Original Research
Orthodontics

The influence of fluoxetine on orthodontic tooth movement in rats

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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https://doi.org/10.1590/1807-3107bor-2023.vol37.0007

Submitted: January 19, 2020
Accepted for publication: December 14, 2021
Last revision: February 17, 2022

Abstract: This study aimed to evaluate the effects of chronic use of fluoxetine on the amount of orthodontic tooth movement and tissue changes in rats. A total of 192 Wistar rats were divided into 4 groups: S, 0.9% saline solution; F, 20 mg/kg of fluoxetine; SM, 0.9% saline solution with orthodontic movement; and FM, 20 mg/kg of fluoxetine with orthodontic movement. After 30 days of daily saline or fluoxetine administration, an orthodontic device (25cN) was used to mesially displace the first molar in animals of the groups SM and FM. The animals were euthanized 2, 7, 14, and 28 days after placement of the orthodontic appliances and animals of groups S and F were euthanized at the same time. The assessment of tooth movement was made in gypsum castings, the collagen neoformation was assessed by polarization microscopy, the number of osteoclasts and root resorption were evaluated using tartrate-resistant acid phosphatase, and presence of hyalinized areas was assessed by hematoxylin-eosin staining. Fluoxetine did not affect the amount of tooth displacement, percentage of collagen, number of osteoclasts, and presence of hyalinized areas ($P>0.05$). There was a higher frequency of root resorption areas in the FM group than in the SM group only on the second day ($P<0.05$). The findings of this study show that chronic use of 20 mg/kg fluoxetine does not affect the amount of tooth movement, collagen neoformation, number of osteoclasts, or hyalinized areas and does not affect root resorption until the last day of orthodontic movement.

Keywords: Fluoxetine; Orthodontics; Root Resorption.

Introduction

The antidepressant fluoxetine, which belongs to the selective serotonin reuptake inhibitors (SSRIs) class, became popular in the United States under the name Prozac® and became one of the most consumed drugs as of 2004.[1] SSRIs inhibit the reuptake of serotonin (5-HT) by blocking its transporter (5-HTT), resulting in high systemic levels of serotonin in the interneuronal space, which relieves the symptoms of mental disorders.[2,3]

Previous studies have identified serotonin receptors and 5-HTT in osteocytes, osteoblasts, and osteoclasts,[4,5] which are related to the potential for alveolar bone remodeling during orthodontic tooth movement.[6] The function of these receptors and serotonin transporters in bone cells is

not well established, but it is known to be associated with bone formation and remodeling.[7-9]

In vitro studies using gene microarrays observed that fluoxetine positively affects bone, reducing osteoclast differentiation.[7] Another *in vitro* study using mesenchymal stem cells (MSC) isolated from the iliac crest found that fluoxetine stimulated proliferation of MSC and murine preosteoblasts.[10] However, many *in vivo* studies have indicated adverse effects of SSRIs on bone quality and quantity, leading to higher risk of both fractures and osteoporosis.[3,5,11-14]

There are few studies in the scientific literature that evaluated chronic use of fluoxetine (10 mg/kg/day) and its impact on tooth movement.[15,16] There are no studies evaluating if 20 mg/kg/day (most used dose in humans) fluoxetine affects orthodontic movement,[17] and since orthodontic movement depends of bone remodeling,[18] it becomes important to evaluate this association.

Therefore, this study aimed to evaluate if chronic use of fluoxetine can interfere with orthodontic tooth movement in rats. The null hypothesis to be tested is that there is no difference in the amount of tooth movement, the number of osteoclasts, collagen formation, and the presence of areas of root resorption and hyalinization between the groups with and without fluoxetine.

Methodology

This research was approved by the Ethics Committee on Animal Use (#795). The sample consisted of 192 male Wistar rats (*Rattus norvegicus* albinos) aged 7 to 8 weeks, weighing approximately 170 g, and housed in a vivarium under photoperiod and temperature control. The supply of water and food was ad libitum. The food was supplied in pellets, and after the orthodontic device was installed, it was supplied in a crushed form. In order to adjust the drug dose, the animals were weighed weekly using an electronic precision scale (Gehaka-BG 4001, São Paulo, Brazil).

Animals were randomly divided into 4 groups: S (n = 48) saline solution without orthodontic movement; F (n = 48) fluoxetine without orthodontic movement; SM (n = 48) saline solution with orthodontic movement; and FM (n = 48) fluoxetine with orthodontic movement. The animals in the S and SM groups received 1 mL of 0.9% saline solution intramuscularly (quadriceps) daily and animals in the F and the FM groups received 20 mg/kg fluoxetine diluted in 53.3% propylene glycol, 0.9% sodium chloride, and 0.1% sodium benzoate (University Pharmaceutical Laboratory, Curitiba, Brazil) intramuscularly daily. The dosage and route of fluoxetine administration were chosen according to Mattioli et al.,[17] as they are compatible with the high dosages used in human clinical prescriptions.[19]

Experiment

The solutions were administered daily in all groups for 30 days in order to characterize chronic use. On the 30th day, the animals in the SM and FM groups were sedated with an intraperitoneal injection of 50 mg/kg of tiletamine/zolazepam (Zoletil®; Virbac Brasil Indústria e Comércio Ltda., Jurubatuba, Brazil). An orthodontic device was installed,[20] consisting of a closed-spring nickel-titanium (G&H Wire, Franklin, IN) attached to the first upper right molar with a 0.010" stainless steel ligature wire (Morelli, São Paulo, Brazil) and connected to the upper central incisors, causing the mesial movement of the molar (25 cN),[21] which was standardized with a tensiometer (Haag-Streit AG, Koeniz, Switzerland) (Figure1).[22] To ensure greater spring stability, the lower incisors were worn down and the upper incisors bonded together with the Charisma composite resin (Heraeus, Hanau, Germany). After installation, the spring was not reactivated.

The administration of solutions continued until the animals were euthanized by intraperitoneal injection of 270 mg/kg ketamine and 30 mg/kg xylazine, 2, 7, 14, and 28 days after placement of the devices. The animals in groups S and F, which were not submitted to orthodontic movement, were euthanized at the same time intervals.

Impressions of the upper dental arches of the animals in the SM and FM groups were taken with condensation silicone (Coltoflax–Vigodent; COLTÈNE SA Indústria e Comércio, Rio de Janeiro, Brazil) before installation of the orthodontic devices and after euthanasia. The impressions were poured with orthodontic plaster (PASOM, São Paulo, Brazil). The

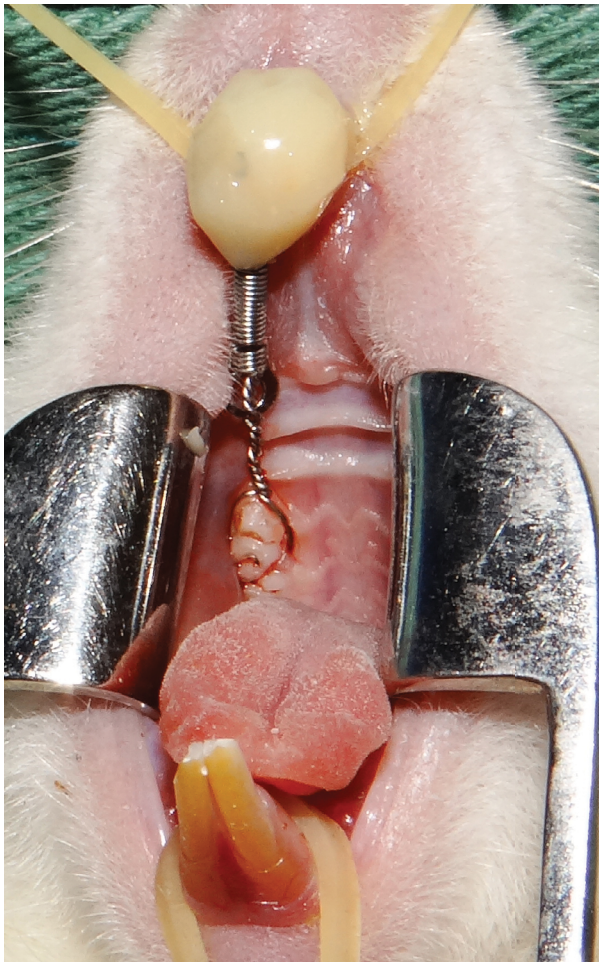


Figure 1. The installed orthodontic device.

amount of tooth movement (final measure minus initial measure) was measured in the gypsum castings using a digital caliper (Absolute; Mitutoyo, Kawasaki-Shi, Japan) from the palatal side of the central incisor to the mesial surface of the first molar of the upper right side.

The right maxillae were dissected and forwarded to the University Experimental Pathology Laboratory for processing of the histological slides. The specimens were fixed in 10% formaldehyde solution for 24 hours and demineralized with 4.13% EDTA (Biotec Reagentes Analíticos, Pinhais, Brazil) for three months and then embedded in paraffin. We obtained 15 cross-sections (5 for evaluation of neoformation of the bone organic matrix, 5 for osteoclast count and evaluation of hyalinized areas, and 5 for evaluation of root resorption) from the cervical third in the apical direction of the mesiobuccal maxillary first molar root, which were cut into 4-µm thick slices, with intervals of 60 µm between each section. The sections were stained using picrosirius red, tartrate-resistant acid phosphatase (TRAP), and hematoxylin-eosin (HE).

Histological analysis

Neoformation of bone organic matrix

Bone neoformation was determined by Picrosirius staining. The organization stage of the bone matrix is indirectly evaluated through the coloration and intensity of the birefringence of the collagen fibers, which reflects the age and diameter of these fibers.[23]

The bone adjacent to the periodontal ligament (PDL) in the distal portion of the mesiobuccal root (tension side) was selected for analysis, because during orthodontic movement, bone is deposited in the alveolar cortex on the tension side.[24] An image of each cross section was captured using an Olympus BX-50 light microscope (Olympus, Tokyo, Japan) and an Olympus U-Pot polarized lens (Olympus) coupled to a Dinolite® AM 423X micro-camera (AmMo Electronics Corporation, New Taipei City, Taiwan) at 200X magnification. The percentage of mature (type I) and immature (type III) collagen areas was calculated through the morphometry program of the Image Pro-Plus 4.5 software (Media Cybernetics Inc., Silver Spring, MD).[25] The average percentage of type I collagen was calculated based on five sections.

Number of osteoclasts

The tartrate-resistant acid phosphatase (TRAP) staining was performed using the TRAP 387A kit (Sigma-Aldrich Co., St. Louis, USA) to identify osteoclasts and cementoclasts. For each of the 5 sections, images from the area around the PDL (tension and pressure sides) of the mesiobuccal root of the first molar were captured, using the same microscope and micro-camera as above. The image acquisition parameters were set during the capture process. For the images with 400x magnification, the osteoclast number count was performed using the Image Pro-Plus 4.5 program, which created a grid for scoring. The osteoclasts were considered

as multinuclear TRAP-positive cells in the PDL adjacent to the alveolar bone.[26] In the images with a magnification of 40x, the area of the PDL was measured using the "create polygon feature" tool to obtain the number of osteoclasts/μm² PDL by averaging the 5 sections.

Root resorption

Evaluation was performed on the entire circumference (tension and pressure sides) of the mesiobuccal root of the first molar with 400x magnification, registering the presence or absence of root craters (cementum or dentin), in which, most of the time, there were cementoclasts in contact with the root.[27] The presence or absence of root resorption was expressed as a percentage.

Hyalinized area

The hyalinized areas were evaluated in HE-stained slides along the PDL (tension and pressure sides) of the mesiobuccal side of the upper first molar root with 40x magnification. Areas of hyalinization were defined as having degenerative changes in the PDL, homogeneous, and free of cells.[28,29] The presence or absence of hyalinized areas was expressed as a percentage.

Statistical analysis

Statistical analysis was performed using the SPSS 22.0 for Windows (SPSS, Inc., Chicago, USA) and Statistica 7 (Statsoft, Inc., Tulsa, USA).

The reproducibility power of the osteoclast count, the measurements of the area of the periodontal ligament, and the hyalinized area were assessed in 36 samples. The maximum Dahlberg error was 5.94% for the osteoclast variable, indicating that the examiner reliably reproduced the measurements.[30,31] The Student *t*-test revealed no systematic error in the counts, at a significance level of 5%.

Data normality and homogeneity were tested with the Kolmogorov-Smirnov test and Levene's test, both with a significance level of 5%.

For continuous variables with normal distribution, or for symmetrical distribution and heterogeneity of variances by group and time, analysis of variance (ANOVA) was applied followed by the Games-Howell multiple comparisons test for heterogeneous variances. For variables with dichotomous categorical scales, analyses of independence were performed using the chi-square test. When there was dependence between the variables, the Z-test for two proportions was applied. The significance level adopted for all tests was 0.05.

A power test was used to calculate the power of the sample size to accept the hypothesis of a difference between the dependent variables, according to time and group.

Results

Tooth movement amount

There was no statistically significant difference ($p > 0.05$) when evaluating the group x time interaction between the SM and FM groups (Table 1). The power of 99.45% was obtained from the test-frame ANOVA.

Neoformation of bone

We found no statistically significant difference in the group x time interaction ($p > 0.05$) (Table 2; Figure 2). The power of the test was 99.99%.

Table 1. Means and standard deviations of tooth movement amount (mm) in saline with tooth movement group (SM) and fluoxetine with tooth movement group (FM).

Comparisons - Games-Howell Test						
Group/	SM		FM		SM X FM	Power test
Time (days)	(n)	(mean ± SD)	(n)	(mean ± SD)	(p)	
2	12	0.36 ± 0.2	10	0.4 ± 0.2	0.9999	0.9945
7	11	0.5 ± 0.29	7	0.85 ± 0.12	0.0526	
14	9	0.6 ± 0.28	7	0.7 ± 0.38	0.9986	
28	9	1.06 ± 0.54	9	0.95 ± 0.59	0.9999	

Table 2. Means and standard deviations of number of osteoclasts/μm² and percentage of type I collagen in the saline (S), fluoxetine (F), saline with tooth movement (SM), and fluoxetine with tooth movement (FM) groups.

Comparisons - Games-Howell Test							
Group/	S	F	SM	FM	S X F	SM X FM	Power test
Variables (days)	(mean ± SD)	(mean ± SD)	(mean ± SD)	(mean ± SD)	(p)	(p)	
% collagen type I							0.9999
2	94.18 ± 2.77	92.79 ± 5.1	64.24 ± 19.07	71.96 ± 14.39	0.9999	0.9986	
7	92.38 ± 3.99	93.53 ± 3.58	98.57 ± 0.37	98.65 ± 0.76	10.000	10.000	
14	95.53 ± 2.5	92.86 ± 3.58	97.91 ± 1.03	97.51 ± 1.97	0.8323	10.000	
28	30.86 ± 11.11	21.6 ± 11.84	20.75 ± 8.69	7.82 ± 5.72	0.8693	0.0922	
N° osteoclasts by μm² of PDL							0.9999
2	0.09 ± 0.04	0.06 ± 0.02	0.17 ± 0.08	0.17 ± 0.06	0.6327	10.000	
7	0.10 ± 0.03	0.10 ± 0.03	0.11 ± 0.03	0.11 ± 0.06	10.000	10.000	
14	0.11 ± 0.04	0.16 ± 0.09	0.11 ± 0.05	0.15 ± 0.05	0.8505	0.9461	
28	0.12 ± 0.05	0.17 ± 0.05	0.07 ± 0.03	0.16 ± 0.08	0.43	0.30	

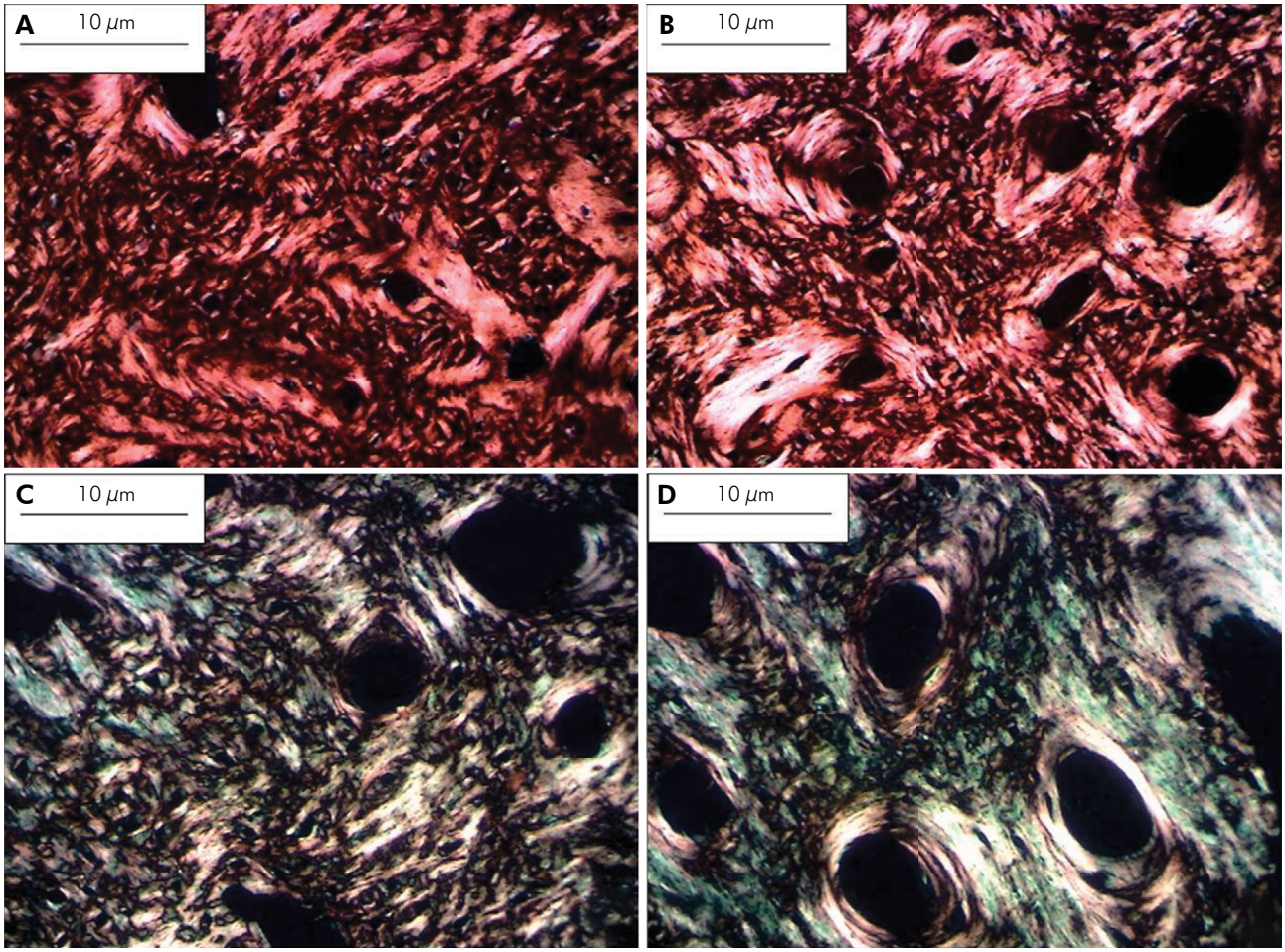


Figure 2. Photomicrographs of the S, F, SM, and FM groups on the 2nd day of orthodontic tooth movement. In groups S (A) and F (B), there is a predominance of type I collagen (mature); in groups SM (C) and FM (D), replacement of collagen type I (mature) for collagen III (immature) is observed (Picrosirius, 200x).

Number of osteoclasts

There was no statistically significant difference ($p > 0.05$) in the group x time interaction (Table 2; Figure 3). The power of the test was 99.99%.

Root resorption

Root resorption was observed in the groups receiving orthodontic movement, except in the SM group on day 2 (Table 3; Figure 4). There was a statistically significant difference between the groups SM and FM on day 2 ($p < 0.05$), with root resorption present only in the FM group. There were no areas of root resorption in the S and F groups.

Hyalinized area

The hyalinized area showed no statistically significant difference for the time x group interaction ($p > 0.05$), and it was present only in the SM and FM groups on days 2 and 7 (Table 3; Figure 5). There were no areas of hyalinization in the S and F groups.

Discussion

In this study, we examined whether the chronic use of fluoxetine in rats could affect induced tooth movement. The null hypothesis tested was accepted, since no differences were seen between the groups with and without fluoxetine at the end of tooth movement.

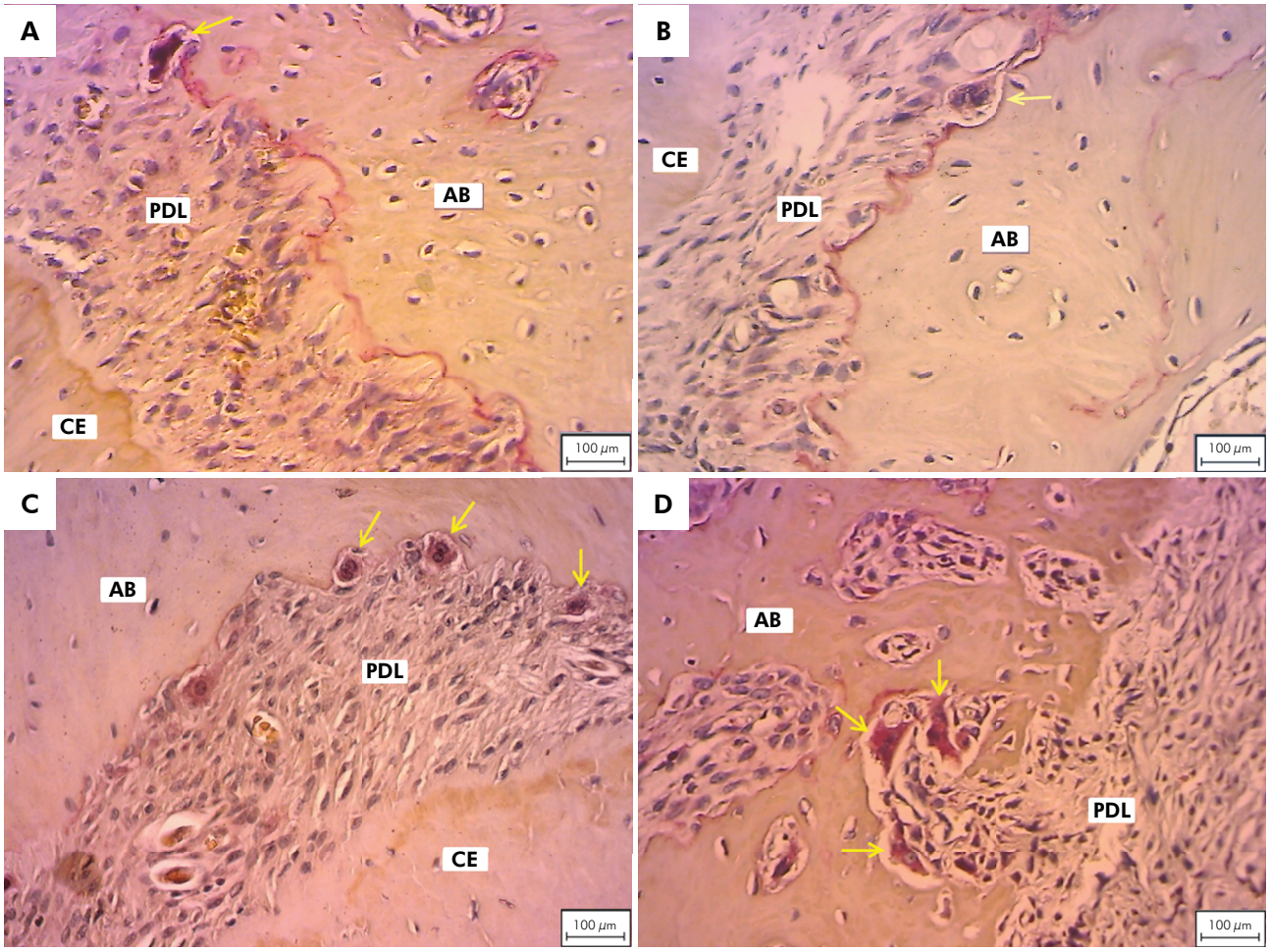
Increasing evidence suggests that daily use of fluoxetine at concentrations ranging from 5 to 20 mg/kg taken orally, intramuscularly, or intraperitoneally, for periods ranging from 4 to 24 weeks, may have an effect on the development of mineralized tissues in rats.[13] The results of the present research (Table 1) showed that the drug used did not affect the amount of tooth movement similarly to the results of Frigotto et al.[15] and Rafiei et al.,[16] in which they used 10 mg/kg/day fluoxetine.

The effect of fluoxetine on bone metabolism was observed by reductions in osteoblastic bone formation and lower bone mineral density.[3,4,12,32] Bonnet et al.[14] observed that doses of 10 mg/kg of fluoxetine decreased the levels of osteocalcin, a marker of bone formation. The present (Table 2) and other studies[11,15,16,33] confirmed that fluoxetine has no effect on the collagen deposition process in bone tissue.

Most *in vivo* evidence indicates that fluoxetine can negatively affect bone quality and quantity by increasing the number of osteoclasts[3,13,14] and decreasing bone mineral density.[3,4,12,13] The adverse effects of this drug are dose-dependent, but the maximum time of application was 4 weeks.[3] In contrast, positive effects of fluoxetine on bone were observed by Battaglino et al.,[34] who found increased trabecular bone volume and bone formation with the chronic use of 10 mg/kg fluoxetine. However, after

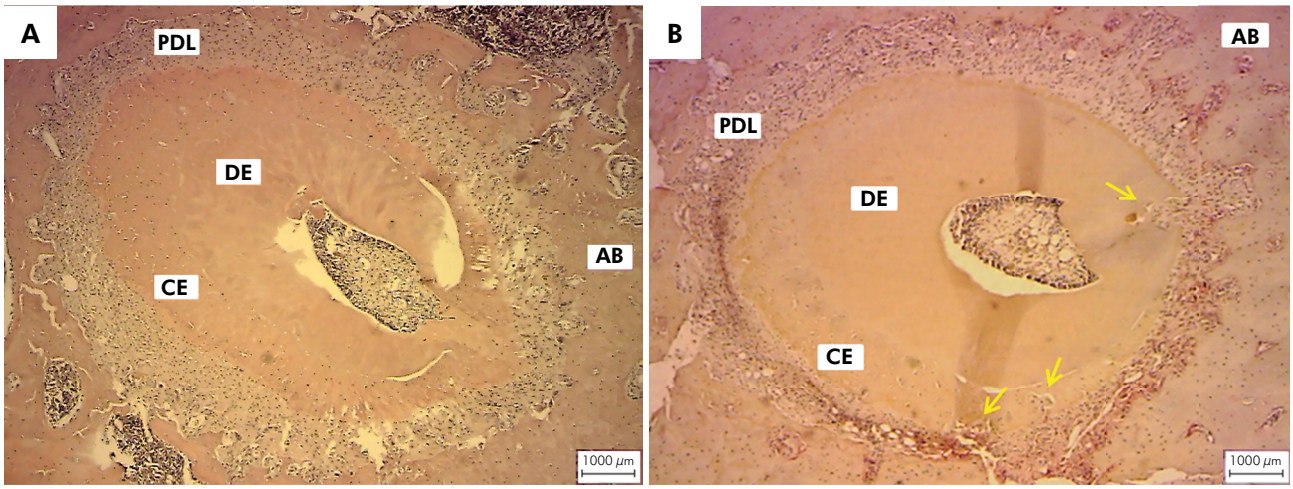
Table 3. Percentage of root resorption and hyalinized areas in saline with tooth movement (SM) and fluoxetine with tooth movement (FM) groups.

Variables	SM		FM		Test for difference between two proportions test
	(n)	(%)	(n)	(%)	SM X FM (p)
Root resorption (days)					
2	12	0.00	10	50.0	0.01
7	11	36.40	7	57.10	0.39
14	9	44.40	7	14.30	0.20
28	9	44.40	9	22.20	0.32
Hyalinized area (days)					
2	12	25.00	10	30.00	0.79
7	11	9.10	7	28.60	0.28
14	9	0.00	7	0.00	1.00
28	9	0.00	9	0.00	1.00



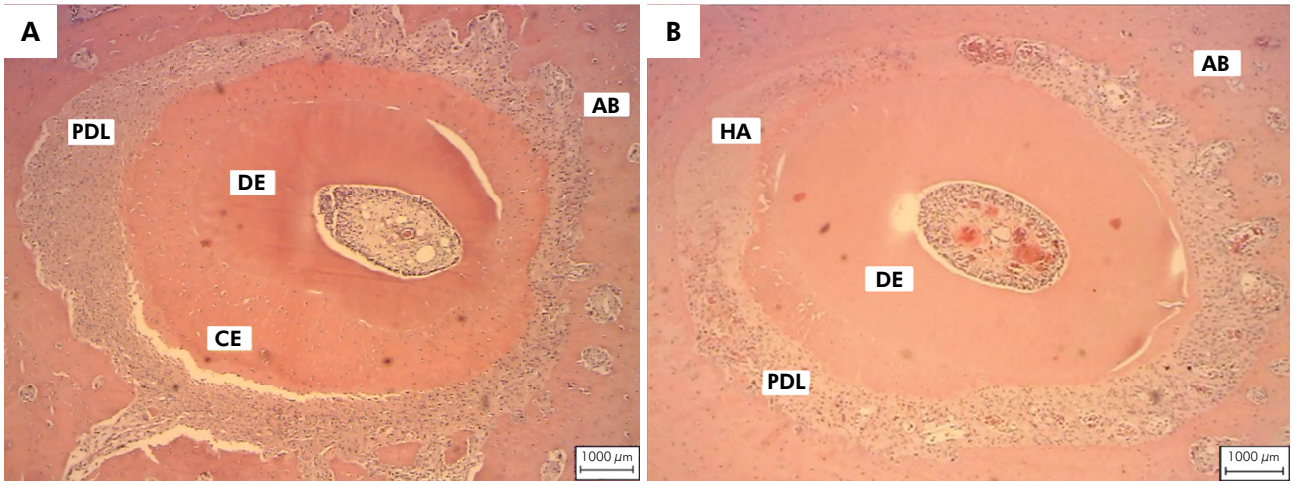
AB: alveolar bone; PDL: periodontal ligament; CE: cement. The yellow arrows indicate osteoclasts. (TRAP, 400x).

Figure 3. Photomicrographs of the S (A), F (B), SM (C), and FM (D) groups on the 28th day of orthodontic tooth movement.



AB: alveolar bone; PDL: periodontal ligament; CE: cement. The yellow arrows indicate osteoclasts. (TRAP, 400x).

Figure 4. Photomicrographs of the SM (A) and FM (B) groups on the 7th day of orthodontic tooth movement.



AB: alveolar bone; PDL: periodontal ligament; CE: cement. The yellow arrows indicate osteoclasts. (TRAP, 400x).

Figure 5. Photomicrographs of the SM (A) and FM (B) groups on the 2nd day.

ovariectomy, fluoxetine did not protect against bone loss. Mortazavi et al.,[35] using 15 mg/kg fluoxetine verified increased trabecular bone volume in rat skulls. Branco-de-Almeida et al.,[36] by inducing periodontal disease in rats treated with fluoxetine 20 mg/kg, observed that fluoxetine suppressed pro-inflammatory responses and protected against bone resorption.

The administration of 20 mg/kg fluoxetine did not influence the process of bone resorption (Table 2), suggesting that fluoxetine had no effect on osteoclast activity, in accordance with the findings of Westbroek et al.[11] In a study associating 10 mg/kg fluoxetine and orthodontic movement, Frigotto et al.[15] observed no significant changes in the bone resorption process and found no differences in the microarchitecture of trabecular bone. So many conflicting results may be explained in part by other mechanisms of blocking 5-HTT,[35-37] high accumulation of fluoxetine in the bone marrow, fluoxetine interference in long-term genetic differences, and the type of bone studied.[13]

The presence of hyalinized areas was observed on days 2 and 7 after induction of orthodontic movement, independent of the administered solution (Table 3). This is similar to the findings of Hamaya et al.,[38] who evaluated orthodontic tooth movement without medication, and also found that hyalinized areas decreased after 7 days of onset of orthodontic movement. Usually, hyalinized areas occur within the first two days after the onset of orthodontic movement, and since the force applied is not reactivated, the tissue tends to repair so that the hyalinized areas decrease after day 7.[38]

Root resorption can be associated with the application of heavy orthodontic forces.[28] It was observed in the SM and the FM groups at all time-points, except on day 2, when only the FM group showed root resorption (Table 3), perhaps due to the association of fluoxetine with the peak of clastic cells that occurs at the very beginning of the tooth movement process. However, in all the other times, all groups presented root resorption, and therefore it is suggested that fluoxetine did not affect root resorption until the last day of tooth movement.[18] Rafiei et al.[16] also did not observe difference in the external root resorption areas when they administered 10 mg/kg fluoxetine for five days a week and applied an orthodontic force of 50 g/f.

The results of our study show that 20 mg/kg fluoxetine does not interfere with tooth movement. Differences in dose, drug administration time, lineage, age, weight, genetic factors, and methodology may justify the different results seen in the literature. In the experimental model and methodology used in our investigation, tissue changes were similar in the groups with and without fluoxetine, suggesting that the chronic use of this medication is safe during tooth movement in Wistar rats. However, caution should be exercised in the orthodontic treatment of patients

until further is conducted research in animals and humans to understand the exact mechanisms of action of serotonin and SSRIs, as they may have antagonistic effects and seem to act through different pathways.

Conclusion

Chronic use of 20 mg/kg fluoxetine had no effect on the amount of tooth movement, collagen neoformation, number of osteoclasts, and presence of hyalinized areas. Additionally, the administration of fluoxetine did not affect root resorption until the last day of tooth movement.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001.

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