

Original Research

Vitamin D and bee propolis: A comparative study about their effect on tooth movement and resorption

Rana Mahdi Alobiedī , Mohannad E. Qazzaz , Ali R. Al-Khatib 

Received (first version): 10-Jun-2024

Accepted: 30-Oct-2024

Published online: 22-Aug-2025

Abstract

The study aimed to evaluate and compare changes in some inflammatory markers involved in the inflammatory pathway associated with tooth movement, such as TRAP (tartrate-resistant acid phosphatase), RANKL (receptor activator of nuclear factor kappa beta ligand), ALP (alkaline phosphatase), and TNF- α (tumor necrosis factor alpha) in gingival tissues of the maxilla for second premolar in a dog's model. The experimental sample consisted of 20 male dogs divided into 4 groups. The study involved dental arch division. Groups included control, injection bee propolis, oral propolis, and Vitamin D3 groups. Control groups received DMSO (Dimethyl sulfoxide) injections, while bee propolis was divided into two sections during dental treatment injected 150 mg and 300 mg of propolis every 3 days. Oral propolis was administered as 100 mg/kg/day daily, while Vitamin D3 was administered as 25 units weekly. Immunohistochemistry expressions with scores were achieved for the 4 markers. Results recorded a significant decrease in the TRAP, RANKL and TNF- α levels in both tension and compression sides in the injected Propolis groups, especially at dose 300 mg, while orally and injected propolis recorded significant increase in ALP in both tooth sides comparing with control groups. The vitamin D3 group revealed significantly increased in the TRAP and RANKL compared with propolis and control groups, in addition to significantly increased in the ALP particularly in the tension side but not like propolis group. In conclusion, vitamin D3 plays a higher role in tooth movement to the compression side than propolis, which is considered more beneficial in terms of time, but it resorption both the alveolar bone and tooth root apex, whereas propolis has very good influencing in the new bone formation comparing with vitamin D3 and well tooth movement to the compression side when comparing with ordinary orthodontic treatment. Both biomaterials, through complicated biological processes, promote bone health and effective orthodontic therapy.

Keywords: TRAP, RANKL, ALP, TNF- α , Propolis, dogs

INTRODUCTION

Malocclusion, or deviated jaws, is a topic within orthodontics, a specialized field of dentistry¹. Through the application of orthodontic forces, local hypoxia and fluid flow are happen, initiating an aseptic inflammatory cascade culminating in osteoclast resorption in areas of compression and osteoblast deposition in areas of tension. On the compression side, regions known as resorption lacunae are created due to orthodontic tooth movement (OTM)². Increased activity of bone resorbing agents is observed in areas subjected to compression force. Regions experiencing Compression can result in bone resorption and cell death. On other hand enhanced blood flow and osteoblastic activity support the creation of bone tissue in tension area³. Tooth movement occurs after bone remodeling as teeth are influenced to shift to their intended positions creating contact between bones

and triggering responses in the ligament and alveolar bone⁴. The RANKL/RANK/OPG system plays a role in stimulating bone remodeling by regulating formation and activation affecting bone resorption through hormones and cytokines⁵. Studies have shown that the expression of RANKL in gingival cells is significant for root resorption during treatment⁶.

Recent research suggests that fibroblasts also produce RANKL when subjected to compression force contributing to root resorption⁷. TRAP (Tartrate Resistant Acid Phosphatase) is an enzyme for breaking down bone tissue produced by osteoclasts during the process of bone resorption and degradation of components, in bones⁸. TRAP affects teeth and bones by breaking down proteins in the bone matrix leading to decreased bone density and increased resorption⁹. Additionally, stimulation of TRAP secretion may result in an increase in bone absorption, leading to bone loss and dental deterioration. Topical related to how vitamin D3 stimulates RANKL expression by local cells, which in turn activates bone-resorbing cells, promotes their growth, and facilitates tissue renewal, particularly alveolar bone¹⁰.

Recently, there has been a growing interest in exploring natural derivatives that may provide apparent therapeutic effects with fewer side effects, given their natural composition and origin. Propolis, an effective antioxidant, antimicrobial, anticancer, and anti-inflammatory agent, may accelerate new bone formation in rodents¹¹. Propolis inhibits the late stages of osteoclast maturation, including fusion of osteoclast precursors to form multinucleated cells and the formation of actin rings¹². This

Rana Mahdi Alobiedī. University of Mosul, College of Dentistry, Mosul, Nineveh province, Iraq. dranaobiedi@gmail.com

Mohannad E. Qazzaz. Assistant Professor, University of Mosul, College of Pharmacy, Mosul, Nineveh province, Department of Pharmacognosy and Medicinal Plants, Iraq. Mohannad.qazzaz@uomosul.edu.iq

Ali R. Al-Khatib. Professor, University of Mosul, College of Dentistry, Mosul, Nineveh province, Department of Pedodontics and Preventative Dentistry Department, Iraq. Alirajih@uomosul.edu.iq



supports the hypothesis that propolis may be beneficial as a drug to reduce orthodontically induced root resorption¹³. Propolis is a complex mixture of various naturally occurring elements, also known as bee glue, a non-toxic, resinous substance produced by honey bees by mixing pharyngeal gland secretions with digested honey¹⁴. Although propolis is not a new drug, there is no valuable previous study that discussed or compared the effect of propolis on root resorption.

The study aimed to evaluate and compare changes in some inflammatory markers involved in the inflammatory pathway associated with tooth absorption, such as TRAP, RANKL, ALP, and TNF- α , in gingival tissues of the maxillary second premolar in dog's model when given different concentrations of propolis and using two different methods.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Ethics Committee of the College of Dentistry at the University of Mosul the code number UOM. Dent 23\22 in date 2\5\ 2023.

Materials

Propolis extract capsule 2000 mg\cap. From COMVITA company in UK. DMSO 99.9% from JINAN BOSS CHEM, Alfaxalone (Alfaxan; Jurox Pty Ltd., Rutherford, New South Wales, Australia), Medetomidine Hydrochloride (Tomidine; Provect Ltd., Istanbul, Turkey), Isoflurane (Fran Lee; Hanah Pharma Ltd., Republic of Korea) and used STEROGYL 15 "H" 600 000 UI/1,5 ml, solution injectable IM en ampoule Ergocalciferol Solution injectable ampoule de 1,5 ml boîte de 1 ampoule

Animals

The experimental sample consisted of 20 healthy adult male local dogs with an average age of 12 months. They were acclimatized for one week before the experiment and housed in metal cages at a temperature of 22°C and light/dark periods of 12 hours to mimic natural conditions as much as possible. They had access to water and a balanced diet.

Experimental Design

A split-mouth experimental design was employed to study the oral cavity in the dog model:

Group 1 (Control): Each dental arch was divided into two sections. One section received only Dimethyl sulfoxide (DMSO) injection, and the other section received orthodontic treatment with DMSO injection.

Group 2 (Bee Propolis): Each dental arch was divided into two sections during orthodontic treatment. One section was injected with 150 mg of propolis every 3 days, and the other section was injected with 300 mg of propolis every 3 days.

Group 3 (Oral Bee Propolis): Orthodontic treatment was performed on dogs receiving oral propolis (100 mg/kg/day)¹⁵.

Group 4: Dogs were given orthodontic treatment with drug which is Vitamin D3. (25 units/week)^{16,17}.

General Anesthesia

General anesthesia was induced by Alfaxalone and Medetomidine Hydrochloride intramuscular, maintained at 2% Isoflurane with simultaneous administration of pure oxygen by inhalation administered according to the animal's body weight and duration of action¹⁸.

Orthodontic Intervention

After general anesthesia of the dogs, impressions were taken using a three-dimensional dental scanner (cosmoLight: Heron IOS/Tscan/Biolase) for the upper dental arches of the dogs. Due to the thin and short roots of the first premolars in these dogs, the second premolars were selected as the movement unit. Additionally, canines (due to their long roots) were selected as anchorage units. Subsequently, the first premolars were extracted due to their proximity to the second premolars and the potential for interference with movement figure (1)^{18,19}.

Therefore, crowns were made for the dogs' upper canine and second premolars. In the dental laboratory, GAC Dentsply tubes sized 0.022 × 0.28 inches were bonded to the surfaces of all upper second premolars crowns and upper canines crowns using straight stainless-steel wires sized 0.021 × 0.025 inches in the tube. Slots were made to secure the tubes in the same vertical and horizontal directions. The crowns were cast in low-melting metal alloys, finished, polished, and then sandblasted with aluminum oxide particles²¹. Once the crowns were received from the dental laboratory, the dogs were pre-anesthetized and anesthetized to insert the crowns into the premolars and canines.

The crowns were reinforced using a third-generation permanent resin cement system (Brea, CA)²². A straight piece of stainless-steel wire sized 0.021 × 0.025 inches was inserted into the attachment tube with a closed-coil spring to reduce the distance between the two tubes (on the premolars and canines)²³. To apply a force of 200 grams per device, which was measured using a force gauge. Orthodontic movements continued for two months²⁴. The coil springs were evaluated and readjusted weekly²³ to maintain a force level of 200 grams²⁴.

Drug Intervention and Dosing

In Group 1 (Control), In 5 dogs, each dental arch was divided into two sections: one receiving only Dimethyl Sulfoxide (DMSO) through injection into the periodontal ligament on the mesial side (the midline was used for lingual/palatal groove measurement) of the upper second premolar, and the other receiving DMSO along with orthodontic intervention via injection. In Group 2 (Propolis), In 5 dogs, bee propolis was available as a solution at 300 mg/ml. This dose was determined based a pilot study, as the previous studies shown that 0.5mg had no significant effect²⁵ Therefore, for a 300 mg dose, 1 mL was injected into the periodontal ligament every 3 days using an insulin syringe, where reconstitution of 0.5 mL from the above stock with 0.5 mL of DMSO provides 150 mg/mL, on the other side of the dental arch after orthodontic intervention. Group 3 (Oral Propolis), 5 dogs with orthodontic intervention received propolis orally (100 mg/kg/day). Finally, Group 4, 5 dogs with



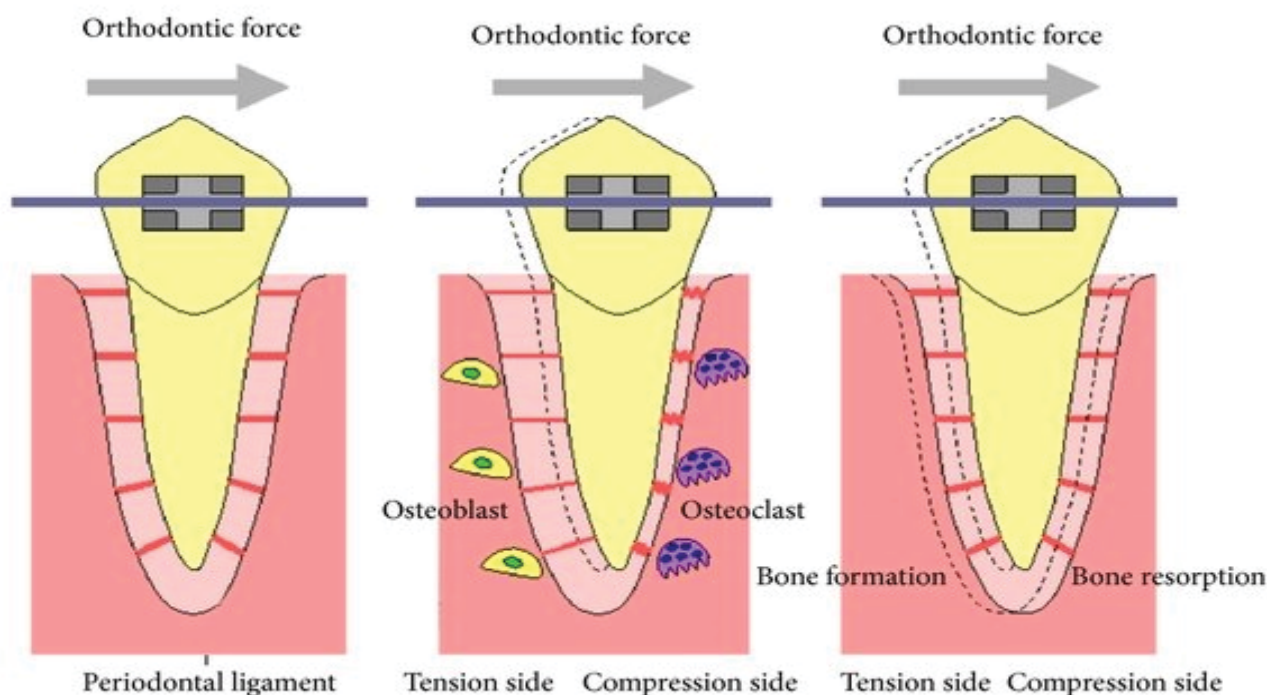


Figure 1. Illustrates orthodontic force Applying orthodontic force to the tooth causes compression of the periodontal ligament. The compressed side of the periodontal ligament is called the compression side and the side where the periodontal ligament is pulled is called the tension side. ²⁰

orthodontic intervention received the reference drug, vitamin D3. (25 units/week). ¹⁷ All drug interventions began one week after the application of orthodontic force.

Immunohistochemistry (IHC)

Sections for the apical, middle and cervical thirds of mesial root of the upper second premolar were stained immunohistochemically. Wax was removed from the prepared sections using xylene and hydrated with water using ethanol. The biological markers studied for tooth movement and root resorption were TRAP, ALP, RANKL and TNF- α ^{21,22}.

Statistical Analysis

All statistical analyses were performed using SPSS 22.0 (Chicago, IL). The Shapiro-Wilk test was applied to assess the normality of the data before choosing the analysis set. Descriptive statistics were performed, comparing between sides for each group and between groups. Statistical significance level was set at $P \leq 0.05$.

RESULTS

The results in the (Table 1) revealed the effect of groups, the tooth part and their interaction on the Tension side (the side being pulled, the periodontal ligament on this side is stretched by which the osteoblasts are highly active. New bone is formed to stabilize the tooth in its new position. of TRAP which shows similar effects of treatments on TRAP side, with no significant differences in tooth parts. DMSO with ortho group and D3 injection group outperformed others in cervical tooth segments,

except for oral propolis group and right-side bee propolis injection 150 mg. it increased significantly ($P \leq 0.05$) compared to DMSO without Ortho on the right-side group (2.18) and in propolis injection 300 mg on the left side group (2.27), where no significant difference was recorded between them. Vitamin D3 injection group significantly increased average tension side in the apical part of root teeth, superior to DMSO treatments without ortho and propolis (Table 1).

The results of the TRAP in the compression side, (the side being pushed): The periodontal ligament on this side is compressed. Osteoclasts are highly activated. Bone is resorbed (broken down) to allow the tooth to move. showed that TRAP increased significantly in left side DMSO with ortho group and D3 injection group while right-side bee propolis injection 150 mg group and oral propolis group had similar effects. Oral Propolis group and propolis injection 150 mg group were less effective compared to previous groups but still significantly higher than right-side DMSO without ortho group. There was significant increase in the TRAP in tooth apical regions of all groups compared with the tooth cervical and middle regions in the compression side (Table 2).

The results of the RANKL revealed that D3 injection group received the highest rating, while oral propolis group showed decreased in RANKL level. However, DMSO without ortho group on the right side had significantly lower ratings. There was a significant increase in the RANKL in tooth apical regions of all groups compared with the cervical and middle regions in the tension side (Table 3).

Groups	Tooth part	Interaction between groups and tooth part	Main effect on tooth part	Main effect for Groups
Right-side DMSO without ortho	Cervical	2.04+1.01 g	5.01+2.71 A	2.18+0.88 C
	Middle	2.34+0.74 fg	4.63+2.36 A	
	Apical	2.16+1.05 g	4.67+2.55 A	
Left -side DMSO with ortho	Cervical	7.24+2.05 a		6.93+1.75 A
	Middle	7.04+1.41 ab		
	Apical	6.52+2.03 abc		
Right-side (bee proplis injection 150 mg)	Cervical	4.44+2.01 cdef		4.36+1.75 B
	Middle	3.72+1.49 defg		
	Apical	4.92+1.89 bcde		
Left side (bee proplis injection 300 mg)	Cervical	2.76+1.49 efg		2.27+1.27 C
	Middle	2.00+1.17 g		
	Apical	2.04+1.25 g		
Orally bee proplis	Cervical	6.12+2.18 abc		5.96+1.87 A
	Middle	7.04+1.10 ab		
	Apical	4.72+1.68 cde		
D3 injection	Cervical	7.44+2.03 a		6.91+1.70 A
	Middle	5.64+0.99 abcd		
	Apical	7.64+1.42 a		

Small different letter refers to significant difference between interaction factors effect at ($P \leq 0.05$). Capital different letter refers to significant difference between main effects of factors at ($P \leq 0.05$).

Groups	Tooth part	Interaction between groups and tooth part	Main effect on tooth part	Main effect for Groups
Right-side DMSO without ortho	Cervical	2.04+0.43 f	8.95+5.50 B	2.11+0.47 C
	Middle	2.00+0.53 f	9.38+5.96 B	
	Apical	2.28+0.48 f	12.13+7.52 A	
Left -side DMSO with ortho	Cervical	14.92+2.39 bc		17.49+4.1 A
	Middle	15.8+1.92 bc		
	Apical	21.76+3.85 a		
Right-side (bee proplis injection 150 mg)	Cervical	8.88+3.04 e		10.76+3.68 B
	Middle	9.32+3.03 e		
	Apical	14.08+2.86 bcd		
Left side (bee proplis injection 300 mg)	Cervical	3.68+0.77 f		3.83+1.51 C
	Middle	3.4+0.94 f		
	Apical	4.4+2.42 f		
Orally bee proplis	Cervical	10.16+4.66 de		11.12+3.47 B
	Middle	10.68+2.84 de		
	Apical	12.52+2.92 cde		
D3 injection	Cervical	14.04+2.9 bcd		15.61+4.21 A
	Middle	15.08+5.34 bc		
	Apical	17.72+4.02 b		

Small different letters refer to significant differences between interaction factors effect at ($P \leq 0.05$). Capital different letters refer to significant differences between main effects of factors at ($P \leq 0.05$).

Table 3: Effect of Treatments, tooth part and their interaction in the RANKL Scoring of Tension side				
Groups	Tooth Part	Interaction between groups and tooth part	Main effect on tooth part	Main effect for Groups
Right-side DMSO without ortho	Cervical	0.40±0.001 j	2.27±1.74 B	0.56±0.14 D
	Middle	0.68±0.11 hij	2.37±1.64 B	
	Apical	0.60±0.002 ij	3.91±2.75 A	
Left-side DMSO with ortho	Cervical	2.48±2.14 defgh		2.87±1.63BC
	Middle	2.32±1.43 defghi		
	Apical	3.80±1.02 bcde		
Right-side (bee propolis injection 150 mg)	Cervical	1.48±0.44 fghij		2.11±1.20 C
	Middle	1.28±0.52 ghij		
	Apical	3.56±0.75 bcde		
Left side (bee propolis injection 300 mg)	Cervical	1.44±1.36 fghij		2.19±1.42 C
	Middle	2.00±0.81 efghij		
	Apical	3.12±1.66 bcdefg		
Orally bee propolis	Cervical	3.24±0.79 bcdef		3.43±1.25 B
	Middle	2.96±0.98 cdefg		
	Apical	4.08±1.73 bcd		
D3 injection	Cervical	4.56±0.98 bc		5.93±2.48 A
	Middle	4.96±1.18 b		
	Apical	8.28±2.97 a		

Small different letter refers to significant difference between interaction factors effect at (P≤0.05). Capital different letter refers to significant difference between main effect of factors at (P≤0.05).

The study found significant differences in RANKL scores between treatment groups in the compression side, with the lowest value being 0.69 for right-sided DMSO without ortho. The study also found that the lowest RANKL Scoring in the cervical part in the tension side of the tooth was achieved with Right-side DMSO without ortho group, while the highest compression was achieved with D3 injection group. The middle tooth part showed the highest RANKL Scoring in the compression side, with the lowest score in the compression side achieved with Right-side DMSO without ortho group. There was significant increase in the RANKL in tooth apical regions of all groups compared with the cervical and middle regions in the compression side (Table 4).

The result of alkaline phosphatase ALP in the tension side found that propolis injection at 300 mg significantly increased compared to vitamin D3 injection group. Vitamin D3 group had a higher effect than propolis 150 mg injection group and oral Propolis group, which were better than DMSO groups.

The study found that D3 injection group significantly improved ALP in the tension side of the cervical tooth part compared to Left-side bee propolis injection 300 mg group. This group also increased in the tension side of the middle part of the tooth compared to D3 injection group and Right-side bee propolis injection 150 mg group. In the apical tooth part, Left-side bee propolis injection 300 mg group had a unique effect of ALP in the tension side, significantly superior to D3 injection (17.76), only on Left-side DMSO with ortho group (15.52), and increased significantly on Right-side DMSO without ortho group (3.32). There were significant increases in the ALP in tooth apical then

middle regions of all groups compared with the cervical regions in the tension side (Table 5).

The results for alkaline phosphatase ALP in the compression side found significant differences in the cervical, middle, and apical tooth parts. Propolis injection group at a 300 mg dose on the left side significantly improved ALP in the compression side compared to oral propolis group and vitamin D3 injections group. In the middle part, these groups, along with vitamin D3 injection, were more effective. There were significant increases in the ALP in tooth apical then middle regions of all groups compared with the cervical regions in the compression side (Table 6).

The results of tumor necrosis factor alpha TNFα scores found that DMSO with ortho group on the left side and D3 injection group significantly increased TNF in the tension side compared to Propolis injections groups and oral Propolis group. The apical part of the tooth had the greatest effect on TNFα in the tension side. The interaction between therapeutic groups and dental parts had no significant difference in cervical treatment. D3 injections group had the most significant effect on TNFα levels.

Results for the effect of groups on TNFα in the compression side: The study found that Left-side DMSO group with ortho and vitamin D3 injection group significantly increase TNFα in the Compression side, but Right-side DMSO without ortho group being the least effective. The most affected tooth parts were the apical, middle, and cervical parts respectively as significant increases in the TNFα in apical then middle regions of all groups comparing with the cervical regions in the Tension side (Table 7).

Table 4: Effect of Treatments, tooth part and their interaction on RANKL Scoring of compression side

Groups	Tooth part	Interaction between groups and tooth part	Main effect on tooth part	Main effect for Groups
Right-side DMSO without ortho	Cervical	0.76±0.33 h	6.16±4.36 B	0.69±0.35 E
	Middle	0.68±0.44 h	6.04±4.43 B	
	Apical	0.64±0.33 h	11.53±7.85 A	
Left -side DMSO with ortho	Cervical	7.36±3.96 ef		9.87±5.04 B
	Middle	7.08±3.62 ef		
	Apical	15.16±2.74 b		
Right-side(bee proplis injection150 mg)	Cervical	5.60±3.2 fg		7.32±3.39 C
	Middle	5.96±2.44 fg		
	Apical	10.40±2.48 cde		
Left side (bee proplis injection300 mg)	Cervical	2.68±1.25 gh		3.48±2.61 D
	Middle	1.88±0.30 h		
	Apical	5.88±3.31 fg		
Orally bee proplis	Cervical	8.76±2.27 def		10.12±3.3 B
	Middle	8.64±2.02 def		
	Apical	12.96±3.69 bc		
D3 injection	Cervical	11.80±1.79 cd		15.97±6.23 A
	Middle	12.00±2.61 bcd		
	Apical	24.12±1.21 a		

Small different letters refer to significant difference between interaction factors effect at (P ≤0.05). Capital different letter refers to significant difference between main effect of factors at (P ≤0.05).

Table 5: Effect of Treatments, tooth part and their interaction on ALP in Tension side

Groups	tooth part	Interaction between groups and tooth part	Main effect on tooth part	Main effect for Groups
Right-side DMSO without ortho	Cervical	3.72±0.78 m	9.92±4.02 C	3.4±0.91 E
	Middle	3.16±0.65 m	12.00±4.59 B	
	Apical	3.32±1.29 m	15.12±5.68 A	
Left -side DMSO with ortho	Cervical	7.04±2.31 L		11.21±3.85 D
	Middle	11.08±1.14 ik		
	Apical	15.52±0.61 cde		
Right-side (bee proplis injection 150 mg)	Cervical	10.28±0.48 k		13.59±2.96 C
	Middle	13.44±0.84 fgh		
	Apical	17.04±1.02 bc		
Left side (bee proplis injection 300 mg)	Cervical	12.92±0.72 gh		17.08±3.41 A
	Middle	17.48±0.83 b		
	Apical	20.84±0.26 a		
Orally bee proplis	Cervical	10.84±0.41 ik		13.13±2.42 C
	Middle	12.32±0.86 hi		
	Apical	16.24±0.43 bcd		
D3 injection	Cervical	14.72±3.25 def		15.67±2.43 B
	Middle	14.52±1.33 efg		
	Apical	17.76±0.43 b		

Small different letter refers to significant difference between interaction factors effect at (P ≤0.05). Capital different letter refers to significant difference between main effect of factors at (P ≤0.05).

Table 6: Effect of Treatments, tooth part and their interaction on ALP in the compression side				
Groups	Tooth part	Interaction between groups and tooth part	Main effect on tooth part	Main effect for Groups
Right-side DMSO without ortho	Cervical	3.20±0.60 g	9.59±4.54 C	3.64±0.80 E
	Middle	3.6±0.32 g	11.35±4.22 B	
	Apical	4.12±1.11 g	13.85±4.93 A	
Left -side DMSO with ort	Cervical	7.24±0.68 f		10.12±2.39 D
	Middle	10.52±0.9 e		
	Apical	12.60±0.71 de		
Right-side (bee proplis injection150 mg)	Cervical	7.88±0.27 f		11.60±3.27 C
	Middle	11.88±2.19 de		
	Apical	15.04±0.59 bc		
Left side (bee proplis injection300 mg)	Cervical	15.68±4.39 bc		17.05±2.86 A
	Middle	16.36±0.93 b		
	Apical	19.12±0.41 a		
Orally bee proplis	Cervical	11.64±1.83 de		13.49±2.04 B
	Middle	13.72±1.75 cd		
	Apical	15.12±0.73 bc		
D3 injection	Cervical	11.92±2.38 de		13.68±3.08 B
	Middle	12.04±2.09 e		
	Apical	17.08±1.20 ab		

This table presents the mean values and standard deviations (Mean ± SD) for the interaction between groups and tooth parts, the main effect for tooth parts, and the main effect for groups. Different letters (e.g., g, f, e) indicate statistically significant differences within each column.

Table 7: Tumor Necrosis Factor-Alpha (TNF-α) Scoring Data in the Tension side.				
Groups	tooth part	Interaction between groups and tooth part	Main effect on tooth part	Main effect for Groups
Right-side DMSO without ortho	Cervical	1.64±0.90 g	5.34±2.81 C	2.19±1.78 C
	Middle	1.60±1.33 g	7.09±4.34 B	
	Apical	3.32±2.46 fg	10.47±4.75 A	
Left -side DMSO with ortho	Cervical	4.48±1.85 efg		10.31±5.21 A
	Middle	12.08±4.09 ab		
	Apical	14.36±2.8 def		
Right-side (bee proplis injection150 mg)	Cervical	5.88±2.88 def		7.63±4.79 B
	Middle	5.80±4.73 abc		
	Apical	11.20±5.05 cdef		
Left side (bee proplis injection300 mg)	Cervical	7.40±2.19 cde		8.21±3 AB
	Middle	7.68±2.92 bcd		
	Apical	9.56±3.85 defg		
Orally bee proplis	Cervical	5.64±1.19 def		7.43±3.12 B
	Middle	6.12±2.30 abc		
	Apical	10.52±3.04 cdef		
D3 injection	Cervical	7.00±3.34 bcd		10.04±3.64 A
	Middle	9.28±1.75 a		
	Apical	13.84±1.34 a		

Small different letters refer to significant difference between interaction factors effect at ($P \leq 0.05$). Capital different letter refers to significant difference between main effect of factors at ($P \leq 0.05$).

The study found that Left-side DMSO with ortho group and D3 injection group significantly increased TNF α in the compression side compared to right-side bee propolis injection 150 mg group, Left-side bee propolis injection 300 mg group, and Orally bee propolis group. The effect of tooth parts of TNF α in the compression side was significant, with the apical part having the most influence. The interaction between groups and tooth parts showed that the effects on TNF α in the compression side in the cervical tooth part were superior to D3 injection group, Left-side DMSO with ortho, and Left-side bee propolis injection 300 mg group. The middle part of the tooth had the highest effect of TNF α in the compression side, outperforming Right-side DMSO without ortho group. There were significant increases in the TNF α in tooth apical then middle regions of all groups comparing with the cervical regions in the compression side (Table 8).

“Ultimately, tooth movement was accelerated in the Vitamin D3 group compared to both the control and propolis groups. Additionally, the Vitamin D3 group exhibited increased resorption in the apical part of the tooth root. Conversely, the propolis group demonstrated an excellent anti-inflammatory response compared to both the control and Vitamin D3 groups.”

DISCUSSION

The significant impact of propolis on TRAP activity can be attributed to its anti-inflammatory, antioxidant, immunomodulatory properties, its direct inhibitory effects on osteoclasts, regulation of bone metabolism, and influence

on hormonal levels²⁶. These combined effects contribute to the reduction of osteoclast activity and TRAP levels, whether propolis is administered particularly via injection. Regarding the effect of propolis on TRAP activity between the tension and compression sides of orthodontic treatment, the difference can be outlined as follows:

Current Study have shown that the use of propolis, taken by injection leads to a significant decline in TRAP and RANKL activity in both tension and compression side. The decrease in TRAP activity in the compression side with the use of propolis can be attributed to its anti-inflammatory and antioxidant properties, its direct inhibitory effects on osteoclasts, modulation of bone remodeling signals, and enhancement of osteoblast activity. These combined effects create a bone environment that favors reduced osteoclast activity and enhanced bone stability, leading to decreased TRAP levels²⁷.

There were significant differences in the TRAP and RANKL in apical regions of all groups comparing with the cervical and middle regions in both sides, which indicating that the osteoblastic activity was high in the apical region of the tooth. This consistency could reflect the bioactivity of osteoblasts in the apical region.

Propolis also decrease TRAP activity on the compression side, although the effect may be less pronounced compared to the tension side. Propolis effectively decreases TRAP activity and thus osteoclast activity, facilitating new bone formation. This suggests a nuanced role of propolis in modulating bone remodeling during orthodontic treatments²⁸. Compounds

Table 8: Tumor Necrosis Factor-Alpha TNF α Scoring Data on compression side.

Groups	Tooth part	Interaction between groups and tooth part	Main effect on tooth part	Main effect for Groups
Right-side DMSO without ortho	Cervical	1.56 $\pm$ 1.77 j	14.64 $\pm$ 7.23 C	2.14 $\pm$ 1.67 C
	Middle	2.27 $\pm$ 1.63 j	17.75 $\pm$ 7.76 B	
	Apical	2.60 $\pm$ 1.80 j	22.13 $\pm$ 9.65 A	
Left -side DMSO with ortho	Cervical	19.96 $\pm$ 4.33 efgh		25.05 $\pm$ 5.83 A
	Middle	23.40 $\pm$ 2.60 bcde		
	Apical	31.80 $\pm$ 0.91 a		
Right-side (bee propolis injection 150 mg)	Cervical	13.08 $\pm$ 2.29 i		19.83 $\pm$ 5.78 B
	Middle	21.8 $\pm$ 2.06 cdef		
	Apical	24.60 $\pm$ 4.14 bcd		
Left side (bee propolis injection 300 mg)	Cervical	16.76 $\pm$ 4.07 ghi		19.01 $\pm$ 3.7 B
	Middle	18.44 $\pm$ 3.79 fgh		
	Apical	21.84 $\pm$ 0.36 cdef		
Orally bee propolis	Cervical	15.96 $\pm$ 5.50 hi		19.45 $\pm$ 5.57 B
	Middle	17.44 $\pm$ 3.23 gh		
	Apical	24.96 $\pm$ 3.14 bc		
D3 injection	Cervical	20.52 $\pm$ 1.43 defg		23.56 $\pm$ 3.02 A
	Middle	23.16 $\pm$ 0.71 bcde		
	Apical	27.00 $\pm$ 1.67 b		

Small different letters refer to significant difference between interaction factors effect at ($P \leq 0.05$). Capital different letter refers to significant difference between main effect of factors at ($P \leq 0.05$).



in propolis have been shown to directly inhibit osteoclast differentiation and function. This can occur through the downregulation of key signaling pathways involved in osteoclastogenesis, such as the RANK/RANKL/OPG pathway. By inhibiting osteoclast formation and activity, propolis directly reduces TRAP activity in bone tissues in the compression side¹².

In the compression side, vitamin D3 also enhances osteoclast activity, but here the primary goal is to resorb compressed bone, allowing new bone formation to align with the required structural changes. The results of the current study in Table 3 and Table 4 indicate that propolis and Vitamin D3 have different effects on RANKL levels between the tension and compression areas of the teeth during orthodontic treatment. The difference in the use of propolis between these areas and their effect on RANKL levels can be summarized as follows:

Propolis reduces the TRAP and RANKL levels which are related to osteoclasts, so it decreases tooth movement towards the compression side, whereas vitamin D3 increases the TRAP and RANKL and thus increases the bone resorption so increase movement of the tooth in the direction of compression side. It also reduces them in the tension side and increases the ALP, which is a marker related to osteoblasts, meaning the creation of new bone. Propolis increases ALP on both sides thus formation of new bone on both sides therefore it is not useful or slow influence in relation to the tooth movement towards the compression side, so it delays the process of tooth movement compared to vitamin D3, which is considered more beneficial in terms of time, as it resorbed the bone on the compression side and increases the bone remodeling around the tooth particularly in the tension side of the tooth, but it is also resorbed the bone in the tooth root apex. This process is a natural part of bone dynamics where a balance occurs between bone formation and resorption²⁹.

One research has shown that using propolis along with Vitamin D3 can have effects on the compression side by boosting RANKL levels. Higher RANKL levels promote activity, which's beneficial for reshaping compressed bone. When Vitamin D3 is administered through injections it notably boosts activity by increasing RANKL levels in the compression area thereby enhancing both osteoblasts and osteoclasts functions to adapt to mechanical compressions. The impacts of propolis on the decrease on TRAP activity could be due to the biological processes and cellular environments present on the two sides during orthodontic treatment. The bone formation is predominant, propolis might exert a stronger influence in reducing osteoclast activity to promote bone formation³⁰.

Propolis reduces TRAP levels on both the tension and compression sides, indicating a decrease in osteoclast activity. The reduction is more significant on the tension side, promoting bone formation, while a more moderate effect on the compression side still supports necessary bone resorption during orthodontic treatment³¹.

The rise in TRAP and osteoclast activity on the tension side could be a reaction to adjustments that necessitate the removal of bone. Conversely reducing RANKL may reduce

stimulation of osteoclasts, helping to maintain a balance between bone formation and resorption^{12,32}. Additionally, propolis may contain elements that directly influence signaling pathways in osteocytes resulting in decrease TRAP production under circumstances and reduced expression of RANKL. This implies context specific effects of propolis on osteocyte signaling³⁰. Propolis also includes substances that can influence the system, which can subsequently affect the equilibrium, between osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells). This delicate equilibrium is part of the bone remodeling process.

In general, the impacts of propolis on TRAP and RANKL demonstrate its involvement in supporting bone health by maintaining a balance between bone formation and resorption³³. When it comes to the compression side Vitamin D3 elevates RANKL levels to boost activity and assist in reshaping compressed bone³⁴. Propolis work to decrease RANKL levels on the compression and tension side promoting bone development. Vitamin D3 in the compression side increase RANKL levels to stimulate activity for remodeling compressed bone. The objective on the tension side is to diminish activity for bone restructuring while on the compression side it is to amplify osteoclast activity for effective resorption and remodeling of compressed bones³⁵. The contrasting effects of propolis and Vitamin D3 on RANKL levels between the tension and compression sides indicate their roles in regulating function based on varying mechanical stresses.

By referring to Table 5 and Table 6 data we can observe how propolis influences alkaline phosphatase ALP activity differently between the tension and compression sides during treatment. The findings suggest that propolis usage in doses of 300 mg leads to a notable increase, in the ALP activity in the tension side. This uptick in ALP activity indicates heightened osteoblast function fostering bone formation in that area. Propolis boosts ALP activity by activating osteoblasts thereby contributing to bone formation and the bones ability to respond to stress³⁶. While propolis also elevates ALP activity on the side, the impact may not be as pronounced as in the tension side. The rise in ALP activity on the side signifies the bones reaction to mechanical compression. Propolis spurs ALP activity on this side aiding in restructuring compressed bone and generating bone tissue.

Propolis exhibits impacts on ALP activity on both sides tension and compression during treatment encouraging both bone formation and reshaping processes. Though the surge in ALP activity is more conspicuous on the tension side propolis remains effective³⁷. According to the data provided in Table 7 and Table 8 the study results suggest that the apical part of the root teeth exhibits levels of tumor necrosis factor alpha (TNF α) regardless of the type of group administered. This implies that cells, in the part are more prone to changes and respond differently to various treatments³⁸.

The injection of 150 mg of Propolis group and oral propolis group had a lesser impact on TNF α levels. This could be due to differences in dosage and bioavailability between these two treatments. The lower propolis dosage may not be adequate,

for achieving desired results resulting in effective outcomes. Furthermore, when oral medications are taken, they have to go through the system before they can reach their intended target. This process can lead to a decrease in the concentration of the ingredients at the target site compared to injections.

The injection with 300 mg of Propolis group and the vitamin D3 group also yielded outcomes. This success can be attributed to propolis well-known antioxidant and anti-inflammatory properties although its impact may be less potent due to mechanisms of action. Vitamin D3 plays a role in regulating responses and might show promising results in boosting TNF α levels. Vitamin D3 influences the immune system by modulating the activity of various immune cells and TNF α levels. The involvement of propolis in controlling TNF α levels on both sides during treatment can be summed up as follows; Propolis demonstrates anti-inflammatory effects on one side resulting in a reduction in TNF α levels. TNF α serves as an indicator of inflammation. Decreasing its levels helps mitigate the inflammatory response that may occur during orthodontic procedures³⁹.

By lowering TNF α levels propolis enhances the wellbeing of tissues. Reduces the chances of chronic or acute inflammation on one side. On the side propolis aids, in reducing TNF α levels well which helps ease the inflammatory response triggered by high mechanical compression⁴⁰. Reducing the levels of TNF α , on the compression side aids in supporting bone adaptation and remodeling facilitating the process of tissue and bone regeneration in that area⁴¹. Propolis plays a role in lowering

TNF α levels on both the tension and compression sides during treatment improving the wellbeing of supporting tissues and reducing inflammation linked to orthodontic procedures⁴². Utilizing propolis as a supplement can enhance treatment results. Contribute to overall oral and dental health.

CONCLUSION

This study determined that vitamin D3 plays a higher role in tooth movement to the compression side than propolis, which is considered more beneficial in terms of time, but its resorption both the alveolar bone and tooth root apex, whereas propolis produce new bone formation while protecting the tooth root from resorption, and well tooth movement to the compression side comparing with ordinary orthodontic treatment which is significant and micro implants stability. Vitamin D3 increases TRAP and RANKL activity during orthodontic therapy so it regulates bone cell function by increasing tooth movement to the compression side. Both biomaterials, through complicated biological processes, promote bone health and effective orthodontic therapy. Here's a revised version: "We recommend a combined treatment of Vitamin D3 and propolis for clinical humans use", however, further studies are required in this context.

CONFLICTS OF INTEREST

None

References

1. Lobprise HB. Occlusion and orthodontics. In: Lobprise HB, Dodd JR, editors. *Wiggs's Veterinary Dentistry: Principles and Practice*. New York: John Wiley & Sons; 2019. p. 411-37.
2. Li Y, Zhan Q, Bao M, Yi J, Li Y. Biomechanical and biological responses of periodontium in orthodontic tooth movement: up-date in a new decade. *Int J Oral Sci*. 2021 Dec;13(1):20. <https://doi.org/10.1038/s41368-021-00125-5>
3. Väänänen K. Mechanism of osteoclast mediated bone resorption—rationale for the design of new therapeutics. *Adv Drug Deliv Rev*. 2005 May 25;57(7):959-71. <https://doi.org/10.1016/j.addr.2004.12.018>
4. Chouksey P, Hazari P, Somkuwar K. *Centric Relation in Prosthodontics*. Telangana: Shineeks Publishers; 2022 Apr 20.
5. Infante M, Fabi A, Cognetti F, Gorini S, Caprio M, Fabbri A. RANKL/RANK/OPG system beyond bone remodeling: Involvement in breast cancer and clinical perspectives. *J Exp Clin Cancer Res*. 2019 Dec;38:1-8. <https://doi.org/10.1186/s13046-018-1001-2>
6. Tyrovolas JB, Spyropoulos MN, Makou M, Perrea D. Root resorption and the OPG/RANKL/RANK system: A mini review. *J Oral Sci*. 2008;50(4):367-76. <https://doi.org/10.2334/josnusd.50.367>
7. Schröder A, Bauer K, Spanier G, Proff P, Wolf M, Kirschneck C. Expression kinetics of human periodontal ligament fibroblasts in the early phases of orthodontic tooth movement. *J Orofac Orthop*. 2018 Sep 1;79(5). <https://doi.org/10.1007/s00056-018-0145-1>
8. Fagerlund K. *Osteoclastic tartrate-resistant acid phosphatase 5b. Diagnostic use and biological significance in bone physiology*. Turku: Turun Yliopisto; 2009.
9. Omi M, Mishina Y. Role of osteoclasts in oral homeostasis and jawbone diseases. *Oral Sci Int*. 2021 Jan;18(1):14-27. <https://doi.org/10.1002/osi2.1078>
10. Krishnan V, Zahrowski JJ, Davidovitch ZE. The effect of drugs, hormones, and diet on orthodontic tooth movement. In: Krishnan V, Davidovitch Z, editors. *Biological Mechanisms of Tooth Movement*. UK: Wiley-Blackwell; 2021 Apr 9. p. 199-215. <https://doi.org/10.1002/9781119608912.ch14>
11. Zulhendri F, Lesmana R, Tandean S, Christoper A, Chandrasekaran K, Irsyam I, Suwantika AA, Abdulah R, Wathoni N. Recent update on the anti-inflammatory activities of propolis. *Molecules*. 2022 Dec 2;27(23):8473. <https://doi.org/10.3390/molecules27238473>
12. Ekeuku SO, Chin KY. Application of propolis in protecting skeletal and periodontal health—A systematic review. *Molecules*.



- 2021 May 25;26(11):3156. <https://doi.org/10.3390/molecules26113156>
13. Yuanita T, Kunarti S, Zubaidah N. East java extract propolis as potential intracanal medicament in experimentally induced chronic apical periodontitis. *Indian J Dent Res.* 2019 May 1;30(3):342-6. https://doi.org/10.4103/ijdr.ijdr_236_17
14. Pileggi R, Antony K, Johnson K, Zuo J, Holliday LS. Propolis inhibits osteoclast maturation. *Dent Traumatol.* 2009 Dec;25(6):584-8. <https://doi.org/10.1111/j.1600-9657.2009.00821.x>
15. Altan BA, Kara IM, Nalcaci R, Ozan F, Erdogan SM, Ozkut MM, Inan S. Systemic propolis stimulates new bone formation at the expanded suture: a histomorphometric study. *Angle Orthod.* 2013 Mar 1;83(2):286-91. <https://doi.org/10.2319/032612-253.1>
16. Al-Hassani AA, Al-Shamma AM. Effect of delayed bonding and different antioxidants on composite restoration microleakage of internally bleached teeth. *Adv Dent Oral Health.* 2018;9(3):555762. <https://doi.org/10.19080/adoh.2018.09.555762>
17. Yogarajah M. *Crash Course Neurology Updated Edition-E-Book*. UK: Mosby; 2015 Nov 12.
18. Aoki M, Wakuno A, Kushiro A, Mae N, Kakizaki M, Nagata SI, Ohta M. Evaluation of total intravenous anesthesia with propofol-guaifenesin-medetomidine and alfaxalone-guaifenesin-medetomidine in Thoroughbred horses undergoing castration. *J Vet Med Sci.* 2017;79(12):2011-8. <https://doi.org/10.1292/jvms.16-0658>
19. You TM, Ban BH, Jeong JS, Huh J, Doh RM, Park W. Effect of premolar extraction and presence of the lower third molar on lower second molar angulation in orthodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2014 Sep 1;118(3):278-83. <https://doi.org/10.1016/j.oooo.2014.05.002>
20. Kitauro H, Kimura K, Ishida M, Sugisawa H, Kohara H, Yoshimatsu M, Takano-Yamamoto T. Effect of cytokines on osteoclast formation and bone resorption during mechanical force loading of the periodontal membrane. *ScientificWorldJournal.* 2014;2014(1):617032. <https://doi.org/10.1155/2014/617032>
21. Al-Dhamadi YA. *Properties of Prosthodontic Resin Composites Veneering Some All-Ceramic and Fibre Substructures [dissertation]*. United Kingdom: The University of Manchester; 2005.
22. Sforcin JM, Bankova V. Propolis: Is there a potential for the development of new drugs?. *J Ethnopharmacol.* 2011 Jan 27;133(2):253-60. <https://doi.org/10.1016/j.jep.2010.10.032>
23. Yee JA, Türk T, Elekdağ-Türk S, Cheng LL, Darendeliler MA. Rate of tooth movement under heavy and light continuous orthodontic forces. *Am J Orthod Dentofacial Orthop.* 2009 Aug 1;136(2):150-e1. <https://doi.org/10.1016/j.ajodo.2008.06.027>
24. Kapoor P, Chowdhry A, Bagga DK, Bhargava D. Biomarkers in external apical root resorption: an evidence-based scoping review in biofluids. *Rambam Maimonides Med J.* 2022 Oct;13(4). <https://doi.org/10.5041/rmmj.10482>
25. Liew KY, Kamise NI, Ong HM, Aw Yong PY, Islam F, Tan JW, Tham CL. Anti-allergic properties of propolis: Evidence from preclinical and clinical studies. *Front Pharmacol.* 2022 Jan 21;12:785371. <https://doi.org/10.3389/fphar.2021.785371>
26. Li B, Zhang Y, Wang Q, Dong Z, Shang L, Wu L, Wang X, Jin Y. Periodontal ligament stem cells modulate root resorption of human primary teeth via Runx2 regulating RANKL/OPG system. *Stem Cells Dev.* 2014 Oct 15;23(20):2524-34. <https://doi.org/10.1089/scd.2014.0127>
27. Zeitoun R, Najjar F, Wehbi B, Khalil A, Fayyad-Kazan M, Dagher-Hamalian C, Faour WH, El-Makhour Y. Chemical composition, antioxidant and anti-inflammatory activity evaluation of the Lebanese propolis extract. *Curr Pharm Biotechnol.* 2019 Jan 1;20(1):84-96. <https://doi.org/10.2174/1389201020666190206201241>
28. Lotif MA, Valadas LA, Fecine FV, Fonseca SG, Bandeira MA, Dantas TC, Neto EM, Squassi A, Fonteles MM. A double-blind randomized clinical trial of Brazilian red propolis dentifrice efficacy in orthodontic patients. *J Oral Sci.* 2022;64(1):28-32. <https://doi.org/10.2334/josnusd.21-0270>
29. Lowe NM, Fraser WD. Vitamin D and bone health. *Proceedings of the Nutrition Society.* 2018;77(2):157-165.
30. Tang M, Wang G, Li J, Wang Y, Peng C, Chang X, Guo J, Gui S. Flavonoid extract from propolis alleviates periodontitis by boosting periodontium regeneration and inflammation resolution via regulating TLR4/MyD88/NF-κB and RANK/NF-κB pathway. *J Ethnopharmacol.* 2024 Jan 30;319:117324. <https://doi.org/10.1016/j.jep.2023.117324>
31. Udagawa N, Koide M, Nakamura M, Nakamichi Y, Yamashita T, Uehara S, Kobayashi Y, Furuya Y, Yasuda H, Fukuda C, Tsuda E. Osteoclast differentiation by RANKL and OPG signaling pathways. *J Bone Miner Metab.* 2021 Jan;39:19-26. <https://doi.org/10.1007/s00774-020-01162-6>
32. Hienz SA, Paliwal S, Ivanovski S. Mechanisms of bone resorption in periodontitis. *J Immunol Res.* 2015;2015(1):615486. <https://doi.org/10.1155/2015/615486>
33. Kamaruzzaman MA, Chin KY, Mohd Ramli ES. A review of potential beneficial effects of honey on bone health. *Evid Based Complement Alternat Med.* 2019;2019(1):8543618. <https://doi.org/10.1155/2019/8543618>
34. Sharma A, Sharma L, Goyal R. Molecular signaling pathways and essential metabolic elements in bone remodeling: An implication of therapeutic targets for bone diseases. *Curr Drug Deliv.* 2021 Jan 1;22(1):77-104. <https://doi.org/10.2174/1389450121666200910160404>
35. Omi M, Mishina Y. Roles of osteoclasts in alveolar bone remodeling. *Genesis.* 2022 Sep;60(8-9):e23490. <https://doi.org/10.1002/dvg.23490>
36. Moghadam ET, Yazdani M, Alam M, Tebyanian H, Tafazoli A, Tahmasebi E, Ranjbar R, Yazdani A, Seifalian A. Current natural bioactive materials in bone and tooth regeneration in dentistry: a comprehensive overview. *J Mater Res Technol.* 2021 Jul 1;13:2078-114. <https://doi.org/10.1016/j.jmrt.2021.05.089>
37. Hamed HS, Abdel-Tawwab M. Ameliorative effect of propolis supplementation on alleviating bisphenol-A toxicity: Growth

- performance, biochemical variables, and oxidative stress biomarkers of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. Comp Biochem Physiol C Toxicol Pharmacol. 2017 Nov 1;202:63-9. <https://doi.org/10.1016/j.cbpc.2017.08.001>
38. Hoang C, Nguyen AK, Nguyen TQ, Fang W, Han B, Hoang BX, Tran HD. Application of dimethyl sulfoxide as a therapeutic agent and drug vehicle for eye diseases. J Ocul Pharmacol Ther. 2021 Oct 1;37(8):441-51. <https://doi.org/10.1089/jop.2021.0043>
39. Yamaguchi M, Fukasawa S. Is inflammation a friend or foe for orthodontic treatment?: Inflammation in orthodontically induced inflammatory root resorption and accelerating tooth movement. Int J Mol Sci. 2021 Feb 27;22(5):2388. <https://doi.org/10.3390/ijms22052388>
40. Lopes D, Martins-Cruz C, Oliveira MB, Mano JF. Bone physiology as inspiration for tissue regenerative therapies. Biomaterials. 2018 Dec 1;185:240-75. <https://doi.org/10.1016/j.biomaterials.2018.09.028>
41. Inchingolo F, Inchingolo AM, Latini G, Ferrante L, Trilli I, Del Vecchio G, Palmieri G, Malcangi G, Inchingolo AD, Dipalma G. Oxidative Stress and Natural Products in Orthodontic Treatment: A Systematic Review. Nutrients. 2023 Dec 28;16(1):113. <https://doi.org/10.3390/nu16010113>
42. Saeed MA, Khabeer A, Faridi MA, Makhdoom G. Effectiveness of propolis in maintaining oral health: A scoping review. Can J Dent Hyg. 2021 Oct;55(3):167. PMID: [34925517](https://pubmed.ncbi.nlm.nih.gov/34925517/)