EFFECTS OF RISEDRONATE SODIUM ON THE TIBIAE OF THE ALBINO RATS ON BETAMETHASONE THERAPY. A LIGHT AND ELECTRON MICROSCOPE STUDY.

By

Gamal A. Mohamed And Adel A. El-hawary

From

Anatomy Department, Faculty Of Medicine,

Mansoura Universty.

ABSTRACT

Glucocorticoids (GCs) are widely utilized in medical practice to combat diseases having an inflammatory element. The aim of the present study is to throw light on the effects of risedronate sodium (Ris)on the architecture of the tibiae of the albino rats on betamethasone (Bet) therapy. Thirty adult male albino rats (200-250 gm) were used in this study. They were randomized into three equal groups:control, Bet-treated and both Bet- and Ris-treated. At sacrifice, small pieces of the decalcified tibiae were processed to prepare paraffin sections stained with haematoxylin and eosin (H&E) and Masson's trichrome. Other ultra-thin sections stained with uranyl acetate and lead citrate for EM study were, also, prepared. The tibiae of the Bet-treated rats showed an apparently marked decrease in the amount of collagenous fibres, separation and thinning of the pesiosteum, notches at the bone surfaces, resorption cavities filled with granulation tissue, irregular sizes and distribution of Haversian canals as well as non-homogenous dark and pale areas of the matrix. Ultra-structurally, the osteoblasts got degenerated. They lost their processes and became smooth. Their cytoplasm lacked its organelles and gained many vacuoles. Similar altera-

tions affected the osteocytes. Following Ris-teatment, the bones of animals on Bet-therapy showed that the above-mentioned affections were lessened. The osteoclasts exhibited marked degeneration. Nearly the histological and ultra-structural features of the control bones were resumed. These data lend support to a protective role of Ris. Consequently, Ris should always be advised for patients during GCs exposure in a trial to alleviate their osseous adverse effects.

INTRODUCTION

GCs are widely used in medicine. They play a pivotal role in the management of numerous diseases that have an inflammatory element such as rheumatoid disorders, collagen diseases, atopic dermatitis, bronchial asthma and sarcoidosis. Their effectiveness as an anti-inflammatory agents is offset by the occurrence of drawbacks (McLaughlin et al., 2002; Lange et al., 2004; Lee et al,2005; Irwin and Richardson, 2006; Summey and Yosipovitch, 2006). The most serious osseous complication of GCs is osteoporosis(OP). This is a public health issue threatening a large proportion of the population over fifty years of age (Watts, 2004). Aside from the post-menopausal OP, the most common secondary cause of OP is the long term use of GCs. It develops in approximately half of patients who take long term GCs and fracture is its first clinical sign (Ramsey-Goldman, 2002; Ward, 2005; Shah and Gecys, 2006; Kanis et al., 2007). In spite of the detrimental role of GCs on bone tissue metabolism, numerous questions remain pertaining the mechanisms of GCs-induced OP (Saag, 2004; Lu et al.,2005; Sedlak et al., 2006). Although GCs-induced OP is common, it is too often unrecognized and its management remains sub-optimal. Many at-risk population do not receive any therapy for this clinical condition (Blalock et al., 2005; Grazio et al., 2005; Orcel,2006; Solomon et al., 2007).

This silent systemic skeletal disorder, OP, has been the target of several researches in the late era in a trial to explain the various mechanisms implicated in its evolution. There are relatively few studies evaluating the process of bone repair in OP. Also, the full impact of OP on the consolidation of bone fracture is still not viv-

idly understood .Thus, performing further investigation for alternative remedies for OP is a must (Larsen et al., 2003; Abadie et al., 2005; Maricic,2005 ; Werkman et al., 2006). Recently, a number of therapies have been introduced for the management of OP. Among these suggested remedies, come the members of biphosphonate group which have a profound effect on calcium metabolism. They have been used for the management of hyper-calcaemia of malignant bone tumours. Also, these drugs are tried in the treatment of many bone diseases associated with bone resorption as Paget's disease, myeloma and bone metastases (Bobba et al., 2006; Giljevic and Volk, 2006; Suzuki,2006; Russell, 2007). Despite the applied clinical efficacy of these medications, the exact mechanism of their action has received a limited attention (Curtis et al., 2006; Takahashi et al., 2006; Reginster et al., 2007; Sato et al., 2007). Consequently, the present study was carried out with an intent to draw a vivid picture for the histological and ultra-structural effects of the latest generation of these biphosphonates, Ris, on the tibiae of the adult male albino rats on treatment with the GC, Bet, utilizing both light and transmission electron microscopes.

MATERIALS AND METHODS

Thirty adult male albino rats (200-250 gm) were used in the present study. They were equally allocated into three groups. Group I rats served as control and were given normal saline 1ml/day orally by a modified plastic syringe. The animals of group II received betamethasone,bet,(a product of Gulf-Pharmaceutical Industries, Ras Al-Khaima, U.A.E.in the form of 0.5 mg tablets under the trade name , betasone) in a dose of 0.45 mg/kg body weight/day, dissolved in 1ml distilled water and given by the same route as in the control group (Takahashi et al., 2006). The rats of group III were given both betamethasone(in an oral dose similar to that given to group II animals) and risedronate sodium, Ris, (a product of Aventis-Pharm, Main, Germany in the form of 5 and 35 mg tablets under the trade name, Actonel) in subcutaneous daily dose of 2.5 micro-gm/kg (Iwamoto et al., 2007). The doses were given for three months. All animals were housed under the same conditions and were allowed food and water ad-

libitum. After the lapse of 24 hours from the last dose, the rats of all groups were anaesthetized by intramuscular ketamine hydrochloride anaesthesia (45mg/kg body weight), perfused through a cardiac puncture by glutaraldehyde 2%, the shaft of each tibia was removed, immersed in glutaraldehyde and after 4 hours, they were put in a buffer for 24 hours. The bones were decalcified by daily exchange of ethylene-diamine tetra-acetic acid (EDTA) for 9 days (Bancroft et al.,1996). A part from the mid-shaft of tibia of each rat was immersed in 10% formalin, dehydrated , cleared and embedded in paraffin . Paraffin sections (6 μm) were prepared and stained with haematoxylin and eosin (H&E) to study the general histological architecture. Other paraffin sections were stained by Masson's trichrome (M.T.) stain for detection of the collagenous fibres (Drury and Wallington,1980; Bancroft and Stevens, 1996).

For electron microscopy, fine fragments of the decalcified mid-shaft of tibiae were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. They were ,then, transferred to 1% osmium tetroxide in the same buffer, dehydrated in ascending grades of alcohol and propylene oxide and embedded in epon. Ultra-thin sections (40-50 nm) were cut using a glass knife,stained with 4% uranyl acetate and 2% lead citrate (Hayat, 1989) and examined by JEOL 100S electron microscope.

RESULTS

Histological changes :

A transverse section in the mid-shaft of the decalcified tibia of the control adult male albino rat (group I)showed that the bone was surrounded from outside by the periosteum. The bone matrix contained many osteons (Haversian systems) composed of central Haversian canals housing blood vessels and connective tissue and were surrounded by concentric lamellae with osteocytes within their lacunae in between. Sub-periosteal cement lines intervened between the newly formed bone lamellae and the old ones (Fig.1). The mid-shaft of the decalcified tibia of the Bet- treated animals (group II) exhibited a separated -occasionally thin- periosteum. Both the outer and inner surfaces of bones exhibited numer-

ous notches. The bone lodged resorption cavities filled with granulation tissue. There were multiple Haversian canals of variable sizes and an irregular distribution. There were, also, non-homogenous dark and pale areas of the matrix with osteoporotic cavities (Figs.2,3). Following therapy with both Bet and Ris in group III rats, the mid-shaft of the decalcified tibiae showed signs of improvement and nearly the control histological configuration was more or less regained. There were, still, non homogenous dark and pale areas of the matrix with small cavities (Figs.4,5). The tibia of group I rats exhibited excessive collagenous fibres in the periosteum, endosteum and in the matrix (Fig.6). There was a remarkable decrease in the amount of collagenous fibres in the thin periosteum and in the matrix of the bones of group II animals (Fig.7). The bones of group III rats presented an apparent increase in the amount of collagenous fibres but it was not identical to the control levels (Fig.8).

Ultrastructural changes

Examination of ultra-thin sections of the decalcified tibiae of group I animals showed the bone cells and matrix. The osteoblast possessed a nucleus with an obvious nucleolus. The collagen fibrils in close vicinity to the cells were pale (unmineralized matrix or prebone). They were ,in turn, surrounded by dark collagen fibrils (mineralized matrix or bone) (Fig. 9). The osteocyte was imprisoned within a lacuna and possessed an oval nucleus and many cytoplasmic processes inside canaliculi. This cell was surrounded by pale (unmineralized) matrix and then a dark (mineralized) one (Fig.10). The osteoclast, in turn, had irregular boundaries and many nuclei. Its cytoplasm lodged many membrane bound vesicles of various sizes and many secondary lysosomes. The encircling demineralized bone looked, more or less, electron pale (Fig.11). Ultra-thin sections of the tibiae of group II rats showed several alterations. The osteoblasts got degenerated . They lost their processes and appeared smooth. Their cytoplasm lacked the organelles and gained many vacuoles. The surrounding matrix looked pale and lodged apparently few collagen fibrils (Fig.12). The osteocytes were ,as well, degenerated with small nuclei and many vacuoles

in their cytoplasm (Fig.13) . The matrix was partly dark with excessive collagen fibrils and partly pale with apparently fewer fibrils (Fig.14). The bones of group III animals showed some sort of improvement. The osteoblasts possessed large nuclei and were surrounded by a pale matrix (prebone) then by a dark one (bone) (Fig.15). The osteoclasts underwent a marked degeneration. They lost their ruffled border and became smooth. Many nuclei exhibited some degeneration and cytoplasm gained many relatively large vacuoles. Such cells were surrounded by non-demineralized electron dense collagen fibrils. The matrix exhibited excessive collagen fibrils (Figs.16,17).

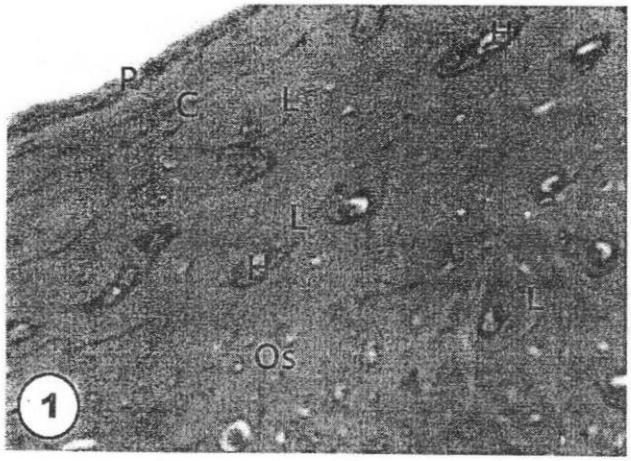

Fig. (1) : A photomicrograph of a transverse section in the mid-shaft of the decalcified tibia of a control adult male albino rat (group I) showing the periosteum (P). The subperiosteal lines (C) intervene between the newly formed bone lamellae and the old ones. The matrix contains many Haversian systems (osteons), each is composed of a central Haversian canal (H) housing blood vessels surrounded by connective tissue lamellae (L) and osteocytes (Os) (H&E x 200).

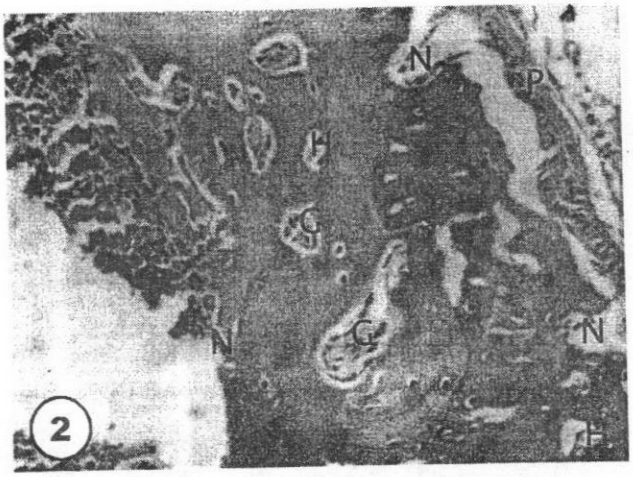

Fig. (2) : A photomicrograph of a transverse section in the mid-shaft of the decalcified tibia of an adult male albino rat treated with Bet (group II) showing separation of the periosteum (P). The outer and inner surfaces of bones exhibit several notches(N).Resorption cavities filled with granulation tissue (G) are encountered. Numerous Haversian canals(H) show apparently variable sizes and an irregular distribution (H&E x200).

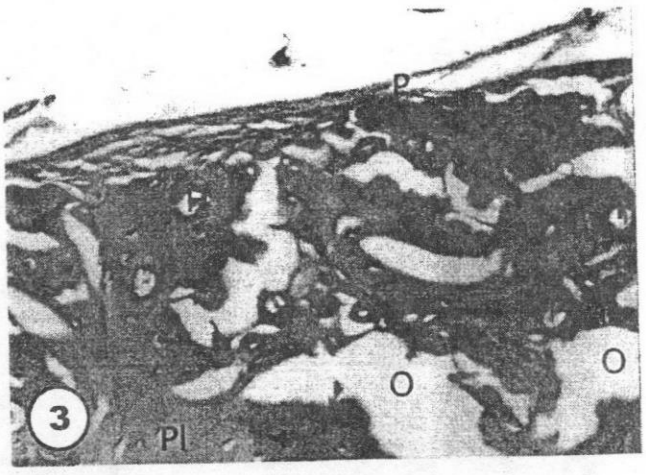

Fig. (3) : A photomicrograph of a transverse section in the mid-shaft of the decalcified tibia of an adult male albino rat of group II showing a thin separated periosteum (P), many dilated Haversian canals(H),several osteoporotic cavities (O) and non homogenous dark (D) and pale (Pl) areas of the matrix (H&E x 200).

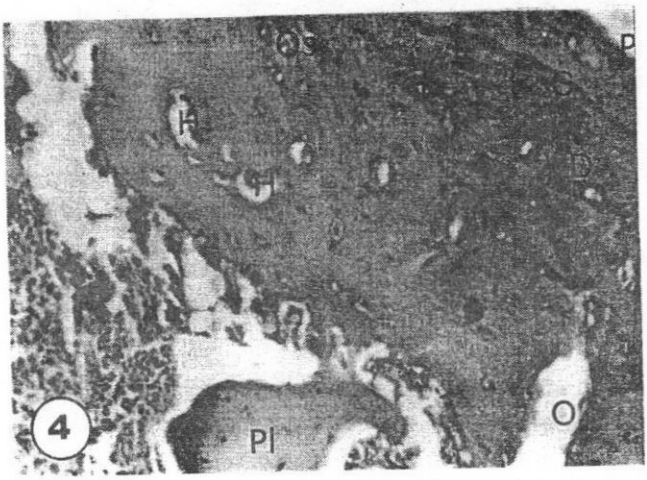

Fig. (4) : A photomicrograph of a transverse section in mid-shaft of the decalcified tibia of an adult male albino rat treated with both Bet and Ris (group III) showing the periosteum (P),the subperiosteal cement lines (C) ,the osteocytes (Os) and Haversian systems (H) resembling nearly that of the control. There are non-homogenous dark (D) and pale(Pl) areas of the matrix and a small osteoporotic cavity (O) (H&E x 200).

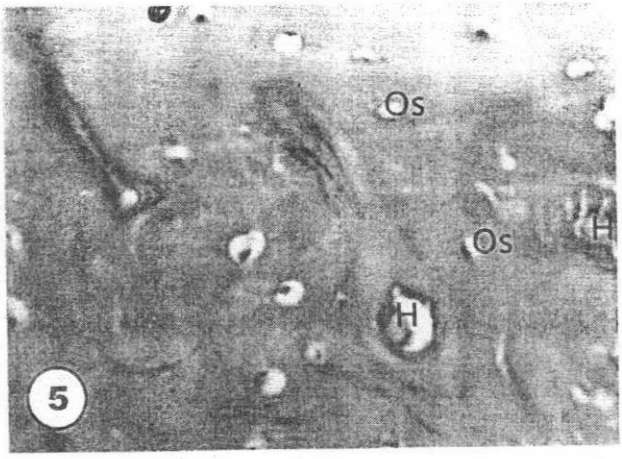

Fig. (5) : A photomicrograph of a transverse section in the mid-shaft of the decalcified tibia of an adult male albino rat of group III showing the homogenous matrix and the apparently normal size and distribution of Haversian canals (H) and osteocytes (Os) (H&E x 200).

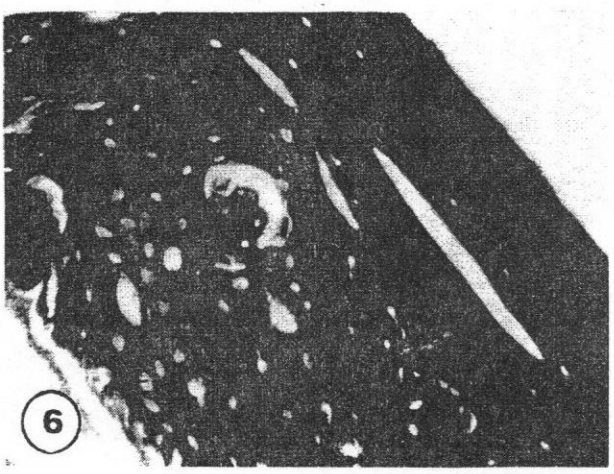

Fig. (6) : A photomicrograph of a transverse section in mid-shaft of the decalcified tibia of a control adult male albino rat of group I showing an excessive amount of collagenous fibres in the periosteum (P), endosteum (E) and in the matrix(M) (Masson's trichrome stain x 200).

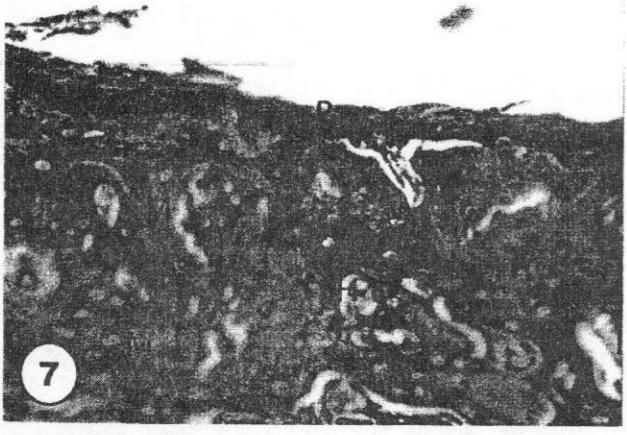

Fig. (7) : A photomicrograph of a transverse section in the mid-shaft of the decalcified tibia of an adult male albino rat of group II showing a remarkable decrease in the amount of collagenous fibres in the thin periosteum(P) and in the matrix(M). Notice the Haversian canals(H) (Masson's trichrome stain x 200).

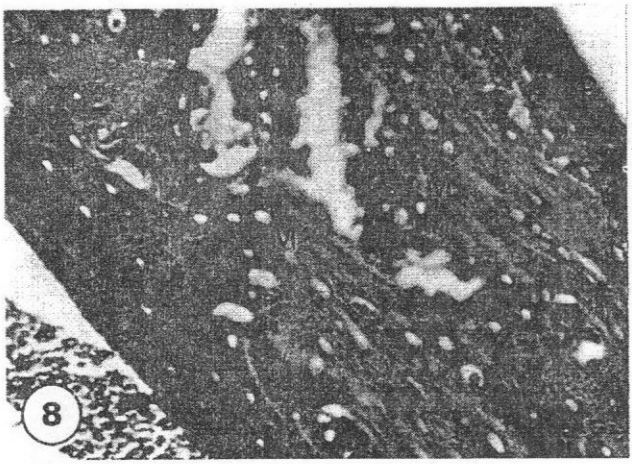

Fig. (8) : A photomicrograph of a transverse section in mid-shaft of the decalcified tibia of an adult male albino rat of group III showing an increase in the amount of collagenous fibres in the periosteum (P), endosteum (E) and in the matrix (M) (Masson's trichrome stain x 200).

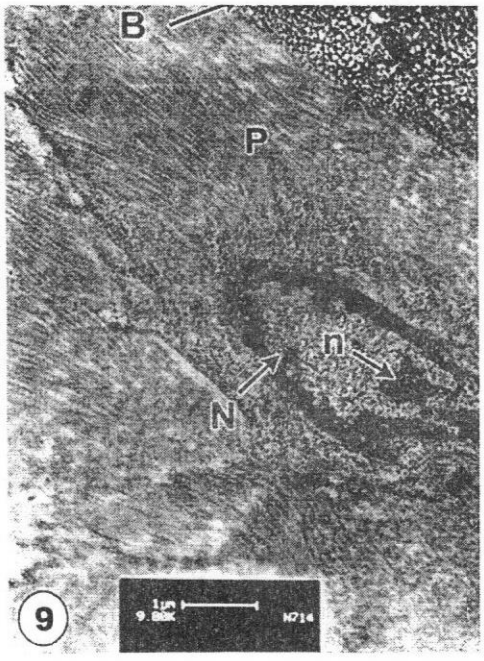

Fig. (9) : An electron micrograph of an ultra-thin section in the mid-shaft tibia of a control adult male albino rat of group I showing an osteoblast .It has a nucleus(N) with an obvious nucleolus(n). In vicinity of this cell, pale collagen fibrils (unmineralized matrix a pre-bone) (P) are visible surrounded, in turn,by dark collagen fibrils (mineralized matrix a bone) (B) (Uranyl acetate / lead citrate x 2000).

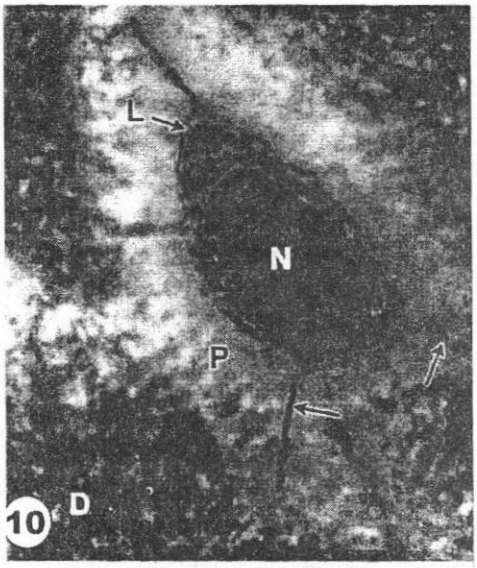

Fig. (10) : An electron micrograph of an ultra-thin section in the mid-shaft of the decalcified tibia of a control adult male albino rat of group I showing an osteocyte imprisoned within a lacuna (L).It has an oval nucleus (N) and cytoplasmic processes (arrows) extending into canaliculi. The cell is surrounded by a pale unmineralized matrix (P) then a dark mineralized one (D) (Uranyl acetate/ lead citrate x 2000).

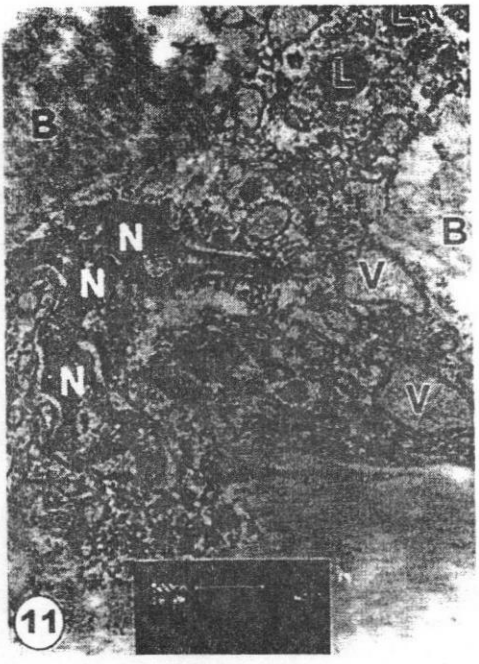

Fig. (11) : An electron micrograph of an ultra-thin section in the mid-shaft of the decalcified tibia of a control adult male albino rat of group I showing an osteoclast. It has irrgular boundaries and many nuclei(N).Its cytoplasm contains many membrane bound vesicles (V) of different sizes that represent the ruffled border of the cell cut in numerous planes and several lysosomes (L).The deminezalized bones(B) around the cell appears more or less electron pale(Uranyl acetate/lead citrate x 10000).

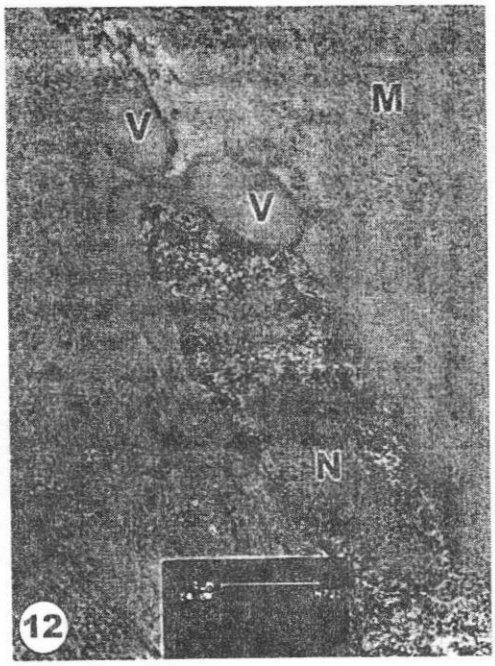

Fig. (12) : An electron micrograph of ultra-thin section in the mid-shaft of the decalcified tibia of an adult male albino rat of group II showing a degenerated osteoblast. It loses its processes and its outline becomes smooth. Its cytoplasm lacks the organelles and lodges many vacuoles (V)and the nucleus(N)looks degenerated. The matrix (M) appears pale lodging apparently few and thin collagen fibrils (Uranly acetate / lead citrate x 3000)

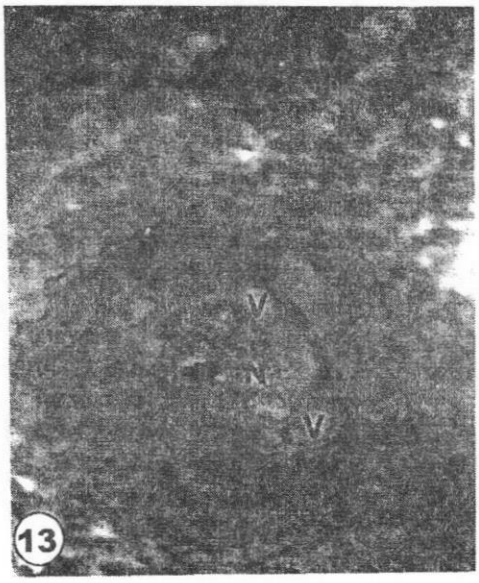

Fig. (13) : An electron micrograph of ultra-thin section in the mid-shaft of the decalcified tibia of an adult male albino rat of group II showing an osteocyte. It has a relatively small nucleus (N) and the cytoplasm houses several vacuoles(V) (Uranyl acetate / lead citrate x 3000).

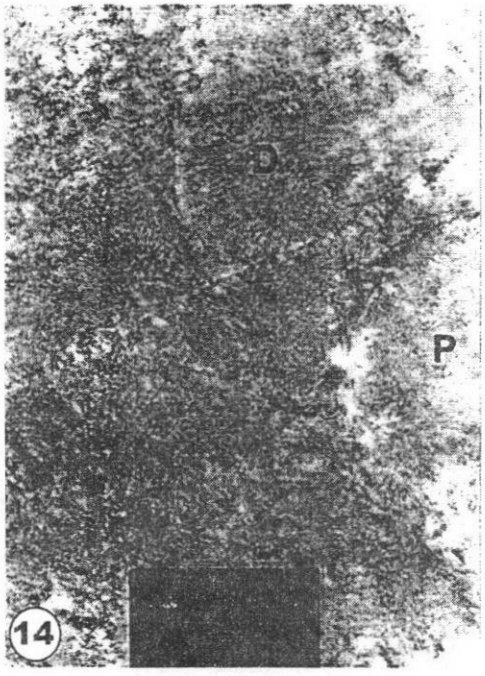

Fig. (14) : An electron micrograph of an ultra-thin section in the mid-shaft of the decalcified tibia of an adult male albino rat of group II showing a part of the matrix possessing dark areas (D) with excess, regular collagen fibrils and pale areas (P) with apparently fewer fibrils (Uranyl acetate / lead citrate x 3000).

Fig. (15) : An electron micrograph of an ultra-thin section in the mid-shaft of the decalcified tibia of an adult male albino rat of group III showing an osteoblast. It has a large nucleus (N) and is surrounded by a pale matrix or prebone(P) and then by dark one or bone (B) (Uranyl acetate / lead citrate x 2000).

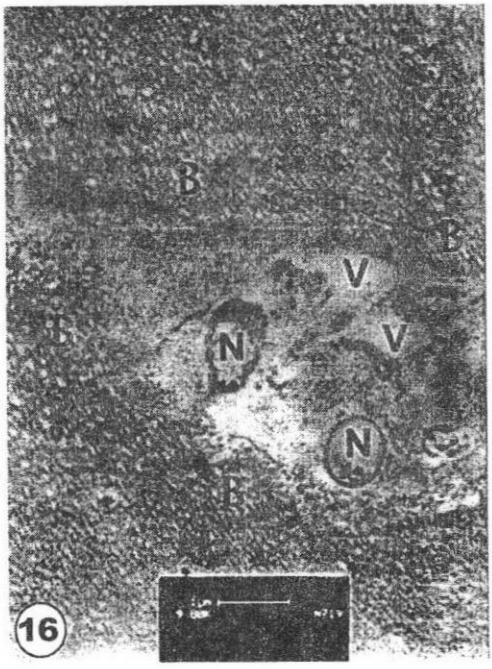

Fig. (16) : An electron micrograph of an ultra-thin section in the mid-shaft of the decalcified tibia of an adult male albino rat of group III showing a degenerated osteoclast. The cell loses its ruffled border which get smooth. The nuclei (N) show some degeneration and the cytoplasm lodges several vacuoles (V).The cell in surrounded by non-demineralized electron dense collagen fibrils (bone) (B) (Uranyl acetate / lead citrate x 5000).

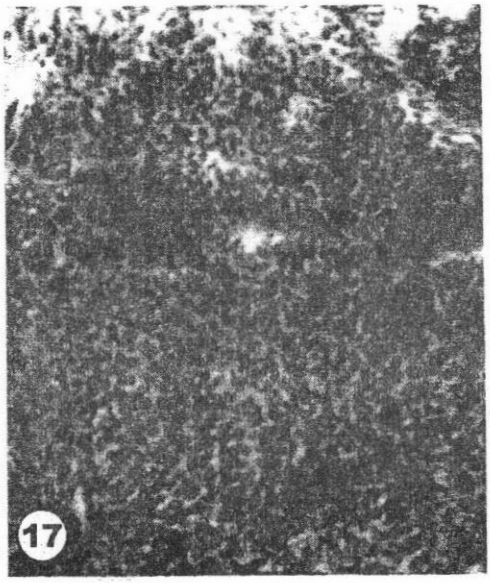

Fig. (17) : An electron micrograph of an ultra-thin section in the mid-shaft of the decalcified tibia of an adult male albino rat of group III showing a part of the matrix containing excessive collagen fibrils (Uranyl acetate lead citrate x 6000).

DISCUSSION

GCs are widely used in medicine. They play a pivotal role in the treatment of diseases that have an inflammatory component. Their effectiveness, in this regard, is limited by the occurrence of side effects (Lee et al., 2005; Irwin and Richardson, 2006; Summey and Yosipovitch, 2006). The present study was accomplished to throw light on the histological and ultra-structural effects of Ris on the tibiae of the Bet-treated albino rats using both light and transmission electron microscopes.

In the present study, the bones of the Bet-treated animals showed a separated or thin periosteum with an apparently marked decrease in the amount of the collagenous fibres. A variation in the sizes and an irregularity in the distribution of the Haversian canals were also noticed. Such alterations might be due to the shadow imparted by the given CS on the bone and paralleled the clinical finding reported by Watts (2004) and Kanis et al. (2007). The surface notches and the resorption cavities encountered, in the current investigation, could be due to the concomitant enhanced bone resorption and the reduced bone formation evoked by the administered GC. Ultrastructurally, the osteoblasts suffered from degenerative alterations in the from of losing both the processes and the organelles and gaining many vacuoles. The osteocytes underwent similar variations. Both types of cells were surrounded by non-homogenous matrix. Again, the GC utilized could be blamed for these afflictions which came in accord with those claimed by Dalle et al.(2005). They declared that the main action of GCs is the osteoblastic dysfunctions by shortening the duration in which the osteoblasts work actively forming the bone matrix. Furthermore ,both Weinstein et al.(2002) and Sedlak et al.(2006) believed that GCs treatment causes alterations in the osteocyte micro-environment (lacunae and canaliculi), demineralization as well as micro-architectural changes in the bone quality. These , in turn, lead to an increased apoptosis of both osteocytes and osteoblasts as well as suppressed turn over of the cell cycle. The present results were also supported by Delany et al.(2001) who announced that proliferation and differentiation of the osteoblasts are

controlled by local growth factors and cytokines produced in bone as well as by systemic hormones. They added that GCs inhibit the synthesis and action of the local growth factors as the insulin like growth factor (IGF) and transforming growth factor beta (TGF-β) which are the important targets of GCs in bone formation.

In the same regard , Massague and Chen (2000) and Sowa et al. (2002a) clarified that TGF-β regulates bone metabolism and modulates both production of proteins of osteoblasts and bone formation. The non-homogenous dark and pale areas of matrix detected in the bones of the Bet-treated rats, in the present study, might be attributed to the structural changes evoked by the given drug. Similar interpretation for the action of prednisolone on bones of rats was delivered by Tkocz-Kiatkowska et al. (1998). They proved that GC-administration results in a reduction in the calcium and other mineral substances in bones. Lu et al.(2005) added that GCs inhibit formation of the bone matrix proteins such as type-I collagen and osteocalcin. Also, Sharla et al. (2005) proposed that GCs decrease the intestinal absorption of calcium and disturb vitamin-D metabolism as they reduce 1,25-di-hydroxy vitamin D (D-hormone) receptors in bone leading to inflammation-induced OP.

The persistence of the small osteoporotic cavities and the heterogenous matrix in the bones of the Bet and Ris-treated albino rats, in the current work, could be owed to the residual harmful effect of Bet. But the over-all scene of the tibial architecture documented that there was an evident improvement so that the bony structure, more or less, resembled that of the control animals. Such alterations could be attributed to the lessening effect of Ris which, in general, seemed to stamp out the negative burden imposed by Bet on bones. As a consequence, the osteoblasts in the present group , were found to possess large nuclei and were surrounded by a pale then a dark matrix. These findings were supported by Iwata et al.(2006) and Follet et al. (2006) who supposed that Ris not only inhibits the apoptosis of both osteoblasts and osteocytes but also stimulates proliferation and differenti-

ation of the former cells. Additionally, Yao et al.(2006) suggested that Ris would restore the bone micro-architecture and improve its strength in the ovariectomized mice via alterations in its mineralization. On the other hand, the osteoclasts, in turn, were affected by the given Ris in the present investigation in a different manner. These cells suffered from degenerative changes .They lost their ruffled border, their nuclei showed some degeneration and the cytoplasm lodged many relatively large vacuoles. Their nearby matrix appeared non-demineralized and electron dense. Such findings denoted that Ris could have a favourable effect on the Bet-stressed bony tissue.

These observations were confirmed by those of von Knoch et al. (2005) and Lambrinouaki et al.(2006) in human bone. They stated that Ris inhibits the osteoclast-mediated bone resorption. Similarly, Boonen et al. (2004) stressed on that, among the biphosphonates, Ris shows a higher anti-resorptive effect. Furthermore,Takahashi et al.(2006), Moreau et al.(2007) and Reginster et al. (2007) hypothesized that Ris inhibits the osteoclastic activity either through its direct cytotoxic effect, thus inducing their apoptosis, or by preventing these cells from removing bone. It was postulated by Werkman et al. (2006) that Ris, in castrated rats alters osteoclasts cytoskeleton proteins or inhibits cholesterol synthesis which are necessary for the formation of the ruffled border, thus interfering with the fixation mechanisms of the osteoclasts to bone matrix. Eventually, Dunford et al.(2006) and Russell (2007) emphasized on that Ris inhibits a key enzyme, farnesyl pyrophoshate synthase,thereby indirectly preventing the biosynthesis of isoprenoid compounds required for both the survival and function of the osteoclasts.

In light of the demonstrated beneficial effects of Ris, in the current investigation, it is advisable to widen the scale of its concurrent administration for patients on GCs therapy to minimize, as possible, their undesirable bony hazards.

REFERENCES

(1) Abadie, E.C.; Devogealer, J.P. and Ringe J.D. (2005) :

Recommendations for the registration and of agents to be used in the prevention and treatment of glucorticoid- induced osteoporosis: updated recommendations from the group for the Respect of Ethics and Excellence in science. Semin Arthritis Rheum,35 (1): 1-4.

(2) **Bancroft, J.D.; Steven, A. and Dawson, M.P. (1996)** : Theory and Practice of Histological Techniques, 4th ed. , Churchill, Livingstone, Edenbrugh, London, New York.

(3) **Blalock, S.J. ; Norton. L.L.; Patel. R.A. and Dooley, M (2005)** : Patient knowledge, beliefs and behaviour concerning the prevention and treatment of glucocorticoid-induced osteoporosis.Arthritis Rheum, 53 (5):732-739.

(4) **Bobba, R.S.; Beattie. K; Parkinson, B.; Kumbhare, D., and Adachi, J.D. (2006)** : Tolerability of different dosing regimens of biphosphonates for the treatment of osteoporosis and malignat bone disease. Drug Saf,29 (1):1133-1152.

(5) **Boonen, S.; Haentjens, P.; Vandenput, L., and Vanderschueren, D. (2004)** : Preventing osteoporotic fractures with anti-resorptive therapy: implications of micro-architectural changes. J Intern Med, 255(1):1-12.

(6) **Curtis, J. R.; Westfall, A.O.; Allison, J. J.; Freeman. A. and Saag, K. G. (2006)** : Channelling and adherence with alendronate and risedronate among glucocorticoid users.Osteoporos Int,17(8): 1268-1274.

(7) **Dalle, C. L.; Bertoldo, F; Valenti, M. T. and Zenari, S. (2005)** : Histomorphic analysis of glucocorticoid induced osteoporosis. Clin Calcium, 6: 783-785.

(8) **Delany; A.M.; Durant, D. And**

Canalis, E. (2001) : Glucocorticoid suppression of IGF-1 transcripition in osteoblasts.Molec Endocrino l, 15:1781-1789.

(9) Drury, R.A.B.and Wallington, E.A. (1980) : Carleton's Histological Techniques. 5th ed. Oxford, NewYork, London:140-141.

(10) Dunford, J. E.; Rogers, M. J.; Ebetino. F. M.; Phipps, R. J. and Coxon F. P. (2006) : Inhibition of protein prenylation by biphosphonates causes sustained activation of Rac, Cdc42,and Rho GTPases. J Bone Miner Res, 21(5): 684-694.

(11)Follet, H.; Li, J.; Phipps, R.J.; Hui, S.; Condon. K, and Burr, D.B. (2007) : Risedraonate and alendronate suppress osteocyte apoptosis following cyclic fatigue loading. Bone, 40 (4) : 1172-1177.

(12) Giljevic Z. and Volk T. (2006) : Treatment of osteoporosis by risedronate -speed, efficacy and safety. Rheum. 53(2): 66-71.

(13) Grazio,S.;Korsic,M.and Anic, B. (2005) : Glucocorticoid ɜ induced osteoporosis : Review and proposition for prevention and treatment guidelines. Lijec Vjesn, 127 (1-2): 36-43.

(14) Hayat, M. (1989) : Principles and Techniques of Electon Microscopy, Biological applications. 3rd ed. , CRC press, NewYork, Florida:4-74.

(15)Irwin, R.S. and Richardson N. D. (2006) : Side effects with inhaled corticosteroids; The physian pereception. Chest, 130 (suppl):415-535.

(16)Iwamoto, J.; Takeda, T.; Sato, Y. and Yeh, J.K, (2007) : Additive effect of vitamin K2 and risedronate on long bone mass in hyphophysectomized young rat. Exp.

Anim, 56 (2): 103-110.

(17) **Iwata, K.; Li, J.; Follet, H.; Phipps, R.J. and Burr, D.B. (2006) :** Biphosphonates suppress periosteal osteoblast activity independently of resorption in rat femur and tibia. Bone, 39 (5) : 1053-1058.

(18) **Kanis, J.A.; Stevenson M.; McCloskey, E.M. and Davis S. (2007) :** Glucocorticoid -induced osteoporosis : a systemic review and cost -utility analysis. Health Technol Assess, 11 (7): (1-7).

(19) **Lambrinouaki, I.; Christodoulakos, G.; and Botsis, D. (2006) :** Biphosphonates. Ann NY Acad Sci, 92: 397-402.

(20) **Lange U.; Illgner, U; Teichmann J. And Schleenbecker H. (2004) :** Skeletal benefits after one year of riedronate therapy in patients with rheumatiod arthritis and glucocorticoid - induced osteoporosis : a prospective study. Int J Clin Pharmacol Res , 24 (2,3): 33-38.

(21) **Larsen, P.R.; Kronenberg H.M; Melmed S.and Polonsky K.S. (2003) :** Osteoporosis in Williams Text book of Endocrinology, 10th ed., Saunders, Philadelphia, 506, 1379-1381, 1389-1390.

(22) **Lee, G.S.; Choi, K.C. and Jeung, E.B. (2005) :** Glucocorticoids differentially regulate expression of duodenal and renal calbindin -Dgk through glucocorticoid receptor mediated pathway in mouse model. Am J Physiol Endocrinol Metab, 290: E 299-E307.

(23) **Lu, M.; Kaji H.; Sowa, H. and Naito J. (2005) :** Dexamethasone suppresses Smad B pathway in osteoblastic cells. J Endocrinol, 185: 131-138.

(24) **Maricic M. (2005) :** Glucocorti-

coid-induced osteoporosis : *treatment options and* guidelines. Curr. Osteoporos Rep, 3 (1): 25-29.

(25) Massagu, J. and Chen, Y.G. (2000) : Controlling TGF-beta signaling. Genes and Develop, 14 : 627-644.

(26) McLaughlin, F.; Mackintosh, J.; Hayes, B.P. and McLaren A. (2002) : Glucocorticoid-induced osteopenia in the mouse as assessed by histomorphometry, microcomputed tomography and biochemical markers. Bone, 30:924-930.

(27) Moreau, M.F.; Guillet, C.; Massin, P. and Chevalier, S. (2007) : Comparative effects of five biphosphonates on apoptosis of macrophage cell in vitro. Biochem Pharmacol, 73(5): 718-723.

(28) Orcel, P. (2006) : Management of corticosteriod -induced osteoporosis. Press Med, 35 (10, pt 2): 1571-1577.

(29) Ramsey-Goldman R. (2002) : Missed opportunities in physician management of glucocorticoid- induced osteoporosis [Review]. Arthritis Rheum, 46: 3115-3120.

(30) Reginster, J.Y.; Malaise, O.; Neuprez, A.; Jouret, V. E. and Close, P. (2007) : Intermittent biphosphonate therapy in post-menopausal osteoporosis: Progress to date. Drugs&Aging, 24(5) : 351-359.

(31) Russell, R.G. (2007) : Biphosphonates:mode of action and pharmacology. Pediatrics, 199(suppl.) 2: 150-162.

(32) Saag, K.G. (2004) : Prevention of glucocorticoid- induced osteoporosis. South Med J , 97(6): 555-558.

(33) Sato, Y.; Honda, Y. and Iwamoto, J. (2007) : Risedronate and ergocalciferol prevent hip fracture in elderly men with Parkinson disease. Neurol, 68(12): 911-915.

(34) **Sedlak, I.K.; Cegiela, U.; Nowinsk, B. and Folwarczna, J. (2006) :** Effect of catecholomines on the intramedullary pressure in the femur in rats with prednisolone-induced osteoporosis. Pharmacol Rep, 58 : 540-550.

(35) **Shah, S.K. and Gecys, G.T. (2006) :** Prednisolone-induced osteoporosis: An over looked and undertreated adverse effect. JAOA, 106 (11): 653-657.

(36) **Sharla, S.H.; Schacht, E. and Lempest, U.G. (2005) :** Alfacalcidol versus plain vitamin D in inflammation-induced bone loss.J Rheumatol (suppl), 76: 26-32.

(37) **Solomon, D.H.; Polinski, J.M.; Stedman, M.and Truppo, C. (2007) :** Improving care of patients at risk for osteoporosis: A randomized controlled trial. Soc of Gen Intern Med, 22:362-367.

(38) **Sowa, H; Kaji H.; Yamaguchi T; Sugimotot and Chichara, K. (2002a) :** Smad3 promotes alkaline phosphatase activity and mineralization of osteoblastic MC3T3-E1 cells. J bone Miner Res, 17:1190-1199.

(39) **Summey, B.T. and Yosipovitch, G. (2006) :** Glucocorticoid-induced bone loss in dermatologic patients:an update. Arch Dermat. 142 (1): 82-90.

(40) **Suzuki, Y. (2006) :** Randomized controlled trials for the prevention and treatment of glucocorticoid-induced osteoporosis.Clin Calcium, 11: 1834-1842.

(41) **Takahashi, M.; Saha, P.K. and Wehrli, F.W. (2006) :** Skeletal effects of short term exposure to dexamethasone and response to risedronate treatment studied in vivo in rabbits by magnetic resonance micro-imaging and

spectroscopy. J Bone Miner Metab, 24 (6): 467-475.

(42) **Tkocz-Kwiatkowska, J.; Kaczmarczyk, S.I. and Folwarczna, J. (1998) :** Effects of pamidronate on the development of changes in bone mechanical properties and bone structure caused by administration of prednisolone in rats. Pol J Pharmacol, 50: 253-258.

(43) **von Knoch F.; Jaquiery, C.; and Kowalsky, M. (2005) :** Effects of biphosphonates on proliferatoin and osteoblast differentiation of human bone marrow stromal cells. Biomaterials, 26(34): 6941-6949.

(44) **Ward, L. (2005) :** Osteoporosis due to glucocorticoids use in children with chronic illness. Horm Res, 64(5):209-221.

(45) **Watts, N.B. (2004) :** Diagnosis and evaluation of patients with osteoporosis. South Med J, 97(6): 540-541.

(46) **Weinstein; R.S.; Chen, J.R.; Powers, C.C. and Stewart, S.A. (2002) :** Promotion of osteoclast survival and antagonism of biophosphonate- induced apoptosis by glucocorticoids. J. Clin. Invest, 109: 1041-1048.

(47) **Wekman, C; Sendra, G.S.; da-Rocha, R.F. and Brandao, A.A. (2006) :** Comparative therapeutic use of Risedronate and calcarea phophoricaallopathy versus homeopathy -in bone repair in castrated rats. Braz oral Res, 20(3): 196-201.

(48) **Yao, W.; Balooch, G.; Balooch, M.; Jiang, Y. and Nalla, R.K. (2006) :** Sequential treatment of ovariectomized mice with b FGF and risedronate restored trabecular bone micro-architecture and mineralization. Bone, 39 (3):460-469.

الملخص العربى

دراسة بالمجهر الضوئى والالكترونى لتأثير عقار ريزيدرونات الصوديوم على عظام القصبة فى الفئران البيضاء المعالجة بعقار البيتاميثازون.

د. جمال ابو الفتوح محمد ، د. عادل عبد المهدى الهوارى

من قسم التشريح - كلية الطب جامعة المنصورة.

أجريت هذة الدراسة فى محاولة لكشف اثر تعاطى عقار ريزيدرونات الصوديوم (اكتونيل) على نسيج عظام القصبة فى الفئران البيضاء المعالجة بعقار البيتاميثازون (بيتازون). وقد أستخدم فيها ثلاثون من ذكور الفئران البيضاء البالغة والتى تراوح وزنها بين ٢٠٠-٢٥٠ جم والتى قسمت بالتساوى الى ثلاثة مجموعات بحيث استخدمت المجموعة الاولى كضابطة ،بينما اعطيت فئران المجموعة الثانية عقار البيتاميثازون عن طريق الفم . ومن ناحية أخرى عولجت حيوانات المجموعة الاخيرة بعقارى البيتاميثازون (كما فى المجموعة الثانية) وريزيدرونات الصوديوم (تحت الجلد) ،واستمر العلاج لكل المجموعات لمدة ثلاث شهور متتالية،بعدئذ تم تخدير الفئران بمخدر الكيتامين(٤٥مجم/كجم) وغمرت من خلال البطين الايسر بمحلول الجلوتارالدهيد ٢%. ثم تم استئصال عظام القصبة حيث فصل االجزء الاوسط (العمود) من كل منها. وغمرت هذه الاجزاء الوسطى فى ذات المحلول آنف الذكر لأربعة ساعات .بعدها تمت عملية ازالة الكالسيوم من تلك العينات العظمية بغمرها فى محلول خاص لذلك الغرض لمدة ٩ ايام . ثم اخذت عينات صغيرة من تلك العظام وتم غمرها فى محلول الفورمالين ١٠% ومن ثم تم تمريرها لتجهيز قطاعات شمعية لصبغها بصبغة الهيماتوكسلين والايوسين و ايضا بصبغة ماسون الثلاثية. وكذلك جرى تجهيز قطاعات رقيقة جدا للفحص بالميكروسكوب الالكترونى النافذ. وقد بدا جليا من نتائج هذه الدراسة أن عظام الفئران التى عولجت بعقار البيتاميثازون قد حاقت بها تغيرات تحللية متفاوتة

فى السمحاق الخارجى والداخلى وكذلك فى كل من الخلايا االعظمية البانية والاساسية مع ازدياد ملحوظ فى الفراغات العظمية التى امتلأت بنسيج حبيبى ضام . كما لوحظ وبشكل واضح اضمحلال فى كمية الالياف الكولاجينية. وحال الفحص بالمجهر الالكترونى شوهدت درجات متباينة من التحلل الخلوى فى الخلايا العظمية المذكورة سالفا. ومن ناحية أخرى عند فحص عينات من عظام الفئران المعالجة بكل من عقار البيتاميثازون وريزيدرونات الصوديوم لوحظ تحسن واضح فى تركيب النسيج العظمى عند مقارنتها بمثيلاتها فى المجموعة الثانية فقد قلت الفراغات العظمية . كما لوحظت علامات زيادة فى نشاط الخلايا العظمية بزيادة كمية الالياف الكولاجينية حولها . بالاضافة الى ذلك اصيبت الخلايا العظمية الهادمة بدرجات متفاوتة من التحلل. وبعد استعراض النتائج السابقة يمكن الاستنتاج ان لعقار ريزيدرونات الصوديوم أثرا ايجابيا على تركيب العظام فى الحالات المعالجة بعقار البتاميثازون اذ أنه يساعد على اعادة تكوين النسيج العظمى المضار.