**Original Research Article**

**Influence of dentoalveolar trauma and orthodontic forces on dentin pulp complex: A experimental study in rats**

**ABSTRACT**

**Aims:** The present study aims to analyze the amount of tooth movement and pulp conditions in teeth with dentoalveolar trauma history (extrusive luxation), when three types of orthodontic forces are applied.

**Study design:** Experimental research

**Methodology:** *Wistar* rats (n = 48) were divided into 8 groups (n = 6), having as variables the three different types of orthodontic forces: continuous (CF), interrupted continuous (ICF) and intermittent (IF), the presence or absence of trauma (PT or AT) and the presence or not orthodontic movement (WM or WOM). The groups were arranged as follows: ATCF, ATICF, ATIF, PTCF, PTICF, PTIF, PTWM and ATWOM (control group). Extrusive dislocation (ED) of the healthy upper right first molar was performed and after 15 days for periodontal reestablishment, the orthodontic springs were installed as devices to promote induced tooth movement and on the 14th day after the first activation, the amount of tooth movement was measured, and euthanasia of the animals was performed for mounting the histological slides and histomorphometric evaluation**. The variables were performed using the parametric analysis of variance (ANOVA) with Tukey’s post-test and Kruskal-Wallis test with Dunn’s post-test, depending on the characteristic of each variable. Differences were considered statistically significant at the 5% level (p<0.05).** The variables analyzed were divided into 4 major groups: cellularity pattern, dystrophic alterations, hemodynamic alterations and dentin alterations. The data obtained were statistically examined by an appropriate test according to the characteristics of each variable.

**Results:** The results showed that there was a statistically significant difference in tooth movement for CF and ICF forces compared with IF (p <0.05). There was a lower amount of inflammatory infiltrate in the control group compared with others. The CF showed a higher resorption area.

**Conclusion:** Pulpal changes are more frequent in relation to hemodynamic alterations, followed by dentin alterations, cellularity alterations, and lastly, dystrophic alterations, but without difference among the groups.

**Keywords:** Traumatized teeth; tooth movement; dental pulp; histology

**1. INTRODUCTION**

In induced tooth movement (ITM), the application of mechanical forces produces regions of stress or tension in the periodontal ligament. In a simple description, the recruitment and activation of osteoclasts induce bone resorption from the area adjacent to the compression of the periodontal tissue, while on the tension side, bone apposition occurs through osteoblasts [1,2]. Thus, through this process of bone resorption and apposition, the tooth is repositioned [3].

There are two different types of forces employed in Orthodontics: continuous and intermittent forces. The use of a continuous force (CF) aims to maintain its initial magnitude over an extended period, being exerted by wires and springs with a high elastic limit. However, when wires or springs with reduced elasticity and shape memory, such as steel wires, are used, the magnitude of the force gradually decreases and reaches a level incapable of establishing continuity in dental movement, thus being classified as interrupted continuous force (ICF) [4]. On the other hand, an intermittent force (IF) is characterized by its action over a reduced period, being eliminated with the removal of the force-generating device. This condition is observed with the use of removable appliances, elastics, and extraoral appliances [5].

The characteristics of a force, such as magnitude, frequency, and duration, have a significant influence on the biological mechanism and can trigger periodontal changes as well as alterations in the dental pulp [3-6].

The dental pulp is a highly vascularized loose connective tissue, containing many cells, extracellular matrix, blood vessels, and nerve fibers. Similar to other connective tissues, the pulp has a high repair capacity, easily recovering under favorable conditions [7]. Thus, in the presence of any physical, chemical, or bacterial aggressor, whose stimuli exceeds the threshold of physiological tolerance, an inflammatory and/or degenerative response of the pulp may occur. When subjected to light and moderate orthodontic forces (physical agent), pulp alterations result in a significant increase in intrapulpal pressure; however, this is transient, with a tendency to return to normalcy [8,9].

In traumatized teeth, damage to the blood vessels of the pulp may be found, along with disturbances in the odontoblastic layer, obliteration of the pulp chamber and the root canal, as well as reduced blood flow and pulp necrosis [6-9]. Therefore, it is recommended to wait for an observation period ranging from 3 to 12 months, depending on the magnitude of the trauma, before initiating orthodontic treatment. This is because teeth with a history of dentoalveolar trauma may exhibit more extensive areas of root resorption during movement compared to initially intact teeth [10,11].

There are still doubts regarding which type of force would be the best for moving a tooth due to the limited number of studies in literature [12]. Even scarcer are the studies that relate the pulp to the movement of teeth that have suffered some type of trauma [13]. The studies present in the literature have only evaluated the alterations of the periodontium in healthy and traumatized teeth [2,7,14], as well as the alterations of the pulp in healthy teeth [6].

Thus, it was considered pertinent to involve dental movement and the pulpal conditions of traumatized teeth in the same study, with the aim of evaluating the amount of movement and the pulp changes, particularly in the cellular pattern, dystrophic changes, hemodynamic alterations, and dentin modifications, when three types of orthodontic forces (CF, ICF, and IF) are applied to teeth with a history of extrusive luxation (EL).

**2. METHODOLOGY**

**2.1 Sample calculation**

For sample size calculation, the GPower 3.1 software (University of Düsseldorf) was used to detect an effect size of 0.6, employing the F-family test (ANOVA) with 7 degrees of freedom (variable: internal resorption area), an alpha error of 0.05, and a test power of 80%, resulting in a sample size of 48 rats (6 per group), ensuring a test power of 80.74%.

**2.2 Animals**

**For this study, fourty-eight (48) male Wistar rats (*Rattus norvegicus albinus*) at the young adult stage (90 days old), weighing between 250 and 350 grams were selected. All rats were originated from the Bioterium of the Center for Biological and Health Sciences – CCBS, State University of Western Paraná, UNIOESTE, Cascavel, State of Paraná, Brazil.** The animals were housed in plastic cages in a controlled environment with a temperature range of 22°C (±2°C) and were fed crushed food and provided with water *ad libitum*. Additionally, the animals were acclimated for 7 days with a 12/12-hour light cycle prior to the experimental procedures. These procedures were in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Animal Ethics Committee (CEUA) at UNIOESTE (Protocol 2112/2017).

**2.3 Dentoalveolar trauma**

To minimize pain and discomfort in the animals, anesthetics based on Ketamine Hydrochloride were used at a dose of 75 mg/kg (Dopalen®, Sespo Ind. e Com. Ltda., Jacareí, SP, Brazil), along with a muscle relaxant at a dose of 15 mg/kg of Xylazine Hydrochloride (Anasedan®, Agribrands do Brasil Ltda., Paulínia, SP, Brazil), both administered intraperitoneally, with prior aseptic preparation of the region using 10% povidone-iodine (Riodeine®; Indústria Farmacêutica Rioquímica Ltda., São José do Rio Preto, SP, Brazil).

For the actual application of the trauma, twenty-four (24) animals, randomly selected, were subjected to an extrusive luxation (EL) dental trauma, performed by the same operator. The rats were held in an anatomical position, and a 0.25 mm diameter ligature wire (Morelli, Sorocaba, SP, Brazil) was tied around the cervical region of the right upper first molar, with an extension. A dynamometer (Morelli, Sorocaba, SP, Brazil) was then attached to this extension to apply dental traction with a force of 1500 cN (1.5 kgf) at an angle of 60º for 5 seconds [15].

**3.3 Induced dental movement (ITM)**

Fifteen (15) days after the trauma, the ITM was initiated, using a 0.25 mm diameter ligature wire, again tied around the same initially traumatized tooth. However, this time, the wire was attached to a closed-coil spring, 7 mm in length, a device designed by Heller and Nanda (1979) **[16],** modified by replacing the steel spring with a nickel-titanium spring (Sentalloy, GAC, NY, USA), as done in the study by Mendonça *et al*., 2018 (Figure 1) [6].

**Rosto de um cachorro com a boca aberta

O conteúdo gerado por IA pode estar incorreto.**

**Figure1**. Closed-coil Spring to perform ITM

Subsequently, the spring was extended using a dynamometer (Zeusan, Campinas, SP, Brazil) until a force of 50 cN (0.05 kgf) was achieved. Once this force was reached, another ligature of the same diameter was attached to the central incisor, which served as an anchor for the ITM. Additionally, a light-cured resin (Z100, 3M, St. Paul, MN, USA) was applied to the cervical region of the incisor to improve the retention of the wire and facilitate the activation and deactivation of the spring, as well as to protect the mucosa of the animals [17].

In the groups represented by continuous force (CF), the springs were installed and removed only on the day of euthanasia. For the groups represented by interrupted continuous force (ICF), the springs were activated and deactivated but were kept in the oral cavity of the animals. Finally, for the groups represented by intermittent force (IF), the springs were removed between activations. Additionally, the activation process was carried out in two cycles for all groups, which ended on the 14th day with the euthanasia of the animals, according to Tondelli *et al*., 2010 [13].

During the experiment, from the installation of the device onwards, the food provided for the animals was moistened and crushed in order to reduce the possibility of it breaking [17]. Additionally, one animal from the PTIF group was lost (n=5).

**3.4** **Euthanasia, Quantitative Analysis of MDI, and Histological Processing**

The euthanasia of the animals occurred on the 14th day after the spring’s installation, through an overdose of anesthetic followed by decapitation using guillotine. Subsequently, the soft tissue of the animals was removed, preserving the entire maxilla, from the incisors to the third molars.

The amount of dental movement on the right side was obtained by comparing it to the left side (non-moved). For this, a digital caliper (Mitutoyo, São Paulo, Brazil) was used to take the measurements, with the reference point being from the mesial of the 1st molar to the distal of the 3rd molar. Thus, the amount of dental movement was determined by the difference between the measurements of the moved and non-moved sides [18,19]. These measurements were recorded in millimeters and performed by one calibrated evaluator.

At the end of this stage, the specimens were fixed in 10% formalin for 24 hours, washed in running water for 48 hours, decalcified in a decalcifying solution (Allkimia®) for 19 hours, and stored in 70% alcohol. After decalcification, the specimens were dehydrated in a graded series of alcohols, cleared in xylene, and embedded in Paraplast. For histological analyses, serial sections were made in the transverse direction of the right upper first molar, with a thickness of 5 μm, using a manual rotary microtome (Olympus 4060) equipped with a steel blade. The obtained sections were dewaxed in xylene, hydrated with distilled water, and subjected to hematoxylin and eosin (H&E) staining for analysis [15-20].

**3.5** **Histological Sections digitalization**

For histological analysis, an optical microscope (Olympus BX61) was used. Photomicrographs at 200x and 400x magnification were captured with a digital camera (Olympus DP71) using the DP Controller 3.2.1.276 software.

The images were analyzed with the assistance of the Image Pro Plus software (Media Cybernetics, USA) at magnifications of 40x, 100x, 200x, and 400x. All pulpal regions of the right upper 1st molar were analyzed, from the coronal portion to the apical region. The slides were identified and coded to ensure examiner blinding [15-17].

**3.6 Histological analysis**

The right upper first molars were evaluated, and the pulpal alterations were divided into four main groups with the following characteristics: cellular pattern (inflammatory infiltration, decreased cellularity, and increased fibrosis), dystrophic changes (hyalinization, vacuolization, nodule, diffuse calcification, and necrosis), hemodynamic changes (vascular congestion, hemorrhage, and thrombosis), and dentin changes (reactive dentin, tubules with nuclei, and internal resorption) [20,21].

Initially, scores ranging from 1 to 4 were assigned for the quantification of the inflammatory infiltrate in the pulp as follows [22]: 1 - Absence of inflammatory cells or a negligible number; 2 - Mild inflammatory infiltrate (less than 25 cells per field); 3 - Moderate inflammatory infiltrate (between 25 and 125 cells per field); 4 - Severe inflammatory infiltrate (more than 125 cells per field).

Secondly, internal resorption was determined with the aid of the Image Pro Plus software to delineate the resorbed area, whether coronal or radicular, with the values expressed in square micrometers (μm²). Additionally, photomicrographs at 400x magnification were used for both the quantification of the inflammatory infiltrate and the internal resorption [15-17].

Finally, the identification of all histological alterations in the four main groups was performed descriptively, using magnifications of 40x, 100x, and 200x, and detected solely by the presence or absence of the alteration, following an adaptation of the method proposed by Mendonça *et al*., 2018 [6].

**3.7 Statistical analysis**

Quantitative data were assessed for distribution patterns using the Shapiro-Wilk test, as well as for homogeneity of variances using the Levene test. Group comparisons for these variables were performed using the parametric analysis of variance (ANOVA) with Tukey’s post-test or the non-parametric Kruskal-Wallis test with Dunn’s post-test, depending on the characteristic of each variable. For qualitative variables, group comparisons were made using the non-parametric Kruskal-Wallis test with Dunn’s post-test. The Chi-square test was used to compare the proportion of occurrence of pulpal alterations. Differences were considered statistically significant at the 5% level (p<0.05). Statistical analyses and graphs were performed using the BioStat 5.3 program (Mamirauá Institute, Belém, Pará, Brazil).

For the assessment of intra-examiner and inter-examiner agreement, the t-test was used for the measurement of resorbed area, which showed p = 0.6277 and p = 0.0706, respectively. The Wilcoxon test was used for the inflammatory infiltrate scores, which showed p = 0.3613 (intra-examiner) and p = 0.3223 (inter-examiner), confirming the reproducibility and reliability of these measurements.

**4. RESULTS**

**4.1 Dental movement**

Table 1 shows the statistically significant difference between the groups regarding the amount of movement. The CF and ICF exhibited greater movement when compared to the IF in the groups subjected to extrusive luxation (EL) (p<0.05). The luxation factor did not promote any difference in this variable, except when the ICF force was applied (ATICF = 0.25 x PTICF = 0.44) (p<0.05).

Table 1. Amount of tooth movement (mm).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **ATCF** | **ATICF** | **ATIF** | **PTCF** | **PTICF** | **PTIF** |
| 0.295 | 0.2533 | 0.2033 | 0.3733 | 0.4417 | 0.2167 |
| (0.083) | 0.1084 | 0.0728 | 0.1786 | 0.1886 | 0.0677 |
| AB | A | A | B | B | A |

Mean (standard deviation), n=6. Different letters indicate a statistical difference between the groups. Same letters indicate no statistical difference between the groups (ANOVA, Tukey's post-test, p<0.05).

**4.2 Inflammatory process**

In the intergroup comparison, a statistically significant difference (p<0.0001) was observed in the amount of inflammatory infiltrate in the ATWOM (control group) compared to all other groups, as shown in Table 2.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ATCF** | **ATICF** | **ATIF** | **PTCF** | **PTICF** | **PTIF** | **PTWM** | **ATWOM** |
|  | A | A | A | A | A | A | A | B |
| **Median** | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 | 0.000 |
| **IQR** | 1.000 | 1.000 | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 |

**Table 2**. Representative results of median (+ interquartile range) for the inflammatory infiltrate (score).

IQR = Interquartile range; Different letters indicate a statistically significant difference (Kruskal-Wallis, Dunn’s post-test, p<0.05).

**4.3 Internal resorption**

The comparison revealed a statistically significant difference between the groups (p=0.0200) for the area of internal resorption.

The trauma factor did not result in a statistically significant difference for the internal resorption variable when compared to the groups without trauma. However, the continuous force exhibited larger areas of resorption when compared to the other types of force (p<0.05).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ATCF** | **ATICF** | **ATIF** | **PTCF** | **PTICF** | **PTIF** | **PTWM** | **ATWOM** |
|  | A | BC | BC | AB | AB | C | C | C |
| **Mean** | 477.333 | 146.6667 | 127.3333 | 366.0000 | 273.5000 | 172.8000 | 131.3333 | 0.000 |
| **S.D.** | 241.071 | 130.6624 | 117.7347 | 338.7760 | 186.4068 | 162.3736 | 138.4163 | 0.000 |

**Table 3**. Representative results of mean (+ standard deviation) for internal resorption area (μm²).

SD = Standard deviation; Different letters indicate a statistically significant difference. At least one identical letter indicates statistical similarity (Kruskal-Wallis, Dunn's post-test, p<0.05).

**4.4 Histological alterations**

Regarding the cellular pattern, the presence of inflammatory infiltrate was observed in all groups, with the highest amounts found in the PTCF and PTIF groups. Additionally, an increase in fibrosis was also observed in all groups, except the control group, although in small quantities. On the other hand, a decrease in cellularity was present only in the ATICF, ATIF, PTICF, and PTIF groups (Figure 2).

Dystrophic changes were also present, although in small quantities. The PTWM and ATWOM groups did not exhibit any dystrophic changes. Furthermore, hyalinization and nodules were not observed in any of the groups. Necrosis was only observed in the PTICF group (Figure 2).

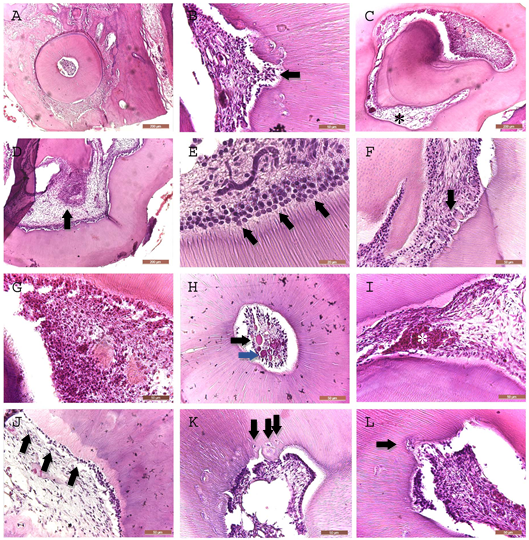
The most frequent event was related to hemodynamic changes. Vascular congestion was found in almost all animals, even in the control group, although in smaller numbers. Additionally, hemorrhage and thrombosis were also observed, with the latter being more prevalent in the groups with trauma and ITM (Figure 2).

Finally, dentin alterations were also observed in all experimental groups, except in the control group. In this comparison, a statistically significant difference was observed between the experimental groups and the control group for the internal resorption and vascular congestion variables, as shown in Table 4. **However, these results should be interpreted with caution, especially in relation to the internal reabsorption variable, since the forces were applied 15 days after the trauma and the results may vary if the trauma was considered chronic.**

**Table 4**. Frequency and inference (Chi-square – p<0.05) of dentinopulpal phenomena observed in the groups.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **PULPAL ALTERATIONS** | **ATCF** | **ATICF** | **ATIF** | **PTCF** | **PTICF** | **PTIF** | **PTWM** | **ATWOM** | ***P value*** |
| **Cellularity pattern** |  |  |  |  |  |  |  |  |  |
| Inflammatory infiltrate | 3/6 | 3/6 | 3/6 | 4/6 | 3/6 | 4/5 | 3/6 | 1/6 | 0.646 |
| Reduced cellularity | 0/6 | 2/6 | 1/6 | 0/6 | 1/6 | 1/5 | 0/6 | 0/6 | 0.426 |
| Increased fibrosis | 1/6 | 2/6 | 2/6 | 2/6 | 3/6 | 3/5 | 1/6 | 0/6 | 0.429 |
| **Dystrophic alterations** |  |  |  |  |  |  |  |  |  |
| Hyalinization | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/5 | 0/6 | 0/6 | 1 |
| Vacuolization | 2/6 | 1/6 | 1/6 | 1/6 | 2/6 | 1/5 | 0/6 | 0/6 | 0.689 |
| Nodules | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/5 | 0/6 | 0/6 | 1 |
| Diffuse calcification | 0/6 | 1/6 | 0/6 | 1/6 | 0/6 | 1/5 | 0/6 | 0/6 | 0.573 |
| Necrosis | 0/6 | 0/6 | 0/6 | 0/6 | 1/6 | 0/5 | 0/6 | 0/6 | 0.431 |
| **Hemodynamic alterations** |  |  |  |  |  |  |  |  |  |
| Vascular congestion | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 5/5 | 5/6 | 3/6 | 0.017 |
| Hemorrhage | 1/6 | 1/6 | 1/6 | 2/6 | 2/6 | 2/5 | 1/6 | 0/6 | 0.778 |
| Thrombosis | 2/6 | 2/6 | 1/6 | 4/6 | 4/6 | 3/5 | 2/6 | 0/6 | 0.178 |
| **Dentin alterations** |  |  |  |  |  |  |  |  |  |
| Reactionary dentin | 2/6 | 2/6 | 3/6 | 2/6 | 2/6 | 2/5 | 1/6 | 0/6 | 0.708 |
| Tubules with nucleuses | 2/6 | 2/6 | 2/6 | 2/6 | 3/6 | 3/5 | 2/6 | 0/6 | 0.625 |
| Internal resorption | 5/6 | 4/6 | 4/6 | 5/6 | 5/6 | 3/5 | 4/6 | 0/6 | 0.058 |

The first number represents in how many rats the alteration was found (n=6). One animal from the PTIF group was lost.



**Figure 2**. Pulpal alterations found in rats subjected to extrusive luxation (LE) for 14 days. A: Pulp with normal appearance; B: Leukocytic inflammatory infiltrate (horizontal arrow); C: Decrease in cellularity (asterisk) found in the coronal region; D: Increase in fibrosis (vertical arrow) in the central region of the pulp; E: Presence of vacuolization (oblique arrows) in the odontoblasts; F: Presence of calcification (vertical arrow) formed by lamellae; G: Pulpal aspect indicative of necrosis with severe inflammatory infiltrate; H: Presence of vascular congestion (black arrow) and thrombosis (blue arrow); I: Extensive hemorrhagic area (white asterisk); J: Formation of reactionary dentin (oblique arrows); K: Formation of reparative dentin with presence of nuclei in the tubules (vertical arrows); L: Presence of internal resorption (horizontal arrow).

**5. DISCUSSION**

In this study, a device was used to induce trauma, which allowed for the standardization of the magnitude and angle of force application. These characteristics were positive and **differentiating for this method**, resulting in similar responses across the entire sample [11,20]. This extrusive luxation method caused a displacement of the dental element in the occlusomesial direction. The effects of the association between extrusive luxation and induced tooth movement were tested and evaluated. Furthermore, in the case of an extrusive trauma, it is recommended to wait 6 months to 1 year before starting orthodontic treatment in humans, **this period aimed periodontal recovery**. In rats, however, the periodontal ligament completes its reorganization in 15 days [9,21,22], which justified the choice of this post-trauma repair period.

The magnitude of the force applied in the IDM experiments on rat molars varies in the literature, ranging from 0.8cN [23] to 100cN [12], with the aim of evaluating the reaction of supporting tissues and the amount of tooth movement**. In this study, a magnitude of 50cN was used and applied through a closed NiTi spring, within the range recommended by specialized literature.** [11,20].

The quantitative analysis of tooth movement in this research followed the method described in the literature by several authors [17,18], through the subtraction of distances from the mesial face of the first upper molar to the distal of the third upper molar on the left (non-moved) and right (moved) sides. When analyzing movement in groups that did not have traumatized teeth, no difference was observed between them. This result supports the findings of Hayashi *et al*., 2004 [24], **who established that the magnitude and duration of the force are important factors in the stimulation of osteoclast recruitment in the periodontal ligament and the initial amount of tooth movement is similar for both continuous and intermittent or interrupted forces.**

Considering the presence of extrusive luxation in the dental element, the PTCF and PTICF groups were similar, but statistically different from the PTIF group. A similar behavior was established by Tondelli *et al*., 2011 [17], evaluating the effect of the same IDM device at different time points. Studies by Zingler *et al*., 2017 showed that biological aspects are closely linked to tooth movement, as the number of molecular factors is massively increased during bone remodeling and influences the amount of movement [25]. This justifies the findings of this study, as prior trauma to the movement would increase the concentration of inflammatory molecular factors, which in turn enhances bone remodeling, influencing the amount of movement.

**Younger dental pulps are larger, displaying a high number of cells with little or no fibrosis. This histological pattern changes over time (natural aging) and with external stimuli such as orthodontic force**, causing the dental pulp to reduce its volume due to the deposition of secondary, reparative, or reactive dentin, which increases fibrosis and cellular density, while reducing blood vessels [8]. This explains the appearance of these alterations in this study. Two factors can explain these findings: the age of the rats (young adults) and the activation regime of the orthodontic springs with two activations of the device (14 days), presenting a greater challenge for pulp repair. Different results were found by Mendonça *et al*., 2018 [6], where the movement period was 9 days (1 activation), and no cellularity alterations were observed. Additionally, nodules and pulp calcifications are also part of the natural "aging" process of the pulp, but they may occur more prematurely due to traumatic processes in dental structure [26], which also justifies the appearance of calcifications and other dystrophic changes in this study.

Neuropeptides, defined as neurotransmitters or neuromodulators, such as calcitonin gene-related peptide (CGRP), can be triggered by caries, trauma, and also by the action of orthodontic forces. Vascular changes in the pulp have been associated with IDM [27-29], and some neuropeptides can induce vasodilation, plasma leakage, activation of the immune system, chemotaxis, and recruitment, as well as the regulation of inflammatory cells [30]. In addition to being involved with hemodynamic changes, CGRP increases the expression of bone morphogenetic protein (BMP) in human pulp cells, stimulating dentin deposition by odontoblasts as a defense mechanism. This event, along with hypoxia, induces degenerative calcification of the dental pulp and can cause obliteration of the root pulp [31-33], which supports this study, as both hemodynamic and dentin changes were found.

**The changes that take longer to be detected, such as dentin deposition, were also found in this study, which agrees with the studies of Aguiar and Arana-Chavez [34], who discovered that after 7 days of mild trauma (extrusion). It was already possible to observe the beginning of the formation of a tubular dentin matrix (reactive dentin) and after 10 days, tubular reactive dentin areas were covered with an original layer of odontoblasts** [34].

The presence of congested thrombotic vessels and some areas of hemorrhage were found in all groups. These events can occur due to changes in the pulpal microcirculation, which increase tissue pressure, leading to the rupture of vessel epithelia and causing hemorrhages [35]. These alterations were described in the studies by Vandevska-Radunovic *et al*., 1994, where the apical neurovascular bundle was evaluated during orthodontic movement, and it was found that there was epithelial disintegration of blood vessels up to the third day, followed by a regenerative process with proliferation of new cellular and vascular elements around the seventh day [36,37]. Furthermore, hemodynamic changes can be triggered by inflammation (irreversible pulpitis) and result in pulp necrosis [7].

Regarding internal resorption, Consolaro *et al*., 2013 [38] stated that only trauma can cause a disorganization of the odontoblastic layer, which activates osteoremodeling units and clasts responsible for this process. **Furthermore, even with high forces, orthodontic treatment is not capable of initiating internal resorption.** However, in this study, internal resorption was identified not only in the trauma groups but also in the groups without trauma.

**In 2003, Weiland [39] found that with the use of super-elastic wires, the possibility of external root resorption is 140% higher compared to steel wires, which corroborates Tondelli study [17],** where continuous forces produced more hyaline areas with a higher potential for generating root resorptions. In our study, continuous force (CF) also showed a higher amount of internal resorption. **The association of these results demonstrates that interrupted continuous force (ICF) provides similar dental displacements to those produced by continuous force (CF), but with a tendency for better biological effects on the pulp**.

Finally, more comprehensive studies are needed to support evidence-based orthodontic treatment, with the aim of consolidating clinical decision-making regarding the ideal type of force for orthodontic movement in teeth with a history of dentoalveolar trauma, without the risk of permanent alterations in the pulp tissues.

**6. CONCLUSION**

Under the conditions of this study, it can be concluded that:

* The amount of dental movement in teeth subjected to extrusive luxation was influenced by the type of force, with greater movement observed in the continuous and interrupted continuous force groups;
* There was a lower amount of inflammatory infiltrate in the control group compared to the other groups;
* The continuous force (CF) showed a greater amount of internal resorption;
* Pulpal alterations were more frequent in relation to hemodynamic alterations, followed by dentin alterations, cellularity, and dystrophic changes.

**ETHICAL APPROVAL**

This study was in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Committee on Ethics in the Use of Animals (CEUA) of UNIOESTE.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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