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AMELIORATING EFFECTS OF BOVINE COLOSTRUM ON EXPERIMENTALLY INDUCED OSTEOPOROTIC LUMBAR VERTEBRAE IN FEMALE ALBINO RATS: A HISTOMORPHOMERIC AND SCANNING ELECTRON MICROSCOPIC STUDY

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Background: Up till now there is no efficient preventive and/or therapeutic treatment of osteoporosis which constitutes a worldwide tragedy especially in women after menopause.

Objectives: Is to investigate the ameliorating effect of the use of bovine colostrums in prevention and treatment of osteoporosis.

Materials and methods: 90 female albino rats were divided into two main groups; a sham operated control group (30 rats) and an experimental group (60 rats). The experimental group was subdivided into 4 subgroups (15 rats each): Subgroup IIa, rats were subjected to bilateral ovariectomy and received daily IM injection of methylprednisolone (1 mg/kg BW) for 3 months; Subgroup IIb, ovx rats received methylprednisolone (1 mg/kg BW) and concomitantly received daily bovine colostrum (1 ml/day) for 3 months; Subgroup IIc ovx corticosteroid-treated rats left for further 3 months to reach 9 months old and Subgroup IId, ovx corticosteroid-treated rats received daily colostrum for 3 months. Lumbar vertebrae were exposed to X-ray and DEXA scan to assess bone densities. Decalcified specimens were processed for histomorphometric assessment as well as scanning electron microscopic evaluation of bone microarchitecture.

Results: Induction of osteoporosis resulted in disruption of ossification zones and significant decrease in BMD. Prophylactic group showed marked improvement of BMD and restoration of bone microarchitecture. Therapeutic group showed reasonable restoration of bone architecture and decrease in percentage of bone loss.

Conclusion: Colostrum significantly prevented bone derangement when used as a prophylactic and reasonably restored bone microarchitecture when used as therapeutic agent for treatment of osteoporosis.

INTRODUCTION

Osteoporosis is a disease characterized by decreased bone mass and architectural deterioration of osseous tissue with a consequent increase in bone fragility and susceptibility to fracture. Osteoporosis has become one of the major health problems worldwide. Increasing life expectancy aggravates the tendency of more complications associated with osteoporosis. Spongy bone appears to be more influenced by osteoporosis than compact bone. Lack of regular lamellae and less mineralized matrix make spongy bone less resistant to resorption than compact bone[1].

It was postulated that cancellous bone loss starts at a younger age in adult men and women and continues throughout life with acceleration

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in women in their postmenopausal period due to lower levels of estrogen. Accordingly, there is a higher risk of vertebral fracture and collapse in aged women compared with aged men[2&3].

Goals of therapy of osteoporosis, in general, non-specific aiming for preventing possible fractures, relieving symptoms of skeletal deformities and stabilizing bone mass. Drugs such as calcitonin, estrogen replacement, fluoride and vitamin supplementation have been tried to stop further bone loss with minimal restorative and healing potentials. Recently, natural products with no side effect have been administrated in order to prevent and treat osteoporosis[4-7].

Colostrum is the pre-milk fluid produced by mammals during the first 24-48 hours after giving birth. Colostrum provides life-supporting immune and growth factors that ensure the health, vitality and protection of all mammalian babies (including humans) and has now been shown to protect and heal adults[8]. The molecular structure of the immune and growth factors in bovine colostrum is very similar to those found in humans. Therefore, bovine colostrum can be safely consumed by humans. The most important components of colostrum can be broken down into three major categories; immune system factors, growth factors, and nutritional components[9].

Bovine colostrum and its extracts have positive effects on bone growth of human beings or experimental rats, such as increasing bone mineral density and promoting calcium absorption[10]. Moreover, colostrum-based dietary supplement enhances bone growth and development in humans[11].

The aim of the present study is to evaluate the effects of colostrums administration in prevention and treatment of osteoporotic-induced female albino rat.

MATERIALS AND METHODS

Chemicals and drugs

Bovine colostrum was obtained from the Animal Farm - Faculty of Veterinary Medicine, Assiut University and stored as frozen cubes that defreezed daily before use. Methylprednisolone ampules were purchased from pharmaceutical source. Other reagents used in this study were of high analytical grade and were obtained from commercial sources.

Materials and animal groups

All animal procedures were according to the guidelines of animal care approved by the Ethical Committee of the Faculty of Medicine, Assiut University.

A total number of 90 female albino rats, 3-months old with average weight 200 gm were used in the present study. These animals were obtained from the Animal House of the Faculty of Medicine, Assiut University. The animals were housed in a wire mesh cages with natural ventilation, at room temperature, nourished by standard laboratory chow and water in the normal daily light and darkness cycle.

The rats were randomly divided into 2 main groups;

Group (I) (30 rats) underwent a sham operation and used as a control group. Animals of the control group were held without any treatment throughout the entire experiment. These rats were subdivided into two subgroups; subgroup Ia (15) rats that were sacrificed after three months (at the age of 6 months) and subgroup Ib (15) rats that were sacrificed after six months (at the age of 9 months).

Group (II) (60 rats) used as an experimental group. This group was further subdivided into 4 subgroups, 15 rats each.

Subgroup IIa (early osteoporotic group); these rats were subjected to bilateral ovariectomy. One week later, the rats received daily IM injection of methylprednisolone (1 mg/kg) for 3 months for enhancing induction of osteoporosis[12].

Subgroup IIb (prophylactic group); these rats were subjected to bilateral ovariectomy. One week later, these rats received daily IM injection of methylprednisolone (1 mg/kg) and concomitantly received daily bovine colostrum (1 ml/day) for 3 months[13]. This group was used to assess the protective effect of colostrums on bone derangement.

Subgroup IIc (late osteoporotic group); these rats were subjected to bilateral ovariectomy. One week later, the rats received daily IM injection of methylprednisolone (1 mg/kg) for 3 months, followed by 3 months latent period to reach the age of 9 months.

Subgroup IId (therapeutic group); these rats were subjected to bilateral ovariectomy. One week later, the rats received daily IM injection of methylprednisolone (1 mg/kg) for 3 months. Thereafter the rats received daily bovine colostrum (1 ml/day) for the following 3 months. This group was used to study the therapeutic effect of colostrum on the induced bone loss.

Procedure of ovariectomy

Bilateral ovariectmy was performed according to the method described by Lasota and Danowska[14].

Bone sample extraction

Animals of both control and experimental groups were sacrificed at; 6 months for subgroups Ia, IIa and IIb; and at 9 months for subgroups Ib, IIc and II.

Animals first anaesthetized by ether, followed by intracardiac perfusion with 10 ml of isotonic solution. This was followed by 10% formalin solution. The lumbar vertebrae were extracted and cleaned from the attached soft tissues. Bony specimens were processed for bone mineral density determination, histomorphometric and electron microscopic techniques.

Bone mineral density determination

A) X-ray technique

A total number of 30 rats were used in this technique, 5 rats for each of the control subgroups and 5 rats for each of the experimental subgroups. The whole lumbar vertebrae of both the experimental subgroups and their corresponding control subgroups were radiographed using digital plain X-ray image device with a dose of 0.5 rad for one second exposure. X-ray images were digitally pictured and a representative picture for the commonly occurring findings was selected for each subgroup. Pictures of each experimental and corresponding control subgroups were compared to detect bone rarefaction.

B) Dual energy X-ray absorptiometry (DEXA) scan

A total number of 30 rats were used in this technique 5 rats for each of the control subgroups and 5 rats for each of the experimental subgroups. The whole lumbar vertebrae of both experimental and their corresponding control subgroups were radiographed using (Unigamma X-ray plus I can) device to give a picture of bone mineral density. Connected to the DEXA machine is a special software computer program that can transfer the bone density into coloured picture which is translated into a qualitative curve displaying the bone mineral density in the form of gm/cm^2.

Histological study

A total number of 30 rats were used in this technique 5 rats from each of the control subgroups and 5 rats from each of the experimental subgroups.

Decalcification

Decalcification was carried out by immersing the specimens in chelating agent, a freshly prepared EDTA solution (5% solution, pH 7.0) for 4 weeks. The volume of decalcifant is about 10 times the volume of the tissue. EDTA solutions were changed every other day. Decalcification process was completed when the specimen was easily bended. Each fifth lumbar vertebra was sagittally cut by a very sharp blade into two equal halves. The right half was used for routine light microscopic examination and the left half for scanning electron microscopic examination.

Light microscopic technique

The right half of the decalcified 5th lumbar vertebra of each of the studied subgroups was processed for histological examination according to Bancroft and Gamble[15]. The paraffin blocks containing the specimens were cut serially in the sagittal plane (7-8 µ thick) using (Lecia 500) microtome. Sections were stained with Haematoxylin and Eosin stain (H&E). Slides were examined for bone cellular assessment and trabecular thickness evaluation.

Scanning electron microscope (SEM)

The left half of each of the decalcified fifth lumbar vertebrae of each of the studied subgroups was processed for SEM in Assuit University Electron Microscopic Unit. Steps of processing for SEM were according to Beane[16].

Morphometric and statistical analysis

Trabecular bone thicknesses was measured from the light microscopic slides using Leica Qwin 500 image analyzer computer system that is calibrated to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units using the interactive measuring menu. The following variables were measured for the lumbar vertebrae:

In each section mean value of bone trabecular thickness was measured using an objective lens of magnification X4. Trabecular thickness was adjusted for magnification and recorded in um.

For each subgroup, four random sections were taken from each bony specimen for the five rats in each subgroup. Mean values ± standard deviations of the mean of the studied variables as well as percentage losses of bone thicknesses were recorded. Basic statistics were also applied for the studied variables in order to test significance of difference among the studied groups using one way analysis of variance (ANOVA test of SPSS 16.0 version).

RESULTS

I) Six-months old lumbar vertebrae

Histological results

Light microscopic examination

Figure 1 illustrated the cranial epiphyses of the 5th lumbar vertebrae of 6 months old control group, ovarictomized corticosteroid-treated rat (osteoporotic group) and ovarictomized corticosteroid-treated rat that received concomitant colostrum (prophylactic group) stained with H&E. The vertebral body of the control group is composed of three parts; cranial and caudal epiphysis that are still ossifying and a middle part that is completely ossified. The epiphysis displayed different zones of endochondral ossification. These zones included Zone of reserve cartilage which is formed of rounded or ovoid cells with vacuolated cytoplasm and deeply stained ovoid nuclei, Zone of proliferation which is formed of closely packed ovoid cells organized into distinct columns. These cells are larger than those in the reserve zone with scanty cytoplasm and large nuclei with variable staining

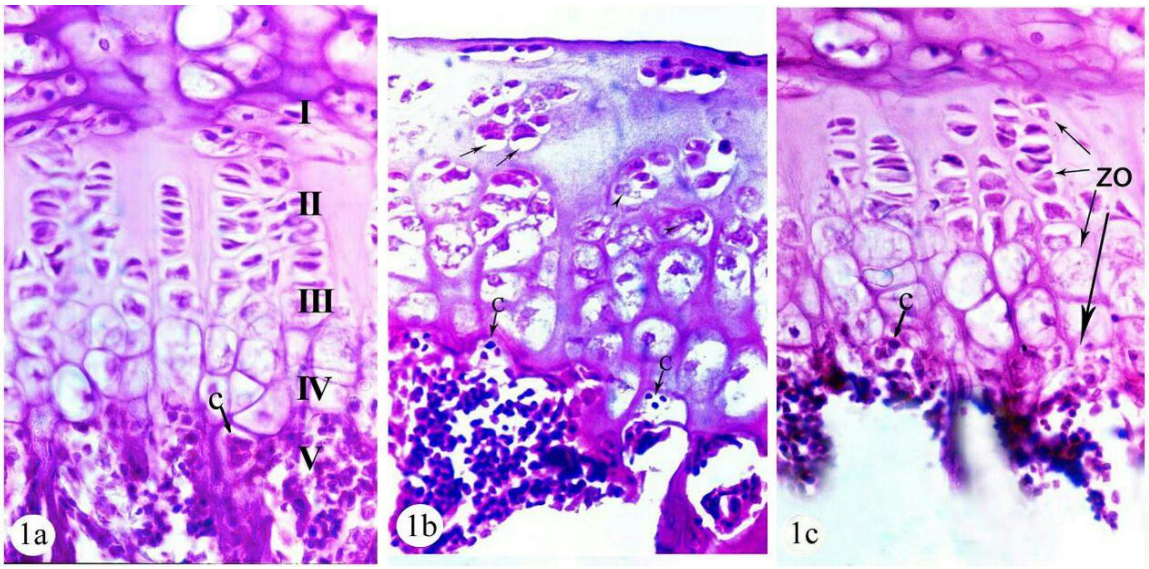


Fig. 1: Photomicrographs of the cranial epiphyses of the 5th lumbar vertebrae of 6 months old control rat, ovarictomized corticosteroid-treated rat (osteoporotic group) and ovarictomized corticosteroid-treated rat that received concomitant colostrum (prophylactic group). (a) Control group showing stages of endochondral ossification; zone of reserve cartilage (I), zone of proliferation (II), zone of hypertrophy (III), zone of calcification (IV) and zone of ossification (V). Osteoclasts appear as multinucleated cells (C →). (b) Osteoporotic group showing disturbed architecture of different zones of ossification with some lacunae containing eccentric chondrocytes (arrows). Other chondrocytes show irregular fragmented nuclei and markedly vacuolated cytoplasm (arrow heads). Note many irregular giant multinucleated osteoclasts (C →). (c) Prophylactic group showing minimal disruption of zones of ossification (zo) with an apparent decreased thickness of zone of proliferation. Osteoclasts (C →) are infrequently recognized. (H&E, X400).

intensities, Zone of hypertrophy which is formed of large rounded cells that are less closely packed as compared with the previous zone. The cells possessed oval or rounded small dense nuclei and pale vacuolated cytoplasm, Zone of calcification which is formed of large irregularly arranged columns with calcified matrix and Zone of ossification which is showing lay down of osteogenic cells and entry of blood vessels. Regular multinucleated osteoclasts were found inside their lacunae (Fig. 1a).

Osteoporotic group revealed that the epiphyses of the lumbar vertebrae showed decreased thickness, disturbed architecture and lack of differentiation between zones of ossification. The proliferating cartilaginous zone was no longer arranged in rows. Some lacunae appeared containing eccentric chondrocytes with darkly stained nuclei. Other chondrocytes showed irregular fragmented nuclei and markedly vacuolated cytoplasm. Many irregular giant multinucleated osteoclasts were recognized (Fig. 1b).

The epiphyses of the lumbar vertebrae of the prophylactic group showed preserved thickness with minimal disruption of zones of ossification. Zone of proliferation though oriented in regular and parallel columns, yet it showed an apparent decrease in thickness in comparison with the control. Compared with the control and osteoporotic groups, osteoclasts were less frequently seen (Fig. 1c).

Figure 2 illustrated the middle part of the vertebral body in the control, osteoporotic and prophylactic groups stained with H&E. In the control group, it was composed of irregular, branching and anastomosing bone trabeculae separated by intercommunicating spaces containing bone marrow. The bone marrow was formed of hematopoietic tissue (Fig. 2a).

The middle part of the vertebral body of the osteoporotic group revealed marked thinning out, fragmentation and discontinuity of bone trabeculae with relative increase in the bone marrow spaces (Fig. 2b).

The prophylactic group showed preservation of the bone trabecular thickness with nearly normal proportion with the intervening marrow spaces. Areas of faintly stained matrix are recognized among bone trabeculae denoting formation of immature ostoid tissue (Fig. 2c).

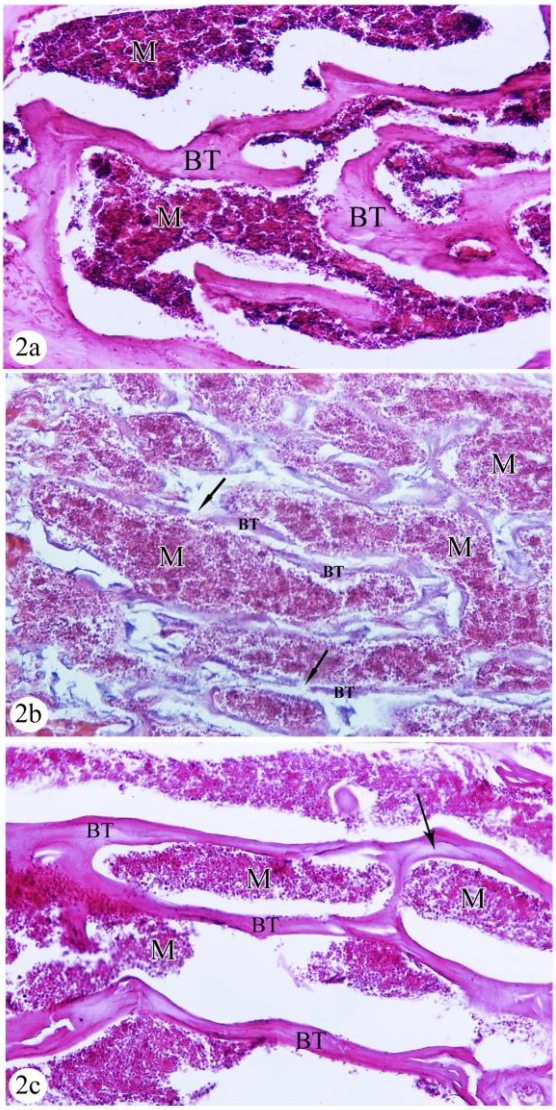


Fig. 2: Photomicrographs of the middle part of the body of the 5th lumbar vertebra of 6 months old control, osteoporotic and prophylactic groups. (a) Control group showing that it is composed of intercommunicating normal dense bone trabeculae (BT) separated by marrow spaces (M). (b) Osteoporotic group showing marked thinning out of bone trabeculae (BT), fragmentation and discontinuity of bone trabeculae are also shown (arrows). Note relative increase in marrow spaces (M). (c) Prophylactic group showing preservation of bone trabecular (BT) thickness and normal marrow spaces (M). Note the areas of faintly stained matrix in bone trabeculae (arrows) indicating formation of immature osteoid. (H&E, X100).

Electron microscopic examination

Figure 3 illustrated scanning electron microscopic (SEM) pictures of the lumbar

vertebral body in the 3 studied groups. In the control group the lumbar vertebral body is composed of an outer shell of compact bone and an inner core of cancellous bone. The outer shell of compact bone showed smooth contour of the outer and inner surfaces. The inner core of cancellous bone was formed of freely intercommunicating bone trabeculae separated by marrow spaces. Bone trabeculae appeared to be branched, anastomosing and mostly of uniform thickness (Fig. 3a).

In the osteoporotic group, the lumbar vertebrae showed apparent thinning of the outer compact bony shell, which appears irregular from its inner surface with multiple tiny cavities. The core of the vertebral bodies showed thinning and irregularity of the bone trabeculae with an apparent increase in the marrow spaces (Fig. 3b).

SEM examination of the prophylactic group showed an outer compact bony shell with nearly normal appearance. The inner core revealed more or less normal architecture of the bone trabeculae with normal proportion between bone trabeculae and marrow spaces (Fig. 3c).

Radiological findings

Figure 4 illustrated X-ray radiograph and DEXA scan photographs of the lumbar vertebrae of 6 months old control, osteoporotic and prophylactic groups. Plain X-ray examination of the whole lumbar vertebrae of the control group revealed that the rat had six lumbar vertebrae with intervening disc spaces. The osteoporotic group showed marked rarefaction of the vertebral bodies specially the cranial vertebrae. The prophylactic group indicated preservation of bone density of the vertebral bodies compared with the osteoporotic group (Fig. 4a).

Bone mineral density of the lumbar vertebrae is depicted by Dual Energy X-ray Absorpitometry (DEXA) scan which shows normal bone density with estimated average Bone Mineral Density (BMD) of 0.90 gm/cm^3 (Fig. 4b). The osteoporotic group showed marked decrease in bone density with estimated average BMD of 0.50 gm/cm^3 (Fig. 4c). The prophylactic group showed substantial preservation of bone density with estimated average BMD of 0.75 gm/cm^3 (Fig. 4d).

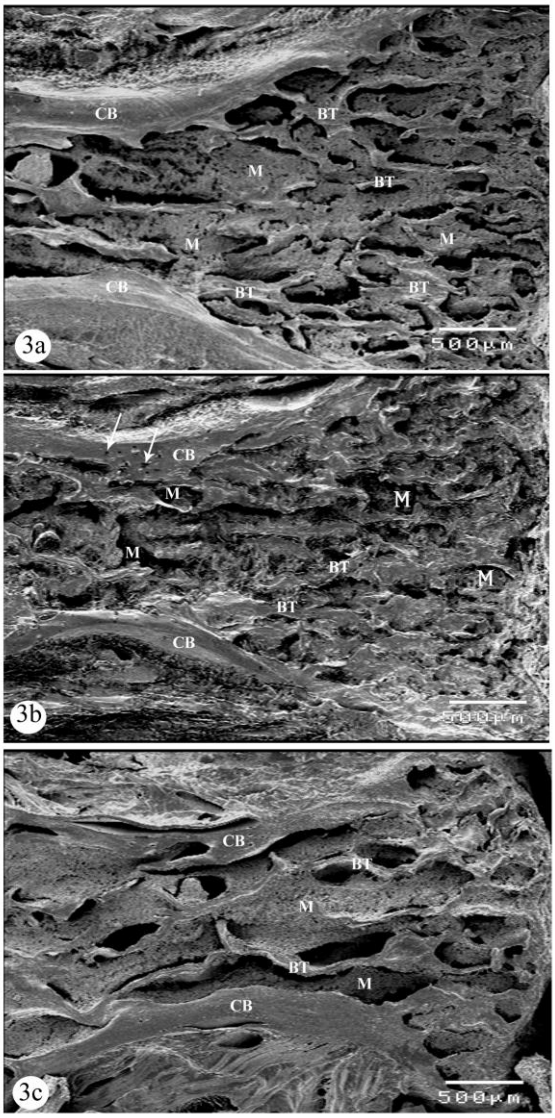


Fig. 3: Scanning electron micrographs of mid-sagittal sections of the 5th lumbar vertebral body of 6 months old control, osteoporotic and prophylactic groups. (a) Control group showing ventral and dorsal shell of compact bone (CB) and inner core of cancellous bone formed of interconnected bone trabeculae (BT) separated by intercommunicating marrow (M) cavities. (b) Osteoporotic group showin an irregular inner surfaces of the outer cortical bone (CB) shell with an apparent decrease in their thickness. Note tiny cavities inside the outer shell (arrows). Markedly diminished thickness of bone trabeculae (BT) with an apparent increase in marrow spaces (M) are observed. (c) Prophylactic group showing thickenings of the compact bony (CB) shells. Notice preserved intact bone trabeculae (BT). Marrow spaces (M). (SEM X35).

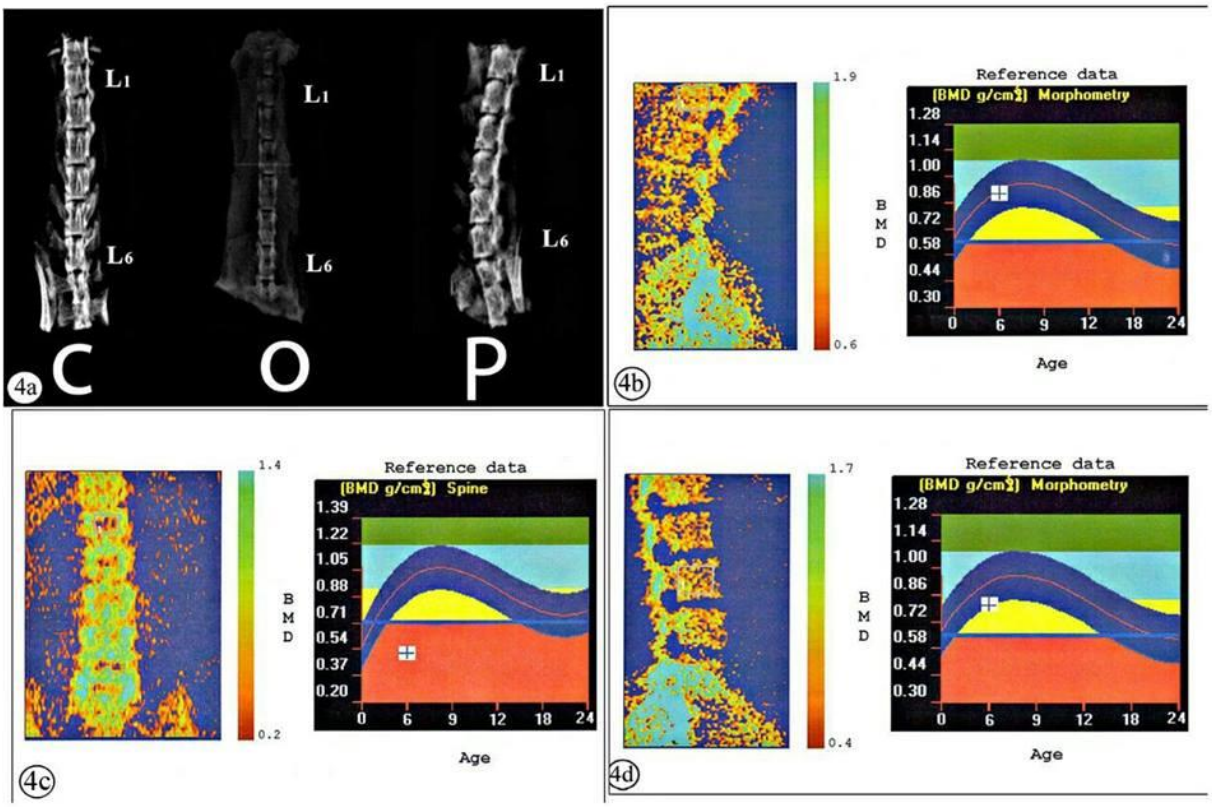


Fig. 4: X-ray radiograph and DEXA scan photographs of the lumbar vertebrae of 6 months old control, osteoporotic and prophylactic groups. (a) X-ray radiograph of the lumbar vertebrae of 6 months old control (C), osteoporotic (O) and prophylactic (P) groups showing marked rarefaction of bone of the osteoporotic group compared with the control group while the prophylactic group shows minimal rarefaction. (b) DEXA scan photograph of a control rat and its representative curve with (□) mark within the blue area indicating normal bone density**.** (c) DEXA scan photograph of an osteoporotic rat and its representative curve with (□) mark within the red area indicating marked decrease in bone density**.** (d) DEXA scan photograph of a prophylactic rat and its representative curve with (□) mark lying low in the blue area indicating preservation of bone density.

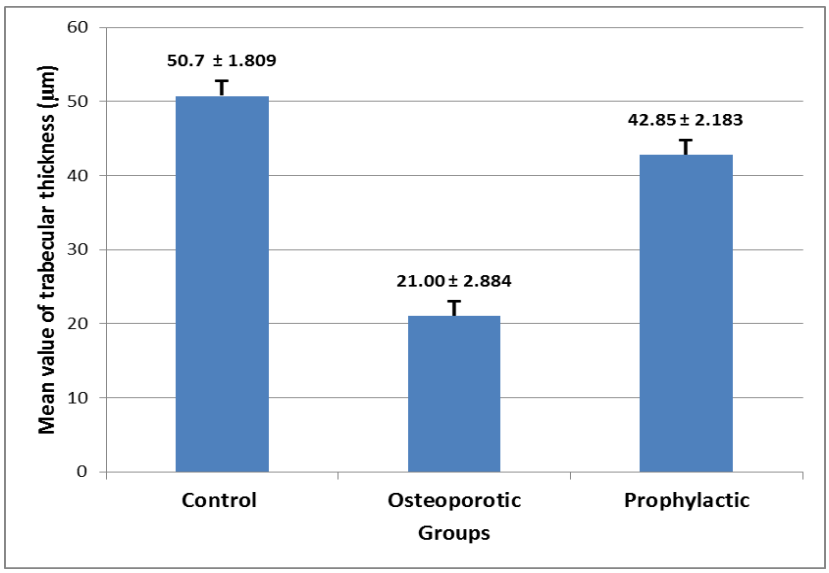
Morphometric analysis

The histological changes were confirmed by morphometric analysis where the trabecular thickness of the 5th lumbar vertebra in the three studied groups at the age of 6 months was shown in table 1 and histogram 1. It appeared that there was a significant decrease in the mean trabecular thickness in the osteoporotic group (21.00±2.88) in comparison with the control group (50.70±1.81). It was also observed that there is a statistically significant difference of the trabecular thickness in the prophylactic group (42.85±2.18) in comparison with osteoporotic group indicating preservation of the trabecular thickness in this group. Three months after induction of osteoporosis there was 58.8% loss of trabecular thickness of the osteoporotic rats. Meanwhile the use of colostrum in parallel with induction of osteoporosis resulted in only 15.6% loss of trabecular thickness in the prophylactic group (Fig. 4e).

Table 1: Trbecular thickness of L5 of the 6-month old groups.

	Group Ia Sham operated control	Group Iia OVX + steroid treated (Osteoporotic)	Bone loss	Group IIb OVX + steroid treated + concomittant colostrum (Prophylactic)	Bone loss
Trabecular thickness (in µm)	50.70 ± 1.81 (n= 20) (R: 47-53)	21.00 ± 2.88*** (n= 20) (R: 18-23)	58.8%	42.85 ±2.18*** (n= 20) (R: 39-47)	15.6%

Changes in bone trabeculae is in the form of percentage loss in comparison with the control.
Each value is represented as the mean ± standered deviation.
n : number of random measurements in the studied rats.
R : range of measurements.
*** $p < 0.001$: differences between the means are highly significant.



Histogram 1: The mean trabecular thickness of the 5th lumbar vertebrae of 6 months old control, osteoporotic and prophylactic groups.

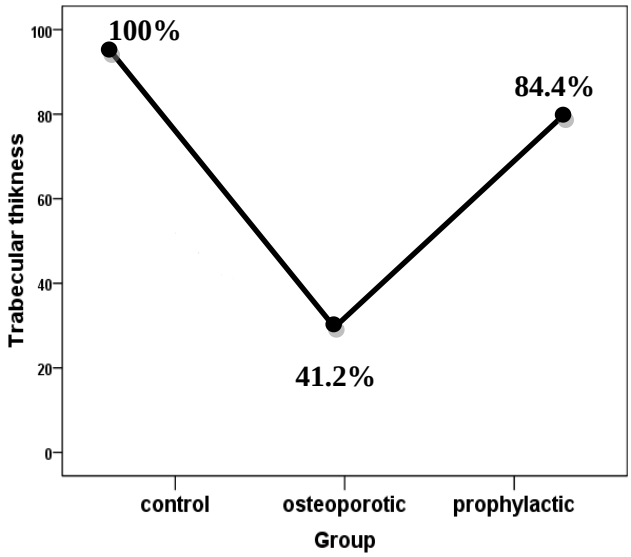


Fig. 4e: Percentage of trabecular thickness of the lumbar vertebrae of the control, osteoporotic and prophylactic groups. Notice trabecular thichkness preservation in the prophylactic group.

II) Nine-months old lumbar vertebrae

Histological results

Light microscopic examination

Figure 5 illustrated the cranial epiphyses of the 5th lumbar vertebrae of 9 months old control, osteoporotic and prophylactic groups stained with H&E. Each vertebral body of the control group is composed of an outer shell of compact bone and an inner core of cancellous bone. The cranial and caudal epiphyses were still ossifying and the middle part was completely ossified. The epiphyses displayed different zones of endochondral ossification (Fig. 5a).

The epiphyses of the lumbar vertebrae of the osteoporotic group showed decreased thickness and disrupted architecture with lack of differentiation of the zones of ossification. The proliferating and hypertrophying cell zones were no longer arranged in rows. Zones of calcification and ossification were poorly formed (Fig. 5b). Some specimens showed multiple osteolytic cavitation within the epiphyses (Fig. 5c).

Therapeutic group revealed that the epiphyses restored thickness with a trial of reorientation of different zones of ossification. However, the regular columns of zone of proliferation were occupied by wide homogenous structureless areas of degenerations in between (Fig. 5d).

Figure 6 illustrated the middle part of the vertebral body in the control, osteoporotic and therapeutic groups stained with H&E. In the control group, it is composed of a network of branching and anatomosing bony trabeculae separated by intercommunicating bone marrow spaces. Bone trabeculae were in the form irregularly arranged lacunae containing osteocytes (Fig. 6a).

The middle part of the vertebral body of the osteoporotic group revealed marked thinning out of the bone trabeculae with relative increase in the bone marrow spaces. Bone trabeculae also show discontinuity and fragmentations (Fig. 6b).

The therapeutic group showed an apparent increase in bone trabecular thickness with less fragmentation compared with the osteoporotic group. There are areas of faint staining in the trabeculae indicating formation of immature osteoid tissue (Fig. 6c).

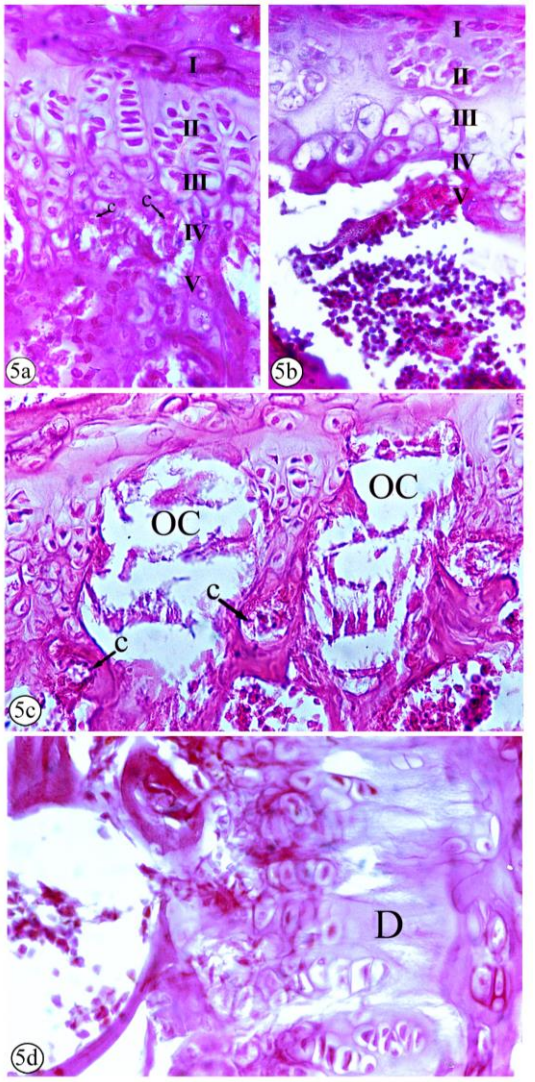


Fig. 5: photomicrographs of the cranial epiphyses of the 5th lumbar vertebrae of 9 months old control, osteoporotic and prophylactic groups. (a) Control group showing stages of endochondral ossification: zone of reserve cartilage (I), zone of proliferation (II), zone of hypertrophy (III), zone of calcification (IV) and zone of ossification (V). osteoclasts (C) are found within their lacunae. (b,c) Osteoporotic group showing markedly disrupted irregular zones of ossification. Zone of reserve cartilage (I), Notice irregular clumps of chondrocytes in the zone of proliferation (II) and zone of hypertrophy (III). Zone of calcification (IV) and zone of ossification (V) are poorly formed. Areas of osteolytic cavtiation (OC) within the epiphyses can be observed. Notice giant multinucleated osteoclasts (C). (d) Therapeutic group showing reorientation of zones of ossification. Areas of degeneration (D) appear in between columns of the proliferating zone. (H&E, X200; 400).

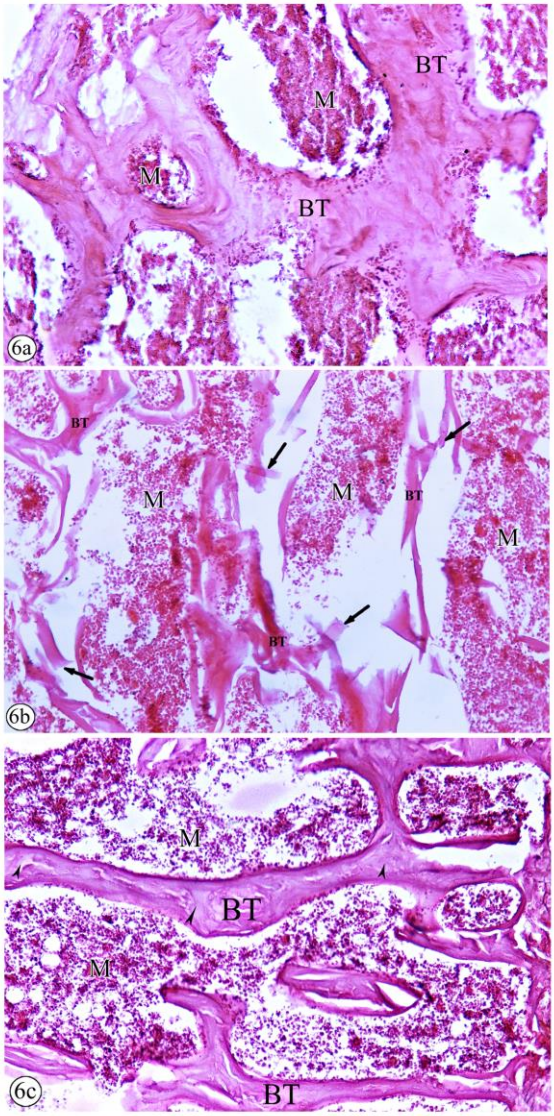


Fig. 6: photomicrographs of the cranial epiphyses of the 5th lumbar vertebrae of 9 months old control, osteoporotic and prophylactic groups. (a) Control group showing intercommunicating bone trabeculae (BT) separated by marrow spaces (M). (b) Osteoporotic group showing marked reduction of bone trabecular (BT) thickness and relative increase in marrow spaces (M). Notice discontinuity and fragmentation of bone trabeculae (arrows). (c) Therapeutic group showing increase in the thickness of bone trabeculae (BT) and normal marrow spaces (M). Notice the areas of faintly stained matrix in the bone trabeculae (arrow heads) indicating formation of immature osteoid tissue. (H&E, X100).

Electron microscopic examination

Figure 7 illustrated scanning electron microscopic (SEM) pictures of the lumbar vertebral body in the 3 studied groups. In the control group the lumbar vertebrae revealed that each lumbar vertebral body is composed of an outer shell of compact bone with smooth contour of the outer and inner surfaces and an inner core of cancellous bone consisting of freely intercommunicating bone trabeculae separated by marrow spaces. Bone trabecular thickness increased as compared with 6-months old control group (Figs. 7a&b).

In the osteoporotic group, the lumbar vertebrae showed thinning out of the outer compact bony shell that appears irregular from its inner surface. The core of the vertebral bodies shows marked loss, breaking and irregularity of the bone trabeculae whose substance is replaced by marrow spaces (Figs. 7c&d).

SEM examination of the therapeutic group showed marked improvement of the thickness of the outer compact bony shell in comparison with that of the osteoporotic group. The inner core of cancellous bone reveals moderate restoration of trabecular architecture and improvement of trabecular thickness (Figs. 7e&f).

Radiological findings

Figure 8 illustrated X-ray radiograph and DEXA scan photographs of the lumbar vertebrae of 9 months old control, osteoporotic and therapeutic groups. Plain X-ray examination of the whole lumbar vertebrae of the control group revealed normal appearance of the lumbar vertebral bodies with the intervening disc spaces. The osteoporotic group revealed marked rarefaction of the vertebral bodies compared with the control group. Therapeutic group showed marked improvement of bone density of the vertebral bodies compared with the osteoporotic group (Fig. 8a).

Bone mineral density of the lumbar vertebrae depicted by DEXA scan showed normal bone density with estimated average BMD of 0.9 gm/cm³ (Fig. 8b). The osteoporotic group showed markedly decreased bone density with estimated average BMD of 0.37 gm/cm³ (Fig. 8c). DEXA scan in the therapeutic group revealed substantial restoration of bone density with estimated average BMD of 0.75 gm/cm³ (Fig. 8d).

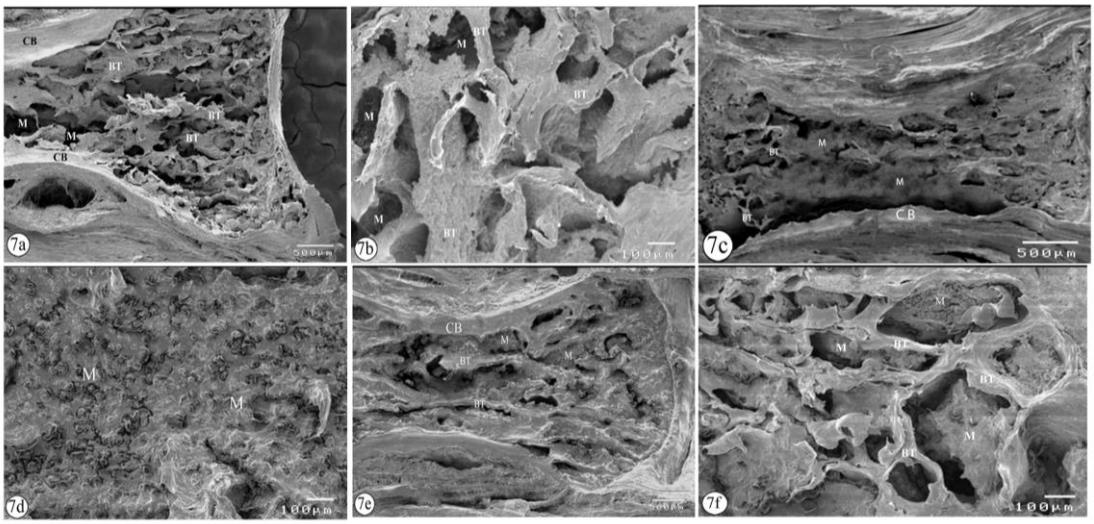


Fig. 7: Scanning electron micrographs of mid-sagittal sections of the 5th lumbar vertebral body of 9 months old control, osteoporotic and prophylactic groups. (a,b) Control group showing an outer shell of compact bone (CB) and inner core of cancellous bone which is formed of interconnected bone trabeculae (BT) separated by intercommunicating marrow cavities (M). (c,d) Osteoporotic group showing decrease in the thickness of the outer cortical shell (CB) and markedly diminished thickness of bone trabeculae (BT) with increase in marrow space (M). Notice the apparent collapse of the lumbar vertebral body. Effacement of the bone trabeculae whose substance is replaced by bone marrow is observed (M). (e,f) Therapeutic group showing moderate restoration of trabecular architecture and improved thickness of bone trabeculae (BT). Notice apparent thicker outer compact bone shell (CB) compared with that of osteoporotic group. Marrow spaces (M) are seen. (SEM X35; 100).

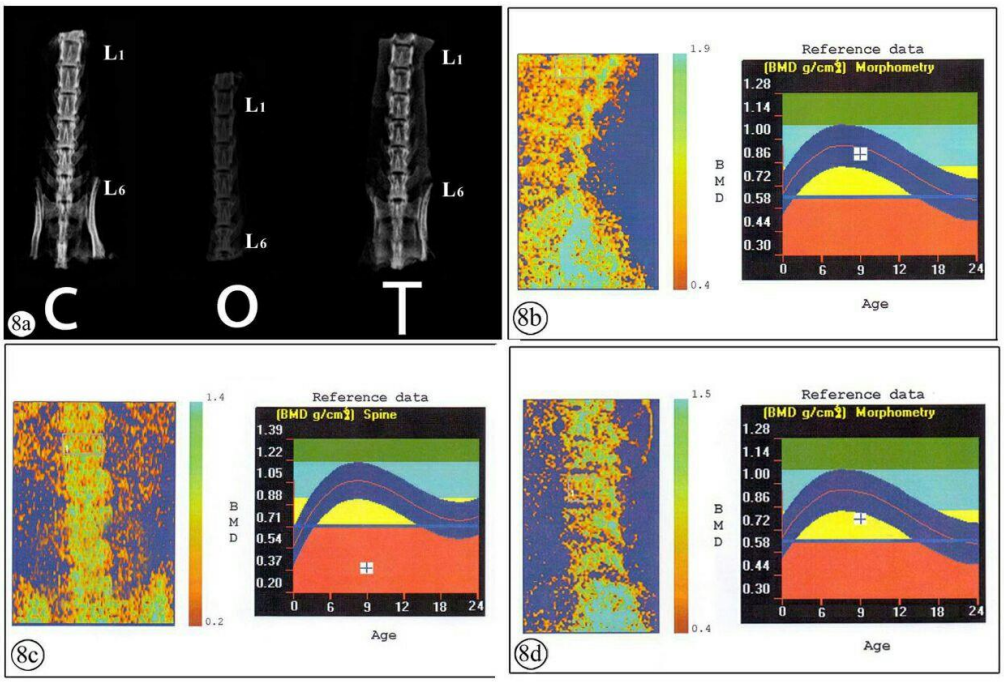


Fig. 8: Antroposterior X-ray radiograph and DEXA scan photographs of the lumbar vertebrae of 9 months old control, osteoporotic and therapeutic groups. (a) X-ray radiograph of the lumbar vertebrae of 9 months old control (C), osteoporotic (O) and therapeutic (T) groups showing marked rarefaction of bone of the osteoporotic group and moderate improvement of bone density in the therapeutic group. (b) DEXA scan photograph of a control rat and its representative curve with (□) mark lying high in the blue area indicating normal bone density. (c) DEXA scan photograph of an osteoporotic rat and its representative curve with (□) mark within the red area indicating marked decrease in bone density**.** (d) DEXA scan photograph of a therapeutic rat and its representative curve with (□) mark high in the yellow area indicating bone density slightly less than normal.

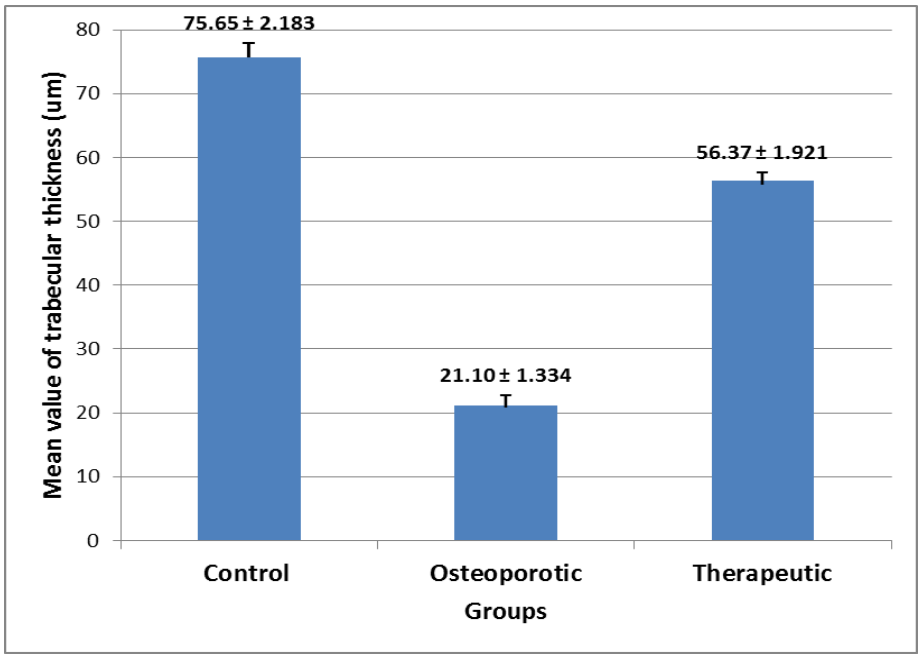
Morphometric analysis

The histological changes were confirmed by morphometric analysis where the trabecular thickness of the 5th lumbar vertebra in the three studied groups at the age of 9 months was shown in table 2 and histogram 2. It was observed that there is a significant decrease in the mean trabecular thickness in the osteoporotic group (21.10±1.33) in comparison with the control group (75.65±2.18). In addition, it was clear that there is a significant improvement of the trabecular thickness in the therapeutic group (56.37±1.19) in comparison with the osteoporotic group. Six months after induction of osteoporosis there is 72.1% loss of trabecular thickness. Therapeutic administration of colostrum for a period of three months (from the sixth to the ninth month) resulted in improvement of bone trabecular thickness from 21.1 to 56.37 µm minimizing bone loss to only 25.5% instead of 72.1% (Fig. 8e).

Table 2: Trbecular thickness of L5 of the 9 month old groups.

	Group Ib Sham operated control	Group Iic OVX + steroid treated (Osteoporotic)	Bone loss	Group IId OVX + steroid treated + therapeutic colostrum (Therapeutic)	Bone loss
Trabecular thickness (in µm)	75.65 ± 2.18 (n= 20) (R: 72-80)	21.10 ± 1.33*** (n= 20) (R: 18-23)	72.1%	56.37 ± 1.19** (n= 20) (R: 53-60)	25.5%

Changes in bone trabeculae are in the form of percentage loss in comparison with the control
Each value is represented as the mean ± standered deviation.
n : number of random measurements in the studied rats.
R : range of measurements.
*** $p < 0.001$: differences between the means is highly significant.
** $p < 0.01$: differences between the means is moderately significant.



Histogram 2: Trabecular thickness of the 5th lumbar vertebra of 9 months old control, osteoporotic and therapeutic groups.

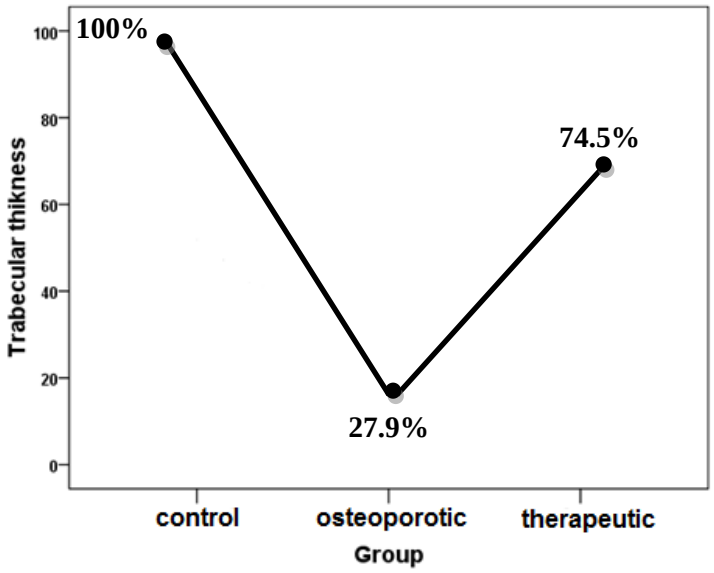


Fig. 8e: Percentage of trabecular thickness of the lumbar vertebra of control, osteoporotic and therapeutic groups. Notice marked improvement in the threapeutic group.

DISCUSSION

Osteoporosis is estimated to affect 200 million women worldwide. The disease and its consequences are regarded as a major source of mortality, morbidity and huge medical expenditure worldwide. The major health consequence of this condition is osteoporotis fracture. The vertebrae are the most common sites of osteoporotic fractures, with one third of women 65 years and older having sustained vertebral fractures, leading to reduction of height, kyphosis and chronic back pain[17].

The actual pathogenesis of osteoporosis is so far not completely understood. Estrogen deficiency is hypothesized to induce bone loss by decrease in bone vascularization[18]. Osteoporosis is likely to be caused by complex interactions of local and systemic regulators of bone cell function[19]. Experimental research can improve our understanding of the pathogenesis and of the activity of agents in the prevention or treatment of the disease. Traditional therapies for postmenopausal osteoporosis have emphasized the use of antiresorptive agents such as calcitonin and bisphophonates. Although these agents may prevent further bone loss in established osteoporosis, they cannot restore bone mass that has been lost already. Hormonal replacement therapy as estrogen is linked with risk for some diseases including cancer, coronary vascular disease, and thrombo-embolis[20].

The present study was conducted on female rats to simulate what is naturally occurring in postmenopausal human females. Three months old female rats were used in the present study because it is important to select skeletally mature animal models to appropriately interpret for postmenopausal women[21].

Rodents do not experience a natural menopause[22&23]. It is mandatory to induce an artificial menopause and this could be achieved by ovariectomy. In the present study ovariectomy was undertaken as a method for induction of osteoporosis as the ovariectomized rat exhibits most of the characteristics of human postmenopausal osteoporosis[24&25].

In the current study, ovariectomy was followed by administration of corticosteroids to ensure the occurrence of osteoporosis. This is in concordance with Yao *et al.*[26] and Lane and Yao[27] who stated that glucocorticoid treatment of rats would induce rapid bone loss and an elevated risk of fractures.

In the present study, bony changes in the osteoporotic cancellous bone was represented by the lumbar vertebrae[28]. Bony specimens were collected 3 months after induction of

osteoporosis as decrease in bone mass induced by ovariectomy occurs predominantly during 90 days post ovariectomy[29].

In the present study, the lumbar vertebra (as a representative of cancellous bone) of the early osteoporotic group (6 months old ovariectomised corticosteroid-treated rats) showed decreased thickness of their epiphyses, disturbed architecture and lack of differentiation between zones of ossification. These findings were in agreement with those of Castaneda *et al.*[30]. These degenerative changes denote disruption of the process of chondrogenesis and in turn inappropriate new bone formation. This might be a leading cause toward the establishment of osteoporosis.

Estrogen is known to increase the secretion of growth hormone (GH) and subsequent insulin like growth factor-1 (IGF-1) synthesis during early puberty. They added that estrogens initiate the pubertal growth spurt and stimulate skeletal growth. Sex steroid-related changes in GH and IGF-1 secretion affect bone size and bone cross-sectional area[31&32].

The present study showed appearance of plenty of irregular giant multinucleated osteoclasts in the epiphyses of the lumbar vertebrae of the osteoporotic group. Consistent with these findings are those noticed by Xia *et al.*[5] who supposed that estrogen deficiency led to increased production of tumor necrosis factor (TNF) by activated T cells which in turn, increased osteoclastic activity and eventually bone resorption.

Within the same group in this work, the middle part of the vertebral body revealed marked thinning out, fragmentation and discontinuity of bone trabeculae with relative increase in the bone marrow spaces. There was a significant decrease in the mean trabecular thickness in comparison with the control group. Trabecular bone loss was about 58.8% compared with the control group. Similar finding was observed by Kalleny[33] who found 52.2% loss of trabecular thickness 8 weeks post ovariectomy without the use of corticosteroids. Lane *et al.*[34] described osteoporosis as a syndrome of excessive skeletal fragility resulted from both the loss of trabecular bone mass and trabecular bone connectivity. They noted that 3 months postovariectomy resulted in approximately 50% loss of trabecular bone volume compared with sham-animals. Zhang *et al.*[35] noted that ovariectomy caused a significant reduction in trabecular bone volume of about 60% compared with sham-operated mice. Variation in the percentages of trabecular bone loss as a result of induction of osteoporosis was assumed to be due to different studied skeletal sites.

Cesnjaj *et al.*[36] investigated the osteoinductive effects of estrogen and postulated that osteogenesis was decreased in ovariectomized rats. The authors explained this finding by hypothesizing that estrogen deficiency altered the production of osteoinductive proteins such as osteogenin and bone morphogenetic protein, thus disturbing bone matrix formation.

Moriishi and Komori[37] claimed that the osteoblast is one of the bone cells principally affected by glucocorticoids. Many of the effects are directly mediated through the osteoblast's glucocorticoid receptors resulting in reduced proliferation and matrix synthesis by mature osteoblasts particularly, the mRNAs for type I collagen and the principal noncollagenous proteins of bone. Styne[38] assumed that it is probably the defective matrix and the slowness of refilling of resorption lacunae as the leading cause of osteoporosis induced by excess glucocorticoids.

In the present study, SEM examination of the early osteoporotic group of the lumbar vertebrae showed thinning of the outer compact bony shell, which appeared irregular from its inner surface with multiple tiny cavities. The core of the vertebral bodies showed thinning, breaking, irregularity of the bone trabeculae with apparent increase in the marrow spaces. These results were in agreement with those found by Chen *et al.*[39] who reported reduced bone volume, trabecular number and connectivity on SEM examination and microCT techniques.

In the current study, the radiologic examination of the lumbar vertebrae of the early osteoporotic group revealed marked rarefaction of the vertebral bodies. Moreover, DEXA scan showed marked decrease in bone density. The bone density fall in the red zone of the DEXA scan representative curve indicating conspicuous osteoporosis. These findings are in harmony with those found by Kamran *et al.*[40]

who X-rayed the lumbar vertebrae of OVX rabbits and observed radiolucent bones.

Villegas *et al.*[41] evaluated the BMD of the third lumbar vertebra in osteoporotic female patients using DEXA scan and X-ray technique. They reported marked decrease in BMD in the osteoporotic vertebrae. Ireland *et al.*[42] speculated that osteoblast surface possesses estrogen receptor alpha that regulates formation of type I collagen which is markedly decreased in case of ovariectomy leading to impairment of formation of osteoid tissue. This would lead to defective mineralization, which appeared as radiolucency in X-ray radiographs and decreased bone density in the DEXA scan.

The late osteoporotic group in the present study showed more pronounced manifestations of bony affection in the form of complete loss of orientation of zones of ossification. Most of the cells appeared as degenerating cells. There were plenty of osteoclast giant cells denoting massive bone resorption. Percentage loss of trabecular thickness progressed from 58.8% in 3 months post ovariectomy to 72.1% 6 months post ovariectomy. These changes coupled with the osteolytic cavitary lesions seen among the epiphyses, and in the vertebral bodies are considered the genuine leading cause of vertebral collapse and kyphosis noticed postmenopausally. In accordance with findings of the present study is the conclusion of David and Alexander[43] who reported that vertebral compression fractures affect at least one-fourth of all postmenopausal women and the most significant risk factor for occurrence of such fractures is osteoporosis.

The present study showed that the rate of bone loss in trabecular bone is 58.8% in the early osteoporotic group and 72.1% in the late osteoporotic group. It appeared that loss in cancellous bone was almost as twice as that of compact bone in the early and late stages of osteoporosis. These findings are in harmony with similar finding reported by Hara *et al.*[44], Kamran *et al.*[40] and Naim[45].

Nevertheless, the randomly arranged trabeculae of spongy bone and the less mineralized matrix make this type of bone more vulnerable to resorption. These observations can explain the higher frequencies of vertebral and femoral neck fractures in osteoporotic patients compared with femoral shaft fractures[1].

Colostrum was administered in this work either in concomitant with induction of osteoporosis to evaluate its protective effects or after a latent period of 3 months to evaluate its therapeutic effects. Few authors used colostrum as a row material to evaluate its beneficial effects. Many other researchers used only one or more of its active ingredients such as lactoferrin, growth factors, acidic or basic protein fractions in order to evaluate its benefits and the underlying mechanism of its actions. In the current study, we used row bovine colostrum orally administered daily for 3 months.

In the present study, the lumbar vertebrae of the prophylactic group showed preserved thickness with minimal disruption of zones of ossification of their epiphyses. Compared with the control and osteoporotic groups, osteoclasts were less frequently seen. The middle part of the vertebral body showed preservation of the bone trabecular thickness with normal proportion with the intervening marrow spaces. Three months after induction of osteoporosis there was 58.8% loss of trabecular thickness of the osteoporotic rats. Meanwhile the use of colostrum in parallel with induction of osteoporosis resulted in only 15.6% loss of trabecular thickness in the prophylactic group. These findings are most probably due to the preservative and bone maintaining properties of the bioactive ingredients of colostrum.

The present findings were similar to those observed by Guo *et al.*[46] who stated that orally administered bovine lactoferrin prevented deterioration of trabecular microarchitecture in 3 and 6 months old OVX rats receiving lactoferrin directly after ovariectomy. The authors indicated that lactoferrin protected rats from the OVX-induced reduction of bone volume/trabecular volume, trabecular thickness, and trabecular number and the elevation of trabecular separation. They found that orally administered lactoferrin in - different doses effectively prevented BMD loss in 3-6 months OVX rats lumbar vertebrae.

It seems that lactoferrin increases osteoblast differentiation and reduces osteoblast apoptosis. Lactoferrin stimulates proliferation of primary chondrocytes, which

may account for the preventive potential of lactoferrin against disruption of different zones of ossification observed in the present osteoporotic group. This explanation is in accordance with Amini and Nair[47] and Włodarski *et al.*[48].

Kruger *et al.*[49] observed that colostrum acidic protein fractions protected against bone loss due to OVX in the mature female rats. Their results of BMD of the spine indicated that the acidic protein fractions reduced bone loss due to OVX and maintained bone density above OVX levels. According to the biomechanical data analysis, they also reported that the acid proteins from colostrum had similar positive effects on bone stiffness, and hence resistance against breaking.

As regard the therapeutic group, the present study showed that the lumbar vertebrae have restored thickness with moderate reorientation of different zones of ossification in their epiphyses. Bone trabecular thickness showed marked improvement with less fragmentation compared with the osteoporotic group. Six months after induction of osteoporosis there was 72.1% loss of trabecular thickness. Therapeutic administration of colostrum for three months resulted in improvement of the mean bone trabecular thickness from 21.1 to 56.37 µm denoting improvement of trabecular thickness by 46.6%. The present findings were supported by those obtained by Hou *et al.*[50] who reported that on treating 6 months old OVX rat with different doses of bovine lactoferrin, the lumbar vertebrae showed marked improvement in trabecular microarchitecture including bone volume and trabecular number. They postulated that lactoferrin, a constituent of bovine colostrum, seemed to partially restore trabecular connectivity by increasing trabecular thickness while reducing trabecular separation.

Legrand *et al.*[51] described a variety of lactoferrin receptors on osteoblast surface. Lactoferrin is endocytosed into cytoplasmic membrane-bound vacuoles by osteoblast cells with subsequent activation of kinase pathway essential for mitogenesis leading to a potent osteoblast proliferation. This theory was supported by *in-vitro* study conducted by Takayama and Mizumachi[52] who noticed that cells of the human osteoblastic line plated on lactoferrin-embedded collagen membranes had increased alkaline phosphatase activity and osteocalcin production. The authors concluded that Lactoferrin potently induces proliferation of primary osteoblasts and osteoblastic-cell lines and increases osteoblast differentiation.

Moreover, Grey *et al.*[53] reported that lactoferrin protect osteoblastic cells from apoptosis induced by estrogen withdrawal. This could be an additional way to expand the population of osteoblasts.

Transforming growth factor-β1 (TGF-β1) is a multifunctional peptide that has a broad range of cellular activities, encompassing control of cellular proliferation of a variety of cells including osteoblasts, osteoclasts and chondrocytes. *In-vivo*, animal studies have demonstrated that TGF-β1, a component of bovine colostrum, is capable of stimulating new bone formation and enhancing fracture healing[54]. In accordance, Jiajia *et al.*[55] reported that TGF-ß1 increased both the rate of DNA synthesis and cell number.

Plain X-ray examination of the lumbar vertebrae of the therapeutic group of the present study revealed marked improvement of bone density of the vertebral bodies compared with the osteoporotic group. DEXA scan estimated the average BMD of the therapeutic group by 0.75 gm/cm³ compared with 0.37 gm/cm³ in the osteoporotic group denoting improvement of BMD by 42.2%. These findings were in harmony with those of Hou *et al.*[50] who reported marked improvement of BMD in the lumbar vertebrae of OVX rats receiving bovine lactoferrin for 6 months.

Insulin-like growth factor (IGF) is one of the bioactive ingredients of bovine colostrum. It is known that the growth hormone induces secretion of IGF. Growth hormone/insulin-like growth factor (GH/IGF) axis is critically important for longitudinal bone growth, skeletal maturation, and bone mass acquisition not only during growth but also in the maintenance of bone in adult life[56&57]. Lindsey and Mohan[58] claimed that estrogen deficiency plays a major role in disturbance of GH/IGF system with subsequent occurrence of osteoporosis. They postulated that lower serum IGF levels in women are correlated with the increased prevalence of osteoporotic fractures.

Disturbances in GH/IGF axis are leading causes of the pathogenesis of osteoporosis. The GH/IGF is regulated by a complex set of hormonal and local factors. Estrogen hormone deficiency and glucocorticoid excess seems to be major factors which disturb GH/IGF system. In the present study, OVX coupled with glucocorticoid administration have documented the establishment of osteoporosis with all its histomorphological features and BMD derangement. The administration of colostrum which is rich in IGF, appeared to restore the architecture and density of bone that was lost as a consequence of osteoporosis.

Conclusion

Colostrum has significantly prevented bone derangement when used as a prophylactic medicine and has reasonably restored bone microarchitecture when used as therapeutic agent for the treatment of osteoporosis.

Conflict of Interests

The authors declare that they have no competing interests.

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دور الكلوسترم في تعديل التغيرات العظمية نتيجة للهشاشة المستحدثة فى الفقرات القطنية في انثى الجرذان: دراسة هستومورفومترية وبالميكروسكوب الإلكترونى الماسح

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المقدمة: حتى الآن لا توجد طريقة علاجية أو وقائية فعالة لمرض هشاشة العظام واللذى يعتبر مأساه في مختلف أنحاء العالم خصوصاً في السيدات بعد انقطاع الطمث.

الهدف من البحث: أجريت هذه الدراسة لتقييم دور الكولوسترم كوسيلة وقائية وعلاجية لمرض هشاشة العظام.

المواد وطرق البحث: أجريت هذه الدراسة على عدد تسعون من إناث الجرذان البيضاء البالغة من العمر ثلاثة أشهر وتم تقسيمها عشوائيًا إلى مجموعتين:-

- المجموعة الأولى: شملت ثلاثين جرذًا واستخدمت كمجموعة ضابطة قسمت بدورها إلى مجموعتين فرعيتين ضمت كل منها عشرين جرذًا ذبحت عند بلوغها الشهر السادس والتاسع على التوالي.
- المجموعة الثانية: شملت ستين جرذًا استخدمت كمجموعة تجريبية قسمت بدورها إلى أربع مجموعات فرعية شملت كل منها خمسة عشر جرذًا.

– المجموعة الأولى : مجموعة الهشاشة في مراحلها المبكرة حيث تم استئصال المبيضين وأعطيت الكورتيزون لمدة ثلاثة أشهر.

– المجموعة الثانية : المجموعة الوقائية حيث أعطيت الجرذان جرعة يومية من الكولسترم لمدة ثلاثة أشهر بالتزامن مع استئصال المبيض والعلاج بالكورتيزون.

– المجموعة الثالثة : مجموعة الهشاشة في مراحلها المتأخرة حيث تم استئصال المبيضين وأعطيت الكورتيزون لمدة ستة أشهر.

– المجموعة الرابعة : المجموعة العلاجية حيث أعطيت الجرذان جرعة يومية من الكولسترم لمدة ثلاثة أشهر بعد استئصال المبيضين والعلاج بالكورتيزون ثم الانتظار لمدة ثلاثة أشهر.

لكل المجموعات تم استخراج الفقرات القطنية وتم تجهيز نصف العينات للتصوير بالأشعة السينية ومسح كثافة العظام. أما النصف الآخر من العينات فقد تم تجهيزه للفحص بالميكروسكوب الضوئي والالكتروني الماسح. كما تم إجراء بعض القياسات وتحليلها إحصائيًا.

النتائج: أوضحت هذه الدراسة أن استئصال المبيضين والمتبوع بتعاطي الكورتزون الذى سبب هشاشة العظام أدى إلى تغيرات ملحوظة في تركيب العظام فى صورة تدمير شديد للجهاز العظمونى. كما لوحظ أيضًا وجود نقص شديد في كثافة العظام. فيما يخص المجموعة الوقائية أظهرت الدراسة حفظًا واضحًا للتركيب الطبيعي للعظام كما ظلت كثافة العظام بحالة أقرب للطبيعي. كذلك بينت الدراسة أن المجموعة العلاجية قد أظهرت تحسنًا واضحًا في تركيب العظام. كذلك تحسنت كثافة العظام حيث وصلت إلى ما يقارب المستوى الطبيعي.

الخلاصة: أظهرت الدراسة أن تعاطي الكولوسترم كوسيلة وقائية أو علاجية هو وسيلة فعالة لمنع وعلاج التغيرات التى تنتج من هشاشة العظام.