Original Article

Anatomy

A comparative Study of Evaluating Healing Effect of Nigella Sativa on Experimentally Induced Skin Wounds in Rabbit Model

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ABSTRACT

Objective: The aim of present study is to observe the effect of Nigella Sativa (NS) on histo-morphological changes on induced skin wound healing in a rabbit model.

Study Design: An experimental study

Place and Duration of Study: This study was conducted on rabbit model at the animal house of Isra University over a period of six months.

Materials and Methods: Thirty male adult wistar rabbits were divided into three groups; Group I. rabbits as control group, Group II. Wound treated with 1% pyodine Group III. Wound treated with Nigella sativa oil extract. Skin was shaved with electrical clipper and anesthetized with 1% intradermal Xylocaine injection. The size of wound was measured on 5th, 9th and 14th days. The animals were sacrificed and the entire wound area of 12 mm along with normal skin was excised. The tissue samples were embedded in paraffin, and stained with Hematoxylin-Eosin and Masson`s trichrome staining. The data was analyzed on SPSS version 16.0. The variable were calculated by one-way analysis of variance (one-way ANOVA) and post hoc Tukey`s HSD testing. A p-value of ≤0.5 was taken statistically significant.

Results: The wounds of rabbits in the control group exhibited severe degree of inflammation compared with the Pyodine and NS groups. The wound size as examined on 5th, 9th, and 14th day revealed significant and highly significant differences among three groups. (p=0.001 and 0.0001), except the control and pyodine on 5th post wounding day (p=0.10). A robust granulation tissue was observed in the NS group which showed accelerated wound healing as compared with either group (p=0.001, p=0.0001). The pyodine group also revealed statistically significant reduction in the wound size and healthier granulation tissue compared with controls (p=0.01). The H & E stained slides revealed that the granulation tissue of Nigella sativa group was having more vascularity and collagen tissue compared to pyodine group and controls during earlier phase of wound healing.

Conclusion: It is conclue That the Nigella sativa has wound healing effect, which is supported by the present study. Histological findings indicate better wound healing in skin of experimental rabbit model.

Key words: Wound healing, Rabbit, Pyodine, Nigella sativa.

INTRODUCTION

Nigella sativa (NS) is an aromatic plant, native to the Mediterranean region and the Southwest Asia. The cultivation of NS plant can be traced back to the kingdom of Assyrians and Ancient Egyptians. These civilizations are more than 3,000 years old.[1] The NS plant grows best in the Egypt because of favorable environmental conditions for its cultivation and the best seeds are imported from the Egypt also.[2] Botanically, the NS 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".[3] 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. The NS seeds and oil are being imported from India, Egypt, Sri Lanka, Iran and few other countries for medicinal purpose. [3,4] 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. [3,4,5] 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.[6] 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.[7]

Currently, the wound infection is very common problem in developing countries like Afghanistan, India, Bangladesh and Pakistan because of poor hygienic conditions. Very few studies are available in the local literature on the effect of Nigella sativa on wound, hence we planned to conduct this study to prove scientifically if Nigella sativa has any effect on wound healing or not. The present study is intended to observe the effect of NS on histo-morphological changes on wound and to confirm the one aspect of wound healing scientifically in a rabbit model.

MATERIALS AND METHODS

An experimental study was conducted on rabbit model at the animal house of Isra University over a period of six months (May-October 2012). Thirty male adult wistar rabbits were recruited for the study. Adult Wistar male rabbits of age 7 months to 1 year, weighing 1.0-1.5 kg were included in the study. Female rabbits, age of <7 months, or >1 years, weight <1.0 Kg or > 1.5 kg and sick rabbits were excluded from the study. The rabbits were divided into three groups; Group I. (n=10) rabbits in which skin wounds were allowed to heal without any dressing material, Group II. (n=10) skin wounds were treated with 1% pyodine solution daily and Group III. (n=10) in which Nigella sativa oil extract was topically applied on the skin wounds. 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*.

Experimental details: 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.

Estimation of Wound healing: The size of wound was observed on 5th, 9th and 14th 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 mm^2 graph sheet and traced out. The squares were counted and were recorded as described by the Chah, et al (2006). [8]

Histology and Photographing: The animals were sacrificed by over-dose of Ketamine and Xylazil as described by Nayak et al. (2006).[9] 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 5 um thick sections and stained with Hematoxylin-Eosin (H & E) and Masson`s trichrome staining for histological examination. Photography was carried out.

Statistical analysis: The data was analysed on SPSS version 16.0 for Windows release (Chicago, IL, USA). The continous variables were presented as mean±SD & range. The differences in continous variables among groups and between groups were calculated by one-way analysis of variance (one-way ANOVA) and post hoc Tukey`s HSD testing. A p-value of ≤0.5 was taken statistically significant.

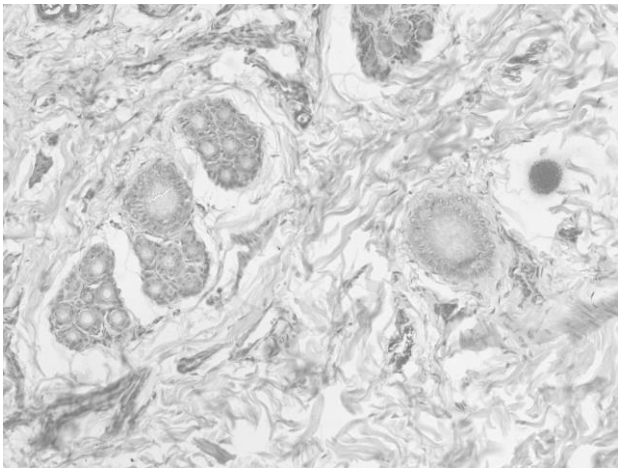
RESULTS

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 Pyodine and NS groups.

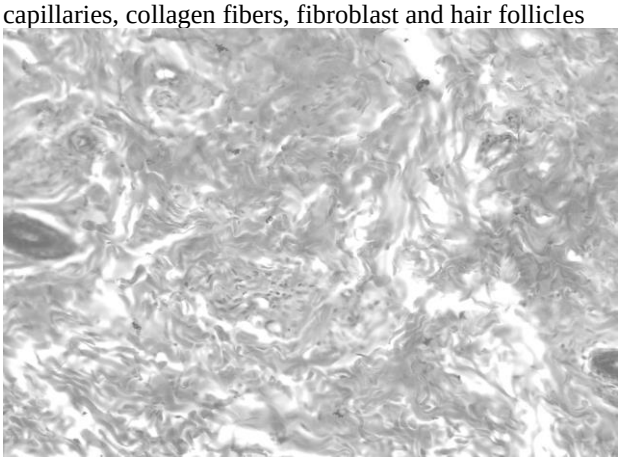
Table. I. Wound size on 5th, 9th and 14th days (mm•)

Days	Group A (Controls) (n=10)	Group B (Pyodine) (n=10)	Group C (Nigella sativa) (n=10)	p-value
5th day	10.89 ± 0.17† (10.60-11.0)*	10.87 ± 0.14† (10.56-11.0) *	10.58 ± 0.07† (8.90-11.0) *	A vs. B 0.10 A vs. C 0.002 B vs. C 0.002
9th day	10.84 ± 0.76† (10.60-10.9) *	10.09± 0.76† (8.90-10.9) *	7.30 ± 2.17† (6.50-10.7)	A vs. B 0.043 A vs. C 0.001 B vs. C 0.001
14th day	10.17 ± 0.77† (8.90-10.9) *	7.48 ± 3.09 (3.50-7.6) *	4.20 ± 0.70† (3.40-5.1) *	A vs. B 0.01 A vs. C 0.0001 B vs. C 0.001

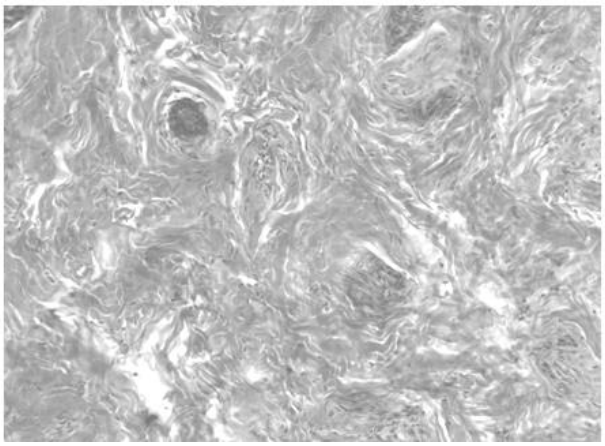
† Mean±S.D *Range •millimeter



Photomicrograph 1. Photomicrograph of control group showing the granulation tissue containing blood capillaries, collagen fibers, fibroblast and hair follicles



Photomicrograph 2. Pyodine group showing the healthier granulation tissue as compared to control group



Photomicrograph 3. Nigella sativa group showing healthier granulation tissue as compared to control groups.

The rate of wound healing as measured by wound size is shown in Table. I. The wound size as examined on 5th, 9th, and 14th day revealed significant and highly significant differences among three groups. (p=0.001 and 0.0001) (Table I.), except the control and pyodine groups which exhibited no significant difference on 5th post wounding (p=0.10) (Table.I). A robust granulation tissue was observed in the NS group which showed accelerated wound healing as compared with either groups. The pyodine 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 histological photomicrograph (I-III) of granulation tissue of Nigella sativa group showed more vascularity and collagen tissue compared to pyodine group and controls.

DISCUSSION

Our present study shows that the rabbits which received NS extract were having better wound healing compared with other two groups and we suggest that the NS has wound healing potential (Table. I). Our results are comparable to studies mentioned in medical literature. The animal models which have been used for the wound healing purpose using NS include rats [10, 11], Guinea pigs [12,13] and the pigs.[14] Different animal models have been used to test the healing effects of not only drugs but also the herbs and herb-derived extracts.[15] Plants have been used for their therapeutic effects since centuries back.[16] There are many studies which have been conducted on animal models to test and compare the antimicrobial agents and herbs in wound healing.[15,17] 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)

The findings of present research work regarding the effect of NS on wound healing are in agreement with previous studies.[15,18] We observed better wound healing in the NS group compared with the pyodine and control groups. (Photomicrographs I-III) The study of Yaman, I et al (2010) [15] 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 many previous studies[15,19], 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.[20] The findings of this previous study are highly consistent with our present observations. The Al-Douri et al. (2010) [21] conducted a prospective study on wound healing 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.[22] This finding is not in consistency with our current and previous studies.[15,18] 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. 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 are of opinion that the effect of NS on inflammation, blood vessels and cell mitosis should be searched at molecular level to reach at proper conclusion.

CONCLUSION

It is concluded that the Nigella sativa possesses wound healing potential. The Nigella sativa has gained popularity in skin wound healing which is supported by the present study. Histological findings indicate better wound healing in skin of experimental rabbit model.

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