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Effects of *Nigella sativa* against osteoporosis

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ABSTRACT

Osteoporosis is a medical condition which affects millions of men and women. People with osteoporosis have low bone mass that places them at increased risk of bone fracture. A promising approach is developed as alternative (non pharmaceutical) strategies for bone maintenance, as well as for the prevention and treatment of osteoporosis. Certain plants from the different families have shown the greatest benefits on bones such as Alliceae, Asteraceae, Thecaceae, Fabaceae, Oleaceae, Rosaceae, Ranunculaceae, Vitaceae, and Zingiberaceae. Among them Nigella sativa L. (Ranunculaceae) is becoming more popular day by day due to its potential and broad spectrum effects on many diseases. Animal studies have shown that Nigella sativa and thymoquinone (active component of Nigella sativa) may be used for the treatment of postmenopausal osteoporosis, 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 Nigella sativa or thymoquinone may play some roles in the treatment of osteoporosis because this bone disease has been linked to oxidative stress and inflammation. Nigella sativa and thymoquinone were found to be safe at the current dosage for supplementation in human. Both Nigella sativa and thymoquinone have shown potential against osteoporosis but more animal and clinical studies are required to further assess their antiosteoporotic efficacies. This review article highlights studies on the antiosteoporotic effects of Nigella sativa and thymoquinone, the mechanisms behind these effects and their safety profiles and their uses in future.

Keywords: *Osteoporosis, Nigella sativa, postmenopausal osteoporosis, thymoquinone, diabetes- induced osteoporosis.*

INTRODUCTION

Osteoporosis is one of the most debilitate medical conditions that develops in humans during aging. Patients with this condition have very fragile bones, which break easily after a minor trauma. Because of the association between decreased postmenopausal estrogen and the onset of drastic bone loss, osteoporosis was thought of as a female disease. More recently, however, studies have shown that men also develop this condition in association with aging. 6 million women and 1–2 million men are currently affected by osteoporosis in the United States[1] and almost $14 billion is spent each year for the treatment of such bone fractures that occur frequently in these individuals[2].

Bone turnover: In the process of bone remodeling, osteoclasts remove bone and osteoblasts form new bone at the same position. Osteoclasts and osteoblasts are differentiated monocyte derived cells. Both cell types arise from hematopoietic stem cell precursors. There are three phases in the process of bone remodeling.

Phase 1: In the first phase, mononuclear osteoclast precursors being recruited to a specific bone site. The old bone is reabsorbed by multinucleated osteoclasts by dissolving the bone minerals and breaking down of the matrix. In this osteoclastogenesis process, several cytokines (interleukin-1 [IL-1], IL-6, IL-7, tumor

necrosis factor-a [TNF-a]), hormones (parathyroid hormone [PTH], estrogen, vitamin D), and prostaglandins are required. After the placement of osteoclasts, proton pumps in their plasma membranes produce H^+ ions, which lower the pH of the local intercellular environment[3]. This is accompanied by the production and release of matrix metalloproteases, lysosomal enzymes (tartrate-resistant acid phosphatase, cathepsin K) and gelatinase[4]. They work omly in acidic environment and dissolve the minerals and matrix of old or injured bone, and leave the cavities in the bone at that place of mineralization. Osteoclasts undergo apoptosis by the end of resorption phase[5].

Phase 2: After the phase 1, a reversal phase begins, with the starting of the layering of glycoproteins on the bone site that is to be rebuilt and the attraction of osteocytes, monocytes, and preosteoblasts, which differentiate into osteoblasts. The differentiation of osteoblast requires hormones (growth hormone, PTH, vitamin D, sex steroids, glucocorticoid, and thyroid stimulating hormone), prostaglandins (PGE1), oncostatin M, adrenomedullin, leptin, growth factors (insulin growth factor-I [IGF-I], connective tissue growth factor), cytokines [IL-18] and transcriptional regulators (homeodomain proteins, surfactant proteins, activating transcription factors and proteins of the Runt homology domain transcriptional factors).

Phase 3: Now these differentiated osteoblasts and monocytes secrete the specific proteins that form a loose collagenous matrix. This matrix is then mineralized with calcium and phosphorous, complete new bone formation. After the mineralization step, approximately half of the osteoblasts undergo apoptosis or are engulfed by the new bone matrix, while the remaining osteoblasts persist as bone-lining cells. These programmed events have to be balanced because increased bone resorption, either alone or in combination with decreased bone formation, results in net loss of bone.

The diagnosis of osteoporosis is done by using dual emission X ray absorptiometry (DEXA) machine but more sophisticated three-dimensional micro-computed tomography (micro- CT) is making way for a better diagnosis.

Nigella sativa is a herbal plant. It is known as black cumin or *habatus sauda*, Kalonji or kalajeera while in China it is referred as Hak Jung Chou[6]. It can grow up to 30 cm and produces pale blue flowers. The seeds of *Nigella sativa*, which have a pungent bitter taste, are used in confectionery and liquors. The seeds of the *Nigella sativa* are the source of the active ingredients and has been used in Islamic medicine for its healing powers[7]. According to Islam; it can cure all diseases except death[8]. Studies on this plant have revealed various medicinal or therapeutic values of *Nigella sativa* such as antioxidant, antinflammatory, anticancerous, antibacterial[9], antifungal, antiparasitic[10–15]. Historically, it has been recorded that *Nigella sativa* seeds were prescribed by ancient Egyptian and Greek physician to treat headache, nasal congestion, toothache, bronchial asthma, infections, obesity, back pain, hypertension and gastrointestinal problems, as well as a diuretic to promote menstruation and increase milk production[7, 16, 17]. *Nigella sativa* seeds mainly contain thymoquinone (TQ), dithymquinone (DTQ), thymohydroquinone (THQ), and thymol (THY) [18].

DIABETES-INDUCED OSTEOPOROSIS

Demographic trends with longer life expectancy and a lifestyle characterized by low physical activity and high energy food intake contribute to an increasing incidence of diabetes mellitus and osteoporosis. In patients with diabetes mellitus, osteoporosis is the most important metabolic bone disease[19-21]. Diabetes could affect the bone of patients through multiple mechanisms such as insulin deficiency, insulin resistance, hyperglycemia, or atherosclerosis. However, the exact mechanism responsible for osteoporosis in diabetes is still unknown[22]. Insulin and insulin-like growth factors (IGF- 1) may have some roles to play in the pathogenesis of diabetic-induced bone loss due to their anabolic effects[23]. In the pathogenesis of diabetic-induced bone loss, insulin and insulin-like growth factors (IGF- 1) may have some roles to play due to their anabolic effects[23]. Currently, *Nigella sativa* has only been tested in animal osteoporosis model of diabetes-induced osteoporosis. There is no study has been conducted on other osteoporotic animal models. *Nigella sativa* seed's oil improved the microarchitecture and biomechanical properties of

the femur in male diabetic rats to a level equivalent to that achieved with parathyroid hormone (PTH) treatment[24, 25].

In a study, the *Nigella sativa* was more effective in reversing the osteoporotic changes and improving the bone strength of steptozocin-induced diabetic rats, when used with parathyroid hormone, than either treatment alone[24]. In another study using the same model, histological assessments found that *Nigella sativa* was able to ameliorate diabetic changes of the bone[26]. All of these findings suggested that *Nigella sativa* has potential to be used for the treatment of diabetic osteoporosis.

But the mechanisms of protective effects of *Nigella sativa* against diabetes induced-osteoporosis are still unclear. In certain parts of the world, *Nigella sativa* is frequently used as the traditional treatment of diabetes[27] because it may have improved the bone metabolism by improving the blood sugar levels. The treatments of diabetic rats with *Nigella sativa* have been shown to increase the area of insulin immune reactive beta-cells. These researches of *Nigella sativa* have shown that it may be used as an effective antidiabetic therapy[24].

POSTMENOPAUSAL OSTEOPOROSIS

The years following menopause are called postmenopause. During this time, many of the symptoms of menopause ease for most women. But, as a result of a lower level of estrogen, postmenopausal women are at increased risk for a number of health conditions, such as osteoporosis, heart disease, and changes in the vagina and bladder.

Several medicinal plants have been studied such as soy, blueberry, *Achyranthes bidentata,* and *Labisia pumila* using postmenopausal osteoporosis animal model[28-30]. The effects of *Nigella sativa* should also be tested in animal osteoporosis models and *in vitro* studies are also required to determine the effects of *Nigella sativa* or thymoquinone on osteoblasts and osteoclasts. A human study was conducted on the effects of *Nigella sativa* supplements on the bone markers of postmenopausal women[31]. In that human study, it was found that *Nigella sativa* was failed to cause any significant changes in the bone markers levels, when supplimented for the duration of 3 months to these postmenopausal women. So the authors of above study were concluded that *Nigella sativa* was not recommended for the treatment of postmenopausal osteoporosis because of its faliure. But, however, there were several weaknesses in the study which may cause for the nonsignificant results. The sample size of the postmenopausal women was only 15 and the duration of study was not longer to obtain the readings of bone markers at several time points and any changes in the bone mineral density. So in the future, a long term study with larger sample size should be planned.

Besides that, previous studies on *Nigella sativa* and thymoquinone have highlighted two properties that may be responsible for their effects against osteoporosis, that is, antoxidative and anti-inflammatory properties.

THE ANTIOXIDATIVE PROPERTY OF *Nigella sativa* AND THYMOQUINONE

In osteoporotic patients, their lipid peroxidation levels were elevated and antioxidant enzymes reduced. So they are found to be under oxidative stress[32, 33] such as hypertension[34], diabetes mellitus[35], and smoking[36]. In oxidative stress, reduction of bone-mineral density occurs[37]. In bone studies, ferric nitrilo-triacetate (FeNTA) was the main cause of induction of osteoporosis via oxidative stress[38]. These ferric ions (Fe^{3+}) in FeNTA generate reactive oxygen species through Fenton reaction[39] and they damage bone cells by lipid peroxidation[40, 41]. These reactive oxygen species could also stimulate the formation and activity of osteoclasts[38, 40, 42], impair the functions of osteoblasts[40, 42], decrease the placement of osteoblasts and collagen synthesis[41], activate nuclear factorkappa B (NFκB) and raised the levels of bone-resorbing cytokines, interleukin-1(IL-1), and interleukin-6 (IL-6)[38, 43]. Since it is considerable that oxidative stress may lead to osteoporosis, antioxidants of *Nigella sativa* may protect the bones against the damaging effects of free-radicals. The potent anti-oxidants such as tocotrienol and tocopherol, were able to protect bone against FeNTA induced osteoporosis, according to some studies [38]. Thymoquinone, which

is present in *Nigella sativa*, is antioxidant. It has been reported that the thymoquinone have the capability to scavange free radicals[44]. Superoxides play an important role in the activation of osteoclasts[37]. Since thymoquinone is a potent antioxidant, it is expected that it may be able to protect bone against osteoporosis due to oxidative stress. According to a cancer study, thymoquinone has been proven to suppress the FeNTA-induced oxidative stress, renal carcinogenesis and hyperproliferative response in rats[45] and in some studies using rheumatoid arthritis model, the serum levels of IL-1 and Tumour Necrosis Factor-α[46, 47], bone turnover markers, alkaline phosphatase and tartrate-resistant acid phosphatase were reduced and activation of nuclear factorkappa B was blocked by thymoquinone[48], that indicated the stable bone formation and resorption activities. So the potent antioxidant *Nigella sativa* or thymoquinone have the effective mechanism against osteoporosis.

ANTI-INFLAMMATORY PROPERTY OF *Nigella sativa* AND THYMOQUINONE

In a patient's body, inflammation is mediated by cyclooxygenase and lipoxygenase enzymes which produce prostaglandins and leukotrienes from arachidonic acid, respectively[49]. Therefore, both prostaglandins and leukotrienes are the main mediators of inflammation[50]. It was found that thymoquinone inhibit the cyclooxygenase and lipoxygenase pathways in a dose-dependent manner of rat peritoneal leukocytes that were stimulated with calcium ionophore A23187[24]. According to that study, the synthesis of prostaglandins and leukotrienes was inhibited by thymoquinone. So it is believed that the thymoquinone has the anti-inflammatory effects[51, 52]. These anti-inflammatory activities of *Nigella sativa* were investigated *in vitro*, using the cyclooxygenase (COX) assay. Thymoquinone was able to inhibit the COX activity at concentrations comparable to indomethacin, a nonsteroidal antiinflammatory drug. Therefore, thymoquinone act as a anti-inflammatory agent and can be used as an alternative to nonsteroidal antiinflammatory drugs[53]. The suppression of nitric oxide production, by macrophages, is also done by anti-inflammatory agent of *Nigella sativa* [54]. Adjuvant-induced arthritis rat model was also used for the demonstration of the anti-inflammatory effect of thymoquinone. *Nigella sativa* -inhibited carrageenan induced paw edema by intraperitoneal injections of *Nigella sativa* in a dose-dependent manner[55] and reduction of formalin-induced paw edema was also observed with oral treatment of *Nigella sativa*[56].

Osteoporosis is known to be caused by various endocrine, metabolic, and mechanical factors and the evidences has led to the opinions that inflammation may contribute to osteoporosis[57, 58]. The incidence of osteoporosis were associated with ankylosing spondylitis, rheumatoid arthritis and systemic lupus erythematosus, which are the inflammatory conditions[59–62]. The bone mineral density is negatively associated with the level of C-reactive protein[63]. The extend of osteoporosis is directly related to the degree of inflammation, whereby local inflammation resulted in bone loss which is restricted to the site of inflammation, while systemic inflammation resulted in general bone loss[61]. Osteoporosis can also be enhanced by elevations of proinflammatory cytokines with aging, psoriatic arthritis, rheumatoid arthritis and gouty arthritis[64–67]. Periodontitis is defined as the inflammation and infection of the ligaments and bones that support the teeth. The results of a study has shown that the gastric feeding of thymoquinone reduce the alveolar bone loss due to periodontitis by reduction in osteoclast number and raised osteoblastic activity in rats[68].

HEALING OF BONE FRACTURE BY *Nigella sativa* OR THYMOQUINONE

There is no study on the effects of *Nigella sativa* or thymoquinone on osteoporotic fracture but in an animal study nonosteoporotic bone used for fracture healing, which resembled traumatic fracture healing[69]. In anatomical observations, sustained delivery of thymoquinone showed positive results in healing of fracture[76]. But so many studies are still required before the effectiveness of *Nigella sativa* or thymoquinone in promoting fracture healing can be declared for human use. In some recent studies, acceleration of bone formation was observed because of thymoquinone[70].

TOXICITY OF *Nigella sativa*

In an acute toxicity study of *Nigella sativa* in mice, toxicity signs (decreased locomotor activity, decreased sensitivity to touch, and jerking) were first noticed, when the median lethal dose (LD50) was about 470 mg/kg body weight, after 4 to 6 hours of *Nigella sativa* extract administration[56]. But in other acute and chronic toxicity studies in mice and rats, *Nigella sativa* was found to have a wide margin of safety at therapeutic doses[70]. In an *in vitro* study, thymoquinone at the concentration of 0 to 10 μM was not cytotoxic to isolated fibroblast-like synoviocytes[47]. The suitable dose for *Nigella sativa* extract in human can be extrapolated from animal studies[71]. Approximately 0.6 mg/kg/day per oral of thymoquinone is suitable for humans[39] or 0.05mL/kg/day per oral of *Nigella sativa* extract for postmenopausal women[32]. *Nigella sativa* extract should be used with precaution in pregnant ladies and children due to its well-known hypoglycemic properties. Diabetic patients should also consult with their physicians before the oral administration of *Nigella sativa*[25, 72]. *Nigella sativa* should be administered according to weight and after meals in children[73]. So the doses below 80 mg/kg of *Nigella sativa* was considered safe in children[74].

CONCLUSION AND FUTURE IMPLICATIONS

There are number of people at the risk of developing osteoporosis. So there is consequently a growing need for therapies that 1) provide a balance between bone resorption and bone formation phases, 2) increase the strength of bone, and 3) have least or no side effects. Therefore A closer look at selection of plant-based agents is needed that may ameliorate age-related and postmenopausal bone loss and studies on the mechanisms of action of these agents should be conducted. Efforts to use nonpharmaceutical agents by themselves, should be made in order to reduce the negative side effects of existing therapies and potentially reduce costs as well.

In more than 150 studies conducted since 1959, *Nigella sativa* or thymoquinone has shown potential in a safe and effective manner against osteoporosis. So many original research articles have shown the potential immunomodulatory and immunotherapeutic potentials of *Nigella sativa* seed active ingredients, in particular thymoquinone. The immunotherapeutic efficacies of thymoquinone contain its antioxidative, anti-histaminic and anti-inflammatory properties and its immunomodulatory efficiacy contain the anti-microbial and anticancerous properties of *Nigella sativa* oil or thymoquinone. The whole seeds, oil or its purified constituents should be administered according to the nature of the disease for being safe from the side effects of it. Therefore more studies are required to explore the specific cellular and molecular targets of *Nigella sativa* constituents. Animal osteoporosis models should be used to test the effects of *Nigella sativa* or thymoquinone. Once the antiosteoporotic effectiveness of *Nigella sativa* or thymoquinone has been established, human studies can be carried out. *In vitro* studies should also be conducted to determine the effects on osteoblasts and osteoclasts of *Nigella sativa*.

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