



## Nigella sativa oil extract in healing of induced skin wound

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### ABSTRACT

The present study was conducted to evaluate wound healing potential of Nigella sativa L. oil extract on induced skin wounds in animal model. The present experimental study was conducted at animal house of Isra University from May to August 2013. A sample of 30 male rabbits was divided into three groups; Group 1. (n=10) controls, Group 2. (n=10) treated with Bacitracin powder and Group 3. (n=10) treated with Nigella sativa (NS) oil extract. The skin of rabbits was shaved after anesthetized with 1% xylocaine subcutaneously. The wound size was observed on days 4, 8 and 12. The tissue samples were embedded in paraffin, and stained with H&E and Masson's trichrome staining for microscopy. Data was analyzed on SPSS 21.0 using ANOVA and post-Hoc Duncan's test. Significant p-value was defined at  $\leq 0.5$ . The controls (Group 1) wounds showed severe inflammatory response compared with Bacitracin (Group 2) and NS (Group 3) L. oil extract groups. The animals showed significant differences in wound size days 4, 8 and 12 days among groups ( $p < 0.001$ ) except for controls and Bacitracin groups on 8th day ( $p = 0.06$ ). The wounds in NS group showed abundant robust granulation tissue formation and accelerated wound healing. The Bacitracin group also showed better wounds compared to controls. The microscopy revealed marked vascularity and collagen fibers in NS group compared to either. It can be concluded from present study that the NS oil extract has wound healing potential as indicated by gross wound size and histological findings of skin wound in rabbit model.

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### Introduction

Nigella sativa (NS) 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".<sup>1</sup> The NS plant as well as its seeds has great importance in the old systems of therapeutics such as Unani and Ayurvedic and also in the Allopathic system of medicine. The NS seeds and oil are being imported from India, Egypt, Sri Lanka, Iran and few other countries for medicinal purpose.<sup>2,3</sup> In Pakistan, the cultivation of NS plant was first time introduced in 2002. In Pakistan the most suitable season for its cultivation is in the months of October-November.<sup>2-4</sup> 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". 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.<sup>5</sup> 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 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.<sup>6</sup>

The wound infection is a common problem in surgical wards in developing countries like Pakistan. From search of local literature, it reveals a few studies are available on the role of NS in wound healing. Therefore the present study was planned to observe wound healing potential of NS oil extract in induced skin wound in rabbit model.

### Materials and methods

An experimental study was conducted on rabbit model at the animal house of Isra University from May to August 2013. A sample of 30 male rabbits was divided into three groups; Group 1. (n=10) controls, Group 2. (n=10) treated with Bacitracin powder and Group 3. (n=10) treated with Nigella sativa (NS) oil extract. Adult Wistar male rabbits of 1 year age having weight of 1.0-1.5 kg were included. The skin of rabbits was shaved after anesthetized with 1% xylocaine subcutaneously. The wound size was observed on days 4, 8 and 12. The tissue samples were embedded in paraffin, and stained with H&E and Masson's trichrome staining for microscopy. The 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*.

The size of wound was observed on 4, 8 and 12 days of induced wound. The wounded area was measured by placing transparent tracing paper over the wound and tracing it out. The tracing paper was placed on 1mm<sup>2</sup> graph sheet and traced out.

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The squares were counted and were recorded as described by the Chah, et al (2006).<sup>7</sup> The animals were sacrificed by over-dose of Ketamine and Xylazil as described by Nayak et al. (2006).<sup>8</sup> The stripped wounded skin samples were collected after sacrificing the rabbits for histological examination on 12 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 5  $\mu$ m thick sections and stained with Hematoxylin-Eosin (H & E) and Masson's trichrome staining for histological examination. Photography was carried out.

Data was analyzed on SPSS 21.0 using ANOVA and post-Hoc Duncan's test. The continuous variables were presented as mean $\pm$ S.D and range. Significant p-value was defined at  $\leq 0.5$ .

### Result

The wounds of controls (Group 1) showed severe inflammatory response compared with Bacitracin (Group 2) and NS (Group 3) L. oil extract groups. The animals showed significant differences in wound size days on 4, 8 and 12 days among groups ( $p < 0.001$ ) except for controls and Bacitracin groups on 8<sup>th</sup> day ( $p = 0.06$ ). During the initial days of post-wounding, the degree of inflammation was observed by simple signs of inflammation viz. calor, rubor and swelling among the three groups. The wounds of rabbits in the control group exhibited severe degree of inflammation compared with the Bacitracin and NS groups. The rate of wound healing as measured by wound size is shown in Table. I. The wound size as examined on 4, 8 and 12 days revealed significant and highly significant differences among three groups. ( $p = 0.001$  and  $0.0001$ ) (Table I.). The Bacitracin group also revealed statistically significant reduction in the wound size and healthier granulation tissue compared with controls (Table. I).

The H & E stained slides of skin were observed under microscope. The Bacitracin group also showed better wounds compared to controls (Figure 1-3). The wounds in NS group showed abundant robust granulation tissue formation and accelerated wound healing. The microscopy revealed marked vascularity and collagen fibers in NS group compared to either.

Groups	Days		
	4 day	8 day	12 day
Group 1. (controls)	10.87 $\pm$ 0.18 (10.61-11.8)	10.87 $\pm$ 0.14 (10.56-11.3)	10.58 $\pm$ 0.07 (8.90-11.1)
Group 2. (Bacitracin)	10.84 $\pm$ 0.76 (10.60-10.9)	10.09 $\pm$ 0.76 (8.90-10.9)	7.30 $\pm$ 2.17 (6.50-10.7)
Group 3. (NS oil extract)	10.17 $\pm$ 0.77 (8.90-10.9)	7.48 $\pm$ 3.09 (3.50-7.6)	4.20 $\pm$ 0.70 (3.40-5.1)

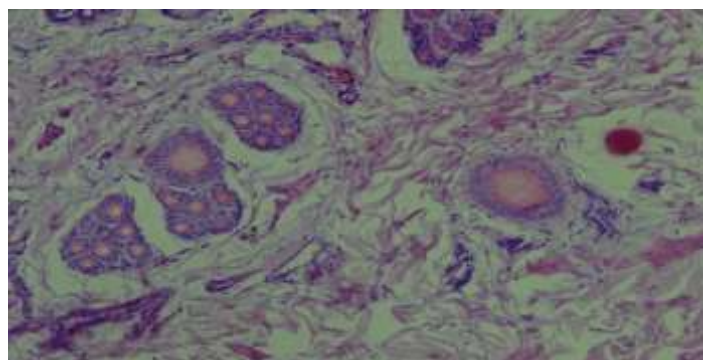


Figure 1. Microscopy of controls

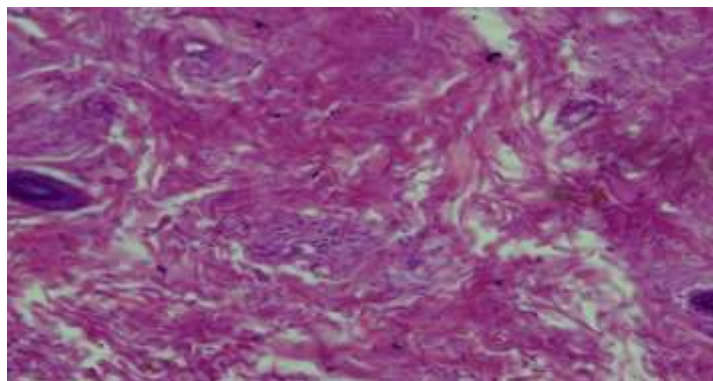


Figure 2. Microscopy of Bacitracin Group

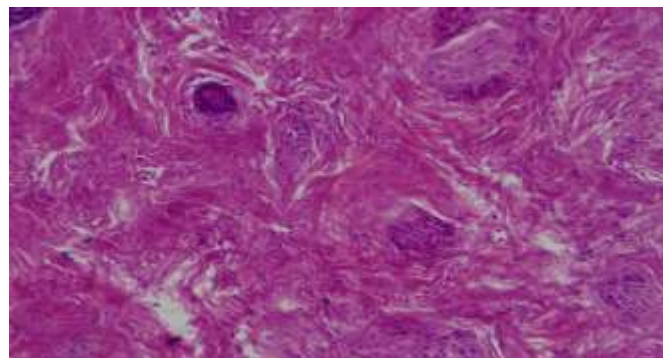


Figure 3. Microscopy of Nigella sativa oil extract group

### Discussion

Our present study shows that the rabbits which received NS L. oil extract were having better wound healing compared with other two groups and we suggest that the NS has wound healing potential (Table. I).

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 Bacitracin powder and control groups. (Table. I)

The findings of present research work regarding the effect of NS on wound healing are in agreement with previous studies.<sup>9,10</sup> We observed better wound healing in the NS group compared with Bacitracin and control groups (Figure 1-3). The study of Yaman, I et al (2010)<sup>9</sup> 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 (Tables. I). According to previous studies<sup>9,11</sup> 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.<sup>12</sup> The findings of this previous study are highly consistent with our present observations. The Al-Douri et al. (2010)<sup>13</sup> conducted a prospective study on wound healing effect of NS on oral ulcers in rabbits and reported that the epithelialization 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.<sup>14</sup> This finding is not in consistency with our current and previous studies.<sup>9, 10</sup> We are of opinion that this might have occurred because of probability of researcher's mistake while applying NS extract or errors in data collection or data analysis.

#### Conclusion

It can be concluded from present study that the *Nigella sativa* L. oil extract has wound healing potential as indicated by gross wound size and histological findings of skin wound in rabbit model.

#### References

- Ahmad Z., Ghafoor, A. and Aslam, M. *Nigella Sativa*. A potential commodity in crop diversification traditionally used in healthcare. *MINFAL* 2004;5:34.
- Kamal A., Arif J M. and Ahmad I Z. Potential of *Nigella sativa* L. seed during different phases of germination on inhibition of bacterial growth. *EJBPR* 2010; 1(1): 009-013.
- Rabbani M A., Ghafoor A. and Masood M. S. (2011). Kalonji; an early maturing and high yielding variety of *Nigella sativa* released for cultivation in Pakistan. *Pakistan Journal of Botany* 2011;43:191-195.
- Najmi A., Haque S.F, Naseeruddin, M.F, Khan, R.A. Effect of *Nigella Sativa* oil on various clinical and biochemical parameters of metabolic syndrome. *International Journal of Diabetes & Metabolism* 2008; 16:85-87.
- El-Din K. and 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; S 1 (1): 1-19.
- 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 - 167.
- 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 Complementary and Alternative Medicine* 2006; 6: 12.
- Yaman I, Durmus A S, Ceribasi S and 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): 619–624.
- Jones FA.: Herbs-useful plants. Their role in history and today. *European Journal of Gastroenterology and Hepatology* 1996; 8:1227–1231.
- Mourad G M, El-Din SGT, Radi SS, Ossman A S, Hassan M A A and Nouh H H. Curcumin versus *Nigella sativa* L: A comparative study of their possible protective effects on experimentally induced liver injury in rats. *J Med Research Inst* 2006; 27 (3):141-151.
- Kanter M, Coskun O, Kalayci M, Buyukbas S, Cagavi F. Neuroprotective effects of *Nigella sativa* on experimental spinal cord injury in rats. *Human and experimental Toxicology* 2006; 25: 127–133.
- 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.
- Al-Douri A S. The effect of honey on the healing process of oral ulcer. (Experimental study). *Iraqi Dent J* 2006; 33: 7.
- Abu-Zinadah OA. Using *Nigella sativa* Oil to Treat and Heal Chemical Induced Wound of Rabbit Skin, *Journal of King Abdul-Aziz University* 2009; 21(2):335-346.