

Effect of Nigella Sativa Extract Oil on Granulation Tissue in Cutaneous Wound: An Experimental Study in a Rabbit Model

1. Muhammad Yaqoob Shahani 2. Samreen Memon 3. Shazia Begum Shahani
4. Umbreen Bano 5. Sana Naz Arain

1. Lecturer, of Anatomy, LUM&HS, Jamshoro 2. Asstt. Prof. of Anatomy, LUM&HS, Jamshoro
3. Lecturer, of Anatomy, Isra University, Hyderabad 4. Lecturer, of Anatomy, LUM&HS, Jamshoro
5. Asstt. Prof. of Anatomy, Isra University, Hyderabad.

ABSTRACT

Objective: The present study aims to observe the effect of Nigella Sativa (NS) oil extract on the granulation tissue formation on induced cutaneous wounds in a rabbit model.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Department of Anatomy, Isra University Hyderabad from May to October, 2012.

Materials and Methods: Twenty male adult Wistar rabbits were studied according to inclusion and exclusion criteria. The rabbits were divided into two groups; Group I. (n=10) skin wounds were treated with 1% pyodine daily and Group II. (n=10) Nigella sativa extract oil was applied on inflicted cutaneous wounds. The animals were sacrificed by over-dose of Ketamine and Xylazil as per protocol and skin wound area was excised. The tissue samples were fixed in 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 & Masson's trichrome and examined for histological findings. The data was analyzed on SPSS version 17.0 (Chicago, IL, USA). The continuous variables were analyzed by independent sample t-test. The p-value was taken at ≤ 0.5 as significant.

Results: The study proves bulky granulation tissue formation in NS extract oil group compared to controls with statistically significant differences on 5th, 9th and 14th days ($p=0.0001$). The overall content of granulation tissue was nearly double on all three days in NS group compared with the controls. Our study shows that NS extract oil induces angiogenesis, fibroblast proliferation and collagen synthesis as observed on the histological examination. The study proves that the blood capillaries are found more in NS oil extract group and similarly more fibroblast and collagen fibers. The findings of present study indicate that NS oil extract has soothing effect on the skin and blood vessels reaction to injury. The findings reveal better healing in NS extract oil group regarding the wound size and healing.

Conclusion: The NS extract oil produces granulation tissue formation as topical agent in cutaneous wounds in rabbit model. It is concluded that the NS extract oil induces angiogenesis, fibroblast proliferation and collagen synthesis, however further studies are recommended.

Key Words: Nigella sativa, Granulation tissue, Pyodine, Rabbit.

INTRODUCTION

Nigella sativa (NS) L. Ranunculaceae, commonly known as black seed or black cumin, is naturally distributed in some regions of Pakistan and other South East Asian countries^{1, 2}. 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.³ 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.⁴ In Pakistan, it is commonly known as "Kalonji".⁵ The NS plant and its seeds has great importance in the old systems of therapeutics such as Unani and Ayurvedic and also in the modern 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.^{5,6}

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 salads⁷. To a common Islamic belief NS seed is a remedy for every disease except death. NS seeds 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. NS oil extract has also antipyretic, analgesic, anti-inflammatory, antimicrobial, and anti-neoplastic activity². The seed contains mucilage, crude fiber, fixed oil, sugars, resins, alkaloids, flavonoids, sterols, tannins, saponins and essential oil⁸⁻¹⁰. 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 actions⁸⁻¹⁰. Healing of wounds remains a challenge to modern medicine. Though many of antiseptic drugs have been used for treatment of wounds but they cause adverse reactions like allergy and toxicity when used for a long period¹¹⁻¹⁴. From a search in local literature revealed a few studies are available on the effect of NS extract oil on granulation tissue in wound healing. The present study aims to observe the effect of NS extract oil on granulation tissue formation in a rabbit model.

MATERIALS AND METHODS

The present study was conducted at the animal house, Department of Anatomy, Isra University Hyderabad from May-October 2012. The animal house is well ventilated, provided with facility of stainless steel cages, normal humidity and temperature with 12 hour light-dark cycles. Animals can dangle freely having free access to animal chaw like fresh alfalfa and water. Twenty male adult Wistar rabbits were included in this experimental study according to inclusion criteria of age 7 month to 1 year having weight of 1.0-1.5 kg. The animals were divided into two groups; Group I. (n=10) rabbits in which skin wounds were treated with 1% pyodine solution daily and Group II. (n=10) in which *Nigella sativa* extract oil was applied on infected skin wounds to examine the granulation tissue.

An electrical clipper was used for skin shaving. 1% intradermal Xylocaine was injected to anesthetize the shaved skin. An area of 12 mm of skin was subjected to repeated adhesive tape stripping until the epidermis and dermis were completely removed. The wounds were left open for regenerative purpose according to protocol in both groups respectively. The granulation tissue was examined and compared on 5th, 9th and 14th days in both groups. The wounded skin samples were collected after sacrificing the rabbits on 5th and 14th days for histological examination. The animals were sacrificed by over-dose of Ketamine and Xylazil as described by Nayak et al. (2006).¹⁵ The tissue samples were fixed in marked containers, containing 10% formaldehyde as preservative. The tissue samples were embedded in paraffin, cut into 5 μ m thick sections and stained with Hematoxylin-Eosin (H & E) and Masson's trichrome staining.

The data was processed on SPSS version 17.0 for Windows release (Chicago, IL, USA). The continuous variables were presented as mean $\pm$ SD and graphs. The continuous variables between groups were calculated by independent sample t-test. A p-value of ≤ 0.5 was taken statistically significant.

RESULTS

In present study, we observe bulky granulation tissue in NS group compared with controls with significant differences on 5th, 9th and 14th days (table. I) with highly significant p-value ($p=0.0001$). The overall content of granulation tissue was nearly double on all three days in NS group compared with the controls. (Table. I) (Graph. 1-3). Our study shows that NS extract oil induces angiogenesis, fibroblast proliferation and collagen synthesis as observed on the histological examination (Figure 2). The study observes that the blood capillaries were found more in NS group compared with controls and similar were the findings for the fibroblast and collagen content. (Figure I. vs. Figure 2). The degree of inflammation as observed by simple signs of inflammation viz; the hotness, redness and swelling of wounded area revealed significant differences between groups. The rabbits in control group show more redness, hot and swollen wounds compared to NS group. The observation of present study indicates that NS extract oil has soothing effect on the skin and blood vessels reaction to injury. We also observed better healing in NS group regarding the wound size and healing.

Table No.1: Granulation tissue formation on 5, 9 and 14 days (mm*)

Granulation tissue (Days)	Group I. (Pyodine) (n=10)	Group II. (<i>Nigella sativa</i>) (n=10)	p-value
Granulation tissue (5 th day)	5.1 $\pm$ 1.63	8.1 $\pm$ 1.10	0.0001
Granulation tissue (9 th day)	5.8 $\pm$ 1.90	10.2 $\pm$ 1.75	0.0001
Granulation tissue (14 th day)	6.8 $\pm$ 1.98	10.9 $\pm$ 0.87	0.0001

*millimeter

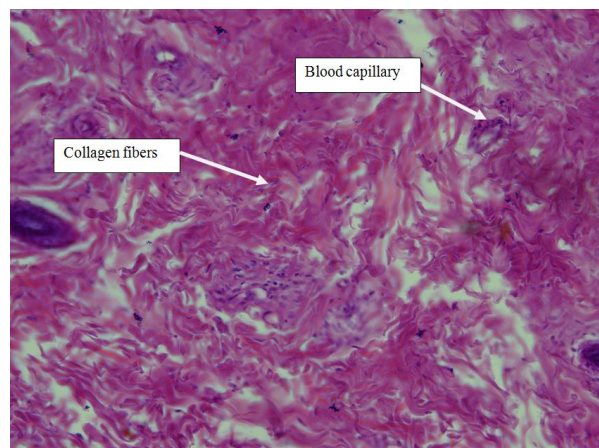
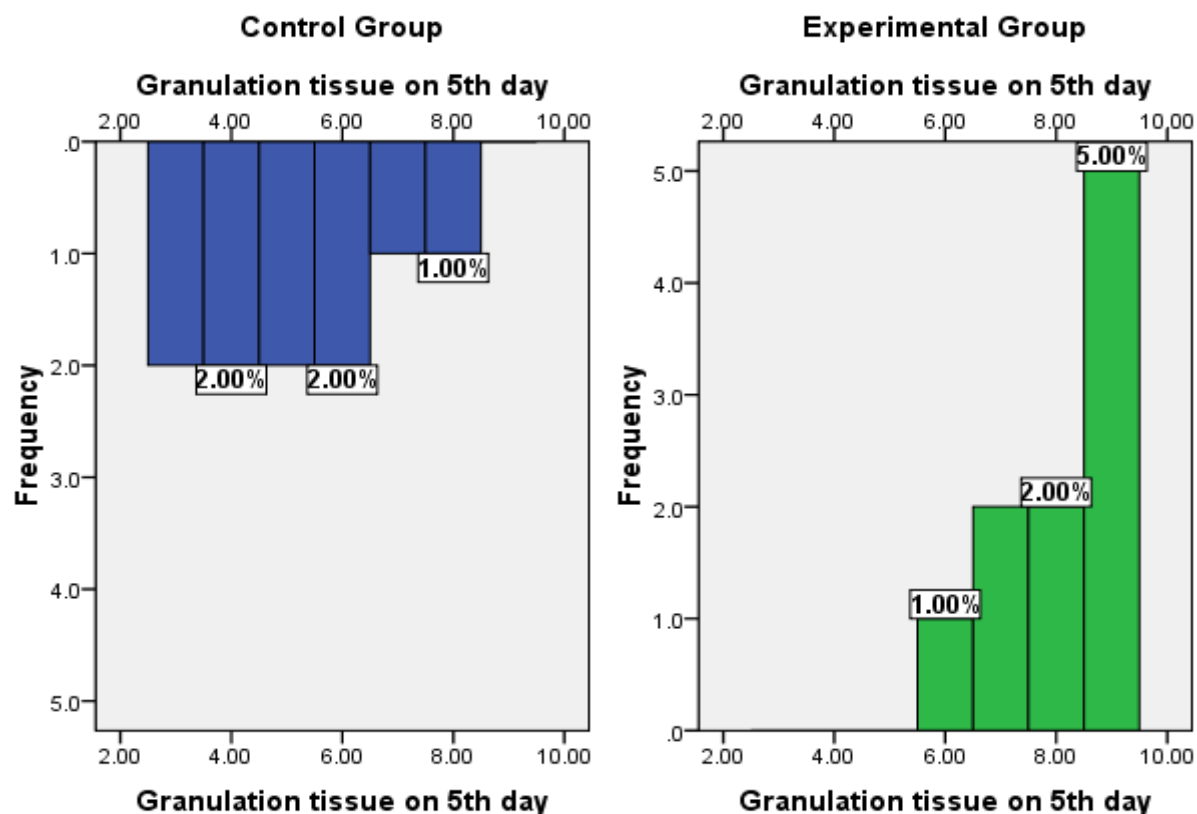
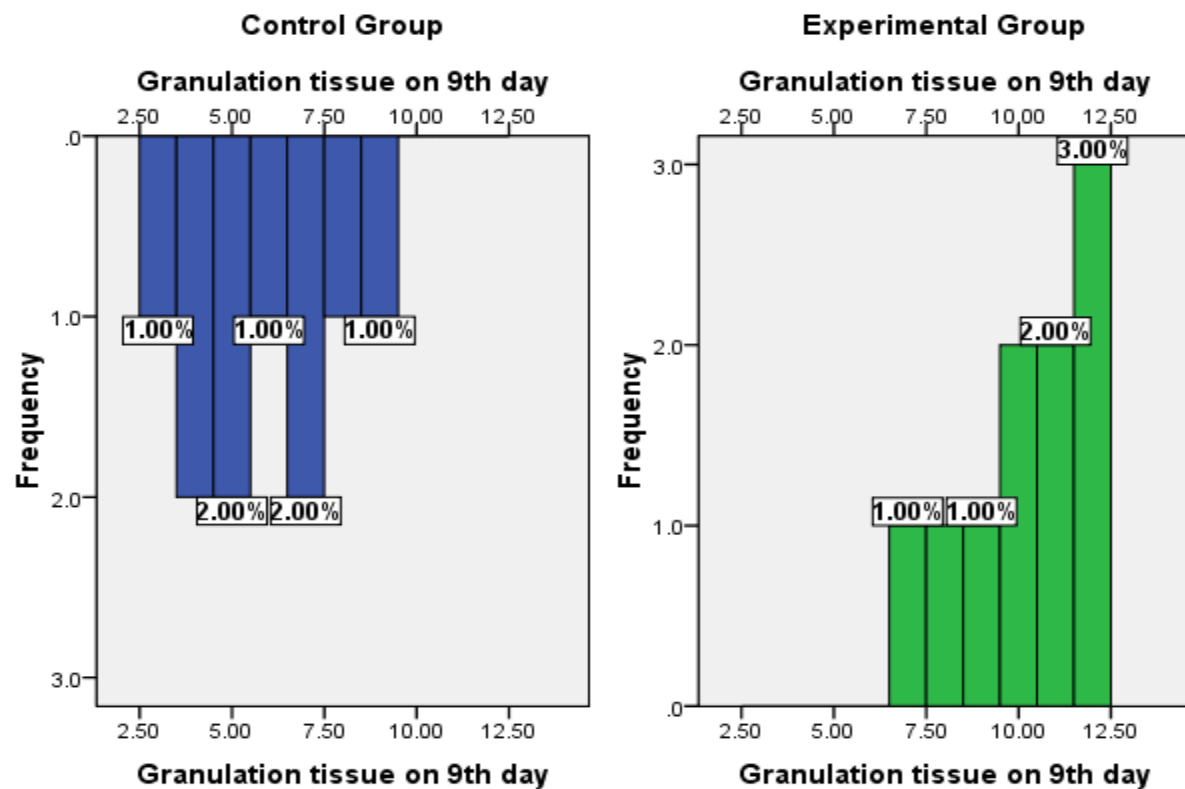


Figure No. 1: Granulation tissue showing a few blood vessels, and collagen fiber in Pyodine group (compare with Figure. 2)

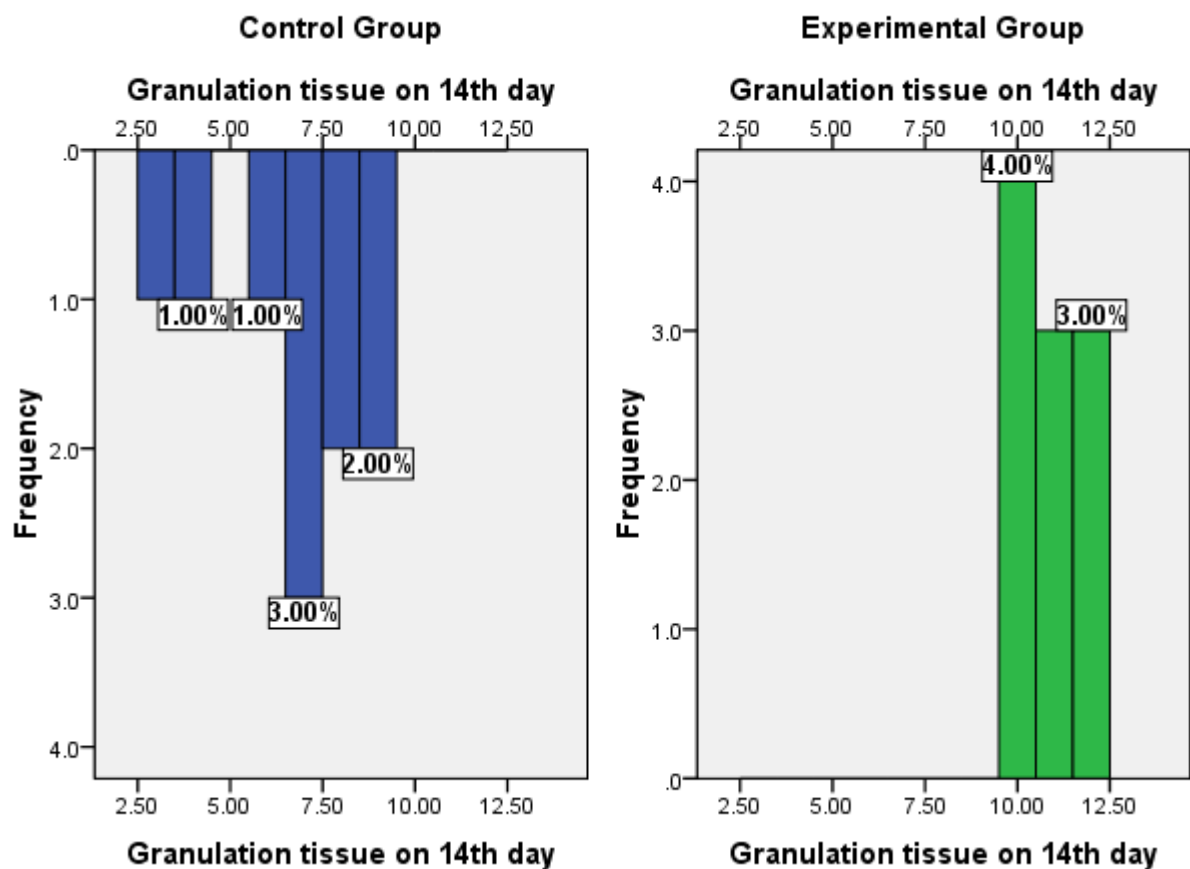
Rabbits

Graph No.1: Granulation tissue in control and experimental (*Nigella sativa*) groups on 5th day

Rabbits

Graph No.2: Granulation tissue in control and experimental (*Nigella sativa*) groups on 9th day

Rabbits



Graph No.2: Granulation tissue in control and experimental (*Nigella sativa*) groups on 14th day

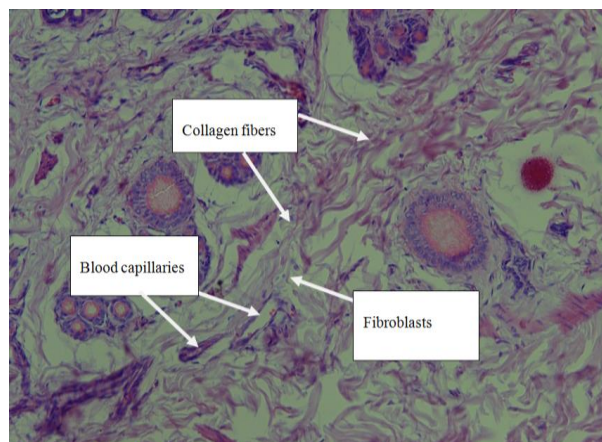


Figure No.2: Granulation tissue showing blood capillaries, fibroblast and collagen fibers in *Nigella sativa* group (compare with Figure 1)

DISCUSSION

Our present study shows that the rabbits which received NS extract oil as topical agent developed bulky granulation tissue formation compared to controls. This shows NS extract oil induces angiogenesis, fibroblast proliferation and collagen synthesis as observed on the histological examination. The Al-Douri et al. (2010)¹⁶

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 controls. It strongly suggests role of NS in angiogenesis and connective tissue formation. Our present experimental study conducted on rabbits as animal models produced equivalent findings to previous study of Al-Douri et al. (2010)¹⁶ and this supports our finding that the NS has granulation tissue inducing effect. The granulation tissue as measured on fifth, ninth and fourteenth days was significantly greater in NS oil extract group compared to controls ($p=0.0001$) (Table. I). Our findings are in agreement to previously published studies mentioned in literature^{17, 18, 19}. Many studies have been conducted on animal models to test and compare the antimicrobial and wound healing potential of NS extract oil with other agents including modern antibiotics.^{17,19} The NS extract oil as wound healing and granulation tissue inducer has been studied in rats^{11, 20}, Guinea pigs^{21,22} and the pigs.²³ The wound healing effect of NS extract oil was compared with control groups and significant differences were observed. (Table. I) The findings of present research work regarding the effect of NS extract oil on wound healing are in agreement with previous studies.^{17,24} We

observed better wound healing in the NS group compared with control group. (Figure I & II) One study from Saudi Arabia reported positive association wound healing effect of NS compared with antibiotic group, but the NS group exhibited a mild retardation in the wound granulation tissue compared controls.²⁵ This finding is not in consistency with our current and previous studies.^{17,24} But, in our opinion this mistake might has been by researcher's bias, quality of NS extract oil, data collection or statistical analysis. Another study¹⁷ reported better wound healing in the NS extract oil group compared to controls as evidenced by bulky granulation tissue formation. Our present study shows comparable findings of granulation tissue in animal groups applied with NS extract oil. Hence, our present study supports previous reports that the NS oil extract has healing effect on cutaneous wounds in animal models. In a study, the NS oil was used to observe wound healing effect in rats. And it was observed that the wounds in NS extract oil groups were healed in a shorter time compared to antimicrobials and pyodine.²⁵ The findings are comparable to our present study. Our findings of NS extract oil as wound healer and inducer of granulation tissue formation is in agreement to studies conducted previously as mentioned above. This proves that the NS extract oil has effect on angiogenesis, fibroblast proliferation and collagen tissue synthesis, which should be searched in further studies.

CONCLUSION

The present study shows that the NS extract oil as topical agent produces granulation tissue formation in induced cutaneous wounds in rabbit model. Hence, it is concluded that the NS extract oil induces angiogenesis, fibroblast proliferation and collagen synthesis, however further studies are recommended to be conducted at molecular level with large sample size, sophisticated instrumentation and modern techniques to elaborate the effects *Nigella sativa*.

REFERENCES

1. Mozaffarian V. A Dictionary of Iranian Plants Names; Tehran: Farhang Moaser Publishers; 1998.p.365.
2. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella Sativa*. *Phytother Res* 2003; 17:299-05.
3. Khan MLA. Kalonji (*Nigella sativa*). *Islamic Voice* 2009;1308 (152): 1-2.
4. Naz H. *Nigella sativa*: the miraculous herb. *Pak. J. Biochem. Mol Biol* 2011; 44(1):44-48.
5. Ahmad Z, Ghafoor A and Aslam M. (2004). *Nigella Sativa*: A potential commodity in crop diversification traditionally used in healthcare. *MINFAL* 2004; 5:34.
6. 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.
7. Ramadan MF. Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): an overview. *Int J Food Sci Tech* 2007; 42:1208-18.
8. Fatah A M, Matsumoto K, Watanake H. Antinociceptive effects of *Nigella sativa* oil and its major components in mice. *Eur J Pharmacol* 2000; 400:89-97.
9. Houghton PJ, Zarka R, Heras B, Hoult JRS. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med* 1995; 61:33-6.
10. Nickavara B, Mojaba F, Javidniab K, Roodgar-Amolia MA. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. *Z Naturforsch* 2003; 58c:629-31.
11. Mokaddas E, Rotimi VO, Sanyal SC. In vitro activity of piperacillin/ tazobactam versus other broad antibiotics against nosocomial gram negative pathogens isolated from burn patients. *J Chemother* 1998; 10:208-14.
12. Hermans MHE, Hutchinson JJ. The incidence of infection under dressings: a prospective comparative trial of hydrocolloid dressing, DuoDERM CGF, versus conventional dressings in the treatment of leg ulcers, burns and donor sites: *Exerpta Med* 1990; 35-41.
13. Boosalis M, McCall J, Ahrenholz D, Solem L, McClain C. Serum and urinary silver levels in thermal injury patients. *Surgery* 1987; 101:40-3.
14. Hutchinson JJ. Prevalence of wound infection under occlusive dressings: a collective survey of reported research. *Wounds* 1989; 1:123-33.
15. 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.
16. Al-Douri AS. The effect of honey on the healing process of oral ulcer. (Experimental study). *Iraqi Dent J* 2006;33: 7.
17. 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.
18. Jones FA. Herbs-useful plants. Their role in history and today. *European J Gastroenterol and Hepatol* 1996; 8:1227–1231.
19. 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.
20. Mekkes JR, Le Poole C, Das PK, Bos JD, Westerhof W. Efficient debridement of necrotic wounds using proteolytic enzymes derived from Antarctic krill: a double-blind, placebo controlled study in a standardized animal wound model. *Wound Repair and Regeneration* 1998; 6: 50–58.
21. Eldad A, Weinberg A, Breiterman S, Chaouat M, Palanker D, Ben-Bassat H. Early nonsurgical removal of chemically injured tissue enhances wound healing in partial thickness burns. *Burns* 1998; 24: 166–172.
22. 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 Research Inst* 2006;27 (3):141-151.
23. Abu-Zinadah OA. Using Nigella sativa Oil to treat and Heal Chemical Induced Wound of Rabbit Skin. *J King Abdul-Aziz University* 2009;21(2):335-346.
24. 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.
25. 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.

Address for Corresponding Author:

Muhammad Yaqoob Shahani,
Lecturer of Anatomy, LUM&HS,
Jamshoro / Hyderabad