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ORIGINAL ARTICLE

Influence of olive oil on alveolar bone response during orthodontic retention period: rabbit model study

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ABSTRACT

Objectives: This study attempted to evaluate clinically and histologically the effects of olive oil (OI) consumption on orthodontic relapse after the retention period.

Design: Thirty apparently healthy female albino rabbits, weight more than 1000 g each was used in this study. The animals were grouped randomly into six groups of five animals each: two control and four experimental groups. In control groups, the relapse was estimated either at zero day, or at the end of the fourth week after orthodontic retention period. In the experimental groups, the animals' groups received OI, 7.7, or 15.4 ml/kg b.w. per day during the orthodontic retention period. The relapse was estimated either at zero day, or at the end of the fourth week after orthodontic retention period for each concentration.

Modified fixed orthodontic appliances were attached to the rabbits' lower central incisors. Each rabbit received orthodontic intervention for one week, followed by six weeks retention period. At the end of the experiments, the clinical and histological investigations were conducted. Data analyses were performed at the level of $p < .05$ for the statistically significant difference.

Results: Clinically, OI high concentration four weeks group showed a significantly lower relapse tendency than control four weeks group. Histologically, OI low concentration zero time group showed significantly higher osteoblasts numbers than control zero time group. Olive oil low and high concentrations four weeks group showed significantly lower fibroblasts count. Moreover, OI high concentration four weeks group revealed significantly higher bone mineralization, osteoblasts and osteocytes counts than control four weeks study group.

Conclusions: Supplementation with OI during an orthodontic retention period, especially at 15.4 ml/kg b.w. per day concentration, clinically reduced orthodontic relapse on rabbit model. Histologically, OI increased osteoblasts and osteocytes counts and the relative amount of bone mineralization of connective tissue layer forming alveolar bone (AB) at the end of four weeks after the orthodontic retention period.

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Alveolar bone; olive oil; orthodontic retention; Remodelling

Introduction

In orthodontics, a tooth can be moved through the periodontal ligament (PDL) when an appropriate orthodontic force is applied. This is based on the principle that a change in mechanical loading of biological results in strain that subsequently leads to cellular responses aiming at the adaptation of the system to the changed conditions. As a result, remodelling of the PDL and the alveolar bone (AB) around a tooth takes place during orthodontic force application [1]. The bone remodelling is a process that couples bone resorption and bone formation and involves a group of different kinds of cells [2]. The bone remodelling follows the coordination of distinct and sequential phases of this process (activation phase, resorption phase, reversal phase, formation phase and termination phase) [3].

After orthodontic treatment (OT), there is both a retention (R) phase and a post-R phase of therapy [4]. One of the challenging problems in the post R phase is orthodontic relapse that may occur complicating the results after an orthodontic R period [4]. Many factors attributed to orthodontic relapse and, on the other hand, many attempts have been made to overcome the problem of orthodontic relapse [5].

Natural products are a chemical compounds or substances produced by a living organism usually, have a biological or pharmacological activities utilize for drug discovery and design [6]. Olive oil (OI) which is one of (*Olea europaea L.*) olive tree natural products (OI, olive mill wastewater and pomace), is the major source of fats in the Mediterranean diet and is considered to be responsible for the health benefits associated with this diet [7]. OI contains a group of related natural products that have potentially anti-inflammatory and antioxidant properties [8]. Olive oil is a source of at least 36 structurally distinct phenolic compounds and has been proved to be with beneficial health effects [8–10].

A number of animal and human studies have established promising-bone health-benefiting properties of OI particularly

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the impact of OI on bone metabolism [11–15]. Fernández-Real et al. [16] examined the longitudinal effects of the diet enriched with OI (in comparison to other non OI-containing diets) on osteocalcin and bone formation markers in elderly men at high cardiovascular risk. They found that consumption of a diet enriched with OI for two years was associated with increased serum osteocalcin and procollagen I N-terminal propeptide concentrations, suggesting protective effects of OI on bone and reduction of age-related bone mass loss. Further study demonstrated the beneficial roles of phenolic compounds in OI in relation to bone health, which would be effective as adjunctive therapy to promote bone formation at the site of surgical dental implants [17].

It is important to mention that limited studies were conducted to assess the effects of the natural products during dental or orthodontic practice [18,19]. Moreover, no previous attempt was made to study the influence of OI during or after the orthodontic intervention, particularly, post-orthodontic R or relapse period. Thus, the main objectives of this study were to:

- Evaluate clinically the effects of OI administration on teeth relapse after an orthodontic R period.
- Examine histologically the effect of OI administration, after an orthodontic R period on collagen arrangement, the relative amount of osteoid tissue formation and bone mineralization of CT layer forming AB addition to the counts of small blood vessel numbers (angiogenesis), fibroblasts, osteoblasts, osteocytes, osteoclasts. This study hypothesized that OI regulates the response of periodontal tissues and reduces orthodontic relapse.

Materials and methods

The ethical approval to perform this study was obtained from the university authorities (No. 351 in 27.1.2013). The sample size was calculated using the following formula: $n = 2\sigma^2/\Delta^2\ (z_\alpha + z_\beta)^2$. The resulted number was adjusted, and the final sample size in each group $= n + (n \times 0.2)$. In this study, n is considered as the number of subjects, $z_\alpha = 1.96$ for $\alpha = 0.05$, $z_\beta = 0.84$ for 80% power, σ (standard deviation) $= 0.38$ mm [19] and (Δ) precision $= 0.8$ unit. According to this, the calculated sample size for each age group was approximately five animals. Information concerning the rabbits' number, rabbit's group, weight, a dose of anaesthesia, a dose of OI, date of orthodontic appliance insertion, date of the beginning of orthodontic R, date of orthodontic appliance removal and date of slaying was recorded for each rabbit.

After sample size calculation, the study was conducted on 30, apparently healthy female albino rabbits of 1000 g each. The rabbits were kept in isolated metallic cages in a well-ventilated room in the animal house of the Dentistry College, Mosul University. Rabbits were kept in the animal house for about two weeks for acclimatization before their use in the experiments.

Diet adjustment of the total sample was made to exclude the possible effects of food type on the rate of tooth movement. Standard concentrated pellet diet plus leafy greens measured per kg per day were given daily to all rabbits during the whole experiment time. Water was available for the animals at any time.

The animals were grouped randomly into six groups of five animals each. Animals received orthodontic intervention for one week, followed by six weeks R period. Animal grouping was as follows:

- C0 group: The relapse was estimated at zero day after orthodontic R period.
- C4 group: The relapse was estimated at the end of the fourth week after orthodontic R period.
- OI L0 group: Each animal received OI, 7.7 ml/kg b.w. per day during the orthodontic R period. The relapse was estimated at zero day after the orthodontic R period.
- OI L4 group: Each animal received OI, 7.7 ml/kg b.w. per day during the orthodontic R period. The relapse was estimated at the end of the fourth week after orthodontic R period.
- OI H0 group: Each animal received OI, 15.4 ml/kg b.w. per day during the orthodontic R period. The relapse was estimated at zero day after orthodontic R period.
- OI H4 group: Each animal received OI, 15.4 ml/kg b.w. per day during the orthodontic R period. The relapse was estimated at the end of the fourth week after orthodontic R period.

All the doses mentioned above were scheduled based on the previous studies regarding the range of OI intake in the diet of human subjects [14,20] and according to guidelines set by the American Food and Drug Administration [21] and dose conversion formula from humans to animals of Shin et al. [22].

Olive oil administration: The OI (Sigma-Aldrich, St. Louis, Missouri, USA) was administered to the study groups via an oral route through mixing to form concentrated pellet diet of the animals (to simulate the natural feeding method in human subjects). The diet was given at two divided meals per day during the whole experiment period. The concentrated pellet mixed with OI was given as one of these two meals. The second meal was not given to the animals unless the first meal containing the OI was administered to them.

Orthodontic appliances: Modified fixed orthodontic appliances were fixed to the rabbits' lower central incisors (Figure 1). Each rabbit received orthodontic intervention for one week, followed by six weeks R period. Each appliance was formed of two modified stainless steel edgewise bands of lower central incisors, size 0 with 0.022×0.030 'slot dimension (Dentaurum company, Germany), cemented on the right and left lower central incisors and 0.017×0.025 'stainless steel wire (International Orthodontic Services, IOS) (USA) sectional arch wire with a nickel titanium open coiled spring (continuous type) (IOS) to achieve 4.5 mm spacing measured at the level of the outer border of upper mesial wing of lower central incisor bracket bands, depending on the amount of inter-bracket distance at the end of active orthodontic intervention. The force exerted by the spring

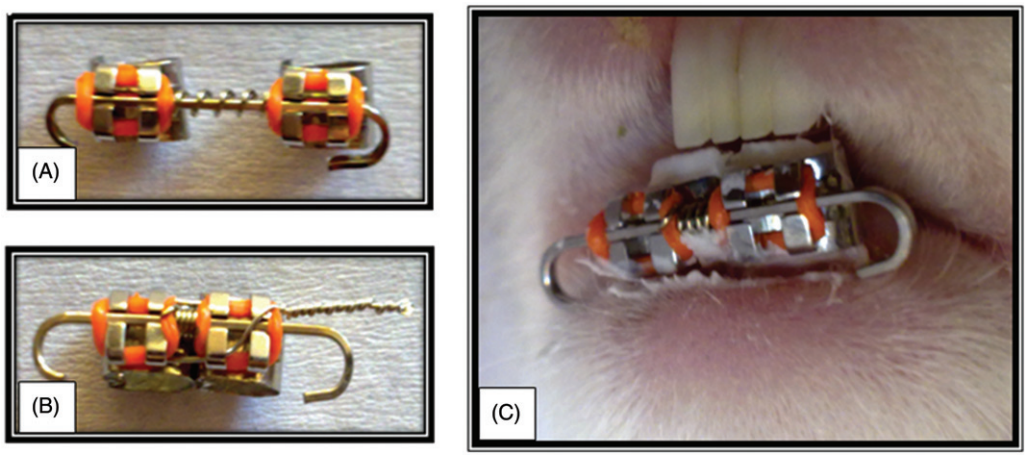

Figure 1. (A) Sectional archwire with a nickel titanium open coiled spring (passive). (B) Orthodontic appliance was activated before cementation and (C) orthodontic appliance after cementation.

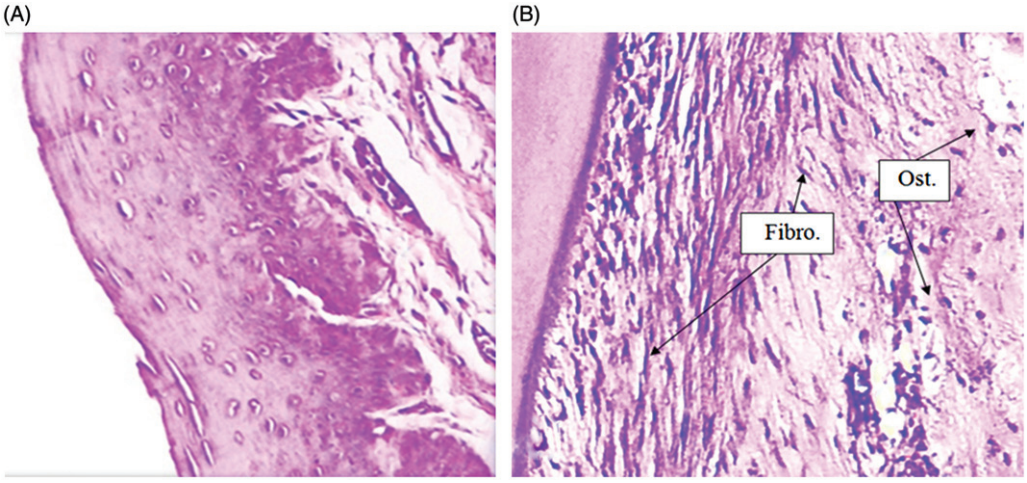

Figure 2. Microphotographs of lower central incisor's AB at (A) cervical third of pressure side of the control group zero time, (B) cervical third of pressure side of the high dose four weeks group showing prominent osteoid tissue formation and fibroblast cells, Ost.: osteoid, Fibro.: fibroblasts, mineral.: mineralization and Osteob.: osteoblasts. Hematoxylin and eosin stain, original magnification 400×.

was determined before insertion, with a tension gauge. The springs were capable of exerting a reciprocal lateral force of 40 ± 2 g so that the tension occurred in PDL of mesial and pressure on distal sides of treated incisors roots. Each wire was secured in the slots of brackets by elastomeric ligatures (Dentaurum, Ispringen, Germany) using mosquito forceps. After anesthetization (Intramuscular injection of a mixture of xylazine 10 mg/kg IM (as a muscle relaxant) and ketamine 35 mg/kg IM (analgesic, hypnotic and amnesic) orthodontic appliance was cemented on the lower incisors of the animal using polycarboxylate cement type (SpofaDental, A Kerr Company; Prague, Czech Republic). The orthodontic appliance was inserted in activated form at the beginning of the experiment with no further activation during the experiment period (Figure 1).

At the end of one week of OT, sufficient amount of cold cure acrylic resin (Major, Moncalieri, Italy) was added to the open coil spring of orthodontic appliance that was served as a retainer during the six weeks orthodontic R period which was required for bone remodelling of rabbits [23]. At the end of the orthodontic R period, the orthodontic appliance was removed using band removing pliers, and the amount of orthodontic relapse was checked using a digital caliper vernier according to the time scheduled for each group. At the end of the experiments, the rabbits were slaughtered, dissected and their lower jaws were used for histopathological slide preparations with haematoxylin and eosin staining.

Histological assessment: The bony lower jaw was dissected out from each rabbit, and was fixed in 10% neutral buffered formalin for at least 48 h. After fixation, the specimens were decalcified, dehydrated, embedded, impregnated with paraffin wax to prepare wax blocks. Longitudinal serial sections were cut for each specimen of 5 μm thickness in a mesiodistal direction parallel to the long axis of the root of the lower incisor. The specimens were prepared for haematoxylin and eosin (H&E) staining was performed according to Luna [23] and Suvarana et al. [24].

Histological examination

Assessment of histological section: The light microscopic images were captured per specimen for the pressure and tension sides of AB of the lower right and left central incisor

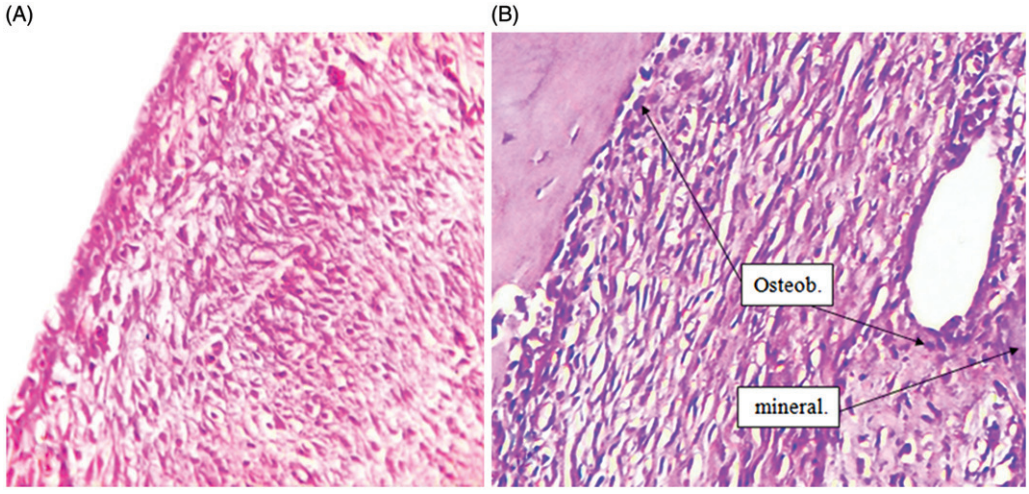

Figure 3. Microphotographs of lower central incisor's AB at (A) apical third of pressure side of the control group zero time, (B) apical third of the high dose four weeks group showing mineralization and osteoblast cells, Ost.: osteoid, Fibro.: fibroblasts, mineral.: mineralization and Osteob.: osteoblasts. Hematoxylin and eosin stain, original magnification 400×.

at the level of cervical, middle and apical thirds of the root of both right and left lower central incisors at a power field (objective lens) 10× and 40×. Determination of the three-thirds of the root was done using the ocular micrometre. The cervical third of the root was determined by the orientation of the ocular micrometre so that the zero line (beginning) of the ocular micrometre was superimposed on the alveolar crest and the ruler representing ocular micrometre was oriented parallel to the long axis of the root and scanned over until the microscope field of view circumscribed by the ocular micrometre was observed. The apical third of the root was determined by the orientation of the ocular micrometre so that the ruler representing ocular micrometre was oriented parallel to the long axis of the root and intersected with a tangent to the root apex at zero line (beginning) of the ocular micrometre and scanned over until the microscope field of view circumscribed by ocular micrometre was observed. The middle third of the root was determined approximately midway between the cervical and apical thirds; first, the approximate whole length of root was determined using the ocular micrometre, from the alveolar crest till the root apex, then dividing the whole length by three yielding the approximate root third length.

Using the ocular micrometre, evaluation of the process of bone remodelling and the cellular activities in bone and PDL was evaluated. Among the serial sections in the mesiodistal direction from each specimen, five central sections were selected for histopathological examination for each specimen. The total slides available for histological examination were 150, divided according to the study groups. Each slide was examined under a light microscope (BH2 Olympus, Levittown, PA, USA) by an experienced histopathologist and then by the researcher.

Qualitative assessment of histological section

General assessment of each histological section was done by examining the lower right and left central incisor at the level of cervical, middle and apical one-third of AB of both pressure and tension sides of the root at a power field (objective lens) 10×. Using an ocular micrometre, evaluation of the process of bone remodelling and the cellular activities in bone and PDL was carried out. The auxiliary histological findings were made by observing it at both 4× and 10× power fields all over the selected areas as follows:

- **Evaluation of Collagen arrangement:** as described by Suvarana et al. [24]. Ordered nominal grades were assigned for vertical (1), oblique (2) or horizontal (3) arrangement in relation to the long axis of the root.
- **Relative amount of Osteoid tissue formation:** as described by Suvarana et al. [24]. Ordered nominal grades were assigned either (0) (when no sign of osteoid is present), low (1) (when localized single area of osteoid is present), moderate (2) (when two to three areas of osteoid are present) or high (3) (when more than three areas of osteoid are present).
- **Relative amount of bone mineralization:** as described by Suvarana et al. [24]. Ordered nominal grades were assigned either (0) (when no sign of mineralization is present), low (1) (when localized single area of mineralization is present), moderate (2) (when two to three areas of mineralization are present) or high (3) (when more than three areas of mineralization are present).

Quantitative assessment of histological section

Precise assessment of each histological section was done by examining AB at the level of cervical, middle and apical one-third of the pressure and tension sides of root of both right and left lower central incisors at a power field (objective lens) 40× and was counted and expressed as cell numbers per microscopic power (40×). Using an ocular micrometre, counting was done as follows:

- **Counting of the Small Blood Vessel Number (Angiogenesis):** as described by Suvarana et al. [24]. The number of small capillaries which were seen within the

microscope field of view at microscopic power (objective lens) 40×, using an ocular micrometre.

- **Counting of the fibroblast number:** Fibroblast was observed as an elongated cell with little cytoplasm and dark staining flatted nucleus [24]. The number of fibroblasts which were seen within the microscope field of view at microscopic power (objective lens) 40×, using an ocular micrometre.
- **Counting of the osteoblast number:** Osteoblast was observed as a plump cell with basophilic cytoplasm and eccentric nucleus found on the surfaces of actively forming bone [24]. The number of osteoblasts which were seen within the microscope field of view at microscopic power (objective lens) 40×, using an ocular micrometre.
- **Counting of the osteocyte number:** Osteocyte was observed residing in a tiny space or lacauna within the bone with projecting canaliculi [24]. The number of osteocytes which was seen within the microscope field of view at microscopic power (objective lens) 40×, using an ocular micrometre.
- **Counting of the osteoclast number:** Osteoclast was observed as large multinucleated giant cell with irregular outline [24]. The number of osteoclasts which was seen within the microscope field of view at microscopic power (objective lens) 40×, using an ocular micrometre.

Data analyses were performed using the Statistical Package for Social Sciences Software (SPSS) for Windows (18.0) (SPSS Incorporated, Chicago, IL, USA). Intra-class correlation was performed to assess the inter and intra-examiner reliabilities of the measurement by examining 10 randomly selected slides. No statistical significant difference detected was among the readings ($p > .05$). The data were checked for normal distribution. Descriptive statistics including median values and interquartile range (IQR) were determined for each group. We also performed Mann–Whitney''s test to investigate difference between the control and OI groups. The *p*-value of less than .05 was considered a statistically significant difference.

Results

Clinical finding

The amount of orthodontic relapse was checked according to the time scheduled for each group. The results showed no clinically recorded relapse in the OI L0 and OI H0 in comparison with C0. However, statistical comparison of C4 with OI L4, OI H4 showed significantly lower relapse in OI H4 than those of C4 (Table 1).

Histological assessment

The histological features of the OI L0 group at the tension side showed more dense, mature and obliquely arranged CT layer, more vascularised and with higher values for osteoid tissue formation and bone mineralization than the pressure side. However, the pressure side being more dense in CT and more vascular apically and rich in fibroblasts and osteoblasts cervically than that of the tension side.

Table 1. Relapse findings and comparisons for the control, olive oil low and high doses groups.

Groups	Median	IQR	Groups' comparison	Cal. Z	*p* Value
C 0	0.00	0.00			
OI L0	0.00	0.00	C0 *vs.* OI L0	0.00	.480
OI H0	0.00	0.00	C0 *vs.* OI H0	0.00	.480
C 4	2.00	1.49			
OI L4	1.88	0.99	C 4 *vs.* OI L4	0.52	.602
OI H4	1.01	0.84	C 4 *vs.* OI H4	2.10	.036*

C 0: control zero time, C 4: control four weeks, OI H0: olive oil high dose zero time, OI H4: olive oil high dose 4 weeks, OI L0: olive oil low dose zero time, OI L4: olive oil low dose four weeks; IQR: interquartile range.
*Significant at $p < .05$. Variable unit for relapse is in mm.

The histological description for OI H0 group at the tension side showed higher values in almost all histological parameters than the pressure side and greater cellular number except for osteoblasts. However, osteoid tissue formation and bone mineralization appear to be similar. For pressure side, the cervical one-third of the root showed higher values in most histological parameters than middle and apical thirds except CT density and vessel number. Fibroblasts number was higher in the middle third, whereas, osteocytes were higher at both cervical and apical thirds than the middle third.

Histological features of the OI L4 group at the pressure side appeared more dense and highly cellular CT layer that was less vascularised with less bone mineralization. The remaining features were relatively similar. The connective tissue was more dense and mature, rich in osteoblasts, osteocytes and osteoclasts apically and fibroblasts cervically at pressure side. The other parameters were equally distributed along the root surface.

The tension side of the OI H4 group showed higher values in almost all histological parameters than the pressure side and greater cellular number except for fibroblasts and osteoblasts. However, CT density and osteocytes number revealed approximately the same value. For the pressure side, the cervical one-third of the root showed higher values in the osteoid tissue formation and fibroblasts number than the middle and apical thirds (Figures 2 and 3). Bone mineralization, vessel number, and osteoblasts number were higher in the apical third. The other parameters were comparable in the three-thirds, but, for tension side, the cervical and middle one-third of the root showed higher bone mineralization and osteocytes than the apical third. Vessels, fibroblasts, and osteoblasts numbers were higher in the apical third.

The median and IQR for the study variables were presented in Tables 2 and 4. The statistical comparisons showed that most of the histological parameters were non-significantly different for both pressure and tension sides in comparison between C0 versus OI L0 groups. However, significant differences were seen for osteoblasts number at three thirds of pressure side and fibroblast number at middle and apical thirds of the tension side (Table 3). Whereas, comparison between C0 versus OI H0 groups showed no significant difference of the histological findings at ($p < .05$) for pressure and tension sides except for fibroblasts apically at tension side (Table 3).

Table 2. Histological findings at pressure and tension side of lower central incisor's root for the control, olive oil low and high doses zero time groups.

	Pressure site						Tension site					
	C0		OI L0		OI H0		C0		OI L0		OI H0	
Parameters	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Cervical region												
Collagen arrangement	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.50	1.00	0.50	1.00	2.00
Osteoid tissue	0.00	0.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00
Bone mineralization	0.00	0.50	0.00	1.00	0.00	1.00	1.00	1.50	0.00	0.50	0.00	1.00
Small blood vessels no.	1.00	1.50	2.00	1.00	2.00	1.00	1.00	2.50	3.00	1.50	2.00	1.00
Fibroblast no.	21.00	7.50	23.00	6.00	22.00	5.00	31.00	7.00	28.00	2.50	27.00	5.50
Osteoblast no.	0.00	0.50	1.00	3.00	1.00	3.00	1.00	2.50	1.00	2.00	1.00	2.00
Osteocyte no.	0.00	0.50	1.00	1.50	1.00	2.50	0.00	1.50	1.00	1.00	1.00	3.50
Osteoclast no.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00
Middle region												
Collagen arrangement	1.00	1.00	0.00	0.00	0.00	0.00	1.00	1.50	1.00	0.50	1.00	2.00
Osteoid tissue	0.00	0.00	0.00	1.00	0.00	0.50	0.00	0.00	0.00	1.00	0.00	1.00
Bone mineralization	0.00	0.50	0.00	1.00	0.00	0.50	1.00	1.00	0.00	0.50	0.00	1.00
Small blood vessels no.	2.00	1.50	2.00	1.00	2.00	1.50	1.00	2.00	2.00	2.00	1.00	1.00
Fibroblast no.	20.00	5.00	25.00	4.50	23.00	5.00	30.00	6.50	25.00	3.00	26.00	5.00
Osteoblast no.	0.00	0.00	1.00	1.50	1.00	1.50	1.00	1.50	1.00	2.00	1.00	1.50
Osteocyte no.	0.00	0.50	1.00	1.00	1.00	1.50	1.00	1.50	1.00	1.00	1.00	2.50
Osteoclast no.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00
Apical region												
Collagen arrangement	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.50	1.00	0.50	0.00	0.00
Osteoid tissue	0.00	0.00	1.00	1.00	0.00	0.50	0.00	0.00	0.00	1.00	1.00	1.00
Bone mineralization	0.00	0.00	0.00	0.50	0.00	0.00	1.00	1.50	0.00	0.50	0.00	1.00
Small blood vessels no.	3.00	1.00	2.00	1.00	2.00	1.50	1.00	2.50	4.00	1.50	2.00	1.00
Fibroblast no.	15.00	10.50	18.00	4.00	13.00	4.00	33.00	4.00	27.00	2.50	28.00	6.50
Osteoblast no.	0.00	0.00	1.00	1.50	0.00	0.50	1.00	2.00	1.00	2.00	1.00	1.50
Osteocyte no.	0.00	0.00	0.00	1.50	1.00	2.50	0.00	1.50	1.00	0.50	1.00	2.50
Osteoclast no.	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00

C0: Control zero time; OI L0: olive oil low dose zero time; OI H0: olive oil high dose zero time; IQR: interquartile range.

Table 3. Comparison of the histological findings at pressure and tension side of lower central incisor's root between the control and olive oil low and high doses zero time groups.

	Pressure site				Tension site			
	C0 *vs.* OI L0		C0 *vs.* OI H0		C0 *vs.* OI L0		C0 *vs.* OI H0	
Parameters	Cal.Z	*p* Value	Cal.Z	*p* Value	Cal.Z	*p* Value	Cal.Z	*p* Value
Cervical region								
Collagen arrangement	0.00	.480	0.00	.480	0.14	.881	0.52	.606
Osteoid tissue	1.96	.500	1.50	.134	1.50	.134	1.50	.134
Bone mineralization	0.65	.513	0.66	.513	1.31	.189	0.81	.419
Small blood vessels no.	0.23	.817	0.23	.817	1.41	.159	0.32	.742
Fibroblast no.	0.10	.916	0.00	1.000	1.48	.138	1.99	.046
Osteoblast no.	2.45	.014*	1.42	.156	0.32	.746	0.21	.827
Osteocyte no.	1.31	.189	1.42	.156	0.23	.817	0.89	.317
Osteoclast no.	0.00	.480	0.00	.480	1.00	.317	1.00	.317
Middle region								
Collagen arrangement	1.50	.134	1.50	.134	0.14	.881	0.51	.606
Osteoid tissue	1.50	.134	1.00	.317	1.50	.134	1.50	.134
Bone mineralization	0.65	.513	0.00	.480	1.22	.221	0.60	.549
Small blood vessels no.	0.45	.650	0.00	.480	0.87	.381	0.47	.635
Fibroblast no.	0.97	.329	0.87	.386	2.10	.036*	1.88	.059
Osteoblast no.	2.82	.005*	1.94	.053	0.87	.381	0.00	.480
Osteocyte no.	1.22	.221	1.32	.189	0.34	.729	0.43	.661
Osteoclast no.	0.00	.480	0.00	.480	1.00	.317	1.00	.317
Apical region								
Collagen arrangement	0.00	.480	0.00	.480	1.00	.317	1.00	.317
Osteoid tissue	1.96	.050	1.00	.317	1.96	.050	1.96	.050
Bone mineralization	1.00	.317	0.00	.480	0.80	.419	0.80	.419
Small blood vessels no.	1.89	.058	1.97	.049	0.32	.742	0.32	.742
Fibroblast no.	0.74	.459	0.95	.343	2.51	.012*	2.51	.012*
Osteoblast no.	2.37	.018*	1.00	.317	0.54	.584	0.54	.584
Osteocyte no.	1.49	.136	1.93	.054	0.78	.432	0.78	.432
Osteoclast no.	1.00	.317	0.00	.480	1.00	.317	1.00	.317

C0: control zero time; OI L0: olive oil low dose zero time; OI H0: olive oil high dose zero time.
*Significant difference ($p < .05$).

Table 4. Histological findings at pressure and tension side of lower central incisor's root for the control, olive oil low and high doses four weeks groups.

	Pressure site						Tension site					
	C4		OI L4		OI H4		C4		OI L4		OI H4	
Parameters	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Cervical region												
Collagen arrangement	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.50	0.00	0.00	2.00	2.00
Osteoid tissue	0.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	0.00	2.00
Bone mineralization	0.00	0.50	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.50	1.00	1.50
Small blood vessels no.	2.00	1.00	1.00	2.00	1.00	0.50	1.00	1.50	1.00	2.00	1.00	1.50
Fibroblast no.	29.00	6.50	25.00	7.00	30.00	3.00	28.00	5.00	20.00	3.5	21.00	4.50
Osteoblast no.	0.00	1.00	1.00	3.00	2.00	1.50	0.00	4.50	1.00	3.00	2.00	3.50
Osteocyte no.	0.00	1.00	1.00	1.50	2.00	3.00	1.00	1.50	1.00	1.50	2.00	3.00
Osteoclast no.	0.00	0.50	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00
Middle region												
Collagen arrangement	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.50	0.00	0.00	2.00	2.00
Osteoid tissue	0.00	1.00	1.00	1.00	0.00	0.50	0.00	1.00	1.00	1.00	0.00	2.00
Bone mineralization	0.00	0.50	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.50	1.00	1.50
Small blood vessels no.	0.00	0.00	1.00	2.00	1.00	1.50	1.00	1.50	1.00	2.00	1.00	1.00
Fibroblast no.	28.00	4.50	25.00	7.00	29.00	3.00	28.00	5.50	21.00	2.50	22.00	4.00
Osteoblast no.	0.00	1.00	1.00	3.00	2.00	1.00	1.00	3.50	1.00	2.00	1.00	2.00
Osteocyte no.	0.00	0.50	1.00	1.00	2.00	2.00	1.00	1.00	1.00	1.00	2.00	2.00
Osteoclast no.	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.50
Apical region												
Collagen arrangement	3.00	2.00	0.00	0.00	1.00	1.00	1.00	0.50	0.00	0.00	1.00	0.50
Osteoid tissue	0.00	1.00	1.00	1.00	0.00	0.50	0.00	1.00	1.00	1.00	0.00	2.00
Bone mineralization	0.00	0.00	0.00	1.00	2.00*	2.00	1.00	1.00	1.00	1.50	1.00	1.00
Small blood vessels no.	0.00	0.00	1.00	2.00	2.00	1.00	1.00	1,50	2.00	1.50	1.00	1.50
Fibroblast no.	28.00	4.00	22.00	11.50	28.00	4.00	30.00	2.50	21.00	2.00	23.00	3.00
Osteoblast no.	1.00	2.00	1.00	6.50	2.00	3.00	1.00	3.50	1.00	2.00	2.00	3.00
Osteocyte no.	0.00	0.00	1.00	3.00	2.00	3.00	1.00	1.00	1.00	1.50	2.00	2.00
Osteoclast no.	0.00	0.50	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

C4: Control four weeks; OI L4: OI low dose four weeks; OI H4: OI high dose four weeks; IQR: interquartile range.

On the other hand, the comparison between C4 and OI L4 groups revealed no significant difference for all histological features studied at cervical, middle and apical thirds for pressure and tension sides except for fibroblast number at tension sides (Table 5). Moreover, significant differences were detected in bone mineralization, fibroblast, osteoblast, osteocyte numbers in comparisons between C4 and OI H4 groups (Table 5).

Discussion

The animal study can provide valuable information and overcomes some of the difficulties associated with human intervention trials. The reasons behind choosing rabbits in this study are standardization of experimental conditions and experiment repeatability, inexpensiveness and high bone turnover rate [25]. Although the rabbit bone shows less similarity to human bone, as it has a different microstructure from humans, it was the most common species used in bone studies [23,26]. Rabbits are accepted as the animal model for studying bone because their bones undergo changes quickly [23]. For example, the bone remodelling period is achieved within six weeks in rabbits in comparison to humans where it lasts 3–6 months [27]. This bone turnover rapidity was behind choosing seven days orthodontic tooth movement. Furthermore, previous studies observed some of the histological changes associated with OT for seven days in addition to less or more than this period [27,28]. The lower incisors were chosen because they are more accessible as they locate anteriorly so any signs of orthodontic movements or orthodontic relapse are easily observed. Orthodontic appliance design for incisors was less traumatic for the animals than other appliances designed between the first molars and lower incisors in the rabbit [27,28], especially, in this study, the same appliance was used to serve as a retainer for the whole six weeks length R period. However, this model may have some limitations as all permanent teeth in rabbits are elodont (i.e. continuously growing, 'open-rooted') [29]. However, this was overcome by minimizing the study period into a total of 11 weeks.

In this study, we used H&E stain which considered as the basic histological staining for the general assessment of cell and tissue morphology and distribution [30]. However, this technique is not without its limitations, such as, dilution and or contamination of stain, reduction of haematoxylin dye content, in addition to uncontrolled stain which may lead to loss of contrast or colour balance between haematoxylin stain and eosin counterstain. Nevertheless, our slide preparation and result interpretation were conducted under the supervision of trained pathologist which thought to be the gold standard for pathology diagnosis [31].

Olive oil was mixed at certain doses with the rabbit's diet. This procedure is an established and simple method for delivering a compound to laboratory animals. It greatly facilitates animal handling and related stress, which can affect their behaviour and metabolism-potentially confounding the study parameters measured and save operator time over other procedures as oral gavages or injections [32].

In the current study, histological reactions in PDL and AB tissue components were checked at both pressure and

Table 5. Comparison of the histological findings at pressure and tension side of lower central incisor's root between the control and olive oil low, high doses four weeks groups.

	Pressure site				Tension site			
	C4 *vs.* Ol L4		C4 *vs.* Ol H4		C4 *vs.* Ol L4		C4 *vs.* Ol H4	
Parameters	Cal.Z	*p* Value	Cal.Z	*p* Value	Cal.Z	*p* Value	Cal.Z	*p* Value
Cervical region								
Collagen arrangement	1.00	.317	0.00	.480	1.00	.317	0.82	.408
Osteoid tissue	0.60	.549	0.00	.480	0.60	.549	0.23	.811
Bone mineralization	0.65	.513	1.22	.221	0.34	.729	0.34	.729
Small blood vessels No.	0.44	.656	1.22	.221	0.87	.381	0.56	.572
Fibroblast No.	0.84	.396	0.42	.671	2.61	.009*	2.30	.021*
Osteoblast No.	1.56	.119	2.47	.013*	0.54	.588	0.54	.589
Osteocyte No.	0.80	.419	1.75	.080	0.00	1.000	1.19	.233
Osteoclast No.	0.00	.000	1.00	.317	1.00	.317	0.00	.480
Middle region								
Collagen arrangement	1.00	.317	0.00	.480	1.00	.317	0.82	.408
Osteoid tissue	0.60	.549	0.65	.513	0.60	.549	0.23	.811
Bone mineralization	0.65	.513	1.22	.221	0.34	.729	0.34	.729
Small blood vessels no.	1.18	.238	1.19	.232	0.87	.381	0.45	.650
Fibroblast no.	0.53	.592	0.84	.396	2.52	.012*	2.21	.027*
Osteoblast no.	1.56	.119	2.49	.013*	0.00	1.000	0.00	.480
Osteocyte no.	1.22	.221	2.01	.043*	0.95	.339	2.13	.033*
Osteoclast no.	1.00	.317	1.00	.317	1.00	.317	1.00	.317
Apical region								
Collagen arrangement	1.96	.050	1.22	.221	1.00	.317	0.00	.480
Osteoid tissue	0.60	.549	0.65	.513	0.60	.549	0.23	.811
Bone mineralization	1.50	.134	3.00	.003*	0.34	.729	0.00	.480
Small blood vessels no.	1.28	.197	0.56	.575	1.64	.100	1.34	.178
Fibroblast no.	1.57	.115	0.21	.833	2.62	.009*	2.62	.009*
Osteoblast no.	0.43	.664	1.73	.083	0.00	1.000	0.63	.523
Osteocyte no.	1.93	.053	2.35	.019*	0.34	.729	1.53	.125
Osteoclast no.	0.65	.513	1.00	.317	0.00	.480	0.00	.480

C4: Control four weeks; Ol L4: Ol low dose four weeks; Ol H4: Ol high dose four weeks.
*Significant difference ($p < .05$).

tension sides to reveal the effect of Ol supplementation on alveolar turnover immediately after the orthodontic R period and four weeks later. The pressure region is an area that is pressed by the orthodontic appliance in the direction of the force. Pressure results in the deformation of blood vessels and disarrangement of tissues surrounding teeth. Subsequently, blood flow and periodontal tissue changes may adapt to the compression force. Metabolic changes can occur in the cells of the PDL as a result of hypoxia and decreased nutrient levels [33]. Mechanical forces often cause hyalinization leading to necrosis in the PDL and lead to osteoplastic activity, thus creating a cavity in bone known as lacunae that later will be filled in by osteoblasts to cover the cavity [34,35]. As soon as the osteoclasts become inactive and move away the pressure areas display bone formation [36,37].

In the present study, tension occurred in PDL of the mesial and pressure on the distal sides of treated incisors roots. The pressure side in C4, Ol L4, and Ol H4 groups represents the mesial side of the lower central incisor roots; this is because, after the removal of the expansive force at the end of R period, the pressure here was as a result of a rebound of the compressed PDL fibres. This viscoelastic behaviour of CT forming the periodontium is generally attributed to the material properties of extracellular matrix (ECM) in addition to cellular activity, mainly fibroblasts. In turn, fibroblasts, through the cytoskeletal remodelling, actively contribute to the viscoelastic behaviour of the whole tissue [38].

Among the various comparisons carried out, a major significant difference was found between C4 and Ol H4 groups this could explain the lower relapse tendency of Ol H4 in comparison to C4 group at four weeks period. This result is supported by previous studies about Ol effects on bone health [12–14,16,17, 20].

Fibroblasts in the study groups seem to be significantly lower than the control group in some areas of pressure and tension sides. Rosa et al. [39] disagree with the present result as they found a positive effect on fibroblast differentiation, proliferations, and activities. Their study adopted diverse Ol concentrations and combinations, different experimental models, different measuring techniques in fibroblast proliferations and activities, various detecting methods and different observational periods in comparison to the present study. Another result in our study is that the remaining areas of the tension and pressure sides of Ol groups showed no significant difference in fibroblasts count in comparison to C0 group. A reasonable explanation for this significantly lower fibroblast count at the tension side could be that fibroblasts that are a member of CT cells family, which are not only related but also unusually inter-convertible, seem to be the least specialized cells and the most versatile cells in the CT family, displaying a remarkable capacity to differentiate into other members of the CT family [40] as bone cells. Cell transformations are important in the natural repair of broken bones [40].

Moreover, it is necessary to mention that after the release of the expansive force, the highly significant differences in the fibroblast number in our study groups may be explained by the remodelling of the ECM, which plays an integral part

in tooth movement in response to the orthodontic relapse forces. This matrix is mainly produced and regulated by fibroblast cells [41,42]. Experimental studies indicate that fibroblasts are not only capable of synthesizing fibrous tissue and ground substance but they also play an important role in the breakdown of CT. These processes occur simultaneously [43].

In comparing C0 with OI L0 group, it was obvious that OI L0 group had significantly higher osteoblasts count in all three-thirds of the pressure side, which indicated the start of the positive bone remodelling process in OI group unlike the C0 group. This result came in agreement with previous literature that emphasized the beneficial roles of OI on osteoblast differentiation, proliferation, and function [17,44,45]. As regards C4 versus OI H4 group, the histological findings for group C4 versus OI H4 group showed a significantly higher difference for bone mineralization apically, osteoblasts and osteocytes at all thirds of the pressure side reflecting the beneficial roles of increasing OI dose (15.4 ml/kg b.w. daily) on AB in the post-orthodontic relapse period.

The greater osteoblasts number and mineralized matrix at the pressure side of the OI H4 group compared to C4 group indicate not only improved osteogenic differentiation but also a greater capacity for ECM synthesis. These facts suggest advanced stages of bone remodelling for the OI-supplemented group. Remodelling or bone turnover is the process of resorption followed by replacement of bone with little change in shape and occurs throughout a person's life [46]. These processes occur at the remodelling sites that are distributed randomly but also are targeted to areas that require repair [47] as that occurring during orthodontic R period, although the associated tissues remodel at a rate above the baseline during this period [48]. The present results are also in agreement with the studies that have proved beneficial bone effects [17,44], though these studies used varied OI concentrations and combinations or even specific phenolic compounds of OI. In addition, those studies used different assessment techniques to observe mineralization process, and different observational periods in comparison to the present study.

In this study, reduction of relapse tendency after completion of orthodontic retention period could be due to OI ability to enhance the remodelling, mineralization and development of bones [12–14,16,44,45]. Previous studies indicated that OI improves intestinal absorption of calcium [49], moreover, OI is an excellent source of gamma linolenic acid, which has been shown to reduce the excretion of calcium and inhibit bone resorption and markers of bone turnover while at the same time increases the calcium content in the bone [50].

During the course of this work, some limitations are encountered. One of these is the difficulty faced during animal selection and collection as well as the difficulty in estimating the exact age of the animals used in the experiment.

Conclusions

Supplementation with OI during an orthodontic retention period, especially at a concentration of 15.4 ml/kg B.w. per day, clinically reduced orthodontic relapse on rabbit model. Furthermore, OI increased osteoblasts and osteocytes counts and the relative amount of bone mineralization of connective tissue layer forming AB at the end of four weeks after the orthodontic retention period.

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Disclosure statement

All authors disclose that there was no conflict of interest that could inappropriately influence this research.

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