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

Rania H. Elsyade [1], Heba Abd Elrazak Abd Elfatah[2], Noha Osama Shawky[3] and Eman A. El-Sawaf [1]

[1]Department of Anatomy and Embryology, Faculty of Medicine, Helwan University, Cairo, Egypt [2]Department of Histology, Faculty of Medicine, Helwan University, Cairo, Egypt [3]Department of Physiology, Faculty of Medicine, Helwan University, Cairo, Egypt

Corresponding author:
Rania Hamdy Elsyade
Mobile: +2 01150461611
E-mail:
rania.elsyade@med.helwan.edu.eg

Received: 14 August 2023
Accepted: 01 September 2023

Online ISSN: 2735-3540

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-3immune 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[1]. 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[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 [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 [4]. After postmenopausal and age-related OP, the third most frequent cause of OP is glucocorticoid-induced osteoporosis (GIO) [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 [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 [7&8]. Reduced BMD would follow exposure to OS [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 [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[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[3&14].

Antioxidants may help shield bone from the harmful effects of free radicals because it is clear that oxidative stress may cause OP [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 [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

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)

NSO: manufactured by Pharco-Company, Alameria, Alexandria, Egypt (EDA.Reg. No.:831/2009)

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 e 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 e 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 periosteum to endosteum) and trabecular bone thickness: were measured by micro-meters (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 deviancies 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 periosteum (outer fibrous layer and inner osteogenic layer) from the outer side and lined by endosteum (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 Haversian 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).

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

*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.

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

*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

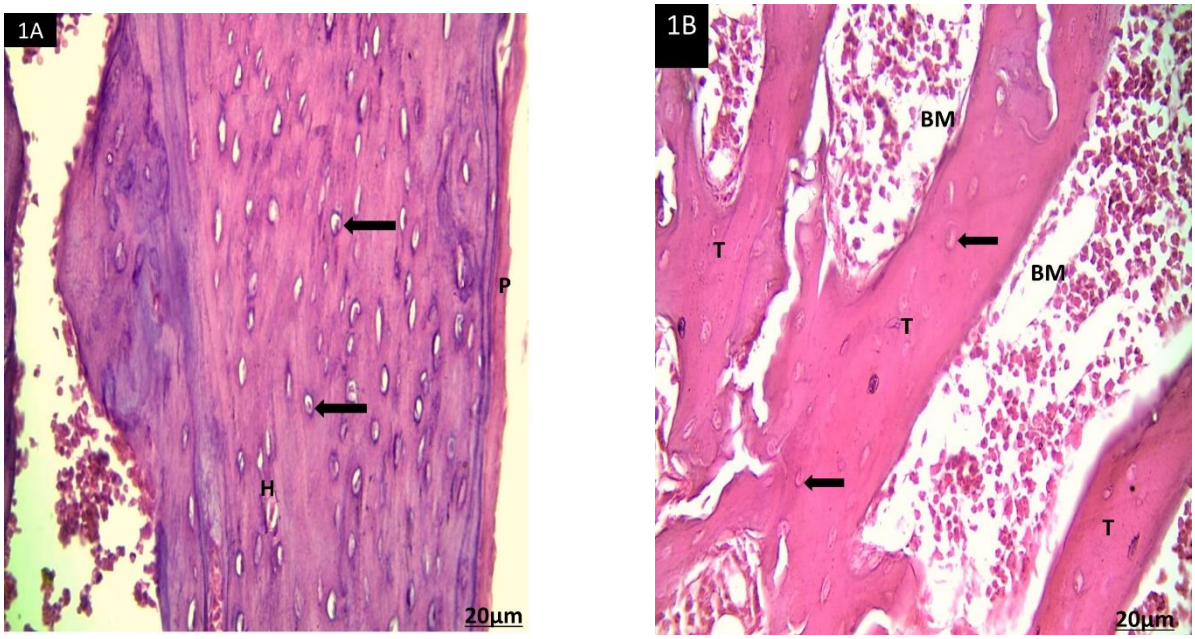


Fig. 1: A photograph of a section of the femur of group I showing: **1A** The compact bone of the shaft covered from the outside by the periosteum (P) and lined from inside by endosteum. The bone matrix contains osteocytes inside their lacunae (arrows) and HC (H). Notice the cement line. **1B** The cancellous bone of metaphysis is formed by bone trabeculae (T) separated by BM cavities (BM). **(H&E, X400).**

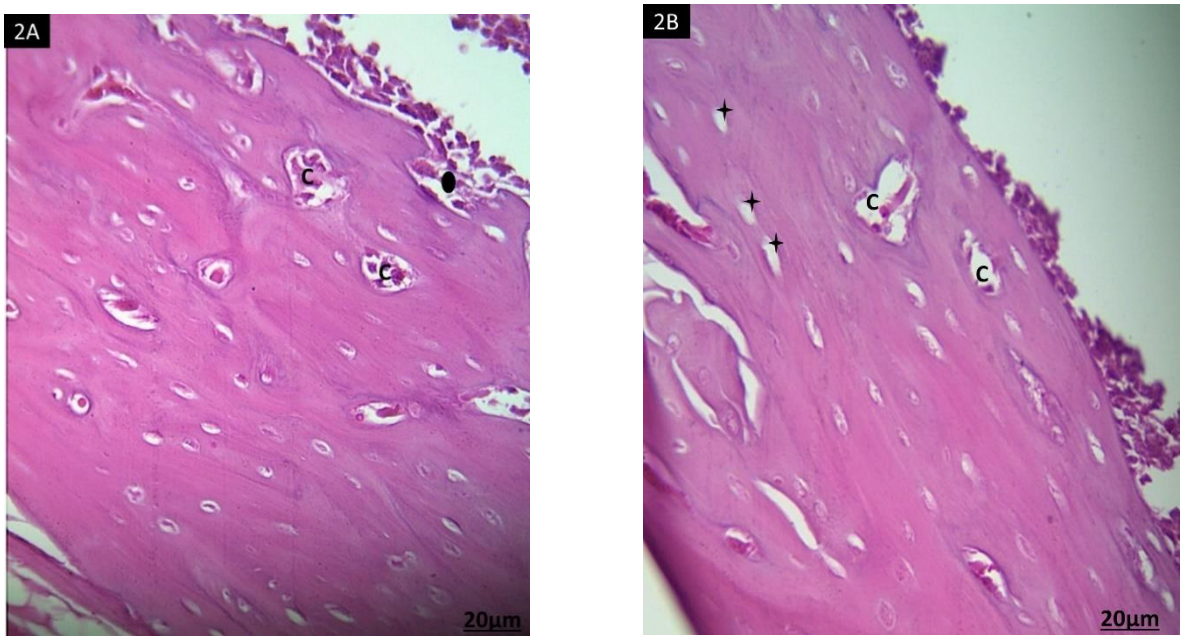


Fig. 2: A photograph of a section of a femoral shaft of group II showing: **2A** erosion cavity inside the bone matrix (C) with eroded endosteal surface (black circles) and few indistinct cement lines. **2B** wide Haversian canal (C) and empty lacunae (stars). **(H&E X400).**

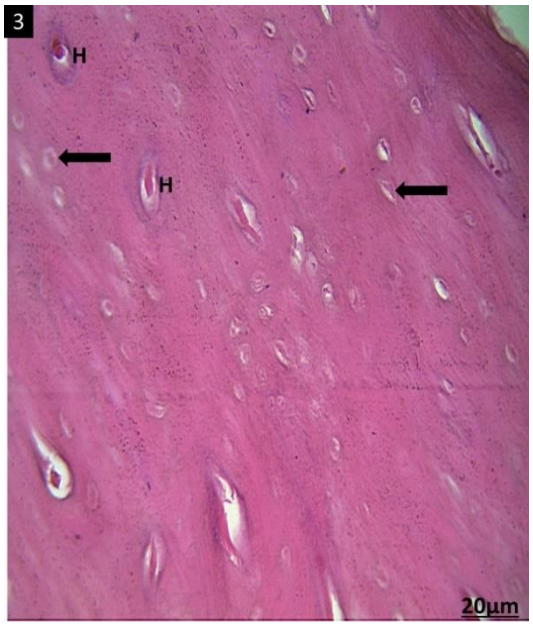


Fig.3: A photograph of a section of the femoral shaft of group III showing apparent normal lacunae enclosing osteocytes (black arrows), and apparent normal size HC (H). **(H&E X400).**

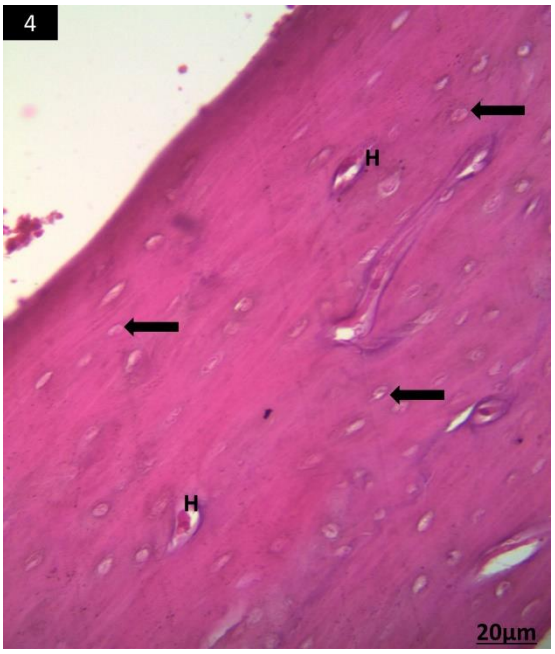


Fig.4: A photograph of a section of the femoral shaft of group IV showing apparent normal lacunae enclosing osteocytes (black arrows) and apparent normal HC (H)**.** Notice the cement line. **(H&E X400).**

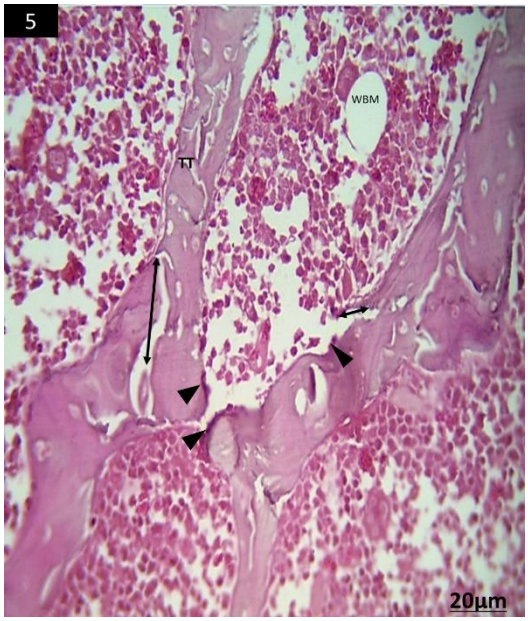


Fig. 5: A photograph of a section of the femoral metaphysis of group II showing apparent thinned-out trabeculae (TT) filled with multiple cavities **(fissures & cracks)** with wide bone marrow spaces (WBM) and eroded trabecular bone surface (double heads arrows). Multiple multinucleated osteoclasts on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

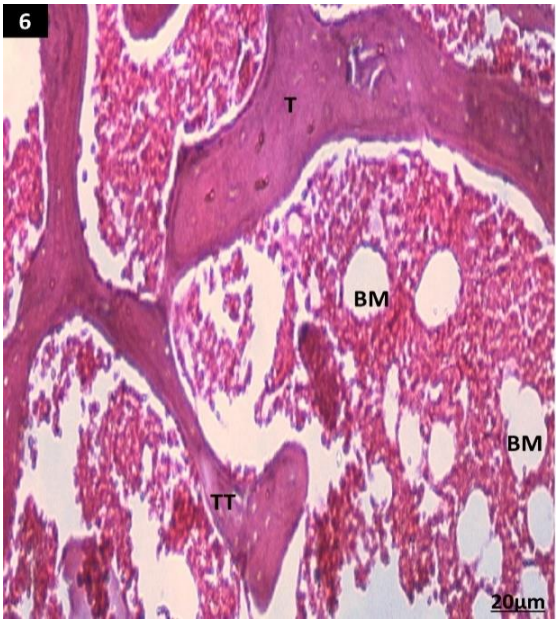


Fig.6: A photograph of a section of the femoral metaphysis of group III showing some trabeculae (T) of nearly apparent normal thickness enclosing bone marrow spaces (BM). But other areas show thin trabeculae (TT). Notice empty osteocyte lacunae. **(H&E X400).**

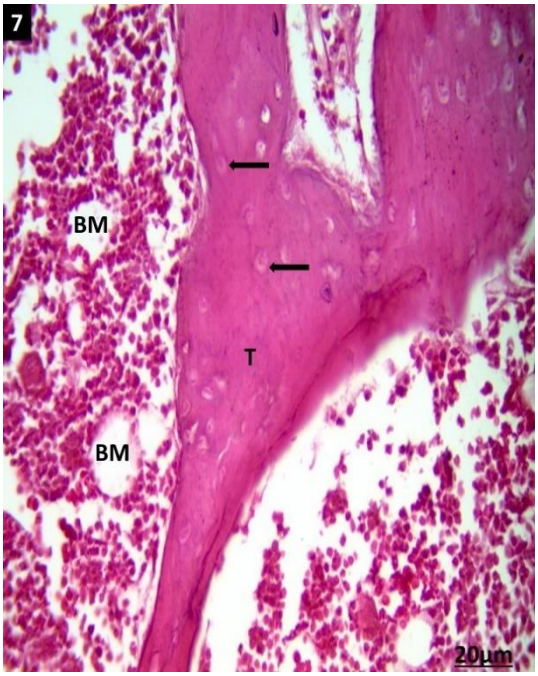
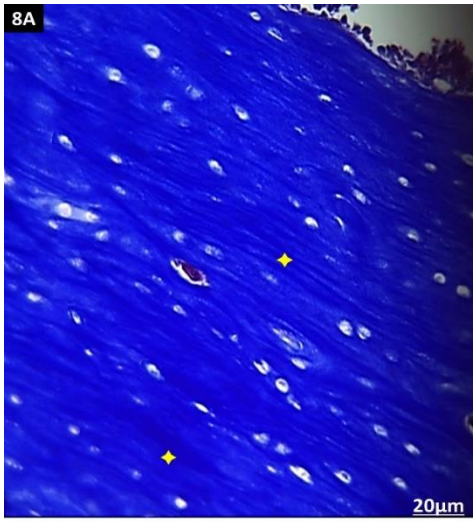


Fig.7: A photograph of a section of the metaphysis of group IV showing trabeculae (T) of nearly normal thickness enclosing bone marrow cavities (BM). Osteocytes (black arrows) osteocytes were also seen.

(H&E X400).



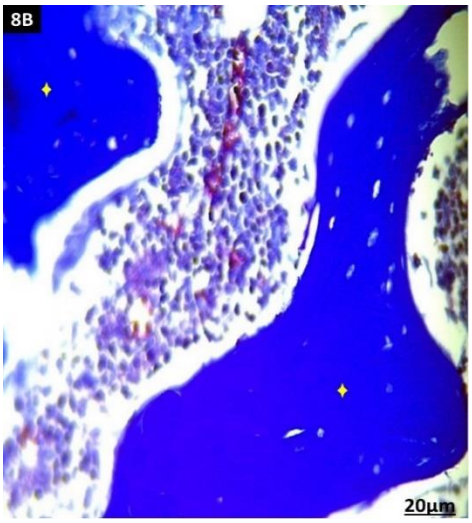
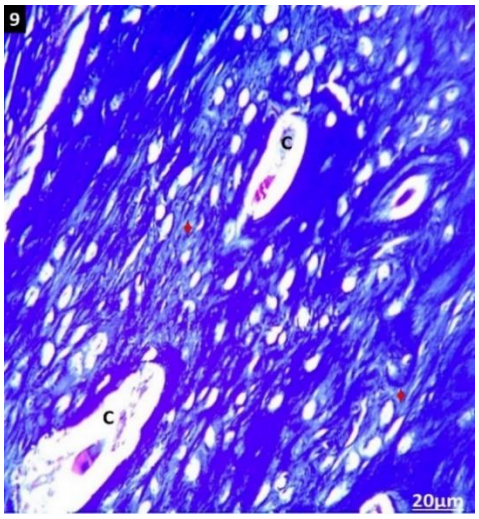
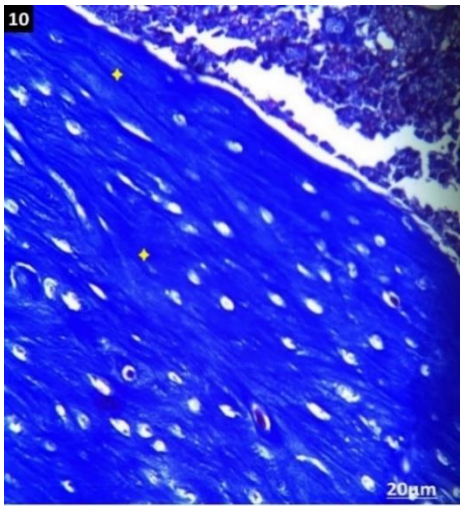
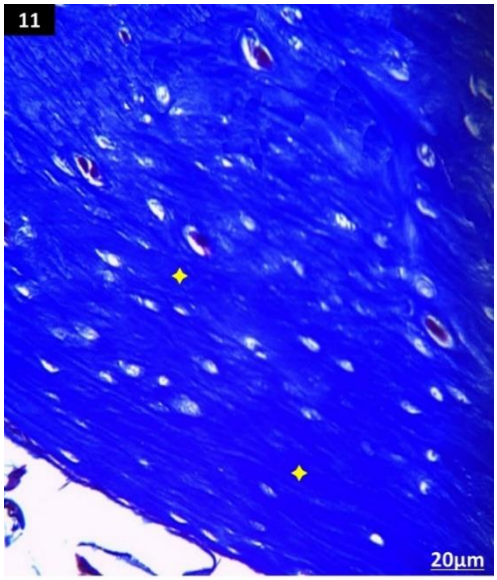


Fig. 8: A photograph of a section of the femur of group I showing intensely blue stained regularly set collagen fibers in the matrix of compact bone **(8A)** and trabeculae of cancellous bone **(8B)** (yellow star). **(MT X400).**

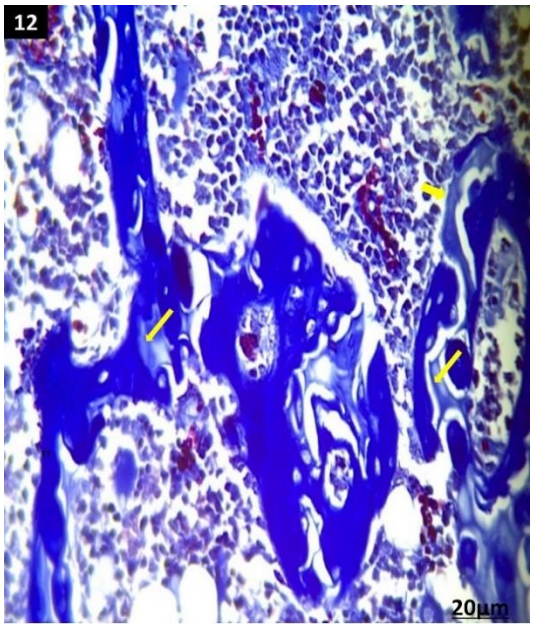


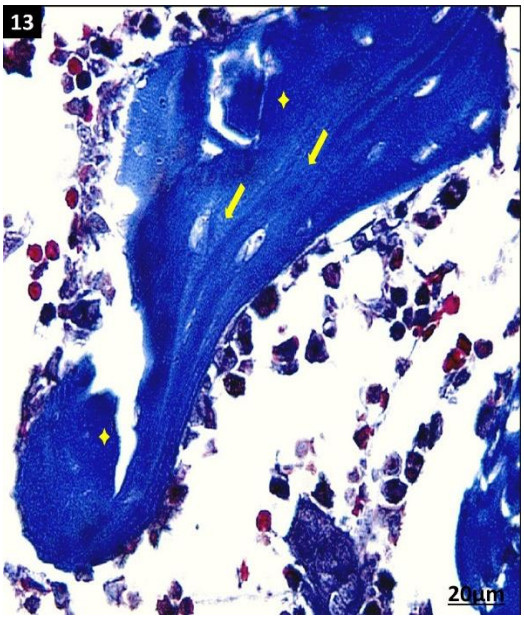


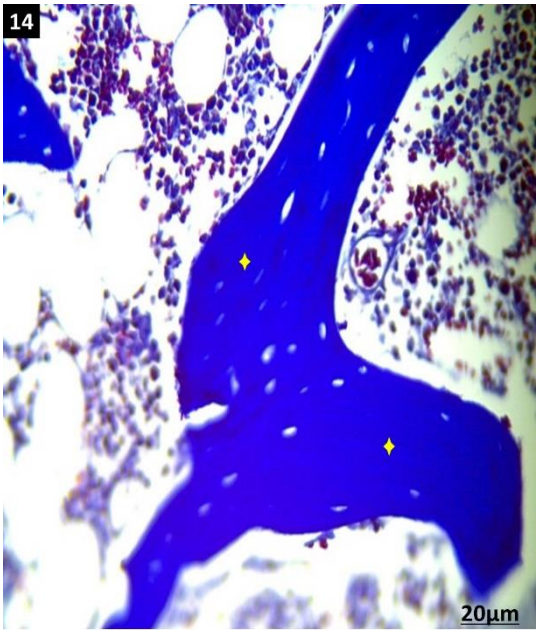


Figs. 9,10,11: A photograph of a section of the femoral shaft of group II **(Fig. 9**) showing intensely blue stained collagen fibers in a few areas & some areas appear pale stained (red stars). Notice, bone cavities (C) are surrounded by intensely stained dense collagen fibers. Groups III **(Fig. 10**) 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).**

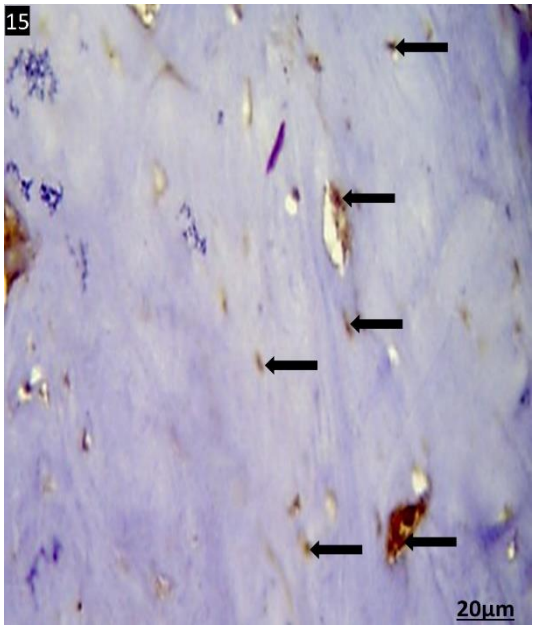


Fig. 15: A photograph of a section of the femoral shaft of group I showing apparent marked positive reactions of osteopontin protein in cells lining the HC, and osteocytes (black arrows).

(OPNX400).

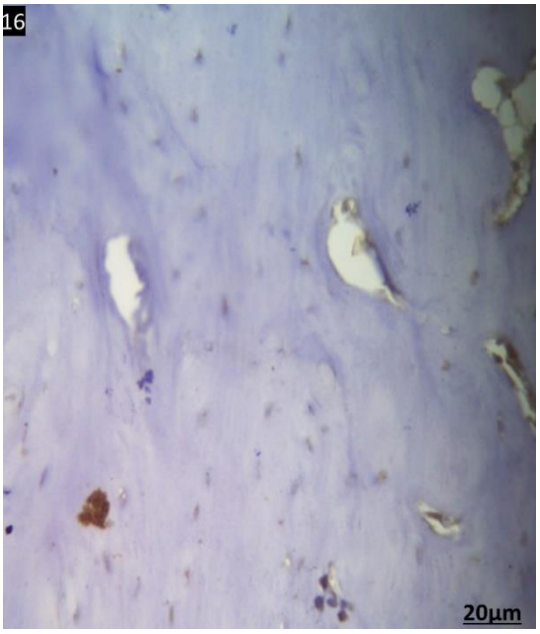


Fig. 16: A photograph of a section of the femoral shaft of group II showing an apparent negative expression of OPN in cells lining the HC, and osteocytes (black arrows). **(OPNX400).**

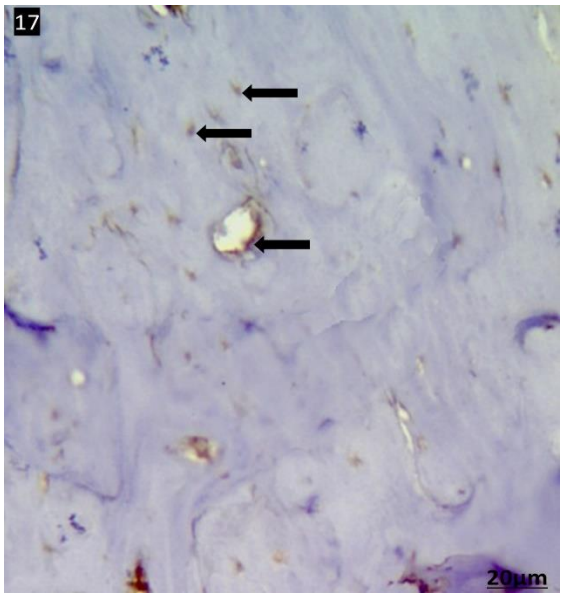


Fig. 17: A photograph of a section of the femoral shaft of group III showing an apparent positive reaction of osteopontin protein in cells lining HC, and osteocytes (black arrows). **(OPNX400).**

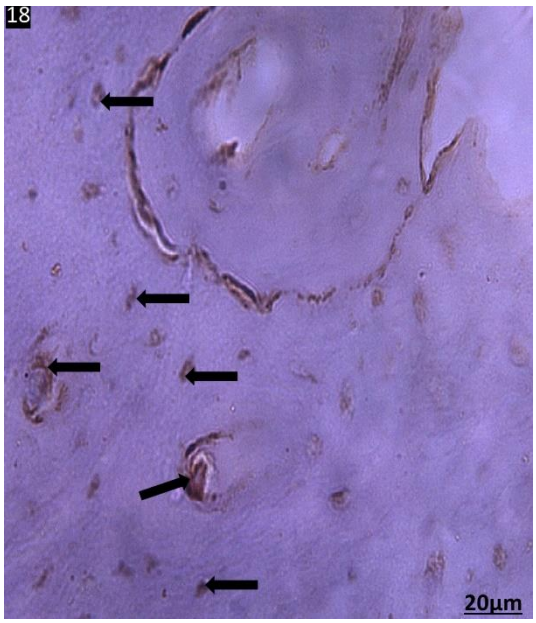


Fig. 18: A photograph of a section of the femoral shaft of group IV showing apparent marked positive reactions of osteopontin protein in cells lining the Haversian canal, osteocytes & cement lines (black arrows). **(OPNX400).**

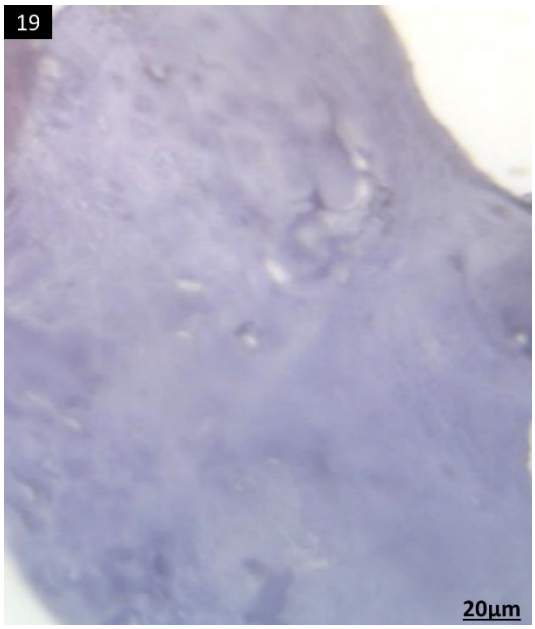


Fig. 19: A photograph of a section of the femoral shaft of group I showing apparent negative immunoreactivity in osteocytes. **(Caspase-3X400).**

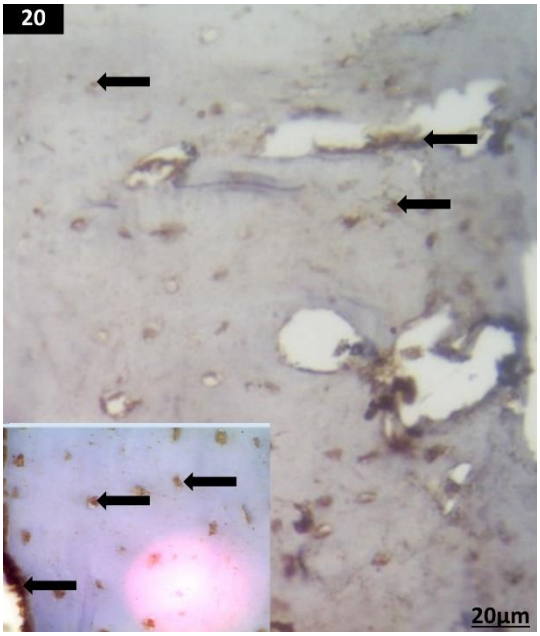


Fig. 20: A photograph of a section of the femoral shaft of group II showing an apparent positive immunoreactivity in osteocytes, cells lining erosion cavities, and bone surface. (arrows). **(Caspase-3X400).**

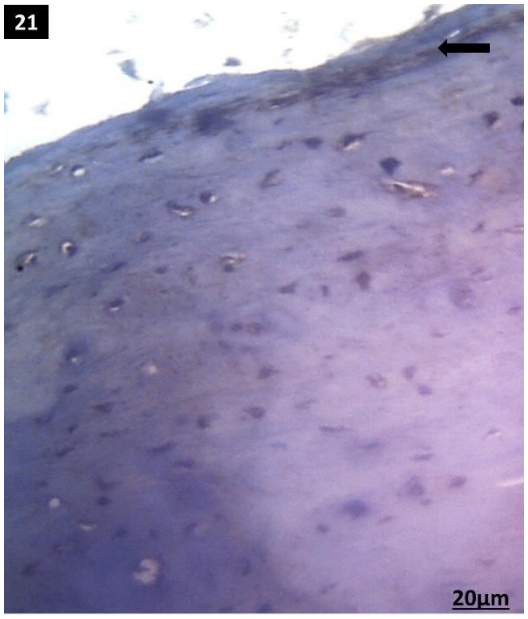


Fig.21: A photograph of a section of the femoral shaft of group III showing mild immunoreactivity in cells lining the bone surface. **(Caspase-3X400).**

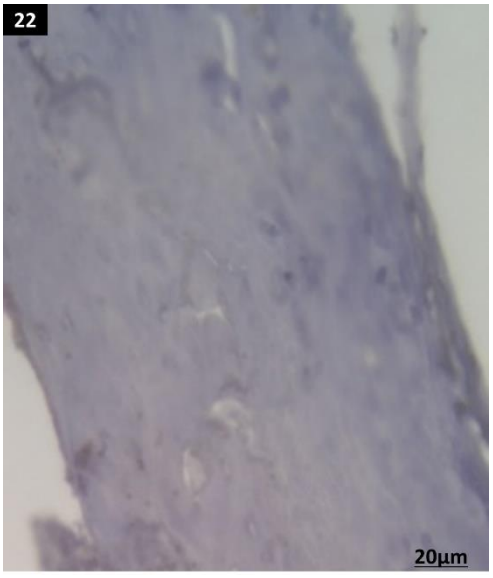


Fig. 22: A photograph of a section of the femoral shaft of group IV showing an apparent negative immunoreactivity in osteocytes. **(Caspase-3X400).**

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 endosteum 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-Mutheffer'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:

1. **Golob AL, Laya MB.** Osteoporosis: Screening, prevention, and management. Med Clin North Am 2015; 99 (3):587-606.
2. **Raisz LG.** Pathogenesis of osteoporosis: Concepts, conflicts, and prospects. J Clin Invest 2005115(12):3318-25.

3. **Ahmad NS, Khalid BA, Luke DA, Ima Nirwana S.** Tocotrienol offers better protection than tocopherol from free radical-induced damage of rat bone. Clinical and Experimental Pharmacology and Physiology. 2005 Sep;32(9):761-70.
4. **Weinstein RS.** Glucocorticoid-induced osteoporosis and osteonecrosis. Endocrinol Metab Clin N Am. 2012;41:595–611.
5. **Kennedy CC, Papaioannou A, Adachi JD.** Glucocorticoid-induced osteoporosis. Women's Health. 2006;2(1):65-74.
6. **Steinbuch M, Youket TE, Cohen S.** Oral glucocorticoid use is associated with an increased risk of fracture. OsteoporosInt. 2004; 15: 323–8. https://doi.org/10.1007/s00198-003-1548-3.
7. **A. N. Sontakke and R. S. Tare**, "A duality in the roles of reactive oxygen species with respect to bone metabolism,"Clinica Chimica Acta, vol. 318, no. 1-2, pp. 145–148, 2002.
8. **Maggio D, Barabani M, Pierandrei M, Polidori MC, Catani M, Mecocci P, *et al.*** Marked decrease in plasma antioxidants in aged osteoporotic women: Results of a cross-sectional study. Journal of Clinical Endocrinology and Metabolism, 2003;88(4):1523-7.
9. **Basu S, Michaëlsson K, Olofsson H, Johansson S, Melhus H.** Association between oxidative stress and bone mineral density. Biochemical and biophysical research communications. 2001 19;288 (1): 275-9.
10. **Callaway DA, Jiang JX.** Reactive oxygen species and oxidative stress in osteo-clastogenesis, skeletal aging and bone diseases. Journal of bone and mineral metabolism. 2015 Jul; 33:359-70.
11. **Dudenkov DV, Yawn BP, Oberhelman SS, Fischer PR, Singh RJ, Cha SS, et al.** Changing incidence of serum 25-hydroxyvitamin D values above 50 ng/ml: a 10-year population-based study. Mayo Clin Proc. (2015) 90:577–86. doi: 10.1016/j. mayocp.2015.02.012
12. **Goreja WG: Black Seed**: Natural Medical Remedy. New York, NY, USA: Amazing Herbs Press; 2003.
13. **Al-Ali A, Alkhawajah AA, Randhawa MA, Shaikh NA.** Oral and intraperitoneal LD50 of thymoquinone, an active principle of *Nigella sativa*, in mice and rats. J Ayub Med Coll Abbottabad 2008;20 (2):25-7
14. **Nader MA, El-Agamy DS, Suddek GM.** Protective effects of propolis and thymoquinone on development of atherosclerosis in cholesterol-fed rabbits. Archives of pharmacal research. 2010; 33:637-43.
15. **Khan N, Sultana S.** Inhibition of two stage renal carcinogenesis, oxidative damage and hyperproliferative response by Nigella sativa. European Journal of Cancer Prevention. 2005 1:159-68.
16. **Mustafa RA, Alfky NA, Hijazi HH, Header EA, Azzeh FS.** Biological effect of calcium and vitamin D dietary supplements against osteoporosis in ovariectomized rats. Progr Nutr [Internet]. 2018;20(1):86-93. Available from: https://www.mattioli1885journals.com/index.php/progressinnutrition/article/view/5223
17. **Abd El-kader M.** Evaluation of Azithromycin Induced Cardiotoxicity in Male Albino Rats and the Possible Protective Role of Nigella Sativa Oil. EJH. 2019;43(2):465-476. ISSN: 1110-0559.
18. **Saad DA, Mohamed AHA, El-Gohary SA.** Green tea extract suppresses osteo-clastogenesis and opposes glucocorticoid-induced osteoporosis in rats. J Arab Soc Med Res 2019;14:33-41
19. **Bancroft JD and Layton, C.** Theory and practice of histological technique, 7th ed., London: Churchill Livingstone. 2010; P 173-214.
20. **Wang YP and Liu BY.** High expression of osteopontin and CD44v6 in odontogenic keratocysts. J. Formos. Med. Assoc. 2009;108(4):286-292.
21. **Kiernan JA.** Immunohistochemistry. In: Kiernan J (ed) Histological and histochemical methods. Theory and

practice, 4th ed., Scion Publishing Ltd, (2015). pp 454´490

22. **Liping An, Liqiang Shi, Yujie Ye, Dankai Wu, Guangkai Ren, Xiao Han, Guangyu Xu, Guangxin Yuan, Peige Du.** Protective effect of Sika Deer bone polypeptide extract on dexamethasone-induced osteoporosis in rats. Electronic Journal of Biotechnology, 2021, Volume 52,,Pages 52-58,ISSN 0717 3458,https://doi.org/10.1016/j.ejbt.2021.04. 002. (https://www.sciencedirect.com/ science/article/pii/S0717345821000178)
23. **Hirayama, T.; Sabokbar, A. and Athanasou, N. (2002).** Effect of corti- costeroids on human osteoclast formation and activity. J. Endocrinol., 175(1):155- 163.
24. **Sivagurunathan, S.; Muir, M.; Brennan, T.; Seale, J. and Mason, R. (2005).** Influence of glucocorticoids on human osteoclast generation and activity. J. Bone Miner Res. 20(3):390- 398.
25. **Kondo, T.; Kitazawa, R.; Yamaguchi, A. and Kitazawa, S. (2008).** Dexamethasone promotes osteoclastogenesis by inhibiting osteoprotegerin through multiple Levels. Cell Biochem. 103(1):335-345.
26. **Bai RJ, Li YS, Zhang FJ.** Osteopontin, a bridge links osteoarthritis and osteoporosis. Front Endocrinol (Lausanne). 2022 Oct 28;13:1012508. doi: 10.3389/fendo. 2022. 1012508. PMID: 36387862; PMCID: PMC9649917.
27. **Vancea A, Serban O, Fodor D.** Relationship between osteopontin and bone mineral density. Acta Endocrinologica (Bucharest). 2021 Oct;17(4):509.
28. **Shady AM, Nooh HZ.** Effect of black seed (*Nigella sativa*) on compact bone of streptozotocin induced diabetic rats. Egypt J Histol 2010;33(1):168-77.
29. **Kara MI, Erciyas K, Altan AB, Ozkut M, Ay S, Inan S.** Thymoquinone accelerates new bone formation in the rapid maxillary expansion procedure. Arch Oral Biol 2012; 57(4):357-63.
30. **Arslan AH, Tomruk CO, MeydanlI EG, Özdemir I, Çapar GD, Kütan E, *et al*.** Histopathological evaluation of the effect of systemic thymoquinone administration on healing of bone defects in rat tibia. Biotechnol Biotechnol Equip 2017; 31(1):175-81.
31. **Al-Mutheffer EA.** The percutaneous effect of black seed (*Nigella sativa*) oil as external topical treatment on bone healing in rabbits. AL-Qadisiya J Vet Med Sci 2014;13 (2):146-54
32. **Corder, C.; Benqhuzzi, H.; Tucci, M. and Cason, Z. (2003).** Delayed apoptosis upon the treatment of Hep- 2 cells with black seed. Bio. Med. Sci. 39:365-370.
33. **Musa, D.; Duisiz, N.; Gumushan, H.; Ulakoglu, G. and Muharrem, B. (2004).** Antitumor activity of an ethanol extract of *Nigella Sativa* seeds. Biologia Bratislava. 59:635- 670
34. **Seif AA**. *Nigella sativa* reverses osteoporosis in ovariectomized rats. BMC Complement Altern Med 2014;14:22.
35. **Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, *et al.*** A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed 2013;3(5):337-52.
36. **Shuid AN, Mohamed N, Mohamed IN, Othman F, Suhaimi F, Ramli ES, *et al*.** *Nigella sativa*: A potential antiosteoporotic agent. Evi Based Complement Alternat Med 2012;2012:Article ID: 696230.
37. **Ansari R, Gheek N.** Effects of *Nigella sativa* against osteoporosis. Int J Pure Appl Biosci 2013;1(2):6-14.
38. **Ali MA, Abu Sayeed M, Shahinur Alam M, Yeasmin Mst S, Khan AM, Muhamad II:** Characteristics of oils and nutrient contents of nigella sativa linn. and trigonella foenum-graecum seeds. Bull Chem Soc Thiop 2012,26(1):55–64.
39. **Winkler, C.; Schroecksnadell, K.; Ledochowski, M.; schennach, H.; Houcher, B. and Fuchs, D. (2008).** Invitro effects of *Nigella Sativa* seeds extracts on stimulated peripheral blood mononuclear cells. Pteridines. 19(4): 101- 106.

40. **Ahmad NS, Khalid BA, Luke DA, Ima Nirwana S.** Tocotrienol offers better protection than tocopherol from free radical-induced damage of rat bone. Clin Exp Pharmacol Physiol 2005;32(9):761-70.

41. **Ebina Y, Okada S, Hamazaki S, Toda Y, Midorikawa O.** Impairment ofbone formation with aluminum and ferric nitrilotriacetate complexes. Calcif Tissue Int 1991;48(1):28-36.

42. **Takeuchi K, Okada S, Yukihiro S, Inoue H:** The inhibitory effects of aluminum and iron on bone formation, in vivo and in vitro study. Pathophysiology 1997, 4(2):97–104.

43. **Al-Mutheffer EA.** The percutaneous effect of black seed (*Nigellasativa*) oil as external topical treatment on bone healing in rabbits.AL-Qadisiya J Vet Med Sci 2014;13(2):146-54.

44. **Ozdemir H, Kara MI, Erciyas K, et al:** Preventive effects of thymoquinone in a rat periodontitis model: a morphometric and histopathological study. J Periodontal Res 2012, 47(1):74–80

45. **Valizadeh N, Zakeri HR, Asnafi GA, Shafiee A, Sarkhail P, Heshmat R, *et al*.** Impact of black seed (*Nigella sativa*) extract on bone turnover markers in postmenopausal women with osteoporosis. DARU J Pharm Sci 2009;17 Suppl 1:20-5.

46. **Zaoui, I.; cherrah, Y.; Mahassini, N.; Alaoui, K.; Amar ouch, H. and Hassarssue, M. (2002).** Acute and chronic toxicity of *Nigella Sativa* fixed oil. Phytomedicine. 9(1):3- 6.

47. **Al-Sa'aidi JA, Dawoodand KA, Latif AD.** Immunomodulatory effect of *Nigella sativa* seed extract in male rabbits treated with dexamethasone.Iraqi J Vet Sci 2012;26 Suppl IV:141-9.

48. **Guo Y, Hao D, Hu H.** High doses of dexamethasone induce endoplasmic reticulum stress-mediated apoptosis by promoting calcium ion influx-dependent CHOP expression in osteoblasts. Molecular Biology Reports. 2021 Dec;48:7841-51.

49. **Jean G, Souberbielle JC, Zaoui E, Lorriaux C, Mayor B, Hurot JM.** Total and bone-specific alkaline phosphatases in haemodialysis patients with chronic liver disease. Clin Biochem. 2012;45(6):436–439. doi: 10.1016/j. clinbiochem. 2012. 01.018.

50. **Buchet R, Millan JL, Magne D.** Multisystemic functions of alkaline phosphatases. Methods Mol Biol. 2013; 1053:27–51. doi:10.1007/978-1-62703-562-0_3

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

رانيا حمدى السيد الصياد١, هبه عبدالرازق عبدالفتاح٢, نهى أسامه شوقى٣, ايمان احمد الصواف١

١ قسم التشريح وعلم الأجنه, ٢ قسم الهستولوجيا،٣ قسم الفسيولوجيا الطبيه- كليه الطب، جامعة حلوان، مصر

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

الهدف من الدراسه: مقارنة دور حبه البركه مقابل الكالسيوم وفيتامين د في الحماية من هشاشة العظام الناجم عن الجلوكوكورتيكويد في نموذج الجرذان.

المواد والطرق: تم تقسيم ٥١ جرذ ذكور من الجرذان البيضاء وزنها ١٢٠-١٥٠ جم إلى أربعه مجموعات: المجموعة الأولى (المجموعة الضابطه) ، المجموعة الثانية (مجموعة الديكساميثازون) ، المجموعة الثالثة (ديكساميثازون بالاضافه الى الكالسيوم + فيتامين د) ، والمجموعة الرابعة (ديكساميثازون بالإضافة إلى زيت حبه البركه) . تم قياس مستويات الكالسيوم المتأين وانترلوكين 6 والفوسفاتيز القلوي. تم تحضير مقاطع البارافين من عظمه الفخذي وتم صبغها باستخدام الهيماتوكسلين والايوسين وصبغه مالورى والصبغات المناعيه اوستيوبونتين وكسباز ٣. بالإضافة إلى ذلك ، تم إجراء التحليلات المورفومترية والإحصائية.

النتائج: كشفت جرذان المجموعة الثانية عن تجاويف ناجمه عن تآكل العظم في العظم المضغوط وأيضا تآكل فى سطح العظم واتساع فى قناة هافيرشن مع وجود واتساع فى الثغرات العظمية. وقد أظهر العظم الإسفنجي ترابيق عظميه رفيعه وغير متصله يحتوي على ثغرات فارغة وتخاويف واسعة من نخاع العظم. وأيضا تظهربعض المناطق ضعيفه الصبغه وكسور طفيفة. تم تأكيد هذه النتائج من خلال انخفاض كبير في سمك وعدد الثغرات التي تحتوي على الخلايا العظمية في العظم المضغوط والإسفنجي. أيضًا ، كان هناك انخفاض واضح في اوستيوبونتين وزيادة في الكسباز ٣. علاوة على ذلك ، تم الكشف عن زيادة احصائية في الفوسفاتيز القلوي وانترلوكين٦. أظهرت المجموعتان الثالثة والرابعة تحسنًا في بنية العظم المضغوط الإسفنجي.ايضا كان هناك تحسن فى الاوستيوبونتين والكسباز ٣ والفوسفاتيز القلوي ووانترلوكين٦. وقدأظهرت المجموعة الرابعة نتائج أفضل من المجموعة الثالثة.

الخلاصة: أظهرت هذه الدراسة أن حبه البركه كانت أفضل من الكالسيوم وفيتامين د في الوقايه من هشاشة العظام لمعظم الجرذان بسبب تأثيرها في إعادة تشكيل العظام. حبه البركه يعمل كمصدر للكالسيوم وله تأثيرات مضادة للأكسدة. لذلك ، يوصى باستخدام حبه البركه في الحماية من هشاشة العظام التي يسببها الجلوكورتيكويد.