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Evaluation of the Effect of Nigella Sativa Oil and Powder on Socket Healing Process

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Abstract
Back ground: Today's world is increasingly seeking ways to replace the synthetic drugs with the therapeutic power of natural products to decrease the percentage of many side effect which result from conventional treatment; one of these products was Nigella sativa (NgS) which was used so extensively that it became known as the seed of blessing "Habbatul Barakah" due to its powerful healing qualities for many ailments.
Aim of the study: This study was performed to evaluate the therapeutic effect of Nigella sativa (powder and oil) on the healing process of extracted teeth sockets.
Materials and Methods: The sample of our study consist of twenty four healthy male New Zealand rabbits ,extracted their upper two central incisors under general anesthesia. The left side filled once with Nigella sativa powder and once with Nigella sativa oil material, and the right side left for normal healing as a control group. The two sockets were sutured. The results were studied radiographically and histologically after 1and 6 weeks postoperatively. The radiographic examination was performed by using parallel technique in a digital radiographic examination and histological examination was performed under light microscope for the section stained with heamatoxiline and eosin.
Results: Radiographically we found that NgS powder showed more radiopacity with complete disappearance of lamina dura in 6 weeks duration compared with NgS oil and control groups, while histologically we found that the Nigella sativa (NgS) groups (powder and oil) illustrate an early apposition of osteoid tissue in 1st week duration with numerous osteoblast and osteocyte in comparison to control group. In six weeks duration well developed bone filled all the portions of the socket in treated socket with NS powder with obvious complete epithelization of socket surface
Conclusion : Nigella sativa (powder or oil) seems to be bioactive materials that enhance differentiation and proliferation of progenitor cells to specialized bone formative cells, with no signs of inflammation.
Key words: Nigella Sativa,Socket healing,Repaired bone,Bone cells

Introduction

Repair of bone tissue is a complex process involving a number of cellular functions directed towards the formation of a scaffold and mineralization of the defect followed by an eventual remodeling of the defect site to attain the original structure .(1)

Today's world is increasingly seeking ways to replace the synthetic drugs with the therapeutic power of natural products **.**

Interest in medicinal plants has burgeoned due to increased efficiency of new plant-derived drugs and the growing interest in natural products. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades.(2)

Many wonderful helping plants surfaced when the cultures of our world started to share trade and teach their herbal medicines to each other. One such plant was *Nigella sativa* commonly known as black seed. This mild aromatic herb is indigenous to the Middle East where it has been used as a traditional remedy for over 2000 years. It was used so extensively that it became known as the seed of blessing "Habbatul Barakah" .(3)

Its therapeutic use was initiated after the advent of Islam since *Prophet Mohammed (peace and pray upon him)* mentioned its therapeutic efficacy and potential of cure, when said that "there is cure for every disease in black seed except death".(4)

Its chemical composition contains volatile and non-volatile oils in addition to many other active ingredients including proteins, alkaloids, coumarines, saponins, minerals, carbohydrates, phenolic compounds, steroidal compounds, and other ingredients .(5)

Many studies have been conducted on the effect of Nagilla sativa seed extracts on varies body systems in vitro or in vivo. The pharmacological investigation of the seed extracts reveal abroad spectrum of activities including immuneopotentiation, antihistaminic, antidiabetic, antihypertensive, antinflammatory, antitumor,(6) antiparasitic, antibacterial, antifungal and antioxidant **(7).** In recent study, the black seed induced bone healing as manifested by faster bone trabeculae formation and mature bone formation (8). And as when a tooth is extracted, the healing process was done by two processes

A.Osteogenesis
B. Epithelization
Therefore the present study was designed to:

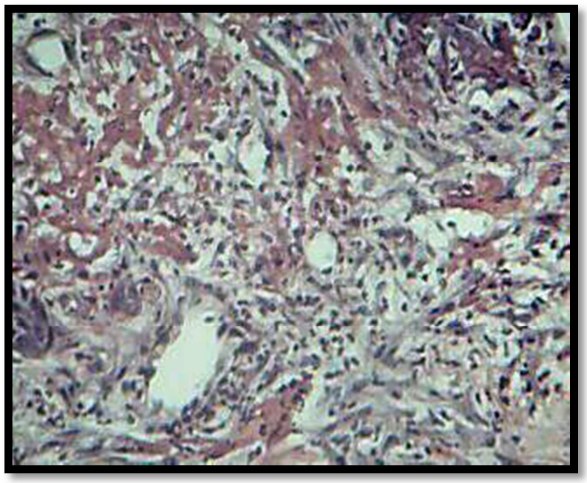
1. Evaluate the effect of Nigella sativa (powder or oil) on healing process of the socket of extracted teeth.
2. Study the comparison effect of Nigella sativa (powder or oil) on healing process in different weeks.

Materials and Methods: Twenty four healthy male New Zealand rabbits ,weighing(2.125-2.25kg) ,aged 14-16 weeks were kept in the animal department of (National Center of Drug Control and Research /Iraq)at a constant humidity and temperature of 23°C according to the National Council's guide for the care of laboratory animals.The rabbits were subjected to extract their upper two central incisors under general anesthesia. The left side filled once with Nigella sativa powder and once with Nigella sativa oil material, and the right side left for normal healing as a control group. The two sockets were sutured. The animals were grouped into:
1st group (12) rabbits, left socket of each rabbit filled by (0.068g) of Nigella sativa powder till 2/3 of socket length which had average length (2.3mm) after removal of blood clot by using blugger instrument.
2nd group (12) rabbits, left socket treated with Nigella sativa oil by using a piece of cotton impregnated in oil then inserted inside the socket till 2/3 of socket length after removal of blood clot and waiting for five minutes then removed it.
The control group of (24) rabbits in which their sockets had been left for normal healing process without removal of blood clot after extraction process.
Each group divided into two subgroup (6 in number); these subgroups are sacrified for each of healing periods; 1week and 6weeks ,and the socket examined radiographically and histologically .
The radiographic examination was performed by using parallel technique in a digital radiographic examination and histological examination was performed under light microscope for the section stained with hematoxiline and eosin .

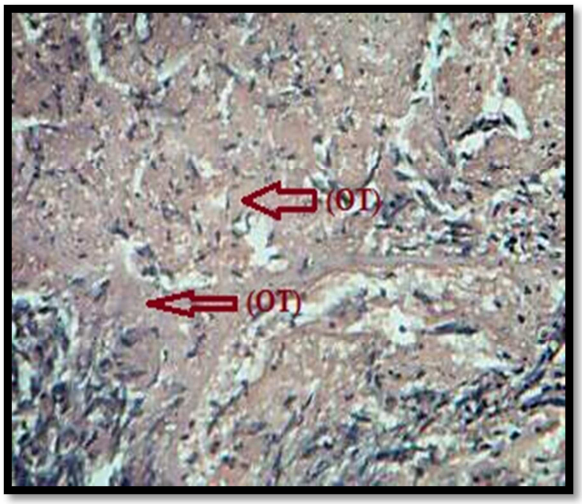
Results
Radiographically. Nigella sativa NgS showed more radiopacity with complete disappearance of lamina dura in 6 weeks duration compared with control group. Figure (1)

Figure(1) Conventional radiographic view of sockets for rabbit's anterior teeth of six weeks duration shows radio-opacity filled coronal, middle and apical portion of experimental (powder and oil) side (left) and the lamina dura shows to be disappear internally and externally partially disappeared, while in the control (right) side the radio-opacity extended to the middle portion and the lamina dura partially disappeared internally and externally

Histologically
The result showed active proliferation of cellular connective tissue with early deposition of osteoid tissue and presence of osteoblast cells in the coronal portion of the experimental socket treated with Nigella sativa powder and oil in 1st week in comparisum to control (Figures 2,3)



Figure(2) Coronal portion of extracted tooth socket of the rabbit (control) one week duration shows formation of granulation tissue H&E×20

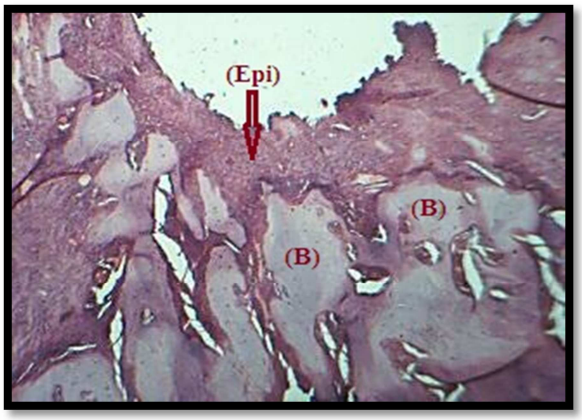


Figure(3)Micrograph view for coronal portion of rabbit left socket treated with NgS , one week duration show active proliferation of osteoid tissue(OT) H&E ×20

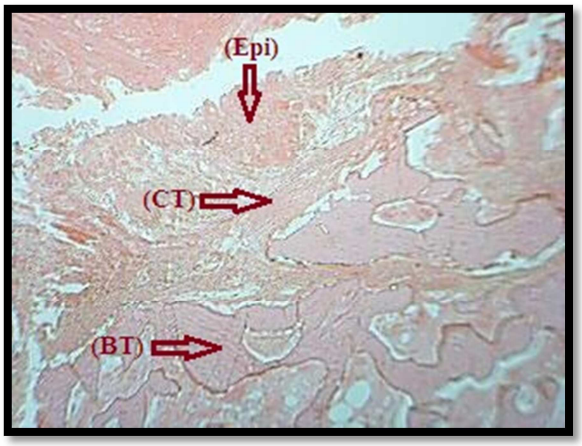
At Six Weeks postoperatively:

Experimental group treated with NgS powder showed that mature bone filled all cervical regions and covered with epithelial layers,Figure(4).On other hand experimental group treated with NgS oil showed that bone trabeculae covered with connective tissue and incomplete epithelization ,Figure(5).

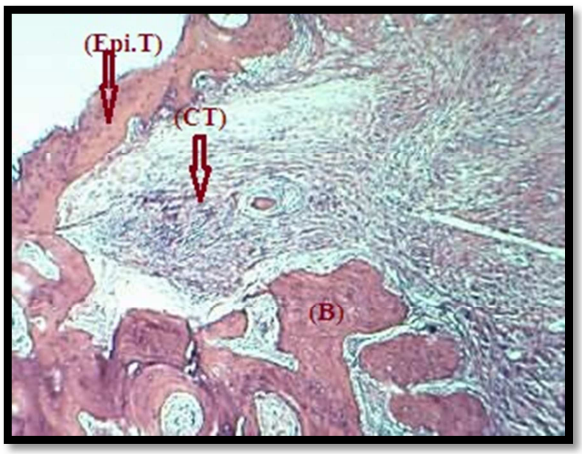
Histological findings for control showed that sparse of bone trabeculae filled cervical portion with fibrous connective tissue lay over it and superficial layer of epithelial tissue Figure(6)



Figure(4)Coronal portion of rabbit left socket treated with (NgS) powder 6weeks duration shows mature bone (B) filled all cervical region and covered with epithelial layers (EPI) H&E×10.



Figure(5)Coronal portion of rabbit left socket treated with (NgS) oil 6weeks duration shows mature bone trabiculae(BT) covered with connective tissue(CT) and epithelia (EPI) H&E×10.



Figure(6) Coronal portion of rabbit right socket(control) 6weeks duration shows bone filled the cervical portion with fibrous connective tissue(CT) lay over it and superficial layer of epithelial tissue (Epi.T) H&E ×10.

Discussion

The results of the present study showed early detection of osteoid formation in 1st week and bone trabeculae formation in 6th weeks in sockets treated with NgS (powder and oil).

Active cell proliferation osteoblast was illustrated in experimental sockets in comparison to control one; it was indicated that NgS acts as bioactive and bioinductive materials that enhance bone formation. This could be attributed to presence of the following components:

1. Protein and amino acids:

Nigella sativa seed had 22.6 % - 26.7 % protein and amino acid , Glutamic acid(Gla,), Arginine(R) and Aspartic acid(D) were the main amino acids present [9] in addition to other like glycine(G), leucine, etc [10]

These mentioned amino acid play important role in the formation and function of the following ECM proteins: -

A. ***Osteocalcin:*** also known as bone Gla protein is one of the most abundant non collagenous proteins (NCPs) in bone (comprising up to 20 % of the NCPs) [11]. It has 49 amino acid residues containing three glutamate (Gla) residues, which are critical for the ability of osteocalcin to bind calcium [12]**.** It's binding to HA through orientation of the Gla residues with the Ca ions in the mineral lattice [11]. It has been reported that osteocalcin involved in bone growth & repair [13]**.**

B. ***Bone Sialoprotein (Bsp):*** comprises 15 % of the total non collagenous protein (NCP) in bone [11]**.**

C. ***Osteopontin:*** One of the important NCPs that includes in bone formation [12]. Bone sialoprotien and osteopontin are belived to play roles in cell adhesion and binding of mineral because they contain Arg – Gly – Asp and poly acidic sequences [14]**.** It was reported that it stimulates the adhesion of osteoblasts and,therefore, to improve the osseointegration process [15,16]**.**

These proteins and amino acid functionated a receptor specificity, binding affinity and signaling of cell responses [17]**.**

2. Minerals:

The induction of bone formation by the black seed oil extract seen in this study as manifested by faster bone formation could be attributed to the presence of calcium and phosphorous in the chemical composition of the black seed oil extract which are necessary for bone formation.

3. Vitamins

Vitamins are low– molecular – mass organic compound that can't be synthesized by humans or are synthesized in inadequate amounts for example:

Vitamin	**Biochemical function**
* Thiamine (B_1)	Co-enzyme in phosphgluconate path way.
* Pyridoxine (B_6)	Amino acid transamination
Niacin	Hydride-transfer reaction.
Foliate	Thymidylate synthase; purine biosynthesis.
Ascorbate (C)	Prolyl and lysyl hydroxylases (collagen); dopamine hydroxylase.
Retinol (A)	Form 11-Cis-retinal with rhodopsin.

It appears that Vitamins are important because they play a central role in metabolism. [18]

NS contain the a above mentioned vitamins so it possess nutritional value [19] and has been reported to possess a favorable effect on growth rate and health of human and animal [20]**.**

In histological study we found that the effect of Nigella sativa (NS) powder more effective than oil in formation of bone inside the socket of extracted tooth because there is no any component of Nigella sativa will be lost such as in oil extract and the minerals present without any defect which help in faster bone formation, also the absorption of Nigella sativa oil require more time for complete absorption which applied by apiece of cotton for only 5 minutes and then removed. This could be attributed to the low percentage of volatile oils (0.4-0.45%) in the chemical composition of the cold-pressed oil as compared with the higher percentage of fixed oils (32-40%) including the unsaturated fatty acids (arachidonic, eicosadienoic, linoleic, linolenic, oleic and almitoleic acid) and the saturated fatty acids (palmitic, stearic and myristic acid, Beta-sitosterol, cycloeucalenol, cycloartenol, sterol esters and sterol glucosides).

From the histological examination of the treated sockets with NgS (specially powder) showed early proliferation of epithelia covered the cervical portion of the socket, compared to control, this can be explained that NgS has inductive power to epithelial cells as if it has induction to connective tissue cells (mesenchymal cells).

Radiographic findings:

Radiographic results of the present study showed radiopaque patches appeared in the middle (specifically) and apical portions of treated sockets with NgS (powder and oil) groups as it supported histological findings which showed trabeculae formation in these portions more than in coronal one and more than in control group.

In 6^{th} week duration NgS powder showed radiopacity filled whole socket (coronal, middle and apical portion) with disappearance of lamina dura, this related to apposition of mature bone filled the socket as it illustrated histologically, and that lamina dura landmark be obscured as the new bone interdigited and coalesce with the original bone. This results in agreement with [21,22] results that found increase in bone formation and reported anabolic effects of NS oil on bone loss radiographically.

Conclusion

Nigella sativa (NgS) seems to be Bioactive materials that enhance differentiation and proliferation of progenitor cells to specialized bone formative cells, with no signs of inflammation the NgS materials close to be inert material.

Nigella sativa powder enhance epithelial proliferation which appeared early compared with NgS oil and control.

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