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THE EFFECT OF ATORVASTATIN ON RELAPSE AFTER ORTHODONTIC TOOTH MOVEMENT

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Abstract

Objective

The causes of relapse are multifaceted and include everything from muscular disorders and bad oral habits to adverse growth patterns and transeptal fiber stretching. This research has been conducted to find a more biological way of controlling the relapse, instead of the classic mechanical retention. For this study we chose the Atorvastatin drug. It's from the statins family which is a commonly used cholesterol lowering drug. Our aim was to study the effect of Atorvastatin on relapse after orthodontic force.

Material and methods

A case control study on 16 healthy male white New Zealand rabbits 16-week-old weighing between 2.1 and 2.6 kg with normally developed dentition. Animals were anesthetized using intramuscular injection of xylazine and ketamine, a closed coil Ni-Ti spring was inserted between the lower incisors and the lower 1st premolar, 150cN of force was measured before using ligature wire and resin cement to hold the appliance in place, the appliance was left for 3 weeks to attain the desired tooth movement. After 3 weeks the appliance was removed, records were taken using rubber base impression materials immediately after. Animals were split randomly into 2 groups; study group was fed 20mg/kg of Atorvastatin Calcium powder suspended in distilled water orally via gavage daily for 21 days. Control group were force fed an equal amount of phosphate-buffered saline solution via gavage also. After 21 days another record was taken using rubber base impression material and 1 animal was randomly selected from both groups for histological analysis. Animals chosen for histological analysis had their mandibles dissected, then sliced in half, fixed, and decalcified. Nine randomly chosen sections per specimen were processed from parasagittal serial sections of 6-mm thickness. Hematoxylin and eosin was used to stain the sections. Under a light microscope, sections of

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the interdental space between the mandibular first and second premolars were photographed. Osteoclastic count, osteoblastic count, cortical thickness, number of bone trabeculae, bone density (intensity), and number of blood vessels were among the histomorphometric characteristics assessed.

Results

The two groups showed significant difference (p=0.002) regarding mean distance before and after treatment. Additionally, control group showed significantly (p<0.001) higher mean relapse (0.79 ± 0.02 mm) than study group (0.28 ± 0.02 mm). The relapse% was significantly (p<0.001) higher in control (67.4%) than study group (29%). The mean number of osteoblasts was insignificantly (p = 0.910) higher in controls (42.2 ± 8.4) than cases (41.8 ± 7.9). The mean number of osteoclasts was significantly (p = 0.004) lower in controls (4.2 ± 1.1) than cases (6.2 ± 1.4). Cases showed significantly (p < 0.009) lower mean bone density (2.2 ± 0.7) compared with controls (3.1 ± 0.6).

Conclusion

Our results show potential positive effect of atorvastatin on decreasing the percentage of relapse after orthodontic tooth movement in animal model, both in the relapse percentage and some of the histomorphometric parameters related to bone remodeling.

Introduction

Successful orthodontic treatment should be characterized by long-term preservation of a healthy and aesthetic dentition, and functional occlusal relationships in tandem with facial esthetics. Sadly, immediately after the removal of orthodontic appliances, the disturbance in the mechanical environment will usually result in teeth moving away from the position acquired at the end of orthodontic tooth movement, trying to attain a new mechanical balance (1,2) Also, supporting tissue are stretched, forces from orofacial muscle, continued craniofacial growth, along with factors related to treatment characteristics, all these factors contribute to moving teeth away from their final, adjusted position. As this phenomenon occurs in a big percentage of orthodontic patients, retention is an essential phase in orthodontic treatment, where retainers are used to preserve the attained result.(3,4).

Studies in animal models have demonstrated that statins (anti hyperlipidemic drugs) like atorvastatin (ATV) (5) and Simvastatin (SMV) (6) reduce OTM and root resorption when given systemically (7,8). According to studies, hypercholesterolemia threatens bone mineral density and accelerates alveolar bone loss, making people more prone to oral inflammatory diseases such chronic periodontitis (9,10). Additionally, it has been suggested that hyperlipidemia causes the gingival crevicular fluid to experience oxidative stress, which is a risk factor for periodontal diseases (10).

The causes of relapse are multifaceted and include everything from muscular disorders and bad oral habits to adverse growth patterns and transeptal fiber stretching (11), It also has

its origin from the form of immature, slightly calcified bone tissue that envelops the moving tooth elements (12).

Studies in animal models have demonstrated that statins (anti hyperlipidemic drugs) like atorvastatin (ATV) (5) and Simvastatin (SMV) (6) reduce OTM and root resorption when given systemically (7,8).

Orthodontic relapse (OR), which is thought to have a complex cause, is a necessary but unfavourable consequence of orthodontic treatment. There are more factors that contribute to this problem besides teeth and supporting structures, include neuromuscular imbalance, continuing craniofacial development, and ingrained habits (13). Some authors stressed the need of recognizing OR as a distinctive manifestation of physiological recovery in response to withdrawal of pressures, distinct from changes brought on by development and ageing (1).

Our aim was to study the effect of Atorvastatin on relapse after orthodontic force.

Materials and Methods

This research was carried out in compliance with the ARRIVE recommendations [133] following the ethics committee's approval, Faculty of Dentistry, Minia University, Egypt, 2019.

Twenty male, healthy New Zealand rabbits (Oryctolagus cuniculus) at the age of sixteen weeks were included in the study. Ten as study group and ten as control group. They were between 2.1 and 2.6 kg in weight, and their incisors, premolars, and molars had developed normally.

Rabbits were anesthetized using intramuscular injection in the thigh of xylazine 0.2 ml/kg (Xyla-ject, manufactured by ADWIA Co. S.A.E, 10th of Ramadan city, Egypt) as a sedative and pre-anethestatic , followed after 5 min by ketamine 0.2 ml/kg (Ketam, manufactured by egyptian int. pharmaceutical industries Co, 10th of Ramadan city, industrial area 81, Egypt) took about 5 minutes for the animal to be ready for appliance installment.

Two assistants were present aside from the researcher, one held the jaws open using tough small ropes, while the other held the tongue using a dental mirror.

Using an insulin needle a pathway was opened for the ligature wire between the 1st and 2nd lower left premolars, wire was then passed through using a Mathew and the 12 mm super elastic nickel titanium closed coil spring with eyelits (Hangzhou DTC medical apparatus co. ltd.) was attached to the ligature wire.

The ligature wire was tied securely using Mathew forceps, making sure there was no mobility in the ligature wire surrounding the 1st premolar and also making sure the coil spring was properly positioned, the extra ligature was cut off using a straight wire cutter and the rest was secured buccally to minimize tissue damage after the animal regains consciousness.

Using a force gauge (Morelli tensiometer 60gf – 60Cgf – color green), 150 cN was measured, and the other end of the 12 mm super elastic nickel titanium closed coil spring was attached

to another ligature wire which was also tied securely to the incisors using the Mathew forceps, the extra wire was cut off using a straight wire cutter and the rest was secured near the incisors to minimize tissue damage

After making sure the appliance was secured and the force on the closed coil spring was 150cN, the incisors and premolars were isolated and dried, and using a dual cure self-bonding resin cement (Overcem SA manufactured by Overfibers via Malatesta, Imola, Italy), the ligature wire surrounding the incisors and the premolars was bonded to the tooth structure to increases the stability of the appliance and reduce the chance of breakage during the tooth movement period. The animals were monitored until they regained full consciousness, they were kept with constant supply of soft food and water to minimize the chance of appliance breakage. They were housed in separate cages with day and night cycle in a well-ventilated room.

They were checked on daily for device breakage or unusual behavior such as violence or not eating, after 10 days 4 random animals were selected and anesthetized using intramuscular injection of xylazine followed by ketamine to ensure the appliance was working and there was no breakage during this period.

The animals were evaluated following 21 days of active orthodontic tooth movement. re-anaesthetized using the same method as before for removal of the appliance and taking impressions of the teeth after the orthodontic tooth movement.

After the animal was unconscious, the ligature wire used to tie the appliance to the teeth was cut using a straight wire cutter and the device was removed from place, any residual wire pieces or broken resin cement was carefully removed using a tweezer and cotton to avoid ingestion after the animal regains consciousness, some of the rabbits had bleeding gums due to inflammation caused by the appliance, bleeding was stopped with a cotton wetted with a hemostatic agent.

When the field was ready, using silicone vinyl polysiloxane injection-type impression material, an impression was taken (Speedix, Coltene dental products) loaded onto special trays that had been previously made, the help retracted the check and the tongue while the operator took the impression of the posterior teeth, same procedures were repeated for all the animals.

Following that, teeth were given 21 days to relapse [134], Each animal was sorted into one of two experimental groups at random. The study group (A) (n=10) allowed the teeth to relapse while being force-fed atorvastatin calcium powder at a rate of 20 mg/kg every day (Borg pharmaceutical industries, S.A.E., El-bostan, Alexandria, Egypt) suspended in distilled water via gavage, and in control group (B)(n=10), the teeth were allowed to relapse with a daily force fed an equal amount of phosphate-buffered saline solution via gavage. At the end of the study, 4 animals from the control group were excluded from the results due to their sickness and death before the end of the second phase of the study.

After that, an improved die stone was used to pour the impressions (Elite Rock Dental Stone; Zhermack, Badia Polesine, Italy), base was added and all casts were trimmed after they set, a 3-dimensional scanner was used to scan the casts (Ceramill400, Amann Girrbach, Herrschaftswiesen 16842 Koblach | Austria.) to create a 3-dimentional model in. stl form for accurate measurements.

Using View box software (View box for Windows, version 4.1.0.12 64 bit; dHal Software, Kifissia, Greece), The cusp tips of the mandibular molars and second premolar were used to identify the mandibular occlusal plane, which was used to align the models. Perpendicular to the mandibular occlusal plane, two planes were drawn. The mandibular first premolar's distal surface's most distal contact area was where the first plane was drawn. The mesial surface of the mandibular second premolar's most mesial contact area was touched by the second plane. The linear distance (perpendicular to the mandibular occlusal reference plane) between the two created planes was measured to estimate the amount of tooth movement.

The View box program's ruler tool was used to take measurements. Calculations were made to determine tooth movement magnitudes, post-orthodontic relapse rates, and relapse percentage.

The researcher conducted the measurements in a blinded manner. The same researcher repeated the measurements of 30 randomly chosen 3-dimensional models two weeks apart in order to assess the intraexaminer errors for tooth movement measurements.

Two rabbits, one from each group, were randomly chosen for histologic examinations at the conclusion of the post-orthodontic relapse phase. The mandibles were dissected, then split in half, fixated, and decalcified after the animals had died. Nine randomly chosen sections per specimen were processed from parasagittal serial sections of 6-mm thickness.

Hematoxylin and eosin was used to stain the sections. Under a light microscope, sections of the interdental space between the mandibular first and second premolars were photographed (*LABOMED Trinocular inverted phase contrast microscope model TCM400 microscope, and the Atlas 16MP Cmos USB Camera software (LABOMED, USA). The magnification power is 40x, scale bar: 50µm*) and photographs of representative regions were described.

On the former tension sides (which turned into compression sides during relapse), histomorphometric examination was carried out. Osteoclastic count, osteoblastic count, cortical thickness, number of bone trabeculae, bone density (intensity), and number of blood vessels were among the histomorphometric characteristics assessed. The lead investigator carried out a blinded histologic study. Histomorphometric measurements were counted using automated algorithms available in the software.

Results

This research was carried out in compliance with the ARRIVE recommendations* following the ethics committee's approval, Faculty of Dentistry, Minia University, Egypt, 2019

Twenty male, healthy New Zealand rabbits (Oryctolagus cuniculus) at the age of sixteen weeks were included in the study .Ten as study group and ten as control group. They were between 2.1 and 2.6 kg in weight, and their incisors, premolars, and molars had developed normally. All experimental procedures were performed under general anesthesia by intramuscular injection of ketamine and xylazin. 4 specimens were excluded from the study, one due to death of the animal, and 3 due to repeated device breakage and getting no orthodontic tooth movement in the first 3 weeks of the experiment.

Table 1 and Fig. 1 showed the difference in the mean distance between 1st and 2nd premolar in mm over the study period before relapse vs. after relapse for the control group specimens. All specimens showed significant reduction regarding mean distance ($p<0.001$).

Table 1: Comparison of Distance between 1st and 2nd premolar (Control)

(Mean ± SD)	Before relapse (n = 8)	After relapse (n = 8)	Mean Difference	P-value*
Distance between 1st and 2nd premolar in mm				
• **Specimen 1**	1.16 ± 0.03	0.39 ± 0.04	**0.77**	< **0.001**
• **Specimen 2**	1.17 ± 0.03	0.37 ± 0.03	**0.80**	< **0.001**
• **Specimen 3**	1.20 ± 0.07	0.39 ± 0.04	**0.81**	< **0.001**
• **Specimen 4**	1.17 ± 0.03	0.38 ± 0.03	**0.79**	< **0.001**
• **Specimen 5**	1.16 ± 0.03	0.39 ± 0.04	**0.77**	< **0.001**
• **Specimen 6**	1.17 ± 0.03	0.37 ± 0.03	**0.80**	< **0.001**
• **Specimen 7**	1.21 ± 0.08	0.39 ± 0.04	**0.82**	< **0.001**
• **Specimen 8**	1.17 ± 0.03	0.38 ± 0.03	**0.79**	< **0.001**

***Paired Sample t-test was used to compare mean before vs after treatment**

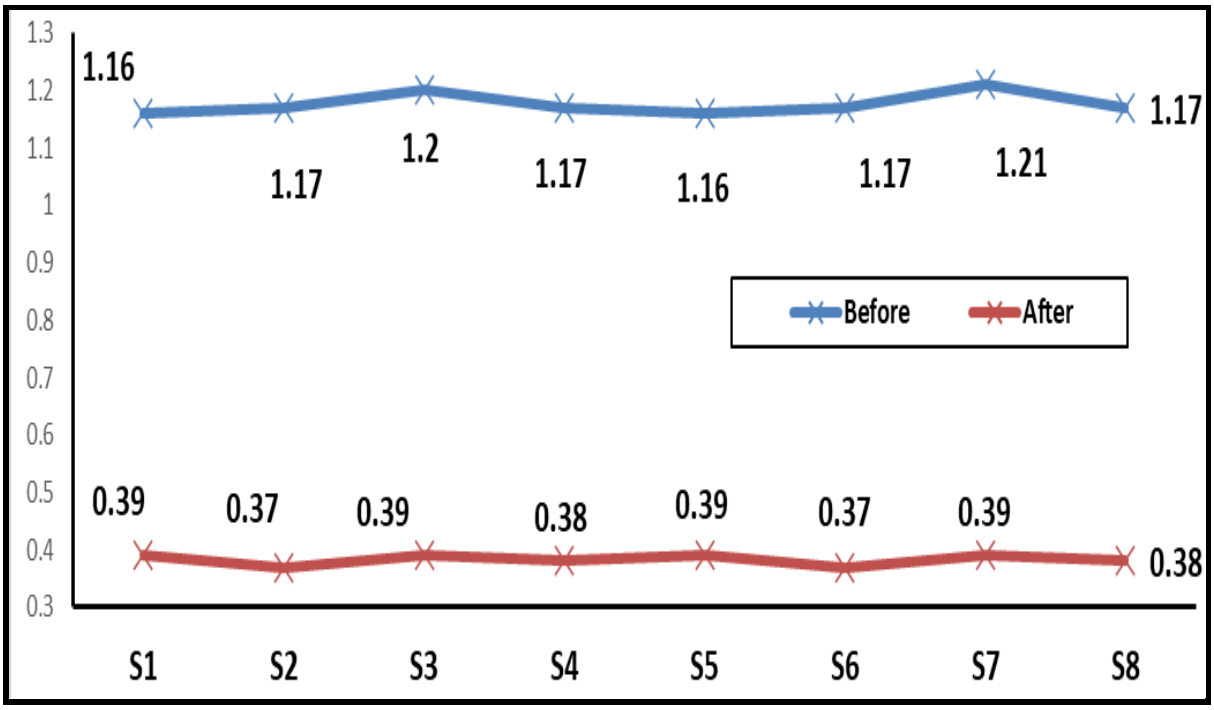

Fig. 1: Mean Distance between 1st and 2nd premolar (Control)

Table 2 and Fig. 2 showed the difference in the mean distance between 1st and 2nd premolar in mm over the study period before relapse vs. after relapse for the study group specimens. All specimens showed significant reduction ($p<0.05$) regarding mean distance.

Table 2: Comparison of Distance between 1st and 2nd premolar (Study)

(Mean ± SD)	Before relapse (n = 8)	After relapse (n = 8)	Mean Difference	P-value*
Distance between 1st and 2nd premolar in mm				
• **Specimen 1**	1.04 ± 0.04	0.79 ± 0.03	**0.25**	< **0.001**
• **Specimen 2**	0.99 ± 0.04	0.87 ± 0.05	**0.12**	= **0.004**
• **Specimen 3**	1.05 ± 0.04	0.49 ± 0.03	**0.56**	< **0.001**
• **Specimen 4**	0.73 ± 0.01	0.54 ± 0.06	**0.19**	= **0.005**
• **Specimen 5**	1.03 ± 0.03	0.78 ± 0.02	**0.25**	= **0.001**
• **Specimen 6**	0.98 ± 0.03	0.86 ± 0.04	**0.12**	= **0.021**
• **Specimen 7**	1.04 ± 0.04	0.48 ± 0.03	**0.56**	< **0.001**
• **Specimen 8**	0.72 ± 0.01	0.53 ± 0.05	**0.19**	= **0.001**

***Paired Sample t-test was used to compare mean before vs after treatment**

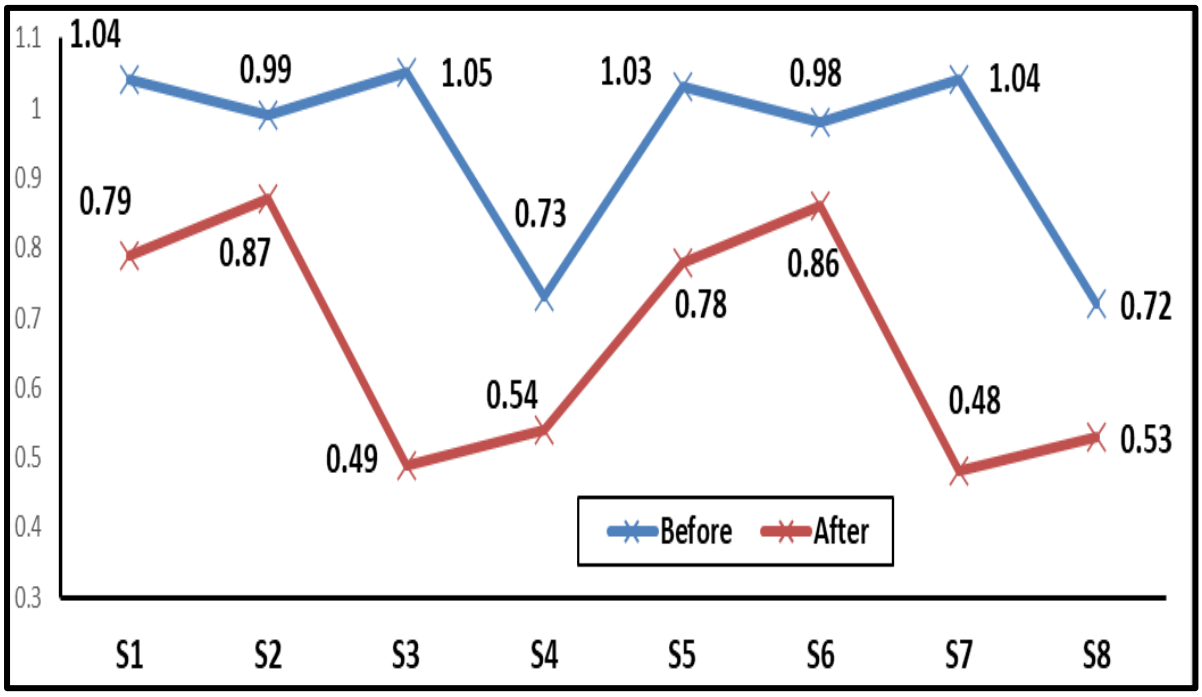

Fig. 2: Mean Distance between 1st and 2nd premolar (Study)

Table 3 presented the comparison of the magnitudes of tooth movement and relapse between groups. The two groups showed significant difference (p=0.002) regarding mean distance before and after treatment.

Additionally, control group showed significantly (p<0.001) higher mean relapse (0.79 ± 0.02 mm) than study group (0.28 ± 0.02 mm). Likewise, the relapse% was significantly (p<0.001) higher in control (67.4%) than study group (29%) **(Fig. 3)**.

Table 3: Magnitudes of Tooth Movement and Relapse, and percentages of relapse in control group and study group

(Mean ± SD)	Control (n = 8)	Study (n = 8)	
• **Mean Distance Before relapse**	1.18 ± 0.02	0.95 ± 0.01	= **0.002**
• **Mean Distance After relapse**	0.38 ± 0.01	0.67 ± 0.02	= **0.002**
• **Absolute Relapse**	0.79 ± 0.02	0.28 ± 0.02	< **0.001**
• **Relative (%) Relapse**	67.44% ± 0.7%	29.01% ± 2.6%	< **0.001**

***Independent Sample t-test was used to compare mean between groups**

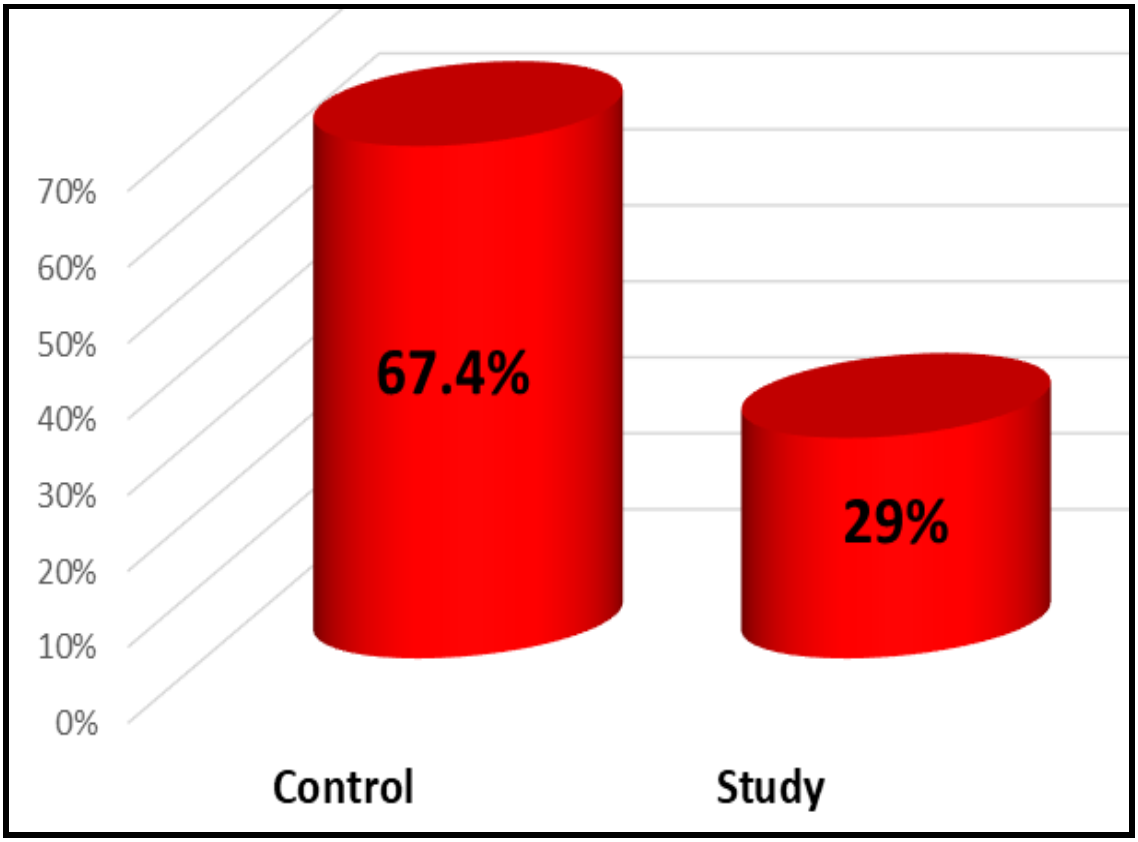

Fig. 3: Mean Difference in Relapse% between groups

Histomorphometry analysis

1. Imaging:

a. A brightfield microscope equipped with a camera was used to capture images of the stained bone sections.

b. The microscope settings adjusted for optimal image quality, including appropriate magnification and lighting conditions.

2. Image Analysis:

a. Image analysis software of Camera software was used, to quantify various histometric parameters. These parameters may include:

1. Bone area: Measure the total area occupied by bone tissue in the section.

2. Trabecular or cortical thickness: Measure the thickness of the trabecular or cortical bone, respectively.

3. Osteoblast or osteoclast number: Count the number of osteoblasts or osteoclasts per unit area.

4. Osteoid volume or mineralization: Assess the amount of unmineralized osteoid or the degree of mineralization in the bone.

5. Vascularization: Quantify the number or density of blood vessels within the bone.

b. The software was calibrated by setting appropriate scale measurements using a reference slide (Control) with a known scale.

c. Multiple fields of view within each section were analyzed to ensure representative sampling.

d. Measurements were counted using automated algorithms available in the software. As showen in figures 4 &5

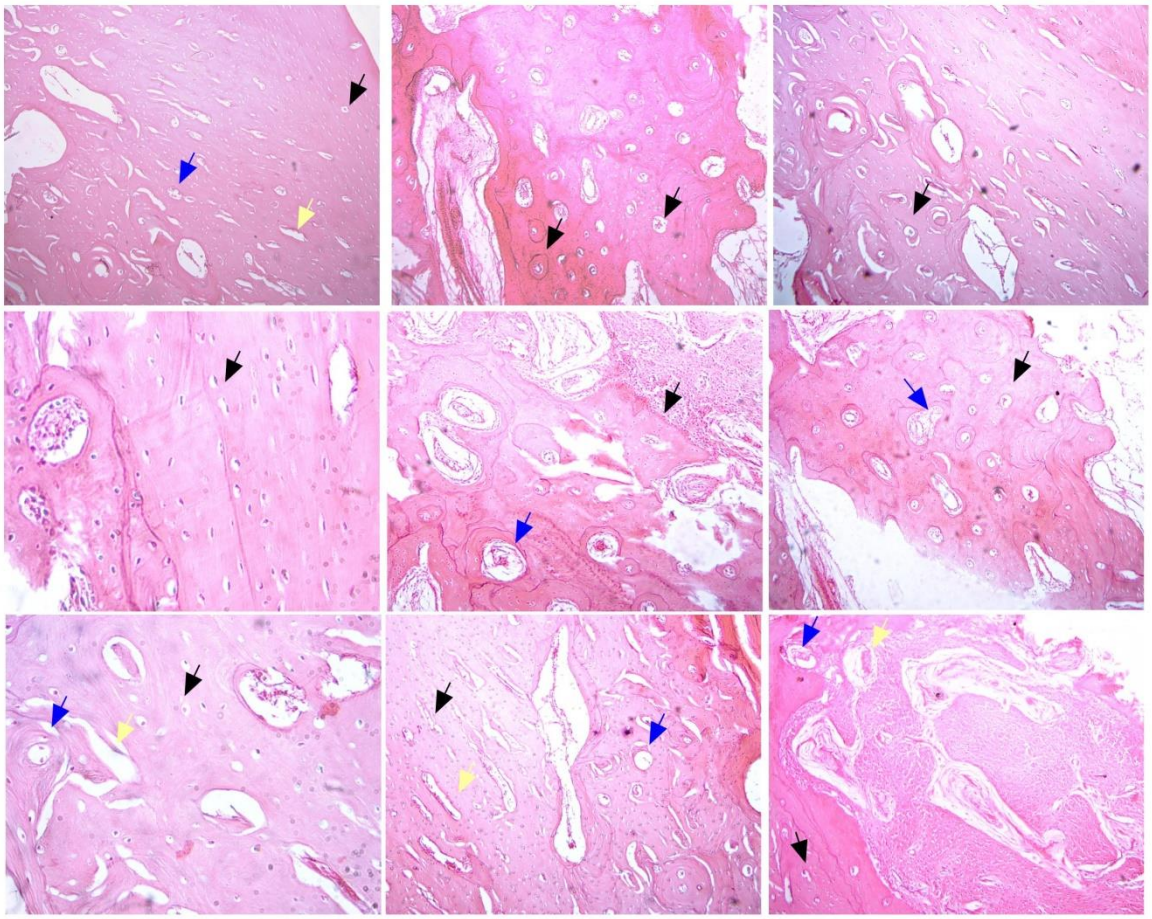

Figure 4. Histomorphometry of rabbit alveolar bone (Control Group): Osteoblasts: black arrow, Osteoclasts: yellow arrow, and Bone marrow trabeculae: yellow arrow. ***Images are captured by LABOMED Trinocular inverted phase contrast microscope model TCM400 microscope, and the Atlas 16MP Cmos USB Camera software (LABOMED, USA). The magnification power is 40x, scale bar: 50µm***

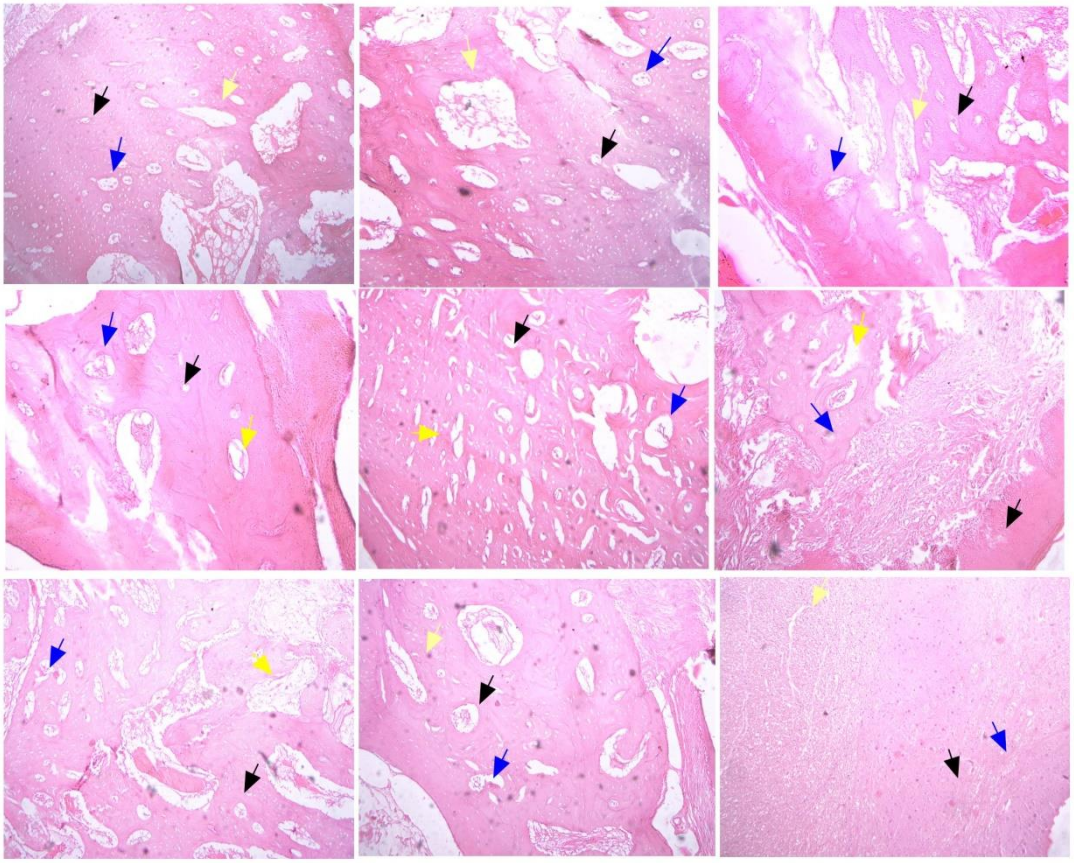

Figure 5. Histomorphometry of rabbit alveolar bone (Study group) (Osteoporosis): exhibited a reduced bone area compared to healthy bone sections, trabecular thickness is decreased in osteoporotic bone, Cortical thickness is reduced in osteoporotic bone samples, Osteoblast numbers were lower in osteoporotic bone sections, and Osteoclast numbers are increased. Osteoblasts: black arrow, Osteoclasts: yellow arrow, and Bone marrow trabeculae: yellow arrow. ***Images are captured by LABOMED Trinocular inverted phase contrast microscope model TCM400 microscope, and the Atlas 16MP Cmos USB Camera software (LABOMED, USA). The magnification power is 40x, scale bar: 50µm***

Table 4 and Fig. 6 showed the difference in the mean histomorphometry analysis parameters between Cases and Control.

Regarding the bone area%, cases showed significantly ($p < 0.001$) lower mean bone area% (52.2 ± 11.2%) compared with controls (82.6 ± 7.4%). Also, cases had significantly ($p = 0.008$) lower mean cortical thickness (1.7 ± 0.4 mm) compared with controls (1.2 ± 0.2 mm).

Additionally, the mean number of bone trabeculae was significantly ($p = 0.014$) higher in controls (4.9 ± 1.6) than cases (3.1 ± 1.1). On the other hands, the mean number of osteoblasts was insignificantly ($p = 0.910$) higher in controls (42.2 ± 8.4) than cases (41.8 ± 7.9). On the contrast, the mean number of osteoclasts was significantly ($p = 0.004$) lower in controls (4.2 ± 1.1) than cases (6.2 ± 1.4).

Respecting the bone density (intensity), cases showed significantly ($p < 0.009$) lower mean bone density (2.2 ± 0.7) compared with controls (3.1 ± 0.6). Likewise, the mean number of vessels was significantly ($p = 0.016$) lower in cases (2.1 ± 0.9) than control (3.1 ± 0.7).

Table 4: Comparison of Histomorphometry Analysis between Cases and Control

(Mean ± SD)	Control (n = 9)	Case (n = 9)	P-value*
Bone area (%)	82.56 ± 7.4	52.22 ± 11.2	**< 0.001**
Cortical thickness (mm)	1.69 ± 0.4	1.18 ± 0.2	**= 0.008**
Bone Trabeculae No.	4.89 ± 1.6	3.11 ± 1.1	**= 0.014**
Osteoblast No.	42.22 ± 8.4	41.78 ± 7.9	= 0.910
Osteoclast No.	4.22 ± 1.1	6.22 ± 1.3	**= 0.004**
Bone density (intensity)	3.11 ± 0.6	2.22 ± 0.7	**= 0.009**
Blood Vessels No.	3.01 ± 0.7	2.01 ± 0.9	**= 0.016**

***Independent Sample t-test was used to compare the difference in Mean between groups**

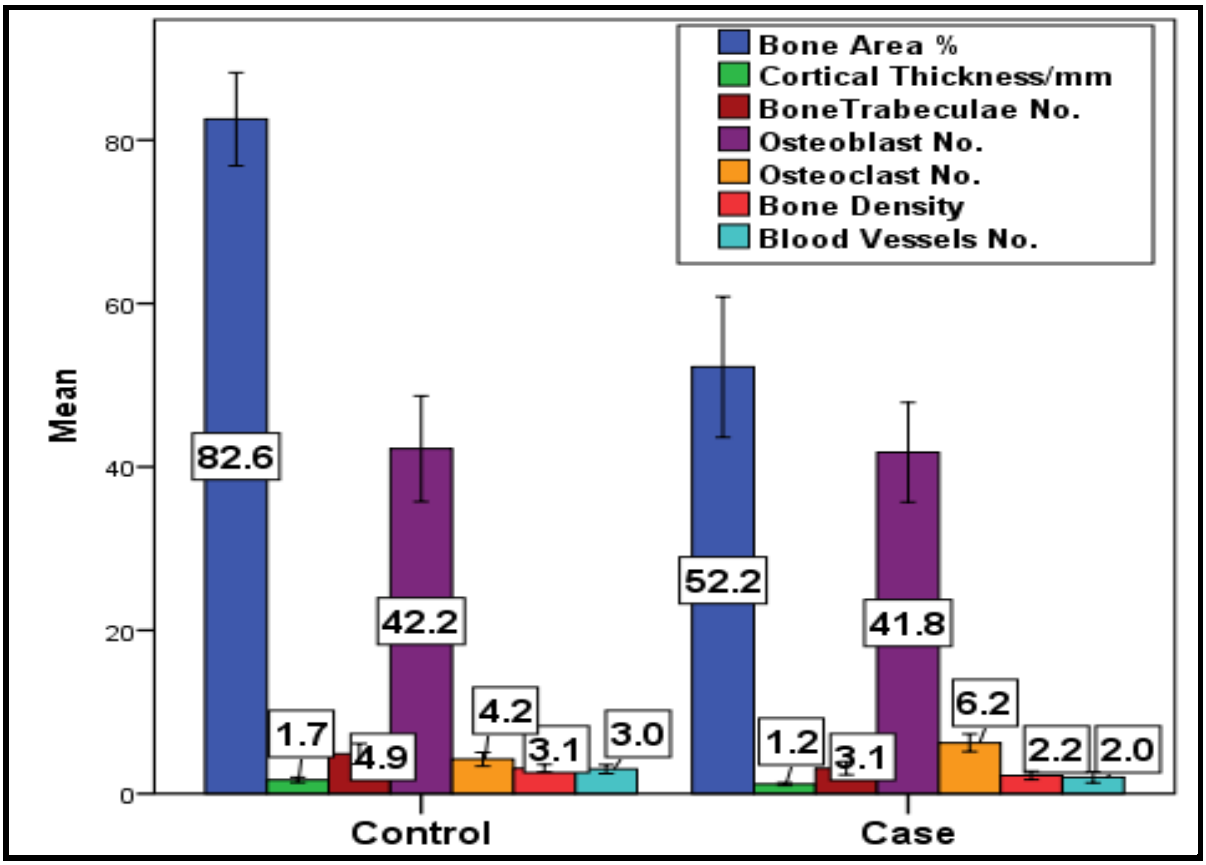

Fig. 6: Difference in the Mean Histomorphometry Analysis Parameters

Statistical analysis

The collected data were verified, coded by the researcher, and analyzed using the Statistical Package for Social Sciences (IBM-SPSS/PC/VER 21) *. Descriptive statistics: Means, standard deviations, medians, ranges, and percentages were calculated. The Shapiro-Wilk test will be used to test data normality. Paired sample t-test was used to test before vs after treatment measure. Independent sample t-test was used to test the difference in the mean parameters. A p-value less than 0.05 was considered significant.

Discussion

The cellular and molecular mechanisms behind orthodontic relapse are not entirely known, despite its relevance(15,16). Yoshida et al (17) suggested that the primary reasons of relapse are remodeling of the alveolar bone and the periodontal ligament fibers. In addition, Franzen et al1 discovered a correlation between orthodontic relapse and orthodontic tooth movement (OTM) and similar cellular changes, namely a rise in osteoclast differentiation in compression regions. The regulation of OTM and relapse may be affected in a clinically significant way by endogenous or pharmacologic bone modulation that inhibits osteoclast resorption and promotes osteoblast neoformation, according to this backdrop(18–22).

Three factors have been proposed as potential explanations for how statins affect bone

anabolism: promoting osteogenesis, preventing osteoblast apoptosis, and suppressing osteoclastogenesis(23). Although statins' impacts on bone anabolism have been amply demonstrated in laboratory studies (5,16,24–26), Their medical results are inconclusive(27).

In a previous study conducted by Dolci, et al[62], They reported that statin administration decreased osteoclast numbers in the rat molars' alveolar bones, which was also associated with a lower rate of orthodontic recurrence. However, they also discovered that ATV can alter long-bone endochondral ossification since, after short-term (7–21 days) treatment, It enhanced the chondrocytic hypertrophic zone and growth plate cartilage in femurs. These results highlight the clinical importance of pharmacologic bone modulation during orthodontic therapy and the negative effects of statins on the femoral growth plate, which seem to limit their potential use as a pharmacologic strategy to enhance paediatric orthodontic treatment.

The literature largely supports statins' ability to inhibit osteoclasts (16,24,25,29). The precise mechanisms driving this process are not known, though. According to a theory, statins' capacity to inhibit hydroxymethylglutaryl-coenzyme A reductase, Their ability to prevent bone resorption is correlated with their ability to inhibit the mevalonate pathway, because of the fact that doing so prevents the activation of guanosine triphosphate binding proteins to molecules essential for osteoclast development and function (actin cytoskeleton regulation, apoptosis, membrane ruffling, and vesicular trafficking) (29,30). Pan et al (31) undertook a study to clarify how the small GTPase-Rho signaling pathway controls periodontal tissues' response to mechanical stimuli. They discovered, in vitro, that Rho is upregulated in human periodontal cells that have undergone a cyclic strain treatment. They propose that this molecular mechanism promotes actin polymerization, which may be responsible for cytoskeletal rearrangement, thereby promoting OTM and alveolar bone remodelling. It makes sense that osteoclast genesis during OTM would be impacted by statin-induced small GTPase downregulation. Blocking the nuclear factor-kappa B pathway's activation is another viable theory(16,24,25), It ultimately encourages suppression of osteoclast genesis by regulation of the extracellular decoy receptor osteoprotegerin, cell receptor activator of nuclear kappa B (RANKL), and cell receptor activator of nuclear kappa B ligand (RANK)(5,16,25).

To our knowledge, this is the first study to use atorvastatin to detect its inhibition on osteoclasts and arrest of tooth movement in rabbits.

Our results showed the difference in the mean distance between 1st and 2nd premolar in mm over the study period before relapse vs. after relapse for the control group specimens. All specimens showed highly significant reduction regarding mean distance ($p<0.001$). As regarding study group specimens, all specimens showed significant reduction ($p<0.05$) regarding mean distance.

Also, our results showed that the comparison of the magnitudes of tooth movement and relapse between the two groups there were significant difference (p=0.002) regarding mean distance before and after treatment.

Additionally, control group showed significantly (p<0.001) higher mean relapse (0.79 ± 0.02 mm) than study group (0.28 ± 0.02 mm). Likewise, the relapse% was significantly (p<0.001) higher in control (67.4%) than study group (29%)

These findings were in accordance with Dolci et al, 2018 (32) who conducted their study on 24 male Wister rats split across 2 groups: experimental (ATV) and control (saline solution [SAL]). The experimental group received atorvastatin, 15 milligram per kilogram per day, administered by gavage, and the control group received 0.1 mL of phosphate buffered saline solution, also administered by gavage. Unlike in previous studies, (5,16,25,33). Because it was anticipated that bile would excrete 73% of the amount taken orally, a high dose was given (34).

Additionally, as reported by Elewa et al, (35) Rats were given 15 mg of ATV per kilogram orally, and the peak plasma concentration was similar to what was seen after giving humans 80 mg of ATV daily. Up until the conclusion of the experiment, when the animals were killed, saline solution or medication delivery was continued. Between the SAL and ATV groups, they discovered a substantial difference in tooth displacement.

Our results also tend to support earlier research that showed significant OTM and relapse prevention following statin administration (5,16). MirHashemi et al, (5) observed that Wistar rats were given 5 mg per kilogram per day of ATV via gavage after 21 days, which decreased the rate of OTM. Han et al11 also reported that rats given simvastatin intraperitoneally showed a substantial decrease in tooth relapse (2.5 mg/kg/day) 7 and 28 days following appliance removal. If we extrapolate this potential action of these drugs to human bone turnover, then impaired OTM should be considered as a possibility in statin users. Our findings may therefore indicate that statins have an anabolic effect on bone, promoting osteogenesis while inhibiting osteoclastogenesis (16,24,25,29,36).

These findings also agree with the results of Han et al, (16) who reported Simvastatin treatment significantly reduced tooth relapse in rats 7- and 28-days following removal of the orthodontic appliance due to increased PDL remodeling and alveolar osteogenesis. The modulation of RANKL and OPG by simvastatin has been postulated to be the cause of both increased bone growth and decreased bone resorption(5).

On the other hand, AlSwafeeri et al 2018 (37) who conducted their study on 10 white New Zealand rabbits but they used simvastatin instead of atorvastatin, who employed simvastatin rather than atorvastatin when conducting their research on 10 white New Zealand rabbits, and whose measurements of tooth movement in the control and

experimental groups showed no significant difference in the magnitude of tooth movement at the conclusion of the experimental tooth movement phase. This result was anticipated given that the same experimental orthodontic appliance was used in the same study circumstances with almost the same force magnitude. The experimental teeth in both the control and experimental groups displayed post-orthodontic regression towards their original positions once the orthodontic appliance was removed. Although the magnitude and percentage of relapses were lower in the experimental group than in the control groups, these differences were not statistically significant. Since the tooth experiences a rebound shift in the tooth socket during the first few days after the appliance is removed, this post-orthodontic relapse was expected. But these post-orthodontic relapse outcomes are in line with those of Vieira et al,, (38) showing that simvastatin did not prevent rats from relapsing into tooth movement after being administered systemically.

The results of histomorphometric analysis parameters between cases and control showed that regarding the bone area%, cases showed significantly ($p < 0.001$) lower mean bone area% (52.2 ± 11.2%) compared with controls (82.6 ± 7.4%). Also, cases had significantly ($p = 0.008$) lower mean cortical thickness (1.7 ± 0.4 mm) compared with controls (1.2 ± 0.2 mm). Additionally, the mean number of bone trabeculae was significantly ($p = 0.014$) higher in controls (4.9 ± 1.6) than cases (3.1 ± 1.1). On the other hands, the mean number of osteoblasts was insignificantly ($p = 0.910$) higher in controls (42.2 ± 8.4) than cases (41.8 ± 7.9). On the contrast, the mean number of osteoclasts was significantly ($p = 0.004$) lower in controls (4.2 ± 1.1) than cases (6.2 ± 1.4).

Respecting the bone density (intensity), cases showed significantly ($p < 0.009$) lower mean bone density (2.2 ± 0.7) compared with controls (3.1 ± 0.6). Likewise, the mean number of vessels was significantly ($p = 0.016$) lower in cases (2.1 ± 0.9) than control (3.1 ± 0.7).

A study by AlSwafeeri et al, 2018 (37) showed signs of bone remodelling 3 weeks after the orthodontic force was removed, with appositional lines indicating an instance of deposition of bone. The extent of bone resorptive lacunae in cases was significantly smaller when controls and cases were compared statistically. Simvastatin treatment was associated with a considerable increase in the area of newly created bone when compared to controls receiving the control vehicle solution. There was a large amount of bone development on the distal side of the experimental teeth in the cases, filling the orthodontically widened periodontal space and returning it to its prior width in just 6 weeks. This supports simvastatin's osteoinductive properties and suggests that it can improve alveolar bone remodeling. The disparity in sensitivity between the cells responsible for creating and destroying bone tissue, with osteoclasts being more sensitive to simvastatin, may be the cause of this divergence in the response of bone remodeling to simvastatin

(39) that would demonstrate a different effect of prenylation from what had previously been observed in the drug's in vitro research (40). These histologic observations concur with those that Choi et al. recorded (41) demonstrating that in rodents, alveolar bone remodeling may begin on day 8 and progress steadily until day 17. Additionally, the outcomes are in line with those of Pavlin et al. (42), who found that in samples from days 8 and 12, gross histologic inspection could also detect new bone growth on the stress side.

According to histologic study of Mundy et al. and Esfahani et al (6,33) the reduction in the number and activity of osteoclasts was observed after taking simvastatin. In contrast, other authors have reported that statins did not affect the number of osteoclasts during OTM or during the relapse phase (5,16).

A study by Dolci et al 2017, (28) They noted that the ATV animals displayed the opposite profile from the SAL animals, which had high osteoclast numbers and low relapse rates. Additionally, they discovered a strong positive association between the number of osteoclasts and the frequency of relapse. In this regard, Yoshida et al (17) suggested that alveolar bone remodeling is a main cause of relapse after tooth movement in rats. Franzen et al (15) reported that, Although the transseptal fibers were normally stretched after seven days, the first molar continued to relapse. They came to the conclusion that while bone remodeling is an important component to take into account, stretching of these fibers may not be a key factor in the etiology of relapse.

Although the exact causes of orthodontic relapse remain unknown, it appears to be a complicated multidimensional process that is difficult to pin down to a single cause. Even while remodeling of the alveolar bone and surrounding periodontal ligament is a crucial step in the relapse process (15,43). Other possible causes could include the normalization of the periodontal vascular system following orthodontic force (44), an increase in the elasticity of the gums when they are retracted and compressed in the direction of tooth movement (45), and stretching of transseptal fibers (46,47). Possible explanation for the considerable post-orthodontic relapse is that relapse energy that had been trapped in the collagenous periodontal and transseptal fiber systems after the orthodontic appliance was removed (48).

A limitation of this study was that We did not incorporate histologic examination of tissues at various stages of the post-orthodontic recurrence. The underlying biologic mechanisms may become clearer with more research into how bone, periodontal ligament, and tissue change over time during post-orthodontic relapse.

Therefore, we recommend that more research on statin effects on OTM be encouraged because it could show whether these clinical effects are long-lasting. Additionally, the contentious use of pharmaceutical suppression of OTM to enhance tooth anchoring during therapy may constitute a therapeutic advance. It is not possible to immediately transfer the outcomes of this animal experiment to a

therapeutic setting. However, they offer fresh perspectives in this field.

Conclusion

- Atorvastatin seems to have a positive effect on reducing the degree of relapse if taken daily in animal models. It also seems to affect the cellular environment in the bone affecting the cellular elements of bone remodeling, thus in turn potentially altering the relapse of teeth after OTM. Medication seemed to be well tolerated by the animals as the feeding cycles and sleep patterns didn't change while taking the ATV, and no changes in behavior were noted during the time of work, and after conclusion of work animals that weren't killed for histological analysis were apparently healthy.

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