THE PUTATIVE ROLE OF NIGELLA SATIVA OIL VERSUS CALCIUM AND VITAMIN D IN MODULATING THE GLUCOCORTICOID-INDUCED OSTEOPOROSIS IN RATS: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY.

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ABSTRACT:

Background: Glucocorticoid is the most common cause of medication-induced osteoporosis.

Aim of the work: Comparing the role of Nigella sativa (NS) versus calcium and vitamin D (Ca+Vit D) in the protection from Glucocorticoid-induced osteoporosis in a rat model.

Material and Methods: Fifty-one male albino rats weighing 120-150 gm were divided into 4 groups: Group I (Control), Group II (dexamethasone group), Group III (dexamethasone plus Ca+Vit D), and Group IV (dexamethasone plus NS oil).

Results: Rats of group II revealed erosion cavities in compact bone with eroded surface and widened Haversian canal and osteocyte lacunae. The cancellous bone showed thin disconnected bone trabeculae containing empty lacunae with wide bone marrow spaces. Areas of faintly stained bone matrix and minor fractures could be detected. These results were confirmed by a significant decrease in the thickness and number of lacunae containing osteocytes in cortical and cancellous bone. Also, there was an apparent decrease in osteopontin and an increase in caspase-3 immune reaction. Moreover, a significant increase in alkaline phosphatase and IL6 was detected. Groups III and IV showed improvement in cortical and trabecular bone structure. The expression of osteopontin, caspase-3, alkaline phosphatase, and IL6 was also improved.

Conclusion: This study demonstrated that supplementation of NS oil was better than calcium and vitamin D in osteoporotic lesions of rat femur due to its effect on bone remodeling. It acts as a calcium source and has antioxidant effects. So, it is recommended to use NS in the protection of glucocorticoid-induced osteoporosis.

Keywords: Osteoporosis, Glucocorticoid, NSO, Osteopontin, Caspase-3

INTRODUCTION:

Osteoporosis (OP) is a widespread bone disease characterized by low bone strength, low bone amount, and microarchitectural changes in the bone cells, with a consequent increase in bone fragility and an increased incidence of a bone fracture. It is the most common reason for bone fracture in old people. The causative mechanism of OP is a lack of balance between bone resorption and its creation. Inadequate bone quantity,
excessive bone resorption, and insufficient production of new bone during remodeling are the pathophysiology by which OP occurs. These three systems work in concert to develop brittle bone tissue\cite{2}. There are two phases for OP induced by dexamethasone in which the bone mineral density (BMD) decreases. A possible cause of decreased BMD in the early phase is massive bone resorption due to increased osteoclast activity. Impairment in bone formation due to the affection of osteoblasts and osteocytes is the reason for decreased BMD in the late progressive phase \cite{3}.

Anti-inflammatory drugs called glucocorticoids (GCs) are frequently used to treat inflammatory, non-infectious conditions like rheumatoid arthritis, organ transplantation, asthma, and malignancies. Although GCs therapy is effective in reducing inflammation and takes effect quickly, it is linked to OP and fractures. Over 30% of people on glucocorticoids get osteoporotic fractures, and more than ten percent of them develop osteonecrosis, making GCs the most prevalent medicine that causes OP \cite{4}. After postmenopausal and age-related OP, the third most frequent cause of OP is glucocorticoid-induced osteoporosis (GIO) \cite{5}. GCs cause fast bone loss from trabecular bone sites and slow, continuous bone loss from cortical bone sites, which over time increases the risk of fracture \cite{6}. Patients with OP were shown to be under oxidative stress (OS) because their levels of lipid peroxidation were higher, and their antioxidant enzyme levels were lower \cite{7,8}. Reduced BMD would follow exposure to OS \cite{9}. Reactive oxygen species (ROS) are produced by OS and have the potential to harm bone cells, increase osteoclast activity and creation and decrease osteoblastic function \cite{10-12}. For the preservation and repair of healthy bones as well as the prevention of OP, calcium, and vitamin D are crucial elements. The hazard of hypervitaminosis D, with warning signs of hypercalcemia could increase as the general population consumes more vitamin D supplements and as more therapeutic doses are prescribed without medical supervision\cite{13}.

In the past several decades, there has been an increase in the use of natural remedies as an alternative to traditional medicine for the healing and treatment of a variety of disorders. Black seed, also known as Nigella sativa (NS) (Family: Ranunculaceae), is a popularly used medical herb worldwide. Thymoquinone (TQ), a key chemical constituent of the essential oil, is responsible for the majority of this plant's therapeutic benefits\cite{3,14}.

Antioxidants may help shield bone from the harmful effects of free radicals because it is clear that oxidative stress may cause OP \cite{15}. It is intriguing to learn that TQ, the active ingredient in NS, is most notable for its antioxidative actions. TQ is a strong antioxidant, therefore it may be able to guard the bone against OP brought on by oxidative stress \cite{16,17}.

**THE AIM OF THE WORK:**

Comparing the role of NS versus calcium & vitamin D in the protection from Glucocorticoid-induced OP in a rat model.

**MATERIAL AND METHODS**

**Ethical Approval**

The Research Ethics Committee (REC) for Human and Animal Research approved the study protocol at the Faculty of Medicine, Helwan University, Cairo Egypt, serial: 68-2022.

**Drugs and chemicals:**

All were purchased from a local pharmacy. Dexamethasone Sodium Phosphate: manufactured by MUP Medical Union.
Glucocorticoid-Induced Osteoporosis and Nigella Sativa Oil

Pharmaceuticals Company, Ismailia, Egypt (M.O.H.Reg. No.:30412/2015)

Calcium: manufactured by DBK Pharma Company, Cairo, Egypt (M.O.H.Reg. No.:398/2018)

Cholecalciferol, vitamin D3: manufactured by MUP Medical Union Pharmaceuticals Company, Ismailia, Egypt (M.O.H.Reg. No.:20680/2011)


Animals:

Fifty-one male albino rats weighing (120-150 gm) were purchased from Helwan farm, Cairo, Egypt to be used in this study. Rats were locally bred at the physiology department, faculty of medicine, Helwan University, Cairo, Egypt. The animals were housed in an ordinary environment regarding the temperature, light exposure, food, and water supply.

Experimental design

The animals were separated into four groups in the following manner:

Group I (Control group) (n=21):

Group Ia (n=7): were injected with normal saline (once a week intramuscular (IM) for 4 weeks)

Group Ib (n=7): received Calcium supplement orally at 210 mg/kg/day [10] and Vitamin D (Cholecalciferol, vitamin D3) orally at a dose of 600 IU/kg/day for 4 weeks [16].

Group Ic (n=7): received Nigella sativa oil (4 ml/ kg/ day) intra-gastric for 4 weeks [17].

Group II (Dexamethasone group) (n=10): injected with dexamethasone Sodium Phosphate (7mg/ kg, once a week IM for 4 weeks) [18].

Group III (Dexamethasone + Calcium and vitamin D) (n=10): injected with dexamethasone Sodium Phosphate as in the second group. Calcium supplements (210 mg/kg/day) [10] and Vitamin D (Cholecalciferol, vitamin D3) (600 IU/kg/day) were given orally for 4 weeks [16].

Group IV (Dexamethasone + Nigella Sativa Oil (NSO)) (n=10): injected with dexamethasone Sodium Phosphate as in the second group. NSO (4 ml/ kg/ day) intra-gastric was given for 4 weeks [17].

Each rat was weighed every week and the dose was adjusted according to its weight.

By the end of the drug supply:

A- The retrobulbar vein was used to gather blood samples for the detection of ionized Calcium (Ca), Interleukin (IL) 6, and Alkaline phosphatase (ALP).

B- Sodium thiopental 50 mg/ kg subcutaneously was used as an anesthetic agent before cervical dislocation. The right femur (upper part including shaft and metaphysis) was dissected to collect the bone samples to be fixed in 10% buffered formalin. Decalcification was done over two weeks using an EDTA solution (chelating agent). After decalcification, five micrometers (µm) thick paraffin sections were prepared to be stained with:

1. Hematoxylin and eosin [19].
2. Mallory’s trichrome (MT) stain [19]: For detection of the collagen fibers.
3. Osteopontin (OPN) immunostaining: Anti-OPN antibody (AB1870, Chemicon®, Sigma-Aldrich, USA) is a rabbit primary polyclonal antibody that possesses species reactivity with rats. Anti-OPN antibody was used at a 1:1500 dilution on paraffin sections. The positive strong reaction appeared in cement lines, osteoblasts, osteocytes, canaliculi, osteoid, and bone matrix. To obtain negative results, substitute the primary antibody with a buffer only [20].
4. Caspase-3 immunostaining: Caspase-3 antibodies are primary rabbit polyclonal antibodies (Thermo Fisher Scientific Corporation, Fremont, CA, USA) with Catalog NO: PA1-29157. They were
used at a dilution of 1:100. Incubation of the slides with antibodies for one hour was done at ordinary temperature, followed by a reaction with a biotinylated secondary antibody. After streptavidin peroxidase conjugation, 3,3- diaminobenzidine (DAB) was used as a chromogen. The positive control was the human tonsils [21].

C- Morphometric study:

All groups were observed under light microscopy by utilizing the interactive measuring menu of the image analyzer (Lecia Q win 500 image analyzer computer system, England) in the Histology and Cell Biology department, faculty of Medicine, Ain Shams University. Ten fields of each specimen were examined and measured for each one of the following parameters. a. The cortical bone thickness (measured from periosteous to endosteous) and trabecular bone thickness; were measured by micrometers (µm) in H&E-stained sections per high power field (HPF) (x10). b. The number of lacunae containing osteocytes in cortical and trabecular bone; was measured in µm in MT-stained sections per HPF (x40).

D- Statistical analysis:

The biochemical and morphometric results were analyzed for significance using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). A one-way analysis of variance (ANOVA) was used followed by a Post Hoc test. The statistical results were listed as mean± standard deviation. The p-value was considered significant if <0.05.

RESULTS

Biochemical results:

The dexamethasone group (Group II) showed a significant decrease in serum ionized Ca and a significant increase in both IL6 and ALP when compared to the other groups. Group III revealed non-significant changes in all these parameters compared to the control group except for IL6 levels which revealed a significant difference in relation to the control group. Group IV also publicized negligible deviances in all these parameters compared to the control group. IL6 showed a significant difference between Group III and Group IV (Table 1).

Histological results:

H&E-stained sections:

The femoral shaft of group I revealed the outer cortical bone covered by the periosteous (outer fibrous layer and inner osteogenic layer) from the outer side and lined by endosteous (single osteogenic layer) from the inner side. Haversion canals (HC) and osteocyte lacunae were seen in the bone matrix. The old and new bones were separated by cement lines (Figure 1A). Group II revealed erosion cavities inside the bone matrix with an eroded endosteal surface and a few indistinct cement lines. Wide Haversion canal and empty lacunae were seen (Figures 2A & 2B). Groups III and IV showed a smooth endosteal surface, apparent normal osteocytes inside their lacunae, and apparent normal-size HC (Figures 3&4) respectively.

The femoral metaphysis of group I revealed multiple bone trabeculae enclosing bone marrow (BM) spaces. The bone matrix contained osteocytes in their lacunae (Figure 1B). The cancellous bone of group II revealed apparent thinned-out trabeculae filled with multiple cavities (fissures &cracks) with wide BM spaces and eroded trabecular bone surface. Multiple multinucleated osteoclasts on the trabecular bone surface were also seen (Figure 5). Group III showed some trabeculae of nearly apparent normal thickness enclosing BM spaces. But other areas show thin trabeculae (Figure 6). Group IV showed trabeculae of nearly normal thickness enclosing BM cavities with a normal structure (Figure 7).
**Mallory’s trichrome-stained sections:**

The femoral shaft and metaphysis of group I showed intensely blue stained regularly set collagen fibers in the matrix of compact bone and trabeculae of cancellous bone (Figures 8A&8B). Group II showed deeply stained collagen fibers in a few areas & some areas appear pale stained. Also, bone cavities were surrounded by deeply stained dense collagen fibers (Figure 9). Groups III & IV showed the same as the control group (Figure 10&11) respectively. Examination of the spongy bone of group II showed several regions of a faintly stained matrix in bone trabeculae (Figure 12). Group III showed the same as the control group with minor faintly stained regions (Figure 13). Group IV showed the same as the control group (Figure 14).

**Immunohistochemical results:**

**Osteopontin immunostaining:**

The femoral shaft of a male albino rat of group I revealed apparent marked positive reactions of OPN protein in cells lining the HC, and osteocytes (Figure 15). Group II showed an apparent negative expression of OPN (Figure 16). Group III showed an apparent positive reaction of OPN protein in cells lining HC, and osteocytes (Figure 17) while Group IV showed apparent marked positive reactions of OPN protein in cells lining the Haversian canal, osteocytes & cement lines (Figure 18).

**Caspase-3 immunostaining:**

The femoral shaft of a male albino rat femur of the control group showed apparent negative immunoreactivity in osteocytes. (Figure 19). Group II showed an apparent positive immunoreactivity in osteocytes, cells lining erosion cavities, and bone surface (Figure 20). Group III showed mild immunoreactivity in cells lining the bone surface (Figure 21) while Group IV showed an apparent negative immunoreactivity in osteocytes (Figure 22).

**Morphometric and Statistical results:**

All measured parameters showed a significant reduction in group II compared to group I. Groups III and IV revealed a significant rise in these parameters. Group III showed no significant difference in comparison with the control group except for the number of lacunae containing osteocytes in both compact and spongy bone. While Group IV revealed no significant changes from the control rats. Group III showed a significant change in the number of lacunae containing osteocytes in both compact and spongy bone compared to Group IV (Table 2).

**Table 1:** Showing the mean ± SD values, results of ANOVA, and post hoc tests for the comparison between different groups regarding ionized calcium/dl”, IL-6 “pg/mL” and alkaline phosphates (IU/ml).

<table>
<thead>
<tr>
<th></th>
<th>Ionized calcium (mg/dl)</th>
<th>IL-6 (pg/mL)</th>
<th>Alkaline phosphates (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>10.80±0.92</td>
<td>17.75±0.53</td>
<td>11.90±0.42</td>
</tr>
<tr>
<td>Group II</td>
<td>9.00±0.18**</td>
<td>21.00±2.83*</td>
<td>16.00±0.71**</td>
</tr>
<tr>
<td>Group III</td>
<td>10.76±0.33@</td>
<td>19.75±0.53 *, @</td>
<td>12.20±0.35@</td>
</tr>
<tr>
<td>Group IV</td>
<td>10.56±0.40@</td>
<td>18.48±0.16 @, &amp;</td>
<td>12.00±0.49@</td>
</tr>
</tbody>
</table>

*Significant compared to group I (**p-value <0.001 is highly significant)
@ Significant compared to group II & Significant decrease compared to group III
Table 2: Showing the mean ± SD values, regarding the thickness of both cortical and trabecular bone and the number of lacunae containing osteocytes in cortical and trabecular bone.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cortical bone thickness in um (H&amp;E X10)</th>
<th>Trabecular bone thickness in um (H&amp;E X10)</th>
<th>The number of lacunae containing osteocytes in cortical bone PHF (MTx40)</th>
<th>The number of lacunae containing osteocytes in trabecular bone PHF (MTx40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>406.03±16.44</td>
<td>68.98±4.69</td>
<td>24.00±2.37</td>
<td>17.67±1.37</td>
</tr>
<tr>
<td>Group II</td>
<td>170.00±4.48**</td>
<td>41.9±6.44**</td>
<td>14.33±1.37**</td>
<td>7.33±1.86**</td>
</tr>
<tr>
<td>Group III</td>
<td>313.57±22.09@</td>
<td>59.06±6.64@</td>
<td>17.00±0.89*, @, &amp;</td>
<td>14.33±1.37*, @, &amp;</td>
</tr>
<tr>
<td>Group IV</td>
<td>389.30±2.03@</td>
<td>66.56±6.86@</td>
<td>22.00±2.00@</td>
<td>16.00±4.24@</td>
</tr>
</tbody>
</table>

*Significant decrease compared to group I (**p-value <0.001 is highly significant)
@ Significant increase compared to group II
& Significant decrease compared to group III

Fig. 1: A photograph of a section of the femur of group I showing: 1A The compact bone of the shaft covered from the outside by the periosteum (P) and lined from inside by endosteum. The bone matrix contains osteocytes inside their lacunae (arrows) and HC (H). Notice the cement line. 1B The cancellous bone of metaphysis is formed by bone trabeculae (T) separated by BM cavities (BM). (H&E, X400).
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**Fig. 2:** A photograph of a section of a femoral shaft of group II showing: 2A erosion cavity inside the bone matrix (C) with eroded endosteal surface (black circles) and few indistinct cement lines. 2B wide Haversian canal (C) and empty lacunae (stars). (H&E X400).

**Fig. 3:** A photograph of a section of the femoral shaft of group III showing apparent normal lacunae enclosing osteocytes (black arrows), and apparent normal size HC (H). (H&E X400).

**Fig. 4:** A photograph of a section of the femoral shaft of group IV showing apparent normal lacunae enclosing osteocytes (black arrows) and apparent normal HC (H). Notice the cement line. (H&E X400).
Fig. 5: A photograph of a section of the femoral metaphysis of group II showing apparent thinned-out trabeculae (TT) filled with multiple cavities (fissures & cracks) with wide bone marrow spaces (WBM) and eroded trabecular bone surface (double heads arrows). Multiple multinucleated osteoclasts on the trabecular bone surface (arrowheads) were also seen. (H&E X400).

Fig. 6: A photograph of a section of the femoral metaphysis of group III showing some trabeculae (T) of nearly apparent normal thickness enclosing bone marrow spaces (BM). But other areas show thin trabeculae (TT). Notice empty osteocyte lacunae. (H&E X400).

Fig. 7: A photograph of a section of the metaphysis of group IV showing trabeculae (T) of nearly normal thickness enclosing bone marrow cavities (BM). Osteocytes (black arrows) osteocytes were also seen. (H&E X400).
Fig. 8: A photograph of a section of the femur of group I showing intensely blue stained regularly set collagen fibers in the matrix of compact bone (8A) and trabeculae of cancellous bone (8B) (yellow star). (MT X400).

Figs. 9, 10, 11: A photograph of a section of the femoral shaft of group II (Fig. 9) showing intensely blue stained collagen fibers in a few areas & some areas appear pale stained (red stars). Notice, bone cavities (C) are surrounded by intensely stained dense collagen fibers. Groups III (Fig. 10) and VI (Fig. 11) show intensely blue stained regularly set collagen fibers in the matrix (yellow stars) (MT X400).
Figs. 12, 13, 14: A photograph of a section of the femoral metaphysis of group II (Fig. 12) showing multiple areas of a faintly stained matrix (yellow arrows) in bone trabeculae. Group III (Fig. 13) shows intensely blue stained regularly set collagen fibers (yellow stars) with small pale stained areas (yellow arrows). Group IV (Fig. 14) shows intensely blue stained regularly set collagen fibers (yellow stars). (MT X400).
**Fig. 15:** A photograph of a section of the femoral shaft of group I showing apparent marked positive reactions of osteopontin protein in cells lining the HC, and osteocytes (black arrows). (OPNX400).

**Fig. 16:** A photograph of a section of the femoral shaft of group II showing an apparent negative expression of OPN in cells lining the HC, and osteocytes (black arrows). (OPNX400).

**Fig. 17:** A photograph of a section of the femoral shaft of group III showing an apparent positive reaction of osteopontin protein in cells lining HC, and osteocytes (black arrows). (OPNX400).

**Fig. 18:** A photograph of a section of the femoral shaft of group IV showing apparent marked positive reactions of osteopontin protein in cells lining the Haversian canal, osteocytes & cement lines (black arrows). (OPNX400).
Fig. 19: A photograph of a section of the femoral shaft of group I showing apparent negative immunoreactivity in osteocytes. (Caspase-3X400).

Fig. 20: A photograph of a section of the femoral shaft of group II showing an apparent positive immunoreactivity in osteocytes, cells lining erosion cavities, and bone surface. (arrows). (Caspase-3X400).

Fig. 21: A photograph of a section of the femoral shaft of group III showing mild immunoreactivity in cells lining the bone surface. (Caspase-3X400).

Fig. 22: A photograph of a section of the femoral shaft of group IV showing an apparent negative immunoreactivity in osteocytes. (Caspase-3X400).
DISCUSSION:

Dexamethasone is a glucocorticoid that has the ability to alter the microarchitecture of bone with long-term use causing OP. This ability was attributed to a decrease in bone formation due to the inhibition of osteoblasts proliferation and the promotion of their apoptosis and an increase in bone loss due to absorption of the bone surface by osteoclasts [22]. Our dexamethasone model showed numerous cavities and an increase in the number of apparent enlarged empty osteocyte lacunae. In addition, an uneven endostium was found, along with an erosion cavity harboring several multinucleated osteoclasts. Also, there were lightly stained collagen fibers by MT staining indicating a decrease in the bone density. These alternations indicate the occurrence of OP. Our findings match the results of Hirayama et al and Sivagurunathan et al who demonstrated that the administration of corticosteroids causes bone fragility and OP due to lowering protein in the bone matrix [23]. Dexamethasone; at a pharmaceutical concentration; increased osteoclast formation as demonstrated by Sivagurunathan et al. [24]. According to earlier research, dexamethasone’s reduction in bone weight was largely caused by hypofunction and apoptosis. This reduction can also be attributed to the quickening of bone resorption and stimulation of osteoclastogenesis [25].

OPN is a 44–75 KD multifunctional phosphoprotein secreted by a variety of cell types including osteoblasts, osteoclasts, and chondrocytes. The extracellular matrix of mineralized tissues contains OPN as cement lines and lamellae. Trabecular bone shows more OPN than cortical bone. OPN has a protective effect on OP [26]. Serum OPN is a diagnostic marker for early OP in postmenopausal women and is inversely correlated with OP severity [27]. In our study OPN expression in dexamethasone-treated rats was decreased confirming the reduced activity of osteoblasts. Also, the expression of the apoptotic marker; caspase-3; was increased. On the contrary, few studies found higher serum OPN levels in postmenopausal groups with fractures, whereas sporadic studies found no difference in OPN levels between postmenopausal groups with or without fractures [27].

Several experiments investigating the influence of NS reported its positive benefits on OP and bone repair [28 - 31]. Our results demonstrated that exposure to NSO improved the altered bone structure, regained OPN immune reactivity, and lowered caspase-3 expression. NS group showed well-distinct cement lines indicating bone remodeling which is an important mechanism of bone strength. Black seed exposure to cells that have been pretreated with cortisol, as demonstrated by Corder et al. and Musa et al., has been shown to protect against progressive apoptosis and can be used therapeutically to reduce anti-inflammatory and antioxidant effects while enhancing the detoxification process [32 & 33]. Similar research on NS’s impact on OP reported that NS can either have an antioxidant effect by scavenging free radicals and/or by inhibiting lipid peroxidation, which both help to mitigate the bone damage brought on by diabetes [28]. The protective effects of NS were also reported with OP produced by ovariectomy in rats [34]. Numerous earlier studies have shown that NS can be used to treat postmenopausal OP, and diabetes-induced OP, and to promote fracture repair [35, 36 & 37].

In our research rats that received NS showed more improvement than those that received Ca and vitamin D. This result can be clarified by the following; first, NS enclose valuable amounts of calcium, so, it is considered a natural source of calcium intake for kids, the elderly, pregnant women,
and breastfeeding mothers. This fact partially explains the increased Ca ions levels observed in ovariectomized NS rats [38]. Another clarification was highlighted by Winkler who reported the biochemical, pharmacological, immunological, and anti-inflammatory effects of NS [39]. Based on the role of oxidative stress in the reduction of BMD [7] as ROS arouses osteoclast creation and activation [40] and weakens osteoblastic tasks [41] and collagen synthesis, NS improves OP through its antioxidant properties [42]. According to Al-Muthheffer’s study in rabbits, applying NS oil extract as a percutaneous therapy improves bone repair by promoting cell migration and differentiation processes. NS increases the production of the extracellular matrix and organizes its calcification [43]. Also, TQ lowered osteoclast number and raised osteoblastic activity [44]. Valizadeh et al studies were unsuccessful in displaying the valuable influences of short-time intake of NS extract on bone turnover, so they did not advise it for remedial use in OP [45]. Regarding the safety of NS, the seed extract played a positive role in the current research without having any negative effects. Previous studies have addressed the safety of NS [46&47].

Endoplasmic reticulum (ER) stress is associated with OP. The ER is a crucial membrane organelle that controls calcium storage, calcium ion balance inside and outside the cell, protein synthesis, and folding. When the environment inside the cell begins to negatively change, the ER loses its typical function, which results in a series of adaptive regulatory mechanisms known as ER stress. When intracellular calcium ion homeostasis is out of balance and unfolded or misfolded proteins accumulate in the ER cavity, the intracellular environment is disrupted [48].

Tissue non-specific ALP is the most prevalent isozyme type of ALP, a homodimeric protein with phosphorylating abilities. The serum contains roughly equal amounts of tissue non-specific ALP isoforms, liver-specific ALP, and bone-specific ALP (BALP). Only a little amount of BALP is physiologically released into the serum before it binds to the osteoblastic cell membrane. Its serum content only increases in situations when bone remodeling is more pronounced [49]. The principal way that BALP stimulates tissue mineralization is by inactivating pyrophosphate and osteopontin, both of which are inhibitors of tissue mineralization [50].

**Conclusion:**

This study demonstrated that supplementation of NS oil was better than calcium and vit D in osteoporotic lesions of rat femur due to its effect on bone remodeling. It acts as a calcium source and has antioxidant effects. So, it is recommended to use NS in the protection of glucocorticoid-induced OP.

**Conflict of interest:**

None

**Funding resources**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Declarations of interest:**

none.

**Conflict of Interest Statement:**

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

**REFERENCES:**


الدور المفترض لزيت حبة البركة مقابل الكالسيوم وفيتامين D في تعديل هشاشة العظام المستحث بالجلوكوكورتيكويد في الجرذان: دراسة نسيجية وكيماوية مناعية.

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الخلفية: تتميز هشاشة العظام بانخفاض قوة العظام. تعد الجلوكوكورتيكويد هو السبب الأكثر شيوعًا لتشكل هشاشة العظام التي يسببها الدواء.

الهدف من الدراسة: مقارنة دور حبة البركة مقابل الكالسيوم وفيتامين D في الحماية من هشاشة العظام الناجم عن الجلوكوكورتيكويد في نموذج الجرذان.

المواد والطرق: تم تقسيم 51 جرذ ذكر من الجرذان البيضاء وزنها 120-150 جم إلى أربعة مجموعات: المجموعة الأولى (المجموعة الضابطة)، المجموعة الثانية (مجموعة الديكساميثازون بالإضافة إلى الكالسيوم + فيتامين D)، المجموعة الثالثة (المجموعة الديكساميثازون بالإضافة إلى زيت حبة البركة) و المجموعة الرابعة (المجموعة الديكساميثازون بالإضافة إلى زيت حبة البركة و الكالسيوم + فيتامين D). تم قياس مستويات الكالسيوم في الدم، والبوتاسيوم، الفوسفاتيات والكالسيوم. تم تحضير جزء من العظام وتم صياغتها في البارافين والصبغة باستخدام الهيماتوكسيلين والإيوسين وماليور وصبغات المناعية أوستيوبونتين وكسباز 3. بالإضافة إلى ذلك، تم إجراء التحليلات المورفومترية والاحصائية.

النتائج: كانت جرذان المجموعة الثانية عرضة للإصابة بالجرذان إلى تأثيرات زيت حبة البركة. تم نشر عظام حية في الأعضاء وتم العثور على ثغرات كبيرة في العظام والميتوكندرنيون. تم العثور على تأثيرات مضادة للأكسدة في المجموعة الأولى. المجموعة الثانية، المجموعة الثالثة، والمجموعة الرابعة، عرضت تأثيرات إيجابية في جميع المراحل. المجموعة الرابعة، كانت الأفضل من جميع المجموعات.

الخاتمة: أظهرت هذه الدراسة أن حبة البركة كانت أفضل من الكالسيوم وفيتامين D في الوقاية من هشاشة العظام الناجم عن الجلوكوكورتيكويد.