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BURN HEALING POTENTIAL OF *NIGELLA SATIVA* SEED OIL IN RATS

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ABSTRACT

Nigella sativa L. (Black cumin) has showed a broad spectrum of pharmacological effects like antipyretic, analgesic, anti-inflammatory and antimicrobial. These activities may be related to the seeds oil. The most important chemical composition of hexanic extract of seeds was unsaturated fatty acids and essential oil. In this investigation the hexanic extract of seeds was topically applied to evaluate the healing efficiency of seeds oil on the second degree burn wound models in rats. Animal were randomly divided into three groups of six for each group. Silver sulfadiazine was applied as an antiseptic standard drug. Wound healing was assessed by the rate of contraction and histological characteristics in treated and untreated groups. On day 12, the extract-treated animals showed 81.20% reduction in the wound area and were significantly (P<0.05) more than control group 63.31%. Histological study showed fully grown regenerated epidermis on day 12 in treated rats. The present study suggests the burn wound healing action of seeds may be due to anti-inflammatory, antioxidant and antimicrobial activities of major compounds oil.

INTRODUCTION: *Nigella sativa* L. Ranunculaceae, commonly known as black seed or black cumin, is naturally distributed in various regions of Iran and other Middle Eastern and Asian countries [1, 2]. Mature seeds are edible and extensively used as a carminative, condiment and as an aromatic spice in cooking and breads making. The ground seed can be mixed with honey or sprinkled on salads [3]. To a common Islamic belief *Nigella sativa* seed is a remedy for every disease except death. *Nigella* seeds (N.s) are used traditionally for treatment of a broad array of diseases and conditions such as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and gastrointestinal disturbances. N.s oil has also antipyretic, analgesic, anti-inflammatory, antimicrobial, and antineoplastic activity [2].

The seed contains mucilage, crude fiber, fixed oil, sugars, resins, alkaloids, flavonoids, sterols, tannins, saponins and essential oil. Some of these activities could be due to the volatile and fixed oils that contain active compounds. Fixed oil is rich of unsaturated fatty acids, especially linoleic and oleic acid. Thymoquinone (2-methyl-5-isopropyl-1,4-benzoquinone, TQ) is main active compound of the essential oil that is considered as a responsible agent for many of pharmacological effects of seeds such as, antioxidant, analgesic and anti-inflammatory actions [4-6] . Healing of burn wounds still remains a challenge to modern medicine. Though many of antiseptic drugs have been used for treatment of burn injury but they may cause adverse reactions like allergy and toxicity when used for a long period [7-10]. Therefore it is important to find more safe and effective drugs. To the best of our knowledge there are no experimental reports on wound healing activity of *N. sativa*. In this study, we report for the first time, the wound healing potential of hexanic extract of N.s.

MATERIALS AND METHODS:

Plant material: *Nigella sativa* L. (Ranunculaceae) seeds were purchased from the local market in Esfahan, Iran; authenticated by Dr. Parisa Sarkhail and a botanically identified voucher specimen (1387/N1) stored in the Herbarium of Pharmacognosy Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Extraction: Dried and milled seeds (200 g) were extracted with n-Hexane for 3h in a Soxhlet apparatus. The extract (oily fraction) was concentrated under reduced pressure and stored in brown bottle in a refrigerator (4 °C). This oil was analyzed by GC using a Shimadzu GC-17A system equipped with a FID detector, a capillary SGE BX-70 column (30 m/0.25 mm) and nitrogen as the carrier gas. The oven temperature was kept at 130˚C for 1 min, programmed to 185˚C at a rate of 5˚C/min and kept at 185˚C for 2 min, then programmed to 220 ˚C at a rate of 15˚C/min and kept at 220˚C for 3 min. The injection volume was 0.1 μl in the split mode. The constituents were identified by comparison of their retention times with those of reference samples.

Extraction and analysis of volatile oil: 5.5 ml of concentrated extract oil (prepared by the above method) were hydrodistilled for 4 h using a Clevenger-type apparatus for essence providing. The oil layer (0.3 ml) was separated and dried over anhydrous sodium sulfate and stored in brown vial in a refrigerator (4 °C). This volatile oil was analyzed by GC/MS using a Hewlett-Packard 6890/5972 system with HP-1MS capillary column (30 m/0.25 mm; 0.25 μm film thickness). The carrier gas was helium with a flow of 1 ml/min. The split ratio was 1:10. The column temperature was programmed from 40-250°C at 3°C/min. Mass spectra were taken at 70 eV. The constituents were identified by matching their mass spectra in the Wiley library [11] and by comparison of their retention indices with the published values [12], the yield was 1%.

Thermal source: For this study a new device was made by attaching a soldering iron with a flat contact surface (diameter 1.5 cm) on top, to a heater (temperature range 50-200 °C) with digital display (**Fig. 1**). The selected temperature (setting point) was attained manually and it was continuously monitored with a temperature sensor was implanted into the device.

FIG. 1: SOLDERING IRON WITH A FLAT CONTACT ON TOP (DIAMETER 1.5CM) ATTACHED TO A HEATER (TEMPERATURE RANGE 50-200°C) WITH DIGITAL DISPLAY

Burn wound model: Male Wistar rats weighting 250-300 g were used to carry out the present experiment. They were housed individually in standardized environmental conditions, feed with normal diet and water *ad libitum*. This study was undertaken after obtaining the approval of institutional animal ethical committee of Tehran University of Medical Sciences (TUMS). Animals were anaesthetized by injection of pentobarbital (50 mg/kg, i.p.). Burn wounds were created on dorsal part of shaved rats and the underlying skin was cleaned with 70% ethanol. Animals bearing the full-thickness second-degree burn wounds by heated to 100°C for 10 seconds were distributed into three groups each containing 24 animals. Control rats (Group 1) were left undressed, while experimental rats were dressed twice daily treatment with the N.s extract (Group 2) and 1% SSD (Silver sulfadiazine, Behvarzan Pharmaceutical Company, Rasht, Iran), as Group 3.

Rate of wound contraction: The rate of wound contraction was measured as percentage reduction in original wound size at every 4 days interval using a millimeter scale graph paper.

Histochemical analysis: Granulation tissue collected at regular intervals of 4 days and preserved in 10% buffered formalin. A 3-4 µm thickness sections were prepared and stained with haematoxylin and eosin [13] and photographed under 200 or 400 × magnification.

Statistical analysis: All values are expressed as means±S.E. Data were analyzed by one-way ANOVA, followed by Tukey's *post hoc* test. The results were considered significantly different at $P < 0.05$.

RESULTS:

Phytochemical analysis: The solvent extraction of *Nigella* seeds gave rich-yellow oil with an aromatic odor (yield 15%). Linoleic acid (55.27%), oleic acid (21.82%) and palmitic acid (14.86%), were identified as major fatty acids in hexanic extract. The hydrodistillation of the hexanic extract from the N.s gave a pale-yellow volatile oil with aromatic odor. Thirty six compounds, constituting 83.76% of the volatile oil, were identified. The volatile oil presented high levels of *p*-cymene (30.35%), thymoquinone (24.33%), *α*-thujene (14.62), *α*-pinene (3.01), *and β*-pinene (2.84).

Rate of wound contraction: The rate of wound healing was determined by the size of lesions at every 4 days interval after burn injury using a graph paper. Table 1 shows the percentage decrease of wound contraction for each group. The percentage of wound contraction in treated groups was significant statistically when compared to control on the 4^{th}, 8^{th}, 12^{th} and 16 days $P<0.05$. On the 8^{th} day, the percentage of wound contraction in treated rats was much faster when compared with control rats. On day 12, the extract-treated animals showed 81.20% reduction in the wound area and were significantly ($P<0.05$) more than control group 63.31%. In this study, there was no a statistically significant difference in the healing time of burn wounds between extract oil and SSD-treated rats within 16 days.

TABLE 1: PERCENTAGE REDUCTION OF WOUND SIZE IN CONTROL AND TREATED RATS

Drug	Wound concentration (%)on days			
	4	8	12	16
Control	32.02±0.62	45.20±1.31	63.31±3.49	88.35 ± 0.25
Extract	55.38 ± 0.95*	72.02 ± 0.47*	81.20 ± 0.58*	96.62 ± 0.16*
SSD	57.45 ± 0.57*	77.27 ± 0.49*	82.91 ± 0.38*	97.00 ± 0.05*

Values are means ± S.E. for each group of six rats; * significantly different from control, $P < 0.05$

Histochemical study: Histological evaluation was studied on the treated and untreated samples at every 4 days interval. Haematoxylin and eosin stained sections of tissue were examined for inflammatory cells, collagen bundles, epidermal regeneration and skin appendages. Comparison of granulation tissue section from extract treated rats with control group showed significant improvement in the wound healing on 4th day after treatment in extract-treated group (**Fig. 2, A and B**). Figure 2A showed burn wound with infiltration of inflammatory cells between viable and non viable tissue and focal epidermal regeneration while in control group there is no epidermal regeneration (Figure 2B). Day 8 sections showed (**Fig. 3, A and B**) increased regenerated epidermis with underlying organizing granulation tissue and new collagen bundles in treated rats than control. A well-advanced collagen bundles in the upper to mid dermis**,** and fully grown regenerated epidermis were observed in treated rats than control on 12th day but cutaneous appendages were appeared only in treated groups (Figure 4, A and B). On the 16th day, complete epithelialisation and skin appendages were observed in treated and untreated rats.

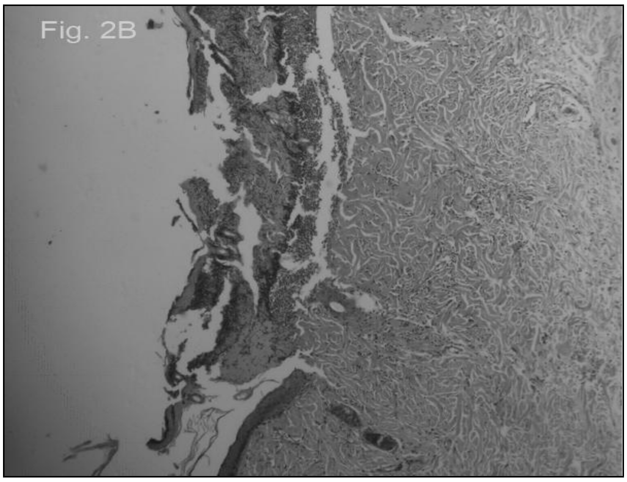
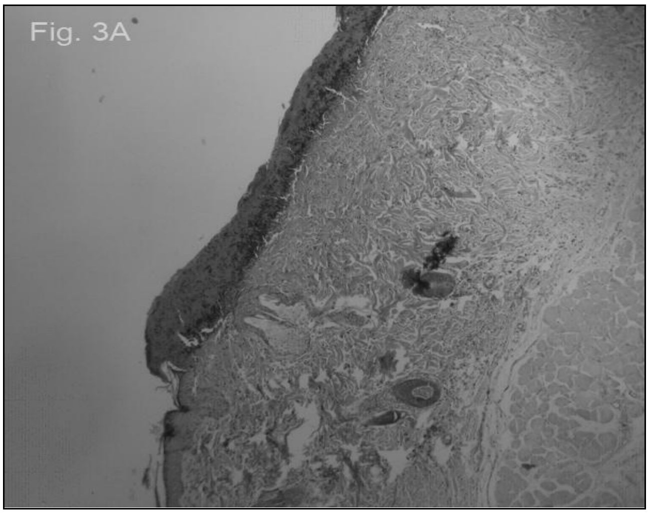
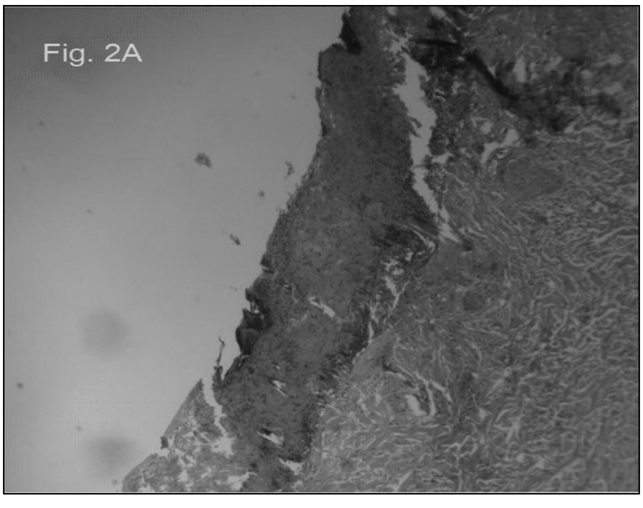


FIG. 2: PHOTOMICROGRAPH OF 4-DAY OLD BURN WOUND OF TOPICAL N.S EXTRACT OIL GROUP (A) AND CONTROL GROUP (B) H & E 200 ×. (A) FOCAL EPIDERMAL REGENERATION, BURN WOUND WITH NEUTROPHILIC INFILTRATION BETWEEN VIABLE AND NON VIABLE TISSUE AND FOCAL EPIDERMAL REGENERATION; (B) BURN WOUND WITH NEUTROPHILIC INFILTRATION BETWEEN VIABLE AND NON VIABLE TISSUE, WITHOUT EPIDERMAL REGENERATION.





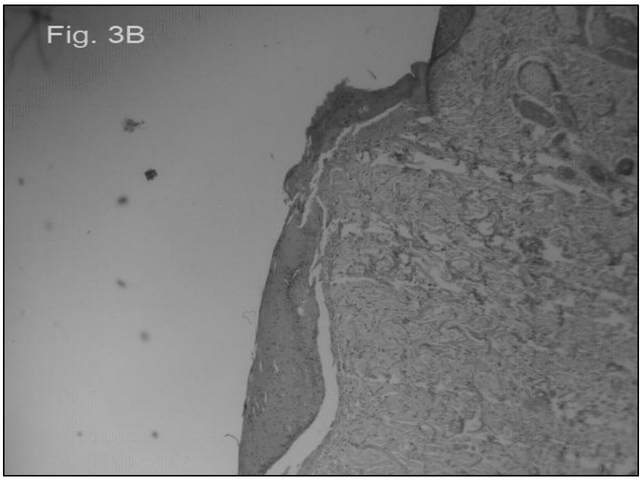
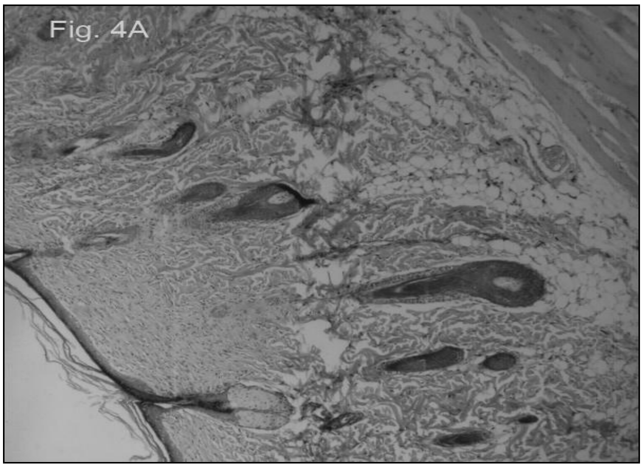


FIG. 3: PHOTOMICROGRAPH OF 8-DAY OLD BURN WOUND OF TOPICAL N.S EXTRACT OIL GROUP (A) AND CONTROL GROUP (B) H & E 200 ×. (A) COMPLETELY REGENERATED EPIDERMIS WITH UNDERLYING ORGANIZING GRANULATION TISSUE AND NEW COLLAGEN BUNDLES, WITHOUT SKIN APPENDAGES; (B) THIN REGENERATED EPIDERMIS (COVERED WITH SCAR) WITH UNDERLYING GRANULATION TISSUE AND NEW COLLAGEN BUNDLES



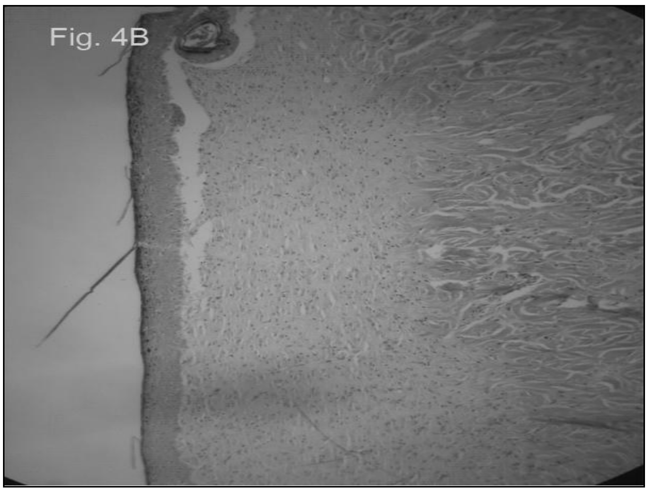


FIG. 4: PHOTOMICROGRAPH OF 12-DAY OLD BURN WOUND OF TOPICAL N.S EXRACT OIL GROUP (A) H & E 400 × AND CONTROL GROUP (B) H & E 200 ×. (A) FULLY GROWN REGENERATED EPIDERMIS. WELL ORGANIZED COLLAGEN BUNDLES IN THE UPPER TO MID DERMIS AND WITH SKIN APPENDAGES WERE OBSERVED; (B) FULLY GROWN REGENERATED EPIDERMIS, WELL ORGANIZED COLLAGEN BUNDLES IN THE UPPER TO MID DERMIS AND FEW INFLAMMATORY CELLS INFILTRATING DEEP DERMIS, WITHOUT CUTANEOUS APPENDAGES.

DISCUSSION: Burn wound healing is a complex process of regenerating dermal and epidermal tissue that some biological mechanisms such as inflammation, granulation tissue formation, collagen synthesis and re-epithelialization involve in healing process. In this study a full-thickness second-degree thermal burn injury was induced on the shaved skin surface by placing a heated soldering iron. Despite the fact that the healing process takes place by itself, it does not require much help, but various risk factors such as infection and delay in healing has brought attention to promote this process. Silver sulfadiazine (SSD), known for its antibacterial effect, is used primarily as a topical burn treatment on second- and third-degree burns but prolonged and excessive application of SSD may results significant risk of systemic absorption and toxicity [8-10].

Results of wound contraction rate, as shown in Table 1, indicated a healing potential for the extract that was comparable to SSD group. On the other hand, the results showed that there was significant difference ($P < 0.05$) between untreated group and treatment groups within 16 days. The healing potential was further confirmed in the histological assay. Histological studies of wound section proved the formation of epidermis and less inflammatory cells infiltrated the dermis in N.s group. After 8 days N.s extract group was close to the normal skin and fully grown regenerated epidermis was observed on 12th day in treated rats. N.s extract oil showed a good potential for acceleration of burn wound healing in rats. These effects might be due to several mechanisms including an increasing collagen synthesis and rate of epithelialization by the effect of an anti-inflammatory, an antimicrobial, and a moisturizing [14, 15].

Nigella sativa seeds have exposed a broad spectrum of pharmacological activities including anti-inflammatory, antidiabetic, antimicrobial [15], antioxidant, immunopotentiation [16], antihistaminic [17] and anti-hypertensive activities [18]. Many of these actions such as anti-inflammatory and antioxidant were due to fixed oil and essential oil compounds of *N. sativa* [2, 5, 19, 20]. Anti-inflammatory and analgesic activities of black cumin seed essential oil may due to the presence of thymoquinone which is an active compound of oil with variety of beneficial effects including anti-oxidative, inhibitory activity against positive and gram-negative bacteria [14] and anti-inflammatory activities [21, 22].

Fatty acids are important components of the cell membrane. Essential fatty acids are required to ensure epidermal integrity and to maintain the water barrier in the skin. On the other hand, fatty acid supplementation like oleic and linoleic acid could stimulate wound healing by enhancement of the total number of cells migrating across the wound line during the reparation process [23]. Additionally, fatty acids have been shown to stimulate neutrophils. These cells play a key role in the healing process by releasing growth factor, inflammatory cytokines, destroy bacteria and remove dead and dying cells from tissue spaces [24]. This may be an important mechanism for the effect of linoleic and oleic acids in acceleration of wound healing process.

In conclusion, the results obtained in this study indicated that topical application of N.s extract oil at the burn wounds significantly stimulated wound contraction and increase wound healing process as compared to control group, which may be due to its anti-inflammatory, antioxidant and antimicrobial activities of N.s oil.

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