ORIGINAL ARTICLE

Nigella Sativa L. Seed Extract Accelerates Granulation Tissue Formation & Wound Healing in Induced Skin Wound

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ABSTRACT

Objective: The study was conducted to evaluate effects of Nigella Sativa L. seed extract on granulation tissue and wound healing in induced skin wound in a rabbit model.

Study Design: Experimental study

Place & Duration: Isra University Hyderabad from July 2013 to December 2013.

Material & Methods: Thirty six male adult rabbits were studied. Group I (n=12) rabbits as control group, Group II (n=12) Wound treated with 1% pyodine Group III (n=12) wound treated with Nigella sativa L. seed extract (NSSE). Wound size was measured on 3rd, 7th and d10th day of experiment. Skin wound tissue samples were embedded in paraffin, and stained with Hematoxylin-Eosin. The data was analyzed on SPSS version 21.0. A p-value of =0.5 was taken statistically significant.

Results: Severe inflammatory reactions were observed at the margins of skin wound in control group compared to pyodine and NSSE. Significant differences in wound size were observed on different days among three groups ($p<0.05$). Robust granulation tissue was seen in the NSSE group with accelerated wound healing compared to control and pyodine group ($p<0.05$). The H & E stained slides showed highly vascular granulation tissue in NSSE group.

Conclusion: The Nigella sativa L. seed extract stimulates granulation tissue formation and accelerates wound healing in induced skin wound in animal model.

Key words: Nigella sativa Granulation tissue Wound healing Skin wound

INTRODUCTION:

Botanically, the Nigella sativa plant belongs to the family of Ranunculaceae. The NS plant is an amazingly spicy herb with historical and religious background. Its dignity as a cure for diseases is mentioned in the religious literature. In Pakistan, it is commonly known as "Kalonji"[1]. The NS plant as well as its seeds has great importance in the old systems of therapeutics such as Unani and Ayurverdic and also in the Allopathic system of medicine. In Southeast Asia, it is publicly known as the Kalonji. In Arabic countries, it is known as the "habat-ul-sauda". The English people call the NS seeds as "black cumin"[2,3]. The NS plant has been a focus of most of the research studies in the modern era. As it has been traditionally used for centuries, hence many studies have been conducted to explore its chemical constituents and biological activities by scientific methods. Several studies on animal models have been conducted to identify the biological activities of N. sativa oil on different components of the metabolic syndrome[4-5]. The most active constituent of NS seeds and oil is the Thymoquinone (TQ). Its chemical name is the "2-isopropyl-5-methyl-benzoquinone" and most of the therapeutic properties are attributed to this constituent. Thymoquinone yields most of the

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bio-therapeutic properties of the NS seeds and oils. Thymoquinone is a promising dietary agent and a chemo- therapeutic and chemo-preventive agent for the treatment of number of diseases[6-7].

The wound infection is very common problem in developing countries Pakistan. Very few studies are available on the effect of Nigella sativa L.Seed extract (NSSE) on granulation tissue formation and wound healing. Therefore, present experimental study was conducted for observing effects of NSSE on granulation tissue and wound healing in a rabbit model.

MATERIAL & METHODS:

An experimental study was conducted on rabbit model at the animal house of Isra University Hyderabad from July 2013 to December 2013. Thirty six male adult rabbits were selected according inclusion and exclusion criteria. Adult male rabbits, weight 1.0-1.5 kilogram were eligible for study protocol. Female and sick rabbits were excluded. Rabbits were kept in stainless steel cages, at room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. Fresh alfalfa and tap water were provided ad-libitum.

Animal Grouping: The experimental animals were randomly divided into three groups; Group I (controls). (n=12) rabbits in which skin wounds were allowed to heal without any dressing material, Group II (experimental group). (n=12) skin wounds were treated with 1% pyodine solution daily and Group III (experimental group). (n=12) in which Nigella sativa L. seed extract (NSSE) was applied on the skin wounds. Experiment Protocol: Skin on the back of rabbits was shaved with electrical clipper and anesthetized with 1% intradermal Xylocaine injection. Tape was applied on skin, and then it was removed by pulling it back quickly on itself from back to forward. The area demarcated (approximately 12 mm) was subjected to repeated adhesive tape stripping until the epidermis and dermis were completely removed. The stripped wounded skin was left open for regenerative purpose according to protocol.

Granulation tissue & wound healing: The size of wound was observed on 3rd , 7th and 10th days of stripped skin. The wounded area was measured by placing transparent tracing paper over the wound and tracing it out. The tracing paper was placed on 1 mm2 graph sheet and traced out. The squares were counted and were recorded.

Animal sacrifice: The animals were sacrificed by over-dose of Ketamine and Xylazil as described by Nayak et al[9]. Histomorphometry examination: The stripped wounded skin samples were collected after sacrificing the rabbits for histological examination on 14th day of stripping. Samples were taken from all the three groups of animals and the entire wound area of 12 mm along with 4-5 mm of surrounding normal skin was excised. The tissue samples were fixed in previously marked containers, containing 10% formaldehyde as preservative. The tissue samples were embedded in paraffin, cut into 4µm thick sections and stained with Hematoxylin-Eosin (H & E) for histological examination.

Data analysis: The data was analyzed on SPSS version 21.0 for Windows release (IBM, Corporation, and USA). The continous variables were presented as mean±SD & range. Analysis of variance and Duncan`s pot Hoc testing were used. p-value of =0.05 was defined significant.

RESULT:

Severe inflammatory reaction was observed as by signs of inflammation hot, red, warm and swollen skin at wound site in controls compared to pyodine and NSSE groups. The rate of wound healing as measured by wound size is shown in Table. I. The wound size as examined on 3rd, 7th, and 10th day showed significant groups. ($p<0.05$) (Table I-III), except the control and pyodine groups which exhibited no significant difference on 5th post wounding ($p>0.05$) (Table I-III). Robust granulation tissue was seen in NSSE groups with accelerated wound healing as compared to other groups. The H & E stained slides of skin were observed under microscope. The histological findings of granulation tissue are shown in figure I-III. Granulation tissue was prominent in the NSSE group.

Table-I: Skin wound size on 3rd day (millimeter)

Group I	Group II	Group III	p-value
10.84±0.15 (10.50-11.1)	10.71±0.13 (10.53-10.8)	10.52±0.06 (9.20 - 10.8)	< 0.041

Table-II: Skin wound size on 7th day (millimeter)

Group I	Group II	Group III	p-value
10.73±0.75 (10.60-10.9)	10.02±0.67 (8.90 - 10.9)	7.40±2.15 (6.30-10.7)	< 0.035

Table-III: Skin wound size on 10th day

Group I	Group II	Group III	p-value
10.16±0.77 (8.80-10.7)	7.38±3.07 (3.50-4.6)	4.10±0.60 (3.50-6.1)	< 0.0.46

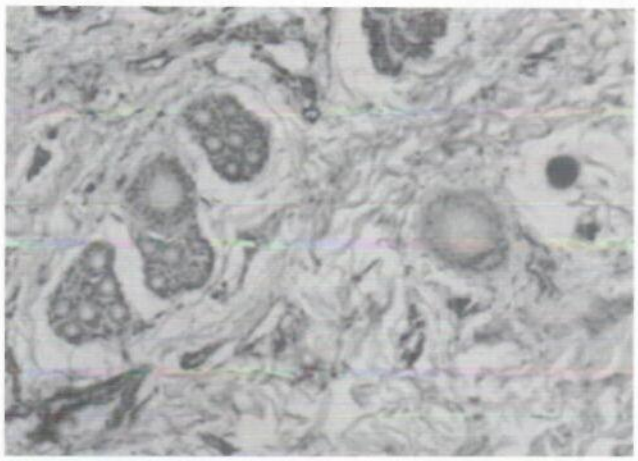

Figure I. Histological slide of control group showing the granulation tissue

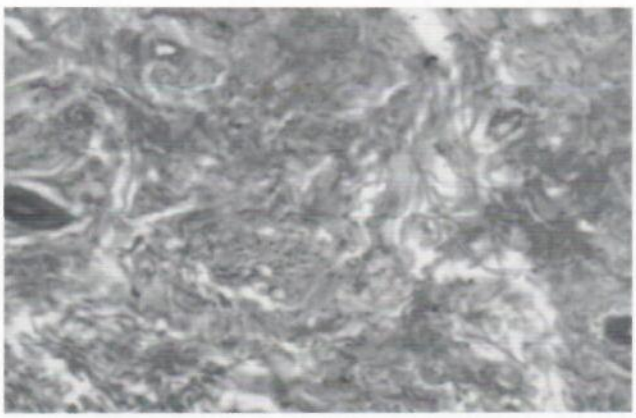

Figure II. Histological slide of pyodine group showing granulation tissue

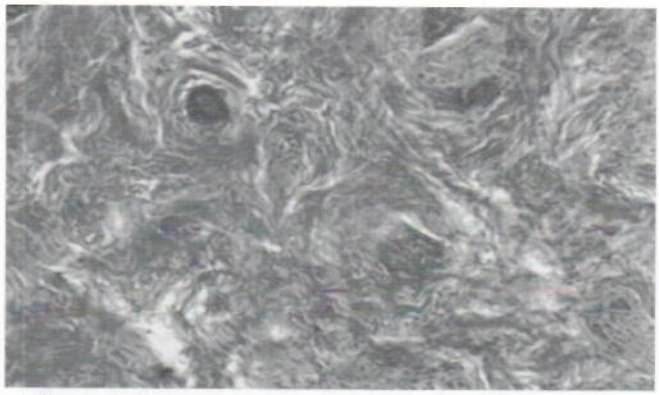

Figure III. Nigella sativa L. seed extract group showed robust granulation tissue

DISCUSSION:

The present research work was undertaken to test previous experiences that whether herbs like NS has any effect on wound healing or not. The present study was purely an experimental study using rabbits as an animal model. The wound healing effect of NS was compared with pyodine and control groups. (Table. I-III) Our results are comparable to studies mentioned in medical literature[10-15]. The findings of present research work regarding the effect of NS on wound healing are in agreement with previous studies[9-10]. We observed better wound healing in the NS group compared with the pyodine and control groups. (Figure I-III). A previous study[9] has reported better wound healing in the NS group compared to pyodine group and controls as evidenced by granulation tissue and histological findings. The findings of our present study are consistent with previous study as accelerated wound healing is attested in NS group in our experimental study. According to many previous studies[11-12], the wound healing effect of NS has been attributed to its antioxidant, antimicrobial and anti-inflammatory effects. In a study, the NS oil was used to observe wound healing effect on wounds in rats. And it was observed that the NS has wound healing enhancing effect as the wounds in NS oil groups were healed in a shorter time compared with antimicrobial creams and pyodine[13]. The findings of this previous study are highly consistent with our present observations. A previous study[14] reported effect of NS on oral ulcers in rabbits and reported that the epithellialization and healing of oral ulcers was completed within

three days in NS group compared with the controls. We also attest enhanced skin wound healing in rabbit model and our findings are parallel to this previous study and this supports our finding that the NS has wound healing effect. One study from Saudi Arabia has reported positive wound healing effect of NS compared with antibiotic group, but the NS group exhibited a mild retardation in the wound granulation tissue compared with other two groups[15]. This finding is not in consistency with our current and previous studies[9-10]. The findings of previous studies helped us to understand the wound healing effect of NS that it might be because of its antimicrobial activity but we are of opinion that this is not a sufficient conclusion. We suggest that the effect of NS on inflammation, blood vessels and cell mitosis should be searched at molecular level to reach at proper conclusion.

CONCLUSION:

The Nigella sativa L. seed extract stimulates granulation tissue formation and accelerates wound healing in induced skin wound in animal model. Histological findings indicate excessive granulation tissue and better wound healing Nigella sativa group.

REFERENCES:

1. Naz H. Nigella sativa: the miraculous herb. Pak J Biochem Mol Biol. 2011; 44(1):44-8.
2. Ahmad Z, Ghafoor A, Aslam M. Nigella Sativa. A potential commodity in crop diversification traditionally used in healthcare. MINFAL/PARC, 2004; 5:34.
3. Kamal A, Arif JM, Ahmad IZ. Potential of Nigella sativa L. seed during different phases of germination on inhibition of bacterial growth. EJBPR. 2010;1(1):9-13.
4. Rabbani MA, Ghafoor A, Masood MS. Kalonji: an early maturing and high yielding variety of Nigella sativa released for cultivation in Pakistan. Pak J Botany.2011; 43:191-5.
5. Najmi A, Haque SF, Naseeruddin MF, Khan RA. Effect of Nigella Sativa oil on various clinical and biochemical parameters of metabolic syndrome. Int J Diabetes & Metabol.2008;16:85-7.
6. El-Din K, El-Tahir H. The Black Seed Nigella sativa Linnaeus - A Mine for Multi Cures: A Plea for Urgent Clinical Evaluation of its Volatile Oil. JTUM.2009;S1(1):1-19.
7. Chah KF, Eze CA, Emuelosi CE, Esimone CO. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. J Ethnopharmacol. 2006;104:164-7.
8. Nayak S, Nalabothu P, Sandiford S, Bhogadi V, Adogwa A. Evaluation of wound healing activity of Allamanda cathartica L. and Laurus nobilis. L. Extracts on rats. BMC Compl Alter Med. 2006;6:12.
9. Yaman I, Durmus AS, Ceribasi S, Yaman M. Effects of Nigella sativa and silver sulfadiazine on burn wound healing in rats. Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey. Veternarni Medicina. 2010;55(12):619-24.
10. Mourad GM, El-Din SGT, Radi SS, Ossman AS, Hassan MAA, Nouh HH. Curcumin versus Nigella sativa L: A comparative study of their possible protective effects on experimentally induced liver injury in rats. J Med Res Inst. 2006;27(3):141-51.
11. Kanter M, Coskun O, Kalayci M, Buyukbas S, Cagavi F. Neuroprotective effects of Nigella sativa on experimental spinal cord injury in rats. Hum Exp Toxicol. 2006;25(3):127-33.
12. Gracia CG. An open study comparing topical silver sulfadiazine and topical silver sulfadiazine-cerium nitrate in the treatment of moderate and severe burns. Burns.2001;27:67-74.
13. Varol Y. Investigation of the effects of Nigella Sativa oil on cutaneous wound healing in rats. [Thesis]. T.C. Marmara Universitesi Saglik Bilimleri Enstitusu, Istanbul 2008.
14. Al-Douri A S. The effect of honey on the healing process of oral ulcer. (Experimental study). Iraqi Dent J.2006;33:7.
15. Abu-Zinadah OA. Using Nigella sativa Oil to treat and Heal Chemical Induced Wound of Rabbit Skin. JKAU Sci.2009;21(2):335-46.