

The effect of local application black seed (*Nigella sativa*) oil on wound healing in rabbits

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Abstract

The objective of this study is to determine the effectiveness of topical application of black seed oil on the wound healing in rabbits. Twenty adult healthy local breed rabbits of both sexes weighing between 1250-1600 gr were used. Animals were divided into two equal groups, under surgical aseptic technique, two surgical skin incisions at length of 3 cm. in the back region were done one each sides of the vertebrae, then they were closed with simple interrupted pattern by silk (2\0). Control group didn't receive any treatment, while in treated group, the wounds were covered with black seed oil twice daily for 14 days. The clinical and histopathological evaluation revealed that black seed oil promote the wound healing by early formation of cellular fibrous connective tissue and granulation tissue and early maturation of fibrous connective tissue, which characterized by regular and less cellular covered by complete layer of epidermis, when compared with control group. In conclusion of this study indicated that the black seed oil was enhanced wound healing, and that may be due to its therapeutic and nutritional activities.

تأثير زيت الحبة السوداء المستخدم موضعياً على التئام الجروح في الأرانب

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الخلاصة

استهدف البحث دراسة تأثير الاستعمال الموضعي لزيت الحبة السوداء على التئام الجروح في الأرانب، استعمل في التجربة عشرون أرنباً بالغاً من كلا الجنسين تراوحت أوزانها ما بين 1250-1600غم. قسمت الحيوانات إلى مجموعتين متساويتين وبعد تحضيرها جراحياً وتخديرها عمل شقين جراحيين في الجلد بطول 3 سنتمتر في منطقة الظهر على جانبي الخط الظهري، خيط الجرح بطريقة المتقطع البسيط باستعمال الحرير قياس 0\2. مجموعة السيطرة تركت للشفاء الطبيعي بدون أي معالجة، في حين استعمل في مجموعة المعالجة زيت الحبة السوداء موضعياً مرتان يومياً لمدة 14 يوم. أظهرت نتائج الفحص العياني والنسجي المرضي ان زيت الحبة السوداء قد ساعد في شفاء الجروح عن طريق التكوين المبكر للنسيج الحبيبي وكذلك نضوج النسيج الضام والذي تميز بانتظام أليافه واكتمال طبقة البشرة التي تغطيه. نستنتج من هذه الدراسة بأن زيت الحبة السوداء يساعد في تسريع التئام الجروح.

Introduction

Nigella sativa (*N.sativa*) seeds, called as Black seeds in English language, *Al-habba Al-sauda* or *Habbatul -barakah* in Arabic and *Kalvanji* in some local languages in the Indian subcontinent, is well known in the Middle East, Middle Asia and Far East; as a natural remedy for many ailments and flavoring agent in bread and pies. There is common Islamic belief that Black seed is a remedy of all ailments but that it cannot prevent aging or death (1). Many studies have been carried out in the last few decades on the pharmacological effect of *N. sativa* and its active principles these studies and the advancements in the methods of analysis have led to discovery of many active principles like; proteins, alkaloids, saponin (melanin), fixed and essential oil (2 and 3). Crude fiber, calcium, iron, sodium, and potassium are also present. Nutritional composition of the seeds has been determined as 21% protein, 35% carbohydrate, and 36% fat (4). It's appear that the compounds in the oil act synergistically so that it is important to use the whole oil or crude extract of the seeds in pharmacological studies (5). The plant extract and its essential oil showed a broad range of pharmacological effects such as: anti-diabetic (6) spasmolytic and bronchodilator (7), antioxidant (4) hepato-protective (8), analgesic and anti-inflammatory antipyretic (9), antitumor (10) anti-bacterial, anti-fungal (11), antiviral (12), immune stimulation (13).

The injury of any type triggers is an organized and complex serial cellular and biochemical events that result in a healed wound. These processes can lead to pathological conditions if healing is excessive or deficient, wound healing failures can pose a significant clinical problem with a large impact on morbidity, mortality and medical costs (14). Thus the healing can be enhanced by controlling the local and systemic factors that influenced it like :- The quality of the vascular supply to the area, the presence of a deleterious infection, mechanical stress on the wound, abrasive or inflammatory suture material, radiation injury, hypoproteinaemia and hypovolaemia, edema, malnutrition and vitamin deficiency, administration of corticosteroids, diabetes mellitus, the administration of cytotoxic drugs, jaundice, uremia and advanced age (15).

Because of the characteristic properties of black seed it has been used in the treatment of many types of wound and trauma (16, 17). In this study black seed oil was topically applied on induced surgical wound in rabbit to evaluate its effect on wound healing clinically and histo-pathologically.

Materials and Methods

Twenty adult healthy local breed rabbits of both sexes weighing between 1250-1600 gr were used. The animals were divided randomly into two groups: control group (n=10) and treated group (n=10). The back region was prepared surgically, then the animals were anesthetized by intra-muscular injection of mixed (40 mg /kg Ketamine HCl, 10 mg Xylazine and 4mg/kg diazepam) (18). Two paravertebral straight incisions of 3 cm length were made through the entire thickness of the skin at a distance of about 4 cm. The skin was closed by simple interrupted sutures using silk (size 2/0). The animals of treated group in addition to the same procedures as in group one they were treated by topical application of black seed oil (Emmad factory for oil production, Mosul, Iraq permit no. 70490) on the wound, twice daily for 14 days. In both groups specimens for histopathological examination were collected at the day 3rd, 5th, 7th and 14th post operation, the specimens were fixed in buffered formalin 10 % and routine preparation of the section, then stained with Hematoxylin and Eosin (19).

Results

Histopathological examination of the control group, revealed that at 3 days post operative the section showed presence of necrosis and inflammatory cells infiltration in the incision site consist mainly from neutrophils (Fig. 1), also in other section a network of fibrin were seen in the dermis and inflammatory cells which was mainly neutrophils and fibroblasts began to replace the fibrin network (Fig.2). At 5 days post-operative the site of incision filled with immature granulation tissue consist from angioblast and fibroblast in the dermis which covered by thick epidermis layer (Fig. 3). The section of 7 days post-operative there is fibrous connective tissue proliferation which characterized by more collagen deposition, moderate irregular direction and sever thickness of epidermis, formed papillae extend to the dermis (Fig.4).While at 14 days post-operative the microscopic lesion showed proliferation of immature fibrous connective tissue which infiltrated by mononuclear cells and proliferation of stratified squamous cell epithelium which extended at bridge into dermis with more basophilic basal cells (Fig.5).

In the treated group, the histopathological examination was revealed that at 3 days postoperative the lesion was characterized by inflammatory cells infiltration mainly neutrophils and in the same section area, there was beginning of the proliferation of fibroblast (Fig. 6), in another section the lesion showed immature cellular fibrous connective tissue, with capillary blood vessels (granulation tissue) which present in the incision site (Fig.7). While at the 5 days, more cellular fibrous connective tissue were present in the sutured area with moderate thickness dermis layer were seen (Fig. 8). At the 7 days postoperative more regular dense collagen fibers with mononuclear cell infiltration were seen in the section area covered by complete epidermal layer (Fig 9).At 14 days ,more mature fibrous connective tissue was present in the section area characterized by regular and less cellular covered by complete layer of epidermis (Fig.10).

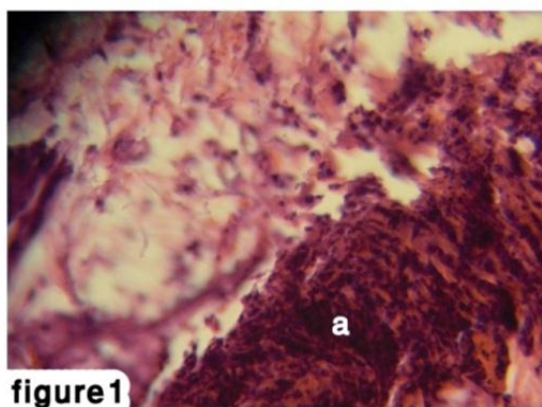


figure1
Fig .(1) Histological section of skin of control group at 3 days post operative showed necrosis and inflammatory cell infiltration in the incision site consist mainly from neutrophil (a) H & E X 40.

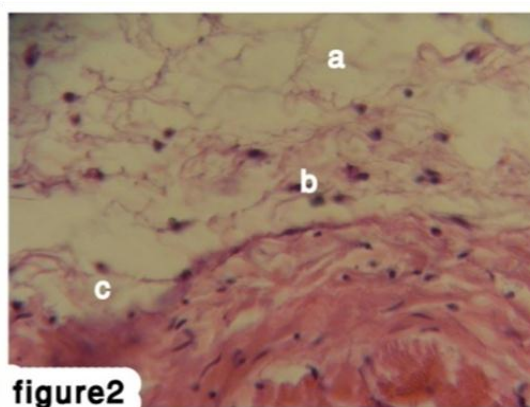


figure2
Fig. (2) At 3 days postoperative, network of fibrin (a) are seen in the dermis, and inflammatory cell mainly neutrophil (b), and fibroblast began to replace the fibrin network (c) H & E X 40.

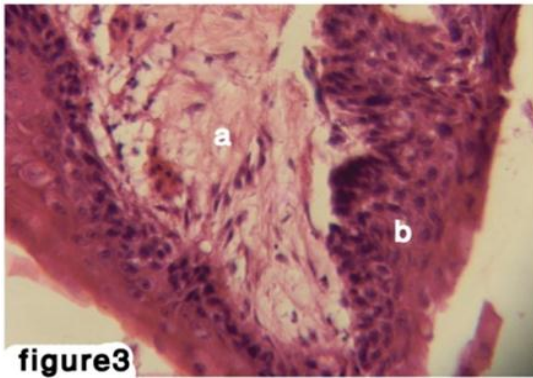


figure3

Fig (3) At 5 days post operative, the site of the incision filled with immature granulation tissue (a), consist from angioblast and fibroblast in the dermis which cover by thick epidermis layer (b) H & E X 40.

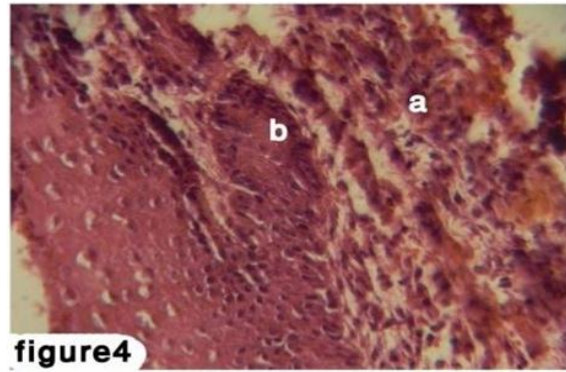


figure4

Fig. (4) At 7 days postoperative there is fibrous connective tissue proliferation characterized by more collagen deposition, moderate irregular direction (a) and sever thickness of epidermis which form papillae (b) extend to the dermis H & E X 40.

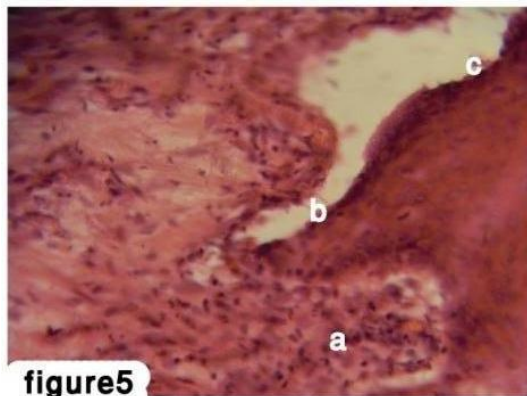


figure5

Fig. (5) At 14 days postoperative, the proliferation of immature fibrous connective tissue which infiltrated by mononuclear cell (a) and proliferation of stratified squamous cell epithelium which extended at bridge into dermis(b) with more basophilic basal cell (c) H & E X 40.

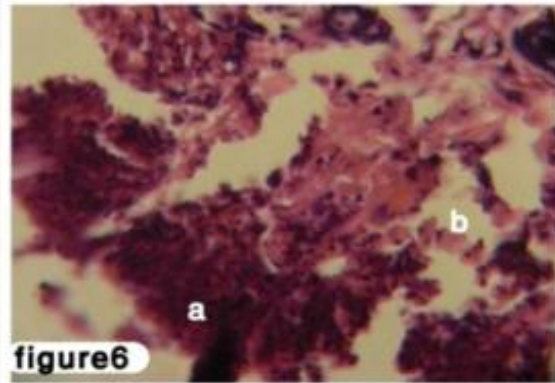


figure6

Fig. (6) At 3 days postoperative of treated group, inflammatory cell infiltration mainly neutrophils (a) with beginning proliferation of fibroblast (b) H & E X 10.

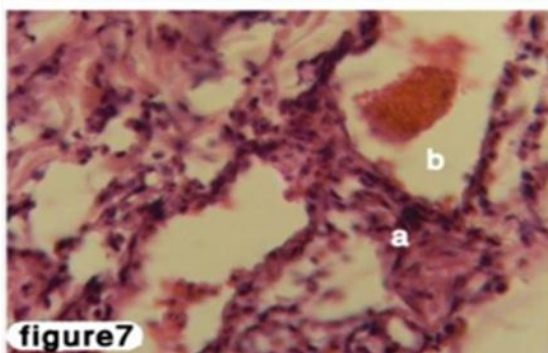


figure7

Fig. (7) At 3 days postoperative of group two immature cellular fibrous connective tissue (a) with capillary blood vessel (b) (granulation tissue) is present in the section area H & E X 40.

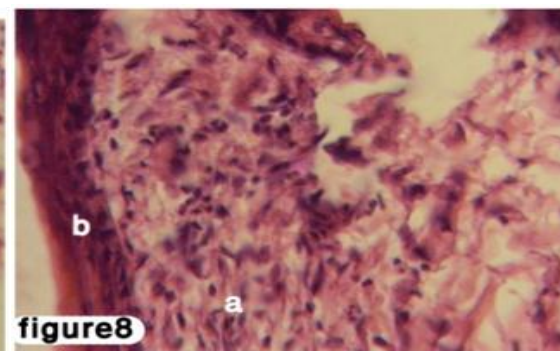


figure8

Fig. (8) At 5 days postoperative: more cellular fibrous connective tissue are present in the sutured area (a) with moderate thickness dermis layer are present (b) H & E X 40.

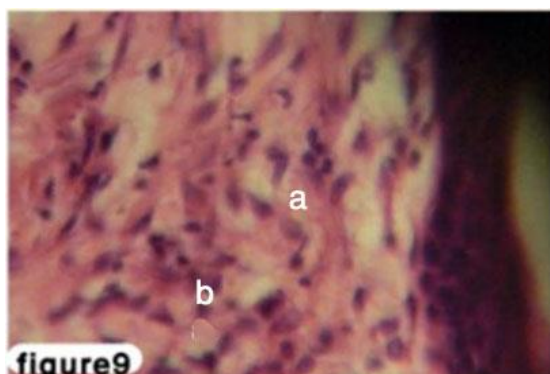


Fig. (9) At 7days postoperative: more regular dens collagen fibers (a) with mononuclear cell (b) infiltration is seen in the section area covered by complete epidermal layer H & E X 40.

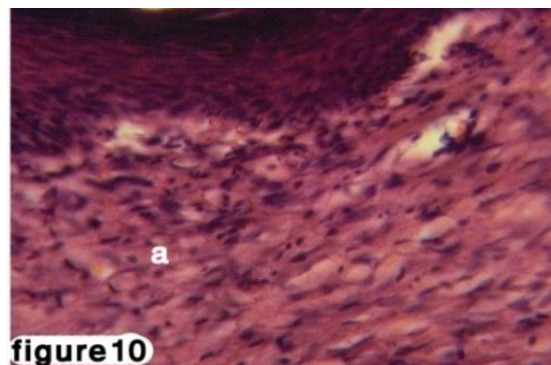


Fig. (10) At 14 days postoperative more mature fibrous connective tissue present in the section area which are regular and less cellular (a) covered by complete layer of epidermis H & E X 40.

Discussion

The goal of wound reconstruction is to return the individual to the best possible function as quickly as possible and with the best cosmetic results (20), innumerable substances and methods have been used, either locally or systemically to achieve this goal. Some examples are: prophylactic administration of antibiotics, medicinal plants (17 and 21), honey (22), lasers (23). Most of these therapies were found ideal and had wide success in promotion of wound healing.

Wound healing generally requires support at three levels: first, improving general resistance and support, second, stimulating the repair and regenerative mechanisms, third, therapeutic and nutritional activities (24). Multitude of these requirements were well provided by *Nigella sativa*.

Histopathological examination of investigate this study that the proliferation phase was began at 3rd day of operation in the treated group, which characterized by granulation tissue formation, while this stage didn't start till 5th day in control group, this confirm that *N sativa* enhance production of human interleukin and alerts macrophages (13).

In otherwise macrophages are able to phagocytes bacteria and provide a second line of defense, they also secrete variety of chemotactic and growth factors such as fibroblasts growth factor (FGF), epidermal growth factor (EGF), transforming growth factor (TGF), and interleukin -1 (IL-1) which appear to direct the proliferative phase (25). Collagen synthesis is stimulating by various growth factors (26), growth hormone is also known to promote the proliferation fibroblasts (27) and fibroblast proliferation form the granulation tissue, so this accelerates two phases of healing epithelization and collagenization; however it retards granulation and scar formation, beside that the *N sativa* oil contains fatty acids which build collagen (4) that's promote wound healing and maintain the skin elasticity.

This appeared histopathologically by epithelized the treated wound faster at 7th day post operative and less thickness dermis layer when compared with control group which was characterized by sever thickness of epidermis.

The black seed oil also act as occlusive dressing with good edge seals and can provide a barrier to migration of micro organisms into the wound, whereas bacteria have been shown to pass through 64 layers of moist gauze (28) and also keep the site moist and give a soft texture to the skin during the healing process, that's described improved wound healing under moist conditions (25). The moisture and nutritional activities of

black seed oil enhance debridement, neutrophils cell life and proteolytic enzymes action which lead to painless debridement (29) further these fibrin degeneration products are factors which stimulating macrophages to release growth factors into wound bed. Finally, when antioxidants can interfere the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers ,oxidative stress also plays an important role in impaired wound healing. Botanical with anti-oxidant or free radical scavenging activity thus can play a significant role in healing of wound (24). It can be suggested that the healing activity of black seed after local and even systemic administration may at least be in part due to its potent antioxidant activity (4). In addition to all above the antimicrobial, antifungal, antiviral activities of *N sativa* oil may lead to a clean wound healing without secondary infection.

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