Journal of Lasers in Medical Sciences

J Lasers Med Sci 2024;15:e39

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.34172/jlms.2024.39

Impact of Photobiomodulation and Melatonin on Periodontal Healing of Periodontitis in Immunosuppressed Rats

Latifa Mohamed Abdelgawad[1*], Yomna Gamal Mahmoud Ibrahim Salem[1], El-Sayed Abd Allah El Tayeb[1]

[1]Medical applications of lasers Department, National Institute of Laser Enhanced Science (NILES), Cairo University, Giza, Egypt

***Correspondence to**
Latifa Mohamed Abdelgawad,
Emails: latifa@niles.edu.eg, latifa.mohamed@cu.edu.eg

Received: January 13, 2024
Accepted: March 9, 2024
ePublished: August 11, 2024

Abstract

Introduction: Periodontitis is an inflammatory disease due to bacterial origin; it has a chronic course and progresses by immunosuppressive therapy. However, adjuvant therapies such as photobiomodulation (PBM) and melatonin can reduce the severity of the inflammation and inhibit the progression of periodontitis. Therefore, the present study evaluated the effects of PBM (PBM) and melatonin, as adjuvant therapies, on periodontal healing in immunosuppressed rats with periodontitis.

Methods: Random allocation was performed on 36 albino Wistar rats, divided into the following groups: control, periodontitis, immunosuppressant only, immunosuppressant + PBM, immunosuppressant + melatonin, and immunosuppressant + melatonin + PBM. Periodontitis caused by ligature in all groups, except for the control group. Subcutaneous administration of dexamethasone was performed in the immunosuppressant groups for immunosuppression. All the groups except the control group received scaling and root planning (SRP). Each group was subdivided into three equal subgroups according to the evaluation period: (A), one week, (B) two weeks (C), 4 weeks. Histological examination was done with haematoxylin & eosin and Masson's Trichrome for inflammation and periodontal healing. Statistical Analysis of the data was done by using the chi-square test. The significance level was set at $P \leq 0.05$.

Results: Regarding the inflammatory response and periodontal healing, histological examination revealed statistically significant difference in all treated groups in comparison with the control untreated immunosuppressed group ($P < 0.001$). The combined application of melatonin and PBM resulted in a best histological response presented by lower inflammatory response and better periodontal healing, when compared with all other treated groups ($P < 0.001$).

Conclusion: After considering the circumstances of this research, the combination of melatonin and PBM by a 650 nm diode laser with output power of 100 mw for one minute for three sessions appeared to be a beneficial adjunct in periodontal healing in immunosuppressed rats with periodontitis.

Keywords: Periodontal healing; Photobiomodulation; Melatonin; Rats; Periodontitis.

Introduction

Immunosuppression can cause different effects on periodontium, including gingival ulceration, epithelial downward migration, attachment loss, and disruption of transseptal fibers due to its effect on different aspects of metabolism such as carbohydrate metabolism, protein catabolism and osteoclastogenesis. This results in higher levels of bone loss and activation of the inflammation within the periodontium.[1-3]

The effects of immunosuppression on periodontium may be due to the reduction of angiogenesis and re-epithelialization, the suppression of fibroblast activity, and the loss of collagen and connective tissue.[3-6]

Non-surgical periodontal treatment is considered the gold standard for the treatment of periodontitis; scaling and root planning (SRP) is often not able to eliminate the infection and associated inflammation, especially in individuals with systemic involvement and those prone to more severe cases of periodontitis.[7-14]

Photobiomodulation (PBM) is a line of treatment that utilizes the photogenic energy of the laser, produced by the electromagnetic spectrum at specific doses, known as "optical therapeutic window", [15-18] which is absorbed by cytochrome C oxidase on the outer membrane of mitochondria.[15-19] This leads to multiple molecular and cellular signalling events, including the activation of DNA/RNA deoxyribonucleic acid (ATP), alteration in membrane activity, and modification of intracellular organelle function. The most important calcium flux and stress protein expression are induced by this. Research has

Please cite this article as follows: Abdelgawad LM, Salem YGMI, Tayeb EAAE. Impact of photobiomodulation and melatonin on periodontal healing of periodontitis in immunosuppressed rats. *J Lasers Med Sci.* 2024;15:e39. doi:10.34172/jlms.2024.39.

indicated that photobiomodulation therapy (PBMT) can enhance the growth and structure of gingival fibroblasts, while also decreasing inflammation with PGE2 and TNF-α. Cementoblast and osteoblast stimulate the regeneration of periodontal tissues.[20-25]

Melatonin (N-acetyl-5-methoxy-tryptamine): The hormone responsible for regulating the sleep-wake cycle, known as an endogenous hormone, has garnered interest for its numerous biological impacts such as functioning as an antioxidant and anti-inflammatory agent, as well as playing a significant role in bone development and reducing bone loss in the mouth.[26-28] It increases bone formation by stimulating the proliferation of osteoblasts. It also has paracrine effects on surrounding cells and promotes other antioxidant enzymes. Melatonin, acting as an immunomodulator, regulates the release of interleukin-2 (IL-2) and interferon-alpha while also promoting the production of type I collagen and bone growth. As a hormone, it has demonstrated potent anti-inflammatory properties with minimal adverse effects.[29-33]

As per the author's knowledge, no study in the literature has been conducted on the Periodontal healing of Periodontitis in immunosuppressed rats when combining the topical application of both melatonin and PBM. Based on previously mentioned properties of melatonin, the aim of the current study was to evaluate the effect of PBM as well as topical application of melatonin as adjunct treatment of immunosuppression-associated periodontitis on rats.

Material and Methods

Selection of the Animal

Thirty-six male three-month-old Wistar rats (Rattus norvegicus, Albinus) weighting 240-260 g were used in the present study. The animals were kept in cages in a suitable animal environment with controlled temperature (22 ± 2 °C), humidity (55 ± 5%.), and light /dark cycles (12/12 hours).

The rats were housed in plastic suitable cages in groups of six and monitored every day, receiving feed and water advert libitum.

Induction of Immunosuppression

Induction of rat immunosuppression was induced according to the protocol of de Almeida et al[34] by using subcutaneous injections of dexamethasone at a concentration of 2 mg/kg body weight/ day (Dexasone® 0.2 %, MEMPHIS pharm & chemical ind. Egypt) three times for three successive days with a total dose of 6 mg/kg.[34]

Experimental Periodontitis Induction

Induction of periodontitis was induced according to Hu et al.[35] The lower incisors were ligated with four sterile silk threads with a non-resorbable quality in a figure of eight shapes, serving as a means to promote the retention of biofilm and accumulation of food particles, while also acting as a nidus for a plaque build-up, ultimately leading to the development of periodontitis.[35]

Anaesthesia of the Rats

According to Barriga-Rivera et al, the rats were anesthetized by a combination of ketamine hydrochloride (70 mg/kg) and xylazine hydrochloride (6 mg/kg) intramuscularly into the femoral musculature of the hind leg, followed by intraorally local anaesthesia using 2 % lidocaine containing 1:100 000 adrenaline.[36]

Melatonin Administration

In the current study, the rats received two melatonin sheets containing 3 mg of melatonin (each sheet contains 1.5 mg). Melatonin (Melacryst, Ora-dispersible films, Crystal pharma, 6th industrial zone, 6th of October, Giza, Egypt) was used locally as a graft in a periodontal defect.[29-32]

Photobiomodulation With a 650 nm Diode Laser

In the current study, the periodontal defect area was irradiated with the gallium aluminum arsenide semiconductor diode laser device (650 nm, P.100 mW, Woodpecker LX 16 plus, China). The laser beam was delivered with a biostimulation tip with a diameter of 8 mm in continuous emission mode in direct contact with the periodontal defect. A low-intensity laser was applied to the labial side and lingual side of the lower incisor in contact with the gingival tissue. The laser was released for 30 seconds for the labial side and 30 seconds for the lingual side, with a total of three laser sessions (day on – day off)[15,25] (Table 1).

Study Groups

Thirty-six rats had been randomly and equally divided into six groups as follows:

- Group I (control): In the control group, the rats were left without periodontitis or immunosuppression or any intervention.
- Group II (periodontitis): The rats in this group

Table 1. Laser Parameters Used in This Study for Photobiomodulation

Parameters	Diode Laser
Wavelength	650 nm (Woodpecker LX 16 plus, China)
Mode	Continuous wave
Power	100 mw
Tip	Biostimulation tip
Position	2 mm from the tooth surface
Exposure time	30 seconds for two times with total exposure time one minute
Frequency (Number) of laser sessions	Three times (day one and day off)

received induced periodontitis and conventional treatment of SRP

- Group III (immunosuppressed with periodontitis): The rats received SRP only.
- Group IV: The immunosuppressed rats received SRP and PBM by irradiation with 100 mw of the 650 nm diode laser for one minute, which was repeated for three sessions.
- Group V: The immunosuppressed rats received SRP and topical melatonin membrane (3 mg).
- Group VI: The immunosuppressed rats with periodontitis received SRP and combined application of topical melatonin, and they were irradiated with 100 mw of the 650 nm diode laser for one minute, which was repeated for three sessions.

Each group was evaluated at one week, two weeks and four weeks.

Histological and Histometric Analysis

After the sacrifice at the assigned dates, the specimens were demineralized for 15 days in a solution of equal parts of 50% formic acid and 20% sodium citrate. Serial sections (6 µm) were obtained in a mesio-distal direction, embedded in paraffin, and stained with haematoxylin and eosin (HE) for descriptive analysis and Masson's trichrome for histochemical analysis, where the newly formed collagen and osteoid were represented with blue or green reaction and cellular cytoplasm was represented by red reaction.[37,38]

Statistical Analysis

Categorical data were presented as frequency and percentage values and were analysed by using the chi-square test. The significance level was set at $P \leq 0.05$ within all tests. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Criteria for Histological Evaluation

A pathologist interpreted the histological slides blindly without knowing the specific periodontal ligament healing technique or time interval related to each slide, for inflammation severity, bone apposition, cementum formation and periodontal ligament formation. The slides were graded according to the criteria and the scoring system developed by Oortgiesen et al,[38] as listed in Table 2, and then histological photographs were examined for inflammatory response and periodontal healing.

Results

In the control group, the periodontal ligament confirmed dense collagen bundles with flattened fibroblasts among them. Few numbers of inflammatory cells infiltrated the connective tissues, with constrained vascularity, and the interstitial spaces were minimal (Figures 1A and B).

The periodontitis group exhibited detachment of periodontal ligament and intense inflammatory cell infiltration with increased vascularity (Figures 2 and 3).

In the immunosuppressant group, disorganized periodontal ligament showed severe infiltration of inflammatory cells and dilatation of blood vessels with extravasated RBCs. The periodontal fibers showed detachments with increased spaces between the cells showing increased vascularity (Figures 2 and 3).

In all experimental groups, there were chronic inflammatory responses in the inflamed periodontium (score 1) that tend to decrease considerably at 4 weeks (Figures 2 and 3). Inflammatory responses were found in immunosuppressed, PBM, melatonin and combined group of PBM and melatonin in descending order. Most of the combined group showed no or very few scattered inflammatory cells at 4 weeks (Figures 1, 2 and 3). Periodontal healing was not observed in any group after one week. At 2 weeks, immature connective tissue showed fibroblasts with few inflammatory cells (Figures 2 and 3). At 4 weeks, tissues showed normal architecture of periodontal healing (Figures 2 and 3).

The histological results were presented in the study as follows:

Histological Results of Inflammation

Intergroup Comparison for Inflammation

For one week, the difference between groups was not

Table 2. Grading System for Histological Scoring of Inflammation and Periodontal Healing by Oortgiesen et al[38]

Response	Score	Description
Inflammation	0	Cannot be evaluated because of infection or other factors not necessarily related to the material.
	1	Masses of inflammatory cells.
	2	Many inflammatory cells showing some fibroblasts.
	3	Immature connective tissue showing fibroblasts with few inflammatory cells.
	4	Normal appearance of connective tissue with few inflammatory cells.
Periodontal healing	0	No PDL regeneration, no connective tissue adhesion, random supracrustal fibber orientation, and epithelial downgrowth
	1	No PDL regeneration but CT adhesion with perpendicular Fiber orientation supracrustal.
	2	Evidence of PDL regeneration limited to the apical part of the defect and CT adhesion with perpendicular Fiber orientation supracrustal.
	3	Complete PDL regeneration

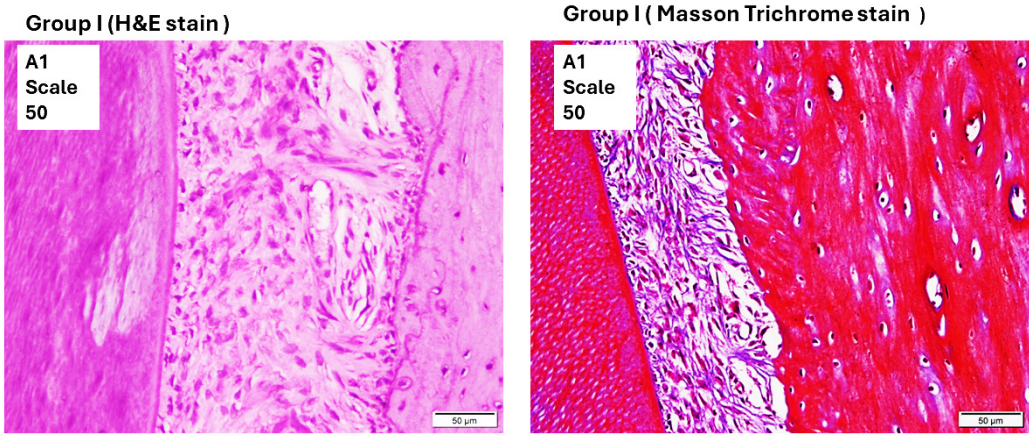

Figure 1. Histological H&E (A1) and Stained Photomicrographs of the Control Group. The control group shows normal alveolar bone architecture, with viable osteocytes and regular outline. The Periodontal ligament reveals dense collagen bundles running in their normal directions, with no inflammatory cells

statistically significant ($p > 0.05$). After two weeks and four weeks, there was a significant difference between score values measured at different intervals ($P = 0.005$) (Table 3; Figures 1 and 2).

In addition, there was a statistically significant difference between different treatment groups and group VI (combination of melatonin and PBM) ($P < 0.05$). Also, there was no statistically significant difference between group IV and group V, as shown in Table 3 and Figures 1 and 2 ($P > 0.05$).

Inter-subgroup Comparison for Inflammation

For group VI, combination of melatonin and PBM, there was a significant difference between scoring values of inflammation at different evaluation periods with significantly higher inflammatory scores after one week in comparison to the total absence of inflammation in 100% of the specimens after four weeks ($P < 0.05$). For group IV and group V, the difference was also statistically significant at different evaluation periods ($P > 0.05$) (Figures 1 and 2; Table 3).

Histological Results of Periodontal Ligament Healing

The comparison of frequency and scoring percentage values in the study groups for the periodontal ligament healing is presented in Table 4.

A. Intergroup Comparison

For one week, the difference between the groups was not statistically significant (P value is more than 0.05), as there was no periodontal ligament formation in all groups after one week. After two weeks, periodontal ligament (PDL) fibers formed and were limited to the apical part of the periodontal defect. After four weeks, there was a significant periodontal healing in all the groups (Figures 1 and 2; Table 4).

Furthermore, there was a statistically significant difference between different treatment groups and group VI (combination of melatonin and PBM) ($P < 0.05$).

Also, there was no statistically significant difference between group IV and group V, as shown in Table 5 ($P > 0.05$), Figures 1 and 2, and Table 4.

B. Inter-subgroup Comparison

For group VI, combination of melatonin and PBM, there was a significant difference at different intervals with significantly highest periodontal ligament formation after four weeks (80% scores 3 and 20% score 2) in comparison to the total absence of the periodontal ligament in 100% of the specimens after one week (the value of P is less than 0.05). For groups IV and V, the difference was not statistically significant (the value of p is more than 0.05) (Figures 1 and 2; Table 4)

Discussion

Periodontitis is a chronic infectious disease characterized by the destruction of periodontal tissues, usually causing gum inflammation, peripheral pocket formation, alveolar bone resorption, tooth loosening, and displacement, and it is the leading cause of tooth loss in adults.[7]

The extent of the periodontal destruction can be influenced by means of different factors affecting the pathogenesis of periodontitis. Systemic diseases might affect the intensity and progression of periodontitis. Hosts' immune and inflammatory response play crucial roles in the progression of periodontal diseases throughout the periodontium.[10]

Glucocorticoids are powerful anti-inflammatory and immunosuppressive medications. Synthetic glucocorticoids (dexamethasone) have been widely used for many years to treat many diseases such as autoimmune, pulmonary, and gastrointestinal (GIT) disorders.[5] However, the non-stop use of glucocorticoids has been considered a predisposing risk factor for other illnesses such as osteoporosis and periodontal diseases.[6]

Various adjunctive therapies accompanying SRP have been proposed as ways to obtain better outcomes. Antibiotics are widely used for adjunctive therapy and

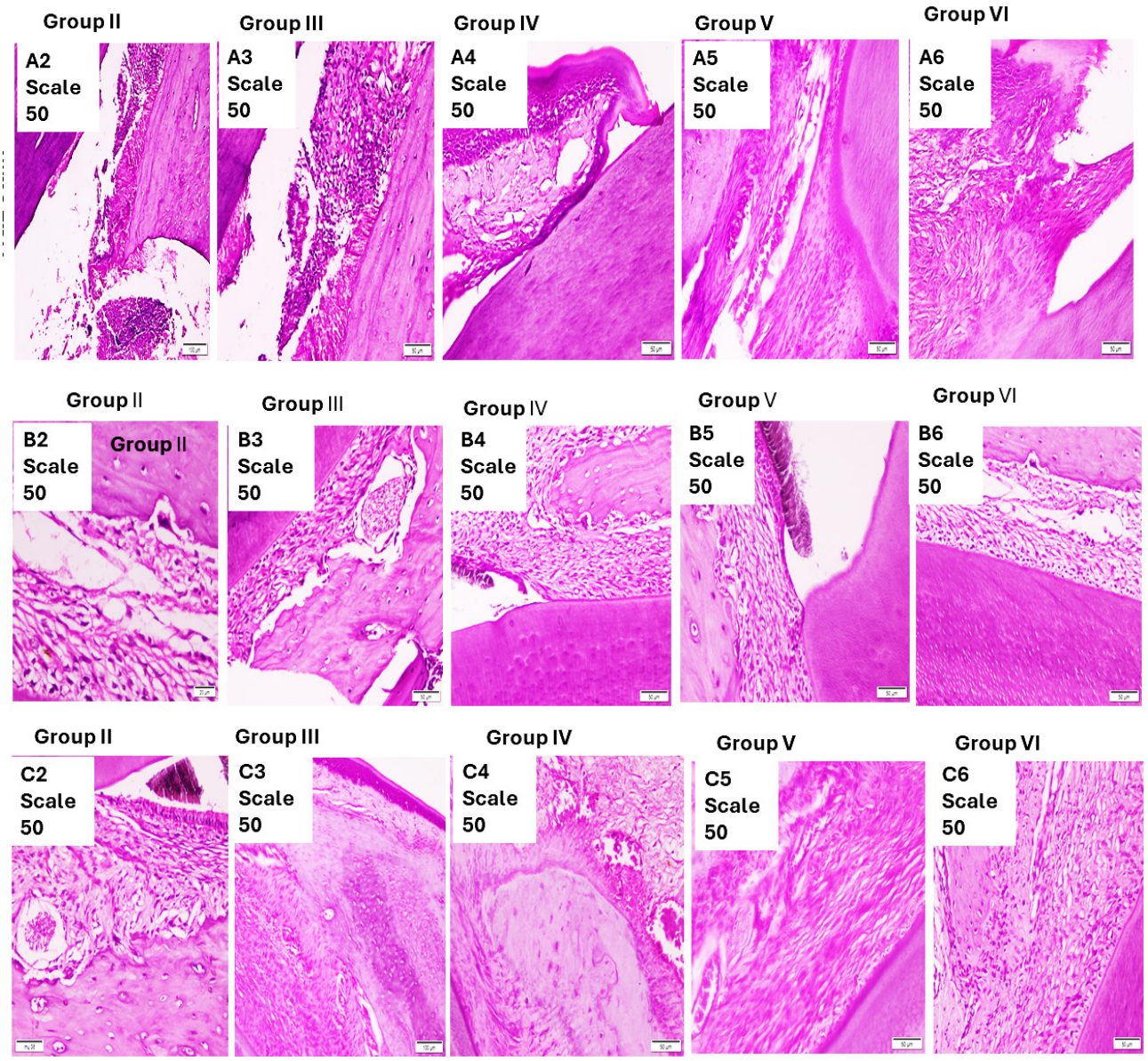

Figure 2 . A. Histological H&E-Stained Photomicrographs of the Treatment Groups, Periodontitis, and Immunosuppressant, PBM, Melatonin, PBM + Melatonin at the End of One Week. The experimental groups reveal thin resorbed bony trabeculae, with massive inflammatory infiltrate in the widened bone marrow spaces and dilated blood vessels (BV) and no periodontal ligament regeneration. B. Histological H&E-stained photomicrographs of the treatment groups, periodontitis, and immunosuppressant, PBM, melatonin, PBM + melatonin at the end of two weeks. The experimental groups reveal new periodontal ligament fibber formation with moderate inflammatory cells and a moderate number of blood vessels (BV). C. Histological H&E-stained photomicrographs of the experimental treated groups at the end of the treatment period (4 weeks) show the active formation of considerable osteoid bone. The periodontal ligament (PDL) reveals collagen fibers running in different directions, with few inflammatory cells., normal alveolar bone architecture, with viable osteocytes and regular outline (BV).

provide an additional effect compared to SRP alone. However, systemic side effects and reinfection are noted as disadvantages of antibiotics. PBM therapy has been used as an adjunctive periodontal treatment.[15-17] Many ranges of laser wavelengths have been used for promoting wound healing.[15] A low-energy red laser was reported to promote the growth of human fibroblasts and enhance blood flow, lowering inflammatory cytokine levels.[19] Moreover, it promoted proliferation and osteogenic differentiation in periodontal ligament stem cells.[25]

In this study, a 650 nm diode laser with 100 mW output power was used three times (working day, resting day) for one minute each. In particular, the data often lack comprehensive information about each usage and number of sessions. Data suggest that low-intensity laser therapy in combination with conventional treatments may be effective in treating periodontal disease.[18]

PBM parameters and values used in this study are from Theodoro et al, and they are the same as those suggested by other authors, who used a low-power diode laser as a treatment modality and adjunctive treatment in periodontal inflammation in mice and concluded that PBM with a low-power laser has a significant impact on the healing process and tissue repair.[39] In addition,

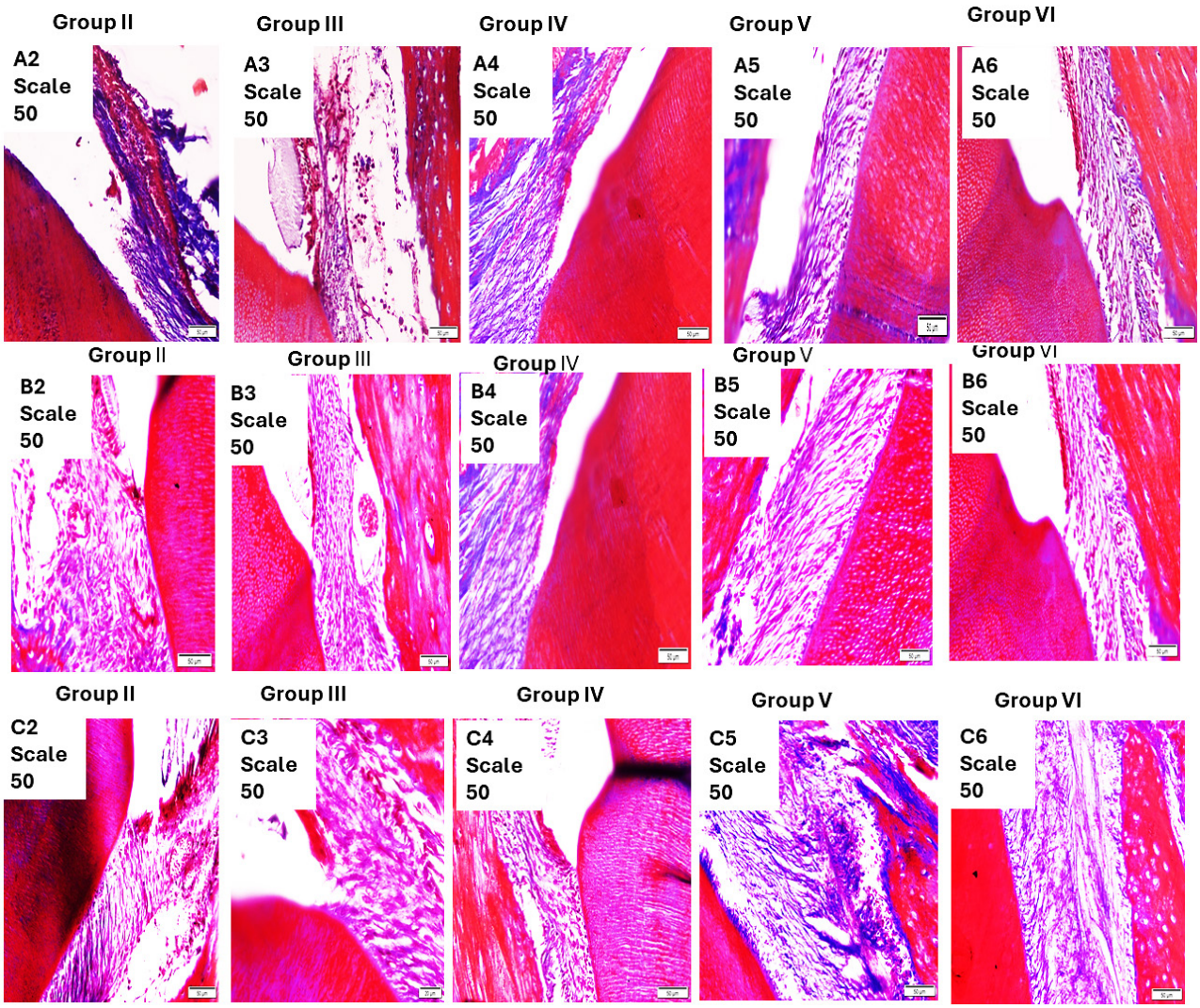

Figure 3. A. Histological Masson trichrome- stained photomicrographs of the treatment groups at the end of one week. The experimental groups reveal thin resorbed bony trabeculae, with massive inflammatory infiltrate in the widened bone marrow spaces and dilated blood vessels (BV) and no periodontal regeneration (scale 100). B. Histological Masson trichrome-stained photomicrographs of the treatment groups, periodontitis, and immunosuppressant, PBM, melatonin, PBM+melatonin at the end of two weeks. The experimental groups reveal new periodontal ligament fibber formation with moderate inflammatory cells and a moderate number of blood vessels (scale 100). C. Histological Masson trichrome-stained photomicrographs of the experimental treated groups at the end of the treatment period (4 weeks) show the active formation of considerable osteoid bone. The periodontal ligament reveals dense collagen bundles running in their normal directions, with no inflammatory cells and few blood vessels (BV) (scale 100).

melatonin is also used in the treatment of periodontal diseases. Melatonin is a pleiotropic substance widely used in sleep therapy and considered as a host modulator during treatment due to its anti-inflammatory, antioxidant and bone-healing properties. There is increasing evidence from clinical and laboratory studies demonstrating the benefits of melatonin for physical health and healthcare. [26-33] However, our current understanding of melatonin is still inadequate. There are no standard guidelines for the clinical use of melatonin in body therapy. Although melatonin is safe for short- and long-term use, some people may experience mild side effects such as dizziness, headache, nausea, and sleepiness.[27-30] Therefore, delivery may be necessary for oral application. Given this, the aim of this study was to evaluate the effect of topical melatonin application and PBM on periodontitis in immunocompromised mice using a 650 nm red diode laser.

In the current study, rats were used as they are considered the desired species for biomedical animal studies because of their anatomical, physiological, and genetic similarity to humans. Similarly, they were included due to their small size, ease of maintenance, and short life cycle.[34]

Periodontitis induced by ligature, a highly advanced model widely used to measure the progression of periodontitis, was used in this study. The pathogenesis of periodontitis in this model is similar to that in humans. In addition, ligatures can cause plaque formation, flattening and displacement of the gingival ridge, proliferation of epithelial cells within the tissue, and initiation of infiltration of mononuclear inflammatory cells.[34] The development and risk of plaque-induced periodontitis has

Table 3. Intragroup and Intergroup Comparison of Histological Percentage of Inflammation at Different Study Periods

Period	Group					*P* Value
	Group II	Group III	Group IV	Group V	Group IV	
At 1 week (mean±SD)	100±3.45.2	80±1.6	60±4.1	65±1.78	60±1.34	0.1
At 2 weeks (mean±SD)	80±2.8	50±3.2	35±1.9	30±2.2	20±1.4	0.01
At 4 weeks (mean±SD)	60±1.5	40±2.6	20±4.2	30±4.8	10±3.8	0.01
P value	0.1	0.01	0.01	0.011	0.01	

Table 4. Intragroup and Intergroup Comparison of the Mean Percentages of Periodontal Healing at Different Study Periods

Period	Group					*P* Value
	Group II	Group III	Group IV	Group V	Group IV	
At 1 week (mean±SD)	0±0	0±0	0±0	0±0	0±0	0.5
At 2 weeks (mean±SD)	10±2.8	30+3.2	35±1.9	40±2.6	50±1.2	0.1
At 4 weeks (mean±SD)	30±1.9	50±2.8	60±3.6	65±2.4	80±3	0.01
P value	0.1	0.01	0.012	0.01	0.001	

Table 5. Results of Periodontal Healing by Grading System According to Oortgiesen et al[38]

Group	Score	One Week		Two Weeks		Four Weeks		*P* Value
		No.	%	No.	%	No.	%	
Group II	0	10	100%	1	10%	0	0	0.01
	1	0	0%	6	60%	1	10%	
	2	0	0%	3	30%	5	60%	
	3	0	0%	0	0%	**3**	30%	
Group III	0	10	100.0%	3	30.0%	**2**	20.0%	0.01
	1	0	0.0%	5	50.0%	**3**	30.0%	
	2	0	0.0%	2	20.0%	5	50.0%	
	3	0	0.0%	0	0.0%	0	0.0%	
Group IV	0	10	100.0%	0	20.0%	0	10.0%	0.03
	1	0	00.0%	6	50.0%	2	20.0%	
	2	0	00.0%	4	30.0%	2	20.0%	
	3	0	00.0%	0	0.0%	6	60.0%	
Group V	0	10	100.0%	1	10.0%	0	10.0%	0.005
	1	0	0.0%	6	60.0%	2	20.0%	
	2	0	0.0%	3	30.0%	3	30.0%	
	3	0	0.0%	0	0.0%	5	50.0%	
Group VI	0	10	100.0%	0	0.0%	0	0.0.0%	0.001*
	1	0	00.0%	6	60.0%	0	20.0%	
	2	0	00.0%	4	40.0%	2	20.0%	
	3	0	0.0%	0	0.0%	8	80.0%	
P value		0.05		0.05		<0.001*		

P>0.05: Non-significant (NS); *P*<0.05: Significant (S); *P*<0.01: Highly significant (HS).
*. Chi-square test.

been reported.[34]

The side effects of periodontitis were more severe in the control group (dexamethasone group). This is consistent with previous studies showing that ligation, which is considered a good method, causes inflammation and alveolar bone loss.[17]

Regarding the inflammatory response in the current study, the combined group of melatonin and PBM showed statistically significant difference when compared with other treatment groups. This was in agreement with previous studies, as PBM can modulate the immune system and decrease the inflammatory response.[15-17] Also, melatonin has anti-inflammatory and antioxidant effects.[30-33,40]

In the current study, there was no significant difference as regards the inflammatory response in the PBM group and in the melatonin group at different evaluation times. This may be due to the anti-inflammatory effect of PBM in group IV (PBM group). Also, melatonin had an anti-inflammatory effect in group V (melatonin group). This result is in accordance with previous studies.[16,30]

In the present study, the immunosuppression group without treatment represented a high intensity of inflammatory cell infiltration with a severe inflammatory response after one week due to the effect of immunosuppression by dexamethasone as it decreased angiogenesis and capillary proliferation, which reduced the blood flow. This is in accordance with previous studies.[3-6]

Regarding the periodontal healing, the superior results of group VI (combination of PBM and melatonin), especially after 4 weeks, with a high periodontal healing score may be mainly related to PBM ability to stimulate gingival fibroblast, periodontal ligament fibroblast proliferation, organization, maturation, and increased production of growth factors, as well as stimulating cementoblast for cementum formation and osteoblast for bone formation, which was in accordance with previous studies.[5-17,25] Also, melatonin promoted the periodontal healing process, which was similar to the results of other studies.[30-36,40]

The periodontal healing in group VI is the best healing in the study; this may be due to the synergistic effect of PBM and melatonin on the stimulation of periodontal tissue regeneration The healing in the control group (dexamethasone without any type of treatment) represents the least healing in the study. This may be because of dexamethasone effects which alter the fibroblast functions, slowing down their migration, disrupting type-I and type-II pro-collagen formation by altering mRNA and mitotic activity. This finding is also consistent with previous studies.[4-6,34]

Many studies have shown that diode laser application has bactericidal and detoxifying properties and this technology has been shown to provide clinical benefits as an adjunct to non-surgical treatments.[18] Low intensity laser therapy promotes healing through collagen synthesis and angiogenesis.[17-20] This study shows that both melatonin and PBM, as an adjunctive treatment for periodontal disease, are effective in reducing inflammation and improving the healing of periodontal disease in immunocompromised mice. These findings support the importance of designing and incorporating the use of PBM therapy and melatonin in dental care in large populations, especially in the presence of chronic pain from infectious diseases. Further research is also needed to evaluate the effects of long-term whole-body PBM. There are also many studies on the standard method for melatonin treatment in medicine.

Limitations

Due to the limitations of an animal experiment, such as differences in metabolism between animals and humans, the data presented in this study should be interpreted with caution. This test may also support further research into specific biomarkers expressed in tissues that are affected by the immune system.

Recommendations

Additional and expiring studies will evaluate treatments or adjunctive treatments that can inhibit the negative effects of the immune system on tissues.

Conclusion

Based on the results of the current study, using PBM with a 650 nm diode laser with output power of 100 mw for one minute for three sessions, combined with topical applications of melatonin, in the treatment of periodontitis of immunosuppressed rats resulted in a significant better histological response (presented by lower inflammatory response and better periodontal healing than using PBM alone or melatonin).

In this way, the combination of PBM and melatonin can be considered a beneficial adjunct in the periodontal regeneration of periodontitis in immunosuppression.

Authors' Contribution

Conceptualization: Latifa Mohamed Abdelgawad, Yomna Gamal Mahmoud Ibrahim Salem**,** El-Sayed Abd Allah El Tayeb.
Data curation: Latifa Mohamed Abdelgawad.
Formal analysis: Latifa Mohamed Abdelgawad, El-Sayed Abd Allah El Tayeb.
Methodology: Latifa Mohamed Abdelgawad, Yomna Gamal Mahmoud Ibrahim Salem and El-Sayed Abd Allah El Tayeb.
Resources: Latifa Mohamed Abdelgawad, Yomna Gamal Mahmoud Ibrahim Salem.
Software: Yomna Gamal Mahmoud Ibrahim Salem.
Supervision: Latifa Mohamed Abdelgawad**,** El-Sayed Abd Allah El Tayeb**.**
Validation: Latifa Mohamed Abdelgawad, Yomna Gamal Mahmoud Ibrahim Salem.
Visualization: Latifa Mohamed Abdelgawad, Yomna Gamal Mahmoud Ibrahim Salem and El-Sayed Abd Allah El Tayeb.
Writing–original draft: Latifa Mohamed Abdelgawad, Yomna Gamal Mahmoud Ibrahim Salem and El-Sayed Abd Allah El Tayeb.
Writing–review & editing: Latifa Mohamed Abdelgawad.

Competing Interests

There are no competing interests.

Ethical Approval

The research was approved by Cairo University's Institutional Animal Care and Use Committee, Cairo University, Egypt, with identification number code (CU-IACUC CU I F 22 21). All the procedures of the study were performed in accordance with the regulation and guidelines of international and institutional animal care, Cairo University. All efforts were made to minimize animal suffering and to reduce the number of animals used in the present study.

Funding
This research received no external funding.

References

1. Shawky HA, Essawy MM. Myricitrin: a promising herbal therapy for periodontitis in immunosuppressed status. Saudi Dent J. 2023;35(5):515-24. doi: 10.1016/j.sdentj.2023.04.002.
2. Sahebari M, Latifian Allaf F, Nasrabadi N, Ghazi A, Khodashahi M, Absalan F, et al. Periodontal disease in patients with newly diagnosed systemic lupus erythematosus. Mediterr J Rheumatol. 2023;34(3):315-21. doi: 10.31138/mjr.20230724.pd.
3. Brasil-Oliveira R, Cruz ÁA, Sarmento VA, Souza-Machado A, Lins-Kusterer L. Corticosteroid use and periodontal disease: a systematic review. Eur J Dent. 2020;14(3):496-501. doi: 10.1055/s-0040-1713954.
4. Schmalz G, Berisha L, Wendorff H, Widmer F, Marcinkowski A, Teschler H, et al. Association of time under immunosuppression and different immunosuppressive medication on periodontal parameters and selected bacteria of patients after solid organ transplantation. Med Oral Patol Oral Cir Bucal. 2018;23(3):e326-34. doi: 10.4317/medoral.22238.
5. Larsen B, Cox A, Colbey C, Drew M, McGuire H, Fazekas de St Groth B, et al. Inflammation and oral contraceptive use in female athletes before the Rio Olympic Games. Front Physiol. 2020;11:497. doi: 10.3389/fphys.2020.00497.
6. Jafri Z, Bhardwaj A, Sawai M, Sultan N. Influence of female sex hormones on periodontium: a case series. J Nat Sci Biol Med. 2015;6(Suppl 1):S146-9. doi: 10.4103/0976-9668.166124.
7. Zhang Y, Jia R, Zhang Y, Sun X, Mei Y, Zou R, et al. Effect of non-surgical periodontal treatment on cytokines/adipocytokines levels among periodontitis patients with or without obesity: a systematic review and meta-analysis. BMC Oral Health. 2023;23(1):717. doi: 10.1186/s12903-023-03383-3.
8. Izidoro C, Botelho J, Machado V, Reis AM, Proença L, Barroso H, et al. Non-surgical periodontal treatment impact on subgingival microbiome and intra-oral halitosis. Int J Mol Sci. 2023;24(3):2518. doi: 10.3390/ijms24032518.
9. Anoixiadou S, Parashis A, Vouros I. Minimally invasive non-surgical technique in the treatment of intrabony defects-a narrative review. Dent J (Basel). 2023;11(1):25. doi: 10.3390/dj11010025.
10. Joseph P, Prabhakar P, Holtfreter B, Pink C, Suvan J, Kocher T, et al. Systematic review and meta-analysis of randomized controlled trials evaluating the efficacy of non-surgical periodontal treatment in patients with concurrent systemic conditions. Clin Oral Investig. 2023;28(1):21. doi: 10.1007/s00784-023-05392-6.
11. Yang X, Liu P, Fan X, Yu S, Chen C, Pan Y, et al. Distribution and influencing factors on residual pockets of the teeth in patients with periodontitis following non-surgical periodontal treatment: a retrospective observational study. BMC Oral Health. 2023;23(1):736. doi: 10.1186/s12903-023-03248-9.
12. Corbella S, Calciolari E, Donos N, Alberti A, Ercal P, Francetti L. Laser treatments as an adjunct to non-surgical periodontal therapy in subjects with periodontitis and type 2 diabetes mellitus: a systematic review and meta-analysis. Clin Oral Investig. 2023;27(4):1311-27. doi: 10.1007/s00784-023-04873-y.
13. Qiu J, Wang X, Zhou H, Zhang C, Wang Y, Huang J, et al. Enhancement of periodontal tissue regeneration by conditioned media from gingiva-derived or periodontal ligament-derived mesenchymal stem cells: a comparative study in rats. Stem Cell Res Ther. 2020;11(1):42. doi: 10.1186/s13287-019-1546-9.
14. Kao RT, Nares S, Reynolds MA. Periodontal regeneration - intrabony defects: a systematic review from the AAP Regeneration Workshop. J Periodontol. 2015;86(2 Suppl):S77-104. doi: 10.1902/jop.2015.130685.
15. Yakout BK, Kamel FR, Khadr M, Heikal LAH, El-Kimary GI. Efficacy of hyaluronic acid gel and photobiomodulation therapy on wound healing after surgical gingivectomy: a randomized controlled clinical trial. BMC Oral Health. 2023;23(1):805. doi: 10.1186/s12903-023-03519-5.
16. Lee D, Lee J, Ahn SH, Song W, Li L, Seol YJ, et al. Adjunctive effect of 470-nm and 630-nm light-emitting diode irradiation in experimental periodontitis treatment: a preclinical study. J Periodontal Implant Sci. 2024;54(1):13-24. doi: 10.5051/jpis.2203580179.
17. Ribeiro IM, de Souza Barroso ME, Kampke EH, Braga LTF, Campagnaro BP, Dos Santos Meyrelles S. Infrared laser therapy decreases systemic oxidative stress and inflammation in hypercholesterolemic mice with periodontitis. Lipids Health Dis. 2023;22(1):171. doi: 10.1186/s12944-023-01934-9.
18. Abesi F, Derikvand N. Efficacy of low-level laser therapy in wound healing and pain reduction after gingivectomy: a systematic review and meta-analysis. J Lasers Med Sci. 2023;14:e17. doi: 10.34172/jlms.2023.17.
19. Misra P, Kalsi R, Anand Arora S, Singh KS, Athar S, Saini A. Effect of low-level laser therapy on early wound healing and levels of inflammatory mediators in gingival crevicular fluid following open flap debridement. Cureus. 2023;15(2):e34755. doi: 10.7759/cureus.34755.
20. AlZoubi IA. An overview of the systematic evidence on the adjunctive use of laser therapy in non-surgical periodontal treatment. Cureus. 2023;15(8):e44268. doi: 10.7759/cureus.44268.
21. Nowak-Terpiłowska A, Zeyland J, Hryhorowicz M, Śledziński P, Wyganowska M. Influence of three laser wavelengths with different power densities on the mitochondrial activity of human gingival fibroblasts in cell culture. Life (Basel). 2023;13(5):1136. doi: 10.3390/life13051136.
22. Abdelgawad LM, Hasan MN, Sabry D, Abdelgwad M. Influence of photobiomodulation and tricalcium silicate-based cement on viability and dentinogenic activity of cultured human dental pulp stem cells. Braz Dent Sci. 2022;25(1):e2497. doi: 10.4322/bds.2022.e2497.
23. Almeida-Junior LA, Teixeira Marques NC, de Oliveira Prado MT, Oliveira TM, Sakai VT. Effect of single and multiple doses of low-level laser therapy on viability and proliferation of stem cells from human exfoliated deciduous teeth (SHED). Lasers Med Sci. 2019;34(9):1917-24. doi: 10.1007/s10103-019-02836-y.
24. Bidar M, Bahlakeh A, Mahmoudi M, Ahrari F, Shahmohammadi R, Jafarzadeh H. Does the application of GaAlAs laser and platelet-rich plasma induce cell proliferation and increase alkaline phosphatase activity in human dental pulp stem cells? Lasers Med Sci. 2021;36(6):1289-95. doi: 10.1007/s10103-020-03239-0.
25. Abdelgawad LM, Abdelaziz AM, Sabry D, Abdelgawad M. Influence of photobiomodulation and vitamin D on osteoblastic differentiation of human periodontal ligament stem cells and bone-like tissue formation through enzymatic activity and gene expression. Biomol Conc. 2020;11(1):172-181.
26. Wang C, Wang L, Wang X, Cao Z. Beneficial effects of melatonin on periodontitis management: far more than oral cavity. Int J Mol Sci. 2022;23(23):14551. doi: 10.3390/ijms232314541.
27. Cho JH, Bhutani S, Kim CH, Irwin MR. Anti-inflammatory effects of melatonin: a systematic review and meta-analysis of clinical trials. Brain Behav Immun. 2021;93:245-53. doi:

10.1016/j.bbi.2021.01.034.
28. Cui Y, Hong S, Xia Y, Li X, He X, Hu X, et al. Melatonin engineering M2 macrophage-derived exosomes mediate endoplasmic reticulum stress and immune reprogramming for periodontitis therapy. Adv Sci (Weinh). 2023;10(27):e2302029. doi: 10.1002/advs.202302029.
29. Konečná B, Chobodová P, Janko J, Baňasová L, Bábíčková J, Celec P, et al. The Effect of Melatonin on Periodontitis. Int J Mol Sci. 2021;22(5):2390. doi: 10.3390/ijms22052390.
30. Balaji TM, Varadarajan S, Jagannathan R, Mahendra J, Fageeh HI, Fageeh HN, et al. Melatonin as a topical/systemic formulation for the management of periodontitis: a systematic review. Materials (Basel). 2021;14(9):2417. doi: 10.3390/ma14092417.
31. Anton DM, Martu MA, Maris M, Maftei GA, Sufaru IG, Tatarciuc D, et al. Study on the effects of melatonin on glycemic control and periodontal parameters in patients with type II diabetes mellitus and periodontal disease. Medicina (Kaunas). 2021;57(2):140. doi: 10.3390/medicina57020140.
32. Bazyar H, Zare Javid A, Zakerkish M, Yousefimanesh HA, Haghighi-Zadeh MH. Effects of melatonin supplementation in patients with type 2 diabetes mellitus and chronic periodontitis under nonsurgical periodontal therapy: a double-blind randomized controlled trial. J Res Med Sci. 2022;27:52. doi: 10.4103/jrms.JRMS_927_19.
33. Yapislar H, Haciosmanoglu E, Sarioglu T, Degirmencioglu S, Sogut I, Poteser M, et al. Anti-inflammatory effects of melatonin in rats with induced type 2 diabetes mellitus. Life (Basel). 2022;12(4):574. doi: 10.3390/life12040574.
34. de Almeida JM, Matheus HR, Fiorin LG, Furquim EM, Gusman DJ. Influence of immunosuppression on the progression of experimental periodontitis and on healthy periodontal tissue: a rat in vivo study. J Dent Res Dent Clin Dent Prospects. 2021;15(2):94-9. doi: 10.34172/joddd.2021.016.
35. Hu Y, Zhang X, Zhang J, Xia X, Li H, Qiu C, et al. Activated STAT3 signaling pathway by ligature-induced periodontitis could contribute to neuroinflammation and cognitive impairment in rats. J Neuroinflammation. 2021;18(1):80. doi: 10.1186/s12974-021-02071-9.
36. Barriga-Rivera A, Tatarinoff V, Lovell NH, Morley JW, Suaning GJ. Long-term anesthetic protocol in rats: feasibility in electrophysiology studies in visual prosthesis. Vet Ophthalmol. 2018;21(3):290-7. doi: 10.1111/vop.12507.
37. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. Methods Mol Biol. 2014;1180:31-43. doi: 10.1007/978-1-4939-1050-2_3.
38. Oortgiesen DA, Meijer GJ, Bronckers AL, Walboomers XF, Jansen JA. Regeneration of the periodontium using enamel matrix derivative in combination with an injectable bone cement. Clin Oral Investig. 2013;17(2):411-21. doi: 10.1007/s00784-012-0743-z.
39. Theodoro LH, Caiado RC, Longo M, Novaes VC, Zanini NA, Ervolino E, et al. Effectiveness of the diode laser in the treatment of ligature-induced periodontitis in rats: a histopathological, histometric, and immunohistochemical study. Lasers Med Sci. 2015;30(4):1209-18. doi: 10.1007/s10103-014-1575-7.
40. Tan YZ, Xu XY, Dai JM, Yin Y, He XT, Zhang YL, et al. Melatonin induces the rejuvenation of long-term ex vivo expanded periodontal ligament stem cells by modulating the autophagic process. Stem Cell Res Ther. 2021;12(1):254. doi: 10.1186/s13287-021-02322-9.