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EVALUATING THE INFLUENCE OF CURCUNIN ON BONE RESPONSE DURING TEETH DISPLACEMENT: AN EXPERMENTAL IN VIVO STUDY

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ABSTRACT
Background and objectives: Orthodontic treatment attempts to improve people's quality of life. A complex and lengthy inflammatory process including simultaneous bone resorption and apposition is required. The length of the treatment process is the most common complaint of patients seeking during treatment. The purpose of this experimental study is to determine the effects of three weeks consumption of curcumin on the rate of orthodontic tooth movement, the level of serum calcium- phosphorous and histomorphometric analysis.
Materials and methods: Twenty healthy male rabbits weighting 1.75 – 2 kg were used; the rabbits were divided in to two groups at random with each group consisting of ten rabbits. Both groups were subjected to orthodontic appliances with the study group receiving a daily dose of curcumin orally for 21 days.
Results: The study group showed tooth movement more than the control group with a decrease in the level of serum calcium and an increase in the level of serum phosphorous, the histomorphometric analysis showed an increase in the number of osteoblasts in the tension side and an increase in the number of osteoclasts in the compression side.
Conclusion: Consumption of curcumin improved the rate of orthodontic tooth movement.

KEY WORDS: Orthodontic treatment, Tooth movement acceleration, curcumin, histomorphometric analysis

1. INTRODUCTION

Orthodontic treatment attempts to improve people's quality of life by correcting malocclusions and improving cosmetic and functional aspects of their teeth. A complex and lengthy inflammatory process including simultaneous bone resorption and apposition is required to achieve tooth movement during this treatment. The length of the treatment process is the most common complaint of patients seeking orthodontic treatment (Anees *et al*., 2014; Demir and Arici, 2021).

The remodeling of alveolar bone in response to mechanical pressure is what allows teeth to move during therapy. When forces are applied to the tooth, load is transferred from the tooth to the alveolar bone via the periodontal ligament (PDL), resulting in minor reversible injury to the periodontium that supports the tooth (Madian *et al*., 2021).

Tooth loading results in local hypoxia and fluid flow, triggering an aseptic inflammatory cascade that results in osteoclast resorption in compression zones and osteoblast deposition in tension areas. Compression and tension linked to specific signaling pathways, resulting in local gradients that regulate bone and periodontal ligament remodeling to allow tooth movement (Arafath, 2012).

Inflammation that occurs during tooth movement must managed carefully, since uncontrolled inflammation leads to tissue death, which manifests itself in orthodontic-induced root resorption and periodontal disease. Understanding biology has significant clinical consequences, particularly in the area of orthodontic tooth movement acceleration (Nakornnoi *et al*., 2019).

Reducing the length of orthodontic treatment is still a difficult task nowadays. It is one of the most prevalent issues orthodontists deal with, and it creates impatient in adults, as

well as an increased risk of cavities, gingival recession, and root resorption (Dai *et al*., 2017).

A number of attempts have made to develop new procedures, both preclinically and clinically, in order to produce faster outcomes and accelerate tooth movement; however, most of these treatments still have a lot of unknowns and unanswered issues. The majority of attempts can categorized into biological, physical, biomechanical, and surgical categories (Wang *et al*., 2016; Abtahi *et al.,* 2018; Al-Khalifa *et al*., 2020).

Now a day worldwide, Attempt have followed for the use of natural products in many aspects as a prophylactic measures, and health improvements. Of these products Curcumin have proven to have many health benefits with interesting anti-inflammatory, antioxidant, antiosteoporosis, and bone health support (Xiong *et al*., 2020; Al-Rubaei *et al.,* 2014; Pizato *et al*., 2018).

The aim of this experimental study was to determine the effect Curcumin on the bone response during orthodontic tooth movement.

MATERIALS AND METHODS

2.1 Experimental Animals:

Twenty healthy 7-10 month-old male rabbits weighing 1.75- 2 kilograms used. The rabbits kept in the metallic boxes in ventilated room in the college of Veterinary medicine at Duhok University. Animal selection, management, and operation protocols have carried out in accordance with the Animal Care Center's policy standard at Duhok University's Basic Science Department. After a one-month adaption period, the study's experimental portion was begin (Huang *et al*., 2021; Abtahi *et al.,* 2018).

2.2 Experimental Designing:

The rabbits divided into two groups at random; each consisting of ten rabbits would house in their own hutch. The first group was control group that subjected to orthodontic appliance but not given natural products. The study group was subjected to orthodontic tooth movement in an experimental setting, with each rabbit received a daily dose of Curcumin 150mg/kg (Sharma, & Ahuja, 2019; Pang *et al.,* 2014).

2.3. Rabbit experimental site:

The two upper incisors used, because it is accessible and there was adequate amount of the bone surrounding these teeth (Anees *et al*., 2014).

Fig. (1): The two upper central incisors of rabbit

2.4 Anesthesia:

Before anesthesia, the rabbits restrained overnight, but they would have access to water until premedication (anesthesia) (Anees *et al*., 2014). Each animal pre-medicated with 0.2 ml/kg b.w (body weight). Ketamine 10% and 0.025 ml/kg b.w. Xylazine 2% intramuscular injections right before the bracketing (Joanne and Marcelo, 2012). Complete anesthesia achieved in as little as 10 minutes, and this dose kept the animal anaesthetized for roughly one hour (Craven R, 2007; National Institute of Health, 2005).

2.5 Orthodontic tooth movement:

The appliance was constructed from molar buccal tube and rectangular stainless steel arch wire, a hook was prepared from rectangular stainless still arch wire and fitted in the hole of molar buccal tube by flowable composite (Anees *et al*., 2014; Pusi *et al*., 2009).

Orthodontic tooth movement achieved after the rabbits anesthetized. The two upper central incisors were prepared for appliance

placement. Firstly the teeth were cleaned with cotton roll and water to remove any derbies, then acid etch were placed for 30 second, cleaning and drying the teeth. The bond was placed and curing with light cured, the bonding materials were placed on the molar buccal tube and fitted the tube on the labial surface of the central incisor and curing with light cure (Anees *et al*., 2014; Pusi *et al*., 2009; Caglaroglu and Erdem, 2012; Kilic *et al*., 2010).

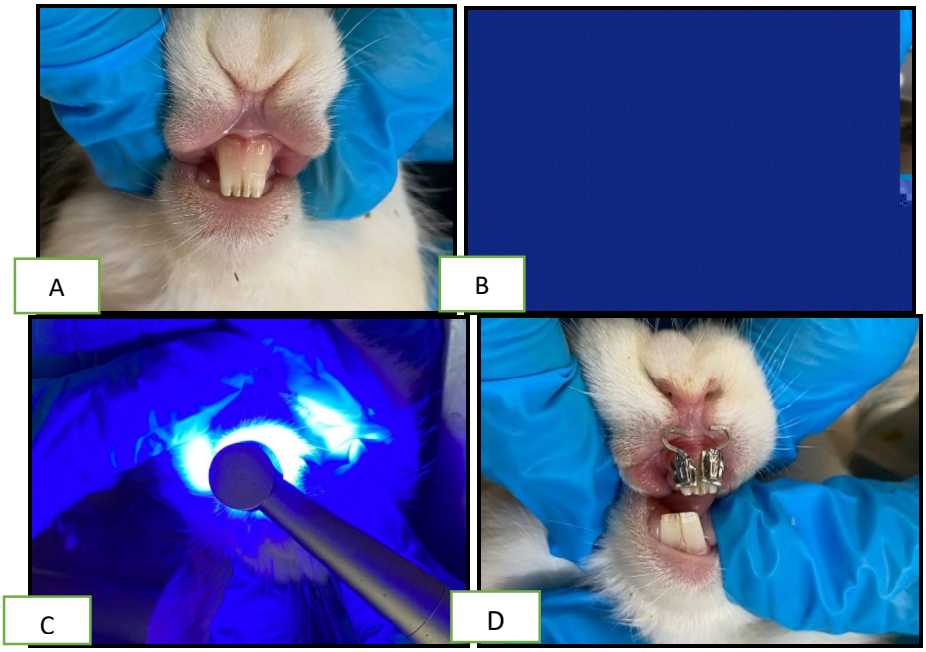

Fig. (2): The steeps of applaince placement: **A-** Cleaning the labial surface of the upper central incisors **B-** Eatching eith acid eatch **C-** Bonding and curing **D-** The final form of the appliance.

Fig. (3): The final form of the appliance with open coil spring in place

2.6 Natural Products Extract loading:

A specially designed oral gavage needle used to load the natural product as a single loading dose (Curcumin 1.5ml/kg body weight) (Chen *et al*., 2021) as possible to the stomach of each rabbits.

2.7. Rabbit Slaughter:

The rabbits slaughtered at the end of the experiment, after leaving few drops of the blood; the blood collected using disposable glass tubes. All of the animals' blood samples taken between 9:00 and 11:00 am; no feeding permitted prior to blood collection. After the rabbit sacrificed, the head separated, and then the premaxila with the two central incisors dissected from the head and preserved in 10% formalin for histological analysis (Anees *et al*., 2014).

2.8 Calcium and phosphorus measurements:

Calcium and phosphorus measured by automated machine known as **Cobas 6000**. The machine made up of three parts, the first part used for preparation of serum the second part known as **Cobas c 501**, which is specialized for biochemistry tests, and the third part known as **Cobas e 601** is specialized for

hormonal tests. All the reagents the required for measuring the rabbits blood calcium and phosphorus are within **Cobas c 501**.

2.9 Histological Examinations:

Steps of histological processing and slide preparation:

The rabbit's premaxilla fixed in 10 percent neutral normal formalin for 48 hours. Following fixation, the specimens decalcified for about 10 days in an incubator at 37 o C using 10% formic acid plus 10% hydrochloric acid. Ethyl alcohol was used to induce dehydration at increasing concentrations, including 70% for 24 hours, 90% for 6 hours, 100% for 24 hours, and 100% for secondary alcohol for 2 to 6 hours. The dehydrated specimen embedded in xylol for 3-5 hs minutes until clearance was completed (Anees *et al*., 2014).

By substituting with paraffin wax, the cleaning agent (xylol) completely removed. Paraffin wax impregnated in an oven that has heated to 56–60°C, depending on the melting point of the wax. The specimen was then oriented in melted paraffin, which when solidified providing a stable medium for maintaining all of the specimen components when sections cut. The wax blocks clamped into a microtome, which used to cut the block into tiny slices or portions. Each longitudinal serial section for each specimen cut on the long axis of the upper incisor root, with a thickness of five µ in the mesiodistal direction. After that, the sectioned films floated onto a water bath (Anees *et al*., 2014).

Using an appropriately sized microscope slide, the smoothed slices are carefully taken from the water surface, dried of excess water, and dried on a hotplate set to between 50 and 60 o C, securely sticking the tissue to the slide so that it can dyed later. The specimens prepared for haematoxylin and eosin staining, which done according to Luna, 1968 and Suvarana *et al*., 2013.

2.10.Histomorphometric evaluation/Quantitative assessment:

- General assessment of each histological section done by examining the upper right and left central incisor of both pressure and tension sides of the root at a power field. Counting the number of osteoblasts, osteoclasts, osteocytes along the root surface.
- The optical dissector method used to determine the osteoblastic and osteoclastic populations in the sections. This method involves counting of units while advancing through virtual optical sections. Hundred 40-µm-thick sections were examined by starting with a random single-digit number and analyzing every 10th section thereafter; for example, beginning with section 7, sections 7, 17, and so on were examined until 10 sections were analyzed.

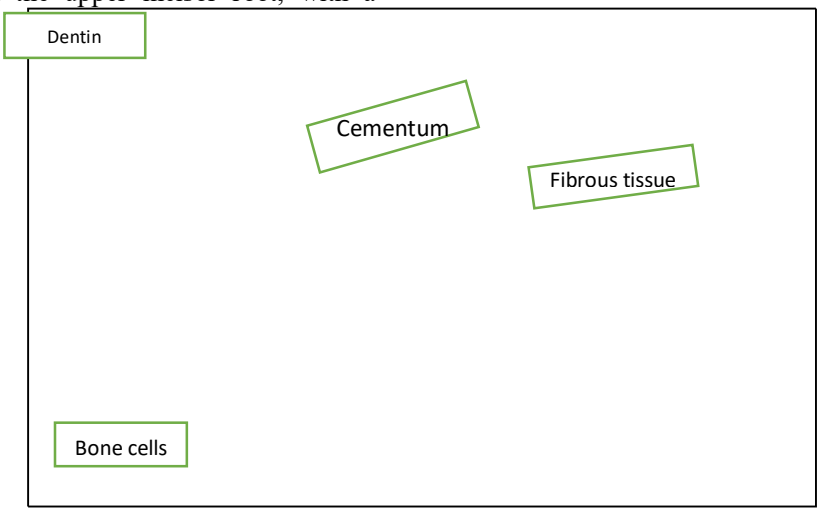

Fig. (4): This figure show the compression zone between the tooth structure and bone

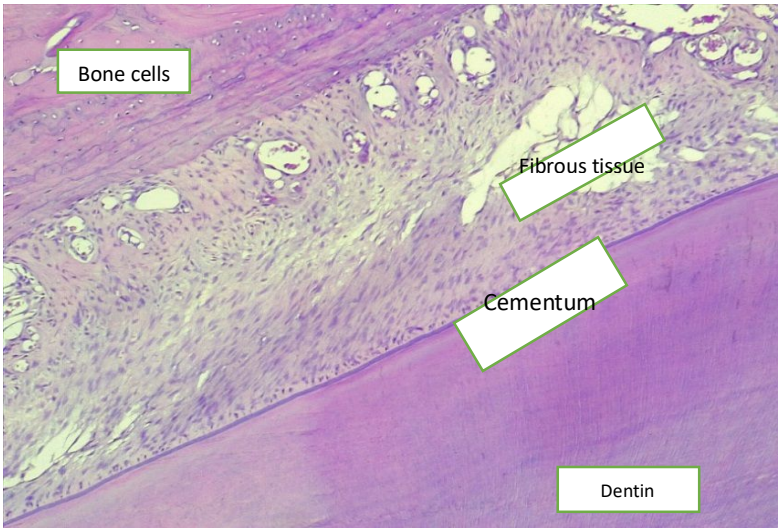

Fig. (5): This figure show the tension zone between the tooth structure and bone

3. Statistical Analysis:

The level of tooth width, calcium, and phosphorus of study groups presented in mean and standard deviation. The comparisons of tooth width of study groups before and after the intervention, calcium, and phosphorus examined in independent t-test. The comparisons of histomorhopmetric findings among and between study groups examined independent t-test, respectively. The comparisons of histomorhopmetric findings of each study group between sides examined in an independent t-test. The significant level of difference was determined in a $p<0.05$. The normality of the outcomes checked through drawing a histogram. The histograms of the outcomes showed that the measurements are approximately normally distributed. The statistical calculations performed by JMP pro 14.3.0.

4. RESULTS

4.1. Comparisons of rabbit's tooth width of study groups before intervention:

The Rabbits tooth width that measured from distal surface of maxillary incisors just before placement of the appliance. It has shown that all the samples shared similar measurements with no significant differences as shown in table below (tab 1).

Table (1): Comparisons of rabbit's tooth width of study groups before intervention

Study groups	Statistics of tooth width before intervention (mm)		p-value
	Mean	Std Dev	
Control (n=10)	5.74	0.15	0.0764 (NS)
Curcumin (n=10)	5.80	0.15	

Independent t-test performed for statistical analysis.

4.2 Comparisons of rabbit's tooth width of study groups after intervention:

The measurements taken 21 days after placement of orthodontic appliance just before rabbits slaughtering. It has shown the tooth movement in curcumin group were significantly higher than control group as shown in table below (tab 2).

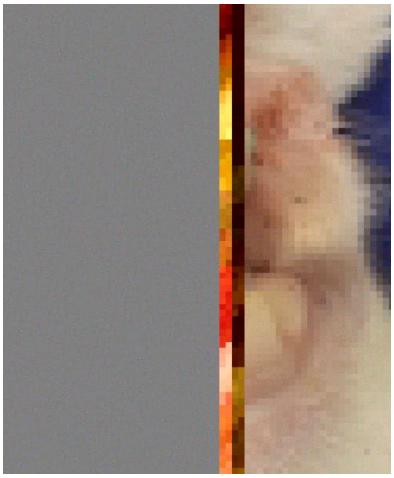

Fig. (6): The final form of the appliance after space opening

Table (2): Comparisons of rabbit's tooth width of study groups after intervention

Study groups	Statistics of tooth width after intervention (mm)		p-value (two-sided)
	Mean	Std Dev	
Control(10)	7.18	0.21	**0.0001 (HS)**
Curcumin(10)	7.77	0.29	

Independent t-test performed for statistical analysis.
The red bold numbers show significant differences.

4.3 Comparisons of rabbit's calcium of study groups after intervention:

The level of the serum calcium measured at the end of the study, all study groups showed decrease in the level of the serum calcium. The level of the serum calcium in curcumin group was significantly lower than control group as shown in table below (tab 3).

Table (3): Comparisons of rabbit's calcium of study groups after intervention

Study groups	Statistics of calcium (mg/dl)		p-value (two-sided)
	Mean	Std Dev	
Control(10)	11.80	0.55	**0.0036**
Curcumin(10)	9.83	0.57	

Independent t-test performed for statistical analysis.
The red bold numbers show significant differences.

4.4. Comparisons of rabbit's phosphorus of study groups after intervention:

The level of the serum phosphorus measured at the end of the study, all study samples showed increase in the level of the serum phosphorus. The level of the serum phosphorus in curcumin group was significantly higher than control group as shown in table below (tab 4).

Table (4): Comparisons of rabbit's phosphorus of study groups after intervention

Study groups	Statistics of phosphorus after intervention (mg/dl)		p-value (two-sided)
	Mean	Std Dev	
Curcumin(10)	5.77	0.42	**<0.0001**
Control(10)	4.55	0.10	

Independent t-test performed for statistical analysis.
The red bold numbers show significant differences.

4.5. Comparisons of histomorphometry among study groups:

- **Tension Osteocytes:** All study samples showed similar results with no significant differences.
- **Tension Osteoblasts:** The number of the osteoblasts in curcumin group was significantly higher than control group.
- **Tension Osteoclasts:** All study samples showed similar results with no significant differences.
- **Compression Osteocytes:** All study samples showed similar results with no significant differences.
- **Compression Osteoblasts:** All study samples showed similar results with no significant differences.
- **Compression Osteoclasts:** The number of the Osteoclasts in curcumin group was significantly higher than control group.

Table (5): Comparisons of histomorphometry among study groups

	Tension Osteocytes				
Study groups	**No.**	**Mean**	**Std Dev**	**P-value**	**Pairwise comparisons**
Control	10	103.00	7.70	**<0.0001**	Control vs. Curcumin (P=0.2600
Curcumin	10	96.14	4.30		
	Tension Osteoblasts				
Study groups	**No.**	**Mean**	**Std Dev**	**P-value**	**Pairwise comparisons**
Control	10	117.14	9.34	**<0.0001**	Control vs. Curcumin (P=0.0018*
Curcumin	10	145.00	6.56		
	Tension Osteoclasts				
Control	10	15.57	3.55	0.0537	Control vs. Curcumin (P=0.1709
Curcumin	10	12.43	1.72		
		12.86			
	Compression Osteocytes				
Control	10	124.14	17.61	**0.0108**	Control vs. Curcumin (P=0.2423
Curcumin	10	105.29	11.83		
	Compression Osteoblasts				
Control	10	21.57	6.02	0.2705	Control vs. Curcumin (P=0.9994
Curcumin	10	21.29	2.06		
	Compression Osteoclasts				
Control	10	31.43	7.21	**0.0143**	Control vs. Curcumin (P=0.0464*
Curcumin	10	41.43	9.85		

Independent t-test performed for statistical analyses.

5. DISCUSSION

Orthodontic treatment attempts to improve people's quality of life by correcting malocclusions and improving cosmetic and functional aspects of their teeth. A complex and lengthy inflammatory process including simultaneous bone resorption and apposition is required to achieve tooth movement during this treatment. The length of the treatment process is the most common complaint of patients seeking orthodontic treatment (Demir and Arici, 2021).

The choice of curcumin in the present study made because many populations' diets are rich in these natural materials. This led to the investigation of their biological effects during orthodontic treatment in the hope of finding natural sources to promote tooth movement, remodeling, and mineralization during this period in order to shorten this period and ultimately the length of orthodontic treatment.

In the present study, the results of orthodontic tooth movement showed the tooth movement in curcumin group were significantly higher than other groups this is may be due to anti- inflammatory effects of curcumin that given a daily dose 150mg/kg of curcumin for 21 days. Curcumin found to be as efficient as cortisone or phenylbutazone in situations of acute inflammation and half as effective in cases of chronic inflammation when taken orally (Chen *et al*., 2021).

The level of the serum calcium and phosphorous measured at the end of the study; all study samples showed decrease in the level of the serum calcium and increase in the level of serum phosphorous. The level of the serum calcium in curcumin group was significantly lower than control group. While the level of serum phosphorous in curcumin group was higher than control group. This may be due to bone remolding process that effect on the level of the calcium and phosphorous in the blood (Midgett *et al*., 1981)

The histomorphometric results of curcumin group showed increase in the number of osteoblasts in the tension side and increase in the number of osteoclasts in compression side when compared with control group.

Curcumin decreased neutrophil aggregation, which linked to inflammation, in monkeys. Curcuma longa's anti-inflammatory benefits are due to its capacity to decrease both inflammatory prostaglandin formation and neutrophil activity during inflammatory conditions (Yang *et al.,* 2020).

An in vitro study published in 2016 found that the antioxidant curcumin, at low concentrations, could protect human adipose-derived mesenchymal stem cells from oxidative stress generated by H2O2 on their survival and osteogenic development. As a result, such antioxidant-based strategy has the potential to improve mesenchymal stem cell therapy for bone regeneration (Wang *et al*., 2016).

Curcumin protects osteoblasts against oxidative stress-induced apoptosis by retaining mitochondrial function and activating the Akt-GSK3 signaling pathway. These findings give experimental support for curcumin's clinical usage in the prevention and treatment of osteoporosis (Dai *et al*., 2017).

Curcumin has no significant inhibitory effect on orthodontic tooth movement in rats in an in vivo study published in 2018. However, the study's practical conclusion was that curcumin might have some effect on tooth movement. Curcumin dramatically reduce bone and/or root resorption. In the realm of orthodontic tooth movement, it also lowered angiogenesis and the amount of osteoclasts. As a result, it suggested as a beneficial local anchoring control approach that is less invasive and has few side effects (Asefi *et al*., 2018).

6. CONCLUSION

As conclusion curcumin improved tooth movement, there were decrease in the level of the serum calcium, increase in the level of the serum phosphorus and improve bone healing by increasing the number of osteoblasts in the tension zone, and increasing the number of osteoclasts in the compression zone.

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