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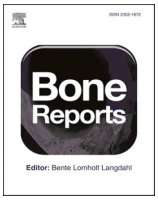

# Therapeutic dosage of isotretinoin in rats may influence orthodontic tooth movement

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ABSTRACT

Objective Isotretinoin, also known as 13-cis-retinoic acid, is an isomer of tretinoin, the oxidized form of Vitamin A. Orthodontic tooth movement (OTM) is the result of a cascade of inflammatory responses stimulated by a physical element that is the force generated by orthodontic appliances. Isotretinoin is mainly used among adolescents and young adults, and coincidentally it is this age group that also undergoes orthodontic treatment. Materials and Methods Fifty-five animals were used, and they were randomly divided into 11 groups, containing 5 animals in each group. Group 1: Control; Group 2: OTM for 7 days; Group 3: OTM for 14 days; Group 4: Treated with isotretinoin for 14 days with a dosage of 7.5 mg/kg/day; Group 5: Treated with isotretinoin for 14 days with a dosage of 1.0 mg/kg/day; Group 6: Treated with isotretinoin for 21 days with a dosage of 7.5 mg/kg/day; Group 7: Treated with isotretinoin for 21 days with a dosage of 1.0 mg/kg/day; Group 8: Treated with isotretinoin for 14 days with a dosage of 7.5 mg/kg/day and undergoing OTM for 7 days; Group 9: Treated with isotretinoin for 14 days with a dosage of 1.0 mg/kg/day and undergoing OTM for 7 days; Group 10: Treated with isotretinoin for 21 days with a dosage of 7.5 mg/kg/day and undergoing OTM for 14 days; Group 11: Treated with isotretinoin for 21 days with a dosage of 1.0 mg/kg/day and undergoing OTM for 14 days. In Groups 8, 9, 10 and 11, the animals were treated with isotretinoin for 7 days before OTM and maintained during the movement period in the respective groups.

Results There was a significant difference in microtomographic parameters, including Trabecular Volume (BV/TV), Trabecular Thickness (Tb.Th), Number of Trabeculae (Tb.N), and Trabecular Separation (Tb.Sp), between the groups. The group that received orthodontic force in conjunction with isotretinoin treatment at a dosage of 7.5 mg/kg/day exhibited lower tooth displacement over a period of 21 days and 14 days. Conclusion Isotretinoin caused a reduction in tooth displacement during OTM when administered at a dose of 7.5 mg/kg/day and isotretinoin did change the microtomographic parameters of treated animals.

## 1. Introduction

Isotretinoin, also known as 13-cis-retinoic acid, is an isomer of Tretinoin, the oxidized form of Vitamin A. It was synthesized for therapeutic use in the treatment of severe inflammatory acne and for cases of acne that are resistance to antibiotics or topical drugs (Vieira et al., 2012). Acne is a common condition that affects a significant percentage of teenagers and young adults in the Western world, with estimates ranging from 79 to 95 % (Vieira et al., 2012; Fleischer Jr et al., 2003; Cordain et al., 2002). Isotretinoin is believed to act on the sebaceous gland by binding to specific retinoid receptors and modifying gene transcription (Vieira et al., 2012). It reduces the activity and size of the sebaceous gland, thereby decreasing sebum production[1]. Some studies have shown that after 8 weeks of isotretinoin treatment in humans, there is a decrease in the genes involved in steroids, cholesterol, and fatty acids metabolism, as well as an increase in genes that encode structural proteins like collagen and fibronectin (Nelson et al., 2009a; Nelson et al., 2009b). Additionally, retinoids, including isotretinoin, normalize sebaceous follicle keratinization and the population of *Propionibacterium acnes* (Nelson et al., 2006).

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Animal studies have demonstrated that an overdose of the active form of Vitamin D, Calcitriol, increases the orthodontic tooth movement (OTM) displacement (Takano-Yamamoto et al., 1992; Kale et al., 2004). Isotretinoin affects Vitamin D metabolism and antagonizes its role in calcium homeostasis (Ertugrul et al., 2011). In rats, an overdose of Vitamin A can lead to accelerated bone resorption due to increased osteoclast activity. Hypercalcemia induced by Vitamin A may cause the removal of calcium ions from bones, increasing their concentration in the bloodstream (Frame et al., 1974; McGuire and Lawson, 1987). A study on the association of isotretinoin and OTM in animals observed that tooth extraction facilitated OTM without interfering with the quality of bone remodeling (Nishio et al., 2017). In a literature review with clinical studies, it was found that, apart from its teratogenic effects, the adverse effects of isotretinoin are generally mucocutaneous, such as dryness of the lips, eyes, and skin. These effects are dose-dependent and reversible upon discontinuation of the drug (Landis, 2020; Vigarios et al., 2019). However, there is a lack of literature regarding reports of intraoral alterations in patients during isotretinoin treatment.

OTM occurs as a result of a cascade of inflammatory responses stimulated by the force generated by orthodontic appliances. It involves remodeling of the alveolar bone, changes in the periodontal ligament, and sometimes in the root cementum (Nishio et al., 2017). Specific factors, such as drug use and changes in metabolism, can influence the cellular events involved in OTM, leading to changes in the quality and quantity of tooth displacement (Nishio et al., 2017). The influence of drug use on the amount of tooth displacement and biological responses has been explored by some authors (Roche et al., 1997; Olteanu et al., 2015).

The interrelation between isotretinoin and tooth movement has been the subject of previous studies, but the results remain inconclusive, and there are still unresolved issues (Nishio et al., 2017; Graciano Parra et al., 2021).Isotretinoin is primarily used among teenagers and young adults, coincidentally the age group that also often undergoes orthodontic treatment (Bergoli et al., 2011; Leachman et al., 1999; Downs, 2003; Erdogan et al., 2006). Considering that there are individuals undergoing orthodontic treatment while also using isotretinoin, and that there is limited information on the effects of this combination in the literature, we are proposing this study to evaluate the influence of isotretinoin on OTM.

## 2. Methodology

### 2.1. Animals and experimental conditions

The project was approved by the Ethics Committee on the Use of Animals under protocol number: 00462–2020. All experiments were conducted in compliance with the Ethical Principles for Animal Experimentation (Percie du Sert et al., 2020). The experiment involved the use of fifty-five male rats (*Rattus norvergicus albinus, Wistar*) aged 75 days old, weighing between 300 g - 350 g at the beginning of the experiments. The rats were housed in a controlled environment with a temperature of 25 ± 2 °C, a 12-h light/12-h dark cycle, and provided with food (Purina®, Paulínia-SP, Brazil) and water ad libitum throughout the experiment.

### 2.2. Experimental delineation

The sample calculation for the experiment was performed based on a previous study, using SigmaPlot 14.0 software (Bertolini et al., 2014). The following test details were used: a significance level ($\alpha$) = 0.05, test power (1- $\beta$) = 0.80, and dropout ($\beta$) = 0.2. Based on these parameters, a minimum sample size of 5 rats per group was determined.

A total of fifty-five animals were used and randomly assigned to 11 groups, with each group consisting of 5 animals. The composition of the groups and the procedures performed will be described in detail below:

**Group 1 (CON)**: Control (gavage with soybean oil);

**Group 2 (OTM7)**: OTM for 7 days;

**Group 3 (OTM14):** OTM for 14 days;

**Group 4 (ISO14a)**: Treated with isotretinoin for 14 days with a dosage of 7.5 mg/kg/day;

**Group 5 (ISO14b)**: Treated with isotretinoin for 14 days with a dosage of 1.0 mg/kg/day;

**Group 6 (ISO21a):** Treated with isotretinoin for 21 days with a dosage of 7.5 mg/kg/day;

**Group 7 (ISO21b)**: Treated with isotretinoin for 21 days with a dosage of 1.0 mg/kg/day;

**Group 8 (ISO14a-OTM7):** Treated with isotretinoin for 14 days with a dosage of 7.5 mg/kg/day and undergoing OTM for 7 days;

**Group 9 (ISO14b-OTM7):** Treated with isotretinoin for 14 days with a dosage of 1.0 mg/kg/day and undergoing OTM for 7 days;

**Group 10 (ISO21a-OTM14):** Treated with isotretinoin for 21 days with a dosage of 7.5 mg/kg/day and undergoing OTM for 14 days;

**Group 11 (ISO21b-OTM14):** Treated with isotretinoin for 21 days with a dosage of 1.0 mg/kg/day and undergoing OTM for 14 days.

In Groups 8, 9, 10 and 11, the animals were treated with isotretinoin for 7 days prior to OTM, and the treatment was continued throughout the movement period in their respective groups. The weight of all animals was measured daily using a precision electronic scale (Bel 0.01 g, 2200 g S2202H, Bel Engineering, Piracicaba, São Paulo, Brazil) until they were euthanized.

### 2.3. Application of isotretinoin

Isotretinoin was obtained in the form of a 20 mg Roaccutane® capsule (Roaccutane, Roche, Basel, Switzerland) and stored at room temperature, between 59 and 86 °F, in its original packaging, protected from light and humidity. Each solution was prepared in the morning, in a dark room, immediately before administration, due to the high sensitivity of Vitamin A to light (Ferguson et al., 2005). The capsule contents were dissolved in soybean oil to facilitate gavage administration. Soybean oil was chosen as it is a component of Roaccutane®. The solution was prepared in an amber bottle, taking into account the animal's weight and the desired concentration. To prepare the solution, a 20 mg Roaccutane® capsule was pierced using a hypodermic needle (30 × 0.70 mm, Descarpack 22G, Santa Catarina, Brazil), and its contents were added to the vial. The recommended daily dose for humans, based on the literature and patient weight, ranges from 0.5 to 2 mg/kg/day (Nair and Jacob, 2016). When adjusting the dosage to the equivalent between animals and humans, a dosage of 1.0 mg/kg/day and 7.5 mg/kg/day was chosen, which closely resembles the recommended human dosage (United States Environmental Protection Agency, 2006; Thomazini, 2016). The vial containing the solution was shaken to ensure proper dilution, and the solution was administered to the animals via oral gavage using a suitable short needle for rats (Bonther, Reference: Steel INOX BD-12, Ribeirão Preto, São Paulo). The dosage was adjusted according to each animal's body weight, which was measured daily until the time of euthanasia.

### 2.4. Orthodontic tooth movement (OTM) protocol

For the installation of the mechanical appliance, the muscle relaxant Xylazine Hydrochloride (DOPASER, Caleir S.A., Barcelona, Spain) was administered at a proportion of 0.03 ml/100 g of body weight, and the anesthetic Ketamine Hydrochloride (VETASET, Fort Dodge Animal Health, Iowa, USA) was administered at a proportion of 0.07 ml/100 g of body weight. Both drugs were administered intramuscularly. In this research, a mechanical system was used based on the method adapted by Cuoghi and colleagues (Cuoghi et al., 2019). The appliance consisted of a nickel-titanium spring (Sentalloy, GAC, USA) with an effective length of 4 mm and a constant force of 50cN. The force magnitude and the amount of spring stretch were predetermined by the manufacturer. To adapt the springs to the molars and incisors, 0.2 mm diameter wires

(Morelli, Sorocaba, SP, Brazil) were utilized. After installing the spring, resin (Z100™ - 3 M, Minnesota, USA) was applied to the incisors to enhance retention. Orthodontic tooth movement (OTM) was performed on the upper right first molar, with the upper right incisor serving as anchorage (Fig. 1).

### 2.5. Euthanasia and specimen preparation

After the experimental periods of each group, the animals were euthanized via transcardiac perfusion. This involved the injection of 100 mL of physiological saline solution containing 0.1 % heparin, followed by 800 mL of 4 % formaldehyde in saline phosphate buffer (PBS) at a temperature of 4 °C and pH 7.4. The purpose of this perfusion was to preserve the tissues for subsequent analysis. The right hemimaxillas (half of the upper jaw) were then dissected and subjected to fixation for 12 h. After fixation, they were washed for 12 h in running water to remove excess chemicals and prepare them for three-dimensional (3D) imaging. The 3D images were obtained using a Skyscan 1272 scanner (Bruker, Billerica, Massachusetts, USA). High-resolution images were captured at 70 KvP (142 μA) with a field of view (FOV) of 20.5 mm. Once the images were acquired, the specimens were returned to the fixative solution and kept there for an additional 72 h. Next, the samples were demineralized in a 10 % ethylenediaminetetraacetic acid (EDTA) solution (Sigma Chemical®) in PBS for a period of 60 days. After demineralization, the samples underwent conventional histological processing, including embedding in paraffin and subsequent microtomy to obtain 5 μm thick sections. Finally, the histological sections were stained with hematoxylin-eosin (HE), a common staining method used in histology to visualize cellular structures and tissue architecture.

### 2.6. Tooth displacement analysis

The 3D image reconstructions were generated using the NRecon Reconstruction Software from Micro Photonics Inc. (Allentown, Pennsylvania). The specific region of interest analyzed was the space between the distal surface of the first molar and the mesial surface of the second molar. The 3D image was obtained with the aim of capturing a sagittal view that allows for the observation of the entire mesiobuccal and distobuccal roots of the first molar (including the cervical, middle, and apical thirds), the pulp, the curvature of the furcation region, and the integrity of the mesial alveolar bone crest. This was achieved to assess the relevant structures and changes in the area (Fig. 2C). To facilitate image orientation and analysis, specific lines were adjusted in the Skyscan Dataviewer Software (Bruker, Billerica, Massachusetts, USA). These lines included the axial line (blue), sagittal line (green), and coronal line (red). In the coronal view, the blue line passed through the long axis of the first molar in the mesiodistal direction (Fig. 2A). In the axial view, the image was appropriately angled (Fig. 2B) to allow for a clear sagittal view (Fig. 2C) that encompassed the mesiobuccal and distobuccal roots of the first molar in their entirety. In the sagittal view, a dashed yellow line was positioned at the tip of the molar cusps, serving as a reference for locating the most convex point on the enamel of the first molar, indicated by the green line. The space between the first molar and the second molar at its greatest convexity on the distal and mesial surface, as determined by the green line, was used for the linear measurement of tooth displacement. This measurement was performed using the Image J Software (National Institute of Health, NIH, U.S).

### 2.7. Analysis of bone morphology

To assess the bone morphology in the region between the roots of the first molar, coronal view images were reconstructed for all groups (Fig. 3). In the coronal plane, the axial line was aligned with the long axis of the maxillary first molar in the mesiodistal direction (Fig. 3A). For bone density analysis, the 3D images were imported into the CT-Analyser Software (CTAn version 1.13, Bruker MicroCT, Kontich, Belgium). To define the area analyzed for bone architecture, the image of the first molar was evaluated in the occlusal-apical direction. The most cervical level analyzed corresponded to the floor of the pulp chamber (Fig. 3B-C), determined by the green line. The most apical level

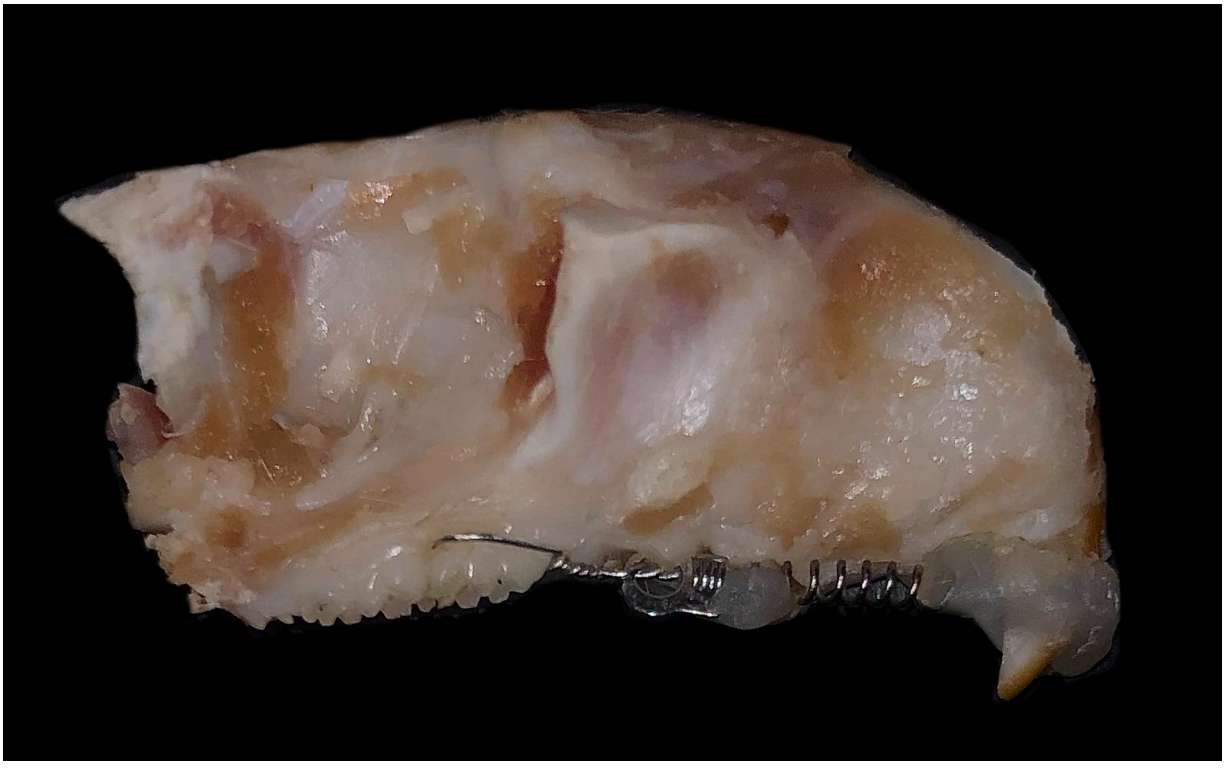

**Fig. 1.** Orthodontic appliance used for orthodontic tooth movement (OTM).

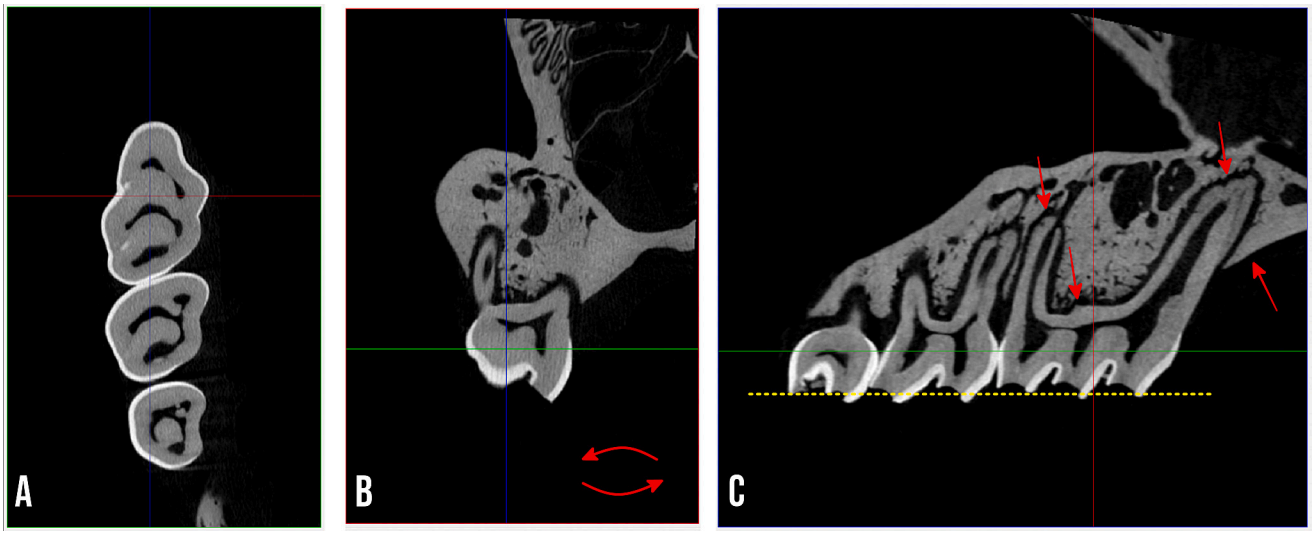


**Fig. 2.** In A, image illustration in coronal view. In B, an axial view and C, a sagittal view.

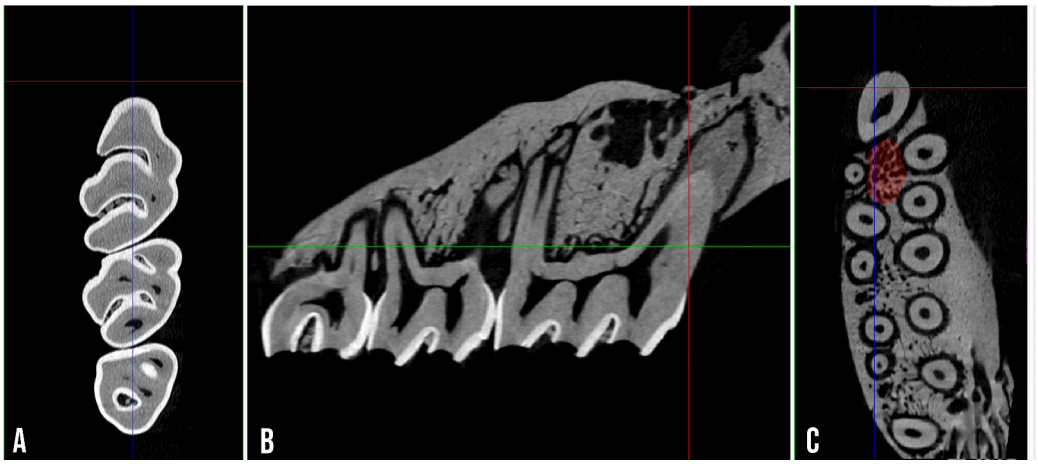


**Fig. 3.** Standardization of sections using the DataViewer software: In A, the illustration of the most cervical portion of the crowns in the coronal view. In B, the illustration of the sagittal view of the same section of the most cervical portion analyzed from the coronal view, in C.

analyzed was close to the middle third of the mesiobuccal root, ensuring that it did not exceed the middle third of the root. A total of 80 image slices were analyzed for each sample. Using the CTAn Software, several parameters related to bone structure were calculated and compared between groups. These parameters included bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp). These measurements provided quantitative information on the bone density and architecture in the analyzed region.

### *2.8. Periodontal ligament and alveolar bone analysis*

The analysis of the periodontal ligament architecture involved the examination of various areas and histological characteristics. These areas included the interradicular septum (the space between the roots), as well as the mesiobuccal and distobuccal roots. For the mesiobuccal root, the analysis covered the mesial and distal surfaces, as well as the cervical, middle, and apical thirds. Similarly, for the distobuccal root, the mesial and distal surfaces, as well as the cervical, middle, and apical thirds, were examined. In each of these areas of the periodontal ligament, several histological features were assessed. These included the presence of hemorrhage (bleeding), inflammatory infiltrate (immune cells present in the tissue), and the arrangement of collagen fiber bundles. These parameters provided insights into the condition and health of the periodontal ligament in the different areas analyzed. Regarding the architecture of the alveolar bone, the analysis focused on the characteristics of the bone located between the mesiobuccal and distobuccal roots. The criteria used for analysis included assessing areas of bone resorption (loss of bone tissue), areas of new bone formation, and overall bone quantity. These assessments helped to understand the changes in alveolar bone structure and remodeling that occurred during the experimental period.

### *2.9. Statistical analysis*

In order to assess the error of the method used to measure tooth displacement, a comparison was made between ten images in the sagittal microtomographic norm and their respective measurements performed by different evaluators. These evaluations were conducted with a 20-day interval between them. The average measurements from the two evaluations were compared using the paired *t*-test, with a significance level of 5 %. The results of this analysis showed no significant differences between the different measurement moments, indicating a low error in the measurement method.

For the statistical analysis of the data, the GraphPad Prism Software (Version 9.5, Boston, MA) was used. The normality and homogeneity of

the data were assessed using the Shapiro-Wilk test. Comparisons between groups and different time points were performed using the Kruskal-Wallis test, followed by Dunn's non-parametric multiple comparison test. The significance level for all statistical tests was set at 5 %. These analyses helped to determine any significant differences between the groups and time points being compared, providing valuable insights into the experimental findings.

## 3. Results

### 3.1. Effects of isotretinoin on bone morphology

Based on the results of the microtomographic parameters (Trabecular Volume - BV/TV, Trabecular Thickness - Tb.Th, Trabecular Number - Tb.N, and Trabecular Separation - Tb.Sp) obtained from groups 1, 4, 5, 6 and 7 the statistical analysis showed significant differences in Tb.N among ISO14a-OTM7 group and ISO14a group, ISO21b group and ISO14a-OTM7 group ($P = 0.0105$; $P = 0.0133$; $P = 0.0143$, respectively), and in Tb.Sp among ISO14a-OTM7 group and CON group, ISO14a group and ISO21b group, ($P = 0.0377$; $P = 0.0180$; $P = 0.0043$, respectively) and between ISO21b-OTM14 group and ISO21b group ($P = 0.0180$). There was no significant difference between the other groups ($P > 0.05$). This indicates that the administration of isotretinoin did have an impact on bone morphology under the specific experimental conditions of this study. The results suggest that the dosage and duration of isotretinoin used in the experiment did lead to changes in the microtomographic parameters measured in the study groups (Table 1; Fig. 4).

### 3.2. Effects of tooth movement on bone morphology

Based on the results of the microtomographic parameters (Trabecular Volume - BV/TV, Trabecular Thickness - Tb.Th, Trabecular Number - Tb.N, and Trabecular Separation - Tb.Sp) obtained from CON, OTM7 and OTM14 groups, the statistical analysis revealed a significant difference in Tb.Th between CON group and OTM14 group ($P = 0.0262$). There was no significant difference between the other groups ($P > 0.05$). (Table 1; Fig. 4).

Effects of tooth movement and isotretinoin on bone morphology.

Based on the results of the microtomographic parameters (Trabecular Volume - BV/TV, Trabecular Thickness - Tb.Th, Trabecular Number - Tb.N, and Trabecular Separation - Tb.Sp), there was no significant difference among the groups ISO14a-OTM7, ISO14b-OTM7, ISO21a-OTM14 and ISO21b-OTM14 groups ($P > 0.05$) (Table 1; Fig. 4).

### 3.3. Effects of isotreinoin on the amount of tooth displacement

The results show there was a significant difference between OTM14 group compared to ISO14a-OTM7 and ISO21a-OTM14 groups ($P = 0.0021$; $P = 0.0405$, respectively). There was no significant difference between the other groups ($P > 0.05$). Therefore, dental displacement was lower in the group where orthodontic force was applied in conjunction with isotretinoin treatment at a dosage of 7.5 mg/kg/day for 14 and 21 days (Table 2; Fig. 5).

### 3.4. Quantitative and qualitative analysis of the effects of isotretinoin on the periodontal ligament

In groups CON, ISO14a, ISO14b, ISO21a and ISO21b (Fig. 6), where no orthodontic dental movement was performed, the histological characteristics of the periodontal tissues were very similar. In these groups, cementum was present in all specimens with no or rare resorption foci. This tissue was covered by cementoblasts and fibroblasts, with the incorporation of a large amount of Sharpey's fibers into the cementum matrix. In the case of cellular cementum, lacunae containing cementocytes were observed. The periodontal ligament was composed of a highly fibrous connective tissue with a large quantity of thick collagen fiber bundles, which constituted the main fibers of the periodontal ligament. In between these fibers, there was a three-dimensional network of finer collagen fibers, the secondary fibers of the periodontal ligament. This tissue contained numerous fibroblasts and blood vessels, with few inflammatory cells found. In the bone tissue, osteoblasts or bone lining cells were observed on the entire surface, along with many Sharpey's fibers incorporated into the bone matrix, rare active osteoclasts, and numerous osteocytes within the matrix.

In groups OTM7, ISO14a-OTM7 and ISO14b-OTM7, stretching of the periodontal ligament fibers was observed on the distal aspect of the disto-vestibular root. On the mesial aspect of the disto-vestibular root, areas of periodontal ligament hyalinization and the presence of a few macrophagic cells were evident. Hyalinization areas were more extensive in the groups treated with isotretinoin. On the distal aspect of the mesio-vestibular root, a slight stretching of the periodontal ligament fibers was observed. On the mesial aspect of the mesio-vestibular root, the periodontal ligament fibers showed slight compression, and active clastic cells were present. Meanwhile, in groups OTM14, ISO21a-OTM14 and ISO21b-OTM14, the distal aspect of the disto-vestibular root exhibited slight stretching of the periodontal ligament fibers. On the mesial aspect of the disto-vestibular root, OTM14 group showed a small area of hyalinization, with periodontal ligament restitution in the surrounding areas, cementum resorption near the hyalinization area, and the presence of macrophagic cells around such areas, along with the presence of clastic cells in the alveolar bone tissue. In contrast, in this same area, ISO21a-OTM14 and ISO21b-OTM14 groups exhibited an extensive area of hyalinization, similar in size to that observed in ISO14a-OTM7 and ISO14b-OTM7 groups. In OTM7, OTM14, ISO14a-OTM7, ISO14b-OTM7, ISO21a-OTM14 and ISO21b-OTM14 groups, both on the distal and mesial aspects of the mesio-vestibular root, intact cementum, periodontal ligament restructuring, and the presence of clastic cells in the alveolar bone tissue were observed, especially on the mesial aspect.

**Table 1**
Bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp) in all groups.

| | Groups | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | CON | OTM7 | OTM14 | ISO14a | ISO14b | ISO21a | ISO21b | ISO14a-OTM7 | ISO14b-OTM7 | ISO21a-OTM14 | ISO21b-OTM14 |
| BV/TV (%) | 15.83 ± 0.6843 | 22.35 ± 2.095 | 22.66 ± 2.415 | 17.92 ± 4.051 | 16.21 ± 0.8499 | 18.96 ± 4.498 | 18.15 ± 3.458 | 20.46 ± 0.9131 | 22.39 ± 0.7087 | 22.55 ± 2.316 | 22.12 ± 1.990 |
| Tb.Th (mm) | 2.291 ± 0.05735 | 2.549 ± 0.1280 | 2.721 ± 0.1446 | 2.948 ± 0.9467 | 2.434 ± 0.1774 | 3.084 ± 0.9503 | 2.930 ± 1.098 | 2.418 ± 0.1600 | 2.453 ± 0.04103 | 2.671 ± 0.1854 | 2.523 ± 0.08242 |
| Tb.N (1/mm) | 0.06834 ± 0.0050 | 0.08754 ± 0.004 | 0.08316 ± 0.006 | 0.06216 ± 0.0055 | 0.06990 ± 0.0063 | 0.06256 ± 0.0059 | 0.06116 ± 0.0083 | 0.08478 ± 0.004 | 0.08912 ± 0.003 | 0.08434 ± 0.004 | 0.008762 ± 0.006 |
| Tb.Sp (mm) | 13.77 ± 0.5575 | 10.23 ± 0.7573 | 10.31 ± 1.043 | 13.68 ± 0.4449 | 13.02 ± 0.9189 | 14.11 ± 1.013 | 14.58 ± 0.9793 | 10.68 ± 0.8367 | 9.458 ± 0.2686 | 10.39 ± 0.9465 | 9.844 ± 0.8079 |

Values are mean ± standard deviation. Significance level of analysis of variance: $P < 0.05$.

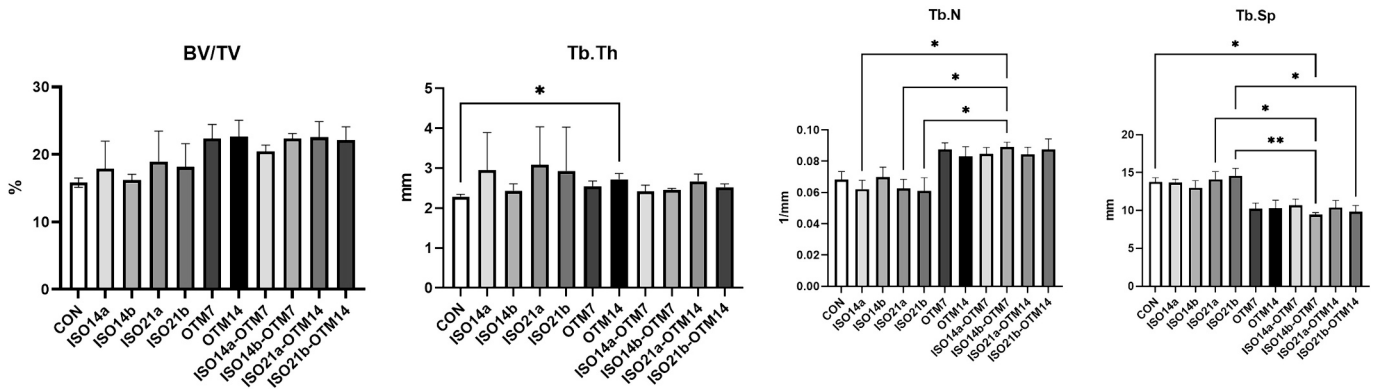


**Fig. 4.** Chart showing Trabecular Volume (BV/TV), Trabecular Thickness (Tb.Th), Trabecular Number (Tb.N) and Trabecular Separation (Tb.Sp) of all groups.

**Table 2**
Amount of tooth displacement in groups 2, 3, 8, 9, 10 and 11, respectively.

| | Groups | | | | | |
|---|---|---|---|---|---|---|
| | OTM7 | OTM14 | ISO14a-OTM7 | ISO14b-OTM7 | ISO21a-OTM14 | ISO21b-OTM14 |
| μm | 53.86 ± 8.294 | 106.0 ± 26.74 | 35.97 ± 9.506 | 76.94 ± 15.13 | 44.97 ± 22.51 | 62.02 ± 20.10 |

Values are mean ± standard deviation. Significance level of analysis of variance: $P < 0.05$.

## 4. Discussion

In this study, isotretinoin was administered at a dosage of 1.0 mg/kg/day and 7.5 mg/kg/day for either 14 or 21 days. The dosage used in the study was determined based on equivalent dosage criteria between animals and humans (Nair and Jacob, 2016; United States Environmental Protection Agency, 2006; Thomazini, 2016). The recommended daily dose of isotretinoin for acne treatment in humans, as stated in the literature and guidelines, is calculated based on the patient's weight and ranges from 0.5 to 2 mg/kg/day for up to 24 weeks (Nair and Jacob, 2016; United States Environmental Protection Agency, 2006; Thomazini, 2016; Brito et al., 2010; Sampaio, 2008). Considered that the side effects of isotretinoin are dose-duration dependent, meaning that higher doses used for longer periods can lead to more significant side effects (Thomazini, 2016; Brito et al., 2010; Sampaio, 2008), in this study, we aimed to evaluate the effects of low and moderate doses of isotretinoin, which correspond to the dosage commonly used in humans, on bone morphology in animals. The results of the study indicated that low-moderate doses of isotretinoin, did not significantly alter bone morphology in the treated animals. This finding is consistent with the findings of Graciano Parra et al., who evaluated the systemic effects of isotretinoin on the femurs of animals and found no relevant effects on bone morphology compared to animals treated only with soy oil (Graciano Parra et al., 2021). Similarly, Nishio et al. did not observe significant changes in bone morphology in the tibia of their animal model with isotretinoin treatment (Nishio et al., 2017). However, it is worth noting that Lind et al. showed a significant reduction in bone density in the calvaria of rats with an overdose of Vitamin A, which is chemically related to isotretinoin (Lind et al., 2017). Additionally, Johansson & Melhus suggested that isotretinoin may indirectly cause a decrease in bone density by interfering with the actions of vitamin D and directly causing bone resorption (Johansson and Melhus, 2001). However, the aforementioned authors analyzed different animal organs, such as the femur, tibia and calvaria, while other authors carried out a biochemical

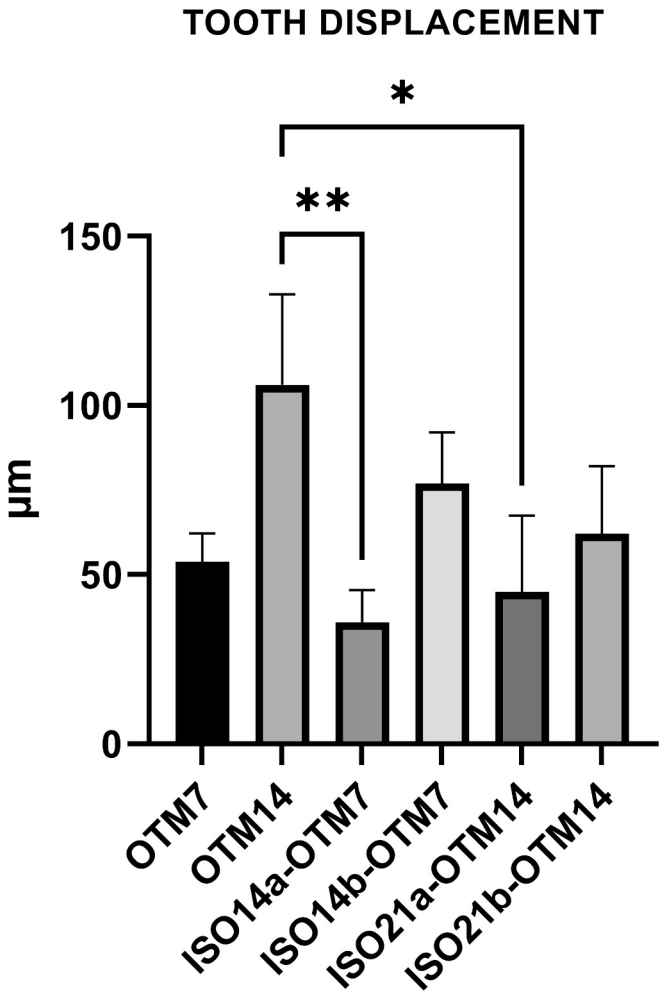


**Fig. 5.** Chart showing the amount of tooth displacement in groups OTM7, OTM14, ISO14a-OTM7, ISO14b-OTM7, ISO21a-OTM14 and ISO21b-OTM14, respectively. Note that OTM14 had significantly more tooth displacement compared to ISO14a-OTM7 and ISO21a-OTM14 groups. ($P = 0.0021$; $P = 0.0405$, respectively).

analysis in humans, unlike our study, which analyzed the furcation region of the rat's first upper molar, so more studies are needed on the effects of isotretinoin on bone morphology in these same experimental

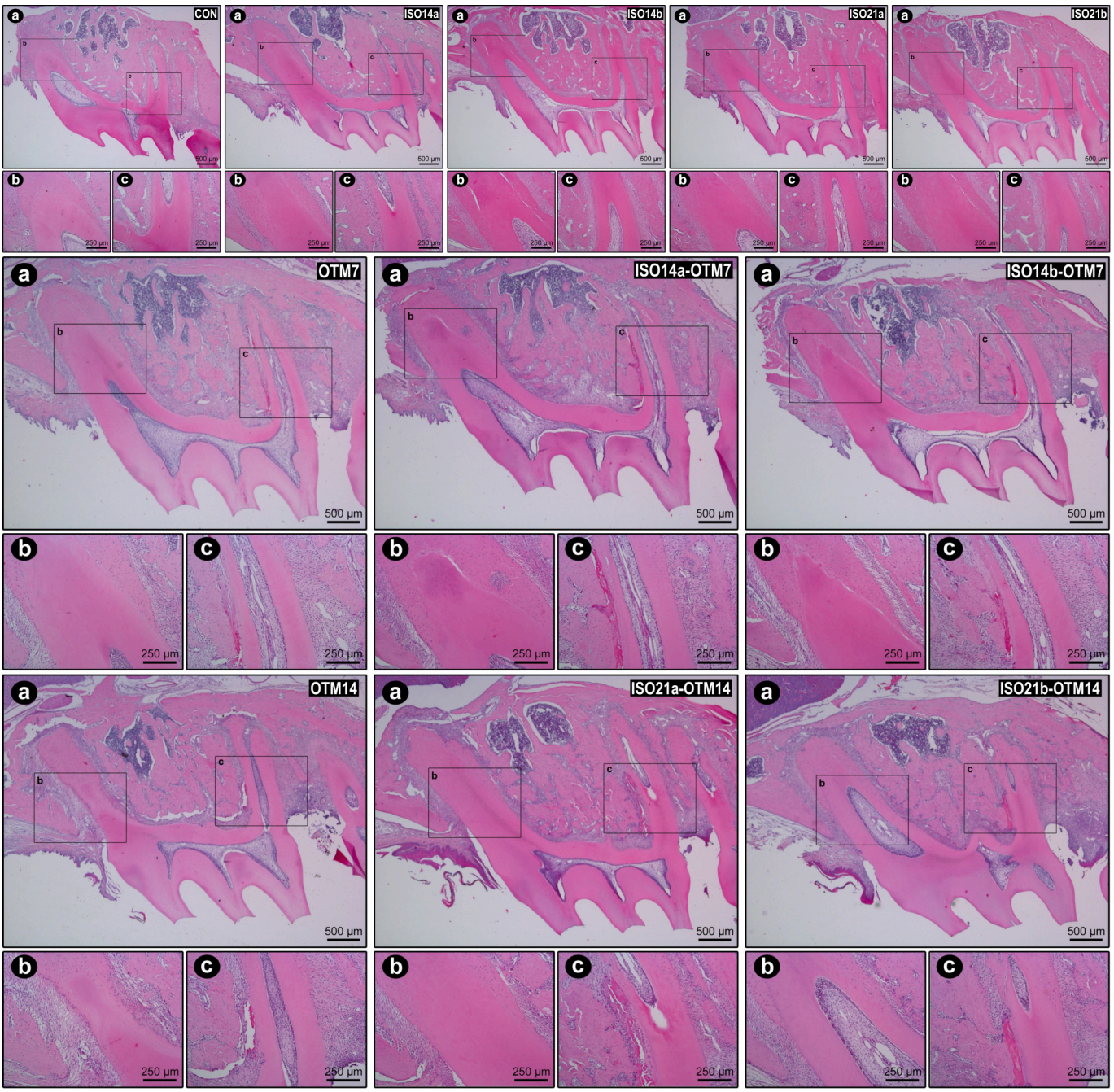


**Fig. 6.** Photomicrographs showing the histological aspect of the maxillary first molar and the supporting periodontal tissues in all groups. In (a) a panoramic view of the maxillary first molar can be observed and in (b) and (c) a greater increase in mesiobuccal root and the distobuccal root. Coloration: HE. Original magnification: (a) 2.5×; (b) and (c) 10×. Scale bars: (a) 500 µm; (b) and (c) 250 µm.

conditions.

In this study, we utilized NiTi helical springs with a constant force of 50cN to perform OTM. The results of this study demonstrated a statistical difference in trabecular thickness parameters when comparing the animals undergoing OTM for 14 days with the control group. Additionally, there was an increase in bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb.N), accompanied by a significant decrease in trabecular separation (Tb.sp) among OTM7 and OTM14 groups when compared to CON group, even that the data was not statistical significantly. Previous research has indicated that different magnitudes of force, ranging from 3 to 100cN, can produce similar tooth movements (Alikhani et al., 2015), knowing that, in a study by Gudhimella et al., using a constant force of 3cN, a decrease in BV/TV was observed during the first 7 days and after 28 days of OTM when compared to a group without OTM (Gudhimella et al., 2019). Another study by Lu et al., employing a constant force of 50cN, revealed a significant decrease in BV/TV in the furcation area of the first molar when comparing a group treated with sclerostin to a control group (Lu et al., 2019). Although there is a lack of studies analyzing microtomographic parameters under the exact experimental conditions described in this study (50cN force), it is crucial to conduct further research where a force of 50cN is exerted and the microtomographic parameters are evaluated. This will allow for a comparison of the results with animals that have not undergone OTM, providing a deeper understanding of the effects of this specific force magnitude on bone morphology during orthodontic tooth movement.

The investigation of the effects of both OTM and isotretinoin on bone morphology showed no significant impact on the microtomographic parameters in groups ISO14a-OTM7, ISO14b-OTM7, ISO21a-OTM14 and ISO21b-OTM14 groups. The only existing study found in the literature that examined OTM with isotretinoin and analyzed the same microtomographic parameters (BV/TV, Tb.Th, Tb.N, and Tb.Sp) also reported no significant difference in bone morphology (Graciano Parra et al., 2021). However, it is important to note that this particular study analyzed the parameters in the distal epiphysis of the femur, rather than between the roots of the maxillary first molar, which was the focus of our study. To gain a better understanding of the effects of OTM and isotretinoin on bone morphology in the specific area between the roots of the moved tooth, further research is necessary. Conducting studies under the same experimental conditions, with a focus on analyzing the microtomographic parameters in the desired area, will provide valuable insights into the potential effects of these interventions on bone morphology. This will contribute to a more comprehensive understanding of the subject matter.

In our study, we found that isotretinoin at a dosage of 7.5 mg/kg/day; for 21 days and 14 days of administration and 14 days and 7 days of OTM did have a significant influence on tooth displacement when compared to animals that underwent tooth movement alone for 14 days. Unlike Graciano Parra et al., being the only study that performed OTM with a force of 30cN in conjunction with treatment with isotretinoin at the same dosage also analyzed in our study – found no significant differences in tooth displacement between animals treated with isotretinoin and undergoing OTM alone (Graciano Parra et al., 2021). These discrepancies findings could be attributed to the difference between forces used at OTM and even the age or weight of the animals during the experimentation. It is important to consider the limitations of available research and the need for further studies to confirm these findings and explore potential dose-dependent effects of isotretinoin on tooth displacement during OTM.

The inflammatory process plays a crucial role in tooth displacement during orthodontic treatment, as it involves new bone formation and remodeling of collagen fibers in the periodontal tissue. Previous studies have indicated that hyalinized areas, which are indicative of tissue remodeling, appear around the third day of induced tooth movement and gradually decrease thereafter (Franzon Frigotto et al., 2015; Holland et al., 2019; Klein et al., 2022). In our study, when examining the periodontal ligament of animals treated with isotretinoin at a dosage of 7.5 mg/kg/day and 1.0 mg/kg/day for 14 days in combination with 7 days of OTM, it was observed a greater expression of hyalinized areas in the mesial and middle thirds of the mesial phase of the distobuccal root of the first molar, compared to the group that underwent tooth movement alone for 7 days. This finding contrasts with the results reported by Graciano Parra et al., who did not find significant differences in the presence of hyalinized areas between their groups treated with isotretinoin and OTM on days 2, 7, 14, and 21. These discrepancies findings regarding the presence of hyalinized areas could be attributed to several factors, including variations in experimental protocols and treatment duration. It is important to further investigate and understand the specific effects of isotretinoin on the inflammatory response and tissue remodeling during orthodontic tooth movement to gain a comprehensive understanding of its impact on treatment outcomes.

## 5. Conclusion

Based on the methodology and results of our study, the following conclusions can be drawn:

- Isotretinoin at a dosage of 7.5 mg/kg/day did reduce tooth displacement during orthodontic tooth movement (OTM). This may suggest that the administration of isotretinoin did have a significant inhibitory effect on the process of tooth movement under the conditions of this study.
- Isotretinoin did result in significant changes in the microtomographic parameters of animals treated with isotretinoin in combination with OTM. This indicates that the use of isotretinoin at the given dosage may have a notable impact on the evaluated bone morphology parameters under the conditions of this study.

## CRediT authorship contribution statement

**Mayra Fernanda Ferreira:** Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Alberto Carlos Botazzo Delbem:** Conceptualization, Data curation, Methodology, Project administration, Resources, Supervision. **Edilson Ervolino:** Methodology, Resources, Writing – review & editing. **Luy de Abreu Costa:** Investigation, Writing – review & editing. **Cristina Antoniali Silva:** Resources, Writing – review & editing. **José Ricardo Prando dos Santos:** Writing – review & editing. **Marcos Rogério de Mendonça:** Conceptualization, Data curation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

## Declaration of competing interest

In the name of all authors, I declare that we have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The authors do not have permission to share data.

*Acknowledgments*

Nothing to declare.

*Ethics approval and consent to participate*

The protocol of this research received the approval of the Ethics Committee on the Use of Animals at the Faculty of Dentistry – campus Aracatuba, Sao Paulo State University (Process FOA n° 2020-0462).

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