Bisphosphonates Reverse the Rapid Deterioration of Bone Cells Following Corticosteroids

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Abstract: To evaluate the ability of Zoledronic acid to prevent and reverse rapid changes of mandibular alveolar bone cells following a whole month of daily injection with Methyl prednisolone. Thus, the purpose of this study was to evaluate these changes histologically, also, immunohistochemical changes of bone cells were screened. [Medhat A. Elzeiny, Reham M.Amin and Dina Mohamed. **Bisphosphonates Reverse the Rapid Deterioration of Bone Cells Following Corticosteroids.** *J Am Sci* 2012;8(12):182-190]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 27

1. Introduction

Osteoporosis is considered one of the most prevalent diseases. Although Glucocorticoids are used in treatment of inflammatory conditions, they have detrimental effects on bones. Researches have indicated numerous detrimental effects resulting from chronic use of these drugs . These include central obesity, hypertension, glucomas and cataracts, impaired wound healing, increased infection rates and impaired growth in children (**Frauman, 1996).** Glucocorticoids have also shown to impair glucose tolerance leading to, or worsening diabetes mellitus and also accelerate bone loss, leading to osteopenia and osteoporosis (**Ravina** *et al.,* **1999**). Furthermore, steroids have a direct negative effect on bone cells, they inhibit osteoblastogenesis and promote apoptosis of osteoblasts and osteocytes (**Weinstein** *et al.,* **1998).** **Li** *et al.***(2008)** stated that the precious mechanisms on how GC can reduce the quality of bone is still veiled, but one possible explanation may be induction of osteocytes' apoptosis.

Zol is considered a recent treatment for male & female osteoporosis because it is used for the prevention and treatment of osteitis deformans, bone metastasis, multiple myeloma, primary hyperparathyroidism and osteogenesis imperfecta (**Brouwers** *et al.,* **2008**). In pharmacology, bisphosphonates (also called diphosphonates) are a class of antiresorptive drugs that prevent the loss of bone mass, used to treat osteoporosis and similar diseases causing bone fragility (**Shapiro** *et al.,* **2009).** They inhibit the digestion of bone by osteoclasts (**Weinstein** *et al.,* **2009**), and are generally considered to act on bone resorption by binding to bone mineral and subsequently inhibiting the activity of the osteoclasts by encouraging osteoclasts to undergo apoptosis.

2. Materials and Methods:

Sixty adult male albino rats weighing about 200 gm each were divided into two main groups:
1- Control group:
Consisted of 20 rats injected with the drugs dissolvent:
2- Experimental group:
- Consisted of 40 rats divided into four groups: The first injected subcutaneously with 5 mg/kg & the second with 20 mg/kg MPSL for one month.
- Half the number of these groups were injected with a single dose of Zol in an attempt to treat the effects of MPSL.
- All groups were housed for additional 2 months.
- After the experimental period (3m) all rats were killed by cervical dislocation. The mandibular molar area was dissected free, kept in formalin then decalcified & prepared for paraffin embedding.
- Sections, 5 mm thick each, were examined by LM using:
1) H & E for histological study.
2) antibodies against active caspase-3 for Immunohistochemically study.

3. Results

A) Histological results

(I) Control group:

Examination of the hematoxylin and eosin (H&E) stained sections demonstrated:

A layer of osteoblasts could be seen lining the socket wall, most of which appeared cuboidal. They exhibited a basophilic cytoplasm and short processes. The prominent, round nucleus was found towards the basal end of the cell. A pale juxtanuclear area indicated the site of Golgi material (Fig. 1).

In other areas, some of the cells lining the alveolar bone surface appeared flattened.

Compact as well as spongy lamellae contained osteocytes present in lacunae inside the bone matrix. The lacunae were filled with a homogenous pale

stained material and are delimited by lamina limitans. Osteocytes were rounded to oval and exhibited a central nucleas and long processes which run in canaliculi in the bone matrix (Fig. 2).

Few osteoclasts were observed in Howship's lacunae in some areas of the socket wall. They are mono or multinucleated and exhibit an eosinophilic cytoplasm (Fig. 3).
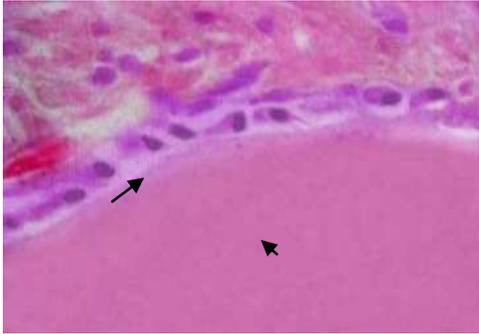

Fig. (1): A photomicrograph of the mandibular molar area from the control group showing osteoblasts lining the bone surface. Note the short processes (arrow) and the pale juxtanuclear areas (arrowheads). (H&E X1000)
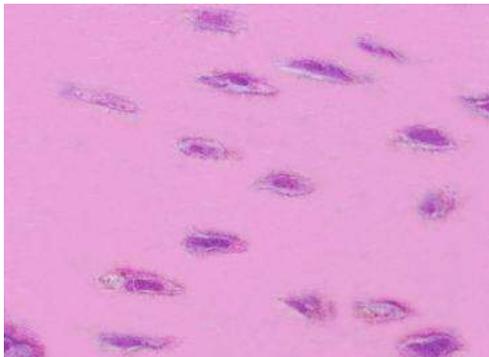
Fig. (2): A photomicrograph of the mandibular molar area from the control group showing osteocytes present in lacunae containing a pale homogenous material and delimited by lamina limitans. (H&E X1000)
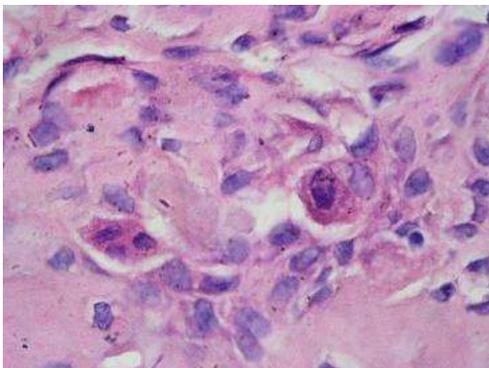

Fig. (3): A photomicrograph of the mandibular molar area from the control group showing mononucleated and multinucleated osteoclasts exhibiting an eosinophilic cytoplasm. (H&E X1000)

II) Experimental groups:
Examination of the hematoxylin and eosin (H&E) stained sections of the group IIA (lower dose +no ttt):

The socket wall is lined by a continous layer of flattened osteoblasts with darkened nuclie, some of which were detached from the surface of alveolar bone (Figs. 4,5).

Some osteocytic lacunae appeared dilated with hyperchromatic and shrunken nuclei compressed to one side. Some lacunae appeared empty (Figs. 6,7).

Apparently, numerous multinucleated, giant osteoclasts lining an irregular bony surface were also observed (Fig. 8).
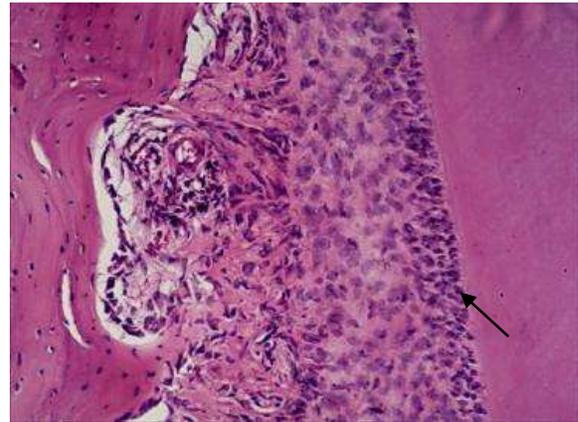

Fig. (4): A higher magnification of the previous fig. showing a continous layer of flattened osteoblasts, detached from the surface of alveolar bone (H&E X200).
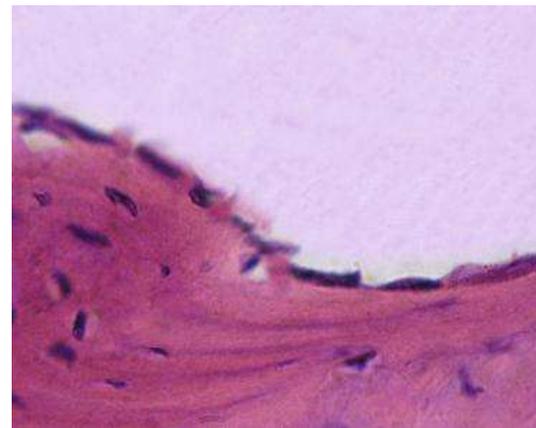

Fig. (5): A higher magnification of the fig. 23 showing a continuous layer of flattened osteoblasts (H&E X1000).
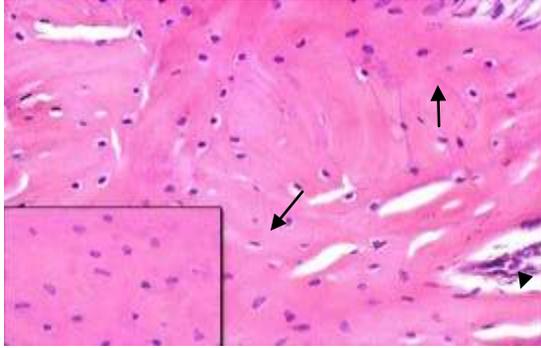
Fig. (6): A photomicrograph of group IIA showing some osteocytic nuclei with no obvious lacunar spaces (arrowheads), others show dilated or empty lacunae (arrows) (H&E X400).
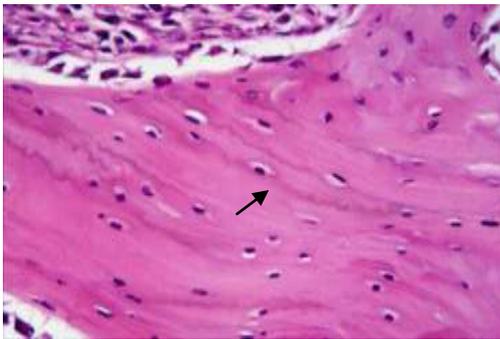

Fig. (7): A photomicrograph of group IIA showing hyperchromatic osteocytic nuclei compressed to one side, multiple reversal lines (H&E X400).
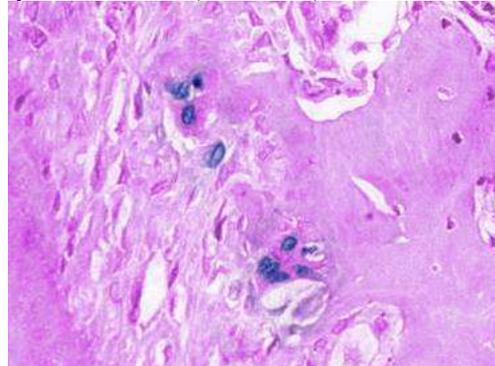

Fig(. 8): A photomicrograph of group IIA showing multinucleated giant osteoclasts (H&E x1000).

Examination of the hematoxylin and eosin (H&E) stained sections of the mandibular molar area of subgroup IIA (lower dose+ ttt):

A layer of osteoblasts could be seen lining the socket wall, some were apparently less flattened (compared to group IIA) depositing osteoid tissue (Fig. 9).

Osteocytes were present in lacunae inside the bone matrix; some show a histological picture that is almost similar to the control group (Fig. 30), while apparently few showed hyperchromatic nuclei compressed to one side. There was widening of the

lacunae around some of them. Only few lacunae appeared empty and some osteocytes were binucleated (Fig. 10,11).

Apparently, few osteoclasts in Howship's lacunae were detected in some areas of the alveolar bone. They appeared giant and multinucleated. These cells lacked the characteristic histological appearance of their highly eosinophilic cytoplasm which appeared vacuolated (Figs. 12,13).
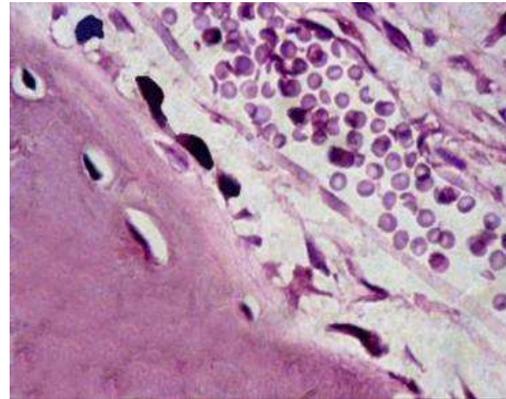

Fig. (9): A photomicrograph of subgroupIIA showing a layer of less flattened osteoblasts on the surface of osteoid tissue. (H&E X1000)
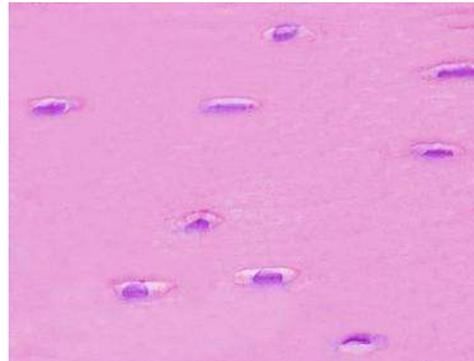

Fig. (10): A photomicrograph of subgroup IIA showing osteocytes in a histological picture almost similar to the control group. (H&E X1000)
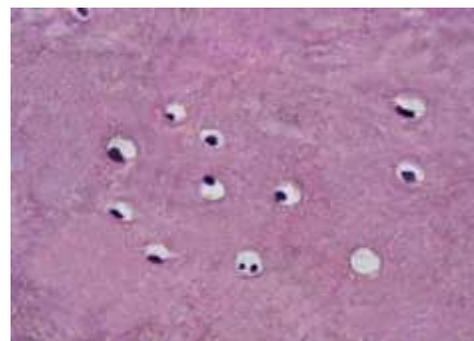

Fig. (11):A photomicrograph of subgroup IIA showing few osteocytes in bone matrix with widened lacunae and displaced hyperchromatic nuclie (H&E X1000)
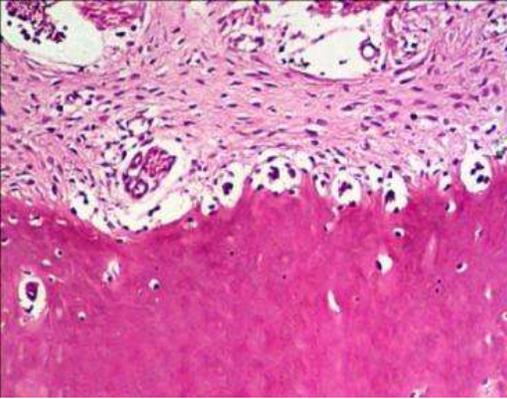
Fig. (12): A photomicrograph of the mandibular molar area of subgroup IIA showing few giant, multinucleated osteoclasts with a vesicular cytoplasm (arrows). Note the irregular bony surface (H&E X200).
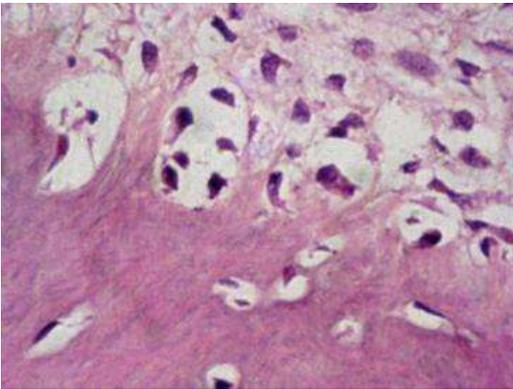

Fig. (13): A higher magnification of the previous figure showing multinucleated osteoclasts lacking their characteristic appearance (H&E X1000).

Examination of the hematoxylin and eosin (H&E) stained sections of the mandibular molar area of group IIB (higher dose + no ttt):

Thin and disorganized bone trabeculae lined by a discontinuous layer of flattened, spaced osteoblasts some of which were detached from the bone surface were also observed (Figs.14,15).

Osteocytes showed dilated or empty lacunae.Their nuclei appeared hyperchromatc. Karyolysis and karyorhexis were seen in some nuclei (Fig. 16).

Osteoclasts showed normal histological features with their intensely eosinophilic cytoplasm indicating their activity (Fig.17).
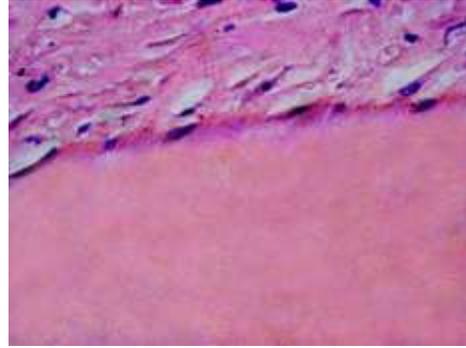

Fig.(14): A higher magnification of the previous figure showing flattened and spaced osteoblasts lining the surface of bone trabecula (H&E X1000).
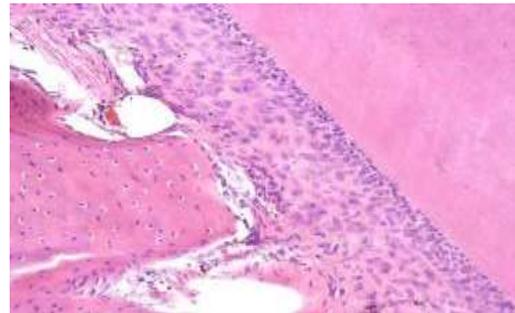

Fig. (15): A photomicrograph of the mandibular molar area of group IIB showing flattened osteoblasts detached from the surface of alveolar bone (H&E X200).
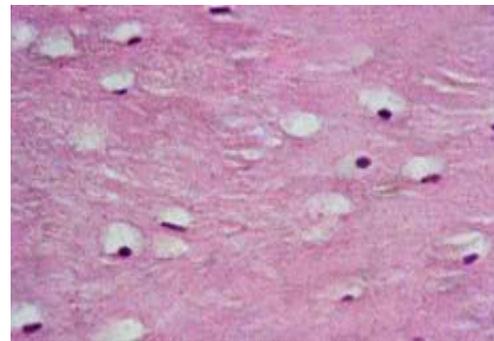

Fig. (16): A photomicrograph of group IIB showing numerous dilated and empty osteocytic lacunae, note karyolysis and karyorhexis of some nuclei. (H&Ex1000.
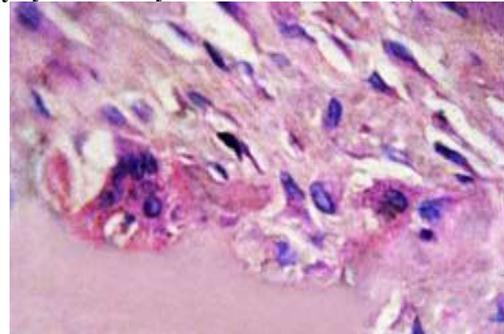

Fig.(17): A photomicrograph of group IIB showing mono and multinucleated giant osteoclasts with charectristic eosinophilic cytoplasm. (H&Ex1000)

Examination of the hematoxylin and eosin (H&E) stained sections of the mandibular molar area of subgroup IIB (higher dose +ttt) revealed the following results:

Apparently thin bone trabeculae lined by flattened and rounded osteoblasts were seen (Figs. 18).

Most osteocytes showed dilated or empty lacunae, while the nuclei of others filled the entire lacunar space (Figs. 19,20).

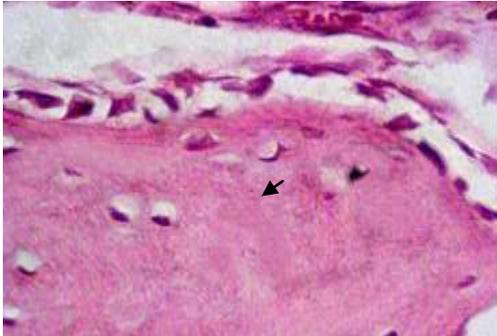Most osteoclasts showed similar histological features to those in subgroup IIA.

Fig. (18): A photomicrograph of subgroup IIB showing bone trabeculae lined by flattened (arrows) and rounded osteoblasts (arrowhead) (H&E X1000).
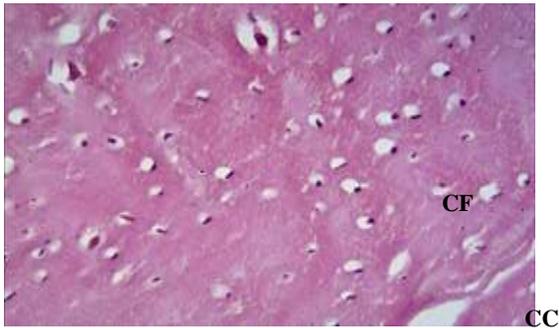

Fig. (19): A photomicrograph of subgroup IIB showing dilated or empty osteocytic lacunae, (H&E X400).
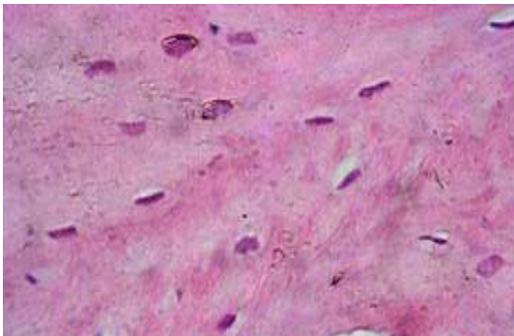

Fig. (20): A photomicrograph of osteocytes of subgroup IIB showing nuclei filling the entire lacunar space (H&E X400).

B) Immunohistochemical results

Using anti-active caspase3, as an apoptotic marker, positive staining reaction is seen as brown nuclear and cytoplasmic staining with different intensities.

Control group

Examination of anti-active caspase3 stained sections of the bone cells of the control group demonstrated:

Apparently, bone cells including osteoblasts, osteocytes as well as osteoclasts show no reaction to anti-active caspase3

Fig(s21,22,23)

Fig. (21): A photomicrograph of osteoblasts from the control group showing almost no reaction to anti-active caspase3 (Antiactive- caspase3 X400) counterstain: Hematoxylin.

Fig. (22): A photomicrograph of osteocytes from the control group showing almost no reaction to anti-active caspase3 (Antiactive- caspase3 X400) counterstain: Hematoxylin
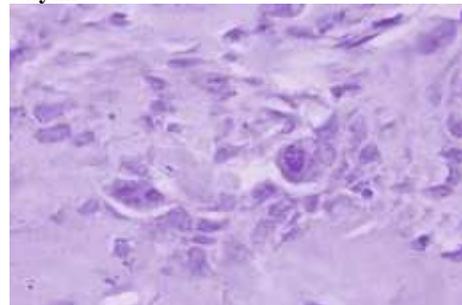

Fig.(23): A photomicrograph of osteoclasts from the control group showing almost no reaction to anti-active caspase3(Antiactive- caspase3 X400) counterstain: Hematoxylin

Experimental groups

Examination of anti-active caspase3 stained sections of the mandibular molar area of group IIA (lower dose +no ttt) revealed the following results:

Apparently in group IIA, most osteoblasts as well as osteocytes show positive reaction. While apparently all osteoclasts seem to show a negative reaction to antiactive- caspase3.

Fig. (24): A photomicrograph of group IIA showing positive reaction of most of the bone cells (Antiactive-caspase3 X400) counterstain: Hematoxylin

Examination of anti-active caspase3 stained sections of the mandibular molar area of subgroup IIA (lower dose+ ttt) revealed the following results:

Apparently, most osteoblasts and osteocytes showed negative reaction, while only relatively few cells showed signs of apoptosis demonstrared as positive staining to anti active- caspase3.

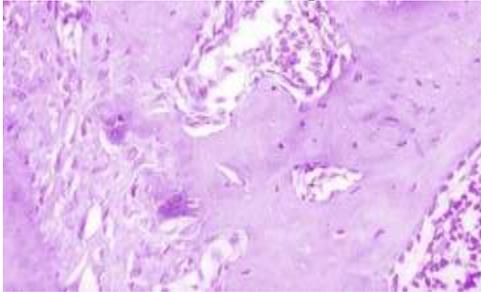Meanwhile, most osteoclasts seem to show positive rection to anti active- caspase3.

Fig.(25): A photomicrograph of osteoclasts of group IIA showing negative reaction (Antiactive- caspase3 X400) counterstain: Hematoxylin
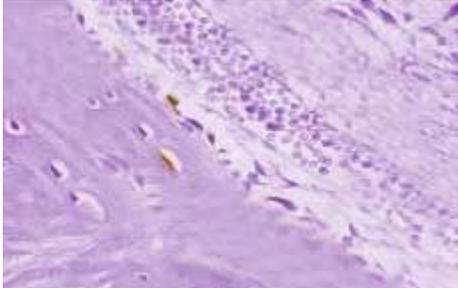

Fig. (26):A photomicrograph of osteoblasts & osteocytes of subgroup IIA showing almost negative reaction (Antiactive- caspase3 X400) counterstain: Hematoxylin

Fig.(27):A photomicrograph of osteocytes of subgroup IIA showing almost negative reaction (Antiactive-caspase3 X400) counterstain: Hematoxylin

Fig. (28): A photomicrograph of osteoclasts of subgroup IIA showing positive reaction (Antiactive- caspase3 X400) counterstain: Hematoxylin.

Examination of anti-active caspase3 stained sections of the mandibular molar area of group IIB (higher dose +no ttt) revealed the following results:

Apparently, all osteoblasts and osteocytes of group IIB show intense positive reaction to anti-active caspase3. Meanwhile all osteoclasts seem to express negative reaction to antiactive-caspase 3.

Fig. (29): A photomicrograph of osteoblasts of group IIB showing intense positive reaction to anti-active caspase3(Antiactive- caspase3 X400) counterstain: Hematoxylin.

Fig.(30): A photomicrograph of osteocytes of group IIB showing intense positive reaction to anti-active caspase3 (Antiactive- caspase3 X400) counterstain: Hematoxylin.

Fig.(31): A photomicrograph of osteoclasts of group IIB showing negative reaction (Antiactive- caspase3 X400) counterstain: Hematoxylin.
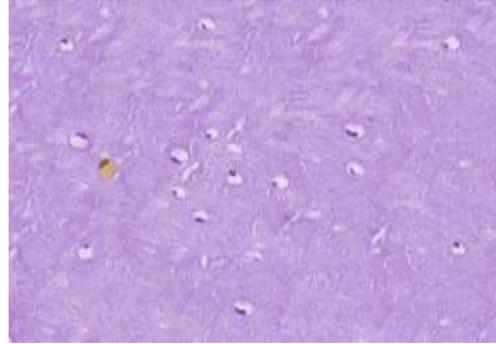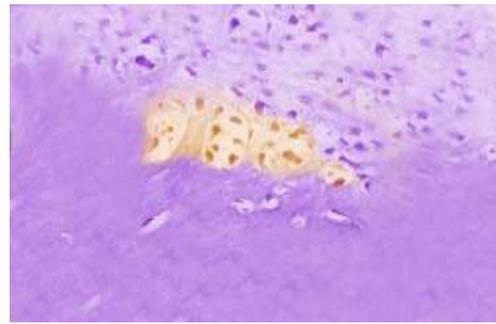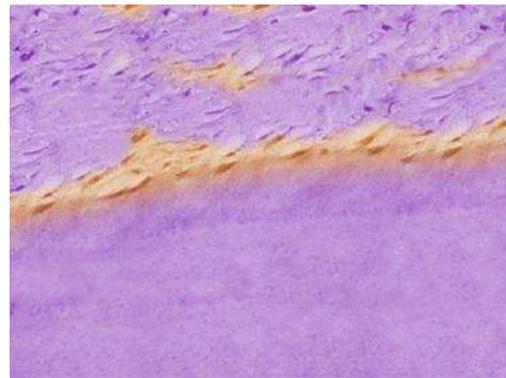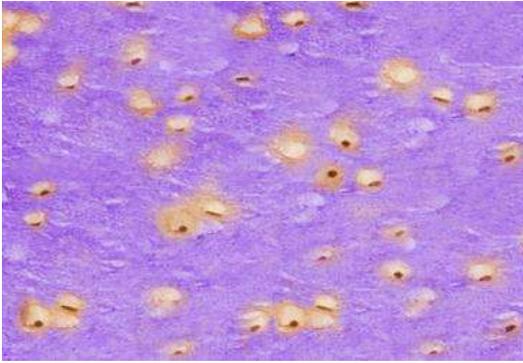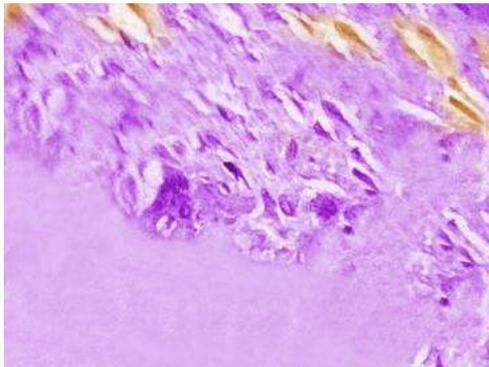
Examination of anti-active caspase3 stained sections of the mandibular molar area of subgroup IIB (higher dose +ttt) revealed the following results:

Apparently, few osteoblasts and osteocytes showed negative reaction while most still showed positive reaction to anti-active caspase3. (Fig. 24).

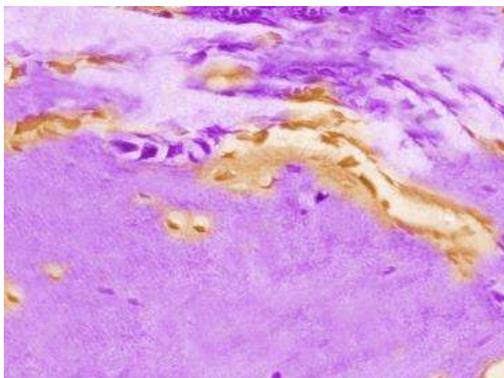Osteoclasts of this group showed a similar immunohistochemical reaction as those of group IIB.

Fig. (32): A photomicrograph of subgroup IIB showing few cells with negative reaction while most show positive reaction to anti-active caspase3 (Antiactive- caspase3 X400) counterstain: Hematoxylin.

4. Discussion

Years ago, many studies have demonstrated the detrimental effects resulting from prolonged use of exogenous GC including loss of skeletal bone mass, reduction in BMD and also avascular osteonecrosis, however, the influence of their use on the alveolar bone is not fully understood.

In this study, we highlighted the histological, histochemical and immunohistochemical alterations that could occur to alveolar bone in the mandibular molar area of albino rats in response to GC. Also, the possible modulation of these alterations by administration of bisphosphonates had been studied.

In the current study, most osteoblasts of GC treated groups were flattened and inactive and only few of them showed signs of activity. This finding is probably related to decreased rate of bone formation with administration of GC which were reported to depress bone formation by their inhibitory effect on the bone cells genesis according to *Albrecht et al. (2006)*. However, in GC +ZOL groups, most osteoblasts appeared rounded and showed signs of activity probably related to new bone formation in response to treatment with bisphosphonates. These findings are in accordance with, *Izu et al. (2011)* reported that osteoblasts become aligned along regions of bone deposition with an epithelioid arrangement. Also, *Maruotti et al. (2012)* reported that bisphosphonates stimulate the activity and the proliferation of osteoblasts.

In the present study, osteocytes of GC administered groups mostly showed shrunken, hyperchromatic nuclei. Others showed dilated or empty lacunae. This finding is probably due to their death by apoptosis in response to GC where it was reported that GC induce apoptosis of osteocytes *Nicholas et al. (2000).* Also, *Emerton et al. (2010)* reported that osteocyte apoptosis may play a central role in signaling bone microdamage. *Tomkinson et al. (1997)* explained that these empty lacunae result from packing of osteocytes as apoptotic bodies available for ingestion by neighbouring osteocytes via canaliculi. Alternatively, apoptotic cells that have not been phagocytosed during resorption, undergo a secondary necrosis with dissolution of their cellular material. It is also possible that osteocytes undergoing apoptosis fragment into apoptotic bodies and remain in their lacunae for extended periods of time. Similarly, loss of osteocyte viability was documented clinically in patients with glucocorticoid-induced osteonecrosis *(Calder et al., 2004)*.

Unlike results reported by **Hamdy (2010)** that ZOL is effective in treatment and prevention of postmenopausal osteoporosis, treatment of osteoporosis in men and treatment of glucocorticoid-induced osteoporosis, most osteocytes in the higher

dose GC+ZOL treated groups in this study also showed similarity to the previously mentioned histological features; could not overcome the deleterious effects of GC usage.

This was explained by **Curtis *et al.* (2005**) who reported that the modulatory effects of bisphosphonates on GC induced male osteoporosis are less pronounced than that on post menopausal osteoporosis.

Also**, Bonewald (2011)** reported that far from being the "passive placeholder in bone," osteocytes have been found to have numerous functions, such as acting as an orchestrator of bone remodeling through regulation of both osteoclast and osteoblast activity, a source of soluble factors on the bone surface and to target distant organs, playing a role in both phosphate metabolism and calcium availability, remodeling its perilacunar matrix and also functioning as an endocrine cell. As a result, it is suggested that, these criteria render osteocytes the most sensitive bone cells and might explain why these cells failed to recover in higher doses of GC even after ZOL therapy.

In our study, osteoclasts showed an apparent increase in number in the alveolar bone of GC treated groups denoting increase in the resorption process. These findings could be related to what was reported about the primary adverse effects of GC excess on the skeleton that act directly on bone cells: decreasing the production of both osteoblasts and osteocytes, increasing the prelevance of their apoptosis while high way up prolonging the life span of osteoclasts *(Weinstein , 2010).*

On the contrary, the number of osteoclasts in ZOL treated groups was apparently decreased, while the few remaining cells lacked their characteristic appearance which might suggest that these cells were undergoing apoptosis. These results are concomittent with those reported by *Bellido and Plotkin (2011)* who reported that bisphosphonates stop bone loss by inhibiting the activity of bone-resorbing osteoclasts and promoting their apoptosis.

Examination of sections from mandibular molar area of GC treated groups, stained with anti active caspase-3 showed an apparent increase in the number of apoptotic cells compared to control and GC+ZOL treated groups. In accordance, *Kim et al. (2006)* have confirmed that glucocorticoid excess prevents osteoclasts apoptosis while promoting osteoblasts and osteocytes apoptosis.

References

Albrecht W. Popp, Juerg Isenegger, Elizabeth M. Buergi, Ulrich Buergi, and Kurt Lippuner (2006): Glucocorticosteroid-induced spinal osteoporosis: scientific update on pathophysiology and treatment Eur Spine J. July; 15(7): 1035–1049.

Bellido T. and Plotkin L.I. (2011): Novel actions of bisphosphonates in bone: preservation of osteoblast and osteocyte viability. Bone. ;49(1):50-5.

Bonewald L.F.(2011): The amazing osteocyte. J Bone Miner Res.; 26(2): 229–238.

Brouwers J.E.M., Lambers F.M., Gasser J.A., van Rietbergen B. and Huiskes R. (2008): Bone Degeneration and Recovery after Early and Late Bisphosphonate Treatment of Ovariectomized Wistar Rats Assessed by *In Vivo* Micro-Computed Tomography Calcif Tissue Int., 82:202–211.

Calder J.D.F., Buttery L., Revell P.A., Pearse M. and Polak J.M. (2004): Apoptosis – a significant cause of bone cell death in osteonecrosis of the femoral head. J Bone Joint Surg.; 86B:1209–1213.

Curtis J.R., Westfall A.O., Allison J.J., Becker A., Casebeer L., Freeman A., Spettell C.M., Weissman N.W., Wilke S. and Saag K.G. (2005): Longitudinal patterns in the prevention of osteoporosis in glucocorticoid-treated patients. Arth Rheum.;52:2485–2494.

Emerton K.B., Hu B., Woo A.A., Sinofsky A., Hernandez C., Majeska R.J., Jepsen K.J. and Schaffler M.B. (2010): Osteocyte apoptosis and control of bone resorption following ovariectomy in mice Bone. March; 46(3): 577–583.

Frauman A.G. (1996): An overview of the adverse reactions to adrenal corticosteroids. Adverse Drug React Toxicol Rev., 15: 203-206.

Hamdy Ronald C. (2010): Zoledronic acid: clinical utility and patient considerations in osteoporosis and low bone mass Drug Des Devel Ther. , 4: 321–335.

Izu Yayoi, Mei Sun, Daniela Zwolanek, Guido Veit, Valerie Williams, Byeong Cha, Karl J. Jepsen, Manuel Koch, and David E. Birk (2011): Type XII collagen regulates osteoblast polarity and communication during bone formation J Cell Biol.; 193(6): 1115–1130.

Kim H.J., Zhao H., Kitaura H., Bhattacharyya S., Brewer J.A., Muglia L.J., Ross F.P. and Teitelbaum S.L. (2006): Glucocorticoids suppress bone formation via the osteoclast. *J Clin Invest.*;116:2152–2160.

Li M., Satio T., Amizuka N. (2008): Assessment of bone quality.Bone quality of steroid-induced osteoporosis. Clin. Calcuim; 18(3):328-35.

Maruotti N., Corrado A., Neve A., Cantatore F.P. (2012): Bisphosphonates: effects on osteoblast Eur J Clin Pharmacol., 27(2):230–238.

Nicholas R.W., Weinstein R.S. and Manolagas S.C. (2000): Apoptosis of osteocytes in glucocorticoid-induced osteonecrosis of the hip .J Clin Endocrinol Metab.; 85(8): 2907-12.

Shapiro J.R., Sponsellor P.D (2009): "Osteogenesisimperfecta: questions and answers". Curr. Opin. Pediatr. 21 (6): 709–16.

Tomkinson A., J. Reeve, R. W. Shaw and B. S. Noble (1997): The Death of Osteocytes via Apoptosis accompanies Estrogen Withdrawal in Human Bone. The Journal of Clinical Endocrinology and Metabolism; 82 (9): 3128.

Weinstein R.S., Robertson P.K. and Manolagas S.C. (2009): Giant osteoclast formation and long-term oral bisphosphonate therapy, N. Engl. J.Med.;360:53-62.

Weinstein Robert S. (2010): Glucocorticoids, Osteocytes, and Skeletal Fragility: The Role of Bone Vascularity Glucocorticoids, Osteocytes, and Skeletal Fragility: Bone march 46(3):564-570.

Weinstein Robert S., Robert L.J., Michael A.P. and Stavros C.M. (1998): Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential Mechanisms of Their Deletrious Effects on Bone. Journal of Clinical Investigation. Vol.102:274-282.

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