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Effect of Olive Oil and Fig Combination on Relapse during Retention Period after Orthodontic Treatment: Animal Model Study

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Aim: to evaluate the effect of olive oil (Ol) and fig combination on relapse during retention phase after orthodontic treatment in animals.

Materials and methods: Forty rabbits recruited for the study in four groups received orthodontic appliance as following: Group 1 (control group) regular diet. Group 2 olive oil (Ol) added to the regular diet. Group 3 Fig added to the regular diet. Group 4 olive oil and fig added to the regular diet. The appliance used was designed to apply force on the lower incisors of rabbits to open a 3-4 mm space. After space opening, the appliance act as a passive retainer for 6 weeks, during which the animal got the olive oil and the fig. After 6 weeks, the appliance was removed and the impression was taken then followed up for 4 weeks to allow the teeth to relapse and taking an impression for post relapse measurement. The space between teeth was measured. Animals were sacrificed and blocks were taken and prepared for histological study.

Results: There was a significant difference between groups indegree of relapse after orthodontic tooth movement. Mixed group had significantly lowest relapse compared with control group, OI group and Fig group.

Conclusion: Olive oil and Fig combination has synergistic effect as each one has a different mechanism for more bone health as increasing the osteoblastic activity or decreasing the osteoclastic activity.

Keywords: Relapse, Fig, Olive oil, Herbal, Retention

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Introduction

The biological reaction of the tissues around a tooth following the application of a suitable mechanical load is known as orthodontic tooth movement.[1] Osteoclasts (bone resorption) on the pressure side and osteoblasts (bone deposition) on the tension side balance the movement of the tooth.

Following orthodontic treatment, there is a retention period during which orthodontists work to keep the final position stable and the results they have achieved for as long as possible. Relapse is the primary issue at this time.[2] Thus, the retention phase which follows orthodontic treatment and involves using a variety of techniques, including biological, physical, and mechanical retainers is important to preserving the tooth in its final position.

One of the most popular natural products with numerous proven health advantages is olive oil (Olea europaea) (Ol). Consuming olive oil has been linked in numerous studies to a lower risk of developing a number of illnesses, such as osteoporosis and breast cancer.[3,4,5] It has many advantages for bone metabolism because it slows down osteoblast aging, which is crucial for the bone remodeling process.[3,4,5,6]

Another natural product with numerous advantages for metabolism and bone health is the fig (Ficus carica). According to earlier research, Ficus carica plays a major part in preventing osteoporosis.[7]

After reviewing the literatures, no studies were found regarding the effects of the olive oil and fig combination on relapse during the retention phase after orthodontic tooth movement. This study was therefore carried out to evaluate this goal.

Materials And Methods

A randomized control trial with equal groups was established to evaluate the effect of Olive oil and Fig combination on relapse during retention period after orthodontic treatment.

Study protocol was approved by the Research Ethics Committee in Faculty of Dentistry, Minia University, Egypt. (Research no: 605 / 2022).

Sample size calculation

Before the study, the number of animals in each group was determined after a power calculation according to data obtained from previous studies.[8] Ten animals in each group were determined to provide 80% power for One-way ANOVA test with post-hoc analysis at the level of 0.05 significance using G Power 3 Software.[9]

They were divided into four equal groups: Group 1 (control group): tooth movement applied with regular diet, Group 2 tooth movement applied with olive oil added to the regular diet, Group 3 tooth movement applied with Fig added to the regular diet and Group 4 tooth movement applied with olive oil and fig added to the regular diet.

The rabbits ranged in age from 8-10 months and 2.5-3 kg weight. The animals were randomly enrolled in four groups using a computer-generated randomization software. Each animal in the experiment was placed in a separate cage.

The management of the animals was carried out by well-trained clinician according to World Organization for Animal Health (OIE). The diet was in soft consistency to decrease the possibility of appliance breakage.

Orthodontic appliance

Orthodontic appliance designed as Al Hamdany description,[8] using bonded brackets rather than cemented band. The appliance consists of orthodontic brackets with MBT 022 Slot (American orthodontics, USA) bonded to lower incisors of the rabbits. 17x25 stainless steel wire (American orthodontics, USA) with a Niti open coil spring (Ormco, England) inserted between the brackets to

apply about 40 gm continuous force to open a 3-4 mm space between the incisors.[8]

The animals were anesthetized in all steps of the experiment using a combination of ketamine hydrochloride 35 mg/kg (KETAMX 50) (Troikaa pharm. India) with xylazine hydrochloride 10 mg/kg (Xyla-Ject) (Adwia pharm. & chemicals co. S.A.E 10th of Ramadan, Egypt) intramuscular.[8]

The mouth of the rabbit was opened then bonding of the appliance. All the animals followed up on a daily basis to check for any deboned appliance or injured animal. After one week of appliance activation, 3-4 mm space opened between the incisors (Figure 1).

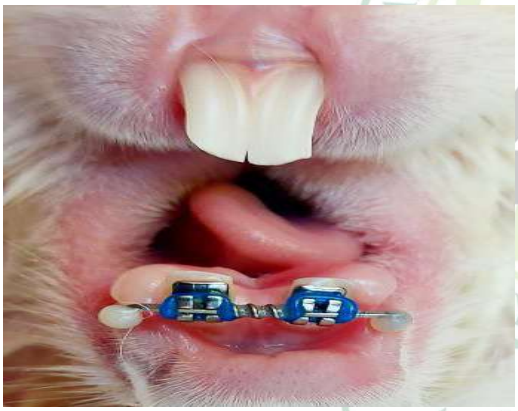

Figure 1: After one week of appliance activation, 3-4 mm space opened between the incisors

Flowable composite added on the coil spring to act as a passive retainer. The space opened measured at the day of appliance removal (T0) and after 4 weeks (T1) to measure the relupse.

Herbal materials feeding was by forced feeding regularly every morning.[10] Olive Oil (Abu Auf company, Egypt) used was Extra virgin olive oil. The dose used was 15.4 ml/kg (14gm/kg) body weight daily.[8] Fig (Abu Auf company, Egypt) used was Dried Turkish Fig, the fig was mixed with water to get a soft mix ,100 gm of fig mixed with 200 ml water to get a soft mix with concentration of 2gm/1ml. The dose used was 2 g/kg body weight daily.[11]

After 6 weeks, the appliance was removed. Impression was taken using condensation silicon impression material (Zetaplus, Zhermack ,Italy) . Taking the impression at T0 (after the retention period) and at T1 after 4 weeks follow up. The casts were scanned using laboratory desktop cast scanner (Ds Mix lab scanner, shining 3D, Hangzhou, China). The space between the incisors was measured between the mesiolabial line angle on the mesial wall of the incisors at the gingival level digitally using Materialize Mimics software (Materialize Mimics, Belgium) (Figure 2) and manually using digital caliper. The space between the incisors was measured two times, the first at T0 after the end of retention period, the second time at T1 following relapse on day 28. At the end of the experiment, animals were sacrificed after general anesthesia and prepared for histological study.

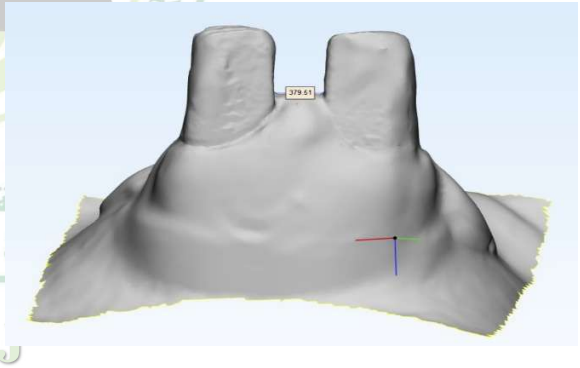

Figure 2: The space between the incisors was measured between the mesiolabial line angle on the mesial wall of the incisors at the gingival level digitally using Materialize Mimics software

Results

Statistical analysis

Statistical analysis was conducted using IBM-SPSS ver. 24. One-way ANOVA test was used to compare the difference in mean between groups. Also, Post-hoc test with Bonferroni Corrections were used to compare the mean difference between groups. The p-value <0.05 was considered significant.

A) Macroscopic findings

1)Digital measurement

According to Table 1, There was a significant difference between groups at post-examination measurements. I.e., mixed group and OI group had significantly higher mean measure compared with control and Fig group.

Table 1: Comparison of Digital Measurements in the studied groups

Digital Measure	Control Group (1) (n = 10)	OI Group (2) (n = 10)	Fig Group (3) (n = 10)	Mix Group (4) (n = 10)	P-value
Pre-examination					= 0.129
Mean ± SD	3.81 ± 0.2	3.87 ± 0.3	3.42 ± 0.4	3.88 ± 0.4	
Median (Range)	3.86 (3.4-4.1)	3.95 (3.4-4.1)	3.26 (3.2-4.1)	3.97 (3.3-4.2)	
Post-examination					= 0.002
Mean ± SD	2.78 ± 0.2	3.31 ± 0.3	2.70 ± 0.5	3.59 ± 0.3	
Median (Range)	2.81 (2.5-2.9)	3.46 (2.8-3.5)	2.52 (2.3-3.3)	3.69 (3.1-3.9)	

Regarding table 2, The digital measurements, mixed group had significantly lower relapse compared with control, OI group and Fig group.

Table 2: Comparison of Differences (relapse) in Digital Measurements in the studied groups

Digital Measure	Control Group (1) (n = 10)	OI Group (2) (n = 10)	Fig Group (3) (n = 10)	Mix Group (4) (n = 10)	P-value
Difference					< 0.001
Mean ± SD	1.02 ± 0.1	0.57 ± 0.05	0.72 ± 0.03	0.30 ± 0.06	
Median (Range)	0.97 (0.9-1.2)	0.59 (0.5-0.6)	0.83 (0.2-0.9)	0.28 (0.3-0.4)	

2) Manual measurement

According to table 3, There was a significant difference between groups at post-examination measurements. I.e., mixed group and OI group had significantly higher mean measure compared with control and Fig group.

Table 3: Comparison of Manual Measurements in the studied groups

Manual Measure	Control Group (1) (n = 10)	OI Group (2) (n = 10)	Fig Group (3) (n = 10)	Mix Group (4) (n = 10)	P-value
Pre-examination					= 0.599
Mean ± SD	3.82 ± 0.2	3.84 ± 0.2	3.06 ± 0.4	3.91 ± 0.2	
Median (Range)	3.92 (3.5-4.1)	3.91 (3.5-4.1)	3.53 (3.2-4.2)	3.91 (3.5-4.2)	
Post-examination					
Mean ± SD	2.71 ± 0.1	3.37 ± 0.3	2.87 ± 0.5	3.61 ± 0.3	= 0.001
Median (Range)	2.70 (2.6-2.9)	3.51 (2.9-3.6)	2.72 (2.4-3.5)	3.70 (3.1-3.9)	

Regarding manual measurements in table 4, mixed group had significantly lower relapse compared with control and Fig group.

Table 4: Comparison of Differences (relapse) in Manual Measure in the studied groups

Manual Measure	Control Group (1) (n = 10)	OI Group (2) (n = 10)	Fig Group (3) (n = 10)	Mix Group (4) (n = 10)	P-value
Difference					< 0.001
Mean ± SD	1.11 ± 0.2	0.47 ± 0.09	0.80 ± 0.08	0.30 ± 0.02	
Median (Range)	1.1 (0.9-1.4)	0.5 (0.4-0.6)	0.79 (0.7-0.9)	0.76 (0.2-0.4)	

3) Comparison between digital and manual measurements

Interclass Correlation Coefficient " ICC" shows there was significant good to excellent agreement "strong correlation" between digital and manual measures for the total sample (Table 5).

Table 5: Agreement between Digital and Manual Measurements

	Control (n = 10)	OI Group (n = 10)	Fig Group (n = 10)	Mix Group (n = 10)	Total (n=40)
ICC (95% CI)					
Pre-exam.	0.89 (0.41-0.98)	0.94 (0.38-0.99)	0.85 (0.24-0.98)	0.95 (0.53-0.98)	0.89 (0.72-0.96)
P-value	= 0.030	= 0.011	= 0.046	= 0.006	< 0.001
Post-exam.	0.31 (-0.74: 0.87)	0.93 (0.34-0.98)	0.99 (0.97-1.00)	0.94 (0.51-0.99)	0.97 (0.93-0.99)
P-value	= 0.366	= 0.012	< 0.001	= 0.003	< 0.001
Difference	0.26 (-0.75: 0.92)	0.04 (-0.82: 0.81)	0.17 (-0.78: 0.84)	0.52 (-0.64: 0.91)	0.91 (0.76-0.96)
P-value	= 0.387	= 0.513	= 0.431	= 0.254	< 0.001

B) Microscopic findings (Histological examination) (H&E stained slide analysis)

According to Table 6, Figure 3 and 4, The highest number of bone forming cells (osteoblasts) and the highest amount of new bone tissues were found in mixed group.

Table 6: Histomorphometry analysis of the studied groups

	Group	Mean new bone area (mm2)	Mean % of new bone formation	Mean No of Osteoblasts	Mean No of Osteoclasts	Mean Bone density
1	Control	1.86	28.4%	36-37	5-6	+1.7
2	Olive Oil	2.33	39%	56-57	1-2	+2.4
3	Fig	2.03	33.9%	45-46	1-2	+1.5
4	Fig and Olive Oil	3.49	58.1%	68-69	0-1	+3.3

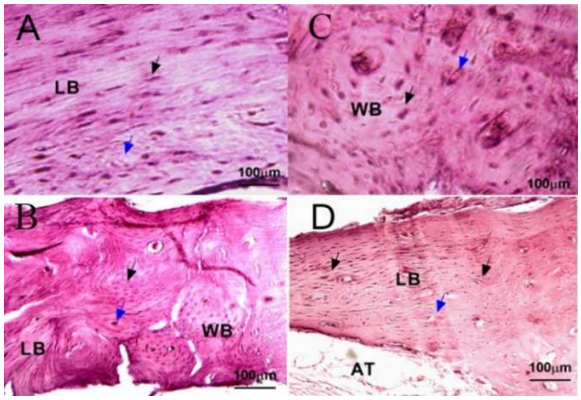

Figure 3: The highest number of bone forming cells

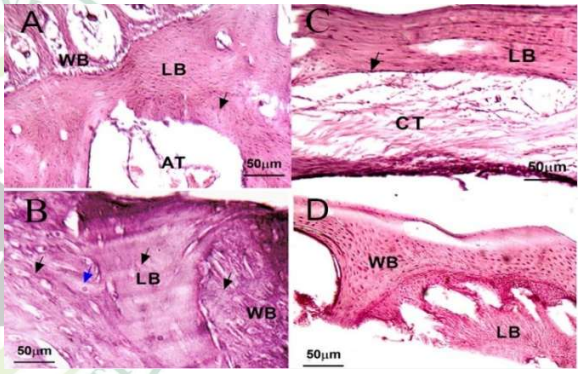

Figure 4: the highest amount of new bone tissues was found in mixed group

Discussion

Following orthodontic treatment, the retention phase is essential for maintaining the desired outcomes and preventing relapse which is the primary issue that arises from orthodontic therapy.[2]

The major goal of this study was to determine how the combination of fig and olive oil affected orthodontic relapse, making use of both resources. Because they are larger than rats and easier to manipulate, rabbits are the most often utilized animal for bone research, which is why they were chosen for this work.[12,13] Additionally, because of their fast bone turnover rate, the bone remodeling stage is completed in just six weeks.[14]

The rabbits' lower incisors were selected because they were more accessible and positioned anteriorly than posterior teeth, making it easier to monitor and see tooth movement.[15] Soft meals were given to the

animals in order to reduce the risk of device damage. and kept in a different cage so that no other animal may damage the appliance.

The appliance used was designed as Al Hamdany et al description but using bonded brackets rather than cemented band as it has smaller size than cemented bands and easier to remove without excess cement on the teeth that facilitate more accurate measurements.[8]

The forced feeding was done regularly every morning daily to be sure the animals were hungry. The animals accepted the olive oil and the fig normally. Extra virgin olive oil dose used was 15.4 ml/kg (14gm/kg) body weight daily which is the recommended dose by Al Hamadany et al. [8] Dried Turkish Fig, the fig was mixed with water to get a soft mix, dose used was 2 g/kg body weight daily using the same ratio suggested by Bashandy et al. as 7:1 olive oil and fig respectively.[11]

Extra virgin type used as it the least proceeded form of the olive oil so it retains its natural contents which are often lost during processing, so that make it healthier.[16] Fig used was dried fig mixed with water to get a soft mix, this form was chosen rather than fig extract as previous studies to simulate the regular use of the fig in daily life.[17]

Digital and manual measurements were used to measure the space opened between teeth. The results showed that Olive oil group had significantly lower relapse compared with control group which agrees with previous study,[8] which can be explained as the Ol is an excellent source of gamma linolenic acid, which has an important role in reducing the excretion of calcium and decrease bone resorption and increases the calcium content in the bone.[18] Also, the phenolic content in virgin olive oil extracts such as apigenin, luteolin, coumaric acid, ferulic acid and affeic acid can increase the proliferation capacity and differentiation of osteoblasts.[19]

Also, the TGF-β1 which can recruit osteoblasts precursors in the area of bone formation and stimulate osteoblasts to differentiate to produce bone matrix.[20] Also, it inhibits the recruitment of osteoclast precursor cells and directly inhibits osteoclast activity in bone resorption.[21,22]

Fig group had significantly lower relapse compared with control group which agrees with previous studies which can be explained as that the hexane soluble fraction of the fig can inhibit osteoclast differentiation by suppression of many factors, as HF6-FC significantly decreased the expression of NFATc1 and c-Fos, which are the major regulator of osteoclast differentiation. Mixed group had significantly lowest relapse compared with control group.[23,24]

OI group and Fig group. Which show a synergistic effect when compared to the use of OI or fig alone. Which can be explained as each one has a different mechanism for more bone health as increasing the osteoblastic activity or decreasing the osteoclastic activity.

The histomorphometry showed that the mixed group had the highest number of osteoblasts, bone density and bone area formed and the lowest number of osteoclastic counts, which justify the positive results of the clinical measurements of mixed group.

Conclusion

Olive oil helps in increasing the osteoblast cells and decreasing the osteoclasts which decreases relapse after orthodontic tooth movement. Also, Fig act as potent inhibitor of osteoclastogenesis so that decreases relapse after orthodontic tooth movement.

Olive oil and Fig mixed group has the lowest relapse compared with olive oil only or fig only which is related to the synergistic effect as each one has a different mechanism for more bone health as increasing the osteoblastic activity or decreasing the osteoclastic activity.

Author Contributions

Conceptualization, M.A. and M.E.; methodology, M.A.; software, M.G., M.E.;

validation, M.G., W.M.; formal analysis, M.E.; investigation, M.E., M.A. and W.M.; resources, M.E, and M.A.; data curation, W.M., M.G.; writing—original draft preparation, M.A., M.E.; writing—review and editing, M.A., M.G. and W.M.; visualization, W.M.; supervision, M.G., W.M.; project administration, M.E. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was approved by Dental Ethics Committee of the Faculty of Dentistry, Minia University. (ID number 605 / 2022).

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

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