Stimulation of rabbit condyle growth by using pulsed therapeutic ultrasound (A radiographical and histological experimental study)

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ABSTRACT

Backgrounds: Many difficulties faced the orthodontic clinician during treatment of class II malocclusion cases in the preadolescence period in which treatment is done by growth modification of condyle , these difficulties are due to the poor cooperation of the patients with the myofunctional appliances. The present research was carried out to evaluate the effect of Low Intensity Pulsed Ultrasound application on mandibular condyle of rabbit radiographically and histologically to evaluate the use of low intensity pulsed ultrasound in condyle growth modification in the treatment of skeletal class II malocclusions in the growth period.

Materials and Methods: The sample was 15 New Zealand male rabbits in which Therapeutic Ultrasound was applied to the left condyle (treated group) for 28 days while the right condyle was without ultrasound application (controlled group), After animal sacrifying , the rabbit mandibles were dissected into two hemi mandible, left (treated) and right (control), radiographic image for each hemi mandible was done and three linear measurements were made, (Ramus height, condylar height and mandibular height). Then these hemi mandibles examined histologically including calculating chondrocyte number, osteocyte number, cartilage area calculation and subchondral bone area measurements.

Results: the results showed: The increasing of all linear measurements as a result of enhancement of chondrocytes, osteocytes, increase of cartilage area and bone area in the treated group. There is significant correlation between all linear measurements and chondrocyte and cartilage area.

Conclusion: low intensity pulsed ultrasound can accelerate condyle cartilage growth.

Key Words: therapeutic ultrasound, Low intensity pulsed ultrasound, condyle, growth modifications. (J Bagh Coll Dentistry 2012;24(2):137-143).

INTRODUCTION

Class II malocclusions of skeletal origin are routinely seen in the orthodontic office. Studies of the etiologic factors of Class II malocclusions recognize that most Class II malocclusions are a result of mandibular deficiency and not of maxillary excess [(1)]. Most Class II patients present with retrognathic mandibles and orthognathic maxillae. Patients with mandibular deficiency and Class II malocclusion have a spectrum of esthetic, skeletal, and occlusal characterstics [(2,3)]. However, treating such malocclusions in growing patients by using bite-jumping appliances is believed to produce satisfactory improvement in facial esthetics and minimize the need for surgical intervention later. There is evidence that compensatory growth occurs at the tempromandibular joint, and especially the mandibular condyle in response to altered occlusal function in growing animals [(4,5)].

Rabie et al. [(6)] studied osteogenesis in the glenoid fossa in response to mandibular advancement. They reported that mandibular protrusion resulted in the osteoprogenitor cells being oriented in the direction of the pull of the posterior fibers of the disk (viscoelastic pull) and also resulted in a considerable increase in bone formation in the glenoid fossa.

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Ultrasound is a form of mechanical energy that is transmitted through and into biological tissues as an acoustic pressure wave at frequencies above the limit of human hearing, is used widely in medicine as a therapeutic, operative, and diagnostic tool. Therapeutic US, and some operative US, use intensities as high as one to three W/cm2 and can cause considerable heating in living tissues. To take full advantage of this energy absorption, physical therapists often use such levels of US acutely to decrease joint stiffness, reduce pain and muscle spasms, and improve muscle mobility [(7,8)]. Low–intensity pulsed US (LIPUS) has been reported to be effective in angiogenesis enhancement during wound healing. Recently, low-level therapeutic-pulsed US was used to enhance bone healing after fracture and after mandibular distraction osteogenesis.[(9-11)].LIPUS is a type of ultrasound that promotes tissue healing. For such use, US is administered in pulses at lower intensity levels than in physiotherapy (0.5 to 3.0 W/cm^2), below 0.1 W/cm^2 [(12)] The mechanisms involved in this process which include mechanotransduction of micromechanical stimuli, will increase local angiogenesis and improved blood supply and aggrecan gene expression [(13-15)]. LIPUS has also been used on growing cartilage. This stimulus has been effective increasing cartilaginous growth potential in primary and secondary cartilage (16,17, 18). ***El-Bialy et al*** (17,18) applied LIPUS (30 mW/cm^2, 1.5 MHz) (Exogen Device) on the temporomandibular joint (TMJ) region of growing rabbits and baboon monkeys for 20 minutes daily. Their results show a significant increase in mandibular cartilaginous growth under LIPUS stimulation, especially under chronic mandibular advancement. The mechanisms that may favor growth could include the same mechanisms involved when bone healing is enhanced with LIPUS.

MATERIALS AND METHODS

The materials used in this study could be classified into three major categories; the pharmacological materials, materials used for radio graphical examination and the materials used for the preparation of histological sections.

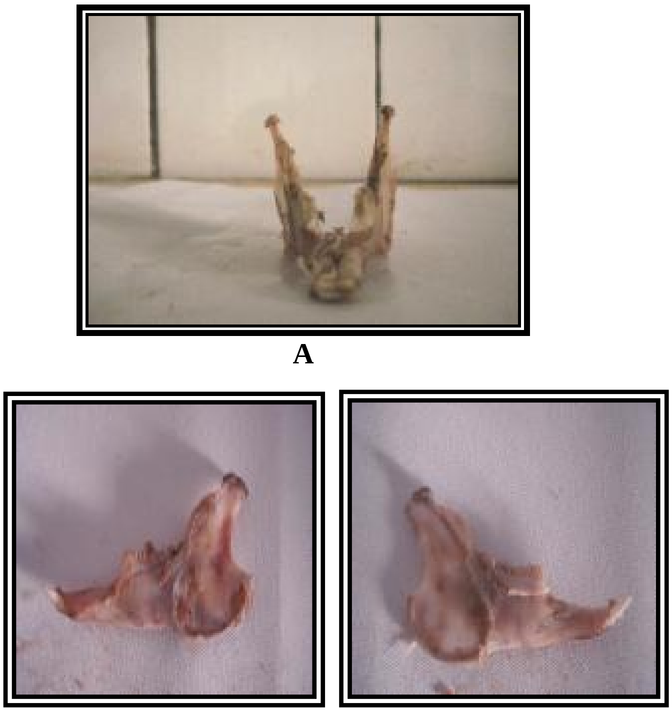
The sample consisted of 15 male New Zealand - white rabbits of 10-11 weeks of age and the rabbits were kept in the animal department of National Center for Drug Control and Research/Baghdad-IRAQ in separate cages in a 12-hour light/dark environment at a constant temperature of 23°C and provided with food and water ad libitum. The health status of each rabbit was evaluated by a day body weight monitoring for two week before start of the experiment as well as during the time of the experiment. According to the ultrasound application protocol, the mandibles in each rabbit were divided into two groups:

1-Control group (non treated group): which was the right side of the mandible (right condyle).

2-Ultrasound group (treated group): in which LIPUS of 50 mW/cm^2 intensity ,1 MHz frequency was applied for 20 minutes /day for four weeks to the left side (left condyle) of the mandible in each rabbit.

All rabbits were adapted to their cages environment for two weeks before experiment. On the day before experiment, each rabbit was shaved in his left condyle region , This procedure was repeated every four days to ensure that the condyle area will be totally shaved along the total period of experiment, the application of LIPUS was done after sedation of rabbits by using intramuscular injections of xylazine (2 mg/kg) , ultrasound transducer was attached securely to the surface of the shaved condyle with turnica, ultrasound gel was used to couple the ultrasound energy between transducer and skin surface, This procedure was repeated for 20 minutes/ day for four weeks in which pulsed ultrasound waves were applied by conventional therapeutic ultrasound device of 3-cm lead zirconate –titnate transducer and consisted of a 200 –microsecond burst of 1 MHz sine that delivered 50 mW/cm^2 (model: HEALOSONIC, New Delhi, India) (17,19,20) (Fig1), After four weeks, all waves animals were sacrificed humanly by intravenous injection of 1 mL/kg sodium pentobarbitone, The mandibles were surgically removed, divided at the symphyseal junction into 2 hemi mandibles by straight hand piece (Fig 2).

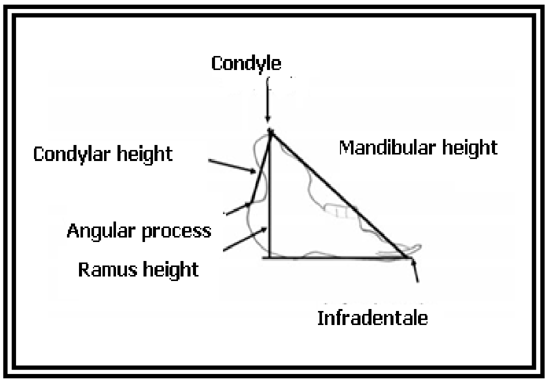
Figure 1: Application of LIPUS



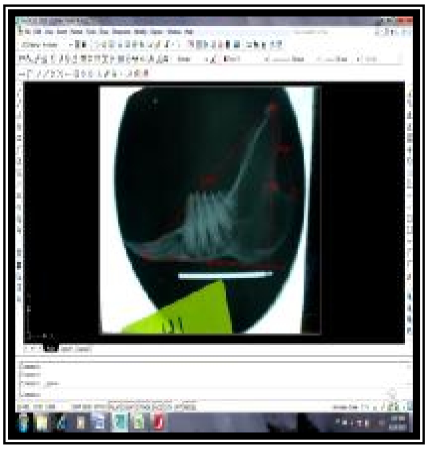
A

B **C**

Figure 2: A-Surgical removal of mandible
B- Right hemi mandible
C- Left hemi mandible



A



B

Figure 3: A- tracing of a hemi mandible showing linearmeasurements taken to evaluate differential mandibular growth changes[17] B- tracing of left hemi mandible by using Auto Cad

All hemi mandibles were radiographed with the X-ray machine, transferred to the laptop by making photographic picture to it using digital camera (Sony Cyber shot) ,then each hemi mandible was traced using (auto cad 2008) program (with control of magnification by rod as standardization).The x ray tracing identified three anatomic points. Three anatomic parameters, two representing anteroposterior mandibular length and one representing mandibular ramus height, were evaluated on the tracing of each hemi mandible .The points and plane and measurements are shown in (Fig3) and are listed below [17]:

1. Measuring points

- ***Infradentale:*** most anterior point on alveolar process below the mandibular central incisor.
- ***Condylar point:*** most superior point on the mandibular condylar summit.
- ***Angular process:*** the most posterior contour on the mandibular ramus.

2. Planes and measurements

- ***Mandibular plane:*** a tangent to the inferior border of the mandible.
- ***Condylar height:*** the distance measured between the condylar point and the angular process.
- ***Ramus height:*** the perpendicular distance from condylar point to the mandibular plane.
- ***Mandibular height:*** the distance from condylar point to infradentale.

After rabbits were sacrified, the surgically dissected hemi mandibles were embedded in 10% buffered formalin for two weeks for fixation and then decalcified using a solution containing 50% formic acid and 20% sodium citrate. The condylar head and necks were embedded in paraffin, then 5 µm thick sections were cut in the sagittal plane with microtome, The tissue sections were mounted on glass slides to be stained with hemotoxylin and eosin, The finished slides were examined using microscope and photomicrographs were taken at 40x power after placing an eye piece with a grid to calibrate the measurements, Then the photomicrographs were transferred to computer software (Auto Cad 2008). A calibration step was performed within the software to get the actual measurements.

At the slide photomicrograph of the condylar head, two sections were chosen for histological examination (anterior and posterior sections for each condyle) in way that ensure about all condyle surface is measured, A subchondral rectangular area of 2 mm^2 was selected for all slides and subsequent measurements and counts were performed within anterior and posterior sections which are represented by (Fig 4,5):-

1- Number of chondrocytes.
2- Number of osteocytes.
3- Cartilage thickness area.
4- Bone area.
5- Bone marrow area.

The first two were counted manually, while the cartilage area, bone area and marrow area were calculated by the (Auto Cad 2008) software after tracing of it manually. From tracing, the cartilage area was obtained, while for bone area it was obtained by subtraction of marrow area form the whole $2mm^2$ subchondral area [18].

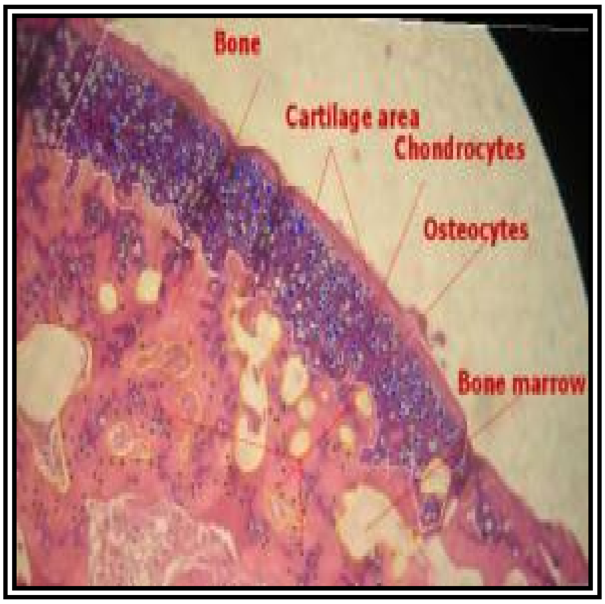


Figure 4: histological examination of left condylar condylar cartilage

Figure 5: histological examination of Right Cartilage

RESULTS

The results included descriptive, comparative and correlation statistics for the anthropometrical and histological variables, the descriptive involved the mean and standard deviation of the three anthropometrical variables and of the four histological variables measured in this study, while for the comparative statistics Student's *t*-tests for independent groups were performed, and a significance level of *P* , .05 was selected. Statistical analysis was done, using SPSS (version 15) software. As shown in (Table 1,2)

1-For the anthropometrical there was a significant increase in the mandibular ramus height condylar height and mandibular height in the US-treated hemi mandibles compared with the untreated hemi mandibles. Enlarged condyles and increased ramal height were clearly observed in the US-treated sides compared with the nontreated sides (Figure 2 A).

2-For the histological variables there was significant increase in the mean values of chondrocytes number, Osteocytes number, Cartilage thickness area and Bone area in the US-treated condyle Compared with the untreated condyles.

For the correlation statistics between histological and anthropometrical variables, there was significant correlation between mandibular height, condylar height and ramus height with chondrocyte numbers and cartilage area in the US treated hemi mandible while the anthropometric parameters did not show significant correlation with histological measurements in the right control side, except there was indirect significant correlation between ramus height and bone area (Table 3, 4).

Table 1: Descriptive & Comparative statistics for saggital jaw linear measurements

Variables		US treated (Left) hemi mandibles (N=15)		Control (Right) hemi mandibles (N=15)		Linear difference (t-test)		
		Mean	±SD	Mean	±SD	t	D.F	P
Sagittal jaw linear parameters (mm)	MH*	52.06	1.33	49.97	1.59	3.889	28	0.001**
	CH*	18.96	0.48	17.18	1.15	5.498	28	0.000***
	RH*	31.89	0.83	29.54	1.755	4.696	28	0.000***

MH= Mandibular Height, CH= Condylar Height, RH= Ramus Height

Table 2: Descriptive & Comparative statistics for Histological examination

Histological variables	US treated (Left) condyle(N=15)		Control (Right) condyle (N=15)		Histology difference (t-test)		
	Mean	±SD	Mean	±SD	t	D.F	p
Chondrocyte No.	473.53	35.49	345.86	58.49	7.227	28	0.000***
Osteocyte No.	363	42.75	221.73	39.73	9.373	28	0.000***
Bone area (mm^2)	1.66	0.036	1.61	0.04	3.193	28	0.003**
Cartilage thickness area ($mm^{2)}$	0.21	0.037	0.16	0.033	3.763	28	0.001**

Table 3: Correlation analysis between the sagittal jaw linear measurements and Histological measurements of the left hemi mandible

Variable		Chondrocyte	Osteocyte	Bone area	Cartilage area
MH	r	0.843***	0.138	-0.131	0.736**
	P	0.000	0.625	0.641	0.002
CH	r	0.565*	0.202	-0.124	0.570*
	P	0.031	0.470	0.659	0.026
RH	r	0.692**	0.406	-0.239	0.661**
	P	0.004	0.133	0.391	0.007

Table 4: Correlation analysis between the sagittal jaw linear measurements and Histological measurements of the right hemi mandible

Variable		Chondrocyte	Osteocyte	Bone area	Cartilage area
MH	r	0.063	0.450	- 0.497	0.101
	p-value	0.823	0.092	0.059	0.720
CH	r	0.061	0.374	- 0.368	- 0.009
	p-value	0.830	0.169	0.177	0.974
RH	r	0.247	0.437	- 0.578*	0.175
	p-value	0.374	0.104	0.024	0.533

DISCUSSION

This study was performed primarily to find out if there is any stimulatory effect of low intensity pulsed ultrasound (LIPUS) on condylar cartilage and on mandibular growth as a whole in growing rabbits. The rabbit model was chosen for this study because of the relatively large mandible and skull. The age of rabbit was 10- 11 weeks because in this age the rabbit is in growth spurt [(21)], the device which was used is the conventional Therapeutic ultrasound apparatus that is adapted for LIPUS emission.

To date, the studies that have been published in the orthodontic literature regarding the use of LIPUS and its influence on condylar growth have been performed using the standard LIPUS device (Exogen, Caldwell, NJ)[(17,18)] , and this device has been extensively proven in humans [(22)] and animals [(16,23)].

Despite this, other LIPUS emission settings have been reported by using conventional ultrasound devices ([19]) in which varying the emission settings within the range of what is defined as LIPUS. The results presented in the use of conventional Ultrasound devices in these animal studies were particularly interesting because they pointed toward the biological effects of LIPUS stimulation, in which using a conventional LIPUS device which was able to produce US emissions of appropriate characteristics, as evaluated from the biological response secondary to its use. This is reported by Rodrigo et al., (2009)[(24)] study in which the results suggested that the biological response may vary and increase when LIPUS was applied for 20 minutes instead of 10 minutes daily. The amount of ultrasound transmission to the control side was negligible in which the intercondylar distance has been reported to be about four cm producing negligible exposure to the condyle on the other side [(17)]

The linear measurements of condylar height, ramal height, and mandibular height were chosen because previous studies on mandibular growth in rabbits showed significant changes in the ramal height and mandibular length in rabbits in which the rabbit condyles growth selectively inhibited by intra-articular papain injection[(25)]. The increase of all anthropometric measurements occurred due to the fact that ultrasound waves can increase chondrogenesis [26, 27]. The greatest increase was in the condylar height measurement and this could be due to the increase of cartilage thickness in vertical dimension more than in anterior and posterior dimension as most of chondrocyte located in the larger upper surface of condyle more than in the anterior and posterior surfaces.

The previous study of the effect of pulsed therapeutic ultrasound on rabbit condyle by [17] revealed only the radiographical effect of

number in the left condyle may be due to the increase in the vascularization which happened due to the minimum thermal effect of ultrasound that increase blood supply by blood vessels dilations and due to non thermal effect in which Ultrasound waves are able to stimulate mandibular Osteoblast to proliferate and produce angiogenesis – related cytokines **[28]**, All of these factors will lead to increase osteocyte nourishment which could increase the osteocyte activity and maturation and formation of other osteocyte .Also it could be as a result to the increase of chondrocyte cells which will pass in the different stages to form osteocytes **[29]**. The significant increase in treated side bone area may be due to the effect of ultrasound which can enhance FGF and VEGF [28], also ultrasound can enhance the process of endochondral ossification [16].Furthermore Ultrasound can affect Osteogenesis in which Osteoblasts can be stimulated to increase collagen production and increase the production of Prostaglandin E2 [30]**.**and all of these factors are responsible for bone matrix formation which will lead to increase of bone area. Also, It probably occur

ultrasound on the condyle of rabbit. In the present study, the aim was not to study the radiographic effect alone but to clarify the quantitive histological effect on rabbit condyle if it present. the significant increase in the number of chondrocytes in the US treated side could be due to ultrasound wave effect by which it can stimulate the chondrocyte proliferation and chondrogenesis-associated gene expression[26,27], which lead to increase the mesenchymal cells differentiations to chondroblasts then chondrocytes. While the increase in osteocytes due ultrasound wave ability to change permeability of chondrocytes leading to the increase of intracellular level of Calcium in the chondrocytes and increase in calcium incorporation into differentiating cartilage and bone cell cultures [31] and this can enhance the mineralization of bone. the significant increase in cartilage area in US treated side may be happened due to the increase of extracellular matrix of cartilage by the action of ultrasound waves in the enhancement of FGF which are responsible for fibroblast growth [28] also due to increase in chondrocytes number , and that will lead to increase collagen II, X type's production **.**
The presence of significant correlation in left condyle may be due to the increase in the chondrocyte number which will lead to the increase of extracellular matrix formation which results in the increase of cartilage area. The increase in cartilage thickness will effect on the condylar point position and this in the end will affect in a direct relation to all linear measurements because all of these measurement share the same condylar point.

REFERENCES

1- McNamara JA. Components of Class II malocclusion in children 8–10 years of age. Angle Orthod 1981; 5: 177–202.

2- Epker NB, Fish LC.The surgical-orthodontic correction of mandibular deficiency. Part I. Am J Orthod Dentofacial Orthop 1983; 106: 408– 21.

3- Charlier JP, Petrovic A, Herrmann-Stutzmann J. Effects of mandibular hyperpropulsion on the prechondroblastic zone of young rat condyle. Am J Orthod 1969; 55: 71–4.

4-Charlier JP, Petrovic A, Herrmann-Stutzmann J. Effects of mandibular hyperpropulsion on the prechondroblastic zone of young rat condyle. Am J Orthod 1969; 55: 71–4.

5-Hinton RJ, McNamara JA Jr. Temporal bone adaptations in response to protrusive function in juvenile and young adult rhesus monkeys (Macaca mulatta). Eur J Orthod 1984; 6: 155-74.

6- Rabie ABM, Zhao Z, Shen G, Hägg EU, Robinson W. Osteogenesis in the glenoid fossa in response to mandibular advancement. Am J Orthod Dentofacial Orthop 2001; 119:390–400.

7- Dyson M. Therapeutic applications of ultrasound. [In: Nyborg WL, Ziskin MC, (Eds), Biological Effects of Ultrasound. New York, NY: Churchill Livingstone; 1985.p. 121– 33.

8- Maylia E, Nokes LD. The use of ultrasonics in orthopaedics—areview. Technol Health Care 1999; 7:1–28.

9-Young SR, Dyson M. The effect of therapeutic ultrasound on Angiogenesis. Ultrasound Med Biol 1990; 16:261– 9.

10- Heckman JD, Ryaby JP, McCabe J, Frey JJ, Kilcoyne RF. Acceleration of tibial fracture-healing by non-invasive, low intensity pulsed ultrasound. J Bone Joint Surg Am 1994; 76:26-34.

11- El-Bialy T, Royston TJ, Magin RL, Evans CA, Zaki AM, Frizzell LA. The effect of pulsed ultrasound on mandibular distraction.Ann Biomed Eng 2002; 30(10):1251– 61.

12- Warden S J. A new direction for ultrasound therapy in sports medicine. Sports Med 2003; 33:95–107.

13- Yang, H. , Parvizi H , Wang SJ , Lewallen DG , Kinnick RR, Greenleaf JF, Bolander ME. Exposure to low-intensity ultrasound increases aggrecan gene expression in a rat femur fracture model. J Orthop Res 1996; 14:802–9.
14- Rubin C, Turner AS, Bain S, Mallinckdrodt C, McLeod K. Low mechanical signals strengthen long bones. Nature 2001; 412:603–4.
15- Rawool N M , Goldberg BB , Forsberg F , Winder AA , Hume E. Power Doppler assessment of vascular changes during fracture treatment with low-intensity ultrasound. J Ultrasound Med 2003; 22:145–53.
16- Nolte PA, Klein-Nulend J, Albers GH, Marti RK, Semeins CM, Goei SA. Low-intensity ultrasound stimulates endochondral ossification in vitro. J Orthop Res 2001b; 19:301-7.
17- El-Bialy T, El-Shamy I, Graber TM. Growth Modification of the Rabbit Mandible Using Therapeutic Ultrasound: Is it Possible to Enhance Functional Appliance Results?. Angle Orthod 2003; 73(6): 631-9.
18- El-Bialy T, Hassan A , Albaghdadi T, Fouad HA ,MaimaniA. Growth modification of the mandible with ultrasound in baboons: A preliminary report. Am J Orthod and Dentofacial Orthop 2006;130 (4):6-14.
19-Omran AA. Acceleration of Bone of Distal Radial Fracture with the use of Low Intensity Pulsed Ultra sound. A Board Thesis for the Iraqi Council of Medical Science (ICMS) 2002
20-Warden S J. A new direction for ultrasound therapy in sports medicine. Sports Med 2003; 33:95–107.
21- Macari M, Machado CR. Sexual maturity in rabbits defined by the physical and chemical characteristics of the semen. Lab Anim 1978;12: 37- 9.
22- Rawool N M, Goldberg BB, Forsberg F, Winder AA, Hume E. Power Doppler assessment of vascular changes during fracture treatment with low-intensity ultrasound. J Ultrasound Med 2003; 22:145–53.
23- Spadaro J, Albanese S. Application of low-intensity ultrasound to growing bone in rats. Ultrasound Med Biol 1998; 24:567– 73.
24- Rodrigo O, Mariana Z, Francisco R .Low – Intensity Pulsed Ultrasound Stimulation of Condylar Growth in Rats. Angle Orthod 2009; 79(5): 964-70.
25- Tingey TF, Shapiro PA. Selective inhibition of condylar growth in rabbit mandible using intra-articular papain. Am J Orthod Dentofacial Orthop1982; 81(6): 455–64.
26-Wiltink A, Nijweide PJ, Oosterbaan WA, Hekkenberg RT, Helders PJM . Effect of therapeutic ultrasound on endochondral ossification. Ultrasound Med Biol 1995; 21:121-7.
27-Wu CC, Lewallen DG, Bolander ME, Bronk J, Kinnick R, Greenleaf JF . Exposure to low intensity ultrasound stimulates aggrecan gene expression by cultured chondrocytes. Trans Orthop Res Soc 1996; 21:622.
28-Doan N, Reher P, Meghji S, Harris M . In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. J Oral Maxillofac Surg 1999; 57:409-19.
29-Nanci A. Ten Cate's Oral Histology: Development, Structre, and Function. 7 ed., Mosby Elsevier, 2008 : 124, 336.
30-Kokubu T, Matsui N, Fujioka H, Tsunoda M, Mizuno K . Lowintensity pulsed ultrasound exposure increases prostaglandinE2 production via the induction of cyclooxygenase-2 mRNA inmouse osteoblasts. Biochem Biophys Res Commun 1999; 256:284-7.
31- Parvizi J, Parpura V, Kinnick RR, Greenleaf JF, Bolander ME. Low intensity ultrasound increases intracellular concentrations of calcium in chondrocytes. Trans Orthop Res Soc 1997; 22:465.