The effects of bisphosphonate administration on teeth development and growth of the jaw bones in neonatal rats (histological and immunohistochemical study)

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ABSTRACT

Background: Bisphosphonates are potent inhibitors of osteoclastic bone resorption and widely used for the treatment of osteoporosis, and osteogenesis imperfecta in children. Clinical and experimental studies have demonstrated that Bisphosphonates delay or inhibit tooth eruption. This study tries to focus on the effect of bisphosphonate on teeth development and jaw bones growth.

Materials and methods: The present study includes 65 neonatal rats during lactation period from 15 Albino Wister rats mother. Alendronate (one type of Bisphosphonates) was administrated orally (15 mg/kg) into 10 pregnant rats two times a week, while other 5 rats regard as control. Then the neonatal rats sacrificed in l, 6, 11, 16 and 21 days. The lower first molar were examined histologically and immunohistochemical for amelogenin expression. Biochemical serum analysis for calcium and alkaline phosphatase level were down for 11, 16 and 21days group. All histological, immunohistochemical, and biochemical results are compare with their controls.

Results: The histological results illustrate retardation in tooth and root development, impairment in maturation of enamel and retardation in tooth eruption of the first molar tooth germ in alendronate treated neonatal rats than their controls. Also immunoreactivity for amelogenin at early stages of tooth development was somewhat more intense in experimental group than that in their controls .Moreover, calcium and alkaline phosphatase serum levels in experimental rats are less than that of their controls.

Conclusion: This study concludes that treatment with alendronate during tooth development has the potential to inhibit tooth eruption, impair tooth formation, may induce some types of dental abnormalities, and increase the bone trabecule thickness by decreasing osteoclastic activity.

Key word: bisphosphonate, tooth development, amelogenin. (J Bagh Coll Dentistry 2013; 25(Special Issue 1):77-82).

INTRODUCTION

Bisphosphonates (BPs) are synthetic, nonhydrolyzable analogues of inorganic pyrophosphate, a naturally occurring compound in which 2 phosphate groups are linked by esterification [(1)]. There are two groups of bisphosphonate both of them work in different way to suppress the osteoclastic activity: Nitrogen containing and Non-nitrogen containing [(2)]. Bisphosphonates promote the apoptosis of osteoclasts, its activity engaged in the degradation of mineral on the bone surface. Such excessive resorption underlies several pathologic conditions for which bisphosphonates are now commonly used, including osteogenesis imperfecta and any other conditions involving fragile, breakable bone such as osteoporosis and malignancy metastatic to bone [(3)]. Alendronate is a potent nitrogen-containing bisphosphonate that become the primary therapy for managing skeletal conditions characterized by increased osteoclast-mediated bone resorption [(4)]. Amelogenin belongs to a family of extracellular matrix proteins.

The function amelogenin is not completely understood, it is believed to be in organizing enamel rods during tooth development. Researches' indicates that this protein regulates the initiation and growth of hydroxyapatite crystals during the mineralization of enamel [(5)]. Although the mode of action of alendronate is mainly being investigated in bone, little is known about its effects on the formation of dental hard tissues.

MATERIALS AND METHODS

The present study includes 65 neonatal rats during lactation period from 15 Albino Wister rats mother which were taken from the animal house of the National Center of Drug Control and Research in Baghdad. The rat's mothers were dividing into two groups: experimental group contain 10 mothers which administrate oral dose (15 mg/kg) [(6)] of sodium alendronate twice a week from first day of gestation to sacrifice day of neonatal rats, while control group contain 5 mothers which administrate with normal saline twice a week. Blood samples were obtained from 11, 16 and 21 days neonatal rats in sacrificing day to find the alkaline phosphatase and calcium levels in both groups. Then the neonatal rats were sacrificed in 1, 6, 11, 16, 21 days. The head separated from the body, blocked, and then processed for sectioning. The sections were

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histologically studied by (H&E) stain and Immunohistochemical study for amelogenin expression by (Anti amelix anti body) from Abcam company (ab59705).

RESULTS

Clinical finding

There are significant increases in size (weight & length) of control group when compared with alendronate-treated group especially in age group 6, 11, 16 days rats **(Figure 1,2).**

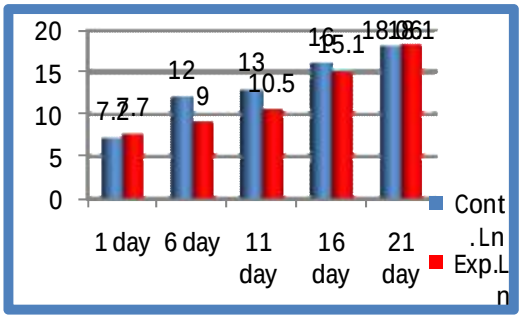

Figure 1: Mean length of neonatal rats in different groups

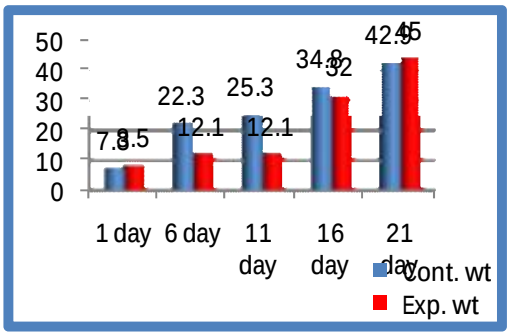

Figure 2: Mean weight of neonatal rats in different groups

Biochemical findings

The biochemical serum analysis revealed that the level of calcium and alkaline phosphatase in alendronate treated rat are lesser than that of control rat **(Figure 3,4).**

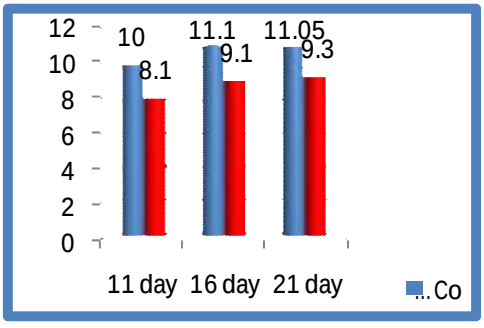

Figure 3: Mean of Calcium serum level of neonatal rats.

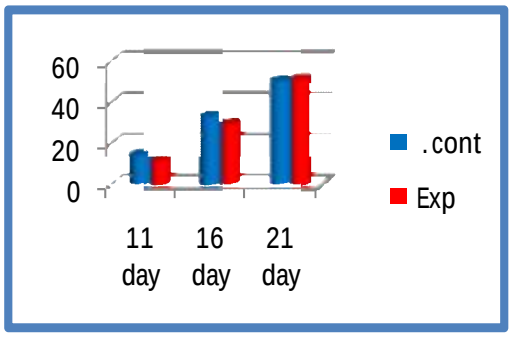

Figure 4: Mean of ALK serum level of neonatal rats in different groups.

Histological & immunohistochemical findings

The histological results of 1 day age group of this study are almost similar in both control and alendronate-treated groups and it shows that the 1st molar teeth germs are at advance bell stage **(Figure 5&6).**

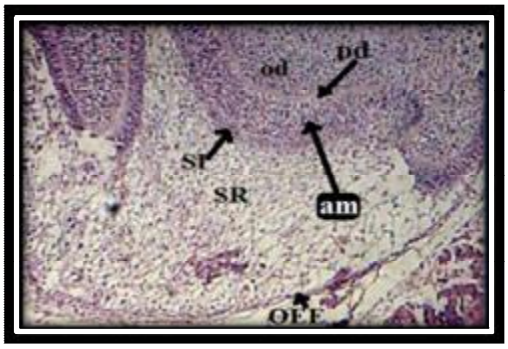

Figure 5: First molar 1 day tooth germ (control) show ameloblasts (am) , stellate reticulum (SR), stratum intermedium (SI) and outer enamel epithelium (OEE). H&E ×200.

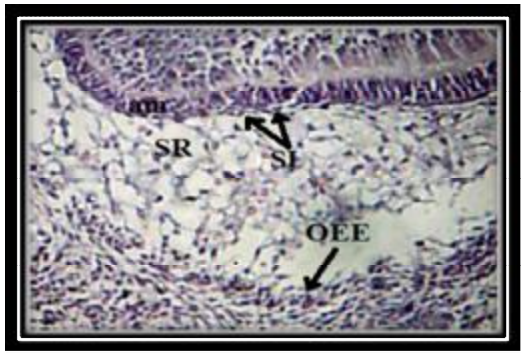

Figure 6: First molar 1 day tooth germ (experimental) show odontoblasts (od), ameloblasts (am), stratum intermedium (SI), stellate reticulum (SR), and outer enamel epithelium (OEE). H&E ×100.

Immunoreactivity for amelogenin illustrate that this protein was expressed in ameloblasts of 1 day experimental group somewhat more intense than that observed in controls (**Figure 7&8).**

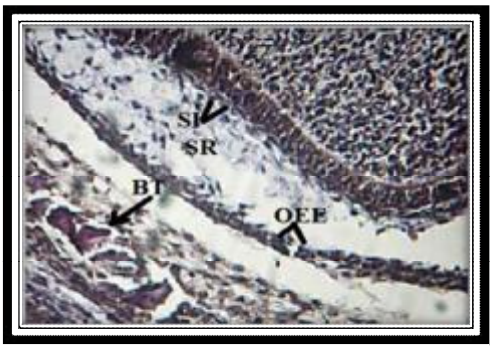

Figure 7: View of 1 day tooth germ (control) show positive expression of amelogenin in ameloblasts. DAB stain with counter stain hematoxylin × 200.

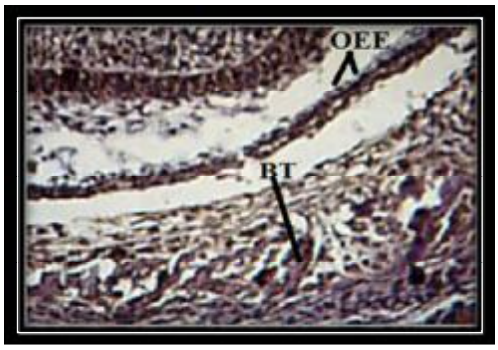

Figure 8: View of 1 day tooth germ (experimental) show strong positive expression in ameloblasts. DAB stain with counter stain hematoxylin ×200.

Histological and immunohistochemical pictures of 6 days group showed retardation in development and impairment in maturation of enamel of first molar tooth germ in experimental rats when compared with their controls (**Figures 9, 10).**

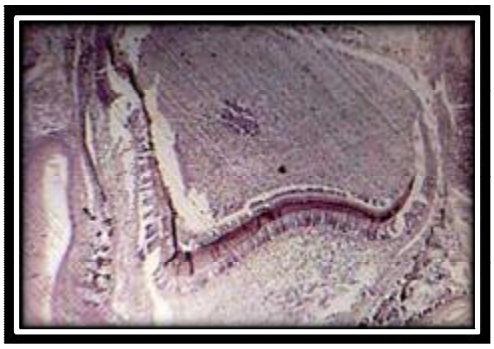

Figure 9: View of the first molar tooth germ of 6 days (control) show hard tissue formation. H&E ×40

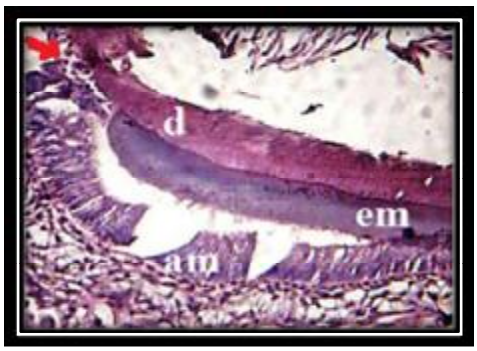

Figure 10: View of 6 days first molar tooth germ (Experimental) show attachment of ameloblasts to the dentin without enamel matrix formation (red arrow). H&E × 200.

The same results of 6 days group are seen in 11 days group in addition to retardation of root formation of first molar tooth germ in experimental rats when compared with their controls **(Figures 11, 12, 13&14).**

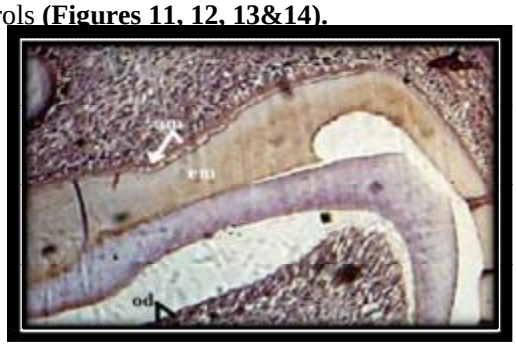

Figure 11: View of 11 day first molar tooth germ (control) show positive expression of amelogenin in ameloblasts (am) and weak expression in enamel matrix (em) . DAB stain with counter stain hematoxylin X200.

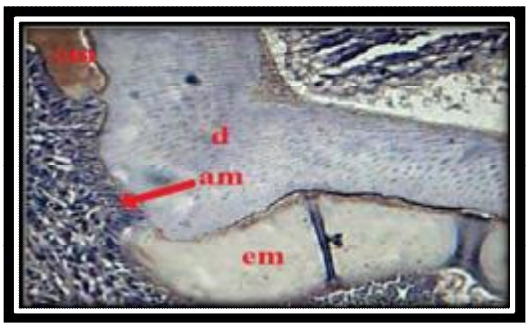

Figure 12: View of 11 days tooth germ (experimental) show negative expression of amelogenin in dentin (d) which attached directly to the ameloblasts (am) without enamel matrix (em) formation (arrow). DAB stain with counter stain hematoxylin ×400.

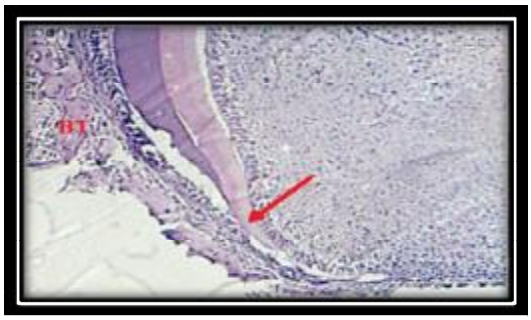

Figure 13: View of first molar tooth germ 11 days (control) show the beginning of root dentin formation (arrow). Bone trabecule (BT) surround the tooth germ. H&E ×100.

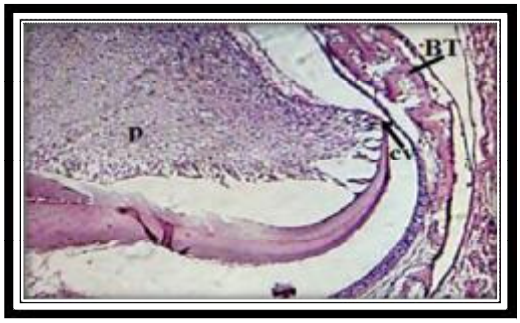

Figure 14: View of first molar tooth germ of 11 day experimental rat show the cervical loop (cv) and bone trabecule (TB) which surround the tooth germ. H&E × 200.

In 16 days control rat show full crown formation, full thickness of dentin with maturation of enamel by presence of enamel space**.** Ameloblasts will fuse with other layer of enamel organs and formed reduced enamel epithelium. While experimental group showed almost full dentin thickness and enamel matrix of the crown were formed, although the enamel matrix was not fully mature yet **Figure 15 & 16**.

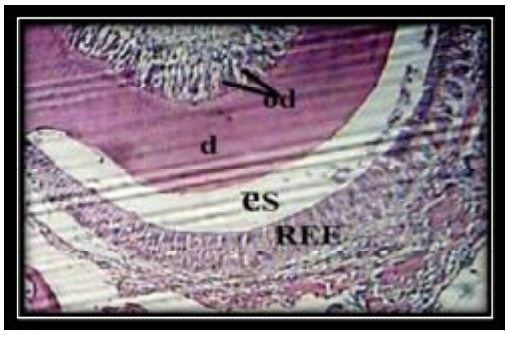

Figure 15: View of first molar tooth germ of 16 days control rat show odontoblasts (od), dentin (d), enamel space (es), reduce enamel epithelium (REE). H&E ×100.

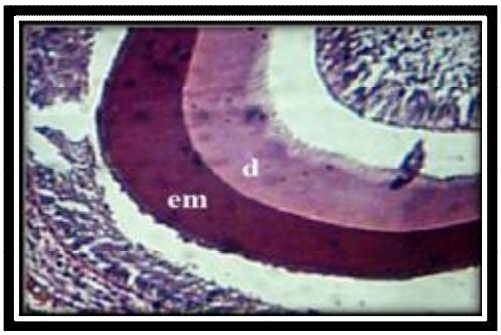

Figure 16: View of tooth germ of 16 days old of experimental group show full formation of dentin (d) and enamel matrix (em). H&E × 200.

The histological picture of the first molar tooth germ of 21 days experimental group illustrate that the tooth germ was still unerupted and root formation was short in length, when compared with their controls (**Figure 17 & 18).**

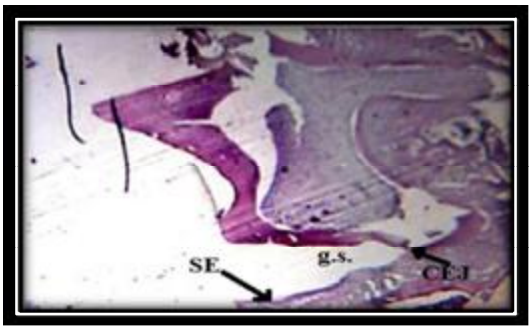

Figure 17: View of first molar tooth of 21 days (control) showed tooth at eruption stage, sulcular epithelium (SE) which surround the gingival sulcus (g.s.). Cementoenamel junction (CEJ). H&E X25.

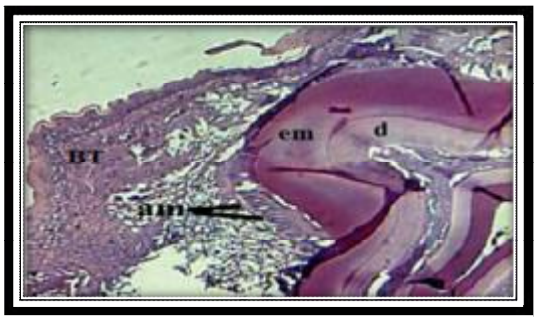

Figure 18: View of first molar tooth germ of 21 days experimental rat show unerupted tooth. Enamel matrix (em) surround by ameloblasts (am), bone trabecule surround the crown (BT). H&E ×100.

There are positive amelogenin expressions in osteoblast, osteocyte and bone matrix of both controls and experimental. The bone sections of control rats express amelogenin more than that of experimental group in almost all age groups (**Figure 19 & 20).**

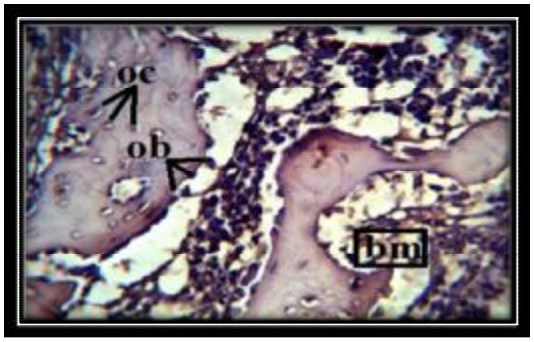

Figure 19: View of bone section (control) show positive expression of amelogenin in bone matrix (bm), osteoblast (ob), osteocyte (oc). DAB stain with counter stain hematoxylin ×400.

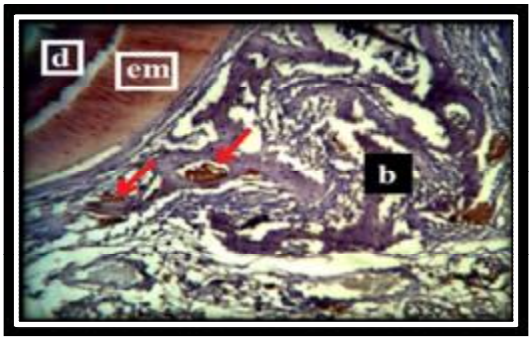

Figure 20: View of first molar tooth germ 21 days (experimental) show positive expression of amelogenin in BV of the pulp (red arrow), cementoblasts (cb) and fibroblasts of PDL (arrow). Negative expression was shown in bone trabecule (BT). DAB stain with counter stain hematoxylin ×200.

In general, the present study showed that the periodontal ligament fibroblasts were positively expresses the amelogenin in both control and alendronate-treated groups especially in 21 days group (**Figure 21 & 22).**

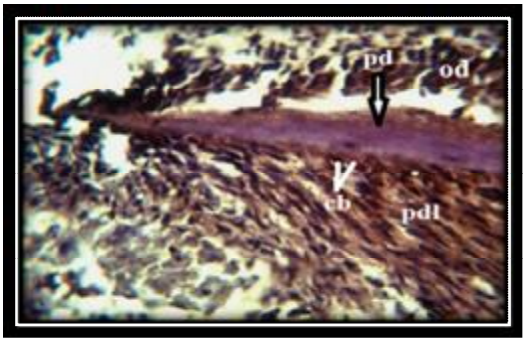

Figure 21: view of first molar erupted tooth 21days control rat show the positive expression of amelogenin in cementoblasts (cb), odontoblasts (od) and predentin (pd), and fibroblasts of periodontal ligament (pdl). DAB stain with counter stain hematoxylin ×400.

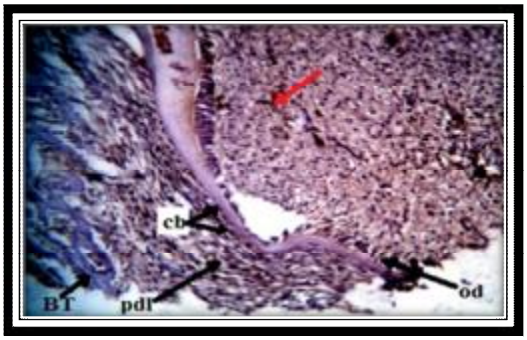

Figure 22: View of first molar tooth germ 16 days experimental rat show positive expression of amelogenin in BV of the pulp (red arrow), odontoblasts (od), cementoblasts (cb) and fibroblasts of PDL (arrow). Negative expression was shown in bone trabecule (BT). DAB stain with counter stain hematoxylin ×200.

DISCUSSION

The clinical findings of the present study showed that the alendronate treated neonatal rats were smaller in length and weight than their controls especially in rats of 6, 11, and 16 days old. These findings are due to decrease mothers activities as side effects of alendronate intake [(7)].

The biochemical serum analysis of the present study revealed reduction in the level of calcium and alkaline phosphatase in alendronate-treated neonatal rats than that of controls This reduction may result from decrease of bone turn over due to inhibition of osteoclastic activity by this medication. This result is in agreement with Iwamoto et al[(8)] . The histological feature of tooth development in one day rats are almost similar in

both alendronate treated and control groups, this agree with Massa et al [9].

Immunoreactivity for amelogenin illustrate that this protein was expressed in ameloblasts of alendronate-treated somewhat more intense than that in their controls [9].

At six days group the ameloblasts in control rats are well differentiated, good layer of enamel matrix. While in alendronate treated rats show absence of enamel matrix in some area due to the effects of alendronate on ameloblasts function during amelogenesis. This finding agrees with Fuangtharnthip et al [10]. Immunoreactivity for amelogenin was somewhat more intense in enamel matrix of alendronate-treated than that in the control. It also diffused through the dentin matrix toward the layer of odontoblasts and accumulated in the predentin, assuming an ectopical deposition clearly visible in the alendronate-treated group [11].

At 11 days old, the control group shows full thickness of enamel matrix formation with the beginning of enamel maturation. While in the alendronate treated rats there was loss of enamel matrix in some area due to the effects of alendronate on ameloblasts function during maturative stage of amelogenesis. This results agree with Hiraga et al[12]. The immunoreactivity shows weak expression of amelogenin in the enamel matrix of control and experimental groups due to maturation of enamel matrix [11].

At 16 day old, the control group show full thickness of crown formation and maturation while in alendronate treated group the enamel matrix is not fully mature yet. This agrees with Massa et al [9]. The amelogenin expression revealed almost the same result for both groups, which agree with previous study [13].

At 21 days old control rat teeth were erupted in the oral cavity and the root development were almost finished, while in alendronate treated rats molar tooth was still unerupted with short root formation Inhibited tooth eruption due to impaired osteoclastic bone resorption by this drug which is an important event in eruption process [12].

The present study concludes that treatment with alendronate during tooth development inhibit tooth eruption, and may induce some types of dental abnormalities.

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