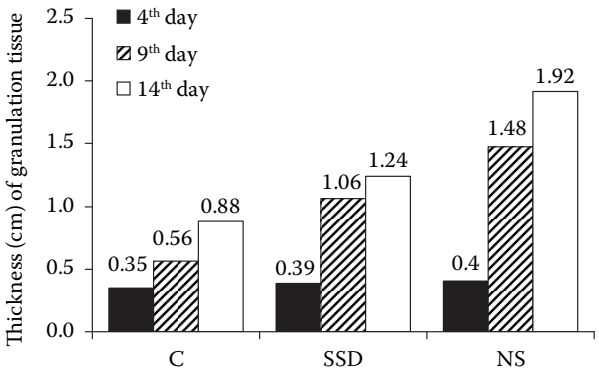

Figure 1. Comparison of the thickness (cm) of granulation tissue in the centre of the wound in experimental groups on the 4th, 9th, and 14th days

A scab formed by necrotic tissue remnants and mononuclear cell infiltration was present in all groups on the 4th day of the trial. Regenerative and reparative attempts in the epidermal layer were also observed. Inflammatory cell infiltration without an epithelial layer was noted under the scab. Vessels were hyperaemic in the dermis and there were no hair follicles, sebaceous or sweat glands in either of the groups (Figure 2)

The scab persisted in all groups until day 9, but was thinner in the NS group. An epithelial layer had partially formed by the same day and regenerative and reparative attempts in the epidermal layer improved substantially in comparison with the 4th day of the trial. Fibrosis in the dermis reached the subcutaneous tissue level (Figure 3).

The scab had fallen off in all groups by the 14th day of the trial, and epidermis was observed to have developed completely. However, the epithelial layer in the NS group had a better appearance when compared to control and SSD groups. Also, granulation tissue in the dermis in the NS group

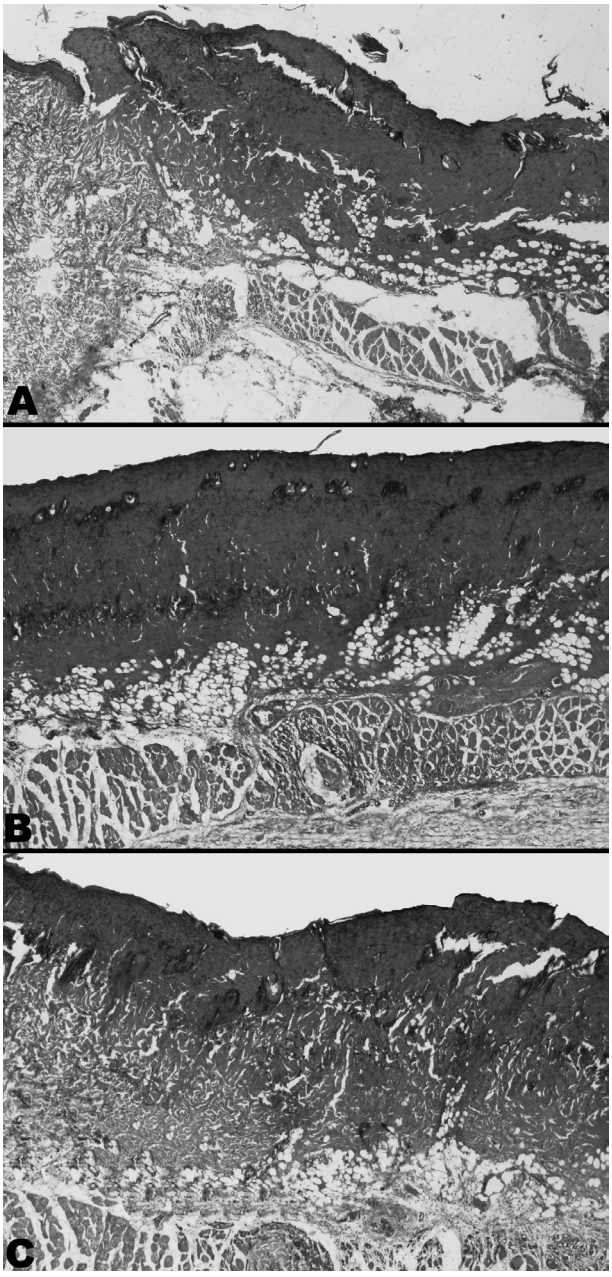

Figure 2. Microscopic appearance of burned skin on the 4th day. A = control group; B = SSD group; C = *Nigella sativa* group (H&E, 40×)

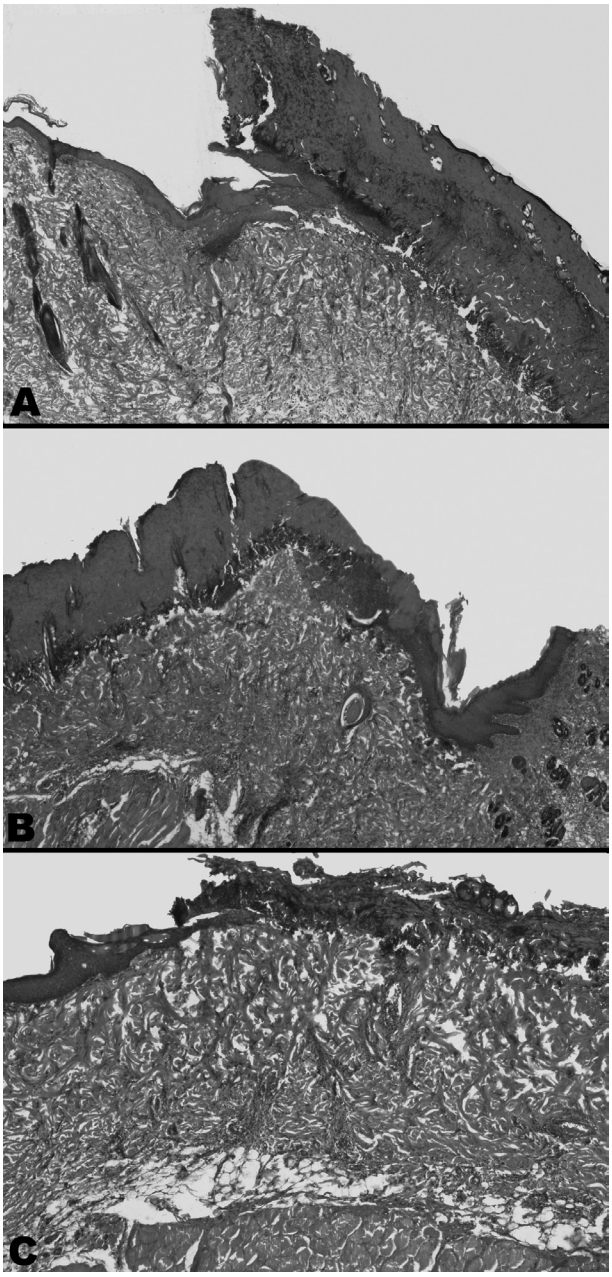

Figure 3. Microscopic appearance of burned skin on the 9th day. A = control group; B = SSD group; C = *Nigella sativa* group (H&E, 40×)

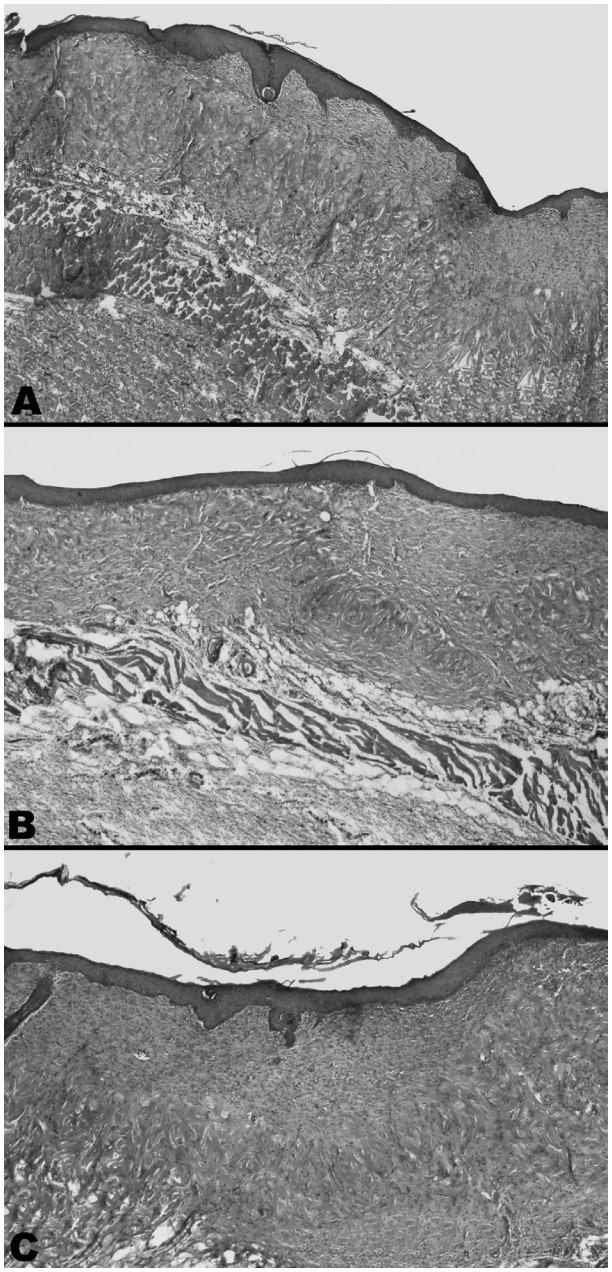

Figure 4. Microscopic appearance of burned skin on the 14th day. A = control group; B = SSD group; C = *Nigella sativa* group (H&E, 40×)

was in better condition when compared with the other groups (Figure 4).

Wound healing was significantly different among the groups at four, nine, and 14 days ($P < 0.001$). On the 4th day of the trial, the thickness of the granulation tissue was almost the same in SSD and NS groups, but statistically differed in the control group ($P < 0.001$). On the 9th and the14th days of the trial, the thickness of the granulation tissue was significantly different among all the groups ($P < 0.001$). The mean values of thickness of granulation tissue in the centre of the wounds for NS, SSD and control are shown in Table 1.

Table 1. Thickness (cm) of granulation tissue in the centre of the wound ($n = 18$).

Days	Groups		
	C	SSD	NS
4	0.35 ± 0.15^{aA}	0.39 ± 0.05^{aB}	0.40 ± 0.04^{aB}
9	0.56 ± 0.66^{bA}	1.06 ± 0.06^{bB}	1.48 ± 0.06^{bC}
14	0.88 ± 0.11^{cA}	1.24 ± 0.02^{cB}	1.92 ± 0.02^{cC}

^{abc}values in the same column with different superscripts are significantly different ($P < 0.001$)
^{ABC}values in the same row with different superscripts are significantly different ($P < 0.001$)

DISCUSSION

Wound healing involves a cascade of events characterized by completion of biological processes in a certain order and a certain time frame. These events represent the restructuring of the damaged tissue in an attempt to restore as normal a condition as is possible. The natural response of a living organism is to repair the wounds in the shortest time possible and to re-establish the normal continuum of the structures (Nayak et al., 2006).

Different test animals such as the pig (Mekkes et al., 1998), Guinea pig (Eldad et al, 1998; Webster et al., 1962) and rat (Hosnuter et al., 2004; Han et al., 2005) have been used in various studies. The model used here is simple and reproducible. The burn wound healing model provides an *in vivo* approach for studying the healing of burn-related wounds in domestic animals.

Topical agents with benefits only as antimicrobials include silver nitrate, sulfamylon and a combination of a sulfonamide and SSD. Sulfamylon has a wide spectrum of activity and as it is easily absorbed systemically, it can result in toxic complications. As a result, SSD became the standard topical treatment for burn wounds (Gracia, 2001). Accordingly, we have chosen SSD in our study.

Plants have been used as therapeutics since ancient times (Jones, 1996). Recently, many therapeutic effects of NS extracts have been documented, including antioxidant (Burits and Bucar, 2000; Yaman and Balikci, 2010), antimicrobial, anti-helmintic (Agarwal et al., 1979), anti-inflammatory

(Houghton et al., 1995), anti-tumour (Worthen et al., 1998), anti-diabetic (Meral et al., 2001) and anti-ulcerogenic (Akhtar et al., 1996) effects in both clinical and experimental studies.

With this study we aimed at comparatively analyzing the effects of NS and SSD on wound healing in a burn wound model. In a study exploring the effects of NS on wound healing in rats, NS oil was found to heal the wound in a significantly shorter period (Varol, 2008). As the ointment applied to the wound was an antimicrobial, the risk of wound infection was reduced to a minimum (Varol, 2008). Likewise in this study, there were no signs of any macroscopic or microscopic infections. We interpret these results to mean that the positive effects of NS on wound healing might be related to its anti-inflammatory, antioxidant and antibacterial properties. In certain studies antimicrobial properties have been reported to accelerate wound healing (Nayak et al., 2006). In a study by Mashhadian and Rakhshandeh (2005), NS seeds were reported to be effective against standard and nosocomial microorganisms like *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In a study investigating the antibacterial effects of NS oil on 24 bacterial organisms (Arici et al., 2005), antibacterial effects were found to originate from thymoquinone, *p*-cymene and carvacrol compounds contained in the oil. In a study on the antidermatophytic effects of NS (Aljabre et al., 2005), the plant was shown to inhibit the growth of eight dermatophyte varieties *in vitro*. This indicates that NS could be used as a antidermatophytic agent in the treatment of fungal skin infections.

Free radicals act on important components of the cell such as lipids, proteins, carbohydrates and DNA resulting in cellular damage and cell death. Free radicals play a role in the pathogenesis of diabetes mellitus, cancer and aging and have unfavourable effects on wound healing, granulation tissue along with collagen and cartilaginous tissues (Keskin et al., 1999). Damage inflicted on skin results in the production of reactive oxygen species and also in a reduction in various enzymatic and non-enzymatic free radical scavengers, which thereby hinders the healing process (Serarslan et al., 2007). Antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) break down free radicals to accelerate wound healing. In a study by Kanter et al. (2006), treatment with NS oil reduced tissue malondialdehyde (MDA) and protein carbonyl levels while preventing the inhibition of SOD, glutathione peroxidase (GSH-Px) and catalase (CAT) enzymes.

In conclusion; in a burn wound model in rats, NS was found to shorten the healing process both histopathologically and statistically as compared to SSD and the control group. Through its antimicrobial, antioxidant, anti-inflammatory and immunomodulatory effects, NS can be used as an adjunctive or alternative agent to existing wound healing therapies in future. However, further studies are certainly needed to shed more light on the healing mechanism of NS.

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