Morphology of Young Male Albino Rats' Epiphyseal Plate after Dexamethasone Administration

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Abstract: Introduction: Corticosteroids are used in the treatment of various clinical conditions affecting children. Continuous corticosteroids therapy imposes a threat to the growth of children. **Aim of work:** This work aimed to study the possible structural changes that may occur in the epiphyseal growth plate of young male albino rats after dexamethasone administration for consecutive 7 days. **Materials and methods:** Ten healthy young male albino rats were equally divided into two groups; a control group and dexamethasone treated one**.** Animals of the treated group were injected with 5 mg/kg body weight dexamethasone subcutaneously once daily for consecutive 7 days. Control rats received an equal volume of saline for the same period and by the same route. Proximal heads of the tibiae were dissected out carefully and processed for light and electron microscope examinations. **Results:** In epiphyseal plate of treated group, an observable reduction thickness, less frequent chondrocytes particularly in resting zone and column organization disruption with wide matrix areas were detected. Matrix had weak staining affinity for alcian blue with deep stained areas around residual chondrocytes. Some resting chondrocytes were shrunken. Most of the light ones showed cytoplasmic vacuoles while dark ones became more electron dense. Some light proliferative chondrocytes had small electron dense nuclei or irregular nuclear envelope and vacuolated cytoplasm while dark ones appeared more electron dense with markedly dilated cisternae of rough endoplasmic reticulum**.** Some hypertrophied light chondrocytes had many vacuoles while some of dark ones showed swollen mitochondria, fragmented cisternae of rough endoplasmic reticulum and progressive fragmentation of cytoplasm. Degenerating hypertrophic chondrocytes (dark and light) were shrunken with few or no electron dense bodies in the matrix. **Conclusion:** Dexamethasone administration led to an observable alteration in the structure of the growth plate of young male albino rats. So, corticosteroids might slow longitudinal bone growth and induced growth retardation.
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Key words: growth plate-dexamethasone-chondrocytes- extracellular matrix.

1. Introduction

The mammalian epiphyseal plate is known as growth plate or physis. It is a thin layer of highly specialized mesodermal derived cartilaginous structure. It is entrapped between the epiphysis and metaphysis of the long bones in young animals and human. The growth plate is avascular, aneural and highly organized stratified growth organ that comprises both chondrocytes and their extracellular matrix (ECM) **[1-4]**.

Growth plate showed ordered columns of chondrocytes that are small and flat at epiphyseal end and become large and round towards the metaphysis. These columns are parallel to longitudinal axis of the bone and showed morphologically distinct zones. The chondrocytes in these zones undergo an orderly serious of structural and functional changes that allow the growth cartilage to drive longitudinal bone growth **[3,5,6]**.

Chondrocytes contributes in longitudinal growth through a combination of proliferation, hypertrophy, ECM synthesis and controlled degradation. Chondrocytic enlargement and ECM synthesis are strongly correlated. Chondrocytes enlarge mostly in the growth direction (longitudinal direction) with little increase in the width. ECM synthesis is required to fill the increased volume laterally. ECM is rich in collagen and proteoglycan. It determines the mechanical properties of the tissue and contributes in structural arrangement of the growth plate by providing a scaffold for chondrocyte attachment and migration **[1,3,7]**.

Longitudinal bone growth is a complex orchestrated event results from progressive replacement of growth plate cartilage by osseous tissue at the metaphysis **[8]**.This complicated process is observed from fetal life, with a rapid deceleration up to about 3 years of age. This dynamic physiological process of proliferation and differentiation is ended by cell death or apoptosis. It is regulated not only by growth factors but also by genetic, environmental, nutritional, hormonal and local factors that may act directly or indirectly on the growth plate **[9-13]**.

Corticosteroids are important regulators of diverse physiological systems. It is estimated that 5-10% of children may require some form of corticosteroids at some point in their childhood. For many decades, corticosteroids have been used as a

therapy to treat diseases that have an inflammatory component as juvenile rheumatoid arthritis, atopic dermatitis, chronic asthma and in organ transplantations. It can be administered through several routes: topically, orally or injected. Prolonged use of corticosteroids may lead to many complications such as: osteoporosis, hypothalamus-pituitary-adrenal (HPA) axis suppression, cataract formation, skin thinning and growth retardation **[4, 14-16]**.

Dexamethasone is a widely used corticosteroid to treat inflammatory diseases, however a multiple undesired effects have been reported specially in children including decreased longitudinal bone growth and growth retardation **[13]**. So, this work was performed aiming to study the possible structural changes that may occur in the epiphyseal growth plate of young male albino rats after dexamethasone administration for consecutive 7 days.

2. Materials and Methods

Ten healthy young male albino rats (aged 4 weeks) weighing 80-90gm were used in this study. They were maintained in room temperature at 23ºC with light-dark cycle. They were equally divided into two groups; a control group and an experimental or dexamethasone treated (Dexa treated) one. The animals of the Dexa treated group were injected with 5 mg/kg body weight dexamethasone subcutaneously once daily for consecutive 7 days. Control rats received an equal volume of saline for the same period and by the same route of administration. All animals were maintained on free access of food and water **[17]**.

At the end of experiment, all animals of both groups were anesthetized with 50 mg/kg body weight sodium pentobarbital intraperitoneally and then intracardiac perfusion was carried out by 2.5% glutaraldehyde buffered with 0.1M phosphate buffer at pH 7.4 for partial fixation of the tibia. The proximal heads of the tibiae were dissected out carefully and processed for light and electron microscope examinations. For light microscope examination, the proximal heads of the tibia were split sagittally and were fixed in a neutral buffered 10% formalin for 24 hours, decalcified by EDTA (Ethylene Diamine Tetra Acetic acid) solution in buffer for 3 successive days at 4ºC and then processed to prepare 5 µm thick paraffin sections for Haematoxylin & Eosin stains and Alcian blue stain **[18]**.

Specimens for electron microscope examination were cut into small pieces. These pieces were immediately fixed in 2.5 % glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4 for 2 hours at 4ºC and then washed with phosphate buffer, postfixed in 1% osmium tetraoxide in the same buffer for one hour at 4ºC. The postfixed specimens were decalcified by EDTA solution in buffer for 3 successive days at 4ºC. Then these specimens were washed in phosphate buffer and were dehydrated with ascending grades of ethanol. They were put in propylene oxide for 30 minutes at room temperature, impregnated in a mixture of propylene oxide and resin (1:1) for 24 hours and in a pure resin for another 24 hours. Then, the specimens were embedded in Embed-812 resin in BEEM capsules at 60ºC for 24 hours **[19]**. Semi-thin sections (1 µm thick) were stained with 1% toluidine blue for light microscope **[18]**. Ultra-thin sections were obtained using Leica ultra cut UCT and stained with uranyl acetate and lead citrate **[19]** and were examined with JEOL JEM 1010 electron microscope in Electron Microscope Research Laboratory (EMRL) of Histology and Cell Biology Department, Faculty of Medicine, Zagazig University.

3. Results

Light microscope examination of the proximal heads of the tibiae of the control young male albino rats showed that the epiphyseal growth plate was formed of highly organized chondrocytes that lies between the epiphysis and the metaphysis. This plate was divided into four well distinct zones; resting, proliferative, hypertrophied and calcified or degenerated zones. All of these zones consisted mainly of chondrocytes embedded in an abundant extracellular matrix **(Figs. 1&2)**. This matrix had strong affinity for alcian blue stain with deep staining areas around chondrocytes **(Fig. 3)**. Within the resting zone, chondrocytes were approximately of uniform size and irregularly scattered in a bed of abundant extracellular matrix. These chondrocytes were ovoid in shape with rounded vesicular nuclei, prominent nucleoli and pale-stained cytoplasm **(Fig. 4)**. Chondrocytes in proliferative, hypertrophic and calcified zones were arranged in columns parallel to the long axis of the bone. Proliferative zone chondrocytes' were flat with pale nuclei and deep basophilic cytoplasm. They were arranged like stacks of coins. Hypertrophic chondrocytes became relatively larger and swollen. They had eccentric nuclei and vacuolated cytoplasm. At the calcified or degenerated zone, chondrocytes were shrunken within the lacunae with deeply stained cytoplasm and nuclei. They had extensive vacuolation. The lacunae gradually increase in size from superficial to deep zones with empty lacunae appeared among the degenerated chondrocytes **(Fig. 5)**.

Electron microscope examination of the ultrathin sections of the epiphyseal growth plate of the same group showed that resting chondrocytes had two morphologically distinct types; light and dark cells. Light chondrocytes were the major population with euchromatic nuclei and electron lucent cytoplasm. Dark chondrocytes contained electron dense nuclei and cytoplasm. Both had irregular plasmalemma

called filopodia **(Fig. 6)**. At the proliferating zone, early chondrocytes were observed as pairs in a single lacuna. This lacuna appeared as dense pericellular matrix formed of many fine granular collagen fibrils **(Fig. 7)**. Late proliferating chondrocytes were aligned closely packed together and became spindle shaped. Most of them were light and few ones were dark. Light chondrocytes contained prominent rough endoplasmic reticulum. Dark chondrocytes had prominent secretory vesicles. Both chondrocytes plasmalemma was irregular (**Fig. 8)**. The hypertrophic light chondrocytes had nuclei with peripheral heterochromatin and voluminous cytoplasm. This cytoplasm was packed with cisternae of rough endoplasmic reticulum, large vacuoles and few mitochondria. Their plasmalemma had a scalloped appearance **(Fig. 9).** The hypertrophic dark chondrocytes had convoluted nuclei with segregation of nucleolus cap, many mitochondria, dispersed rough endoplasmic reticulum, plenty of glycogen, numerous vacuoles, secretory vesicles and blebs of plasmalemma **(Fig. 10)**. Degenerating light hypertrophic chondrocytes had extremely irregular nuclear outlines. Their cytoplasm was disintegrated within cell membrane. They contained only strands of rough endoplasmic reticulum **(Fig. 11)**. Degenerating dark hypertrophic chondrocytes had nuclei with patchy chromatin condensation and dilated cisternae of rough endoplasmic reticulum **(Fig. 12)**. Degenerated chondrocytes were shrunken leaving a space between cell membrane and lacunae. The wall of these lacunae was composed of microfibrils oriented parallel to cellular margin and showed electron dense calcified bodies (materials) **(Figs. 11&12)**. At the deepest area of calcified zone, these bodies became extensive in between almost empty lacunae contained only cellular remnants **(Fig.13)**.

Light microscope examination of the proximal heads of the tibiae of the Dexa treated young male albino rats showed a relatively observable reduction in the thickness of the epiphyseal growth plate in comparison with that observed in the control group in Fig. 1. Less frequent cells were observed particularly in the resting zone. The column organization showed disruption with wide matrix areas between cells **(Figs. 14&15)**. The extracellular matrix had weak staining affinity for alcian blue in comparison with that observed in the control group in Fig. 3. Slightly deep stained matrix was still present around the residual chondrocytes **(Fig. 16)**. Many chondrocytes in resting zone exhibited deeply stained nuclei and vacuolated cytoplasm **(Fig. 17)**. Longitudinal columns in proliferative and hypertrophied zones became disarranged with some deeply stained chondrocytes were observed (**Fig. 18)**.

Electron microscope examination of the ultrathin sections of the epiphyseal growth plate of the same group showed that some resting chondrocytes were shrunken within their lacunae. Most of the light ones showed cytoplasmic vacuoles while dark ones became more electron dense **(Fig. 19)**. At the proliferative zone, some light chondrocytes had small electron dense nuclei. Others showed irregular nuclear envelope and vacuolated cytoplasm. Dark proliferative chondrocytes appeared more electron dense with markedly dilated cisternae of rough endoplasmic reticulum **(Fig. 20).** Some hypertrophied light chondrocytes had many vacuoles while some of dark ones showed swollen mitochondria, fragmented cisternae of rough endoplasmic reticulum and progressive fragmentation of cytoplasm **(Fig. 21)**. Degenerating hypertrophic chondrocytes (dark and light) were shrunken with almost no electron dense bodies in the matrix between the lacunae **(Fig. 22).** Deeply, few electron dense bodies appeared and many lacunae were noticed with cellular remnants **(Fig. 23)**.

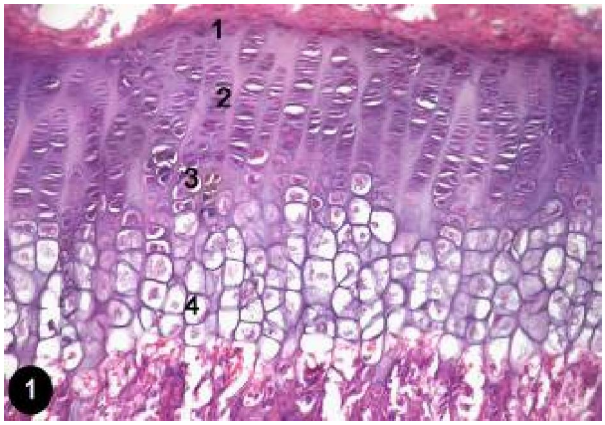

Figure (1): A section in the control tibia proximal head showing the four well distinct zones of the epiphyseal growth plate; resting (1), proliferative (2), hypertrophied (3) and calcified or degenerated zones (4). (H&E: X 200).

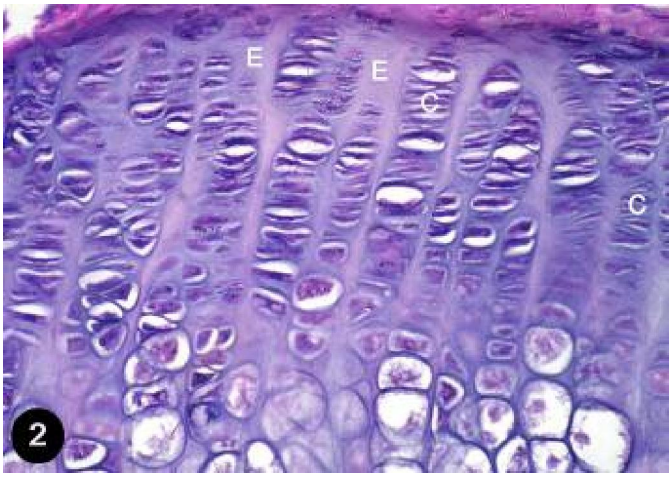

Figure (2): A section in the control tibia proximal head showing that all zones is consisted mainly of highly organized chondrocytes (C) embedded in an abundant extracellular matrix (E). (H&E: X 400).

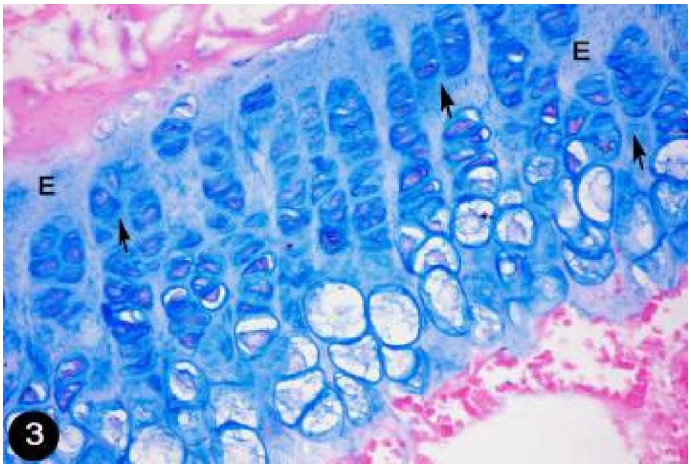

Figure (3): A section in the control tibia proximal head showing the strong affinity of the extracellular matrix (E) with deep staining areas (arrows) around chondrocytes. (Alcian blue stain: X 400).

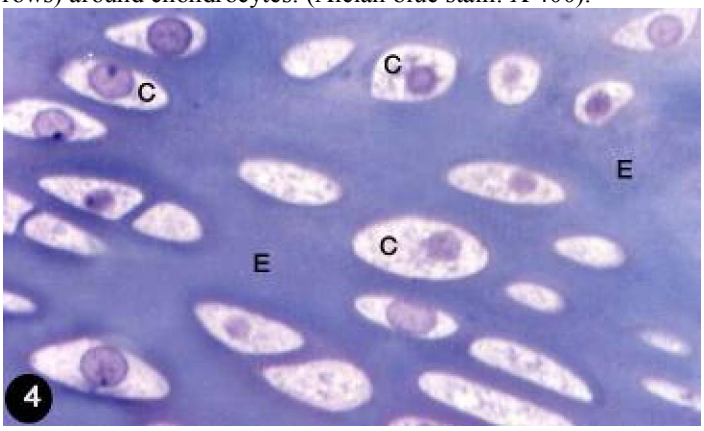

Figure (4): A semithin section (1µm thick) in the control tibia proximal head showing that resting chondrocytes (C) are approximately of uniform size and irregularly scattered in a bed of abundant extracellular matrix (E). Chondrocytes are ovoid in shape with rounded vesicular nuclei, prominent nucleoli and pale-stained cytoplasm. (Toluidine blue: X 1000).

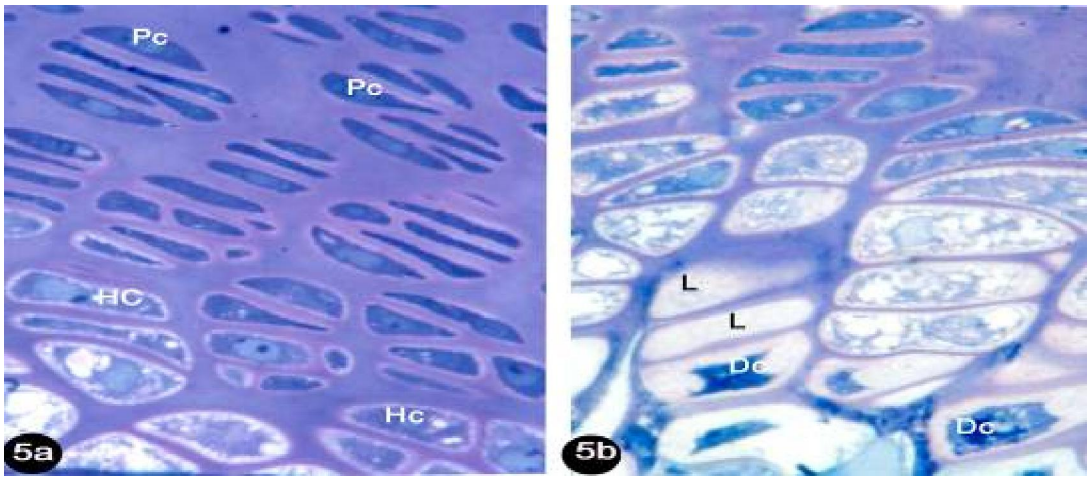

Figure (5): A semithin section (1µm thick) in the control tibia proximal head showing chondrocytes columns in proliferative, hypertrophic and calcified zones. **a-** Proliferative chondrocytes (Pc) are flat with pale nuclei and deep basophilic cytoplasm. Hypertrophic chondrocytes (Hc) are larger, swollen with eccentric nuclei and vacuolated cytoplasm. **b-** Degenerated chondrocytes (Dc) are shrunken and deeply stained with extensive vacuolation. Gradual increase in the size of the lacunae from superficial to deep zones is noticed with empty lacunae (L) appeared among the degenerated chondrocytes. (Toluidine blue: a&b X1000).

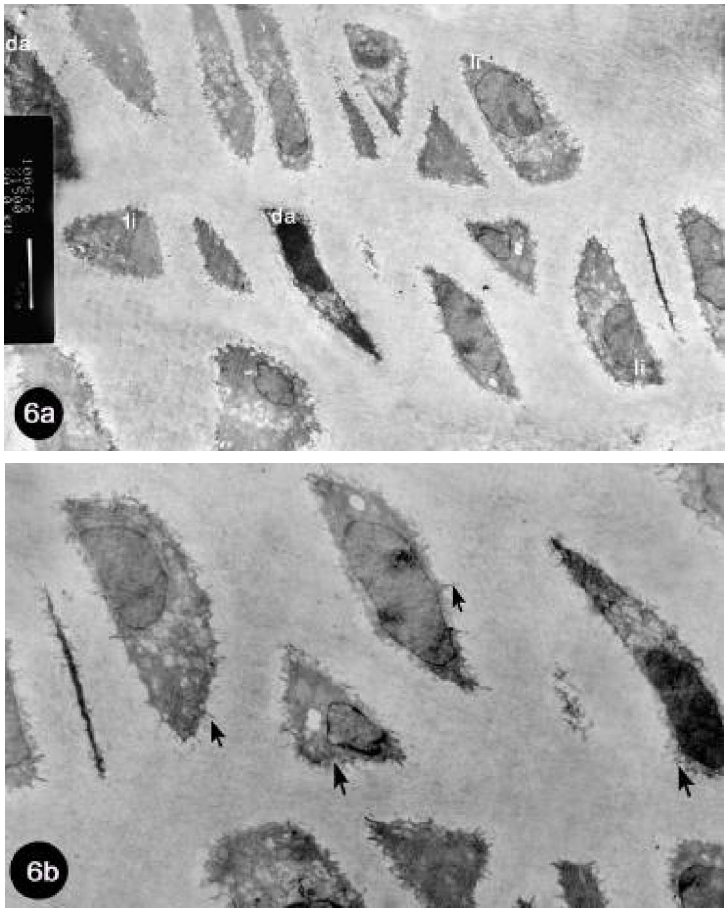

Figure (6): An electron micrograph from the control tibia proximal head showing: **a-**resting light (li) and dark (da) chondrocytes. Light chondrocytes are the major population. **b-** Light cells have euchromatic nuclei and electron lucent cytoplasm while dark ones contain electron dense nuclei and cytoplasm. Both have irregular plasmalemma or filopodia (arrows). (a: X 3400 & b: X5500).

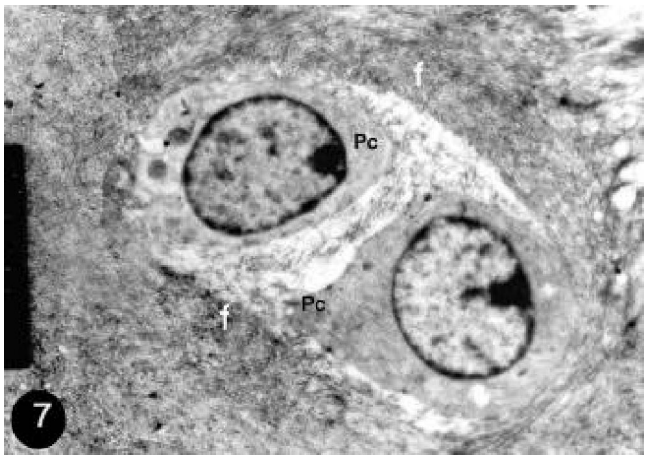

Figure (7): An electron micrograph from the control tibia proximal head showing a pair of early proliferating chondrocytes (Pc) in a single lacuna. The latter is dense pericellular matrix formed of many fine granular collagen fibrils (f). (X 8500).

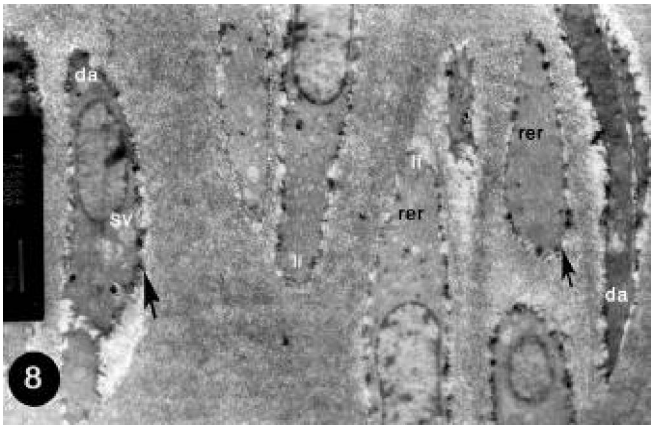

Figure (8): An electron micrograph from the control tibia proximal head showing that late proliferating chondrocytes are mostly of light ones, spindle shaped, closely packed together and with irregular plasmalemma (arrows). Light chondrocytes (li) contain prominent rough endoplasmic reticulum (rer). Dark chondrocytes (da) have prominent secretory vesicles (sv). (X 6500).

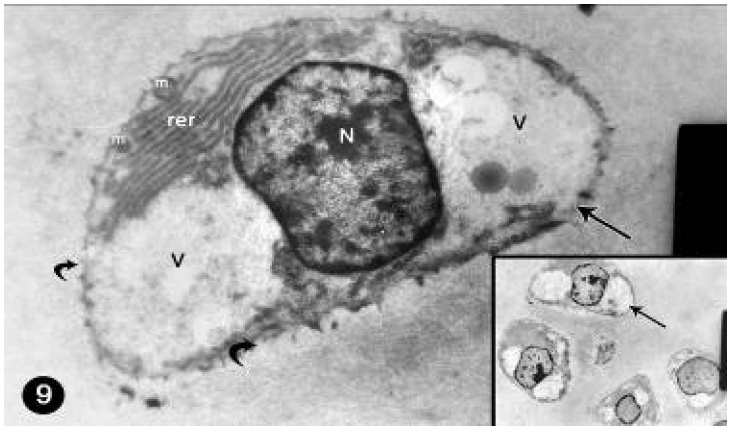

Figure (9): An electron micrograph from the control tibia proximal head showing some hypertrophic light chondrocytes (inset X5500). A higher magnification of one of them (arrow) shows nucleus with peripheral heterochromatin (N) and voluminous cytoplasm packed with cisternae of rough endoplasmic reticulum (rer), large vacuoles (v) and few mitochondria (m). Their plasmalemma has a scalloped appearance (curved arrows). (X 11000).

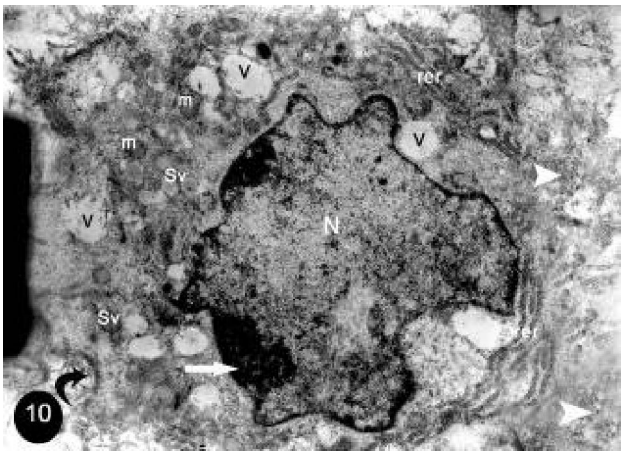

Figure (10): An electron micrograph from the control tibia proximal head showing that hypertrophic dark chondrocyte has convoluted nucleus (N) with segregation of nucleolus cap (arrow), many mitochondria (m), dispersed rough endoplasmic reticulumn (rer), plenty of glycogen (arrow heads), numerous vacuoles (v), secretory vesicles (sv) and blebs of plasmalemma (curved arrow). (X 4500).

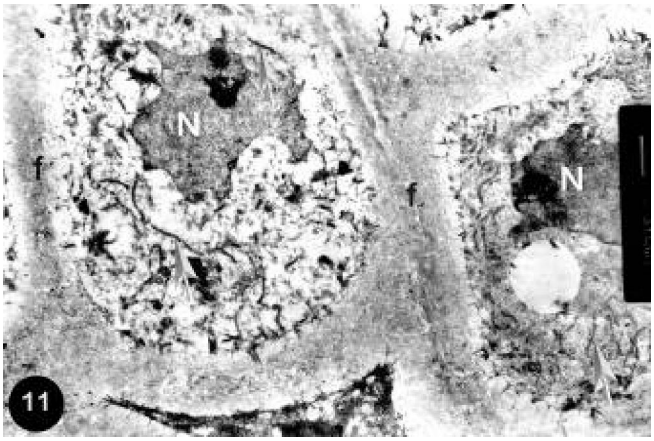

Figure (11): An electron micrograph from the control tibia proximal head showing degenerating light hypertrophic chondrocyte with extremely irregular nuclear outlines (N). Its cytoplasm disintegrates within cell membrane. It contains only strands of rough endoplasmic reticulum (arrows). Microfibrils (f) are noticed in the wall of the lacunae. (X 5500).

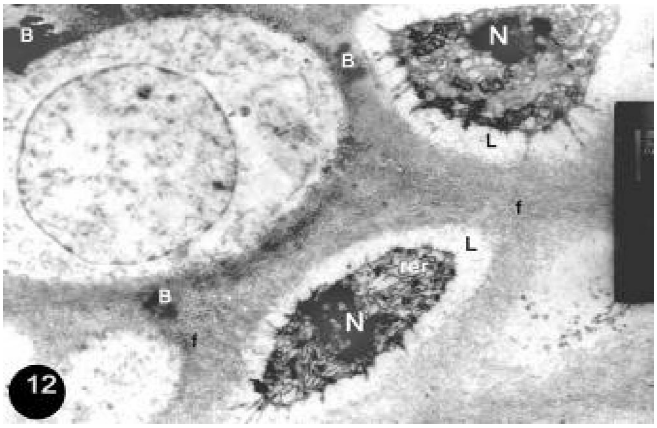

Figure (12): An electron micrograph from the control tibia proximal head showing that degenerating dark hypertrophic chondrocytes have nuclei (N) with patchy chromatin condensation and dilated cisternae of rough endoplasmic reticulum (rer). They are shrunken within their lacunae (L). The wall of these lacunae is composed of microfibrils (f) oriented parallel to cellular margin and shows electron dense calcified bodies (B). (X 6500).

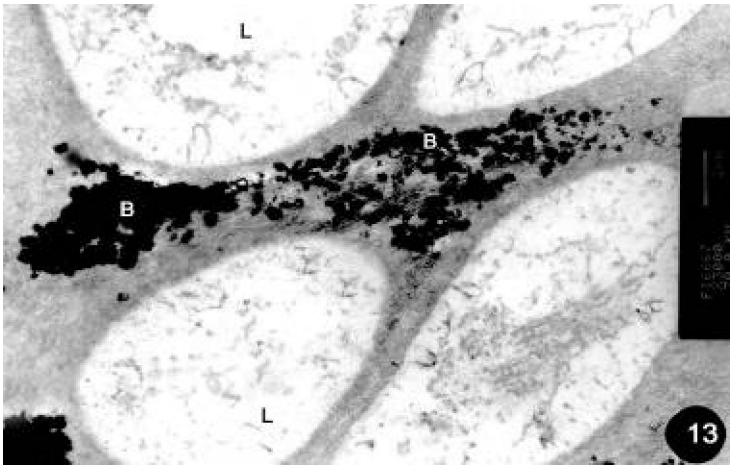

Figure (13): An electron micrograph from the control tibia proximal head showing extensive electron dense bodies (B) at the deepest area of calcified zone in between almost empty lacunae (L) contained only cellular remnants. (X 6500).

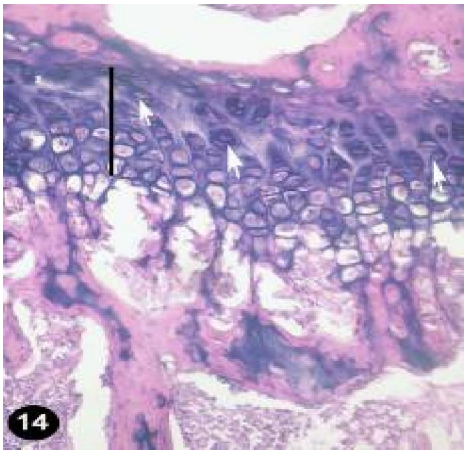

Figure (14): A section in the tibia proximal head of Dexa treated group showing a relatively observable reduction in the thickness of the epiphyseal growth plate in comparison with that observed in Fig. 1. Disruption in the column organization (arrows) is observed (H&E: X 200).

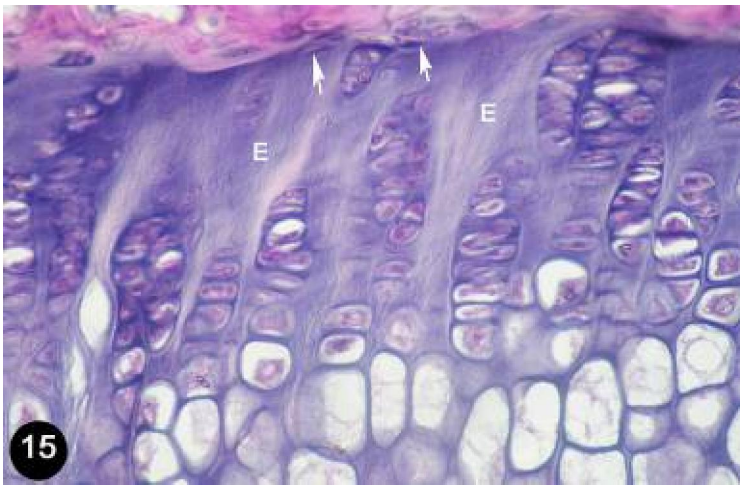

Figure (15): A section in the tibia proximal head of Dexa treated group showing that less frequent cells (arrows) are observed particularly in the resting zone and wide matrix areas (E) between cells. (H&E: X 400).

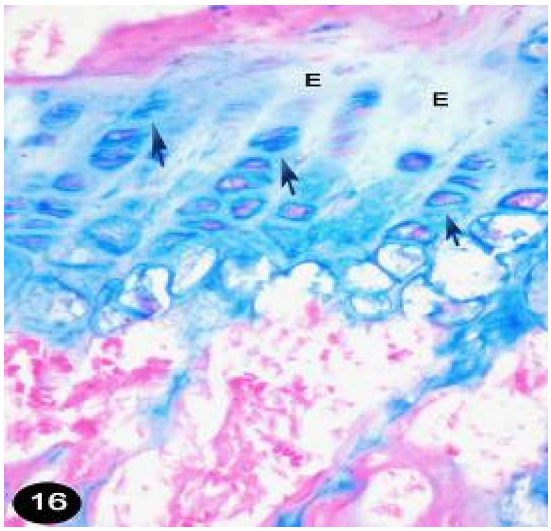

Figure (16): A section in the tibia proximal head of Dexa treated group showing weak staining affinity of extracellular matrix (E) for alcian blue in comparison with that observed in Fig. 3. Slightly deep stained matrix (arrows) is still present around the residual chondrocytes (Alcian blue stain: X 400).

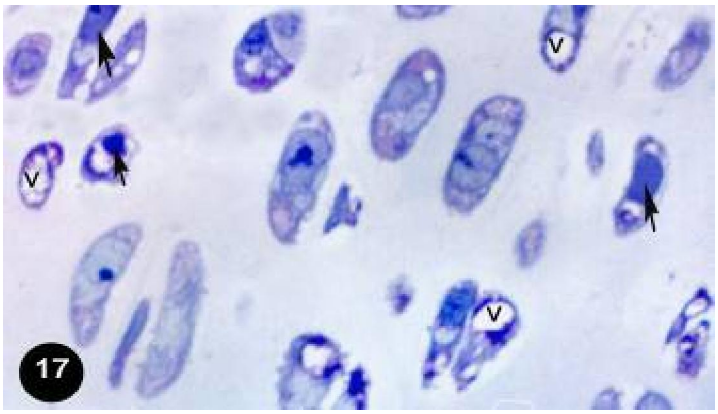

Figure (17): A semithin section (1µm thick) in the tibia proximal head of Dexa treated group showing that many resting chondrocytes exhibit deeply stained nuclei (arrows) and vacuolated cytoplasm (v). (Toluidine blue: X 1000).

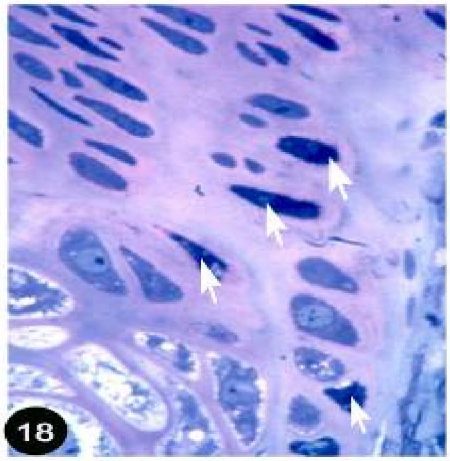

Figure (18): A semithin section (1µm thick) in the tibia proximal head of Dexa treated group showing disarrangement of longitudinal columns in proliferative and hypertrophied zones with some observed deeply stained chondrocytes (arrows). (Toluidine blue: X 1000).

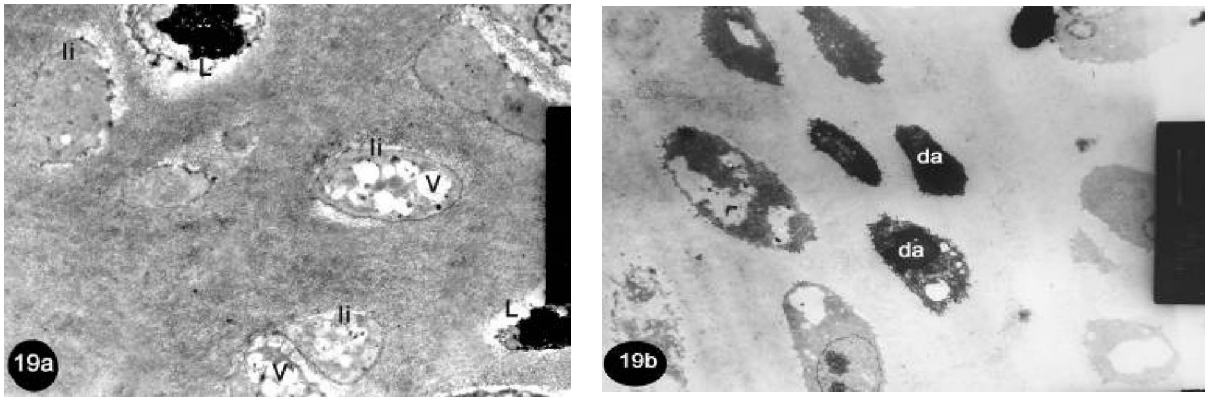

Figure (19): An electron micrograph from the tibia proximal head of Dexa treated group showing that: **a-** some resting chondrocytes are shrunken within their lacunae (L). Most of the light (li) ones show cytoplasmic vacuoles (v). **b-** dark (da) ones become more electron dense. (a: X 5500 & b: X3400).

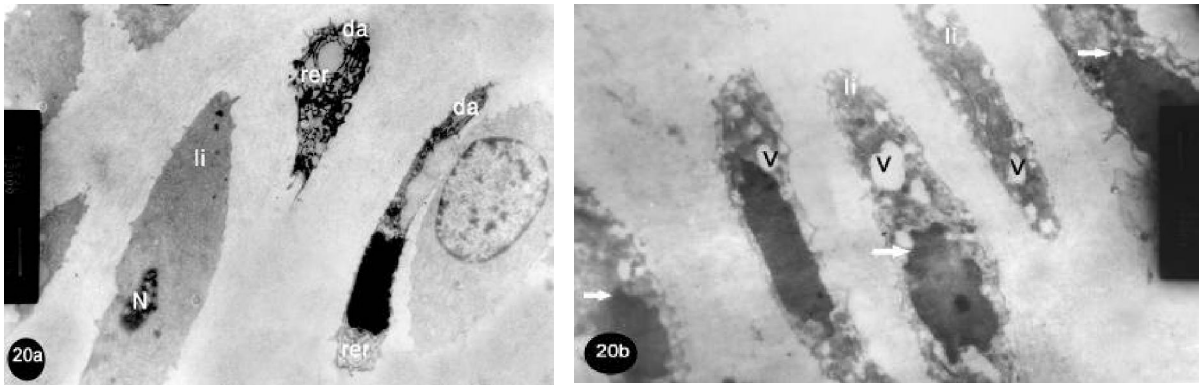

Figure (20): An electron micrograph from the tibia proximal head of Dexa treated group showing that: **a-** a proliferative light chondrocyte (li) has small electron dense nucleus (N). Dark proliferative chondrocytes (da) appear more electron dense with markedly dilated cisternae of rough endoplasmic reticulum (rer). **b-** Other light chondrocytes (li) show irregular nuclear envelope (arrows) and vacuolated cytoplasm (v). (a: X 6500 & b: X5500).

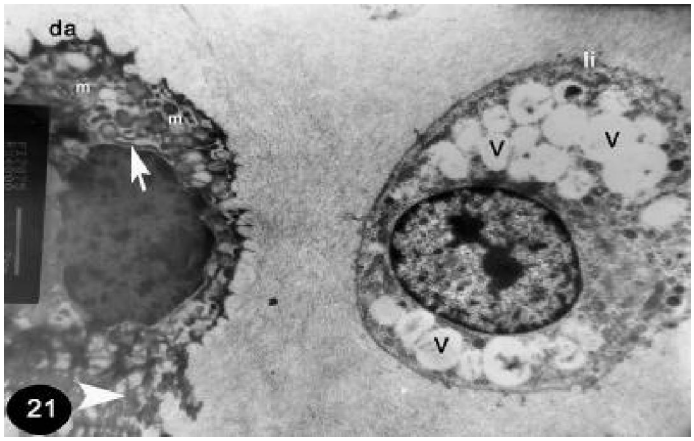

Figure (21): An electron micrograph from the tibia proximal head of Dexa treated group showing that hypertrophied light chondrocyte (li) has many vacuoles (v) while dark one (da) shows swollen mitochondria (m), fragmented cisternae of rough endoplasmic reticulum (arrow) and progressive fragmentation of cytoplasm (arrow head). (X 8500).

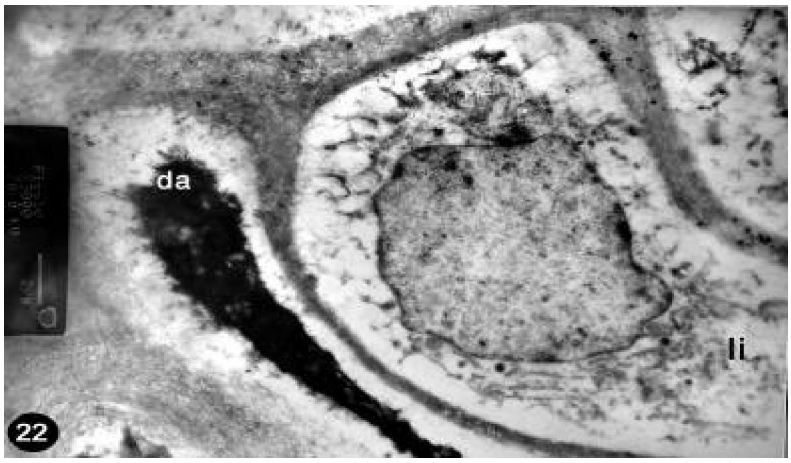

Figure (22): An electron micrograph from the tibia proximal head of Dexa treated group showing that degenerating hypertrophic chondrocytes [dark (da) and light (li)] are shrunken with almost no electron dense bodies in the matrix between the lacunae (X 6500).

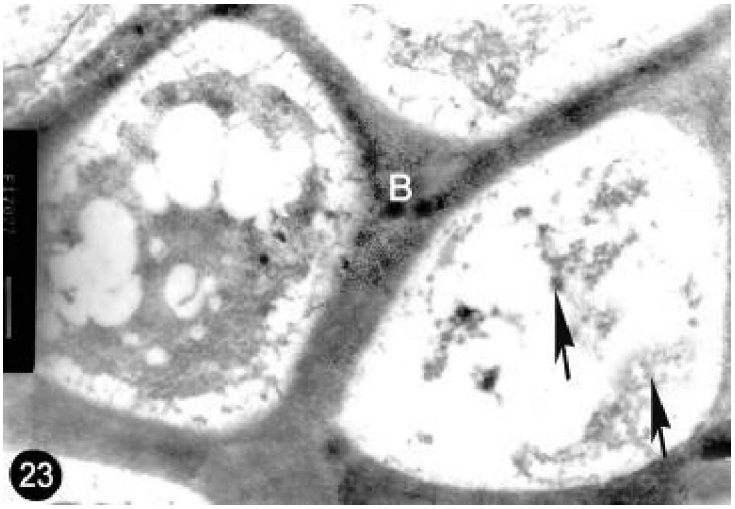

Figure (23): An electron micrograph from the tibia proximal head of Dexa treated group showing that few electron dense bodies (B) and many lacunae with cellular remnants (arrows) are observed deeply. (X 6500).

4. Discussion

Growth is commonly understood as a progressive increase in the size of various parts and organs of the body. It is characterized by changing height velocity from infancy to adulthood. The normal growth is regulated by complex interactions between various hormones and the tissue responsiveness depending upon nutritional state, genetic factors, socioeconomic status and season. Growth is controlled by dynamic and complex multisystem interactions that make the child return to its path of growth after any deviation. Although height and weight are sensitive indicators of overall health, height is considered to be a more accurate measure of growth process **[20,21]**.

Growth failure is a distinctive feature of many chronic diseases in children as chronic renal failure, inflammatory bowel diseases, rheumatoid arthritis and cystic fibrosis as well as some drugs as corticosteroids. Various clinical conditions, such as juvenile rheumatoid arthritis, chronic asthma and post-renal transplantation, require prolonged corticosteroids therapy which is associated with marked skeletal growth retardation in children **[15,16,22]**.

In the present work, examination of the proximal heads of the tibia of the Dexa treated young male albino rats showed an observable reduction in the thickness of the epiphyseal growth plate in comparison with that observed in the control group. Less frequent cells were observed particularly in the resting zone. The column organization showed disruption with wide matrix areas between cells. It has been reported **[8]** that the reduction in growth plate height (thickness) indicate a disruption of the equilibrium between cartilage production and bone formation. Several different theories have been

suggested in an attempt to explain the cause of these changes in growth plate; not only the reduction in the width but also the alterations in its architecture. Certain study **[23]** in nepherctomized rats treated with methylprednisolone attributed these changes to the ability of corticosteroids to reduce the expression of growth hormone receptors on chondrocytes and consequently inhibit the activity of growth hormone. Others **[24]** claimed that corticosteroids decreased chondrocytes proliferation and increased apoptosis. This premature loss of chondrocytes diminished the final height of the plate. Other scientists **[16]** demonstrated that growth plate insulin-like growth factor I (IGF-I) expression and production by chondrocytes have a crucial role in longitudinal skeletal growth. Corticosteroid have been shown to suppress or down-regulate IGF-I gene expression. Other group of researches focused on which zone(s) have been affected. Some authors **[25]** attributed the decrease in overall height of the growth plate to decrease in the number of resting, proliferative and hypertrophic chondrocytes. Others**, [26]** reported that the growth plate became thinner because of prominent decreased in the thickness of the proliferative zone. In contrary, certain research **[27]** documented that total growth plate width was not affected. Although dexamethasone administration led to marked reduction in the proliferative zone, it was compensated by prominent increase in the hypertrophic zone. This increase of the hypertrophic zone could be caused by an accelerated differentiation of proliferative chondrocytes under the effect of IGF-I. This premature maturation of chondrocytes has previously been shown to lead to growth retardation.

In this study, the extracellular matrix (ECM) of Dexa treated group showed weak staining affinity for alcian blue in comparison with that observed in the control group. Slightly deep stained matrix was still present around the residual chondrocytes. It was known **[1,28,29]** that chondrocytes can produce large amount of strong basophilic extracellular matrix rich in collagen fibers and acid proteoglycan molecules that consist of proteins with attached chains of polysaccharides called glycosaminoglycans. This matrix determines the mechanical properties of the tissue and contributes to the structural arrangement of the growth plate. Additionally, it was reported **[30-32]** that corticosteroids injection had deleterious effect on various tissues, including cartilage. They can act on cartilage tissue by inhibiting certain processes as proliferation and maturation of chondrocytes or impairment in their synthetic capability and deposition of important matrix constituents. These changes in the matrix were in concomitant with alteration of the rough endoplasmic reticulum and the Golgi apparatus of chondrocytes. The impairment may be just by marked reduction in proteoglycan synthesis only **[33]** or with also reduction in collagen and protein synthesis **[34]**. Vitro studies **[28],** had been showed that various corticosteroids, including dexamethasone, hydrocortisone, and betamethasone, inhibit human glycosaminoglycan biosynthsis by reduction in the number and function of chondrocytes. Other study **[35]** claimed that corticosteroids decreased IGF-I production, induced IGF-I resistance and reduced the production of C-type natriuretic peptide which resulted in a reduction of chondrocyte proliferation and also matrix synthesis.

The result of this search revealed many chondrocytes in resting zone exhibited deeply stained nuclei, vacuolated cytoplasm and were shrunken within lacunae. Longitudinal columns in proliferative and hypertrophied zones became disarranged with some deeply stained chondrocytes were observed in both zones. Some of light chondrocytes had small electron dense nuclei. Others showed irregular nuclear envelope and vacuolated cytoplasm. Previous study **[36]** reported that corticosteroids not only suppress the proliferation of chondrocytes in the proliferative zone but also increasing the number of apoptotic chondrocytes in the hypertrophic zone. Another study **[17]** clarified that darkly-stained chondrocytes were demonstrated mainly in terminally differentiated hypertrophic chondrocytes. Chondrocytes similar to most other mammalian cells are physiologically eliminated via programmed cell death. Apoptotic cells are characterized by nuclear chromatin condensation into dark crescents, caps, spheres and so called shrinks size. Such changes are secondary to DNA fragmentation and intracellular disintegration **[2]**. Some authors **[24]** explained the presence of these apoptotic chondrocytes in both resting and proliferative zones by down-regulation of anti-apoptotic proteins Bcl-2 and Bcl-x as well as an increase in capase-3. Other scientists **[4,37]** attributed the dexamethasone induced apoptosis to activation of caspases and suppression of the phosphatidylinositol 3'-kinase (PI3K) signaling pathway; a family of enzymes involved in cellular functions as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. Also, PI3K can delay apoptosis by inactivating a number of pro-apoptotic proteins, including pro-apoptotic Bax protein, caspase-9 and glycogen synthase kinase-3 beta. However, others **[13]** stated that Dexa activates the pro-apoptotic protein Bax in growth plate chondrocytes and that Bax plays a key role in the pathogenesis of Dexa induced bone growth impairment by triggering apoptosis and premature loss of growth plate chondrocytes. They also added that Bax-deficient mice were significantly protected from Dexa-induced chondrocyte apoptosis and bone growth

impairment. Another opinion **[38]** focused in growth hormone (GH) and its central role in the promotion of growth of various tissues through stimulation of IGF-I production from the liver and growth plate. In the epiphyseal plate, IGF-I acts in an autocrine or paracrine manner by stimulating the expansion of proliferating chondrocytes. They attributed the inhibitory effect of corticosteroids on chondrocyte proliferation through interference with the GH/IGF-I axis. In addition, to the previous indirect effect of corticosteroids, corticosteroids reduce the expression of the growth hormone receptors on chondrocytes **[39]**.

In this work, dark chondrocytes with more electron density were observed in resting, proliferative and hypertrophic zones. Some of these cells had markedly dilated cisternae of rough endoplasmic reticulum. Others showed swollen mitochondria, fragmented cisternae of rough endoplasmic reticulum and progressive fragmentation of cytoplasm. The dilated cell organelles had been attributed to greater secretory function of these dark cells than the light ones **[40]**. However, some researchers **[41]** reported that dark chondrocytes appeared to eliminate themselves by forming vacuoles with digesting and expelling their cellular content. The lysosomal acid phosphatase has been claimed to play a role in this digestion and that cytoplasmic digestion preceded any nuclear changes. Furthermore, **[42]** the mechanism of death that involving 'dark chondrocytes' seems to be rather difference than classical apoptosis. In 'dark chondrocytes', cell death was preceded or accompanied by a massive expansion of the endoplasmic reticulum, suggesting an increased rate of protein synthesis and/or secretion. So when the 'dark chondrocytes' shrunk to the centre of the lacunae, the space between the cell and the lacunar wall was filled by a matrix which consisted, at least partly, of proteoglycans. It was stated **[43]** that dexamethasone administration led to down-regulation or affection of expression of vascular endothelial growth factor (VEGF) by epiphyseal chondrocytes that have the ability to synthesize and secrete this factor. VEGF is an important angiogenic factor responsible for new blood vessel formation which highly concentrated in hypertrophic zone. So, subsequently it led to delay bone formation.

In the present study, almost no electron dense bodies between the lacunae of shrunken degenerating hypertrophic chondrocytes (dark and light) were observed**.** Deeply, few electron dense bodies appeared and many lacunae were noticed with cellular remnants. It had been reported **[44,45,46]** that corticosteroids increase renal calcium excretion and decrease gastrointestinal calcium absorption in part by opposing the action of vitamin D as well as by decreasing the expression of calcium channels in the duodenum resulting in reduced serum calcium. This resulted in decreasing transcellular active calcium transport and normal calcium uptake by brush-border membrane vesicles and decrease synthesis of calcium-binding proteins as well.

In summary, our study revealed that dexamethasone has a damaging effect on growth plate. Firstly, it inhibited chondrocytes proliferation which indicated by reduction in the growth plate thickness, less frequent cells with wide areas in between and disruption of column organization. Secondly, it impaired matrix synthesis which indicated by weak staining affinity for alcian blue and almost few or no electron dense bodies in the matrix between the lacunae of calcified zone. Thirdly, dexamethasone induced apoptosis of many chondrocytes starting even from resting zone and increasing progressively in other zones.

From these results we concluded that dexamethasone administration led to an observable alteration in the structure of the growth plate of young male albino rats. So, corticosteroids might slow longitudinal bone growth and induced growth retardation. We recommended that corticosteroids need to be used judiciously and cautiously.

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References

1. **MacRae VE, Farquharson C and Ahmed SF.** The pathophysiology of the growth plate in juvenile idiopathic arthritis. Rheumatology 2006; 45:11-19.
2. **Burdan F, Szumilo J, Korobowicz A, Farooquee R, Patel A, Dave A, Szumilo M, Solecki M, Klepacz R and Dudka J.** Morphology and physiology of the epiphyseal growth plate. Folia Histochemica Et Cytobiologica 2009; 47: 5-16.
3. **Villemure I and Stokes IAF.** Growth plate mechanics and mechanobiology. A survey of present understanding. Journal of Biomechanics 2009; 42: 1793-1803.
4. **Jabłoński M, Turzanska K, Gorzelak M and Orlicz-Szczesna G.** Histological changes in the epiphyseal plate of growing young adult male rats after long-term hydrocortisone and salmon calcitonin administration. Endokrynol Pol 2011; 62: 18-23.
5. **Ahamed YA, Tatarczuch L, Pagel CN, Davies HMS, Mirams M and Mackie EJ.** Physiological

death of hypertrophic chondrocytes. Osteoarthritis and Cartilage 2007; 15: 575-586.

6. **Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS and Mirams M.** Endochondral ossification: How cartilage is converted into bone in the developing skeleton. The international Journal of Biochemistry and Cell Biology 2008; 40: 46-62.
7. **Mackie EJ, Tatarczuch L and Mirams M.** The skeleton: A multi-functional complex organ. The growth plate chondrocyte and endochondral ossification. Journal of Endocrinology 2011; 211: 109–121.
8. **Carbajo E, Lopez JM, Santos F, Ordonez F, Nino P and Rodriguez J.** Histological and dynamic changes induced by chronic metabolic acidosis in rat growth plate. J Am Soc Nephrol 2001; 12: 1228–1234.
9. **Farnum CE and Wilsman NJ.** Converting a differentiation cascade into longitudinal growth stereology and analysis of transgenic animals as tools for understanding growth plate function. Curr Opin Orthop 2001; 12:428-433.
10. **Ballock RT and O'Keefe RJ.** Physiology and pathophysiology of the growth plate. Birth Defects Res 2003; 69(2): 123-143.
11. **Atabek ME, Pirgon O, Kurtoglu S, Tavli L, Esen HH, Koylu O and Erkul I.** Effect of phototherapy on growth plate in newborn rats. J Pediatr Orthop 2006; 26:144–147.
12. **Emons J, Chagin AS, Savendahl L, Karperien M and Wit JM.** Mechanisms of growth plate maturation and epiphyseal fusion. Horm Res Paediatr 2011; 75:383–391.
13. **Zaman F, Chrysis D, Huntjens K, Fadeel B and Sävendahl L.** Ablation of pro-apoptotic protein Bax protect mice from corticosteroids-induced bone growth impairment. PLoS One 2012; 7(3):1-12.
14. **Mclaughlin F, Mackintosh J, Hayes BP, Mclaren A, Uings IJ, Salmon P, Humphreys J, Meldrum E and Farrow SN.** Corticosteroids-induced osteopenia in the mouse as assessed by histomorphometry, microcomputed tomography and biochemical markers. Bone 2002; 30:924 – 930.
15. **Mushtaq T and Ahmed SF.** The impact of corticosteroids on growth and bone health. Arch Dis Child 2002; 87:93–96.
16. **Fernandez-Cancio M, Esteban C, Carrascosa A, Andaluz TN and Audi L.** IGF-I and not IGF-II expression is regulated by corticosteroid in human fetal epiphyseal chondrocytes. Growth Hormone and IGF Research 2008; 18: 497–505.
17. **Chrysis D, Ritzen EM and Sävendahl L.** Growth retardation induced by dexamethasone is associated with increased apoptosis of the growth plate chondrocytes. Journal of Endocrinology 2003; 176: 331–337.
18. **Bancroft J. and Gamble A.** Theory and Practice of Histology Techniques. 6th ed., pp: 83-92, Churchil Livingstone, New York and London, 2008.
19. **Singh D.** Principle and Techniques. In Histology, Microscopy and Photography. 1st ed., Pp: 679-699, CBS Puplishers and Distributers. New Delhy, Bangalore (India), 2003.
20. **Saxena A, Phadke SR and Agarwal SS.** Linear catch-up growth. Indian J. Pediatrics 2000; 67(3): 225-230**.**
21. **Wolthers OD.** Impact of inhaled and intranasal corticosteroids on the growth of children. Bio Drugs 2000; 13(5): 347-357.
22. **Luca FD.** Impaired growth plate chondrogenesis in children with chronic illnesses. Pediatric research 2006; 59:625-629
23. **Sanchez CP and He YZ.** Alterations in the growth plate cartilage of rats with renal failure receiving corticosteroid therapy. Bone 2002; 30(5): 692-698.
24. **Chrysis D, Zaman F, Chagin AS, Takigawa M and Sävendahl L.** Dexamethasone induces apoptosis in proliferative chondrocytes through activation of capases and suppression of the AKt-phosphatidylinositol 3-kinase signaling pathway. Endocrinology 2005; 146(3): 1391-1397.
25. **Lui JC and Baron J.** Effect of corticosteroid on growth plate. Endocr Dev 2011; 20: 187–193.
26. **Tomaszewska E, Dobrowolski P and Puzio I.** Morphological changes of the cartilage and bone in newborn piglets evoked by experimentally induced corticosteroids excess during pregnancy. J Anim Physiol Anim Nutr 2012; 21:1-12.
27. **Smink JJ, Koster JG, Gresnigt MG, Rooman R, Koedam JA and Van Buul-offers SC.** IGF and IGF-binding protein expression in the growth plate of normal, dexamethasone-treated and human IGF-II transgenic mice. Journal of Endocrinology 2002; 175: 143-153.
28. **Hauser RA.** The deterioration of articular cartilage in osteoarthritis by corticosteroids injections. Journal of Prolotherapy 2009; 2: 107-123.
29. **Halawa AM.** Effect of ciprofloxacin on the articular cartilage and epiphyseal growth plate cartilage in the growing albino rats and the possible protective role of vitamin E (α-tocopherol): A histological and mophometric study. Egypt J Histol 2010; 33(3): 569-582.
30. **Mcdonough AL.** Effects of corticosteroids on articular cartilage: A review of literature. Phys Ther 1982; 62: 835-839**.**

31. **Ranz FB, Aceitero J and Gaytant F.** Morphometric study of cartilage dynamics in the chick embryo tibia. II Dexamethasone-treated embryos. J Anatomy 1987; 154: 73-79.
32. **Annefeld M.** Changes in rat epiphyseal cartilage after treatment with dexamethasone and glycosaminoglycan-peptide complex Path Res Pract 1992; 188: 649-652.
33. **Mushtaq T, Farquharson C, Seawright E and Ahmed SF.** Corticosteroids effects on chondrogenesis, differentiation and apoptosis in the murine ATDC5 chondrocyte cell line. Journal of Endocrinology 2002; 175: 705-713.
34. **Shapiro PS, Rohde RS, Froimson MI, Lash RH, Postak P and Greenwald AS.** Effect of local corticosteroids or ketolac exposure on histologic and biochemical properties of rabbit tendon and cartilage. HAND 2007; 2:165–172.
35. **Olney RC.** Mechanism of impaired growth: effect of steroids on bone and cartilage. Horm Res 2009; 72: 30-35.
36. **Smink JJ, Buchholz IM, Hamers N, van Tilburg CM, Christis C, Sakkers RJB, de Meer K, van Buul-Offers SC and Koedam JA.** Short-term corticosteroids treatment of piglets causes changes in growth plate morphology and angiogenesis. Osteo Arthritis and Cartilage 2003; 11:864-871.
37. **Peng L, Wu TT, Tchieu JH, Feng J, Brown HJ, Feng J, Li X, Qi J, Deng H, Vivanco I, Mellinghoff IK, Jamieson C and Sun R.** Inhibition of phosphatidylinositol 3-kinase-Akt pathway enhances gamma-2 herpes virus lytic replication and facilitates reactivation from latency. Journal of General Virology 2010; 91: 463-469.
38. **Barbosa APF, Silva JDP, Fonseca EC, Lopez PM, Fernandes MBC, Balduino A and Duarte MEL.** Response of the growth plate of uremic rats to human growth hormone and corticosteroids. Brazilian Journal of Medical and Biological Research 2007; 40: 1101-1109.
39. **James CG, Ulici V, Tuckermann J, Underhill TM and Beier F.** Expression profiling of dexamethasone-treated primary chondrocytes identifies targets of corticosteroids signaling in endochondral bone development. BMC Genomics 2007; 8: 205-232.
40. **Erenpreisa J and Roach H.** Aberrant death in dark chondrocytes of the avian growth plate. Cell death and Differentiation 1998; 5: 60-66.
41. **Bowen ID, Mullarkey K and Morgan SM.** Programmed cell death during metamorphosis in the blow-fly, Calliphora Vomitoria. Microsc. Res. Tech. 1996; 34: 202-219.
42. **Roach HI and Clarke NMP.** Physiological cell death of chondrocytes in vivo is not confined to apoptosis: New observations on the mammalian growth plate. J Bone Joint Surg. 2000; 82: 601-614.
43. **Koedam JA, Smink JJ and Van Buul-Offers SC.** Corticosteroid inhibits vascular endothelial growth factor expression in growth plate chondrocytes. Molecular and Cellular Endocrinology 2002; 197: 35-44.
44. **Chan-Lam D, Prentice AG, Copplestone JA, Weston M, Williams M and Hutton CW.** A vascular necrosis of bone following intensified steroid therapy for acute lymphoblastic leukaemia and high-grade malignant lymphoma. Br J Haematol 1994; 86: 227-230.
45. **Robson H, Siebler T, Shalet SM and Williams GR.** Interactions between GH, IGF-I, corticosteroid and thyroid hormones during skeletal growth. Pediatric Research 2002; 52(2):137-147.
46. **Donatti TL, Koch VHK, Takayama L and Pereira RMR.** Effects of glucocorticoids on growth and bone mineralization**.** Jornal de Pediatria 2011; 87(1): 4-12.

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