



EFFECT OF HOT METHANOLIC EXTRACT OF *NIGELLA SATIVA* ON THE HEALING OF INFECTED CUTANEOUS WOUNDS IN RABBITS

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Abstract

The study about the preparation of hot methanol extract (70%) of *Nigella sativa* seeds. The diagnosis of perpetrated two extractions by the used preliminary qualitative reagent. The antimicrobial activity of these two extracts on *Staphylococcus aureus*, which plays two roles; as antimicrobial and a stimulator to tissue repair. In this experiment, we used a twelve male mature rabbits. Divided of these rabbits into three groups, four rabbits in each group. In group1 treated the wounds with 500 mg concentration of Cefotaxime. Wounds of the two groups were treated with hot methanol extract (70%) of *Nigella sativa* seeds. Wounds of the three groups were treated with Cefotaxime with *Nigella sativa* seeds. Clinically, the clinical signs of wounds were recorded throw 10 days post induce wound. The results of this study indicated that using hot methanolic extract (70%) obtained a similar results to those antibiotic used (same bacterial reduction rates). Moreover, combination used of apple cider hot methanol extract (70%) and the antibiotic in equal amounts will get to more better and rapid healing than using one alone of them. histopathological indicated that the hot methanol extract (70%) with Cefotaxime accelerates the healing. Additionally, there are differences in healing response among groups.

Keywords: *Nigella sativa* seeds, Hot methanolic extract, Wound healing, Infected wound.

Introduction

Nigella sativa belonging to Ranunculaceae family is an annual herb the which grows in bordering of the Mediterranean Sea countries, India, and Pakistan. This plant is distributed widely in the Mediterranean region and Arab countries (Jansen, 1981). Different pharmacological effects such as antimicrobial effect (Abu-Al-Basalc, 2009), anticancer activity (Shafi *et al.*, 2009), antitussive effect, immunomodulatory and anti-inflammatory effects (Hosseinzadeh *et al.*, 2008; Majdalawieh *et al.*, 2010). *N. Sativa* crude extracts can effect on multi drug resistance to organisms such as gram- negative and gram positive bacteria (Sokmen *et al.*, 1999; Hasso and Al-Janabi, 2019). The healing wound is an extremely complex and dynamic tissue which is come way could be regarded as an organ. Normal wound healing occurs in recognizable, usual progressive though overlapping (Kindlen and Morison, 1997). The quality of wound healing is depending on another factor such as bacteria, hot, chemicals ...etc, therefore, there are three types of the wound; acute, sub-acute, and chronic (Harari, 1996). The type of bacteria may influence on wound healing. Many chronic wounds are colonized with *Staphylococcus aureus* (Emmerson *et al.*, 1996). Bacteria are present in the most wound, the numbers, virulence, and host defense are determined the stage of wound inflammation (Scanlon, 2005), therefore most bacteria cause odor, dehydration, local cellulitis, and death.

The present study aimed to prepare extracts of *Nigella sativa* seeds by using hot 70% alcohol and studied the biological activity of prepared extracts of *Nigella sativa* seeds on bacteria which contaminated the wounds.

Materials and Methods

Animals: Twelve males adult healthy rabbits were used in the present study weight ranged from 1.5 to 2 kg from Basra city (local market).

Plant Material: Black Cumin (*Nigella sativa*) was purchased from the local market in Basrah. The plant was brought to the laboratory and washed with distilled water and room temperature make a shade dried then plastic bag used for storing until use.

Preparation of 70% hot methanolic extract: The *Nigella sativa* was finely ground to powder by using a blender. Fifty grams of plant material in powder form was dissolved in 500 ml (70%) methanol then the solution was put in flask in 50°C for 24 hours. Filtered the extract using filter paper (Whatman No.1), the supernatant concentrated and dried to a constant weight. The color of the extract was dark brown with a semi-solid consistency and weight 1.6gm (Abdulzahraa, 2011).

Preliminary qualitative chemical tests of 70% hot methanolic extract: Used a qualitative chemical tests for the *Nigella sativa* seeds extraction (7-9) Table (1).

Table 1 : Preliminary qualitative chemical tests for 70% hot methanolic extract of *Nigella sativa* seeds.

Phytoconstitute	Reagents	Results
Phenoles	1ml extract + 1ml FeCl ₃	Brown precipitate
Flavonoids	1ml extract + 1ml KOH alcoholic	Yellow precipitate
Tannins	1ml extract + 1ml lead acetate	Brown to Yellow precipitate
Saponins	1ml extract + 1ml HgCl ₂	Yellow precipitate
Glycosides	1ml extract + 1ml Bendict	Blue coloration
Alkaloids	1ml extract + 1ml Mayers	Turbidity of solution
Proteins	1ml extract + 1ml Biurete	Green to yellow coloration
Amino acids	1ml extract + 1ml ninhydrine	Violet coloration
Steroids	1ml extract + 1ml CHCl ₃ + 1ml H ₂ SO ₄ (Conc)	Raddish Brown ring at the junction

Testing the antibacterial activity of the extract: Agar – well diffusion was used according to (Pérez Rodríguez *et al.*, 1990). In this experiment, *Staphylococcus aureus* was cultured on nutrient agar by streak method in order to obtain overnight contains which were used to prepare bacterial suspension as described by (Piddock and Wise, 1989), Bacterial suspension of *Staphylococcus aureus* was prepared from fresh colonies, this suspension was contained 106 bacteria/ml. One hundred microliters of bacterial suspension was used to spread on Muller-Hinton agar which previously prepared. The Muller-Hinton agar was left for 15 mint to dry after that 4 wells with diameter 5mm were done per each plate. Four dilutions (100, 500, 700, 1000 mg/ml) of the extract was prepared. One hundred microliters of the extract were put in the wells. One well was used for each dilution. The plates incubated at 37C overnight after that the inhibition zones were measured.

Treatment protocol: Ketamine- Xylazine (15 mg⁻⁵ mg) /kg of B.W IM (PANAPHARMA S.A France) were given to the animals. The rabbits were randomly divided into three equal groups. Obtained the used bacteria in this study from the microbiology department, college of veterinary medicine, university of Basrah. Prepared the animals for surgery Firstly, prepared the left thigh under aseptic technique, the skin incision was made in lateral view of thigh superficial layer of skin equal 4cm. These wounds were exposed to infection with bacteria. The follow-up animal daily until the infection takes place then the wound was treated as follows: The first group, infected wound treated with topical antibiotic cefotaxime at a concentration of 500 mg daily for 10 days. This antibiotic is widely used in the treatment of the infected wound. The second group infected wound treated with the topical hot methanolic extract (70%) of *Nigella sativa* seeds daily for 10 days. The third group infected wound treated by a mixture of topical antibiotic cefotaxime with hot methanolic extract (70%) of *Nigella sativa* seeds daily for 10 days.

Results and Discussion

Skin wounds affected on the life quality of patients due to long healing processes. The short time of wounds healing without associated of scars or infections to be the wanted results. Wound healing treated by several alternative methods according to different studies (Jagetia and Ravikiran, 2015; Abdulsamad *et al.*, 2017). In human and animals, the wounds are commonly contaminated; then required to complicated treatments and prolong duration, so that, our study aimed to investigate an alternative contaminated wounds treatment that speed up the healing wounds using natural compounds. *Staphylococcus aureus* is considered the most common cause of infected wounds and burns (Molina *et al.*, 1991; Todar, 2004), due to their ability to diffuses in lacerated tissue, disturbance the function of the host and produce pus. The inflammation of the wound occurs directly after bacterial infection of the open wound (Giacometti *et al.*, 2000). The inflammation occurs due to tissue damage bay these bacteria, which carry many virulence factors (Dubouix *et al.*, 2004).

Clinical results

During the study period, no rabbits mortality rate recorded and no wounds damage of the rabbits by each other, this agree with many studies (Han *et al.*, 2017). An

inflammation signs were recorded the lower degree in groups that were treated with the hot methanolic extract (70%) of *Nigella sativa* seeds and wounds of the three groups were treated with Cefotaxime with *Nigella sativa* seeds compared to the control group groups were treated with Cefotaxime. An infected wound in rabbits injection with *Staphylococcus aureus* after 3 day shows inflammatory swelling with signs of bleeding with the presence of pus and perfusion in the wound site with the occurrence of necrosis in the wound. The infected wound was examined grossly after the use of treatment occurrence of healing of a wound and through mature granulation tissue formation during (2-3) days. The healing of infected wound by topical hot methanolic extract (70%) of *Nigella sativa* seeds, marked scab formation and granulation tissue formation in the wound margins While the third group, revealed healthy surface, no infection, cleanness and decrease the wound surface, these facts may due to *Nigella sativa* seeds were reported to have anti-inflammatory, antimicrobial, immunomodulatory and antioxidant properties, and the *Nigella sativa* used to fast the process of burn healing a histological manner (Yaman *et al.*, 2010). Wound healing is a concerted effort of a sequence of various physiological processes including inflammation, metabolism, regeneration, and remodeling leading to complete wound closure (Haubner *et al.*, 2012). The wound areas measurements showed that the *Nigella sativa* seeds with Cefotaxime and *Nigella sativa* seeds groups were a better on the wound healing when compared to the control group (Figure 1). During the postoperative period there are no significant difference among the groups in terms of colors of wound. The wound areas measurements showed that the *Nigella sativa* seeds with Cefotaxime was better in the wound healing. Another key feature of wound healing is wound contraction, that reduce infection and promotes a rapid closure of wound (Erpek *et al.*, 2006).

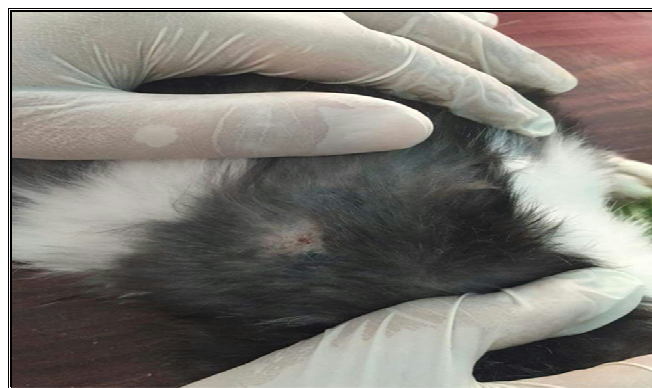


Fig 1 : Showed the healing of infected wound by topical hot methanolic extract (70%) of *Nigella sativa* seeds with Cefotaxime.

Chemical results

The results of chemical tests (table 2) were showed the presence of glycosides, saponin, phenolics, flavonoids, tannins, protein, steroids, alkaloids and amino acid in the 70% hot methanolic extract of *Nigella sativa* these ideas may be agreed with (Chakravarty, 1976; Atta-ur-Rahman *et al.*, 1995). Atta-ur-Rahman *et al.* (1995) the *Nigella sativa* contain special colloids (Nigellimine-N-oxid and Nigellicine) which is not present in other medicinal plants, the seeds containing tannins also confirm by other (Chakravarty, 1976).

Table 2 : Qualitative chemical tests performed in the 70% hot methanolic extract of *Nigella sativa*

Phytoconstitute	Results	Color
Phenoles	++	Brown precipitate
Tannins	++	Brown to Yellow precipitate
Glycosides	++	Blue coloration
Saponins	+	Yellow precipitate
Proteins	++	Green to yellow coloration
Steroids	+	Reddish Brown ring at the junction
Alkaloids	+	Turbidity of solution
Flavonoids	++	Yellow precipitate
Amino acids	++	Violet coloration

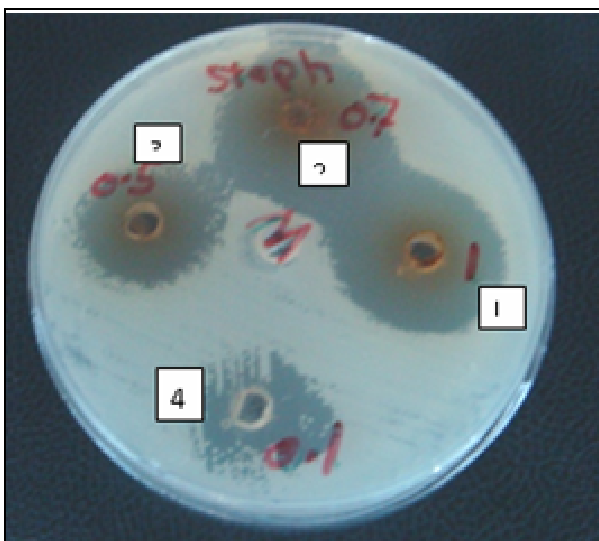
Tannins compound contain tannic acid act against growth of bacterial through the bacterial fatty layer wall were dissolved by these compounds causes leakage out of cell fluid and destroyed; also it can loss the vital bacterial action and destroys by a form the hydrogen bond between group in phenol compound in bacterial cell (Aqeel *et al.*, 1989; Mohammed *et al.*, 2008).

The biological activity of the extracts :

The hot methanolic extract has high inhibition ability against *Staphylococcus aureus* as shown in figure 1 and table 2 that may be due to present high percent (20-21%) of protein with other active materials other studies showed similar results of Ali *et al.* (2014) from this study we conclude the extraction method effects on the active materials. Also, we recommended using the hot methanolic extract of *Nigella sativa* as the pharmaceutical form to treat the diseases caused by a microorganism, a hot methanolic extract shows high antibacterial activity against gram-positive bacteria, these result agreement with other studies (Anwar and Farooqi, 2005; Salem, 2005).

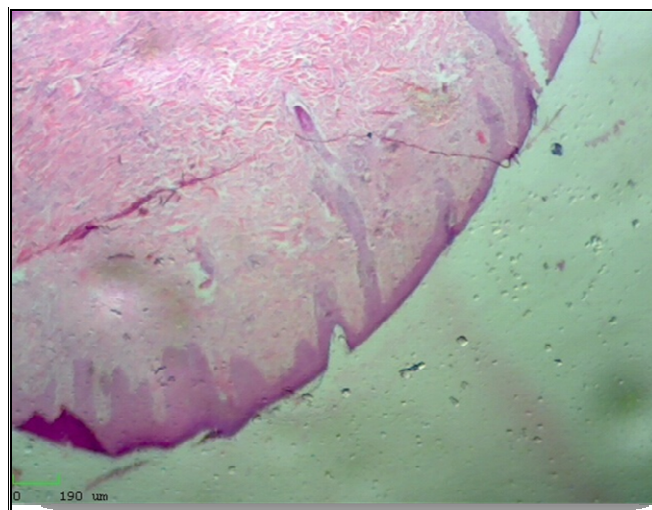
Table 2 : The sizes of inhibition zones against *Staphylococcus aureus* by using different concentration of 70% hot methanolic extract of *Nigella sativa*:

Extract concentration mg/ml	Inhibition zone against <i>Staphylococcus aureus</i>
1000	30
700	25
500	23
100	20

**Fig. 2 :** The inhibition zones against *Staphylococcus aureus* by use 70% hot methanolic extract.

Histological results

Histopathological results after 10 days of treatment, in the first group, were treated with Cefotaxime showed a poor re-epithelization and healing area, area of bleeding in the surface of the wound, excessive amount of collagen and vacuoles of epithelium cell around the wound with little inflammatory cells (Figure 3, 4), delayed of the healing in this group resulting in closure of the patent wound. Our results showed a higher collagen accumulation and granulation tissue levels in the rabbit in the second and third groups after the 10th-day group, the wound healing indicates when collagen deposition in the proliferative stage of the process of healing (Guo and DiPietro, 2010). In addition, the present results revealed no differences among the groups in terms of inflammatory cell infiltration, angiogenesis and epithelialization. Moreover, the third group treated with the hot methanolic extract (70%) of *Nigella sativa* seeds with Cefotaxime show complete wound healing, also the presence of inflammatory cells infiltration with fibroplasia, it is considered the excellent and optimal wound healing group of another groups (Figure 5,6,7). The granulation tissue of the control group showed a severe edema and less in the third group. Our results revealed a constantly increased the collagen deposition and granulation tissue in the wound of the third group after 10 days of injury compared to another groups. These results were agreed with those of the past studies (Guo and DiPietro, 2010).

**Fig. 3 :** Histopathological section of first group showed poor area of re-epithelization and healing, with amount of collagen and glands and vacuoles of epithelium cell with few inflammatory .H&E stain. 40X.

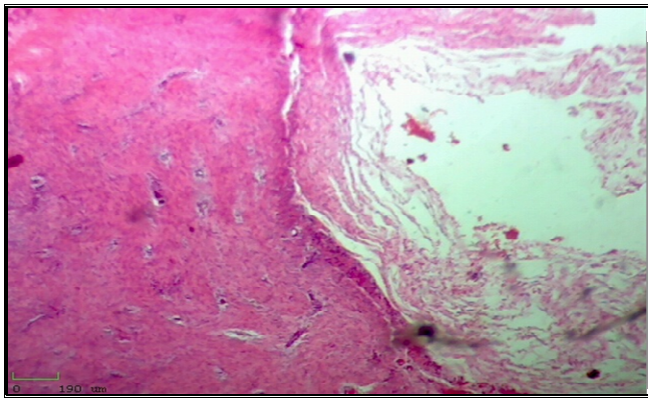


Fig. 4 : Histopathological section of first group show area of bleeding in the wound surface, also presence of few inflammatory cells H&E stain. 10X.



Fig. 5 : Histopathological section of second group showed complete wound healing, with fibroplasia. H&E stain. 40X.

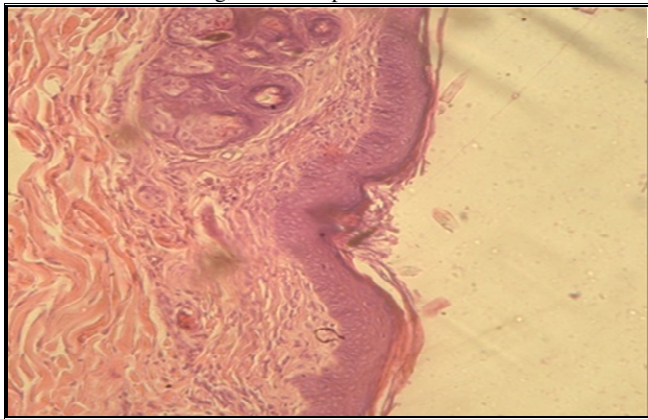


Fig. 6 : Histopathological section of third group showed complete re-epithelization area H&E stain. 40X.

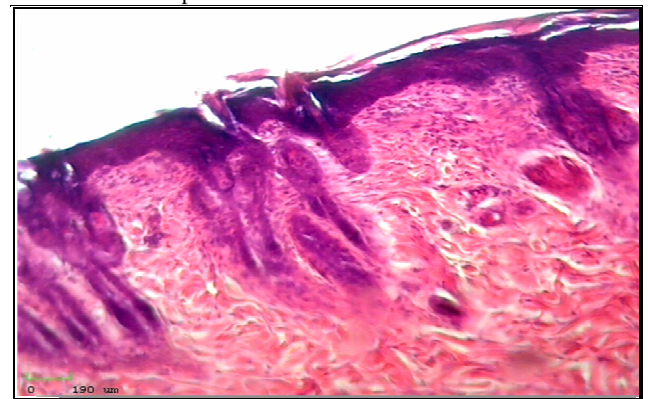


Fig. 7 : Histopathological section of third group showed complete wound healing, also presence of inflammatory cells infiltration. H&E stain. 40X.

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