REPARATIVE EFFECT OF MELATONIN ON THE MANDIBULAR ALVEOLAR BONE AND GINGIVA OF RATS WITH INDUCED PERIODONTITIS

Nouran A. El-Amrawy[1]* BDS, Hanaa M. Aly[2] PhD, Maha R. Taalab[3] PhD, Nesma M. Khalil[4] PhD

ABSTRACT

BACKGROUND: Periodontitis is a chronic inflammatory disease marked by intricate interactions between periodonto-pathogenic bacteria and the host's immunity. It has a complicated etiology including bacterial colonization, excessive inflammation, and increased oxidative stress. Due to its antioxidant properties, stimulation of fibroblast proliferation, anti-inflammatory capacity, and bone remodeling capability; melatonin can contribute to ameliorating chronic conditions and can have potential dental applications such as in periodontal disease.

AIM OF THE STUDY: To evaluate the effect of oral administration of melatonin on the mandibular alveolar bone and gingiva of albino rats with ligature-induced periodontitis.

MATERIALS AND METHODS: 42 healthy adult male albino rats were divided into 3 equal groups. Group A (negative control group), group B (LIG), and group C (LIG+MEL). In groups B and C, periodontitis was induced by the subgingival placement of a silk ligature around the right mandibular first molar tooth for 15 days and then removed. After ligature removal, only group C was treated by oral administration of melatonin. Clinical evaluation of modified gingival index (MGI) was made before treatment and repeated just before euthanasia. Animals were euthanized after 30 days from the start of the experiment and the mandibles' right side was dissected out and prepared for scanning electron microscopic (SEM) examination.

RESULTS: MGI scores showed a significant enhancement in the gingival condition of group C in comparison to group B. SEM results showed significantly less alveolar bone loss with a decrease in the surface porosity in the treated group C compared to the untreated group B.

CONCLUSIONS: Melatonin administration has a positive biological impact on the alveolar bone and gingiva in rats with induced periodontitis.

KEYWORDS: Melatonin, Periodontitis, Alveolar bone, Gingiva, Ligature-induced Periodontitis

----

1. Demonstrator of Oral Biology -Faculty of Dentistry –Alexandria University
2. Professor of Oral Biology -Faculty of Dentistry –Alexandria University
3. Assistant Professor of Oral Medicine, Periodontology, Oral Diagnosis, and Oral Radiology -Faculty of Dentistry– Alexandria University
4. Assistant professor of Oral Biology- Faculty of Dentistry – Alexandria University

Corresponding author:
Nouran.elamrawy@gmail.com

INTRODUCTION

Periodontitis is an infectious chronic inflammatory disease that destroys healthy dental tissues leading to gradual alveolar bone destruction and periodontal ligament deterioration together with increased gingival recession, probing depth, or both. Periodontal disease causes soft and hard tissue destruction due to the direct influence of bacterial-derived toxic products, overactivation of the host immuno-inflammatory response against pathogenic bacterial plaque, and the excessive production of reactive oxygen and nitrogen species (1).

The key features of periodontitis are host inflammatory processes that include the production and activation of cytokines and inflammatory mediators such as interleukins (ILs) and tumor necrosis factor α (TNF-α), in addition to proteolytic enzymes such as matrix metalloproteinases (MMPs). Likewise, inflammatory cell infiltration is a key characteristic feature of the chronic inflammatory process (2).

These mediators stimulate catabolic activity and promote the interaction between receptor activator of nuclear factor-kappa B (RANK) and its ligand (RANKL). RANKL is expressed by several cells including osteoblastic, stromal, as well as activated B and T lymphocyte cells where it interacts with RANK present on osteoclast precursors promoting the fusion and differentiation of these precursors into multinucleated osteoclasts, and hence RANKL-mediated osteoclastogenesis is activated (3). Therefore, the repression of inflammatory mediators

by bioactive compounds may be a potential technique for periodontitis alleviation and optimal periodontal health (4).

Several biological compounds have antibacterial action which enhances periodontal tissue repair and regeneration. The use of anti-inflammatory agents or MMPs and pro-inflammatory cytokines inhibitors is considered to be advantageous for treating periodontal diseases. However, prolonged use and/or overuse of antibiotics can develop antibiotic resistance (5).

Melatonin, also referred to as N-acetyl-5-methoxytryptamine, is a hormone produced and released by the pineal gland. It is made from tryptophan and derived from serotonin via two enzymatic reactions, in addition to the pineal synthesis that occurs in the gastrointestinal tract, lymphocytes, skin, retina, and stem cells (6).

In 1917, the effects of melatonin were first discussed. Nevertheless, it wasn't isolated and recognized until 1958 (7). Being a nontoxic highly lipophilic indole, melatonin has the ability to penetrate the cell membrane and its compartments (8). It gets into the oral cavity through passive diffusion into the saliva (9).

Melatonin's anti-inflammatory effects are based on its antioxidant and free radical scavenging abilities. It has a very potent affinity towards hydroxyl group (OH⁻); therefore, it shields cells from free radical damage and promotes bone formation by stimulating Type I collagen fiber production and modulating osteoblastic and osteoclastic activity (10). It also suppresses bone resorption by down regulating RANKL-mediated osteoclast activation (11).

Owing to its anti-inflammatory, antioxidant and bone remodeling capabilities, several studies started to test the effect of using melatonin as an adjunctive therapy to protect alveolar bone and periodontal tissues in cases of periodontitis (12-14). According to Montero et al., (15) the pocket depth, gingival index, as well as IL-1, IL-6, and prostaglandin E2 (PGE2) concentrations in the patients' gingival sulcular fluid all significantly improved after topical melatonin treatment. The administration of melatonin has been used in bone healing, wound healing, as well as osseointegration around dental implants (16). There are few literatures about the impact of systemic administration of melatonin on the mandibular alveolar bone regeneration and gingival healing. Therefore, the present study aims to assess the impact of melatonin on the alveolar bone structure and gingiva of rats with ligature induced periodontitis.

MATERIAL AND METHODS

The Ethical Committee of Faculty of Dentistry, Alexandria University approved the study design. The approval number by the ethical committee is 0232-03/2021

Experimental animals

Forty-two healthy adult male eight-week-old Albino rats weighing (250-300g) were used. The experiment was conducted at the animal house of Medical Research Institute, Alexandria University; where the rats were kept under the same environmental conditions(17). All animal procedures were conducted in accordance with the National Research Council guidelines for the care and use of laboratory animals (18).

Grouping (Randomization technique)

Rats were randomly assigned (by using computer generated random numbers) into three equal groups (fourteen rabbits each) (19)

Group A (CONTROL): Negative control group.

Group B (LIG): ligature induced periodontitis for 15 days and then no treatment for 15 more days.

Group C (LIG+MEL): ligature induced periodontitis for 15 days and then treatment with 10 mg/kg body weight of melatonin for 15 more days.

Materials

Ketamine (SIGMA TEC CO, Egypt); Xylazine hydrochloride (Xyla Ject®) (ADWIA Co, 10th of Ramadan City, Egypt); Melatonin (SIGMA TEC CO, Egypt); and 4-0 silk ligature wire (GHATWARY MEDICAL GMS, Alexandria, Egypt).

Induction of periodontitis

General anesthesia was established by an intramuscular injection of a combination of 70mg/kg body weight ketamine and 30mg/kg body weight xylazine prior to periodontitis induction. Once adequate anesthesia was attained, a 4-0 silk ligature was secured around the mandibular right first molars at the level of the gingival sulcus of the animals in groups B and C (20).

Clinical Evaluation

MGI was used twice to assess the clinical status of the periodontal tissues, once directly after ligature removal and then repeated again after treatment administration in all groups. The Lobene MGI was used to record 4 categories of visual inflammatory changes where 0 = normal gingiva and absence of inflammation; 1= mild inflammation with little changes in color and minimal changes in texture of any portion of the gingiva; 2 = mild inflammation of the whole gingiva; 3 = moderate inflammation of the gingiva and 4 = severe inflammation of the gingiva (21).

Melatonin Administration

Fifteen days after its application, the silk ligature wire was removed in both groups B and C. Melatonin was then orally administrated to group C only at a dose of 10 mg/kg using gastric gavage. Melatonin solution was prepared by dissolving melatonin in distilled water using ultrasonicator. Only distilled water was orally

administered using the same procedure to groups A and B (22,23).

Animal euthanasia

After 30 days from the beginning of the experiment, all rats were euthanized by intravenous injection with a fatal dose (100 mg/kg) of pentobarbital sodium. The rats were decapitated, and their mandibles were dissected out (24). In each group, the right mandibular molar regions were prepared for examination of their buccal surface topography via the scanning electron microscope. Rats were then disposed off through burning by special authorities (25).

Scanning Electron Microscope (SEM)

Specimens were examined by SEM at the SEM unit in the Faculty of Science Alexandria University to study the surface topography of alveolar bone in different groups. The specimens were fixed in 4% formaldehyde with 1% glutaraldehyde. They were rinsed afterwards in phosphate buffer for 10 minutes before being dehydrated by passing them through ascending concentrations of ethyl alcohol (50%, 70% and 95%) for 10 minutes each and then in 100% alcohol for two changes of one-hour period.

After dehydration, the specimens were dried at the critical point and mounted using silver paint on the specimen holder. Afterwards, they were coated with gold through ion sputtering device to be equipped for SEM examination. After coating, the samples were examined by the Jeol scanning microscope (JSM-5300). The applied accelerating voltage changed from one specimen to another depending on received picture on display screen, which was usually 25 Kv (26).

Statistical analysis

Kruskal Wallis test was applied to make a comparison between groups followed by a post hoc test with Bonferroni correction. Wilcoxon Sign Rank was performed for within-group comparisons. The level of significance was set at a p-value of 0.05. All tests were two-tailed. Data analysis was carried out using IBM SPSS Statistics for Macintosh, Version 28.0. Armonk, NY: IBM Corp.

RESULTS

Modified gingival index statistical analysis

A comparison between the MGI among different study groups before and after treatment was demonstrated in Table (1). MGI scores before treatment confirmed the induction of periodontitis in groups B and C where the MGI score showed a median value 4.00 with no significant difference between both groups. In group A, the MGI showed a median value 0.50 with a statistically significant difference between groups A and B as well as between groups A and C.

MGI was repeated after treating group C with 10 mg/kg melatonin and leaving group B untreated. The results after treatment showed statistically significant amelioration in the scores for both groups B and C with a greater improvement in the treated group C in comparison to the untreated group B. These results show that in group B, there was an enhancement in the gingival condition where the median score was 2.00 after the cause(ligature) was removed and gingiva left to recover without treatment for 15 days. However, the melatonin treated group C showed a greater improvement in the gingival condition with a median value 1.00. Groups A and B showed statistically significant difference between their MGI scores after treatment. There was a statistically significant difference also between the MGI score of groups A and C after treatment.

SEM results

SEM results of the bone surfaces revealed variations between the different groups. However, the findings were similar in all specimens from the same group, and the buccal cortical plates' morphological characteristics were consistent from the apical to the cervical region.

Group A (control group)

The surface topography of the alveolar bone's buccal cortical plate displayed a generalized smooth, uniform, and homogenous surface. In all the examined specimens, the alveolar bone was intact with a relatively normal level around mandibular first molar (Fig.1a). The surface of cortical bone displayed well-defined and regularly bordered nutritive canals (Fig.2a).

Group B (LIG)

The buccal cortical plate's surface topography at the site of the first molar exhibited significant generalized roughness with irregular resorptive craters, porosities, and prominent deep areas of erosions and pits indicating active osteoclastic bone resorption. Severe resorption at the level of the alveolar bone around the mandibular first molar that extended to the root apical area was also seen (Fig.1b). At a higher magnification, irregular roughly bordered nutritive canals with less defined edges were also visible (Fig.2b).

Group C (LIG+MEL)

In comparison to the untreated group B, the alveolar bone's buccal cortical plate surface topography demonstrated a significant improvement in surface texture. A generalized uniform, smooth, and homogenous pattern was present in the first molar region, recreating the regular architecture seen in Group A.

The alveolar bone surface displayed marked masking of almost all resorptive changes related to the periodontitis group B. Considerable restoration in the level of the alveolar bone around mandibular first molar was revealed (Fig.1c). Relatively well-defined nutritive canals with regular borders were also noticed (Fig. 2c).
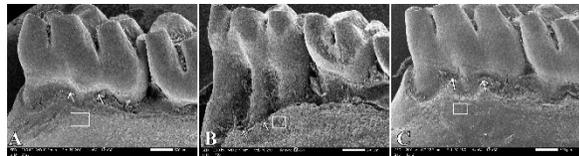
Figure 1: (A) SEM of group A (control group) revealing intact alveolar bone with normal level around mandibular first molar (arrows). The buccal cortical plate of the alveolar bone shows a smooth and uniform surface topography (x30). **(B)** SEM of group B (LIG group) showing severe resorption in the level of the alveolar bone leading to root exposure (arrows). Buccal cortical plate showed generalized surface roughness with severe porosity. (x30). **(C)** SEM of group C (LIG+MEL) showing obvious partial restoration of the level of the alveolar bone around mandibular first molar with a relatively smooth surface topography of the buccal cortical plate of the alveolar bone (arrows). (x30)
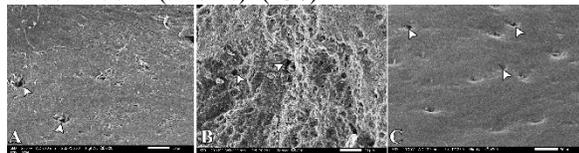

Figure 2: Higher magnifications of the SEM insets of (Fig.1). **(A)** showing smooth & regular buccal cortical plate surface with nutritive canals having regular borders (arrow heads) (x300). **(B)** showing pronounced generalized roughness, porosity, and irregularity of the alveolar bone surface with areas of erosion. Nutritive canals also show rough borders (arrow heads). (x300). **(C)** showing a smooth regular buccal cortical plate with uniform surface and regular borders of nutritive canals (arrow heads). (x300)

Table 1: Comparison of MGI among the study groups before and after treatment

		Group A (n=14)	Group B (n=14)	Group C (n=14)	P value
Before	Median (IQR)	0.50 (1.00) a	4.00 (0.00) b	4.00 (0.00) b	<0.001*
	Min - Max	0.00 – 2.00	3.00 – 4.00	3.00 – 4.00	
After	Median (IQR)	1.00 (1.00) a	2.00 (1.00) b	1.00 (1.00) c	<0.001*
	Min - Max	0.00 – 2.00	1.00 – 3.00	0.00 – 3.00	
P value		0.317	<0.001	<0.001	

*Statistically significant at p-value <0.05
Different superscript lowercase letters denote statistical significance differences between groups

DISCUSSION

Periodontal damage has been linked to the influence of an oxidant-antioxidant imbalance in patients with periodontal disease (27,28). An increase in ROS, caused primarily by PMN during an inflammatory response where many of the immune cells are activated and can release a multitude of proinflammatory cytokines as, for instance, IL-1b and TNF-a. The produced cytokines can trigger inflammatory enzymes and mediators resulting in periodontal tissue destruction (28).

Beside regulating the circadian rhythm, melatonin has anti-oncotic, anti-inflammatory, and immunomodulatory properties due to its ability to act as a free radical scavenger and interact with cell membranes and intracellular proteins (29). Melatonin is thought to have a very promising role in dentistry where it could be employed therapeutically in bacterial, viral, fungal, or mechanical oral cavity damage, in post-extraction wounds and several other oral procedures, in assessing bone formation in multiple autoimmunological diseases such as Sjogren syndrome, in dental implants, and in oral malignancies (16,29,30).

The present experiment assessed the efficacy of melatonin administrated orally for 15 days on the alveolar bone and gingiva of rats with ligature induced periodontitis using MGI and SEM. Melatonin was administrated by oral intra gavage at a dose of (10 mg/kg/day). This dose has proved to be efficient in regulating bone metabolism, inhibition of osteoclastogenesis, acceleration of osteoblastogenesis, and the stimulation of apoptotic cell death in mature osteoclasts, in addition to the repression of osteolytic metastasis of bone as reported by MacDonald et al., (31).

In the current study, induction of periodontitis was carried out by the subgingival placement of a 4-0 silk ligature wire for 15 days around the right mandibular first molars. This is commensurate with the findings of Moraes et al., (32), as well as other researchers (33,34) who used silk ligatures to establish experimental periodontitis. This was the method of choice because it is a simple, effective, inexpensive and was regarded as a useful periodontitis experimental model with alveolar bone resorption; moreover, it creates scenarios that resemble bacterial colonization and food impaction between teeth (33). Kurt et al., (20) also proved that tying silk ligatures around the experimental rats' mandibular first molars for 2 weeks was capable of creating a model for experimental periodontitis.

The results of this study were not in favor of the null hypothesis since the melatonin had a favorable impact on the reconstruction of the alveolar bone and

minimizing alveolar bone resorption in comparison to the untreated group.

The MGI results of this work revealed a significant reduction in gingival inflammation in group C than the periodontitis group B. These results are consistent with those of Gómez-Florit et al., who proposed that melatonin contributed to the preservation and retrieval of the gingival integrity and therefore exhibit a potential use for the treatment of periodontal diseases and improving soft tissue integration in implantology (35).

The SEM results of the control group in the current experiment demonstrated generalized smooth and uniform surface topography of the buccal cortical plate of the alveolar bone. Moreover, normal level of intact alveolar bone was observed around mandibular first molar of the control group with multiple nutritive canals exhibiting smooth regular borders. These findings were consistent with those of de Souz a et al., (36) who verified the presence of uniform intact alveolar bone in the control group in their research on the consequence of prolonged alcohol consumption at varied frequencies on adult rats' periodontal bone loss. This is also comparable to the results of Abu Ayana et al., (37) who studied the impact of quercetin treatment on the alveolar bone in diabetic rats.

As for the periodontitis group (B), the SEM surface topography revealed pronounced generalized roughness of the surface of the buccal cortical plate with severe porosity and deep areas of erosions. Roughly bordered nutritive canals were also detected denoting the resorption process. This was explained by Silva et al.,(38) who proved that there was an increase in the alveolar bone loss in animals with periodontal disease due to stimulation of inflammatory cytokines. Moreover, this bone loss was due to increased expression of the osteoclastogenic mediator RANKL and reduced expression of the osteogenesis related factor RUNX2, as well as the anti-osteoclastogenic factor OPG(39).

Concerning group C, relative restoration of the level of the alveolar bone was presented. The alveolar bone surface showed marked improvement of the bone architecture with a relatively smooth surface and well-defined nutritive canals. These results are in accordance with Arabacı et al., who found that melatonin treatment dramatically reduces alveolar bone resorption and promotes periodontal healing in a rats with experimentally induced periodontitis(13).

All the previous MGI and SEM findings of the deteriorated bone tissue of periodontitis group revealed remarkable changes from those of the melatonin treated and control groups. The detected results of the current experiment showed the beneficial effect of melatonin administration on the re-

establishment of the alveolar bone structure in rats with induced periodontitis.

Even with the limitations of the study, such as the short duration of treatment and the relatively low dose of melatonin chosen to match the animal models, melatonin administration still promoted a reparative effect on the alveolar bone tissue of rats with experimentally induced periodontitis. However, milder forms of periodontitis may show a better response to the treatment and the administration of larger doses may shown a greater reparative influence. Limitations of the study also include the lack of measurement of some important parameters as serum melatonin, serum cytokines, as well as analysis of oxidative stress markers which are other means to measure the efficacy of melatonin when used as a treatment for rats with induced periodontitis.

Whether a prolonged treatment or even higher dose of melatonin would have a beneficial effect in periodontitis is currently unknown and should be further studied.

CONCLUSION

This study verifies that melatonin administration promotes a reparative influence on the alveolar bone and gingival tissues of rat models with induced experimental periodontitis.

CONFLICT OF INTEREST

We proclaim that we don't have any conflicts of interest.

FUNDING

The presented work received no specific fundings.

REFERENCES

1. Glimvall P, Wickström C, Jansson H. Elevated levels of salivary lactoferrin, a marker for chronic periodontitis? J Periodontal Res. 2012;47:655-60.
2. Yucel-Lindberg T, Båge T. Inflammatory mediators in the pathogenesis of periodontitis. Expert Rev Mol Med. 2013;15:e7.
3. Tobón-Arroyave SI, Isaza-Guzmán DM, Restrepo-Cadavid EM, Zapata-Molina SM, Martínez-Pabón MC. Association of salivary levels of the bone remodelling regulators RANKL and OPG with periodontal clinical status. J Clin Periodontol. 2012;39:1132-40.
4. Araújo AA, Souza TO, Moura LM, Brito GA, Aragão KS, Araújo LS, et al. Effect of telmisartan on levels of IL-1, TNF-α, down-regulated COX-2, MMP-2, MMP-9 and RANKL/RANK in an experimental periodontitis model. J Clin Periodontol. 2013;40:1104-11.
5. Bhattarai G, Poudel SB, Kook SH, Lee JC. Resveratrol prevents alveolar bone loss in an experimental rat model of periodontitis. Acta Biomater. 2016;29:398-408.

6.   Acuña-Castroviejo D, Escames G, Venegas C, Díaz-Casado ME, Lima-Cabello E, López LC, et al. Extrapineal melatonin: sources, regulation, and potential functions. Cell Mol Life Sci. 2014;71:2997-3025.

7.   Lerner AB, Case JD, Takahashi Y. Isolation of melatonin and 5-methoxyindole-3-acetic acid from bovine pineal glands. J Biol Chem. 1960;235:1992-7.

8.   Hacışevki A, Baba B. An overview of melatonin as an antioxidant molecule: a biochemical approach. Melatonin molecular biology ,clinical and pharmaceutical approaches. 2018;5:59-85.

9.   Reiter RJ. Normal patterns of melatonin levels in the pineal gland and body fluids of humans and experimental animals. J Neural Transm Suppl. 1986;21:35-54.

10. Cutando A, Arana C, Gómez-Moreno G, Escames G, López A, Ferrera MJ, et al. Local application of melatonin into alveolar sockets of beagle dogs reduces tooth removal-induced oxidative stress. J Periodontol. 2007;78:576-83.

11. Calvo-Guirado JL, Gómez-Moreno G, Barone A, Cutando A, Alcaraz-Baños M, Chiva F, et al. Melatonin plus porcine bone on discrete calcium deposit implant surface stimulates osteointegration in dental implants. J Pineal Res. 2009;47:164-172.

12. Virto L, Cano P, Jiménez-Ortega V, Fernández-Mateos P, González J, Haugen HJ, Esquifino AI, Sanz M. Melatonin as adjunctive therapy in the treatment of periodontitis associated with obesity. J Clin Periodontol. 2018;45:1336-46.

13. Arabacı T, Kermen E, Özkanlar S, Köse O, Kara A, Kızıldağ A, et al. Therapeutic Effects of Melatonin on Alveolar Bone Resorption After Experimental Periodontitis in Rats: A Biochemical and Immunohistochemical Study. J Periodontol. 2015;86:874-81.

14. Kose O, Arabaci T, Kara A, Yemenoglu H, Kermen E, Kizildag A, et al. Effects of Melatonin on Oxidative Stress Index and Alveolar Bone Loss in Diabetic Rats With Periodontitis. J Periodontol. 2016;87:e82-90.

15. Montero J, López-Valverde N, Ferrera MJ, López-Valverde A. Changes in crevicular cytokines after application of melatonin in patients with periodontal disease. J Clin Exp Dent. 2017;9:e1081-7.

16. Permuy M, López-Peña M, González-Cantalapiedra A, Muñoz F. Melatonin: A Review of Its Potential Functions and Effects on Dental Diseases. Int J Mol Sci. 2017;18:865.

17. Oz HS, Puleo DA. Animal models for periodontal disease. J Biomed Biotechnol. 2011;2011:754857.

18. Council NR. Guide for the care and use of laboratory animals.8th ed. Washington, D.C. National Academies Press;2010.

19. Suresh K. An overview of randomization techniques: An unbiased assessment of outcome in clinical research. J Hum Reprod Sci. 2011;4:8-11.

20. Kurt S, Gürkan ÇG, Keleş Tezal GÇ, Çiftçi A, Gürgör PN, et al. Histopathological and biochemical evaluation of the effect of Paeoniflorin on the periodontium during and after periodontitis formation in rats. Arch Oral Biol. 2019;102:135-40.

21. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in clinical trials. Clin Prev Dent. 1986;8:3-6.

22. Mahmoud GS, El-Deek HE. Melatonin modulates inflammatory mediators and improves olanzapine-induced hepatic steatosis in rat model of schizophrenia. Int J Physiol Pathophysiol Pharmacol. 2019;11:64-75.

23. Dos Santos BC, Gomes JC, Miola AE, Rothen SK, Rodrigues CP, Costa RM, et al. Evaluation of the Effect of Melatonin on Periodontal Tissues in Rats with Periodontitis Induced ExperimentallyJ Adv Med Med Res. 2020:115-26.

24. Özdoğan AI, İlarslan YD, Kösemehmetoğlu K, Akca G, Kutlu HB, Comerdov E, et al. In vivo evaluation of chitosan based local delivery systems for atorvastatin in treatment of periodontitis. Int J Pharm. 2018;550:470-476.

25. Council NR. Guide for the care and use of laboratory animals.8th ed. Washington, D.C. : National Academies Press; 2010.

26. Goldstein JI, Newbury DE, Michael JR, Ritchie NWM, Scott JHJ, Joy DC. Scanning electron microscopy and X-ray microanalysis. 4th ed. New York, NY: Springer; 2017.

27. Waddington RJ, Moseley R, Embery G. Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. Oral Dis. 2000;6:138-51.

28. Borges I Jr, Moreira EA, Filho DW, de Oliveira TB, da Silva MB, Fröde TS. Proinflammatory and oxidative stress markers in patients with periodontal disease. Mediators Inflamm. 2007;2007:45794.

29. Najeeb S, Khurshid Z, Zohaib S, Zafar MS. Therapeutic potential of melatonin in oral medicine and periodontology. Kaohsiung J Med Sci. 2016;32:391-6.

30. Cengiz Mİ, Cengiz S, Wang HL. Melatonin and oral cavity. Int J Dent. 2012;2012:491872.

31. MacDonald IJ, Tsai HC, Chang AC, Huang CC, Yang SF, Tang CH. Melatonin Inhibits Osteoclastogenesis and Osteolytic Bone Metastasis: Implications for Osteoporosis. Int J Mol Sci. 2021;22:9435.

32. Moraes RM, Lescura CM, Milhan NVM, Ribeiro JL, Silva FA, Anbinder AL. Live and heat-killed Lactobacillus reuteri reduce alveolar bone loss on induced periodontitis in rats. Arch Oral Biol. 2020;119:104894.

33. Toker H, Ozan F, Ozer H, Ozdemir H, Eren K, Yeler H. A morphometric and histopathologic evaluation of the effects of propolis on alveolar bone loss in experimental periodontitis in rats. J Periodontol. 2008;79:1089-94.

34. Lee HJ, Lee DR, Choi BK, Yang SH. Antiperiodontitis Effects of Magnolia biondii Extract on Ligature-Induced Periodontitis in Rats. Nutrients. 2019;11:934.

35. Gómez-Florit M, Ramis JM, Monjo M. Anti-fibrotic and anti-inflammatory properties of melatonin on human gingival fibroblasts in vitro. Biochem Pharmacol. 2013;86:1784-90.

36. De Souza DM, Rodrigues VA, Silva AA, Gonsalves VS, Pereira KA, Nishioka RS, et al. Influence of different alcohol intake frequencies on alveolar bone loss in adult rats: A sem study. J Clin Exp Dent. 2018;10:e852-57.

37. Abu Ayana MA, Elmasry NA, Shehata FI, Khalil NM. Efficacy of quercetin on alveolar bone structure of rats with induced diabetes. Alex Dent J. 2017;42:141-6.

38. Silva VD, Lobato RV, Andrade EF, Orlando DR, Borges BDB, Zangeronimo MG, et al. Effects of β-Glucans Ingestion on Alveolar Bone Loss, Intestinal Morphology, Systemic Inflammatory Profile, and Pancreatic β-Cell Function in Rats with Periodontitis and Diabetes. Nutrients. 2017;9:1016.

39. Gennaro G, Claudino M, Cestari TM, Ceolin D, Germino P, Garlet GP, et al. Green Tea Modulates Cytokine Expression in the Periodontium and Attenuates Alveolar Bone Resorption in Type 1 Diabetic Rats. PLoS One. 2015;10:e0134784.