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Morpho-histological assessment of the periodontal support structures under the action of excessive occlusal forces and under the influence of nicotine

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Abstract

Smoking and occlusal trauma are two factors that can interfere with bone homeostasis. The aim of this study was to evaluate the histocellular changes occurring in the periodontal ligament and alveolar bone during the action of excessive occlusal forces, and to assess the influence of nicotine on the alveolar bone loss in teeth subjected to occlusal trauma. *Materials and Methods*: Fifty-six Wistar rats were randomized into seven groups (*n*=8). Animals were exposed to nicotine and occlusal trauma for 7, 14 and 30 days. Three groups were exposed to occlusal trauma alone, another three groups were exposed to occlusal trauma and nicotine, and one group was not exposed to any treatment. *Results*: Periodontal lesions induced in the first stage (7–14 days) manifested by a moderate increase of the periodontal space, a multiplication, thickening and elongation of periodontal fibers, as well as their condensation in the middle area of the periradicular space. Regarding bone changes induced by occlusal trauma, groups 5 and 7 (occlusal trauma and nicotine administration) had higher bone losses compared to groups 1, 2, 3, 4 and 6. This study demonstrated that nicotine significantly affected the alveolar bone. *Conclusions*: The induced occlusal trauma caused obvious tissue damage. At the same time, it was found that nicotine enhanced alveolar bone resorption, increased tooth mobility and induced an exacerbation of inflammatory processes.

Keywords: occlusal trauma, periodontal support structures, nicotine, alveolar bone loss, histological modifications.

⊡ Introduction

Smoking and occlusal trauma are two factors that can interfere with bone homeostasis. They may act synergistically on alveolar bone loss [1].

Cigarette smoking significantly increases the risk of developing various diseases including cancer, vascular disease, chronic obstructive pulmonary disease, as well as periodontal diseases [2–5]. It has been suggested that the increased incidence of these diseases in smokers may be due to chronic inhalation of chemicals from cigarette smoke that eventually alters the immune response [4].

Several *in vitro* studies have shown that nicotine can impair the migration, attachment and proliferation of gingival fibroblasts and periodontal ligament cells [6, 7]. It has also been reported that nicotine can alter the expression of neutrophil adhesion molecules [8]. Additionally, we previously reported that nicotine modulates the immunological characteristics of macrophages and dendritic cells [9, 10] and that it inhibits the differentiation of periodontal ligament cells [11].

Animal models are useful for investigating and understanding the pathogenic mechanisms of human diseases; however, smoking-associated periodontitis has not been investigated using mouse models. To investigate the effects of smoking on periodontal diseases in mouse models, we used nicotine, which has been thoroughly investigated and represents one of the main constituents of cigarette smoke [12].

The occlusal trauma is a lesion that develops in the periodontium, following the action of excessive forces that surpass the functional adaptation potential of periodontal structures [13–16].

The position, integrity and functionality of the teeth and periodontium do not change without a cause produced by one or more dysfunctional factors. The determining factors induce an imbalance of the occlusal forces by altering their value, duration, frequency, direction and point of application. Precipitating factors change the type of occlusal contacts, the resistance of periodontal structures and their recovery capacity [17]. The symptoms of occlusal trauma only occur in situations where the magnitude of the occlusal forces is such that the periodontium surrounding the targeted tooth cannot adequately support the forces and cannot distribute the resultant force without altering the position and stability of the tooth [18].

Excessive occlusal forces are considered major etiological factors with implications in periodontal destruction. Lesions caused by occlusal trauma are not inflammatory in nature. They take the form of compression atrophy, with the possible mortification or necrosis of the altered areas [19].

Histological studies [18] conducted in animals have led to contradictory conclusions.

In accordance with some authors [20, 21], who reported a greater reduction of bone mineral content in rats exposed to tobacco as compared to rats that inhaled cigarette smoke or those unexposed to tobacco, we designed an *in vivo* experimental model. Our aim was to study the

histocellular changes that occur in the periodontal ligament and alveolar bone during the action of excessive occlusal forces, along with the assessment of the influence of nicotine on alveolar bone loss in teeth subjected to occlusal trauma.

⊟ Materials and Methods

The experimental rat model developed at the Animal Facility of the Department of Physiology, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, included 56 white female Wistar rats (mean weight 300 g), randomized into seven groups (*n*=8). The animals were placed in type II L polycarbonate cages, in a room with a constant temperature (21.5–23^{0}C) and 65% relative humidity. The animals were exposed to a standard 12-hour light/dark cycle. The rats were supplied with a light diet and water *ad libitum*.

The research protocol was approved by the Ethics Committee of "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, and was registered under No. 39/16.

The animals were randomized into seven groups, as follows:

1 – control group;

2 – group with occlusal trauma (OT) for a 7-day period;

3 – group with occlusal trauma and nicotine administration (OT + Nic) for a 7-day period;

4 – group with occlusal trauma (OT) for a 14-day period;

5 – group with occlusal trauma and nicotine administration (OT + Nic) for a 14-day period;

6 – group with occlusal trauma (OT + Nic) for a 30-day period;

7 – group with occlusal trauma and nicotine administration (OT + Nic) for a 30-day period.

The occlusal trauma was induced by placement of 0.5 mm thick Ni–Cr metal crowns on the lower first molar of the right quadrant in 48 animals (Figure 1). The 0.5 mm thick metal crowns were made in the dental laboratory, taking into consideration the small size of dental crowns for experimental animals. The rats were anesthetized with intramuscular Ketamine 0.3 mg/g body weight (b.w.) and Narcoxyl 0.1 mg/g b.w. Before placement of the metal crown, the occlusal surface was thoroughly cleaned. The crowns were cemented with dual cement (BisCem®).

Figure 1 – The rat mandibular model, with the occlusal trauma-inducing crown in place in the fourth quadrant, ×50.

Nicotine (Glentham Life Sciences Ltd., Unit 5 Ingoldmells Court, Edinburgh Way, UK) 6 mg/kg b.w. was administered by application to the gingival mucosa of the lower first molar, three times a day. The daily dose of nicotine was 0.18 mg, which diluted in 25 mL physiological saline solution.

After 7, 14 and 30 days of the occlusion being increased, the animals were anesthetized with Ketamine 0.3 mg/g b.w. and Narcoxyl 0.1 mg/g b.w., and were subsequently sacrificed. Then, the tooth, periodontal ligament and alveolar bone were removed en bloc for histopathological examination.

The collected samples were fixed in 10% buffered formalin solution. Decalcification of the samples was obtained by immersing the rat heads into a mixture of 8% formic acid and 8% hydrochloric acid for 48 hours.

After fixation and decalcification, the samples were processed using the paraffin embedding technique for histopathological examination.

Serial 4-μm thick sections cut with a Leica RM 2125 RT microtome were mounted on histological slides for histopathological examination by Hematoxylin–Eosin (HE) staining. Mesiodistal, transverse and longitudinal sections of both periodontal tissue and bone were performed.

The preparations were examined under an Olympus BX 51 microscope. Images were taken with an Olympus UC 30 digital camera and they were processed using the Olympus Stream Basic image acquisition and processing software.

The morphological aspects monitored in the tooth–periodontium complex were related to the periodontal ligament: ligament width, ligament blood vessels, fiber appearance, and presence/absence of alveolar bone resorption.

⊟ Results

Macroscopic examination of the heads and decalcification of the samples were performed in order to detect potential changes visible with the naked eye. We also aimed to identify the metal prosthesis, which was used to induce occlusal trauma, checking whether the operative technique was correctly performed or not.

Following the macroscopic analysis of the samples, it was found that the metal prosthesis was correctly seated in the case of all the animals included in the study (Figure 1).

Macroscopically, grade 1 mesiodistal mobility of the right lower molar was observed after seven days. Grade 2 mobility of the molar was seen after 14 days.

The microscopic examination of the mandibular sections revealed significant differences between the animals of the different experimental groups. In the mesiodistal sections, we aimed to identify bone resorption lesions, periodontal ligament vascularization and the presence of inflammatory infiltrate in the alveolar bone and periodontal ligament.

The observations of the study involved the four components of the tooth supporting system: cementum, alveolar–dental ligament, alveolar bone and gingiva. The most important morphological changes found in the experimental groups were the increase in the width of the periodontal ligament, the increase in the number of osteoclasts and the intensification of the bone resorption processes, particularly in the inter-radicular septum.

In the control group, the periodontal ligament maintained the same width, and the main fibers maintained their direction between the cementum and the alveolar bone. The number and diameter of the blood vessels in the ligament were situated towards the alveolar bone. The alveolar bone morphology was found normal. A few osteoclasts, which are characteristic of bone remodeling, were noticed on the inner side of the alveolar bone (Figure 2).

In the group subjected to occlusal trauma for seven days (OT=7), an increase in the width of the periodontal ligament and in the diameter of periodontal ligament capillaries was found. The main periodontal ligament fibers had an orderly appearance, without any damage found at this level (Figure 3). Bone resorption could also be seen, the apical margin of the inter-radicular alveolar bone being uneven. Alveolar bone resorption (Figure 3) by osteoclasts was present, so that numerous syncytial cells were evidenced near the alveolar bone.

In group 3, with occlusal trauma and nicotine administration for seven days (OT + Nic=7), we found an increase in the width of the periodontal ligament, a reduction in the number and diameter of blood capillaries in the ligament and a decrease in the number of osteoclasts adjacent to the alveolar bone (Figure 4). Discrete mononuclear inflammatory cell infiltrate in the junctional epithelium was also present (Figure 4).

In group 4, the animals subjected to occlusal trauma for 14 days (OT=14) presented a relative normalization of the periodontal ligament morphology, with an appearance similar to that found in the control group. In addition, a reduction occurred in the number of osteoclasts on the alveolar bone surface and a decrease in the number of blood capillaries was found (Figure 5).

In group 5, the animals with occlusal trauma and nicotine administration for 14 days (OT + Nic=14) showed an intensification of bone resorption processes and an increase in the number of blood capillaries. Near the alveolar bone, osteoclasts could be seen particularly in the inter-radicular area, and few mononuclear inflammatory cells were found to be present (Figure 6). After 14 days, the animals showed significantly higher bone loss compared to the previous groups (1, 2 and 3), and in the inter-radicular area, hyalinized tissue and root cementum resorption were noticed.

In group 6, animals subjected to occlusal trauma for 30 days without any other treatments (OT=30) presented a normal periodontal ligament and adjacent structure morphology, as well as a marked reduction in bone resorption compared to the previous groups. The number of osteoclasts was found significantly diminished as compared to the previously mentioned groups (Figure 7).

The examination of the animals from group 7, the ones with occlusal trauma and nicotine administration for 30 days, revealed the presence of bone resorption processes, with few osteoclasts and a reduction in the number of blood capillaries. Rare mononuclear inflammatory cells and isolated mast cells were also noticed (Figure 8). In this group, the highest bone loss was found by examining the presence of the two previously-mentioned processes, namely: root cementum resorption and hyalinized inter-radicular tissue.
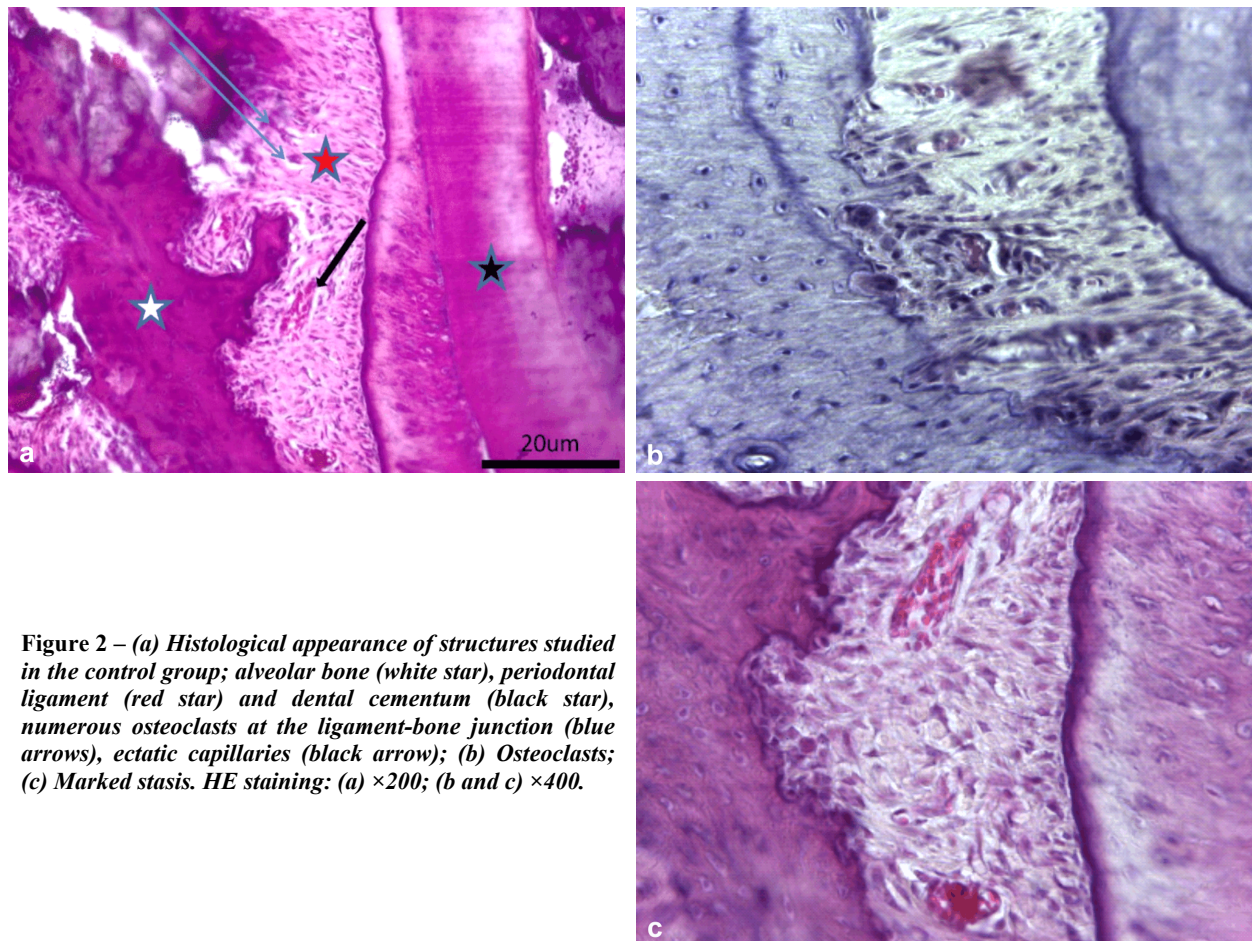

Figure 2 – *(a) Histological appearance of structures studied in the control group; alveolar bone (white star), periodontal ligament (red star) and dental cementum (black star), numerous osteoclasts at the ligament-bone junction (blue arrows), ectatic capillaries (black arrow); (b) Osteoclasts; (c) Marked stasis. HE staining: (a) ×200; (b and c) ×400.*
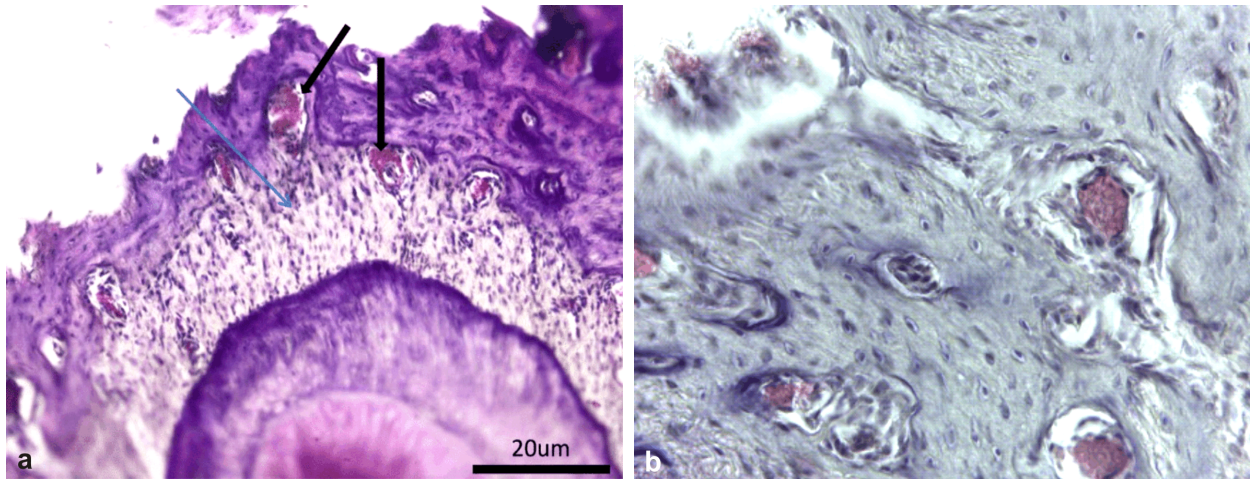
Figure 3 – *(a) Histological appearance of structures studied in group 2, numerous ectatic blood capillaries (black arrows), bone resorption with the presence of osteoclasts (blue arrow); (b) Osteoclasts. HE staining: (a) ×200; (b) ×400.*
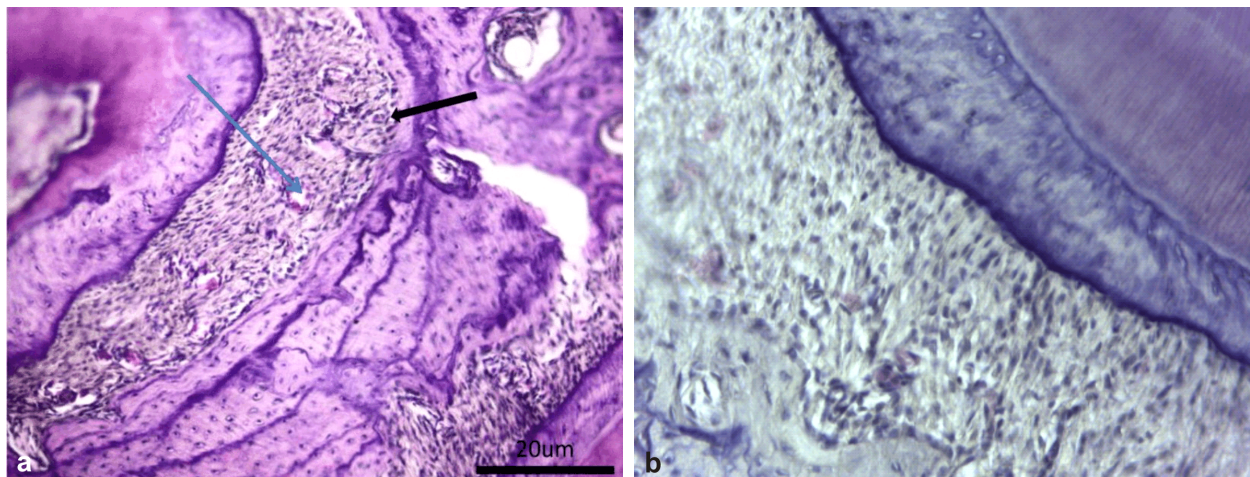

Figure 4 – *(a and b) Histological appearance of structures studied in group 3, rare blood capillaries (blue arrow), rare mononuclear inflammatory cells (black arrow). HE staining: (a) ×200; (b) ×400.*
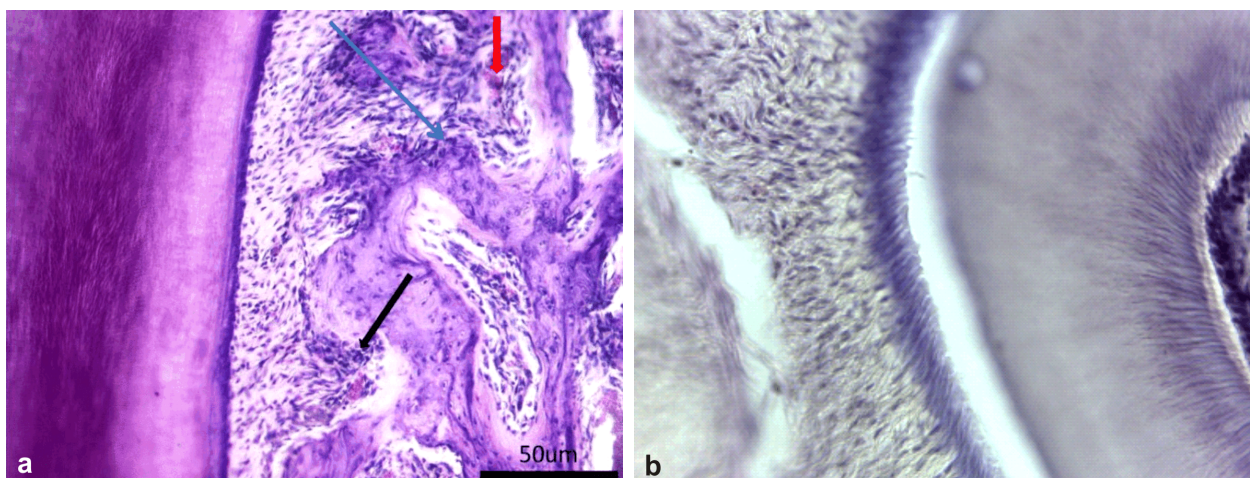

Figure 5 – *(a and b) Histological appearance of structures studied in group 4, rare blood capillaries (black arrow), rare osteoclasts (blue arrow). HE staining: (a) ×200; (b) ×400.*
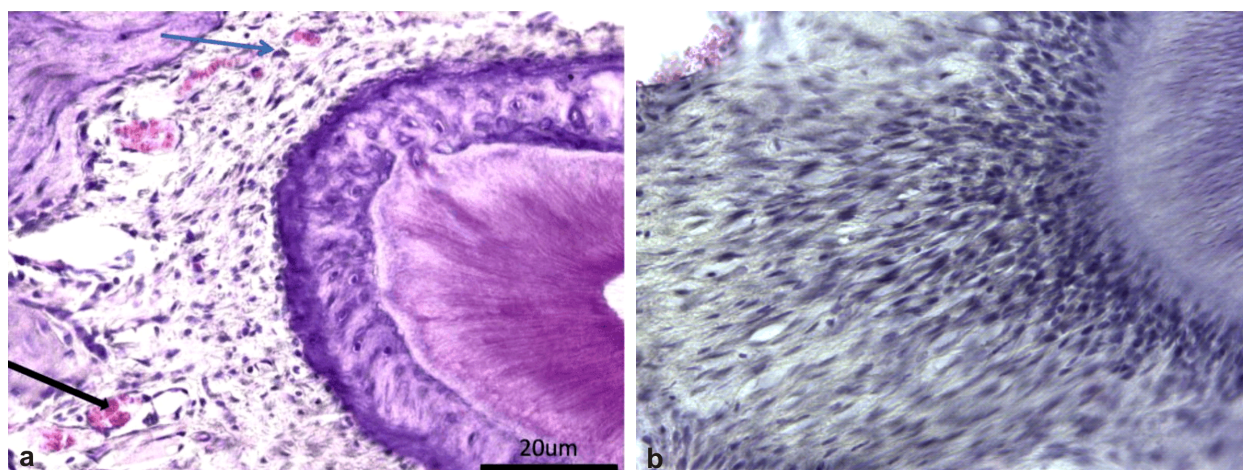
Figure 6 – *(a and b) Histological appearance of structures studied in group 5, rare blood capillaries (black arrow), rare osteoclasts, mononuclear inflammatory infiltrate (blue arrow). HE staining: (a) ×200; (b) ×400.*
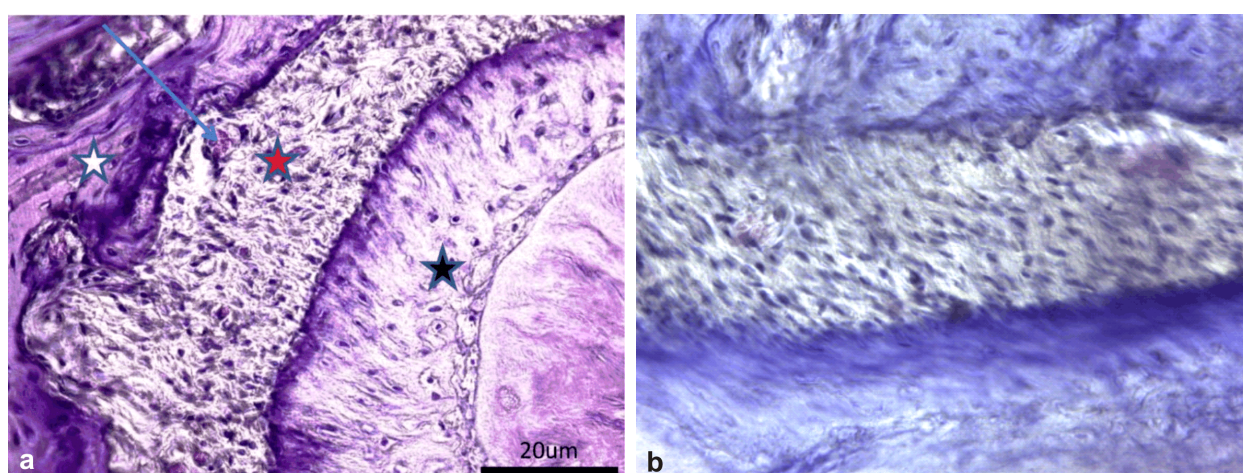

Figure 7 – *(a and b) Histological appearance of structures studied in group 6, alveolar bone (white star), periodontal ligament (red star) and dental cementum (black star), rare blood capillaries (blue arrow). HE staining: (a) ×200; (b) ×400.*
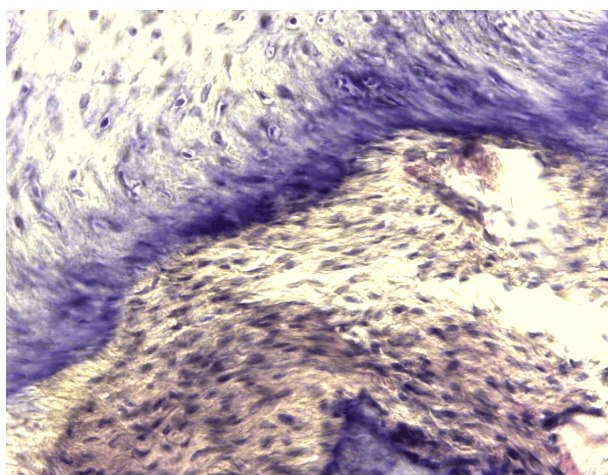

Figure 8 – *Histological appearance of structures studied in group 7, rare ectatic blood capillaries, rare osteoclasts (HE staining, ×400).*

⊟ Discussions

We investigated the histological changes which occurred in the periodontal ligament (PDL) and the alveolar bone under the action of excessive occlusal forces and nicotine.

We found that PDL plays an essential role in the preservation of the alveolar bone. Literature studies have suggested that PDL represents an environment for the transfer of forces and directs the remodeling process of the alveolar bone. It also distributes forces to the alveolar bone; the frequency, direction, action duration and magnitude of the applied forces can affect the rate and amplitude of the bone remodeling process [22–24].

A number of studies [17] have investigated the deterioration of the periodontal ligament *in vivo*. Glickman [13, 18] reported that teeth with occlusal trauma show a different progression of periodontal tissue loss, compared to those without occlusal trauma, and concluded that trauma from occlusion is an aggravating factor. Lindhe *et al.* [18] suggested that experimentally induced trauma does not lead to the development of periodontal pockets. However, Kem & Wagner [25] concluded that dental practitioners should consider occlusal trauma as a co-factor with a role in the progression of periodontal insertion loss. These authors reported cases of periodontal tissue destruction caused by excessive forces.

We studied the effect of mechanical strains on the periodontal ligament from the perspective of a constant mechanical strain. Establishing an experimental model that can be subjected to intense and intermittent forces is

indispensable. This would allow a detailed investigation of periodontal ligament damage from mechanical strain. In the current study, occlusal trauma was induced through the cementation of a metal crown for a certain time period (7, 14 and 30 days).

Periodontal lesions that occurred in the first stage (7–14 days) revealed a moderate increase in the periodontal space while the periodontal fibers showed a multiplication, thickening and elongation as well as a condensation in the middle area of the inter-radicular space. This is the result of the adaptation effort of the periodontal ligament system after being subjected to functional mechanical stress.

These changes correspond to the adaptation reaction of the periodontium, in response to the overload of one or more teeth after the placement of oversized prosthetic restorations. The effects of this stage are reversible by restoring the functional occlusion after the removal of the traumatic factor [17].

Regarding bone changes induced by occlusal trauma, groups 5 and 7 (occlusal trauma and nicotine administration) had higher bone loss compared to groups 1, 2, 3, 4 and 6. This study demonstrated that nicotine significantly affected the alveolar bone.

Some authors [26, 27] identified changes in the sequence of bone resorption and apposition after experimentally-induced occlusal trauma. Bone resorption induced by occlusal trauma was increased under the influence of nicotine and estrogen deficiency [28–30].

Nicotine can inhibit bone cell proliferation and extracellular matrix production, slowing down the bone remodeling process [31–33].

Nicotine, as a vasoconstrictor, affects gingival blood flow. Smoking affects the revascularization of soft and hard tissues in the oral cavity. In smokers, nicotine may cause a decrease in the collagen production by stimulating collagenase production, which is equivalent to a reduction of periodontal ligament width [34].

Other authors [34, 35] have assessed the influence of diabetes on bone response in the inter-radicular teeth subjected to occlusal trauma, in the presence or absence of the experimentally-induced periodontal disease in rats.

In the studied animals, nicotine negatively affected bone healing, by inhibiting some growth factors and delaying revascularization.

The rats, which were continuously exposed to cigarette smoke, had a smaller amount of mineralized tissue in the inter-radicular area. Thus, the presence of low-density alveolar bone suggests an exacerbated response to the action of forces exceeding the tolerated limits, and an increase of bone loss in the context of cigarette smoke inhalation [29, 30].

We found that nicotine significantly affects the repair and molecular remodeling processes. Exposure to nicotine for seven days negatively influenced bone formation during the initial stages of healing of the dental alveolus, while the 14-day exposure almost completely suppressed the presence of alkaline phosphatase during the experimental period [36].

These results were selectively interpreted because experimental models do not necessarily reproduce masticatory dynamics. These models are essential instruments for understanding the physiological and pathological mechanisms, allowing the development of prevention, diagnosis and treatment methods applicable to human disorders.

⊟ Conclusions

The induced occlusal trauma caused obvious tissue damage. It also led to a functional adaptation of the periodontium within certain limits of occlusal force values. At the same time, we found that nicotine enhanced alveolar bone resorption and induced an exacerbation of the inflammatory processes.

Conflict of interests

The authors declare that they have no conflict of interests.

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