Influence of Experimental Periodontitis on Periodontal Tissues and Penis of Rats

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Abstract

This study aimed to investigate the effect of experimental periodontitis on rat penis. Eighteen Wistar rats underwent the placement of a cotton ligature around the first molars to induce periodontitis. It was confirmed that periodontitis was induced in the group with ligature due to the greater number of osteoclasts, decreased osteocytes, greater distance from the cementoenamel junction to the alveolar crest, increase in gingival swelling ($p < 0.05$) and intense bone resorption. Furthermore, there was a higher average concentration of IL-6 in the gingival samples and of TNF- α in the penile samples of the rats with ligature ($p < 0.05$). There was a statistically significant thicker and smaller area of the dorsal penile arteries in the experimental group, as well as a smaller area of the vascular spaces of the corpora cavernosa ($p<0.05$). Therefore, the systemic inflammation caused by periodontal disease can be an important risk factor for erectile dysfunction.

Keywords: Periodontal disease; erectile dysfunction; endothelial dysfunction

Introduction

Periodontitis is a chronic infectious disease that is initiated by microbial bio film, which causes an immune inflammatory process in periodontal tissues, leading to their destruction and in some cases the consequent loss of teeth. Several clinical and epidemiological studies have suggested that periodontal disease is associated with increased risk of cardiovascular pathologies (Friedewald et al., 2009; Kebschull et al., 2010; Scheinken & Loss, 2013; Tonetti & Van Dyke, 2013). According to Janket et al. (2003) this risk is 19% higher in an individual presenting periodontal disease compared to a healthy person and it is most common in those under the age of 65, in which the increase reached 44% (Jamket et al., 2003).

Proinflammatory mediators, such as interleukin-1beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), which are produced locally in inflamed gingival tissues, can dissipate to the circulation, and the result of this spread of bacteria and inflammatory mediators is the promotion of systemic impact, such as the induction of endothelial dysfunction, which is usually accompanied by impaired production of nitric oxide (NO) through endothelial nitric oxide synthase (eNOS) (Amar et al., 2003; Elter et al., 2006). Consequently, systemic inflammatory status, oxidative stress and endothelial cell injury are common pathophysiological factors among patients with periodontitis and cardiovascular diseases (Friedewald et al., 2009; Kebschull et al., 2010; Scheinken & Loss, 2013; Tonetti & Van Dyke, 2013).

Furthermore, there is a co-occurrence of cardiovascular disease and erectile dysfunction. Recently, dysfunctional erection has been recognized as a biological and physiological abnormality that affects the penile circulation as part of a generalized vascular disease (Johannes et al., 2000). Therefore, impaired erectile function and coronary atherosclerosis are closely linked, as both are consequences of endothelial dysfunction, leading to restrictions in blood flow (Chiurlia et al., 2005; Vlachopoulos et al., 2007). Based on the above information, i.e. that cardiovascular disease has path physiological relationships with periodontitis and erectile dysfunction, it is suggested that periodontal disease may be correlated with erectile difficulty. The evidence that the underlying pathogenic mechanisms of endothelial dysfunction in penile corpora cavernosa seem to contribute to the onset and progression of erectile dysfunction, and that chronic periodontitis may contribute to the development of endothelial disorder; provide evidence that this correlation is possible (Zadik et al., 2009).

Consequently, it is reasonable to infer that the systemic inflammation induced by periodontal pathogens may be associated with endothelial dysfunction and atherosclerosis, firstly in the small blood vessels, such as the vascular in the penis, and later in major arteries such as coronaries (zadik et al., 2009). Therefore, the present study aimed to investigate the effect of experimental periodontitis on periodontal tissues and penis of rats.

2. Methodology

2.1 Animals

Pregnant rats were obtained from the Central Animal Facility of the State University of West Paraná (UNIOESTE), Cascavel campus, and kept in the vivarium of the Laboratory of Endocrine Physiology and Metabolism under controlled conditions of temperature (23 ± 2 °C) and light (12-hour cycle of light and 12 hours of darkness - 07:00 to 19:00). At birth, the pups were separated by sex, with only 9 males for each litter, making a total of 18 animals, which were divided into 2 groups and all the animals received standard diet and water at will throughout the experimental period. The sample was based on previous studies as the research group, and 9 rats were included in each group (Nassar et al., 2009). All the experimental protocols were approved by the Ethics Committee on Animal Experiments and Practical Classes (CEEAAP) of UNIOESTE

2.2 Induction of Periodontal Disease

At 70 days of age, the animals were anesthetized (xylazine 0.04 mL/100 g and ketamine 0.08 mL/100 g), and placed on an appropriate operating table, which permitted the opening of the mouths of the rats, facilitating access to the posterior mandible teeth. With the aid of modified forceps and an exploratory probe, a number 40 cotton thread was tied around the first right and left lower molars. Two groups were then designated: the first being control without ligature (CON) and the second being experimental with ligature (LIG). This ligature acted as a gingival irritant for 30 days, which favored the accumulation of bacterial plaque and the subsequent development of periodontal disease (Nassar et al., 2009) .

2.3 Sacrifice and samples obtained for immunologic and morphological analysis

Thirty days after the induction of periodontal disease, the rats in the control and experimental groups were fasted for eight hours with only unlimited water. They were then desensitized with carbon dioxide and when it was observed that they were sedated they were decapitated. In the 18 hemimandibles of the left antimere, the gengiva that was firmly attached to the alveolar process was removed. Samples were also collected from the glans of the penis of the rats from each group and all of these tissues were stored in eppendorfs in a freezer at -80ºC to analyze the interleukin-6 and tumor necrosis factor-alpha. After this, the left hemimandible was completely dissected and fixed in 10% formalin solution for subsequent radiographic examination. The 18 hemimandibles of the right antimere, as well as the penis of the rats from each group were collected, reduced and fixed in methacarn (70% methanol, 20% chloroform and 10% glacial acetic acid) for 24 hours; they were subsequently immersed in 10% formalin solution.

2.4. Histological processing

The hemimandibles of the right antimere and the penis were decalcified by immersion in 5% trichloroacetic acid (TCA) at 4 °C for 27 and 10 days, respectively. The samples were evaluated on a daily basis in order to verify the expected degree of decalcification, and the performing TCA was changed every 5 days. After decalcification, the tissues were immersed in 5% sodium sulfate for 2 hours to neutralize the TCA, washed in tap water (for 2 hours) and stored in 70% alcohol until routine histological processing for paraffin embedding. In the latter process, the tissues were dehydrated in ascending alcohol series, diaphanized in xylene and embedded in paraffin (Purified Paraffin, code 1228, lot 1008459, Vetec Química Fina, Rio de Janeiro, Brazil). Then, histological sections of 5 µm thickness, which were obtained using a manual microtome (Olympus, CUT 4055), were stained using the hematoxylin and eosin (HE) technique.

2.5. Microscopic observations

The microscopic analyses were performed by a single examiner, light microscope (Leica Microsystems®, Switzerland), through the evaluation of the stained histological sections. The slides were examined for the morphological features of the gingiva and alveolar process of the hemimandibles, such as epithelial integrity, inflammatory infiltration, and characteristics of the bone tissue. The overall morphological characteristics of the rat penises from all groups were analyzed in the same manner.

2.6. Histomorphometry of gingiva

The histomorphometric measurements were performed on the marginal vestibular gingiva and the right buccal gingiva in all the groups using an image analysis program (LAS V.4.2 – Leica Microsystems® software) coupled to a light microscope with 40x magnification, at intervals of 10 cuts between one count and the next in a series of cuts (approximately 50 µm). Measurements were taken from predetermined morphological points on marginal gingiva, as illustrated in Figure 1. The results were expressed in mm.

2.7. Bone histomorphometry

The quantification of osteoblasts, osteocytes and osteoclasts present in five consecutive fields of the alveolar bone from the highest point of the bone crest was performed. For this observation we used the 400 times magnification in the light microscope (Leica Microsystems®, Switzerland). There were two observations per field, and then the average value for each rat and for each group was calculated. The measurement of the alveolar bone crest was conducted through a microscope (Leica Microsystems®, Switzerland) coupled to a computer, which made it possible to capture images using LAS V.4.2® software. Measurements were made of the shortest distance between the apex of the buccal bone crest and the cementoenamel junction. The measurements were repeated twice a day, on three different days, and then the average of the values was calculated.

2.8. Radiographic analysis

The hemimandible from the left side of each animal was fixed in buffered formaldehyde (pH 7.2) for 48 hours and was then subjected to radiographic analysis. This entailed placing the hemimandibles with the lingual side on periapical X-ray film (AGFA DENTUS®, Ultraspeed) positioned so that the buccal and lingual cusps of the first molars stayed in the same vertical plane. A GE – 1000 X-ray machine was used, set to 15mA, 65Vp, 18 pulses, focus/film distance of 50 cm with perpendicular incidence of X-rays to the pieces. For the processing of the films Kodak® developer and fixer was used in the respective processing of time/temperature and the images were scanned through a scanner for slides (Polaroid Sprint Scan 35 Plus, Polaroid). The scanned images were analyzed in three measures using the Image Tools 3.0 program and an average was taken from them by linear measure, which covered the distance from the cementoenamel junction to the alveolar bone crest of the mesial side of the first lower left molar of the rat, with the measurements in pixels (Nassar et al., 2014).

2.9. Analysis of expression of IL-6 and TNF-α

The gingiva around the first lower molar on the left side of the hemimandible, subjected or not to the placement of ligation, was analyzed by the Enzyme-Linked Immunosorbent Assay (ELISA) for the presence of IL-6 cytokines. Likewise, portions of glans from the rat penis from all the groups, which were obtained at the time of sacrifice, were analyzed by ELISA for the presence of the TNF-α cytokine. The total protein was extracted from the samples of gingival tissue and from the rat penile tissue using an extraction buffer containing a detergent base of a protease inhibitor cocktail. Subsequently, the proteins were quantified using the Bradford method.

2.10. Histomorphometric analyses of penis

The histomorphometric analyses were performed on cross sections (5 µm) to obtain the ventral-dorsal (VD) and lateral-lateral (LL) dimensions at 40x magnification. The area of the vascular spaces of the corpora cavernosa was also measured, as well as the luminal area and the wall thickness of the right dorsal artery of the penis at 100x magnification. The analyses were performed using a microscope (Leica Microsystems®, Switzerland) coupled to a computer, which made it possible to take the pictures and make the measurements using LAS V.4.2® software (Figure 2). For the statistical analysis, all the numerical values were expressed as mean ± standard deviation. After checking the normal distribution of the data through the Bioestat 5.3 program (Mamiraua Institute, Amazonas, Brazil) Student's t test was performed with $p < 0.05$ to evaluate the difference between the groups.

3. Results

3.1. Morphological descriptive analysis

3.1.1. Hemi mandible

Figure 3A is representative of the control group. It was observed that the gingival tissues, the sulcular epithelium, the junctional epithelium and the underlying connective tissue in the region of the first molar showed normal characteristics. No inflammation was observed in the tissues. The alveolar bone was integral, compact and regular, with the central spongy portion of normal appearance. The alveolar bone crest showed average thickness and height to the level of the third cervical root. Moreover, no significant osteoclast activity was observed. The appearance of incremental lines, the organization of some Haversian canals, and the presence of osteoblasts aligned adjacent to the bone crest was observed, indicating bone formation activity in all the groups. It was also found that the cementoenamel junction and the periodontal ligament had normal characteristics. In some cases it was observed that the cement was thickened in the apical third, suggesting local hypercementosis.

Figure 3B represents the group with induced periodontitis. The junctional epithelium presented an apical migration and a discrete inflammatory infiltrate in the connective tissue; intense bone resorption was also observed. Thus, the alveolar process was compact, with a spongy central area; however, there were conical and low bone crests so that there was exposure of the cervical third. Expressive osteoclastic activity was observed in these areas, as shown in Figure 4. Bone neoformation was found, shown by incremental lines in the region of the periodontal ligament adjacent to the alveolar bone in some regions. Furthermore, it was found that the cement was thickened in the apical third, suggesting local hypercementosis.

3.1.2. Penis

Figure 2 is representative of the general organization, in cross section, of the rat penis. In the control group, there were three cylindrical and erectile bodies (a pair of corpora cavernosa and the spongy body). The corpora cavernosa consisted of numerous vascular spaces (cavernous sinuses), separated by trabeculae, lined by endothelial cells and smooth muscle cells that were restricted to the subendothelial area. It was found that the trabecular of the corpora cavernosa consisted of connective tissue containing dispersed fibroblasts and a dense extracellular matrix that contained mainly fibrillar proteins such as collagen and elastic. The spongy body was housed inside the urethra. All these structures were surrounded by a fibrous connective tissue layer, the tunica albuginea. The deep dorsal vein and dorsal artery, as well as branches of the dorsal nerve of the penis were also observed. Figure 5B shows the cross section of an animal from the experimental group. It was observed that the trabecular were organized typically but they were apparently larger, and the presence of narrower vascular spaces compared to those of the control animals was verified (Figure 5A).

3.2. Histomorphometry of gingiva

Table 1 show that the height of the crest of the gingival epithelium was significantly higher in the experimental group, by about 26%. Likewise, there was a significant difference in the width of the buccal epithelium, which for the group with induced periodontitis was 25% higher. The height of the connective tissue in the middle region in the rats with ligation showed an increase of 72% compared to the control group, with statistically significant differences. Similarly, the experimental group showed an increase of 82% in the width of the connective tissue in the basal region, compared with the control group.

3.3. Bone histomorphometry

Table 2 shows that the mandibular alveolar bone surrounding the right first molar showed more statistically significant osteoclasts in the experimental group. The number of osteocytes was significantly higher in the control group. As regards osteoblast counts, significant differences were not observed between the groups.

3.4. Histomorphometric and radiographic analysis of the distance between the apex of the buccal bone crest and the cement enamel junction

Table 3 shows that the histomorphometric analysis revealed that the experimental group showed an increase in this distance of 75% compared with the control group, indicating that periodontitis caused a greater alveolar bone loss. The radiographic analysis of the first lower molar showed that the experimental group had significantly higher alveolar bone loss than the control group ($p < 0.05$), indicating the action of induced periodontitis on the alveolar bone tissue.

3.5. Analysis of the presence of IL-6 cytokine in the gingival samples and the cytokine TNF-α in penis samples

Table 4 shows that there was a higher average concentration of IL-6 in the experimental group, i.e. there was a significant increase of this cytokine in the rats with induced periodontitis, proving its higher production when in the presence of a chronic inflammation. The results also showed a statistically significant increased concentration of TNF-α cytokine in the penile samples from rats with induced periodontitis compared with the control group.

3.6. Analysis of the dimensions of the dorsal penile artery and area of vascular spaces of the corpora cavernosa

The results in Table 5 show that there was no statistically significant difference in terms of both the ventral-dorsal and the lateral-lateral dimensions between the groups. Table 5 also shows that the experimental group presented a higher wall thickness and smaller area of the dorsal penile artery compared with the control group. As for the area of the vascular spaces, this was significantly higher in the control group compared to the experimental group.

4. Discussion

Periodontal disease is an inflammatory condition caused by bacterial biofilm, which produces infection, the destruction of periodontal supporting tissue, and subsequent loss of teeth (Piconi et al., 2009). Periodontitis is one of the most common chronic inflammatory diseases in the world. The prevalence of mild and moderate forms ranges from 20 to 50%, reaching 85% in the elderly population (Balan, 2010). Serious forms of periodontitis affect 30% of adults over the age of 50 (Paizan & Martim, 2009; Ramirez et al., 2011).

Periodontal infections can promote a negative effect on the systemic health of the body. Periodontitis is associated with systemic diseases, such as coronary heart disease, cerebrovascular disease, diabetes, chronic obstructive pulmonary disease, and endothelial dysfunction, the latter being the pathophysiological link between periodontal disease and erectile dysfunction (Aversa et al., 2010; Ceriello et al., 2002; Gulati et al., 2013).

In the present study, rats with ligature showed typical features of periodontitis, as can be seen by the larger number of osteoclasts and a decrease in the number of osteocytes compared with the control group (Table 2). According to Spolidorio et al. (2007) increased numbers of osteoclasts are associated with bone resorption and according to Kurikchy et al. (2013) an increased amount of osteocytes indicates maturity. Thus, we would suggest that the placement of the ligature produced alveolar bone loss. In addition, there is a positive relationship between the number of active osteoclasts in the bone surface and the distance from the cementoenamel junction to the alveolar bone crest. This can be used as a starting point for diagnosing the alveolar bone height, because by measuring the distance from the cementoenamel junction to the alveolar bone crest it is possible to estimate whether there is loss or not (Kurikchy et al., 2013). The measurement of this distance was significantly greater in the experimental group, so it is clear that alveolar bone loss was induced by the use of the ligature. The results of the radiographic analysis of the height of the cementoenamel junction to the alveolar bone crest strengthened this hypothesis, since this measurement was also higher in this group (Table 3).

The descriptive microscopic evaluation of histological sections from the group with periodontitis revealed changes in the junctional epithelium and osteoclastic resorption activity, i.e. morphological features which are typical of periodontitis. These results were in accordance with the results shown in the alveolar bone histomorphometric analysis (Table 2).

In the experimental group, the height and gingival thickness showed greater statistical significance, thereby demonstrating not only histopathological changes, but also an increase in gingival volume, which is typical in the presence of periodontitis, due to the existence of bacterial biofilm (Table 1). Based on these results, it is possible to say that the model of this study complied with the experimental requirements of periodontitis and that it was possible to investigate its effect on penile structures.

Several mechanisms may be involved in penile erection, which is determined by pressure changes in the cavernous sinuses. In the absence of excitation stimuli, cavernous vasoconstriction maintains the penis in the non-erect state. The contraction of the smooth cavernosal muscle, mainly in response to norepinephrine which is released from sympathetic nerve endings, closes the arteriolar lumen and the sinusoidal cavities, restricting the flow of blood, maintaining low intracavernous pressure and a penis without erection (flaccid). During sexual arousal or nocturnal tumescence the release of nitric oxide (NO) occurs, predominantly via the activation of the enzyme neuronal nitric oxide synthase (nNOS) in non-adrenergic non-cholinergic nerves, and endothelial nitric oxide synthase (eNOS) in local endothelial cells. Nitric oxide stimulates the enzyme guanylate cyclase, which then converts the GTP to cGMP, inducing a substantial increase of this within the cell. This causes the relaxation of the smooth muscle, which results in dilation of the arterioles and cavernous sinuses, thus causing increased blood flow (which is driven by the force of mean arterial pressure) and a subsequent increase in intracavernous pressure. Because of the increased pressure, the erectile response expands the tunica albuginea, resulting in an increase in the length and diameter of the penis, which is characteristic of erection (Andersson & Stief, 1997; Andersson, 2001; Burnett, 1995). Erection essentially depends on the production of NO by the vascular endothelium, and for this to happen it is essential to have normal endothelial function. Endothelial dysfunction is defined as a change in vascular relaxation due to a decrease in the bioavailability of the relaxing factors derived from the endothelium, principally NO (Gimbrone et al., 1995; Ross, 1993).

Chronic periodontitis is associated with an increase in reactive oxygen species, a process referred to as oxidative stress (Dijhorst-Oei et al., 1999; Higashi et al., 2002). Excess production of these species leads to an increased inactivation of NO, and the damage to the antioxidant system may contribute to endothelial dysfunction in patients with periodontitis (Higashi et al., 2008). In addition, raised concentrations of inflammatory mediators such as TNF-α, IL-6, interleukin-8 (IL-8) and interleukin-18 (IL-18) may be associated with an increased risk of endothelial dysfunction (Eaton et al., 2007; Vlachopoulos et al., 2006), since these cytokines reduce the bioavailability of NO in endothelial cells and prevent endothelium-dependent vasodilation.

The results of this study showed that the concentration of the cytokine IL-6 in the gingival samples from the experimental group was significantly increased when compared with the control group (Table 4). Another study that also showed a higher concentration of inflammatory markers in animals with chronic periodontal disease was conducted by Zuo et al. (2011), who showed that serum concentrations of CRP and TNF-α were higher in the group with periodontitis. In the present study, the experimental group also showed increased TNF-α expression in the penile samples, suggesting that these increased concentrations could contribute to endothelial dysfunction (Table 4).

Thus, periodontal disease can directly or indirectly inhibit the expression and activity of eNOS and the production of cytokines, such as TNF-α and IL-6. This was confirmed by a study by Zuo et al.(2011) in which experimentally induced periodontitis resulted in impaired penile erection and decreased eNOS protein expression in the corpus cavernosum of rats. Similarly, a study by Andrukhov et al. (2013) demonstrated that patients with periodontitis had significantly lower concentrations of NO metabolites in comparison with the control group and concluded that the production of NO was reduced in patients with periodontitis, especially in the male population. The authors (Andrukhov et al., 2013) suggested that this decrease in NO in males could explain the association between periodontitis and erectile dysfunction. Based on these studies (Andrukhov et al.,2013; Zuo et al., 2011) we would propose the hypothesis that periodontitis, which is known to induce endothelial dysfunction, causes erectile dysfunction due to a decrease in the activity of nitric oxide synthase in the corpus cavernosum, resulting in the reduction of NO production and subsequent reduction of cGMP activity, thereby inhibiting the endothelium-dependent relaxation of the smooth muscle of the corpus cavernosum.

This study also evaluated the effect of periodontitis on the structure of the penis. The penile erection is a neurovascular phenomenon involving increased arterial inflow, the relaxation of the smooth subendothelial muscle, and decreased shedding of blood in the vascular spaces (veno-occlusion of the cavernous spaces).

Any change in these hemodynamic components, or in the interaction of the nervous system, can cause erectile dysfunction (Wespes, 2002). In the morphological analysis, it can be seen that the most marked structural change in the experimental group was vascular, with wall thickening and a concomitant decrease in the lumen area of the dorsal arteries of the penis (Table 5). Therefore, it is suggested that there may have been an increase in the resistance to the outflow of arterial blood, causing local tissue ischemia and fibrosis and affecting erectile function by interfering with veno-occlusion. Villalba et al.(2009) observed structural and functional changes in the penile arteries of obese Zucker rats, including the thickening of the tunica media and decreased diameter of the penile arteries, suggesting internal vascular remodeling; these animals also showed functional abnormalities such as abnormal vascular reactivity and endothelial dysfunction. According to these authors (Villalba et al., 2009), structural changes in penile arteries may explain the reported erectile dysfunction in obese Zucker rats.

Moreover, the morphological descriptive analysis of the penis in the experimental group of this study showed that the trabecular of the corpus cavernosum were apparently larger and with narrower vascular spaces (Table 5), which allowed us to infer that these spaces were unable to retain the blood, with a consequent reduction of the influx that is essential to increase the pressure within the penis and cause erection. According to Jiang et al. (Jiang et al., 2005), hyperplasia of the interstitial collagen fiber of trabeculae may result in reduced cell to cell contact. The intercellular communication through this contact is the main modulator of the tone of smooth muscles of the corpora cavernosa. Its decrease may restrict the release of neurotransmitters that participate in the modulation of smooth muscle tone to cause sinusoidal relaxation and the development of erection. Thus, it is proposed that the animals in the experimental group of this study may have presented physiological changes in the erection mechanisms due to a possible increase in the extracellular matrix of the trabeculae of the corpora cavernosa (Figure 5B).

5. Conclusion

The results of this study provided evidence that periodontal disease may be associated with erectile dysfunction. First, because the experimental group showed higher concentrations of IL-6 in the gingiva and TNF-α in the penis, and because high concentrations of these inflammatory mediators can induce endothelial dysfunction and impair erection. Second, this study showed significant histopathological changes in the penis of rats with experimentally induced periodontitis, such as functional and structural changes in the penile arteries, which may have been caused by chronic endothelial dysfunction promoted by periodontal disease, and a smaller area of the vascular spaces in the group with ligation, suggesting decreased intra-cavernous pressure, which is essential for erection of the penis. Given the impact that periodontal disease can cause in both the erectile function and the structure of the penis, prevention of this disease can be effective in reducing the incidence of erectile dysfunction.

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7. Competing Interests

The authors declare that there are no conflicts of interest in this study.

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Figure Legends

C - Height of the epithelium of the gingival crest
E - Width of the buccal epithelium
H - Height of connective tissue in the middle region
L - Width of connective tissue in the basal region

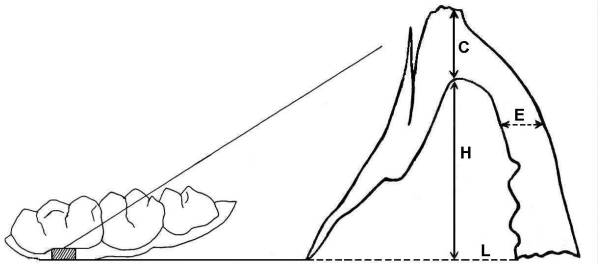

Figure 1: Scheme of marginal gingiva of rat, showing the reference points used for morphometric measurements of the oral epithelium, epithelial crest and connective tissue.

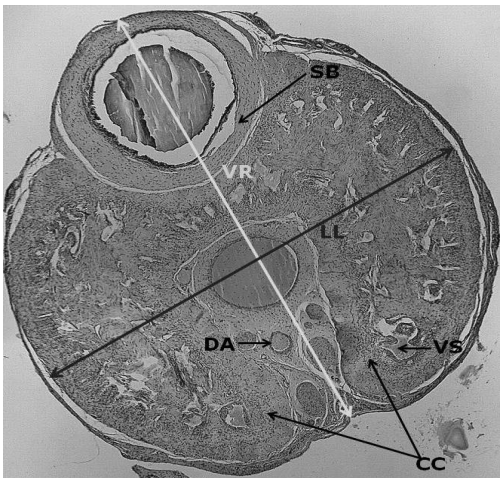

Figure 2: Representative photomicrograph of the general organization and analysis of dimensions in cross-section, of rat penis. The following are shown: VR, ventral-dorsal dimension; LL, lateral-to-lateral dimension; VS, vascular space; DA, dorsal artery of the penis; SB, spongy body; CC, corpus cavernosum. Hematoxylin and eosin, 40x.

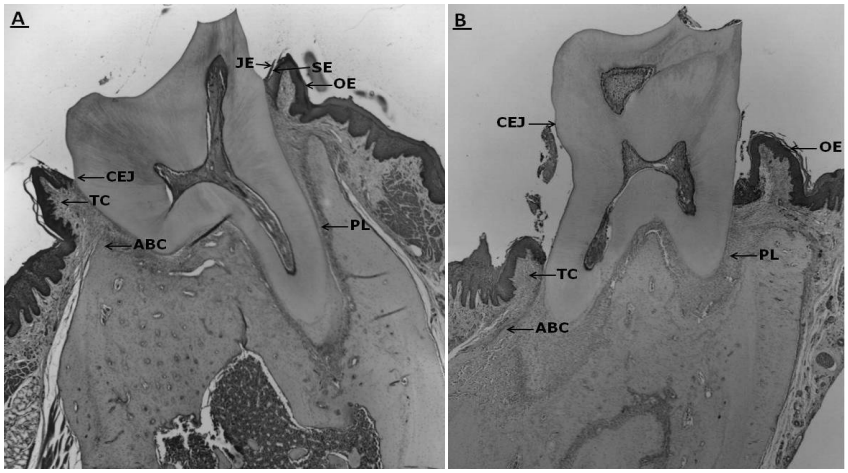

Figure 3: Representative photomicrographs of sagittal section of the mandibular right first molar for a rat from the control group (A) and a rat from the experimental group (B). The following are shown: JE, junctional epithelium; SE sulcular epithelium; OE, oral epithelium; PL, periodontal ligament; CEJ, cementoenamel junction; TC, connective tissue; ABC, alveolar bone crest. Hematoxylin and eosin, 40x.

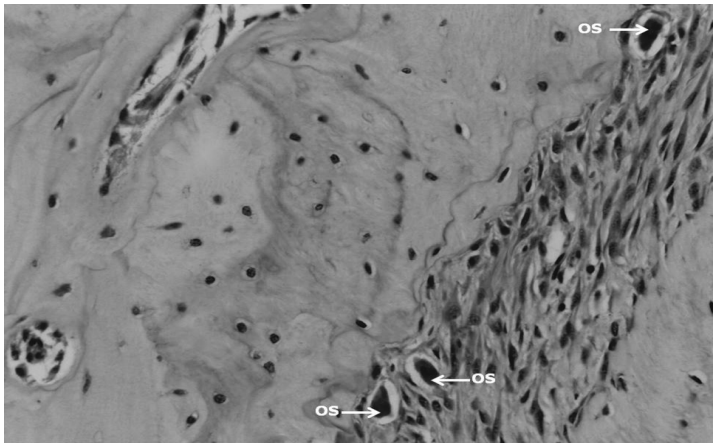

Figure 4 Representative photomicrograph of evidence of bone resorption of the mandibular alveolar bone surrounding the first right molar of a rat from the experimental group. The following is shown: OS, osteoclast. Hematoxylin and eosin, 400x.

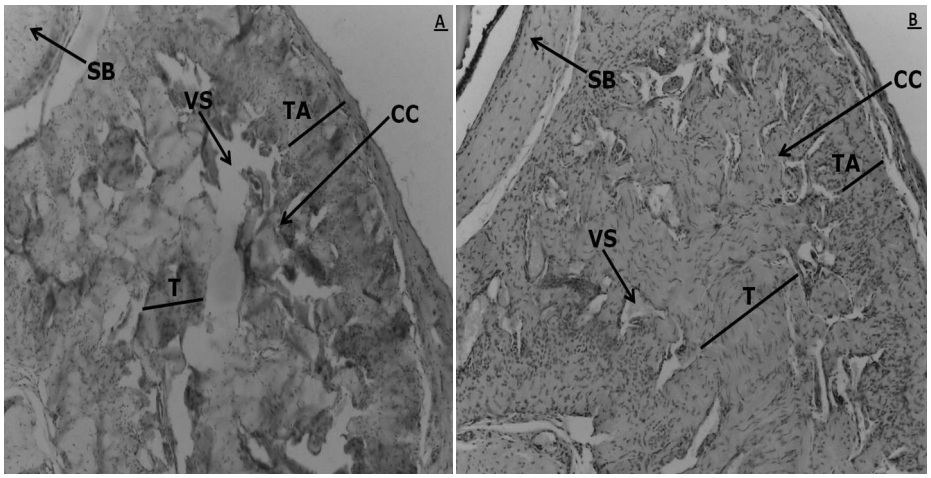

Figure 5: Representative photomicrographs of cross section of the penis of a rat from the control group (A) and a rat from the experimental group (B). The following are shown: SB, spongy body; CC, corpus cavernosum; VS, vascular space; T, trabeculae; TA, tunica albuginea. Hematoxylin and eosin, 100x.

Tables

Table 1: Histomorphometric analysis of gingiva of right hemimandible of rats in both groups. Values represent mean ± standard deviation and are expressed in mm.

Groups	C	E	H	L
CONTROL	0.0832 ±0.0114	0.067 ±0.0073	0.230 ±0.0548	0.125 ±0.033
EXPERIMENTAL	0.1051 ±0.0186*	0.084 ±0.0130*	0.396 ±0.1249*	0.228 ±0.067*

* Statistically significant within the same parameter, with $p < 0.05$.

Table 2: Histomorphometric analysis of right hemimandible of rats from both groups, for the quantification of osteoblasts, osteocytes and osteoclasts. Values represent mean ± standard deviation and are expressed in units.

Groups	Osteoblasts	Osteocytes	Osteoclasts
CONTROL	10.600 ±6.557	37.833 ±11.570	0.033 ±0.182
EXPERIMENTAL	10.333 ±6.249	25.633 ±10.420*	0.800 ±1.063*

* Statistically significant within the same parameter, with $p < 0.05$.

Table 3: Histomorphometric analysis of the lower right first molar of rats, and radiographic analysis of the mesial side of the first lower left molar of rats with distance of the cementoenamel junction to the alveolar bone crest of the control and experimental groups. Values represent mean ± standard deviation and are expressed in mm for histomorphometric analysis and pixels for the radiographic analysis.

Groups	Histomorphometric analysis	Radiographic Analysis
CONTROL	0,690 ±0,233	57.07 ±9.67
EXPERIMENTAL	1.206 ±0,620*	93.43 ±5.51*

* Statistically significant within the same parameter, with $p < 0.05$.

Table 4 Analysis of the concentrations of IL-6 cytokine in gingival samples and of TNF-α cytokine in the rat penis samples in the control and experimental groups. Values represent mean ± standard deviation and are expressed in pg/ml.

Groups	Gingiva (IL-6)	Penis (TNF-α)
CONTROL	26.56 ±13.16	1.57 ±0.40
EXPERIMENTAL	50.65 ±20.92*	5.35 ±0.32*

* Statistically significant within the same parameter, with $p < 0.05$.

Table 5: Values of the ventral-dorsal and lateral-lateral dimensions of cross sections of the penis, wall thickness and area of the dorsal penile artery, as well as the area of the vascular spaces of the corpora cavernosa. Values represent mean ± standard deviation and are expressed in mm for the ventral-dorsal and lateral-lateral dimensions of cross sections of the penis and wall thickness, and mm^2 for the area of the dorsal artery of the penis and the area of the vascular spaces of the corpora cavernosa, respectively.

Groups	Ventral-dorsal dimension (penis)	Lateral – lateral dimension (penis)	Wall thickness of the dorsal artery (penis)	Dorsal artery area (penis)	Vascular spaces area (corpora cavernosa)
CONTROL	3.279 ±0.8354	3.378 ±0.6365	0.0169 $\pm$0.004	0.007 $\pm$0.0008	0.0140 $\pm$0.0058
EXPERIMENTAL	2.845 ±0.3984	3.079 ±0.1627	0.0201 $\pm$0.004 *	0.004 $\pm$0.0007*	0.0071 $\pm$0.0014 *

* Statistically significant within the same parameter, with $p < 0.05$. Without the symbol * the values are statistically similar, with $p > 0.05$.