The Saudi Dental Journal (2023) **35**, 515–524

King Saud University

The Saudi Dental Journal

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Myricitrin: A promising herbal therapy for periodontitis in immunosuppressed status

Heba A. Shawky [a,*], **Marwa M. Essawy** [b,c]

[a] *Department of Preventive Dental Sciences, Periodontics Division, College of Dentistry, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia*
[b] *Oral Pathology Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt*
[c] *Center of Excellence for Research in Regenerative Medicine and Applications (CERRMA), Faculty of Medicine, Alexandria University, Alexandria, Egypt*

Received 25 December 2022; revised 8 April 2023; accepted 10 April 2023
Available online 17 April 2023

KEYWORDS

Adjunctive periodontal therapy;
Alendronate;
Bone histomorphometry;
Cyclosporin A;
Immunosuppression;
Myricitrin

Abstract *Background:* Periodontitis is a complex chronic inflammatory disease aggravated in immunosuppressed patients. However, adjuvant therapies can alleviate severe inflammation and slow down disease progression.

Objective: To evaluate the efficacy of myricitrin, a herbal flavonoid glycoside, in reducing immunosuppression-associated periodontitis and compare its effects with that of alendronate on alveolar bone regeneration.

Methods: Fifty albino Wistar rats were randomly allocated to the control, periodontitis, immunosuppressant, myricitrin, and alendronate groups. Ligature-associated periodontitis was induced in all groups, except the control group. Cyclosporin A (CsA) was administered subcutaneously in the immunosuppressant group for immunosuppression. The myricitrin group received CsA and myricitrin, whereas the alendronate group received CsA and alendronate. The therapeutic efficacies of myricitrin and alendronate were compared histologically, morphometrically, and biochemically.

Results: Myricitrin reversed bone destruction in the periodontitis and immunosuppressant groups. Morphometrically, myricitrin showed comparable improvements to alendronate in terms of gaining more bone area to 49.4 ± 4.6 and 59.5 ± 2%, respectively ($P < 0.001$ in relation to

* Corresponding author at: College of Dentistry, Princess Nourah bint Abdulrahman University, King Khalid International Airport road, Riyadh 13414, Saudi Arabia.
E-mail address: haelsabagh@pnu.edu.sa (H.A. Shawky).

Peer review under responsibility of King Saud University. Production and hosting by Elsevier.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sdentj.2023.04.002

the untreated periodontitis group). Concomitantly, myricitrin increased osteoblast count significantly to 28.4 ± 4.7 closer to the 34.5 ± 2.4 count in the alendronate group ($P < 0.001$ compared with 22.5 ± 2.6 count of the immunosuppressant group). Moreover, myricitrin restored the serum calcium to 9.4 ± 0.6 mg/dL and alkaline phosphatase up to 112.9 ± 2.9 IU/L, which were almost normal levels similar to the control cohort ($P > 0.05$).

Conclusion: Myricitrin showed beneficial effects in counteracting bone resorption in subjects with immunosuppression-associated periodontitis. Its efficacy in slowing down disease progression was comparable to that of alendronate.

1. Introduction

Periodontitis, a chronic inflammation of the teeth-supporting tissues, results in progressive bone resorption and pocket formation with/without gingival recession. It involves interactions between the host immune system and periodontopathogens, which are affected by various systemic and environmental factors (Saini et al., 2009). Local inflammation is initiated by the dysbiosis of the microbial biofilm. However, increased activity of the host-immune response directly triggers osteoclastic bone resorption, with the failure of the inflammation-resolving mechanisms (Pan et al., 2019).

Various medications may also affect the progression of periodontal disease. Cyclosporin A (CsA) negatively affects the periodontium. It is an immunosuppressive agent administered in transplantation patients to prevent organ rejection. Furthermore, CsA is the core treatment for several immunologically mediated disorders, such as rheumatoid arthritis, Behçet disease, systemic lupus erythematosus, and insulin-dependent diabetes mellitus. However, it usually has adverse effects, such as hepatotoxicity and nephrotoxicity (Spolidorio et al., 2005).

CsA negatively affects the periodontium throughout the treatment course, inducing gingival overgrowth and cementum formation on the root surfaces (Shen et al., 2005). Although CsA's effect on alveolar bone resorption remains controversial, low concentrations can stimulate osteoblastic differentiation and induce bone formation (Yeo et al., 2007). Notably, CsA administration increased alveolar bone resorption in rats with induced periodontitis (Spolidorio et al., 2004, Nassar et al., 2013). Previous studies have advocated the concomitant administration of antiresorptive drugs to counterbalance the resulting bone resorption (Woo et al., 2008, Rejnmark and Mosekilde, 2011).

Alendronate is an amino-bisphosphonate drug used in immunosuppression to reduce osteoclastic activity. Therefore, it counteracts alveolar bone resorption in periodontitis (Veena and Prasad, 2010). However, alendronate has various short- and long-term side effects. The early side effects include gastroesophageal irritation, musculoskeletal pain, and transient hypocalcemia with secondary hyperparathyroidism. More serious adverse effects include osteonecrosis of the jaw (Kennel and Drake, 2009). Therefore, there is an increased demand for substitutional treatments having minimal side effects.

Different natural pharmaceutical agents have shown positive effects on bone formation and remodeling with minimal adverse effects. These natural therapies improve bone metabolism and induce osteoblastic activity by suppressing osteoclastogenesis. They prevent osteoclast differentiation, thereby downregulating bone resorption (He et al., 2019).

Myricetin-3-O-a-rhamnoside (myricitrin) is a botanical flavone extracted from Chinese bayberries (*Myrica rubra*, *Myrica cerifera*, *Myrica esculenta*, etc.). Myricitrin exerts potent antioxidant activity with robust scavenging of free radicals compared to other flavonol rhamnosides and quercetin (Zhu et al., 2013, Chen et al., 2013). Moreover, it inhibits protein kinase C and nitric oxide with considerable antinociceptive effects (Domitrovic et al., 2015). It hinders the macrophages' production of tumor necrosis factor-alpha (a pro-inflammatory cytokine) (Shimosaki et al., 2011) in addition to its documented anti-myeloperoxidase activity (Meotti et al., 2008).

This *in vivo* study evaluated the therapeutic efficacy of myricitrin in immunosuppression-associated periodontitis. Myricitrin showed an inductive effect on bone formation as a novel approach for managing ligature-induced periodontitis receiving CsA. Its bone regenerative capacity was comparable to that of the well-established alendronate therapy, with a safe biochemical profile.

2. Materials and methods

2.1. Animals

This *in vivo* study included 50 male Albino Wistar rats weighing 280–300 g. The animals were acclimatized for 2 weeks in the animal house of the Medical Research Institute, Alexandria University before the experiment. They were kept in polypropylene cages under 12-h light–dark periods at 24 ± 2 °C with free water and food access. This study was approved by the Alexandria University Ethics Committee for Animal Experimentation. The procedures were performed according to the institutional guidelines (IRB No.: 00010556-IORG0008839) complying with the ARRIVE reporting guidelines.

2.2. Study design

The grouping and methodology are presented in a flow chart (Fig. 1). The rats were randomly and equally divided into five cohorts (n = 10 in each). In the control group, the rats were injected subcutaneously with normal saline daily. Meanwhile, periodontitis was induced in the remaining animals. For this, the rats were first sedated by injecting a combination of 0.1 mL/100 g ketamine hydrochloride (Pharmazeutische Prä-

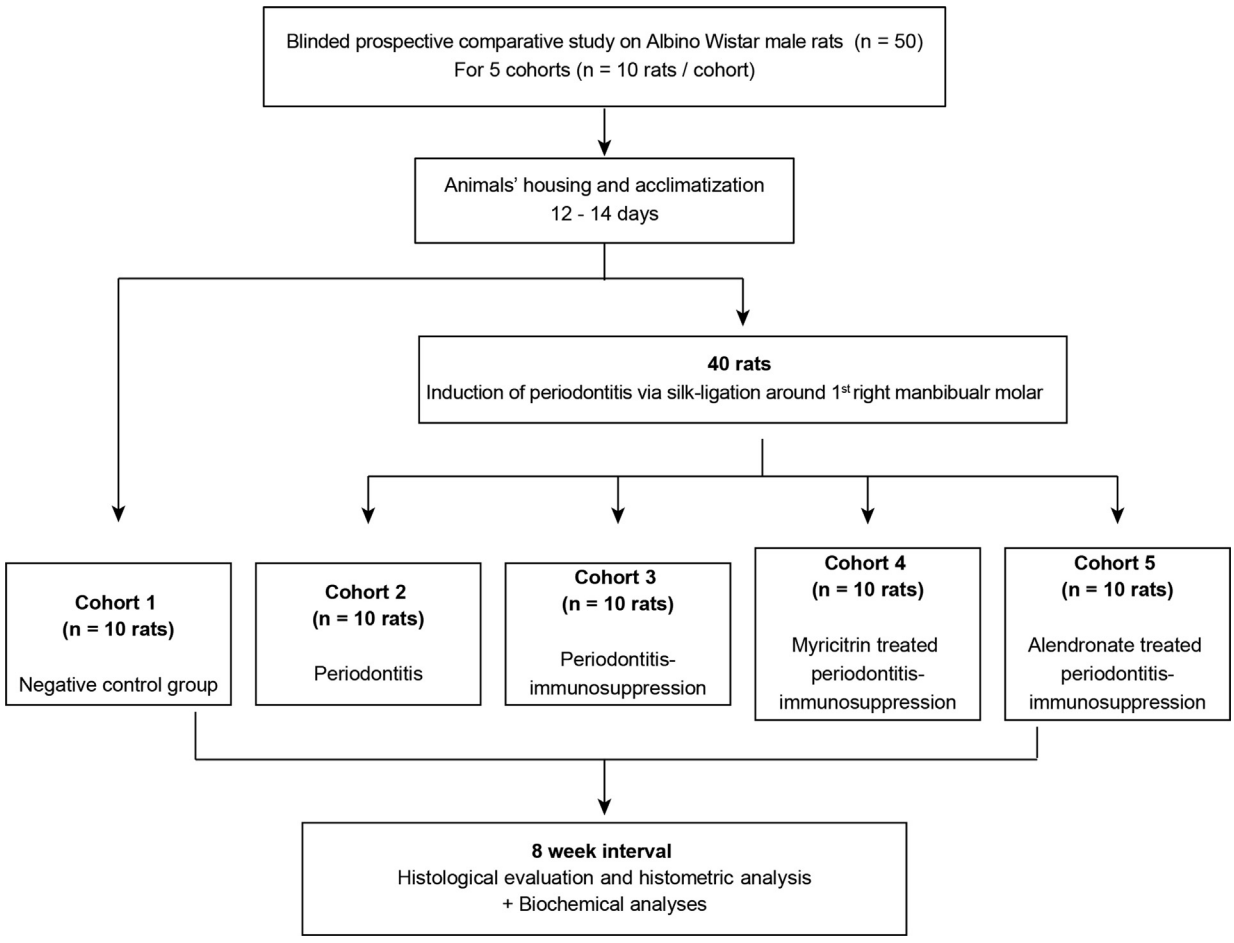

Fig. 1 Schematic diagram of the procedures sequence with the time frame of the study design.

parate, Germany) and 0.05 mL/100 g xylazine hydrochloride (Chanazine, Ireland) intramuscularly. Following this, 4/0 silk (Foosin Medical Supplies Inc., China) ligatures were applied around the lower right first molars to act as a nidus for plaque accumulation to induce periodontitis (Semenoff et al., 2008).

After 2 weeks, these rats were randomly allocated into four cohorts. The periodontitis group received subcutaneous injections of saline daily. The immunosuppressant group received subcutaneous injections of 10 mg/kg CsA (Novartis Pharma AG, Switzerland) daily (Hagar et al., 2015). The myricitrin group received CsA injections and oral administrations of 100 mg/kg of water-dissolved myricitrin (Source Naturals, USA) daily (Godse et al., 2010). The alendronate group received CsA injections and subcutaneous injections of 0.3 mg/kg alendronate (Merck Sharp and Dohme, USA) weekly (Spolidorio et al., 2007).

The treatment period was 8 weeks, after which the animals were sacrificed by overdosing with ketamine and xylazine mixture (100 mg/mL of each) (Schoell et al., 2009).

2.3. Histological examination

The mandibular region bearing the ligated teeth was dissected for histological examination, followed by 48-h fixation in 10% buffered formalin and decalcification for 8 weeks using 10% ethylenediaminetetraacetic acid (pH 7.8). After gradient dehydration, the paraffin-embedded specimens were sectioned with their buccal surfaces oriented toward the cutting plane to obtain serial mesiodistal sections of 4–5 μm from three planes: center of the tooth and 120–180 μm on either side of the center. The slide sections were hematoxylin and eosin (H&E) stained (Nassar et al., 2009).

For gross histological and morphological examination of the periodontium (mainly from the centered cut plane), digital images were captured at × 100 magnification including the mesial, distal, and furcation aspects of the examined tooth (one image per aspect). For detailed qualitative and morphometric evaluation of the alveolar bone and periodontal ligament (PDL), the three predetermined aspects were further divided into six high-power digital images captured at × 400. The images were captured using digital Olympus DP20 camera connected to Olympus BX41 microscope.

2.4. Histomorphometric analysis

The × 400 photomicrographs were interpreted in a double-blind manner using Image J analysis software (1.52p software 32, NIH, USA). The histomorphometric parameters assessed for each section were structural parameters (bone area, osteoblast count, osteoclast count, trabecular width, trabecular number, and trabecular separation), static bone-formation parameters (osteoblast perimeter, osteoid perimeter, and

osteoid area), and static resorption parameters (eroded perimeter and osteoclast perimeter) (Vidal et al., 2012, Dempster et al., 2013).

2.5. Biochemical analysis

For biochemical analysis, blood samples were collected by cardiac puncture during sacrifice. The analysis was performed using the serum stored at − 20 °C. Serum calcium, phosphorus, and alkaline phosphatase levels were analyzed using Siemens Dimension (Germany); colorimetric tests were performed using Dimension Reagents (Siemens, Germany) (Popović et al., 2016).

2.6. Statistical analysis

For histomorphometric parameters, the data retrieved from each sacrificed rat was calculated as the mean of the six high-power photomicrographs. Data were analyzed using Statistical Product and Service Solutions, version 20.0 (IBM Corp., Armonk, NY, USA). For normally distributed quantitative variables, one-way ANOVA was applied, followed by post-hoc Tukey test for pairwise comparison. For non-normally distributed quantitative variables, the Kruskal–Wallis test was applied, followed by a post-hoc Dunn test for multiple comparisons. Data are expressed as means ± standard deviations, with P-values < 0.05 considered statistically significant.

3. Results

3.1. Histological evaluation

The alveolar bone in the control group showed typical architecture, with a smooth border lined with resting flat-shaped osteoblasts and multiple living osteocytes within their lacunae. The PDL showed dense collagen bundles with flattened fibroblasts between them. Overall, the stroma was free of inflammatory cells, with limited vascularity and minimal interstitial spaces (Fig. 2 A and B).

The periodontitis cohort exhibited massive alveolar bone destruction and intense inflammatory cell infiltration. The resorbed bone appeared as thin osteolytic trabeculae with irregular outlines and empty lacunae. An increased osteoclast count was a prominent feature with decreased osteoblastic activity (Fig. 2 C and D).

The immunosuppressant group showed disorganized alveolar bone architecture with osteoclastic activity. The bone marrow showed moderate inflammatory cell infiltration and dilated blood vessels with extravasated red blood cells. The PDL fibers revealed detachments with increased intercellular spaces showing increased vascularity (Fig. 2 E and F).

The myricitrin group showed a newly formed osteoid rim lined with plump active osteoblasts. The accentuated incremental lines—resting lines alternating with reversal lines—showed a mosaic pattern appearance, indicating an active bone remodeling process. Both bone marrow and PDL showed fibroblastic activity with increased vascularity in some sections. However, scattered inflammatory cells were seen within the disorganized PDL fibers. Moreover, some osteoclasts were observed within their lacunae (Fig. 3 A-C).

The alendronate group showed well-organized alveolar bone mimicking the typical architecture with a less prominent mosaic appearance. The bony trabeculae showed increased osteoblastic activity with an almost regular smooth border and living osteocytes. Although newly formed fibroblasts remained evident in some PDL areas, they showed diminished vascularity and minimal interstitial spaces. Neither osteoclasts nor inflammatory cells were detected (Fig. 3 D-F).

3.2. Histomorphometric analysis

The myricitrin and alendronate groups revealed significant differences in the structural parameters when compared with the periodontitis group. The alendronate group recorded a significant gain in the bone area when compared with the periodontitis group (59.5 ± 2% vs. 33.8 ± 4.03%; $P < 0.001$). The myricitrin group showed a similar significant increase in the mean bone area percent (49.4 ± 4.6%; $P < 0.001$), without a significant difference from that of the immunosuppressant group (48.1 ± 6.6%; $P > 0.05$).

The osteoblast counts in the periodontitis (13.6 ± 1.5) and immunosuppressant (22.5 ± 2.6) groups were significantly lower than that in the control group (42.3 ± 5.1). The osteoblast count increased significantly to 28.4 ± 4.7 and 34.5 ± 2.4 in the myricitrin and alendronate groups, respectively ($P < 0.001$). Consequently, the static bone-formation parameters reflected a similar picture, especially the osteoblast perimeter. The osteoblast-lined bone proportion increased significantly in the myricitrin and alendronate groups (64.6 ± 9.7 and 78.2 ± 9.2%, respectively) when compared with the periodontitis group (31.1 ± 7.9%; $P < 0.001$). Although the myricitrin and alendronate groups had similar osteoid perimeter ($P > 0.05$), their values were significantly higher than that of the untreated groups ($P < 0.001$).

Regarding the osteoclasts, massive bone destruction due to periodontitis was reflected by the highest osteoclast count of 3 ± 0.67 in the periodontitis group, which differed significantly from that of the control group (0.7 ± 0.67; $P < 0.001$). The bone resorption slowed down by CsA in the immunosuppressant group significantly decreased the osteoclast count to 1.7 ± 0.8 ($P < 0.001$). In the myricitrin and alendronate groups, the osteoclast count significantly decreased to 1.2 ± 0.9 and 0.8 ± 0.79, respectively ($P < 0.001$). However, the difference in the osteoclast count and static resorption parameters between the myricitrin and alendronate groups was not significant ($P > 0.05$). Both the osteoclast count and eroded perimeters, indicating Howship lacunae, were almost similar in the myricitrin and alendronate groups (Table 1; Fig. 4).

3.3. Biochemical analysis

The efficacy of myricitrin was systematically evaluated by measuring three serum markers (calcium, phosphorus, and alkaline phosphatase).

The serum calcium levels significantly increased in the periodontitis and immunosuppressant groups (10.7 ± 0.4 and 9.8 ± 0.5 mg/dL, respectively) when compared with the control group (9.1 ± 0.5 mg/dL; $P < 0.001$); however, the levels were significantly lower in the immunosuppressant group than in

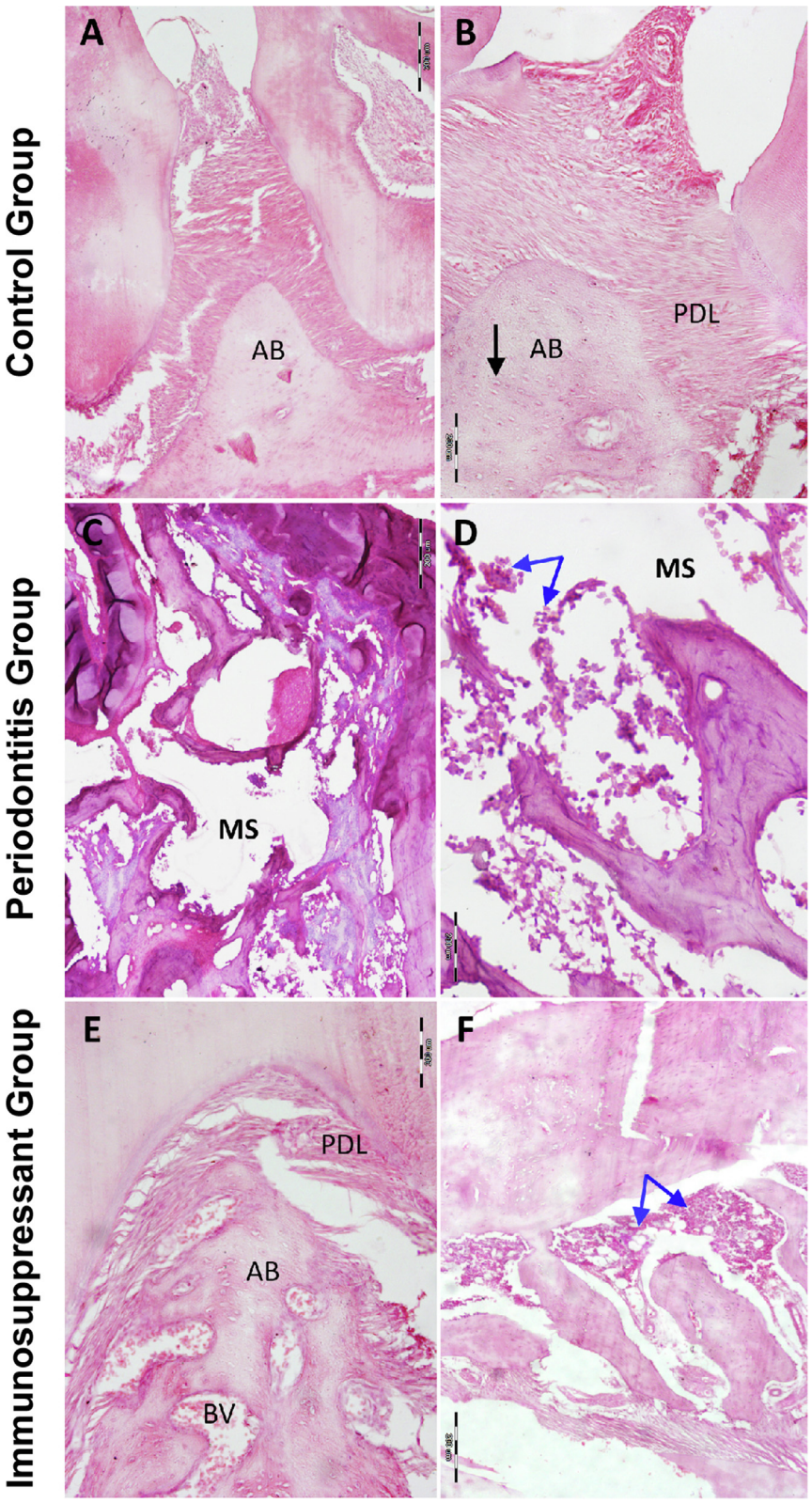

Fig. 2 Histological H&E-stained photomicrographs of the control, periodontitis, and immunosuppressant untreated groups at the end of the treatment period (8 weeks). The control group **(A and B)** shows normal alveolar bone (AB) architecture, with viable osteocytes (black arrow) and regular outline. The PDL reveals dense collagen bundles running in their normal directions, with no inflammatory cells. The periodontitis group **(C and D)** reveals thin resorbed bony trabeculae, with massive inflammatory infiltrate (blue arrows) in the widened bone marrow spaces (MS). The immunosuppressant group **(E and F)** shows irregular outline of alveolar bone architecture, with moderate inflammation in the bone marrow (blue arrows) and dilated blood vessels (BV). (A-F: H&E).

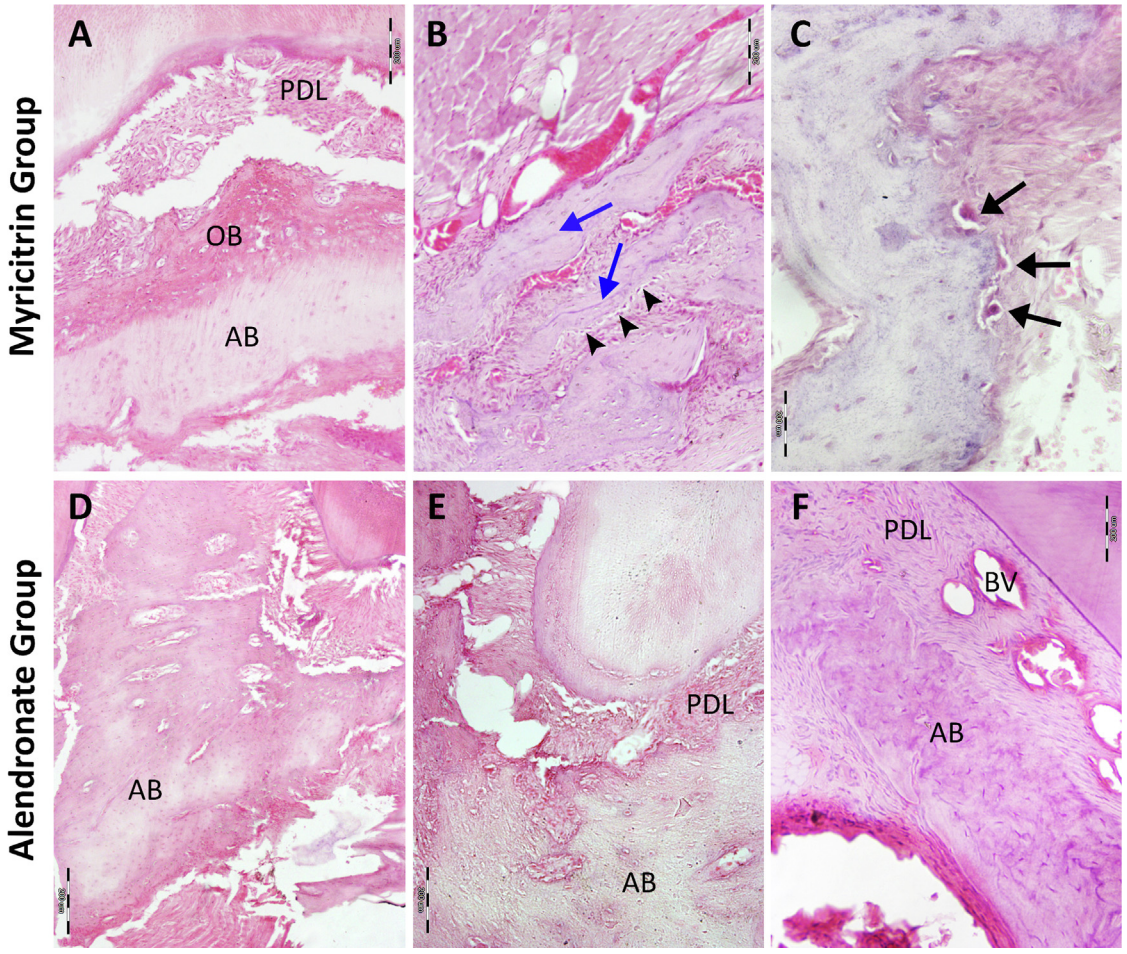

Fig. 3 Histological H&E-stained photomicrographs of the myricitrin and alendronate treated groups at the end of the treatment period (8 weeks). Myricitrin treated group **(A-C)** shows active alveolar bone (AB) formation with considerable osteoid bone (OB). The AB is lined with plump osteoblasts (arrow heads) and reveals prominent incremental lines (blue arrows) and few osteoclasts (black arrows). The PDL reveals collagen fibers running in different directions, with few inflammatory cells. Alendronate treated group **(D-F)** shows normal alveolar bone architecture, with viable osteocytes and regular outline. The PDL reveals dense collagen bundles running in their normal directions, with no inflammatory cells and few blood vessels (BV). (A-F: H&E).

Table 1 The histomorphometric parameters of the different studied groups.

Parameter	Control	Periodontitis	Immune-suppressant	Myricitrin	Alendronate	p
Structural parameters						
Bone area (%)	$71.3^{a} \pm 12.2$	$33.8^{d} \pm 4.03$	$48.1^{c} \pm 6.6$	$49.4^{c} \pm 4.6$	$59.5^{b} \pm 2$	<0.001*
Osteoblast count	$42.3^{a} \pm 5.1$	$13.6^{e} \pm 1.5$	$22.5^{d} \pm 2.6$	$28.4^{c} \pm 4.7$	$34.5^{b} \pm 2.4$	<0.001*
Osteoclast count	$0.70^{c} \pm 0.67$	$3^{a} \pm 0.67$	$1.7^{b} \pm 0.8$	$1.2^{bc} \pm 0.9$	$0.80^{bc} \pm 0.79$	<0.001*
Trabecular thickness (μm)	$0.12^{a} \pm 0.01$	$0.04^{e} \pm 0.01$	$0.05^{d} \pm 0.01$	$0.08^{c} \pm 0.01$	$0.11^{b} \pm 0.01$	<0.001*
Trabecular number (/mm)	$7.8^{d} \pm 0.57$	$26.2^{a} \pm 6.1$	$18.3^{b} \pm 1.4$	$12.2^{c} \pm 0.7$	$9.1^{cd} \pm 0.8$	<0.001*
Trabecular separation (mm)	$0.01^{a} \pm 0.01$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$-0.01^{a} \pm 0.01$	0.110
Static formation parameters						
Osteoblast perimeter (%)	$97.7^{a} \pm 12.03$	$31.1^{d} \pm 7.9$	$58.6^{c} \pm 12.4$	$64.6^{c} \pm 9.7$	$78.2^{b} \pm 9.2$	<0.001*
Osteoid perimeter (%)	$3.2^{b} \pm 2.8$	$5^{b} \pm 4.9$	$9.1^{b} \pm 5.7$	$26.3^{a} \pm 8.4$	$44.8^{a} \pm 8.2$	<0.001*
Osteoid area (%)	$0.13^{cd} \pm 0.09$	$0.08^{d} \pm 0.08$	$0.62^{bc} \pm 0.57$	$1.23^{ab} \pm 0.66$	$2.4^{a} \pm 1.3$	<0.001*
Static resorption parameters						
Eroded perimeter (%)	$15.1^{c} \pm 10.3$	$93.4^{a} \pm 24.01$	$62.4^{b} \pm 4.1$	$58.5^{b} \pm 10.3$	$24.2^{c} \pm 5.01$	<0.001*
Osteoclast perimeter	$11.1^{b} \pm 9.9$	$80.5^{a} \pm 29.1$	$28.2^{b} \pm 12.4$	$28.9^{b} \pm 8.4$	$20.3^{b} \pm 19.7$	<0.001*

Means in the same **raw** with **small common letters** are not significant (i.e., Means with **Different letters** are significant).
Statistical significance is of *P*-value of < 0.05.

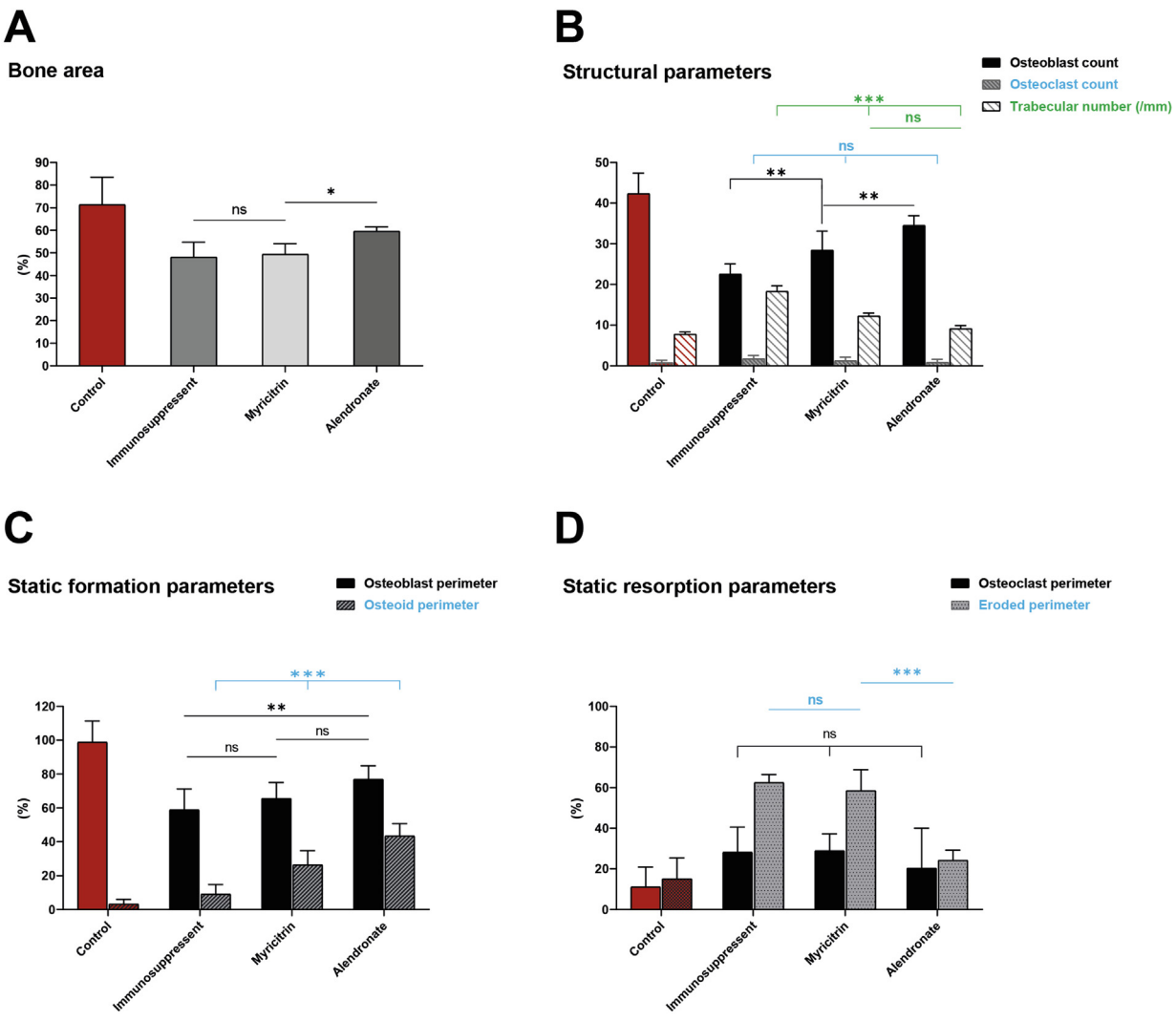

Fig. 4 Bar charts of the different bone histomorphometric parameters show the efficacy of myricitrin in treating periodontitis in immunosuppressant rats. **(A)** In the bone area percent, myricitrin is closer to alendronate with a low significant difference*. **(B and C)** The structural and static formation parameters reveal active bone formation in myricitrin- and alendronate-treated groups, compared with immunosuppressant groups. Myricitrin is more significant than the immunosuppressant group in increasing the osteoblast counts** (shown in B), with concomitant increasing in the percentage of newly bone formed*** (osteoid perimeter shown in C). Furthermore, myricitrin's ability to lay down primitive reparative bone is similar to alendronate (ns displayed in C). **(D)** The static resorption parameters denote the efficacy of myricitrin is similar to alendronate in decreasing the percentage of bone surface lined with osteoclast (osteoclast perimeter, ns). The bone-forming effect of myricitrin and alendronate is further supported by the significant decreases in number of eroded thin bony trabeculae (shown in B), compared with immunosuppressant groups***. Data in all charts are expressed in mean ± SD (error bars). Statistical significance is denoted by an asterisk, where adj-P value of * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Meanwhile ns denotes insignificant adj-$P > 0.05$.

the periodontitis group. The levels in the myricitrin and alendronate groups significantly decreased to 9.4 ± 0.6 and 9.3 ± 0.5 mg/dL, respectively, when compared with the untreated groups ($P < 0.001$). However, the levels in the myricitrin and alendronate groups were similar to those in the control group ($P > 0.05$).

In contrast to the serum calcium levels, the phosphorus levels significantly decreased in the periodontitis and immunosuppressant groups ($P < 0.001$). The phosphorus and calcium levels showed a contrasting picture in the myricitrin and alendronate cohorts. Myricitrin significantly restored the phosphorus level to 8.6 ± 0.6 mg/dL ($P < 0.001$), which was similar to the increase in the alendronate group (8.9 ± 0.9 mg/dL; $P > 0.05$).

The serum alkaline phosphatase levels significantly decreased in the periodontitis and immunosuppressant groups (96.2 ± 3.3 and 102.5 ± 2.0 IU/L, respectively) when compared with the control group (119.9 ± 2.0 IU/L; $P < 0.001$); however, the levels were significantly higher in the immunosuppressant group than in the periodontitis group. Myricitrin elevated the serum alkaline phosphatase significantly to 112.9 ± 2.9 IU/L, which was similar to that in the alendronate group (116.2 ± 3.7 IU/L). Table 2 summarizes the biochemical results of the collected blood samples.

Table 2 The biochemical analysis results of the different studied groups.

	Control	Periodontitis	Immune-suppressant	Myricitrin	Alendronate	p
Serum calcium level (mg/dl)						
Mean ± SD.	9.1^{c} ± 0.5	10.7^{a} ± 0.4	9.8^{b} ± 0.5	9.4^{bc} ± 0.6	9.3^{c} ± 0.5	< 0.001*
Median (Min. – Max.)	9.1 (8.4 – 9.7)	10.6 (10.1 – 11.2)	9.8 (8.9 – 10.5)	9.5 (9.1 – 10)	9.4 (9 – 9.9)	
Serum phosphorus level (mg/dl)						
Mean ± SD.	9.0^{a} ± 1.1	5.3^{b} ± 0.6	6.4^{b} ± 0.8	8.6^{a} ± 0.6	8.9^{a} ± 0.9	< 0.001*
Median (Min. – Max.)	8.9 (7.2 – 10.8)	5.3 (4.3 – 6.1)	6.7 (4.9 – 7.1)	8.5 (7.7 – 9.3)	8.8 (7.4 – 10.0)	
Serum alkaline phosphatase (IU/l)						
Mean ± SD.	119.9^{a} ± 2.0	96.2^{d} ± 3.3	102.5^{c} ± 2.0	112.9^{b} ± 2.9	116.2^{ab} ± 3.7	< 0.001*
Median (Min. – Max.)	119.2 (117.2–122.9)	96.8 (89.4–99.3)	102.6 (99.9–105.3)	112.9 (109.2–116.6)	117.3 (108.4–119.7)	

Means in the same **raw** with **small common letters** are not significant (i.e., Means with **Different letters** are significant).
Statistical significance is of *P*-value of < 0.05.

4. Discussion

CsA is an immunosuppressive drug widely used in transplant patients. This *in vivo* study revealed the effects of CsA on ligature-induced periodontitis. In the periodontitis group, the inflammatory process revealed massive bone destruction. Morphometrically, the eroded bony trabeculae count was 26.2 ± 6. However, CsA administration in the immunosuppressant group downregulated periodontal destruction. The slowdown in the osteoclastic activity and slight increase in the osteoblast count with a more organized alveolar bone architecture indicates that CsA inhibited the immune response. Similarly, other studies have shown that CsA reduced bone resorption and promoted new bone formation in rats with ligature-induced periodontitis (Nassar et al., 2004, Cetinkaya et al., 2006). In contrast, in another study, CsA administration has revealed bone resorption similar to the extensive bone loss in the periodontitis cohort (Goncalves et al., 2003). Moreover, another study has reported pronounced resorption sites in CsA-treated specimens, even though new cementum formation was observed (Jayasheela and Mehta, 2013).

This study compared the effects of alendronate and myricitrin in the management of immunosuppression-associated periodontitis. The results showed that both treated groups exhibited new bone formation with increased bone area and trabecular thickness compared to the untreated groups. Similarly, Sass *et al.* have demonstrated the ability of alendronate to counteract the adverse effects of CsA on the alveolar bone. Alendronate use has revealed a low amount of bone loss in a CsA-induced immunosuppression group (Sass et al., 1997).

Histomorphometrically, alendronate has stimulated new bone formation by increasing the osteoblast proliferation and inhibiting osteoclastic activity (Komatsu et al., 2013). Furthermore, alendronate has improved trabecular structure by increasing its number and thickness. Consequently, the space between bony trabeculae have decreased (Tokmak Ozsahin et al., 2017).

Alendronate belongs to the bisphosphonate class and is a pyrophosphate analog. It has a high affinity for hydroxyapatite crystals and hinders their breakdown. Thus, alendronate inhibits osteoclast-mediated bone resorption. Moreover, bisphosphonates impede the apoptosis of osteoblasts and osteocytes (Drake et al., 2008). However, serious complications occur with the prolonged use of alendronate. Jaw osteonecrosis induced by bisphosphonates (Ruggiero et al., 2014) and the risk of gastrointestinal lesions are among these drawbacks (Vestergaard, 2011).

Nowadays, herbal adjuvants have gained immense attention in replacing pharmacological drugs to minimize such side effects. Thus, myricitrin has been utilized as a promising alternative to alendronate. Myricitrin is one of the natural flavonoid glycoside herbs with potent antioxidant and anti-inflammatory activities (Hobbs et al., 2015).

Myricitrin showed promising histological outcomes confirmed morphometrically. Compared with the untreated periodontitis and immunosuppressant groups, the myricitrin group showed a significant increase in the bone area, osteoblastic count, and trabecular thickness. Furthermore, the osteoclast count was lower in the myricitrin group than in the immunosuppressant group and significantly lower than that in the periodontitis group. The ability of myricitrin to alleviate the oxidative stress by quenching the reactive oxygen species and cytokines responsible for bone resorption could explain its positive effects in reducing bone resorption and inducing bone formation (Wang et al., 2013).

In addition to the ability of myricitrin to reduce serum malondialdehyde and increase the low glutathione levels, it has antiresorptive properties. It can reduce the interleukin-6 levels, a pro-inflammatory cytokine promoting osteoclastogenesis (Huang et al., 2014). Additionally, myricitrin downregulates the expression of the receptor activator of nuclear factor kappa-B ligand (RANK-L), which is a well-known potentiator of osteoclast activity and a potent stimulator of the differentiation of macrophages into osteoclast phenotypes (Wang et al., 2018). Thus, myricitrin reducing RANK-L expression decreases the bone resorption process. Furthermore, myricitrin increases bone morphogenetic protein-2 synthesis, thus promoting the maturation and differentiation of bone-forming cells (Hsu et al., 2007).

Both myricitrin and alendronate groups showed equivalent bone remodeling with new bone formation. However, the alveolar bone was better organized in the alendronate group, similar to the typical bone architecture. Among the histomorphometric parameters, the improvement in the bone

area, osteoblast count, and trabecular thickness was more significant in the alendronate group than in the other groups.

The biochemical analysis in this study supported these histological and morphometric results. In the periodontitis cohort, the elevated serum calcium levels and the reduced serum phosphorus and alkaline phosphatase levels reflected significant resorptive activity. Meanwhile, the slowdown in the osteoclastic activity after CsA administration conserved the mineralized bone content. This was evident by the reduced serum calcium levels and slight elevation in the serum alkaline phosphatase levels in the treated groups contrasting the findings of the periodontitis group. Similar biochemical analyses reported by Cetinkaya *et al*. have indicated bone formation in the CsA-treated group compared with periodontitis one (Cetinkaya et al., 2006). Moreover, periodontitis and CsA-induced immunosuppression previously have revealed a marked reduction of alkaline phosphatase (Nassar et al., 2004).

Myricitrin and alendronate showed comparable advantageous effects, as demonstrated by the decreased calcium levels and the increased phosphorus and alkaline phosphatase levels when compared with the untreated groups. Alendronate administration in induced periodontitis has elevated the bone-specific alkaline phosphatase (Goes et al., 2012). In this study, the calcium and phosphorus levels in the treated groups were similar to those in the control group. The non-significant biomedical results emphasize the similar therapeutic efficacies of myricitrin and alendronate. Nevertheless, the results of the alendronate group were more analogous to those of the control group.

5. Conclusions

This study demonstrates that the natural myricitrin herb counteracts bone resorption in immunosuppressant-associated periodontitis. Although CsA administration in the immunosuppressant group downregulated the bone resorption, the periodontal destruction remained significantly apparent when compared with the control group. Therefore, it is suggested that subjects with periodontitis taking immunosuppressants require adjuvant therapies. The beneficial effects of myricitrin in improving the periodontitis parameters were comparable to those of alendronate. Compared with the immunosuppressant group, the myricitrin group showed significant improvements in the osteoblast count, trabecular thickness, number of spicules, and osteoid perimeter.

The safer biochemical profile of the herbal remedy over the long-term shortcomings of alendronate fortifies the substitution of alendronate with myricitrin. Future studies are recommended to evaluate the therapeutic effects of myricitrin in periodontitis subjects without immunosuppression. However, the transition of this herb to the clinical stage requires further studies to screen its pharmacokinetics. Additionally, studying the molecular mechanism of the osteoregenerative power of myricitrin would be a step forward, potentiating the upgrade of this herbal therapy from the preclinical to clinical stage. Investigating both arms of bone homeostasis, osteoblast activation and osteoclast hampering (osteocalcin, RANK-L, tartrate-resistant acid phosphatase, etc.), is crucial, particularly when complicated with immunosuppression. Furthermore, radiographic studies on larger animal models are warranted for assessing the quality of the regenerated bone.

Ethics approval and consent to participate

The study was approved by Alexandria University Ethics Committee for Animal Experimentation (IRBNO:00010556-IORG0008839) and the followed procedures were in accordance with guidelines implemented by the institution.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

A deep gratitude is expressed to Center of Excellence for Research in Regenerative Medicine and Applications (CERRMA), Faculty of Medicine, Alexandria University, Egypt (STDF-Funded) for providing the facility in the microscopic imaging unit.

References

Cetinkaya, B.O., Acikgoz, G., Keles, G.C., Ayas, B., Korkmaz, A., 2006. The effect of cyclosporin A on alveolar bone in rats subjected to experimental periodontal disease. Toxicol. Pathol. 34, 716–722.

Chen, W., Zhuang, J., Li, Y., Shen, Y., Zheng, X., 2013. Myricitrin protects against peroxynitrite-mediated DNA damage and cytotoxicity in astrocytes. Food. Chem. 141, 927–933.

Dempster, D.W., Compston, J.E., Drezner, M.K., Glorieux, F.H., Kanis, J.A., Malluche, H., Meunier, P.J., Ott, S.M., Recker, R.R., Parfitt, A.M., 2013. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J. Bone. Miner. Res. 28, 2–17.

Domitrovic, R., Rashed, K., Cvijanovic, O., Vladimir-Knezevic, S., Skoda, M., Visnic, A., 2015. Myricitrin exhibits antioxidant, anti-inflammatory and antifibrotic activity in carbon tetrachloride-intoxicated mice. Chem. Biol. Interact. 230, 21–29.

Drake, M.T., Clarke, B.L., Khosla, S., 2008. Bisphosphonates: mechanism of action and role in clinical practice. Mayo. Clin. Proc. 83, 1032–1045.

Godse, S., Mohan, M., Kasture, V., Kasture, S., 2010. Effect of myricetin on blood pressure and metabolic alterations in fructose hypertensive rats. Pharm. Biol. 48, 494–498.

Goes, P., Melo, I.M., Dutra, C.S., Lima, A.P., Lima, V., 2012. Effect of alendronate on bone-specific alkaline phosphatase on periodontal bone loss in rats. Arch. Oral. Biol. 57, 1537–1544.

Goncalves, P. F., Nogueira Filho Gda, R., Sallum, E. A., Sallum, A. W. & Nociti Junior, F. H. 2003. Immunosuppressant therapy and bone loss in ligature-induced periodontitis–a study in rats. Pesqui. Odontol. Bras., 17, 46-50.

Hagar, S., Sahar, S., Mona, Y., Nancy, M., 2015. Histomorphometric and histological evaluations of the simvastatin effect on alveolar bone loss induced by cyclosporine A in rats. Indian J. Multidiscip. Dent. 5, 2.

He, J., Li, X., Wang, Z., Bennett, S., Chen, K., Xiao, Z., Zhan, J., Chen, S., Hou, Y., Chen, J., Wang, S., Xu, J., Lin, D., 2019. Therapeutic Anabolic and Anticatabolic Benefits of Natural Chinese Medicines for the Treatment of Osteoporosis. Front. Pharmacol. 10, 1344.

Hobbs, C.A., Swartz, C., Maronpot, R., Davis, J., Recio, L., Koyanagi, M., Hayashi, S.M., 2015. Genotoxicity evaluation of the flavonoid, myricitrin, and its aglycone, myricetin. Food. Chem. Toxicol. 83, 283–292.

Hsu, Y.-L., Chang, J.-K., Tsai, C.-H., Chien, T.-T.-C., Kuo, P.-L., 2007. Myricetin induces human osteoblast differentiation through bone morphogenetic protein-2/p38 mitogen-activated protein kinase pathway. Biochem. Pharmacol. 73, 504–514.

Huang, Q., Gao, B., Wang, L., Hu, Y.Q., Lu, W.G., Yang, L., Luo, Z. J., Liu, J., 2014. Protective effects of myricitrin against osteoporosis via reducing reactive oxygen species and bone-resorbing cytokines. Toxicol. Appl. Pharmacol. 280, 550–560.

Jayasheela, M., Mehta, D.S., 2013. The role of cyclosporine A on the periodontal tissues. Dent. Res. J. (Isfahan) 10, 802–808.

Kennel, K.A., Drake, M.T., 2009. Adverse effects of bisphosphonates: implications for osteoporosis management. Mayo. Clin. Proc. 84, 632–637. quiz 638.

Komatsu, K., Shimada, A., Shibata, T., Wada, S., Ideno, H., Nakashima, K., Amizuka, N., Noda, M., Nifuji, A., 2013. Alendronate promotes bone formation by inhibiting protein prenylation in osteoblasts in rat tooth replantation model. J. Endocrinol. 219, 145–158.

Meotti, F.C., Senthilmohan, R., Harwood, D.T., Missau, F.C., Pizzolatti, M.G., Kettle, A.J., 2008. Myricitrin as a substrate and inhibitor of myeloperoxidase: implications for the pharmacological effects of flavonoids. Free. Radic. Biol. Med. 44, 109–120.

Nassar, P.O., Felipetti, F.A., Nassar, C.A., Spolidorio, L.C., 2013. Evaluation of effect of cyclosporine A on the bone tissue with induced periodontal disease to ligature in rats. Transplant. Proc. 45, 778–782.

Nassar, C.A., Nassar, P.O., Abi Rached, R.S., Holzhausen, M., Marcantonio Jr., E., Spolidorio, L.C., 2004. Effect of cyclosporin A on alveolar bone homeostasis in a rat periodontitis model. J. Periodontal. Res. 39, 143–148.

Nassar, P.O., Nassar, C.A., Guimaraes, M.R., Aquino, S.G., Andia, D.C., Muscara, M.N., Spolidorio, D.M., Rossa Jr., C., Spolidorio, L.C., 2009. Simvastatin therapy in cyclosporine A-induced alveolar bone loss in rats. J. Periodontal. Res. 44, 479–488.

Pan, W., Wang, Q., Chen, Q., 2019. The cytokine network involved in the host immune response to periodontitis. Int. J. Oral. Sci. 11, 30.

Popović, T., Šrbić, R., Matavulj, M., Obradović, Z. & Sibinčić, S. 2016. Experimental model of osteoporosis on 14 weeks old ovariectomised rats: biochemical, histological and biomechanical study. Biologia. Serbica., 38.

Rejnmark, L., Mosekilde, L., 2011. New and emerging antiresorptive treatments in osteoporosis. Curr. Drug. Saf. 6, 75–88.

Ruggiero, S.L., Dodson, T.B., Fantasia, J., Goodday, R., Aghaloo, T., Mehrotra, B., O'ryan, F., 2014. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw—2014 update. J. Oral. Maxillofac. Surg. 72, 1938–1956.

Saini, R., Marawar, P.P., Shete, S., Saini, S., 2009. Periodontitis, a true infection. J. Glob. Infect. Dis. 1, 149–150.

Sass, D.A., Bowman, A.R., Yuan, Z., Ma, Y., Jee, W.S., Epstein, S., 1997. Alendronate prevents cyclosporin A-induced osteopenia in the rat. Bone. 21, 65–70.

Schoell, A.R., Heyde, B.R., Weir, D.E., Chiang, P.C., Hu, Y., Tung, D.K., 2009. Euthanasia method for mice in rapid time-course pulmonary pharmacokinetic studies. J. Am. Assoc. Lab. Anim. Sci. 48, 506–511.

Semenoff, T.A., Semenoff-Segundo, A., Bosco, A.F., Nagata, M.J., Garcia, V.G., Biasoli, E.R., 2008. Histometric analysis of ligature-induced periodontitis in rats: a comparison of histological section planes. J. Appl. Oral Sci. 16, 251–256.

Shen, E.C., Fu, E., Gau, C.H., Hsieh, Y.D., Chiang, C.Y., 2005. Effect of cyclosporin A on the mineral apposition rate of cementum and dentin in growing rats. J. Periodontol. 76, 936–940.

Shimosaki, S., Tsurunaga, Y., Itamura, H., Nakamura, M., 2011. Anti-allergic effect of the flavonoid myricitrin from Myrica rubra leaf extracts in vitro and in vivo. Nat. Prod. Res. 25, 374–380.

Spolidorio, L.C., Spolidorio, D.M., Holzhausen, M., 2004. Effects of long-term cyclosporin therapy on the periodontium of rats. J. Periodontal. Res. 39, 257–262.

Spolidorio, L.C., Spolidorio, D.M., Holzhausen, M., Nassar, P.O., Nassar, C.A., 2005. Effects of long-term cyclosporin therapy on gingiva of rats–analysis by stereological and biochemical estimation. Braz. Oral. Res. 19, 112–118.

Spolidorio, L.C., Marcantonio Jr., E., Spolidorio, D.M., Nassar, C.A., Nassar, P.O., Marcantonio, R.A., Rossa Jr., C., 2007. Alendronate therapy in cyclosporine-induced alveolar bone loss in rats. J. Periodontal. Res. 42, 466–473.

Tokmak Ozsahin, E.T., Cam, B., Dere, F., Kurkcu, M., Evruke, C., Soames, R., Oguz, O., 2017. The effect of alendronate sodium on trabecular bone structure in an osteoporotic rat model. Turk. J. Phys. Med. Rehabil. 63, 165–173.

Veena, H.R., Prasad, D., 2010. Evaluation of an aminobisphosphonate (alendronate) in the management of periodontal osseous defects. J. Indian. Soc. Periodontol. 14, 40–45.

Vestergaard, P., 2011. Occurrence of gastrointestinal cancer in users of bisphosphonates and other antiresorptive drugs against osteoporosis. Calcif. Tissue. Int. 89, 434–441.

Vidal, B., Pinto, A., Galvao, M.J., Santos, A.R., Rodrigues, A., Cascao, R., Abdulghani, S., Caetano-Lopes, J., Ferreira, A., Fonseca, J.E., Canhao, H., 2012. Bone histomorphometry revisited. Acta. Reumatol. Port. 37, 294–300.

Wang, B., Hao, D., Zhang, Z., Gao, W., Pan, H., Xiao, Y., He, B., Kong, L., 2018. Inhibition effects of a natural inhibitor on RANKL downstream cellular signalling cascades cross-talking. J. Cell. Mol. Med. 22, 4236–4242.

Wang, Y.-H., Xuan, Z.-H., Tian, S., He, G.-R., Du, G.-H., 2013. Myricitrin attenuates 6-hydroxydopamine-induced mitochondrial damage and apoptosis in PC12 cells via inhibition of mitochondrial oxidation. J. Funct. Foods. 5, 337–345.

Woo, J.-T., Yonezawa, T., Cha, B.-Y., Teruya, T., Nagai, K., 2008. Pharmacological topics of bone metabolism: antiresorptive microbial compounds that inhibit osteoclast differentiation, function, and survival. J. Pharmacol. Sci. 106, 547–554.

Yeo, H., Beck, L.H., Mcdonald, J.M., Zayzafoon, M., 2007. Cyclosporin A elicits dose-dependent biphasic effects on osteoblast differentiation and bone formation. Bone. 40, 1502–1516.

Zhu, H.J., Brinda, B.J., Chavin, K.D., Bernstein, H.J., Patrick, K.S., Markowitz, J.S., 2013. An assessment of pharmacokinetics and antioxidant activity of free silymarin flavonolignans in healthy volunteers: a dose escalation study. Drug Metab. Dispos. 41, 1679–1685.