Biological evaluation of alveolar bone remodeling in methylprednisolone treated –rats during orthodontic tooth movement

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ABSTRACT

Background: Bone remodeling and metabolism associated with orthodontic tooth movement are regulated by a large number of local and systemic factors. The widespread useof therapeutic corticosteroids (GCs) today raise concerns with regard to their effects on mineralized tissue metabolism. This study aimed to investigate the effect of Methylprednisolone treatment on alveolar bone remodeling during orthodontic tooth movement.

Materials and Methods: A twenty-six 12-weeks old male Wistar albino rats were divided into 2 groups; control group (n = 13) without any drug administration during the study and steroidal group (n = 13) which received 5 mg/kg/day of methylprednisolone for 3 weeks. A split- mouth design was used performing orthodontic tooth movement on the upper right 1st molar by applying 20 g of mesial force using superelastic closed-coil spring attached to the incisors for 21 days while the upper left side served as the non-appliance side. Orthodontic tooth movement was evaluated on weekly basis using digital caliber. The rats were sacrificed after 3 weeks and alveolar bone remodeling process was evaluated by counting the number of osteoblast and osteoclast cells at the compression and tension sites at the coronal and apical levels of the mesiobuccal root of upper 1st molar in both appliance and non-appliance sides using digital microscope at 400× magnification. At day of sacrifice serum measurements for alkaline phosphatase (ALP) and acid phosphatase (ACP) activity were carried out.

Results: Showed that in the steroid group there was significantly greater amount of orthodontic tooth movement, greater reduction of bone formation and an increase in bone resorption with the presence of orthodontic appliance, increase in serum ACP activity and reduction of serum ALP activity as compared with the control group,($P \leq 0.05$).

Conclusion: The Methylprednisolone therapy in low-medium doses elicits a noticeable change in the bone turnover rate during orthodontic tooth movement.

Keywords: methylprednisolone, tooth movement, bone remodeling. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):133-142).

INTRODUCTION

Tooth movement during orthodontic treatment is achieved by the remodeling of the alveolar bone in response to mechanical loading as the forces of orthodontic appliances applied to the teeth are transmitted through the periodontal ligament (PDL) to the supporting alveolar bone, leading to deposition or resorption depending upon whether the tissues are exposed to a tensile or compressive mechanical strain.The transduction of mechanical forces to the cells triggers a biological response, which has been described as an aseptic inflammation because it is mediated by a variety of inflammatory cytokines. [1, 2]

Investigations of the actions of hormones on bone have revealed that glucocorticoids cause marked effects on bone metabolism and that continued exposure of skeletal tissue to excessive amounts of glucocorticoids results in osteoporosis. However, the exact mechanisms by which glucocorticoids act on bone are unknown. [3]

It has been shown that orthodontic tooth movement may be influenced by general and local administration of pharmaceutical agents.[4-9]As the prevalence of allergies and diseases that need corticosteroidtreatment is on the increase, it can be anticipated that an important number of orthodonticpatients can present variations from normal boneremodeling because of this steroid.[10] In most of thepublished animal experiments that studied glucocorticoidadministration and orthodontic tooth movement, the glucocorticosteroid dose has been high. These high doses made the animals osteoporotic. Daily injections (15 mg/kg) of glucocorticosteroid drug caused amarked state of osteoporosis in a short time period inthe rabbit [11, 12] and even higher doses (25 mg/kg) have been used in cats.[13]The dosages used in theabove-mentioned studies, however, are not compatiblewith the

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concentrations recommended for use inhumans, either for short or long durations. Yamane*et al.*[14]used a dosage of 10 mg/kg for only 7 days. Ong*et al.*[15] used a therapeutic dosage of 1 mg/kg in young rats for short-term, thus avoiding the risk ofsecondary hyperparathyroidism. Whereas a study performed by Kalia*et al.*[5] used a dosage of 8 mg/kg/day for shortand long-term administration, showed the mechanical load induced an enlargement of the alveolar wall that was less pronounced in both medicated groups, and in the short-term group the drug suppressed bone resorption and formation without mechanical stimulus. Force application resulted in significant increase in the relative extension of resorption and formation in both drug groups; it was particularly pronounced in the long-term group due to the secondary hyperparathyroidism state that the animals reached. The differences in the results of these studies probably reflect the combined effects of the dosages, the induction periods, and the amount of orthodontic force applied and the relative anti-inflammatory activity of the glucocorticoids tested.

In the present study, the effect of methylprednisolone (one of the most widely used corticosteroids) on bone metabolism in a rat model was tested with therapeutic dosages of 5 mg/kg/day to examine the effect of low dose prednisolone treatment on bone remodeling during orthodontic tooth movement.

The effect of treatment was evaluated by measuring the rate oforthodontic tooth movement, and analysis of bone remodeling patterns through thequantification of both the resorptive and formative components of the remodeling cycle (osteoclast and osteoblast cells counting), and by biochemical investigation of both alkaline phosphatase and acid phosphatase enzymes activity as the alkaline phosphatase enzyme is observed to be associated with osteoblastic activity whereas acid phosphatase enzyme is observed to be associated with osteoclastic activity. [16-18]

MATERIALS AND METHODS

Animals and Steroid treatment

Twenty-six 12-week-old adult male Wistar albino rats (average weight270.5 g) obtained from the animal department of (High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain university/Baghdad-IRAQ) were used in this study. Animals were acclimatized for 5 days in plastic cages (two per cage) with a standard 12-hour light/ dark cycle at a constant humidity and temperature of 25°C according to the National Research Council's guide for the care and use of laboratory animals and accessed to drinking water *ad libitum* with standard laboratory rat pellets. Body weights of all rats were measured daily.All rats received orthodontic treatment for 3 weeks and were divided randomly in two groups: control group (n=13) without corticosteroid treatment and steroid group (n = 13) administered daily doses of 5 mg/kg/day of methylprednisolone (Solu-medrol; Pharmacia NV/SA, Puurs - Belgium) intramuscularly for the prescribed number of days.

Orthodontic appliance treatment

Following acclimatization, an orthodontic appliance was inserted on the maxillary right first molar, and a mesially directed force of 20 g was applied. The orthodontic appliance consisted of a stretched superelastic (rematitan®)closed coil spring (9 mm in length, Dentaurum, Germany) ligated between the maxillary right first molar and 2 maxillary central incisors as described previously byMohammed-Salih[19]. The molar on the left side was used as the non-appliance side, (Fig. 1). The magnitude of tooth movement was determined by measuring the relative separation between the first andsecond maxillary molar using digital vernier calipers with sharpenedtips inserted into occlusal pits as the procedure modified by Ong*et al.*[15]. The distance betweenthe mesial occlusal pits on the first and second molars was measured intraorally before appliance insertion and at the end of the first, second and third week of the study (immediately after sacrifice). All appliances were checked weekly and at the time of sacrifice and all appliances were still in place and in good order.Measurements were performed by thesame operator and were repeated five times foreach side of the maxilla.Rats were sedated during appliance insertion using intramuscular injection of a mixture of ketamine (90 mg/kg body weight) and xylazine (10mg/kg body weight).

Histological Preparation

At 21 days post-appliance insertion, rats were sacrificed humanly under general anesthesia.Maxillae were immediatelyremoved, (Fig. 2) and dissected into halves, fixed in 10% neutral-buffered formalin solution for 24-48 hours and all the specimens from each group were decalcified by 10% formic acid for 3-4 weeks; it was checked every 4 days with changing of the acid, after that dehydration were done and paraffin cross- sections of 5 µm thick were prepared(parallel to the occlusal plane of molar teeth) with microtome. At the coronal and apical level two 5 µm thick horizontal sections, 150 µm apart, were cut. The coronal and apical levels

were defined using as a start the first section showing bone on the non-appliance side. Distance from the lower coronal section to the first apical section was 1150 µm.[20]The sections were stained with hematoxylin and eosin (H&E stain). Then sections were photographed by a photomicroscope (Olympus-Japan).

For evaluation of pathological changes consistent with the experiment. Tissues surrounding the mesiobuccal root were investigated on the appliance and contralateral non-appliance sides under digital light microscope atboth compression and tension sites and the following histomorphometric parameters were determined:

Evaluation of the Bone formation

Bone formation was evaluated at both compression/mesial andtension/distalsites at the coronal and apical levels on both appliance and contralateral non-appliance sides by estimating the number of osteoblasts cells were examined at ×400 magnification by the inbuilt image processing software of digital microscope (Micros Crocus II MCX100LCD Produktions und HandelsgmbH) that was fed directly to a TV monitor with a real time live camera.One area from each section was selected for the evaluation of bone neoformation.[21]

Evaluation of Bone Resorption

Bone resorption was evaluated at both compression/mesial and tension/distal sites at the coronal and apical levels on both appliance and contralateral non-appliance sides by estimating the number of osteoclasts cells were examinedinactive Howship's lacunae at ×400 magnification by the inbuilt image processing software of digital microscope (Micros Crocus II MCX100LCD Produktions und HandelsgmbH) that was fed directly to a TV monitor with a real time live camera. The histological criterion used to identify the osteoclast-like cells was the presence of multinuclear and eosinophilic cells on the bone surface or in bone resorptivelacunea. [6]

Serum Measurements

At sacrifice, blood was collected by cardiac puncture (2ml from each animal) after thoracotomy, into glass tubes and allowed to coagulate for30 minutes on ice. After centrifugation at ×3000 g for 20 minutes at 4C°, the serum was transferred to new tubes and frozen at − 20C°. Alkaline phosphatase (ALP) and acid phosphatase (ACP) activity were measured using method of determination as described previously by*Milne et al.* [22]

Statistical Methods

Data were expressed as the mean ± standard deviation of the mean (SD).The statistical analysis was carried out using SPSS version 15 computer program and the following tests were used:

-ANOVA test was used to determine if significant differences exist between the groups in the amount of tooth movement followed by least significant difference (LSD) test between each two groups.

-Mann-Whitney U test was used to compare between the two independent groups (control and steroid) for bone resorption and bone formation activity.

-T-test was used to compare between the means of the control and steroid groups for the serum level of ALP and ACP enzymes.

P value of ($P \leq 0.05$) was regarded as statistically significant.

RESULTS

Rate of tooth movement

On the basis of the weekly measurements, the pharmacological treatment resulted in a highly significant difference in the rate of orthodontic tooth movement which was faster in the steroid group than in the controlgroup by nearly two times after the 1st ,2nd and 3rd weeks post-appliance insertion ($p \leq 0.01$) ,(Table 1, Fig.3).

Histology

The alveolar bone remodeling process wasaffected dramatically inmedicated group than in control group with the presence of orthodontic appliance. Medicated rats differed from the controlson both the appliance and the non-appliancesides. Alveolar bone formation in the appliance sideat the compression site was significantlyreduced in the steroid group than in the control group at both levels (coronal and apical)($p \leq 0.05$),whereas non-significantly at the tension site ($p \geq 0.05$).At the non-appliance side although the results indicate there was a reduction in bone formation in the steroid group compared with the control group at both sites (mesial and distal sites)but non-significantly($p \geq 0.05$), (Table 2).

Alveolar bone resorption in the appliance side was significantly increased at both sites (compressionand tension sites) in the steroid group than in the control group at both levels (coronal and apical) ($p \leq 0.05$), except at the coronal level of the tension site was increased non-significantly($p \geq 0.05$).Also at the non-appliance side there was an increase in bone resorption in the steroid group than in the control group at both mesial anddistal sites but non-significantly($p \geq 0.05$), except there was a significant difference between them at the coronal levelof the mesial site($p \leq 0.05$), (Table 2).

Serum ALP and ACP levels

Serum ALP activity was found to have reducedsignificantly in the steroid group compared with the control group ($p \leq 0.01$), (Fig. 4). Whereas serum ACP activity showed a significantincrease in the steroid groupcompared with the control group($p \leq 0.01$), (Fig. 4).

DISCUSSION

Most in vivostudies of orthodontic tooth movement have concentrated on changes occurring within the PDL. However, the PDL can only provide a partial explanation for the mechanisms involved in dentoalveolar remodeling, and more attention has focused lately on the wider response of the alveolar bone. [23-26]Previous proposals have suggested that orthodontic loading may trigger bone remodeling by producing microdamage[27] or by stimulating the induction of a regional acceleratory phenomenon [23,25](a reaction to trauma in which the rate of bone remodeling exceeds normal tissue activity).

In the present study changes in the remodeling of alveolar boneupon 21 days of systemicglucocorticoidadministration were carried out in a rat model with and without orthodonticforces. The experimental model for mesial movement of rat molar has been repeatedly used in previous studies [5, 19, 25, 28-30]. The rat model is the standard method for the study of skeletal adaptation to mechanical stimuli[31]and to impaired metabolic conditions.[32-34]The total treatment duration of 3 weeks (pharmacological and orthodontic treatment) was chosen in order to interfere with bone metabolism for a minimumof one remodeling cycle (sigma), ranging according to various authors between 10 and 31 days [32]. According to Li *et al.*[35]the sigma of a rat changes as a function of age and at 6 months it is considered to beapproximately 21 days.

The effects of physiological and therapeutic doses of glucocorticosteroid administration (5 mg/kg/day) on alveolar bone as specified in this study with and without orthodontic movement have not been previously investigated which is comparable with low-oral dosesrecommended for more common diseases and to keep thedetrimental effects of bone loss minimal[36]. The short duration of corticosteroid administration in the present study makes the possibility of iatrogenic hypercortisonism and hyperparathyroidism remote.

The results showed ahigher rate of tooth movement was in steroid group than in control animals. This finding is consistent with a more rapid tooth movement found in animals in the acute phase of corticosteroid treatment [11, 19] and also with high bone turnover caused by secondary hyperparathyroidism during orthodontic tooth movement. [37]However, normal bone remodeling process is a fundamental to orthodontics; this increase could be explained by the effect of GCs on bone remodeling process. There is evidence that during the initial administration of corticosteroids, a period of very rapid bone loss occurs. This could be ascribed to the lack of balance between formation activities (inhibited or reduced by the drug) and the resorption activities (enhanced by drug administration) occurring in the initial phase of drug administration[19, 38, 39]. However, controversy exists as to the effects of corticosteroids on tooth movement. As noted previously, Ashcraft *et al.*[11] induced orthodontic molar tooth movement for 14 days in corticosteroid-induced osteoporotic rabbits, and showed a greater rate of tooth movement in steroid-treated rabbits. In contrast, Yamane *et al.*[14] reported that tooth movement in rats was inhibited by 10 mg/kg per day of hydrocortisone, while Davidovitch*et al.*[13] showed slower tooth movement in cats treated with cortisone acetate (12.5to 25 mg/day). These differences may be explained by variationswithin animal species studied, forces used to move teeth, duration of the experiment, dosage and time interval of administration, and potency of the steroid used. The present study used a standardized technique for inducing orthodontic tooth movement in rats as described previously by Brudvik and Rygh[40]. This technique mimics orthodontic tooth movement in humans.Experimental studies on tooth movement are often difficult to compare because of the use of different orthodontic appliances and different magnitudes, types, and duration of forces.

However, normally, a balance exists between the amount of bone resorbed by osteoclasts and the amount formed by osteoblasts to maintain a constant bone mass; in other words, bone resorption and formation are said to be coupled.

In the present study, the results showed that the steroid treatment disturbed the normal bone remodeling process in the presence of mechanical stimuli (at the appliance side) as the bone formation wasreduced at the compression (Fig.5) and tension sites (Fig.6).Alsoat the non-appliance side bone formation was reduced,but this is a reflection of steroid effect on bone, these findings consiestance with a decreased percentage of bone formation in the acute group carried by Kalia*et al.*[5] but in association with a decreased percentage of resorption activity. Such reduction of bone formation might be due to at least two different mechanisms, i.e., inhibition of osteoblast function

and inhibition of the proliferation or differentiation of precursor cells to osteoblasts. GCs have also been reported to promote the apoptosis of osteoblasts and osteocytes [41].GCs are known to have various effects on osteoblast gene expression, including down-regulation of type I collagen and osteocalcin. The expression of IGF-1, which is an important stimulator of osteoblast function, is also known to be decreased by GCs. [3]GCs at physiological concentrations are known to inducethe proliferation and differentiation of bone marrow stromalcells into cells that express a mature osteoblast phenotype,whereas GCs at higher concentrations or pharmacologicaldoses drastically reduce the proliferation ofosteoblast precursors [42]and inhibit the differentiationto mature osteoblasts.[43]

Bone resorption was increased at both appliance and non-appliance sides (Fig.7), when comparing scientific studies in the literature, itwas observed that glucocorticoids may produce antagonistic effects upon bone resorption during tooth movement. Hofbauer*et al.*[44]and Swanson *et al.*[45] affirm that corticosteroids stimulate *in vitro* bone resorption by osteoclast activity and/or formation increased, whileKalia*et al.*[5] used methylprednisolone 8 mg/kg/day under chronic and acute treatment and observed different results between the groups. In the acute, it was observed reduction on resorption percentage, while in the chronic, the tooth movement rate increased, due to secondary hyperparathyroidism. Ashcraft *et al.*[11] evaluated the effect of cortisone acetate on orthodontic movement in rabbits and observed a decrease in the mean incremental active tooth movement. Ong *et al.*[15] observed lower tartrate-resistant acid phosphatase-positive cells on the compression side after prednisolone administration. It is important to note that the glucocorticosteroid therapy is not only dose dependent but also time dependent. Many previous studies performed at 3, 14 and 21 days; there was a significant difference in the number of Howship'slacunae,therefore in the present study the use of steroid therapy for 21 days can be considered as a transition point from short to long-term of drug administration.

Studies testing the effect of glucocorticoids onbone resorption in vitro have not yielded uniformconclusions due to differences in the systems,culture conditions, and length of glucocorticoidtreatment used. Some researchers foundthat glucocorticoids inhibited PTH-stimulatedbone resorption in vitro.[46, 47]However; more recent studies havedemonstrated that glucocorticoids stimulatebone resorption in cultured calvaria.[48, 49]The effects ofglucocorticoids on osteoclast recruitment /differentiationand activity have been dissociated usingthe model system of bone chips implantedsubcutaneously into rats.[50]It was shown that glucocorticoids inhibited therecruitment and differentiation of bone resorbingcells, but stimulated the bone resorbing activity.This may be related to the hypothesized“coupling” of osteoblastic activity to bone resorption.[51]

An important interaction was noted betweenmechanical perturbation and the drug, leading to anincrease in the extension of mineralizing surfacesexceeding what was seen in the control animals.On the mesial aspect we might have generated a localized rapid acceleration phenomenon, where bone surface was subjected to a high local stress by the orthodontic appliance. This could lead to decreased resorption in some sites because of ischemia and increased in others reflecting a local repair process.

Biochemical markers of bone metabolism such as ALPand ACP levels in serum are frequently employed asadjuncts to bone mass measurements to detect systemicchanges of bone turnover in metabolic bone diseases. Eventhough serum ALP consists of several isoforms that originatefrom various tissues such as bone, liver, and kidney, it iscommonly used as a clinical marker for measuring osteoblastactivity and bone formation.[52]Thedecrease in serum ALP activity detected in the steroid group compared withthe controls was consistent with the reduction inbone formation capacity (no. of osteoblast cells) observed histologically in the present study. Since serum markers of bone metabolismreflect whole-body rates of bone formation and resorption,the loss of alveolar bone was clearly of rapid onset, resultinginsignificant osteopenia after just 2-4 days. Evidence frommicrogravity studies suggests that in addition to reducedosteoblast differentiation and function, [53, 54]osteoblast apoptosis[55] may have contributed to the osteopenia,although more recently, Bucaro*et al.*[56] reported thatthe effect of microgravity on osteoblasts was independentof the induction of apoptosis.

Theincrease in serum ACP activity suggests that bone resorption exceeds bone formation[57] may therefore be a reflection of the fact that bone formation and resorption, although both down-regulated by reduced mechanical loading,remained coupled, the outcome being a localized negativeskeletal balance of the tooth-supporting bone. Nevertheless, confirmation of this observation will require future assays of serum for the tartrate-resistant ACP5b isoform, a

unique bone resorption marker released fromresorbing osteoclast cells. [58]

Histological analyses in this study confirmed that the glucocorticoid drug (methylprednisolone) used under the conditions of this study elicits a noticeable change in the bone turnover rate. The effects on bone remodeling indicated a reduction of bone formation and increase in bone resorption and this effect was greater with the presence of theprocess of orthodontic tooth movement.

Clinically, it is fair to say that patients who are within the low-medium doses of this drug who are already undergoing orthodontic treatment should have their appointments scheduled with shorter intervals, as bone turnover will be enhanced and tooth movement would be faster to avoid and prevent any unwanted tooth movement.

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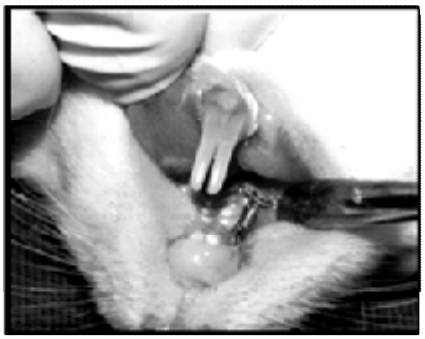

Fig.1: Experimental appliance inducing mesial traction of the rat molar (right) by a closed coil spring producing a force of 20g.

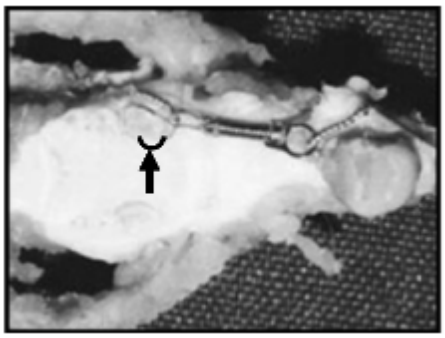

Fig.2: The steroid rat whole maxilla at sacrifice showing the distance formed at the appliance side between 1st and 2nd molar (arrow).

Table 1: The rate of orthodontic tooth movement (mm) after 1st, 2nd and 3rd weeks between the studied groups

		Control	Steroid	ANOVA	LSD
after 1 week	Range	(0.94 - 0.52)	(1.9 - 1.17)	P ≤0.01	1 week x 2 weeks P ≤0.01
	Mean	0.8	1.6		
	SD	0.1	0.3		
	SE	0.04	0.08		
	t-test	P ≤0.01			
after 2 weeks	Range	(1.24 - 0.82)	(2.62 - 1.54)		1 week x 3 weeks P ≤0.01
	Mean	1.1	2.2		
	SD	0.1	0.4		
	SE	0.04	0.11		
	t-test	P ≤0.01			
after 3 weeks	Range	(1.77 - 1.43)	(3.35 - 2.62)		2 week x 3 weeks P ≤0.01
	Mean	1.6	3.1		
	SD	0.1	0.3		
	SE	0.03	0.08		
	t-test	P ≤0.01			

Values are given as Range, mean, standard deviation (SD), and standard error (SE). P≤ 0.01: Highly Significant Difference.

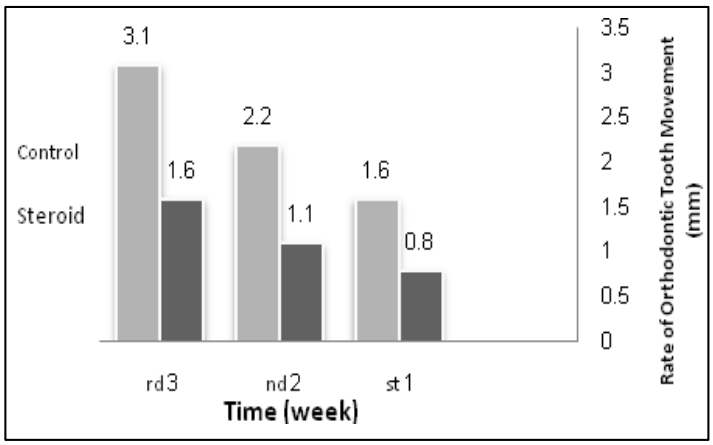

Fig. 3: The rate of orthodontic tooth movement (mm) after 1st, 2nd and 3rd weeks between the studied groups.

Table 2: Mann-Whitney U-test of groups for comparison of bone formation and resorption at different sides, sites and levels

				Control		Steroid		Mann-Whitney U- test	
				Mean	SD	Mean	SD	p	Sig.
Bone formation	Appliance	Compression	coronal	2.000	1.069	0.625	0.518	0.007	**
			apical	2.125	0.641	1.375	0.744	0.050	*
		Tension	coronal	4.750	1.909	3.375	1.302	0.130	NS
			apical	4.125	1.553	3.750	0.463	0.878	NS
	Non-Appliance	Compression	coronal	0.250	0.463	0.250	0.463	1.000	NS
			apical	0.625	0.518	0.250	0.463	0.234	NS
		Tension	coronal	1.125	0.835	0.750	1.165	0.382	NS
			apical	1.250	0.707	0.625	0.744	0.130	NS
Bone resorption	Appliance	Compression	coronal	3.375	1.302	6.250	1.669	0.001	**
			apical	2.375	1.061	5.250	1.282	0.001	**
		Tension	coronal	1.000	0.535	1.250	0.707	0.505	NS
			apical	0.250	0.463	1.000	0.535	0.028	*
	Non-Appliance	Compression	coronal	0.625	0.518	2.000	1.069	0.007	**
			apical	0.750	0.707	1.125	0.835	0.382	NS
		Tension	coronal	0.375	0.518	0.875	0.835	0.279	NS
			apical	0.250	0.463	0.625	0.744	0.382	NS

The values are given as mean and Standard Deviation (SD). (NS): Non-Significant ($p \geq 0.05$), (*): Significant Difference ($p \leq 0.05$), (**): Highly Significant Difference ($p \leq 0.01$).

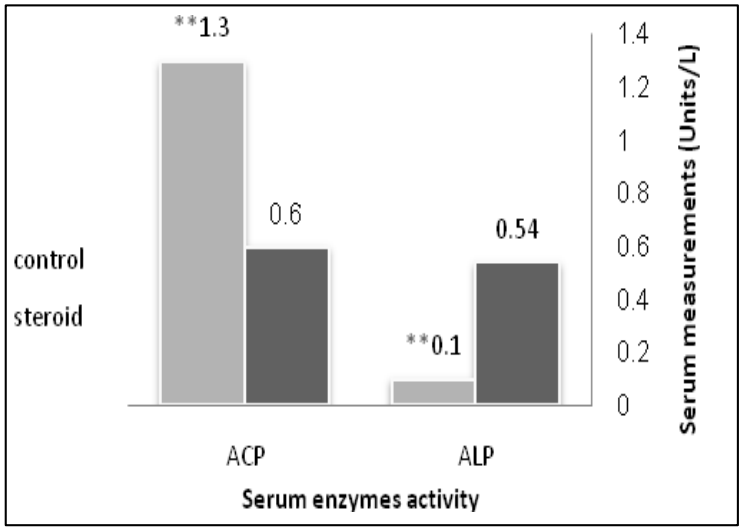

Fig. 4: Alkaline (ALP) and acid phosphatase (ACP) activity in serum (Units/L) between controls and steroid groups. **ALP significantly less in steroid than controls, P ≤ 0.01. While **ACP significantly higher in steroid than controls, P ≤ 0.01.

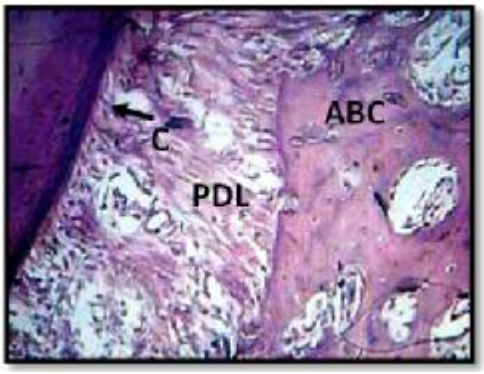

Fig. 5: Microphotograph view for the coronal portion of a steroidal rat tooth treated orthodontically at the compression site shows alveolar bone crest (ABC), cementum (C), and in between principle fibers of periodontal ligament (PDL). H&E, X200.

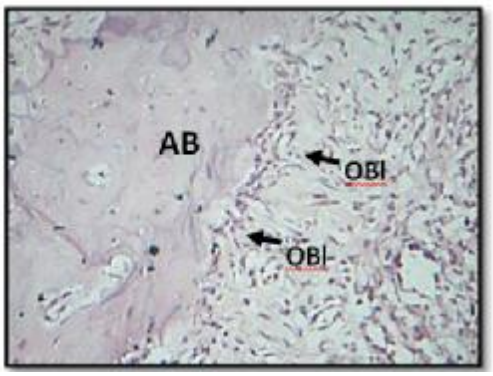

Fig. 6: Microphotograph view for the coronal portion of a steroidal rat tooth treated orthodontically at the tension site shows less no. of activated osteoblast cells (OBl) with minor apposition of alveolar bone (AB). H&E, X200.

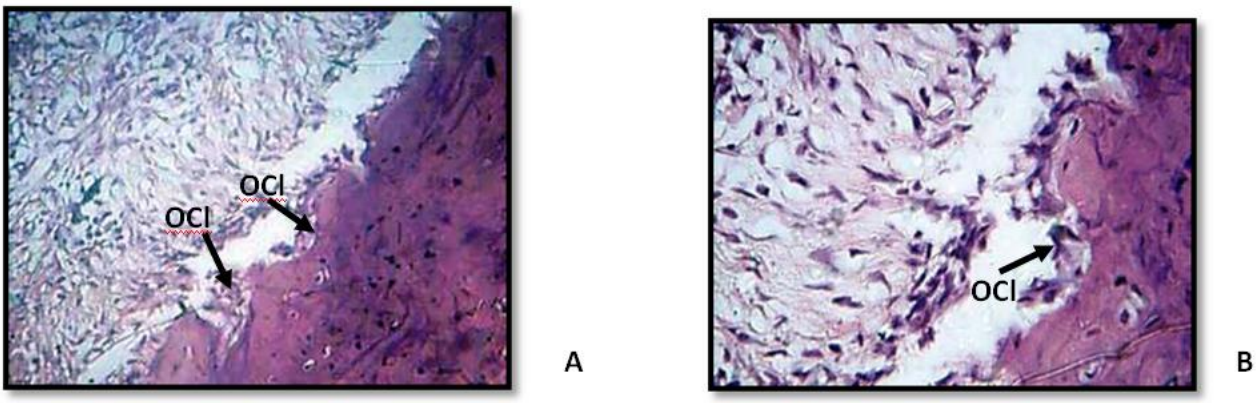

Fig. 7: Microphotograph view for the coronal portion of a steroidal rat tooth treated orthodontically at the compression site shows alveolar bone resorption. Note: proliferation of osteoclast cells (OCl) as multinucleated giant cells occupies Howship's lacunae. H&E, (A) X200, (B) X400.