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 perpetuated two extractions by theused 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 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-Basale, 2009), antitumor activity (Abu-Al-Basale, 2009), antioxidant (Shafi et al., 2009), antitussive, 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.

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 in500Ctor 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.

<table>
<thead>
<tr>
<th>Phytoconstitute</th>
<th>Reagents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>1ml extract + 1ml FeCl₃</td>
<td>Brown precipitate</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1ml extract + 1ml KOH alcoholic</td>
<td>Yellow precipitate</td>
</tr>
<tr>
<td>Tannins</td>
<td>1ml extract + 1ml lead acetate</td>
<td>Brown to Yellow precipitate</td>
</tr>
<tr>
<td>Saponins</td>
<td>1ml extract + 1ml HgCl₂</td>
<td>Yellow precipitate</td>
</tr>
<tr>
<td>Glycosides</td>
<td>1ml extract + 1ml Bendict</td>
<td>Blue coloration</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>1ml extract + 1ml Mayers</td>
<td>Turbidity of solution</td>
</tr>
<tr>
<td>Proteins</td>
<td>1ml extract + 1ml Biurete</td>
<td>Green to yellow coloration</td>
</tr>
<tr>
<td>Amino acids</td>
<td>1ml extract + 1ml ninhydrine</td>
<td>Violet coloration</td>
</tr>
<tr>
<td>Steroids</td>
<td>1ml extract + 1ml CHCl₃ + 1ml H₂SO₄(Conc)</td>
<td>Raddish Brown ring at the junction</td>
</tr>
</tbody>
</table>
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 min 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 37°C overnight after that the inhibition zones were measured.

Treatment protocol: Ketamine- Xylazine (15 mg<sup>5</sup> 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 is 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 by these bacteria, which carry many virulence factors (Duboux 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 Nigelicicine) which is not present in other medicinal plants, the seeds containing tannins also confirm by other (Chakravarty, 1976).
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 (Aqel et al., 1989; Mohammed et al., 2008).

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

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<td>Glycosides</td>
<td>++</td>
<td>Blue coloration</td>
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<tr>
<td>Saponins</td>
<td>+</td>
<td>Yellow precipitate</td>
</tr>
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<td>Proteins</td>
<td>++</td>
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<td>Steroids</td>
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<td>Amino acids</td>
<td>++</td>
<td>Violet coloration</td>
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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).
References


Effect of hot methanolic extract of *Nigella sativa* on the healing of infected cutaneous wounds in rabbits


