

NIGELLA SATIVA HAS BENEFICIAL EFFECT ON OSTEOPOROSIS AND BONE HEALING: IS IT A FACT OR FICTION? A SYSTEMATIC REVIEW

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ABSTRACT

Osteoporosis is the most common reason for a broken bone among the elderly. Several experimental studies have been reported that *Nigella sativa* (NS) and/or its major component thymoquinone (TQ) have good effects on osteoporosis and bone healing. We conducted this systematic review to evaluate these relevant studies to prove whether NS and/or TQ has potential effect on osteoporosis and can stop pathogenesis of this disease or this matter still just a fiction. A search on published studies was done using databases including Scopus, PubMed, Google Scholar, Web of Science, and CINAHL. Terms searched included "*Nigella sativa*, black seed, TQ, osteoporosis, bone healing." Initially, 213 articles were extracted. After reviewing their titles and abstracts, 124 articles (Medline, 43; Scopus, 67; EBSCO, 14) were retrieved for further evaluation. However, after excluding the clinical trial studies, human reviews, removal of abstracts and unrelated studies, seven studies were considered finally as eligible for our review. Finally, seven studies were considered eligible for our review. The total number of animals used was 220 (150 rats and 70 rabbits) from different experimental study. Based on the results of this systematic review, we conclude that NS or TQ extract therapy for osteoporosis cannot be recommended yet and these data will not suffice to exclude the beneficial effects of NS on bone turnover reliably. Therefore, more studies are required to explore the specific cellular and molecular targets of NS or TQ using animal osteoporosis models. Once the anti-osteoporotic effectiveness of NS or TQ will be established, human studies can be carried out.

Keywords: *Nigella sativa*, Black seed, Thymoquinone, Osteoporosis, Bone healing.

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INTRODUCTION

Osteoporosis is a disease where decreased bone strength increases the risk of a broken bone. It is the most common reason for a broken bone among the elderly [1]. The underlying mechanism in all cases of osteoporosis is an imbalance between bone resorption and bone formation [1].

The mechanisms of pathogenesis by which osteoporosis develops are inadequate peak bone mass, excessive bone resorption, and inadequate formation of new bone during remodeling. The interplay of these three mechanisms underlies the development of fragile bone tissue [2]. Hormonal factors strongly determine the rate of bone resorption; lack of estrogen (e.g., as a result of menopause) increases bone resorption as well as decreasing the deposition of new bone that normally takes place in weight-bearing bones. The amount of estrogen needed to suppress this process is lower than that normally needed to stimulate the uterus and breast gland. The α -form of the estrogen receptor appears to be the most important in regulating bone turnover [2]. In addition to estrogen, calcium metabolism plays a significant role in bone turnover, and deficiency of calcium and vitamin D leads to impaired bone deposition; in addition, the parathyroid glands react to low calcium levels by secreting parathyroid hormone (PTH), which increases bone resorption to ensure sufficient calcium in the blood. The role of calcitonin, a hormone generated by the thyroid that increases bone deposition, is less clear and probably not as significant as that of PTH [2].

Regarding prevention of osteoporosis, it has been suggested that lifestyle prevention in many aspects can inverse of the potentially modifiable risk factors of the disease [3]. As tobacco smoking and high alcohol intake have been linked with osteoporosis, smoking cessation and moderation of alcohol intake are commonly recommended as ways to help prevent it [4]. Studies of the benefits of supplementation

with calcium and vitamin D are conflicting possibly because most studies did not have people with low dietary intakes [5]. A 2015 review found little data that supplementation of calcium decreases the risk of fractures [6]. However, a systematic review has found a benefit of vitamin D supplements combined with calcium for fractures, and they did not find a benefit of vitamin D supplements alone [7].

Different types of medicinal plants approach osteoporosis in different manner means that some provide dietary calcium, some regulate body's use of calcium, and some increase the level of certain hormones in the body. Among these plants is *Nigella sativa* (NS).

NS (Family: Ranunculaceae) or black seed is a widely used medicinal plant around the world. It is very popular in various traditional systems of medicine such as Unani, Ayurveda, Arabic, Siddha, and Chinese. Seeds and oil have a long history of folklore usage in various systems of medicines and food. It has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesic, anti-bacterial and in skin disorders [8]. Extensive studies on NS have been carried out by various groups of researchers, and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic [9,10], anticancer, immunomodulator [10], analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastro-protective, antioxidant properties [8]. The seeds of NS are widely used in the treatment of numerous diseases such as bronchitis, asthma, diarrhea, rheumatism, and skin disorders. In addition to that, many literatures reported that NS was used as liver tonic, digestive, antidiarrheal, appetite stimulant, emmenagogue, and supportive of immune system [11-13]. Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ), which is a major active chemical component of the essential oil. Black seeds are also used in food like flavoring additive in the breads and pickles because it has very low level of toxicity [14].

Several experimental studies have been conducted to study the effect of NS as a whole or only its major component TQ reported that NS and/or TQ have beneficial effects on osteoporosis and bone healing [15-21]. Besides that, some few review articles that have surveyed the effect of NS on osteoporosis and bone healing. However, we are going to conduct a systematic review of the literature to identify these relevant studies to prove whether the NS and/or TQ have potential effect on osteoporosis and can stop pathogenesis of this disease or this matter still just a fiction.

METHODOLOGY

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist as a protocol in this review. A search of published studies was performed by using computer databases including Google Scholar, Scopus, PubMed, Web of Science, and CINAHL.

Literature review

A systematic review of the literature was conducted to identify relevant studies about NS and osteoporosis. To conduct a comprehensive search of health science journals, we used Medline through Ovid Medline (published between 1980 and January 2017) and CINAHL through EBSCO host (published between 1980 and 2017). The search strategy involved a combination of the following two sets of key words: (1) *Nigella sativa* OR black seeds OR habbatus sauda, black cumin OR TQ and (2) osteoporosis OR bone healing. The inclusion criteria were (1) the effect of NS on osteoporosis and bone healing; (2) the effect of TQ on osteoporosis and bone healing. However, the exclusion criteria were (1) clinical trial studies, (2) review studies, (3) the effect of NS and TQ on unrelated clinical parameters, (4) the effect of NS and TQ in combination with other plants, and (5) duplicated studies.

Selection of research articles

The results were limited to the studies that were published in English language that included abstracts. To be included, studies had to (1) report the association or effect of NS and TQ on bone changes. Papers were excluded if the studies were related to reviews, news, letter, editorials, or case studies.

Data extraction and management

We selected papers to be included in the review in three phases. First, we excluded any paper that did not match the inclusion criteria based solely on the title. Second, we screened all the abstracts of the remaining papers and then excluded a second group of papers that did not meet our inclusion criteria. Finally, we read the remaining papers from the second phase to exclude any paper that did not meet our inclusion criteria.

After the initial screening of the titles and abstracts, duplicates were removed and the remaining papers were again screened by at least two reviewers. The inclusion of the full papers into the review had to be agreed by at least two reviewers before the data extraction phase. Initially, 213 articles were extracted. After reviewing their titles and abstracts and removing unrelated, human, and review studies, 124 articles (Medline; 43, Scopus; 67, EBSCO; 14) were retrieved for further evaluation. Seven studies were considered eligible for our review after excluding all clinical trial studies, reviews, duplicate abstracts and unrelated studies (Table 1).

RESULTS

Data extraction was performed independently from seven studies and in a standardized manner with the use of a data collection form. We recorded the following data from the studies: (1) the type of study, (2) a brief description of the sample/population of the study; (3) a brief description of the methods used in the study; (4) the brief description of the results of the study; (5) comments and conclusion of the study; (6) complications if any.

Table 1: Process of study selection

Process	Selected studies
Initial search	213 papers
After exclusion of studies that were unrelated to this study	124 papers
After exclusion of human studies	47 papers
After exclusion of review studies	22 papers
After exclusion of combined studies	13 papers
After exclusion of duplicated studies (eligible for our review)	7 papers

All studies were animal experimental studies and have been conducted either on rats or rabbits [15-21]. Information of these studies is summarized in the Table 2.

The total number of animals used was 220 (150 rats and 70 rabbits), from different experimental studies. The parameters that have been determined in rat studies were different such as serum alkaline phosphatase (ALP) level, radiological, histological, immunohistochemical, and morphometrical studies [15], other study measured plasma levels of calcium (Ca^{2+}), phosphorous (Pi), ALP, amino terminal collagen Type 1 telopeptide, malondialdehyde (MDA), nitrates, nitric oxide surrogate, tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) in addition to istomorphometric analysis of the tibia [16]. Moreover, systemic quantitative measurement for new bone formation, osteoblast lining, and semi-quantitative measurement of capillary intensities were examined in another study [18]. However, in the studies that have been used rabbits in their experiments determined radiological and histopathological effect on the bones [20,21].

The duration of the trials was between 5 days and 10 weeks. The dose of NS and TQ was between 2.5, 5, and 10 mg/kg/day and was administered orally in all studies, except one external.

Rats treated with NS showed remodeling of bone with slight decrease in cortical thickness, while sections from treated rats with NS showed nearly normal osteoblasts. Most of osteocytes were normal, but a few cells were degenerated. There were few small osteoporotic cavities and some cement lines indicating bone repair [15].

Ovariectomized (OVX) rats showed significant decrease in plasma Ca^{2+} , accompanied by a significant increase in plasma ALP, amino-terminal collagen Type 1 telopeptide, MDA, nitrates, TNF- α , and IL-6. These changes were reversed by NS supplementation in OVX-NS group to be near-normal levels in addition to that histological changes were also noticed on tibia in OVX-NS rats [16]. These results reported also in studies that been used rabbits [20,21]. They confirmed that NS provided important factors which contributed to the 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 [21]. However, no complications caused by NS were observed in all studies (Table 2).

DISCUSSION

We evaluated the results of seven experimental studies that have been conducted to evaluate the potential effects of NS and or TQ and whether these studies succeeded to explain why NS and TQ have anti-osteoporotic effects on animal's bones according to underlying mechanism of this disease which is mainly an imbalance between bone resorption and bone formation. Moreover, we analyzed the studies to notice the three main mechanisms by which osteoporosis develops which are an inadequate peak bone mass, excessive bone resorption, and inadequate formation of new bone during remodeling [2].

Shady and Nooh, 2010 [15], tried to clarify changes of the compact bone of streptozotocin-induced diabetic rats and the possible role

Table 2: Characteristics of studies that eligible to the study

S.No.	Study	Type of study	Animal sample	Methodology	Results	Comments or outcomes	Complications
1.	Shady and Nooh, 2010	Experimental study	40 adult male rats	Four groups: Control, NS treated; diabetic and diabetic with NS. On sacrifice, blood samples were drawn to determine serum ALP level. Both femora of each animal were processed for radiological, histological, immunohistochemical and morphometrical studies	Treatment of diabetic rats with NS resulted in improvement of body weight, biochemical, radiographical, histological, morphometrical and immunohistochemical pictures	NS could be considered as a curative measure of diabetic osteoporosis	Nil
2.	Seif, 2014	Experimental study	30 female Wistar rats aged 12-14 months	Three groups: Sham-operated control, OVX, and OVX supplemented with NS orally for 12 weeks; After 12 weeks, plasma levels of calcium (Ca^{+2}), phosphorous (Pi), ALP, amino terminal collagen Type 1 telopeptide, MDA, nitrates, nitric oxide surrogate, TNF- α , and IL-6 were measured. Histomorphometric analysis of the tibia was also performed.	Changes were reversed by NS supplementation in OVX-NS group to be near SHAM levels. All parameters were markedly reversed in OVX-NS rats	NS reverses osteoporosis in OVX rats	Nil
3.	Kara <i>et al.</i> , 2011	Experimental study	32 Wistar albino rats	Four equal groups: OE, expansion plus TQ (TQ1 group, expansion plus TQ (TQ2 group, TQ given to the rats only during the retention period, and CG (no procedure done). Expansion appliances were placed on the maxillary incisors of all animals for 5 days. Histomorphometric evaluation was carried out	New bone formation, number of capillaries and the ratio of intensities of inflammatory cells in maxillary sutures was higher in the TQ groups than in the other groups. Statistical analysis also demonstrated that osteoblast and osteoclast numbers were also highest in the TQ1 group	Histomorphometric analysis demonstrated that systemic use of TQ may be effective in accelerating new bone formation in the RME procedure	Nil
4.	Arslan <i>et al.</i> , 2016	Experimental study	18 Wistar rats	Three groups and defects were created in their tibias. CG (C): No systemic drug administration; Test Group 1 (T1): Systemic TQ was administered daily starting 40 days before creation of the defect and additionally during the post-operative healing period of 28 days; Test Group 2 (T2): Systemic quantitative measurement for new bone formation, osteoblast lining and semi-quantitative measurement of capillary intensities were examined	There was a significant increase in the ratio of new bone per total defect area and new bone trabeculae lined by active osteoblasts in both test groups (T1 and T2) compared to CG (p<0.05)	Systemic administration of TQ either starting 40 days before or on the day of surgery accelerated new bone formation in a rat model and can be advocated as an adjunct to expedite bone healing	Nil
5.	Al-Enazi, 2015	Experimental study	30 female Wistar albino rats	The OVX rats were treated with different doses (2.5, 5 and 10 mg/kg/day) of TQ for 10 weeks. Bone turnover-biomarkers and inflammatory cytokines were determined using ELISA techniques. Changes in bones cortical and trabecular morphometric parameters were determined using micro-CT scan.	TQ treatments inhibited the increased bone turnover biomarkers and inflammatory cytokines in dose-dependent manner. The altered morphometric parameters of the femoral bones were also markedly attenuated by TQ	TQ has osteoprotective properties may be due to its ability to inhibit osteoclastogenesis inducing factors and inflammation	Nil

(Contd...)

Table 2: (Continued)

S.No.	Study	Type of study	Animal sample	Methodology	Results	Comments or outcomes	Complications
6.	Al-Sa'aidi <i>et al.</i> , 2012	Experimental study	50 mature male rabbits	Rabbits were randomly assigned into five equal groups (control and four treated groups [TGs]). Animals were daily treated, for 42 days, as follow: C was orally administered with drinking water; T1 was orally administered with NSSE (1.5 g/kg, b.w.); T2 was injected with dexamethasone (2 mg/kg, b.w., i.m.); T3 was combined treated concomitantly with NSSE and dexamethasone; T4: was treated with dexamethasone for 21 days followed by NSSE for 21 days	Bone weight in T1 and T4 groups was significantly higher than that of other groups. Phagocytes activity and bone marrow mitotic index were significantly reduced in T2 group, while returned to normal in T1, T3 and T4 groups compared with control. Titers of IgM, IgA, C3, and C4 showed no significant differences among groups, while IgG titer was increased in T1 and T4 and decreased in T2	The examined extract showed a certain immunomodulating effect. Of the immunological aspects, cellular immunity was potentially ameliorated in intact and dexamethasone-induced immunosuppressed- male rabbits	Nil
7.	Al-Mutheffer, 2014	Experimental study	20 adult domestic male rabbits	Two equal groups. Radius bone of both forelimbs was chosen for making a defect (2 mm cavitation using electrical drill) in the middle shaft, the animals of CG were left to heal normally, while the animals of TG were treated by rubbing the operated limb by oil extraction of black seed twice a day	NS provided important factors which contributed to enhancement the healing process of the bone defect. However, the X-ray and histopathology section of the bone of the TG showed enhancement of bone healing in deposition, resorption, angiogenesis and remodeling stages, when compared to the control group	NS as percutaneous therapy enhances bone healing by enhancing different processes of cell migration and differentiation, extracellular matrix formation and organization towards calcification	Nil

NS: Nigella sativa, ALP: Alkaline phosphatase, OVX: Ovariectomized, MDA: Malondialdehyde, TNF- α : Tumor necrosis factor- α , IL-6: Interleukin-6, OE: Only-expansion, TQ: Thymoquinone, ELISA: Enzyme-linked immunosorbent assay, CT: Computed tomography, SE: Seed extract, IgM: Immunoglobulin M, CG: Control group, TG: Treated group

of NS. They reported that NS could ameliorate bone damage caused by diabetes through either its antioxidant effect by its ability to scavenge free radicals and/or inhibit lipid peroxidation with consistent with previous reported results [22]. However, they have not clarified the exact explanation related to mechanism of osteoporosis.

Seif, 2014 [16], objected to elucidate the protective effect of NS on osteoporosis produced by ovariectomy in rats as a model of postmenopausal osteoporosis. She reported that ovariectomy rats showed significant decrease in plasma Ca^{2+} , accompanied by a significant increase in plasma ALP, TNF- α , and IL-6. These changes were reversed by NS supplementation near-normal levels. Similar to biochemical effects, same reported about the effect on histology of the tibias. Examination of tibias in OVX-NS has initially revealed discontinuous eroded bone trabeculae with widened bone marrow spaces in ovariectomy rats accompanied by a significant decrease in both cortical and trabecular bone thickness compared to normal rats. These changes were markedly reversed in after the treatment with NS [16]. These parameters were markedly reversed in after the treatment with NS [16]. These results, especially significant hypocalcemia, are in agreement with previous report by Mattix Kramer *et al.*, 2003 [23], who reported that OVX rats had impaired Ca^{2+} balance that could have contributed to ovariectomy-induced osteoporosis.

Anti-inflammatory effects of NS were clarified by the significant decrease in plasma TNF- α and plasma IL-6 levels in the menopausal-induced rats which treated by NS [16,18,19]. Indeed, there are many studies reported that the osteoporotic patients were found to be under oxidative stress as their lipid peroxidation levels were elevated and antioxidant enzymes reduced [24] and exposure to oxidative stress would result in reduction of bone-mineral density [25]. However, majority of risk factors for osteoporosis were associated with oxidative stress such as diabetes mellitus [26-28] and smoking [29]. Reactive oxygen species could also stimulate osteoclast formation and activity [30] and impair osteoblastic function [31].

Al-Mutheffer, 2014 [21], evaluated the percutaneous effect of NS oil as external topical treatment on bone healing in rabbits. She reported that using of oil extract of NS as percutaneous therapy enhances bone healing by enhancing different processes of cell migration and differentiation, extracellular matrix formation, and organization toward calcification. These results are consistent with other same reports [32-35].

Besides the studies which included in this systematic review, there are many previous reviews on NS and TQ which also discussed the potential effect of NS and TQ on osteoporosis [8,36-39]. These reviews have highlighted that NS and TQ may be used for the treatment of postmenopausal osteoporosis and diabetes-induced osteoporosis and for the promotion of fracture healing. The mechanism involved in the treatment of osteoporosis is unclear, but it was postulated that the antioxidative and anti-inflammatory activities of NS or TQ may play some roles in the treatment of osteoporosis because this bone disease has been linked to oxidative stress and inflammation [39]. However, there is one study conducted as a clinical trial on 15 postmenopausal women with osteoporosis [40]. Researchers performed Ca, P, and plasma bone formation and resorption markers including osteocalcin, bone-ALP, and carboxy-terminal cross-linked telopeptide. The study failed to show beneficial impact of NS extract administration for a short time on bone turnover, so they did not suggest it for medicinal application in the osteoporosis [40].

CONCLUSIONS

Based on the results of this systematic review, we conclude that NS or TQ extract therapy for osteoporosis cannot be recommended yet and these data will not suffice to exclude the beneficial effects of NS on bone turnover reliably. However, there is consequently a growing need for osteoporosis therapies that provide a balance between bone resorption and bone formation phases and increase the strength of bone with least or no side effects. Therefore, more studies are required to explore

the specific cellular and molecular targets of NS or TQ using animal osteoporosis models. Once the anti-osteoporotic effectiveness of NS or TQ will be established, human studies can be carried out since there is only one clinical trial that has been conducted.

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