The percutaneous effect of black seed (Nigella sativa) oil as external topical treatment on bone healing in rabbits

E’atelaf A. Al-Mutheffer  
Coll. of Vet. Med./ Univ. of Baghdad  
email: eatelaf23@gmail.com  
(Received 1 April 2014, Accepted 11 May 2014)

Abstract

This experiment was designed to evaluate the effect of black seed oil extraction as external topical treatment on bone healing. Twenty domestic rabbits were used; the animals were divided into two equal groups. Radius bone of both forelimbs were chosen for making a defect (2mm cavitation using electrical drill) in the middle shaft, the animals of control group (CG) were leaved to heal normally, while the animals of treated group (TG) were treated by rubbing the operated limb by oil extraction of black seed twice a day. The results of this study confirmed that N. sativa provided important factors which contributed in enhancement the healing process of the bone defect. However, the X-ray and histopathology section of the bone of the treated group showed enhancement of bone healing in: deposition, resorption, angiogenesis and remodeling stages, when compared to the control group. In conclusion the using of oil extract of N. sativa as percutaneous therapy enhances bone healing by enhancing different processes of cell migration and differentiation, extracellular matrix formation and organization towards calcification.

Keywords: Nigella sativa, oil extraction, percutaneous therapy, bone healing.

Introduction

Bone is a dynamic biological tissue composed of metabolically active cells that are integrated into a rigid framework. The healing potential of bone is influenced by a variety of biochemical, biomechanical, cellular, hormonal, and pathological mechanisms (1). The bone healing process was continuously occurring state of bone deposition, resorption and remodeling. Different modalities have been advocated for fracture repair, these modalities may be physical modalities like low-intensity pulsed ultrasound, electrical stimulation and laser (2-4), chemical modalities like bio-ceramic, and chitosan (5 and 6) or biological modalities like bone morphogenetic protein,
Materials and methods

Twenty adult domestic male rabbits weighing 1.5-2 kg were used for this experiment. The rabbits were caged under identical conditions in animal house of the department of surgery, College of Veterinary Medicine, Baghdad University, and had free accesses to water and food. The animals were anesthetized by intra-muscular injection of 40 mg /kg Ketamine HCl, 35 mg Xylazine and 4mg/kg diazepam, as an anesthetic protocol (18), and were operated upon under sterile conditions. Surgical wound was made on the medial side of both forelimbs with about a 3 cm incision, after careful dissection, the radius was exposed and a 2 mm bony defect (cavitation) was made using a small electrical drill. All bone debris were meticulously washed and wiped away by sterile distilled water, then the wound was routinely closed by several stitches of simple interrupted sutures by surgical silk 2/0. Procaine pencilin mixed with streptomycin (Pen-strepto Norbrook Company, England) was given post operatively as an infection prophylaxis. The animals were divided in two equal groups of 10 animals each. First group was control group (CG) returned to their cages after operations, while the second group was treatment group (TG) which treated by rubbing the operated limbs at the radial site percutaneous by Nigella sativa oil extract (Emmad factory for oil production, Mosul, Iraq permit NO, 70490) immediately after operation and then twice daily with intervals 8 hours for four weeks after operation. Daily clinical signs were recorded and radiographs were obtained after 1st, 2nd, 3rd and 4th week postoperative on medio-lateral position of the limb. The histopathological samples were collected after euthanasia of two animals of each group at 1st, 2nd, 3rd, 4th and 5th week postoperatively. Animals were euthanized by overdose of thiopental sodium. The samples were fixed in 10% buffered formalin then the specimens preserved in the formalin and nitric acid (1:3) over night for decalcification process, then were embedded in paraffin, sectioned and stained with Hematoxylin–Eosin stain (19).
Fig. 1: CG 1st week appear clear radiolucent circle defect in the dorsal aspect of the radius, with signs of breaking in the cortex.

Fig. 2: CG 2nd week, showed the defect is still clear, with some turbidity in the center of it, there are signs of high radiographic opacity at the margin of the defect.

Fig. 3: CG 3rd week, marked clear in the radiolucent circle defect, with sign of turbidity in the center of it, thickening in the endostium.

Fig. 4: CG 4th week, marked less demarcation of the defect, high radiographic opacity around the defect with thickening in the endostium.

Fig. 5: TG at 1st week appear radiolucent circle defect in the dorsal aspect of the radius.

Fig. 6: TG at 2nd show less margined of the defect, clear sclerotic area around, especially in the endostium, with signs of periosteal reaction.

osteotomies were at different stages of bone healing according to dating of scarification.

The radiographic picture in control group, at 1st week showed clear radiolucent circle defect in the dorsal aspect of the radius, with signs of breaking in the cortex (Fig. 1). In the 2nd week, the defect was still cleared, with some turbidity in the center of bone’s defect,
Fig. 7: TG at 3rd week appears turbidity in the center of the defect, high radiographic opacity at the margin, especially at the endostium away from the defect especially toward the proximal aspect of the radius, filled marrow canal with new bone formation, there is signs of periosteal reaction.

Fig. 8: TG at 4th week showed the radiolucent defect is obscure, with enlargement of the bone; the cortex is more dense compare with the adjacent area, the marrow canal still narrow distally to the defect while the proximal area is filled with new bone formation.

Fig. 9: Photomicrographs at 1st week of CG showed hemorrhage (arrow) and hypercellularity in bone defect (arrowhead) (H&E stain 40X).

Fig. 10: Photomicrographs at 1st week of TG showed congested sinusoid in bone marrow and dilated Haversian canal with mononuclear cells in their lumen (H&E stain 40X).

Fig. 11: Photomicrographs at 2nd week of CG showed necrotic fragment bone (arrowheads) surrounded by inflammatory cells and fibrous connective tissue extended to periosteum (arrow) and necrosis of endosteum (H&E stain 40X).

Fig. 12: Photomicrographs at 2nd week of TG showed, mature trabecular bone with narrow Haversian canal (arrow) (H&E stain 40X).
Fig. 13: Photomicrographs at 3rd week of CG showed, fragment of new bone tissue (arrow) surrounded with mononuclear cells (arrowhead) in the artificial cavity (H&E stain 40X).

Fig. 14: TG at 3rd wk. see the bone cavity filled with primary and secondary spongiosa (thin arrow) the bone cavity filled with fibrous connective tissue (thick arrow) with mature trabecular bone surrounded by active osteoblasts (arrowhead) (H&E stain 40X).

Fig. 15: Photomicrographs at 4th week of CG of bone showed narrow trabicul surrounded large cavities (arrow) extended to the lumen of the space (H&E stain 40X).

Fig. 16: Photomicrographs at 4th week of treated group of bone showed compact bone with Haversian canals filled the bone defect (arrows) (H&E stain 40X).

Fig. 17: Photomicrographs at 5th week of CG showed woven bone surrounded wide Haversian canals (arrow) (H&E stain 40X).

Fig. 18: Photomicrographs at 5th week of TG showed bone cavity filled with compact bone with congested Haversian canals (arrow) (H&E stain 40X).
thickening in the endostium and high radiographic opacity at the margin of the defect (Fig. 2). While in the 3rd week, there was less demarcation in the defect, high radiographic opacity around the defect area with thickening in the endostium, periosteal reaction was seen as an elevated area around the defect due to the new bone formation (Fig. 3). Also, in the 4th week, showed less demarcation of the defect, thickening in the endostium from defect toward the proximal and distal aspect of the bone with close of the medullary canal beside the defect due to the new bone formation from the endostium were seen the signs of periosteal reaction was seen as an elevated area around the defect due to the new bone formation, the cortex is not completely incorporated (Fig. 4). The radiographic picture of the treated group at 1st week, show clear mark radiolucent circular defect in the dorsal aspect of the radius (Fig. 5). In the 2nd week, there was showed less margin defect, clear sclerotic area around especially in the endostium, with the signs of periosteal reaction (Fig. 6). At the 3rd week, there was turbidity in the center of the defect, high margin radiographic opacity especially at the endostium away from the defect toward the proximal aspect of radius, the marrow canal filled with new bone formation, and there was a sign of periosteal reaction (Fig. 7). In the 4th week, the radiolucent defect was obscure, with enlargement of the bone. The bone cortex was more dense compare with the adjacent area. The marrow canal was still narrow distally to the defect while the proximal area was filled with new bone formation due to endostium reaction. The cortex of the both side of the defect was completely corporate with the adjacent part of the bone (Fig. 8). The histopathological sections of CG was demonstrate hemorrhage and hypercellularity were found between periosteum and the bone defect at 1st week postoperatively (Fig. 9), while at the same period in the TG showed congested sinusoid in bone marrow and dilated haversian canal with mononuclear cells in their lumen were seen (Fig 10). At the 2nd week necrotic fragment surrounded by inflammatory cells and fibrous connective tissue extended to periosteum and necrosis of endostium in CG were seen (Fig. 11), whilst TG showed, mature trabecular bone with narrow Haversian canal (Fig 12). At 3rd week, CG were showed, fragment of new bone tissue surrounded by mononuclear cells in the induced cavity (Fig.13), while the TG was appeared bone cavity filled with primary and secondary spongiosa, fibrous connective tissue with mature trabecular bone surrounded by active osteoblasts (Fig.14). At 4th week, CG exposed narrow trabiculi surrounded by large cavities extended to the lumen of the space (Fig.15), while TG were displayed compact bone with Haversian canals filled the bone defect (Fig. 16). At 5th week, the bone in the CG was showed woven bone surrounded by wide Haversian canals (Fig. 17), while TG was exhibited cavity filled with compact bone with congested Haversian canals (Fig. 18).

Discussion

In this study, there was increasing in the density of callus in the defect site of the radius shafts of treated groups. Despite both control and treated group were radiographically similar in 1st, 2nd and 3rd week. But in 4th week the bone cortex in TG was more dense when compare with the adjacent area than the CG, and the cortex at the both sides is completely regenerated compared with the adjacent part of the bone. This result may mean that there was an effect from the treatment which stimulates the callus formation and enhance bone healing process, and didn’t appear radiographically, because the callus was radiolucent until the hard callus was formed at the 4th week. This consequence could be attributed to the active components of black seed like protein, amino acid, fatty acids, minerals especially (calcium), vitamins (20), which absorbed from skin during massage by black seed oil and reached the bone defect through blood circulation. The histopathological evaluation showed enhancement in healing process of the bone defect early at 1st week reaching the 5th week in comparison with control group. The result of histopathological sections of first week agree with Travlos (21) who discussed that increases in nucleated cells in histological sections of bone marrow usually
indicates a response to increased cell indigence and is most clear in the hematopoietic cell. *N. sativa* have beneficial role for improving the blood flow (22) that’s lead to increasing of the blood amount that reach the fracture or defect area and thereby increase angiogenesis, which is a key aspect of fracture healing, and also regulated at the molecular level. An angiopoietin pathway has been described in the early stages of the healing process as has a vascular endothelial growth factor (VEGF)-dependent pathway related to endochondral bone formation; in addition to that, optimal bone healing is dependent on adequate vascularization and therefore requires the development of new sinusoid (23). This appeared histopathologically when the treatment group showed congested sinusoid in bone marrow faster at 1st post-operative week when compared with control group. The presence of mononuclear cells at the section has been found to be more accountable an activation of host cellular and humoral immune system response (24). The anti-inflammatory effect of *N. sativa* oil could be credited to the inhibition of production of mediators (25 and 26), Antioxidant or free radical scavenging (27-29) and modification of trafficking of the inflammatory cells into inflammatory lesion (30). This confirms that *N. sativa* enhances production of human interleukin and alerts macrophages (31). That’s made the significant differences in histopathological evaluation between experimental and control group at the end of 2nd week. The tissue develops from granulation tissue to mature bone in the sequence of fibrous tissue, cartilage, immature bone and intermediate bone based on the endochondral ossification process (32). Histopathological examination of this study shows good reaction without complications in treated groups. Beside that *N. sativa* oil contains fatty acids which build collagen and that is the main continence of cartilage (33). The treated group at 3rd week showed the bone cavity filled with fibrous connective tissue with mature trabecular bone surrounded by active osteoblasts. The findings in this study support the healing performance which illustrated as the ratio of the average callus modulus to the initial modulus of the mature bone. Developed bone tissue was assorted into three phases; the granulation phase composed of granulation tissue and fibrous tissue; the immature phase composed of cartilage, immature bone and intermediate bone, and finally the mature phase composed of the mature bone (34). In 4th and 5th week the histopathological change was more clear of treated group that compact bone with Haversian canals filled the bone cavity at a faster pace than control group, this information herein illustrates presence of many important factors which the black seed shown to be rich sources with, like potassium, calcium, sodium and other elements, and that was essential for bone formation and regeneration (35).These results were agreed with Suh et al., (34) who revealed that the increased amount of Ca may facilitate integrin mediated attachment of bone forming cells through enhanced ligand binding of receptors. Valizadeh et al., (36) who was demonstrated that administration of *N. sativa* excreted more anabolic effects on bone by increasing the rate of bone formation and improving biomechanical bone strength, whilst Alnajar and Mohammed (37) showed that the implants (titanium screw) coated with *N. sativa* oil produced a significant increase in torque value when compared to the uncoated ones within different time period, and emphasize that the reasons can be generally interpreted as an increase in bone healing around the implant and improvement in the strength of bony integration at the bone–implant interface. This supports the incidence of ossification process in treatment group in a faster pace because of the presence of black seed oil treatment. In addition to all above epicutaneous therapy onto intact skin has proved to be an effective and safe alternative remedy of both local and systemic conditions of animal as well as human (38).

**In conclusion** the using of oil extract of *N. sativa* as epicutaneous therapy enhances bone healing by enhancing different processes of cell migration and differentiation, extracellular matrix formation and organization towards calcification, and this study findings support the common folk idea that the external rubbing and lubrication
of the area adjacent to the fracture around the splint and after plucking out the splint, by warm black seed oil daily during the course of fracture healing for enough time will enhance the fracture healing process.

Acknowledgement
Special thanks for Prof. Dr. Mohammed Jawed, Assist. Prof. Dr. Enam Falah Bader and Assist. Prof. Dr. Hemmed Ali Altememe.

References
derivatives on reactions generating reactive oxygen species. J. Chemosphere, 41:1059-64