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Effect of bromelain on periodontal destruction and alveolar bone in rats with experimental periodontitis

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ABSTRACT

Purpose: Several substances that have anti-inflammatory, antiproteinase, and anti-infective properties have been evaluated as modulators of the inflammatory response in periodontal disease. However, evidence for the anti-inflammatory and antioxidative activities of bromelain is limited. This study evaluated the impact of systemically administered bromelain on the progression of experimental periodontitis.

Methods: Four equal groups of 32 Wistar albino rats were created as follows (n = 8): control, periodontitis + saline, periodontitis + 5 mg/kg/day bromelain, and periodontitis + 10 mg/kg/day bromelain. To quantify the resorption of bone and bone volume/tissue volume, bone surface / bone volume, and connectivity, lower jawbones were fixed and then scanned using microcomputed tomography (micro CT). Blood samples were taken to measure the macrophage colony-stimulating factor(M-CSF) concentrations, receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), tumor necrosis factor-alpha (TNF-α), matrix metalloproteinase-8 (MMP-8), interleukin-6(IL-6), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA). Histopathological assessments were made to examine the tissue.

Results: Treatment with bromelain improved the healing of the periodontium by decreasing the number of leukocytes and ligament deterioration in the gingival connective tissue and by supporting reintegration with alveolar bone. Bromelain used in ligature-induced periodontitis reduced alveolar bone (AB) resorption as measured by microCT; reduced inflammatory parameters such as IL-6 and TNF-α; regulated oxidative-antioxidative processes by increasing GPx and SOD and reducing MDA levels; and regulated AB modeling by decreasing M-CSF, RANKL, and MMP-8 and increasing OPG levels.

Conclusion: Bromelain may be an option in periodontal therapy by regulating cytokine levels, improving the healing process, and reducing bone resorption and oxidative stress.

1. Introduction

Periodontitis occurs in response to periodontal pathogens followed by periodontal tissue destruction in response to proinflammatory mediator production, which regulates the activation of osteoclast origination by the receptor activator of nuclear factor-κB ligand (RANKL) and promotes the resorption of bone [1]. Recovery from this pathological condition depends on preventing the initiating agents of the

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dysbiotic microbiota or remediating inflammation [2,3]. Recently, there have been investigations on several anti-infective, anti-inflammatory, and anti-proteinase substances that alter the inflammatory response in periodontal disease [4,5].

Bromelain is a natural blend of proteolytic enzymes generated from both stem and fructus of the pineapple (*Ananas comosus*) plant that prevents the spread of inflammation by blocking proinflammatory metabolites. Bromelain is commonly used to treat arthritis, trauma, and other inflammatory processes [6,7]. It reduces the interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) secretion when immune cells caused by inflammation are already stimulated by the overproduction of cytokines [8]. Bromelain inhibits the growth of microorganisms that play a role in periodontitis advancement and reduces neutrophil chemotaxis by 40% in the periodontal region [4]. Bromelain performs both anti-inflammatory and antioxidant roles in nonsurgical intervention and thus lowers the effect of periodontitis [9]. In particular, oral bromelain effectively reduces pain at the donor site after free gingival grafting and enhances wound healing with no increased risk of postoperative bleeding [10].

This study examined the effect of systemically administered bromelain on the progression of experimental periodontitis in rats. For this purpose, we examined the activation of antioxidant enzymes and the levels of proinflammatory cytokines in the blood and performed both micro CT and histopathological evaluations of the tissues.

2. Materials and methods

2.1. Reagents

Bromelain procured from Cayman Chemical (Item No. 17337, Cayman chemical, USA) and its aqueous solution was prepared following manufacturer's procedure. ELISA assay kits of Elabscience (M-CSF Cat. E-EL-R0601; RANKL Cat. E-EL-R0841; OPG Cat. E-EL-R0050; Elabscience, Houstan, TX, USA), ThermoFisher Scientific (MMP8 Cat. ERMMP8, TNF alpha Cat. BMS622; IL-6 Cat. BMS625, Invitrogen-ThermoFischer Scientific, Cincinnati, OH, USA), enzymatic assay kits of Randox (RANSEL and RANSOD, Randox Laboratories, Crumlin, County Antrim, UK), and Cayman Chemical's TBARS assay kit (Item No. 10009055, Cayman Chemical, Ann Arbor, MI, USA) were used in the study and purchased through dealers in Turkey.

2.2. Design of the study

This study used 32 Wistar albino rats that weighed 220–240 g at the onset of the experiment. They were separated equally (n = 8) into the control group (CG), periodontitis + saline group (PSG), periodontitis + 5 mg/kg/day bromelain, and periodontitis + 10 mg/kg/day bromelain groups.

A power calculation was carried out [11], and the sample size was calculated to be at least 3 rats for identification of a substantial difference in terms of BV/TV% between groups at a power level of 95% and an alpha error of 5%, and the effect size is 6.1176 (G*Power 3.1 software; Heinrich Heine University, Dusseldorf, Germany). Among these groups, 8 rats were collected for each group as a precaution in case of loss due to any reason and insufficient sample size.

During the procedure, the animals were kept in pairs in a silent space at a controlled temperature (21–22 °C) and a reversed cycle of light and dark (12 h/12 h). Usual food and water were available to the animals. The experimental protocol was analyzed and accepted by the Near East University Animal Experiment Ethics Committee (number: 2019/05–79).

2.3. The placement of ligature around the mandibular incisors' cervical region

A mixture of xylazine (Rompun, Bayer, Istanbul, Turkey) (10 mg/kg i.p.) and ketamine (Ketalar, Pfizer, New York, NY, USA) (90 mg/kg i.p.) was employed to narcotize the animals following overnight fasting. Alveolar bone (AB) loss was induced by the placement of 4.0 silk ligatures in the periodontitis groups. The ligatures were knotted buccally after being tied in an '8′ shape around the left mandibular incisors and right cervix [11]. Any ligatures that were missing or loose were immediately replaced following a daily inspection.

2.4. Bromelain administration

Wistar albino rats were intraperitoneally exposed to bromelain dissolved in physiological saline at a dose of 5 mg/kg/day or 10 mg/kg/day for 14 days [12].

2.5. Euthanasia and collection of specimens

The rats were put to death two weeks following the launch of experimental periodontitis. The lower jawbones were cut out, and three millimeters of gingival tissue were attained from the zone enclosing the lower incisors that were influenced by experimental periodontitis for micro-CT and histopathology examination. For biochemical analysis, blood was collected.

2.6. Micro CT analysis

2.6.1. Scanning of micro CT

A micro CT device with high resolution (Bruker Skyscan 1172, Kontich, Belgium) was used to scan the samples at 13.73 μm voxel size, 100 kV, 100 μA, with 360° rotation, 0.5 mm Al filter, 0.7° rotation step, and 250 ms exposure. The detector was air-calibrated to minimize ring artefacts prior to every scan. Each sample was rotated 360° within 5 min of integration time. The total duration of the sample scan was approximately one hour. In accordance with the instructions of the manufacturer and the initial scanning and reconstruction tests, beam hardening correction and best contrast limits were employed [13].

2.6.2. Analysis of micro CT imaging

The study used NRecon software (v. 1.6.10.4, SkyScan, Kontich, Belgium) and CTAn (v. 1.17.7.2 SkyScan, Kontich, Belgium) to visualize and quantify the specimens by having two-dimensional (2D), 1000 × 1000-pixel axial images. The ring artefact correction and smoothing were set to zero, and the beam artefact correction was set to 50% during the reconstruction. The CTAn (v.1.17.7.2, Bruker micro-CT, Kontich, Belgium) and the DataViewer program (v1.5.6.2; Bruker Micro CT) were used to analyze the division scans.

For volumetric assessments, connectivity, bone volume/tissue volume (bone volume fraction, BV/TV), and bone surface/bone volume (BS/BV) were examined (v. 1.17.7.2 SkyScan, Kontich, Belgium). An interpolated region of interest (ROI) using CTAn comprised the zone from the incisor root apex to the incisal edge roof. Binarizing the images through grayscale (limits from 50 to 140; threshold from 0 to 140) allowed the distinction of bone and dental structures in line with the differences in the density (Fig. 1-a, b, c, d, e).

For linear assessments, Dataviewer was used to acquire multiplanar reconstruction (MPR) images in the sagittal and coronal directions through the center of the related zone (x-z and z-y axis of Dataviewer). By use of this software, the user could designate an ROI to define the slice number within it. As a result, a standard slices were obtained for all samples in every direction. All measurements were carried out manually by a single examiner using the software because automated measurements were impossible due to small radiographic artefacts. Measurement points are standards for minimizing errors. The mean loss of AB was linearly calculated from the cementoenamel junction through the AB crest at the incisors' mesial, buccal, and distal surface root (Fig. 1-f, g, h).

The reconstructed illustrations were treated by the use of Skyscan

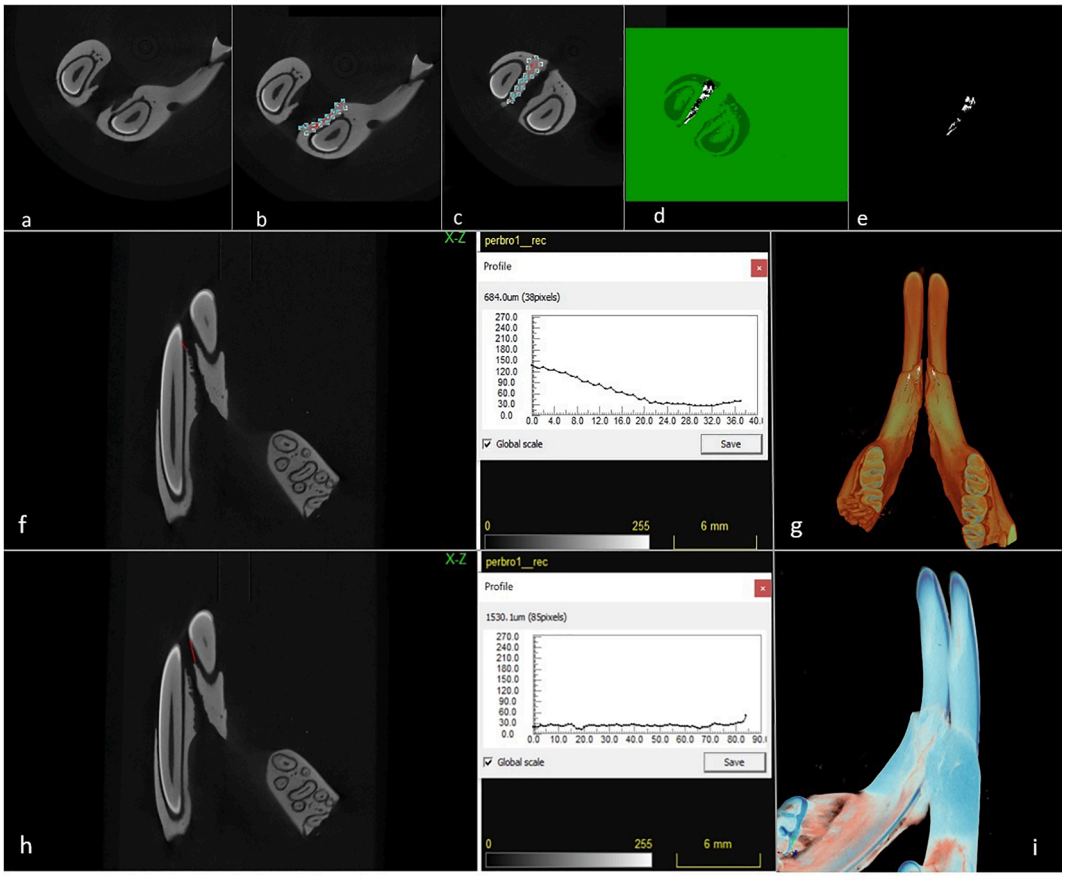

Fig. 1. Diagram of linear and volumetric measurements. **A, B, C, D, E. Diagram of the volumetric measurements.** Binarization on images was performed to differentiate the bone and dental structures, as per the divergence in the density, by using a grayscale (limits 50 to 140 with threshold 0 to 140) transformation. **F, G, H. Diagram of the linear measurements**. The mean loss of AB was calculated linearly from the cementoenamel junction through the AB crest at the mesial, buccal, and distal surface roots of the incisors. **I. Diagram of volumetric image.** The reconstructed illustrations were visualized after further processing using Skyscan CTVox (ver. 3.3.0, SkyScan) (Skyscan, Kontich, Belgium).

CTVox (v. 3.3.0, SkyScan) for screening (Skyscan, Kontich, Belgium). All image reconstructions and measurements were conducted by a dento-maxillofacial radiologist with 20 years of experience (KO) (Fig. 1-i).

2.7. Biochemical analysis

The collected blood samples were placed into serum separator tubes (SST). Then, sera were split by centrifugation at 1500 g × 10 min following coagulation (K241, BRK5324, Centurion Scientific, West Sussex, UK) and stored at −80 °C until analysis.

The M-CSF, RANKL, OPG, and MMP8 concentrations, signs of AB remodelling, and improvement and function of not only osteoclasts but also TNF-α and IL-6, which are proinflammatory cytokines, were determined through samples of serum employing rat specific ELISA kits. After consideration of the manufacturers' instructions, trials were performed, and the results were calculated.

To assess oxidative-antioxidative processes, glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were quantified using commercially available test kits (RANSEL and RANSOD) and an auto-analyzer (BS120, Mindray, Shenzhen, China).

The most stable product of lipid peroxidation, malondialdehyde (MDA), was measured in sera (TBARS, Cayman Chemical). The test was carried out with the help of a spectrophotometry technique on the basis of reaction with thiobarbituric acid (TBA) at 100 °C under acidic conditions and by measuring the absorbance of the reaction mixture at 530–540 nm [14].

2.8. Histopathological analysis

One hundred percent neutral formaldehyde solution was used to fix the samples for 24–72 h. Decalcification solution including formic acid-decalcified bone tissue (Merck Formic acid 98%-100% for analysis EMSURE® ACS) was added till the tissue came to an ideal limpness for analysis. Tissues were embedded in paraffin, subdivided into parts of 4-μm-thick by a microtome (Leica RM 2125, Wetzlar, Germany) in the mesiodistal orientation on the sagittal axis; besides they were stained for histopathology (eosin and hematoxylin) to determine the congestion, accumulation of leukocytes and disorganization of periodontal ligaments (PL). The parameters were analyzed and scored as; 0, none; 1, mild; 2, moderate; and 3, severe using a light microscope (Olympus BX51, Tokyo, Japan).

2.9. Statistical analysis

The analyses were conducted by use of GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). Whether there was a normal distribution was checked with the Shapiro-Wilk test, which showed a normal distribution. Further, the data were evaluated using a one-way analysis of variance (ANOVA), and paired comparisons were assessed with Tukey's test. Followed by Dunn's test, the Kruskal-Wallis test served analyze the data nonnormally distributed. A p value that was$<$0.05 was deemed significant.

3. Results

3.1. Histopathological results

Table 1 shows the outcomes of the histopathological parameters.

CG had regularly structured dentin, gingiva, AB, and periodontal ligaments (PL) (Fig. 2-A). Congestion and increased leukocytes were observed in the gingival connective tissue (GCT) in the PSG, and disorganization and degradation were observed in the PL (Fig. 2-B). In the periodontitis + 5 mg/kg bromelain group, ligament deterioration decreased, reintegration with the AB increased, and leukocytes in the GCT decreased (Fig. 2-C). The PL, alveolar integration, and dentin were improved in the Periodontitis + 10 mg/kg bromelain group (Fig. 2-D).

3.2. Microtomography results

The PSG ($p < 0.001$) and the periodontitis + 5 mg/kg bromelain group ($p < 0.01$) had significantly higher bone resorption (BR) in the lingual zone than the CG. The PSG ($p < 0.0001$) and periodontitis + 5 mg/kg bromelain group ($p < 0.01$) had higher BR in the mesial region than the CG. The periodontitis + 10 mg/kg bromelain group ($p < 0.01$) had significantly less BR in the mesial and lingual regions than the PSG (Fig. 3-A).

In the distal zone, the BR of the PSG ($p < 0.001$) and periodontitis + 5 mg/kg bromelain group ($p < 0.05$) was significantly higher than that of the CG. However, resorption in the Periodontitis + 10 mg/kg bromelain group was significantly less than that in the PSG ($p < 0.05$) (Fig. 3-A).

The results showed no considerable difference between the CG and PSG in BV/TV ratio values ($p > 0.05$). Both the periodontitis + 5 mg/kg bromelain and periodontitis + 10 mg/kg bromelain groups had significantly higher ratios than the control ($p < 0.0001$) and PSG ($p < 0.0001$) (Fig. 3-B).

The CG had a similar BS/VB ratio as the PSG ($p > 0.05$). However, the ratios of the periodontitis groups treated with 5 mg/kg bromelain ($p < 0.01$) and 10 mg/kg bromelain ($p < 0.001$) were significantly higher than those of the CG. The periodontitis + 10 mg/kg bromelain group was significantly higher than the PSG ($p < 0.05$) (Fig. 3-B).

The CG had greater connectivity than the PSG ($p < 0.0001$) and Periodontitis + 5 mg/kg bromelain ($p < 0.05$) groups, while Periodontitis + 5 mg/kg Bromelain ($p < 0.0001$) and Periodontitis + 10 mg/kg Bromelain ($p < 0.0001$) groups had significantly higher connectivity than the PSG (Fig. 3-B).

The bromelain groups (Periodontitis + 5 mg/kg bromelain and Periodontitis + 10 mg/kg bromelain groups) did not have a significant difference in all parameters shown above ($p > 0.05$) (Fig. 3-A, 3-B).

3.3. Biochemical results

Table 2 shows the outcomes of the biochemical parameters.

TNF-α value was considerably higher in the PSG than in the CG ($p < 0.01$) and lower in both bromelain groups than in the PSG ($p < 0.05$).

The PSG ($p < 0.0001$) and periodontitis + 5 mg/kg bromelain ($p < 0.01$) group had considerably higher IL-6 values than the CG. This was found to be lower in the Periodontitis + 10 mg/kg Bromelain group than in the PSG ($p < 0.001$).

The RANKL value was considerably higher in the PSG ($p < 0.0001$) and Periodontitis + 5 mg/kg bromelain ($p < 0.05$) groups than in the CG, and it was significantly lower in the Periodontitis + 5 mg/kg bromelain ($p < 0.05$) and periodontitis + 10 mg/kg bromelain ($p < 0.01$) groups than in the PSG. The OPG value was considerably lower in the PSG ($p < 0.0001$) and Periodontitis + 5 mg/kg Bromelain ($p < 0.01$) group than in the CG, and it was higher in the Periodontitis + 5 mg/kg bromelain ($p < 0.05$) and Periodontitis + 10 mg/kg Bromelain ($p < 0.001$) groups than in the PSG ($p < 0.001$).

Not only GPx values but also SOD values were considerably lower in the PSG than in the CG and were found to be higher in the Periodontitis + 10 mg/kg Bromelain group than in the PSG.

The M-CSF was considerably higher in the PSG than in the CG ($p < 0.0001$). The PSG ($p < 0.0001$) and Periodontitis + 5 mg/kg bromelain ($p < 0.05$) groups had higher MMP-8 than the CG. When compared to the CG, the PSG ($p < 0.0001$), Periodontitis + 5 mg/kg Bromelain ($p < 0.01$), and periodontitis + 10 mg/kg Bromelain ($p < 0.05$) groups had higher MDA values.

Compared to the PSG, the M-CSF, MMP-8, and MDA were found to be lower in the periodontitis + 5 mg/kg bromelain and periodontitis + 10 mg/kg bromelain groups.

4. Discussion

Studies of bromelain due to its anti-inflammatory [15], antioxidant [5,16], fibrinolytic, and antithrombotic activities [17] have recently become popular in periodontal treatment. Therefore, this study hypothesized that bromelain could downregulate inflammation by modulating the host response in periodontitis. Thus, the current study analyzed the effects of bromelain using different aspects of periodontitis, such as cytokine regulation, histopathological changes, oxidative-antioxidative processes, and loss of alveolar bone.

There are few studies on bromelain's impact on periodontal tissues, periodontal treatment, or the response of the immune system to specific periodontal pathogens [4,9,10]. Bromelain plays a role in the reduction of neutrophil migration to sites of inflammation [18] and has an antibacterial effect against periodontopathogens [19]. However, further studies should be conducted to elucidate how bromelain affects the immune response.

The optimal efficacy of bromelain for pain reduction and enhanced wound healing was considered in previous studies [20–23]. These studies assessed the efficacy of different dosages of bromelain for pain control after third molar extraction surgery. As demonstrated by Soheilifar et al. [10], oral bromelain effectively reduces pain at the donor site after free gingival grafting and enhances wound healing. To date, however, only one recent indexed study [9] has evaluated its efficacy in periodontitis due to ligature in rats. Thus, the evidence for the anti-inflammatory and antioxidative activity of bromelain is limited and only reported from studies in animal models.

Periodontitis is a multifaceted illness in which inflammatory

Table 1
Evaluation of the histopathological parameters.

	Control	Periodontitis + saline	Periodontitis + 5 mg/kg/day bromelain	Periodontitis + 10 mg/kg/day bromelain
Congestion	0.46 ± 0.07	2.43 ± 0.09 ****	1.63 ± 0.08 **	1.31 ± 0.05 $^{++}$
Leukocyte accumulation	0.41 ± 0.06	2.51 ± 0.09 ****	1.61 ± 0.08 ****, $^{++++}$	1.30 ± 0.06 **** $^{++++}$, $^{\gamma\gamma}$
Periodontal ligaments (PL) disorganization	0.46 ± 0.07	2.43 ± 0.09 ****	1.63 ± 0.08 ****, $^{++++}$	1.31 ± 0.05 ****, $^{++++}$,$^{\gamma}$

Data are expressed as Mean ± Standard error of mean (SEM).
** $p < 0.01$, **** $p < 0.0001$ comparison with control group.
$^{++}$ $p < 0.01$, $^{++++}$ $p < 0.0001$ comparison with Periodontitis $^{+}$ Saline group.
$\gamma p < 0.05$, $^{\gamma\gamma}$ $p < 0.01$ Periodontitis + 5 mg/kg bromelain versus Periodontitis + 10 mg/kg bromelain.

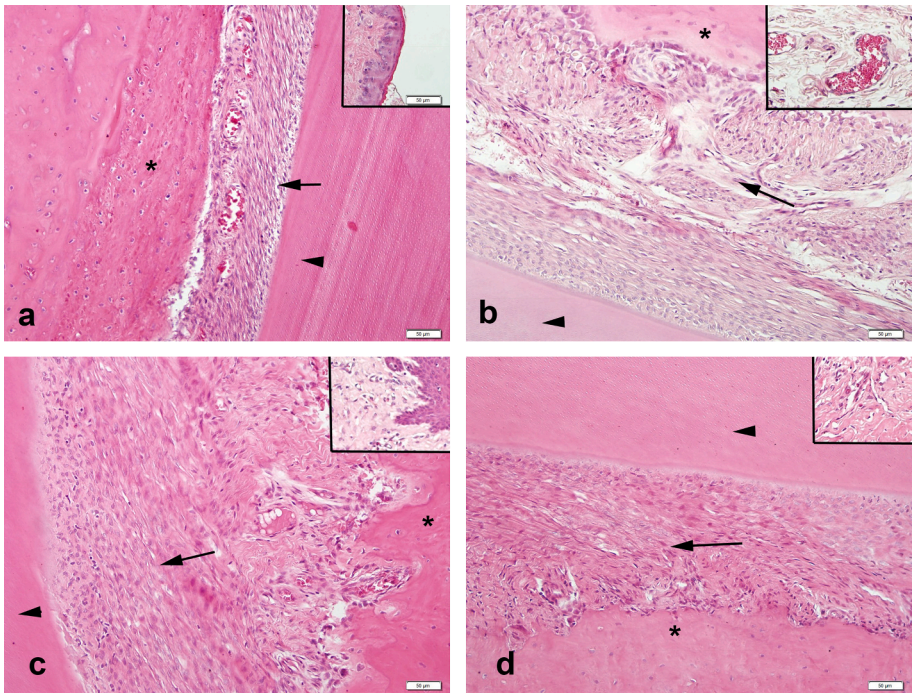

Fig. 2. The groups' representative histological illustrations. A, Control group; normal histopathological findings. Intact AB (*), PL (arrow), dentin (arrowhead), and gingiva (inset-small illustration). B, Periodontitis + saline group; disorganization and degradation in the PL (arrow), dentin (arrowhead), and AB (*), dense congestion and increased leukocytes in the gingival mucosa (inset-small illustration). C, Periodontitis + 5 mg/kg bromelain group; the AB and PL reintegration (arrow), new lines of ossification appear darker in the AB and dentin (arrowhead) and decreased leukocytes in the gingival mucosa (inset-small illustration). D, Periodontitis + 10 mg/kg bromelain group; the PL (arrow) and AB integration, dentin (arrowhead), new lines of ossification in the AB (*), and decreased leukocytes in the gingival mucosa (inset-small illustration).

infiltration is induced by periodontopathogens with the employment of leukocytes, polymorphonuclear neutrophils (PMNs), and macrophages [24]. Neutrophil degranulates considerable amounts of enzymes that destroy tissue such as matrix metalloproteinases. They also generate reactive oxygen species, and these cells discharge numerous proinflammatory cytokines, such as TNF–α, IL-6, and M-CSF, that are engaged in the inflammatory response and BR [25], acting via the pathway of RANK/RANKL/OPG [26]. In the present study, the levels of TNF-α, IL-6, and M-CSF were significantly higher in the PSG than in the CG. In regard to the levels of IL-6 and TNF-α, the Periodontitis + 10 mg/kg bromelain group had lower levels than the PSG. All bromelain groups had lower levels of M-CSF than the PSG. All these parameters showed levels similar to those in group C. Similarly, bromelain lowers IL-1β, IL-6, and TNF-α secretion in stimulated immune cells during inflammation-induced cytokine overproduction [8,27]. Therefore, bromelain may regulate the cytokine response by restraining TNF-α, IL-6, and M-CSF overexpression [4,9].

Overexpression of proinflammatory cytokines leads to the upregulation of other inflammatory enzymes and mediators, such as matrix metalloproteinases (MMPs) and RANKL, which can irreversibly damage soft and hard tissues [8]. Proinflammatory cytokines contribute to BR through osteoclast differentiation by the MAP kinase–JAK–STAT signaling pathway, as well as by interfering with the mechanism of osteoclastogenesis [28]. Bone loss in periodontal disease is mediated by the RANK/RANKL/osteoprotegerin (OPG) system, which regulates osteoclast development, differentiation, activation, and function. During periodontitis, RANK and RANKL are involved in BR because differentiation of osteoclast and stimulation of osteoclastogenesis are increased [29]. Bromelain inhibits the phosphorylation of the Ser–32 residue of phosphor–inhibitor kappa B (IkB), an important molecule for the translocation and function of nuclear factor–kappa B (NF–kB), consequently blocking NF–kB action [30,31]. In the current study, RANKL, OPG, and MMP-8 levels were measured in serum, and micro-CT images were examined from mandibular samples to appraise the influence of bromelain on AB turnover and loss.

In this study, BR at all sites was significantly lower in the periodontitis + 10 mg/kg bromelain group than in the PSG, while it was similar to that in the healthy CG. In terms of connectivity and BV/TV ratio values, both the Periodontitis + 5 mg/kg Bromelain and Periodontitis + 10 mg/kg Bromelain groups were significantly higher than the PSG. Furthermore, RANKL and MMP-8 levels were lower in the bromelain groups than in the PSG, and OPG was higher in the bromelain groups than in the PSG. de Silva et al. [4] hypothesized that bromelain can decrease the osteoclastogenesis process with a decrease in AB loss in periodontitis. This hypothesis is supported by our study results. Consequently, both doses of bromelain might effectively prevent AB loss.

Patients with periodontitis had a high level of oxidative stress markers in their gingival crevicular fluid, saliva, and plasma, further supporting a link between oxidative stress and periodontal inflammation [32,33]. Under normal physiological conditions, reactive oxygen species (ROS) and antioxidants are balanced. Oxidative stress occurs when the antioxidant defense system is unable to neutralize the elevated ROS production [34].

In our study, the PSG had considerably lower GPx and SOD than the CG, and the Periodontitis + 10 mg/kg bromelain group had higher values than the PSG. The MDA levels were lower in the bromelain groups than in the PSG. Similarly, bromelain is an antioxidant that induces the secretion of SOD and GPx [35,36]. Thus, bromelain may also inhibit periodontal tissue destruction in experimental periodontitis by mediating oxidative stress.

The histopathological evaluation in this research showed that in addition to degradation in the PL, congestion and increased leukocytes in the GCT were observed in the PSG, whereas there was a decrease in the deterioration of the ligament, increase in the alveolar bone reintegration, and leukocytes decrease in the GCT of the Bromelain groups. These results corroborate other studies demonstrating that bromelain causes specific proteolytic removal of enzymes that inhibit leukocyte migration, particularly neutrophils, in response to IL-6, and thus lessen acute responses to inflammatory stimuli [18]. Additionally, bromelain removes several cell facet molecules that have an important role in leukocyte adhesion and activation by its proteolytic activity [37]. Based on the data from this research and literature on this subject, it is possible that bromelain improves the healing process.

Considering our study findings, we propose that bromelain may be an option in periodontal therapy. However, more clinical trials must validate this hypothesis.

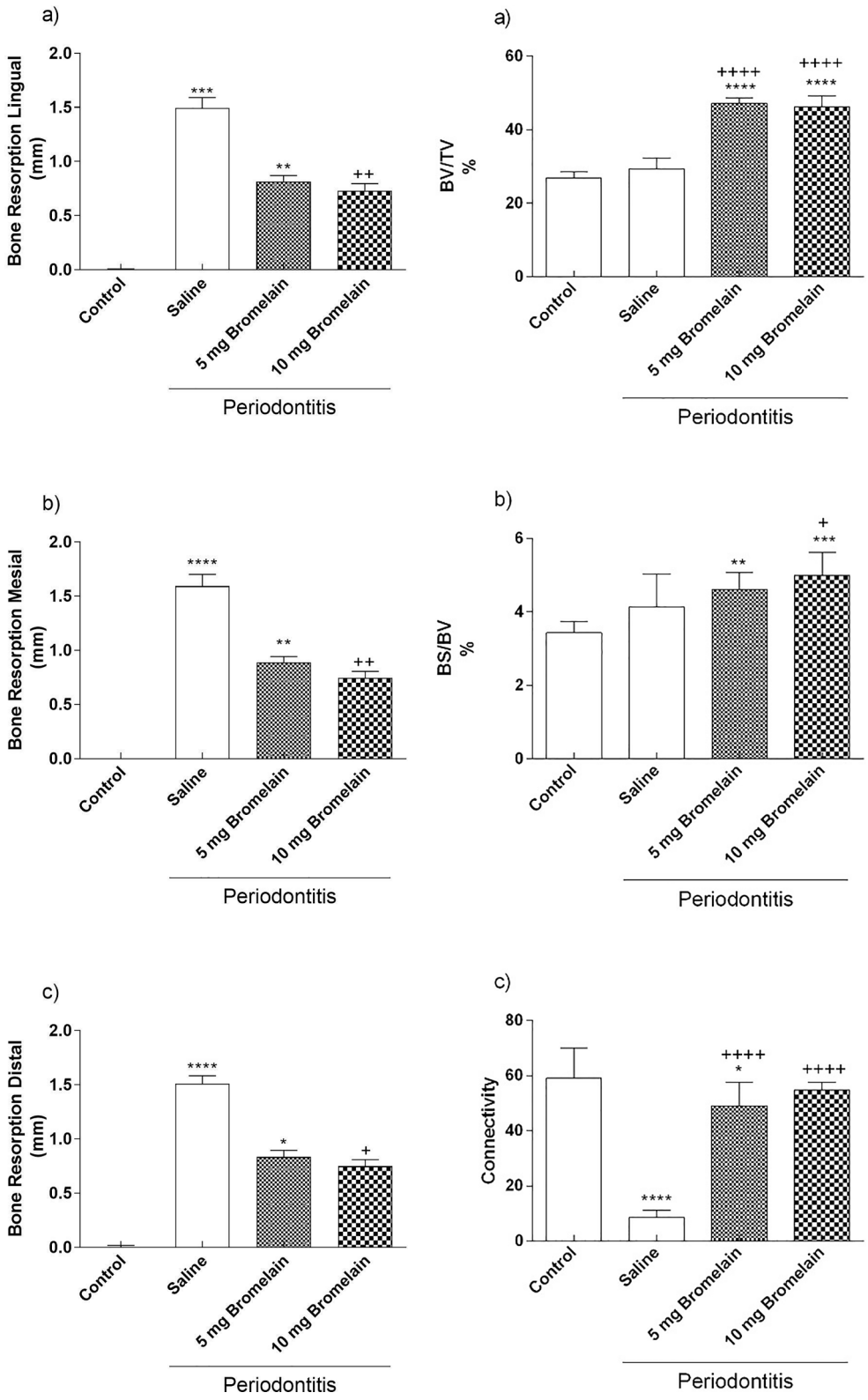

Fig. 3. Micro-CT assessment. A. The bone resorption of the study groups. B. The bone volume fraction (BV/TV), bone surface/bone volume (BS/BV), and connectivity of the study groups.

5. Conclusion

Drawing upon the data presented, it appears that bromelain - a compound known for its dual antioxidant and anti-inflammatory properties - holds significant promise in the battle against periodontitis. Utilizing a trio of investigative techniques - histopathology, microtomography, and biochemical assays - we observed that bromelain can effectively tackle inflammation, halt bone loss, and foster a harmonious balance between oxidative and antioxidative processes. This, in turn, safeguards vital structures such as the alveolar bone, periodontal

Table 2
Mean and standard error of mean with regard to the mean of blood biomarkers.

	Control	Periodontitis	Periodontitis + 5 mg/kg/day Bromelain	Periodontitis + 10 mg/kg/day Bromelain
TNF-α (pg/ml)	34.05 ± 4.57	116 ± 23.55 **	62.88 ± 11.01 $^{+}$	50.33 ± 4.49 $^{+}$
IL-6 (pg/ml)	16.46 ± 3.11	256.30 ± 49.21****	114.10 ± 10.40 $^{++}$	65.94 ± 11.05 $^{+++}$
RANKL (pg/ml)	50.56 ± 7.86	128.9 ± 9.98 ****	86.56 ± 8.54 *, $^{+}$	82.18 ± 7.29 $^{++}$
OPG (pg/ml)	519.1 ± 35.4	246.5 ± 26.2 ****	361.1 ± 26.5 **, $^{+}$	421.3 ± 15.6 $^{+++}$
M-CSF (pg/ml)	38.24 ± 2.96	104.1 ± 11.93****	60.36 ± 6.96 $^{++}$	65.03 ± 6.02 $^{++}$
MMP-8 (pg/ml)	55.86 ± 5.86	172.7 ± 15.3 ****	105.6 ± 13.4 *, $^{++}$	89.6 ± 7.6 $^{+++}$
MDA (μmol/l)	3.75 ± 0.35	8.61 ± 0.61 ****	6.49 ± 0.54 **, $^{+}$	6.01 ± 0.44 *, $^{++}$
GPx (U/l)	2815 ± 78	1540 ± 99 **	2325 ± 108	2697 ± 225 $^{++}$
SOD (U/ml)	0.34 ± 0.02	0.19 ± 0.01 ***	0.27 ± 0.01	0.28 ± 0.02 $^{+}$

TNF-α (tumor necrosis factor-alpha), IL-6 (interleukin-6), RANKL (receptor activator of nuclear factor kappa-B ligand), OPG (osteoprotegerin), M-CSF (macrophage colony-stimulating factor), MMP-8 (matrix metalloproteinase), MDA (malondialdehyde), GPx (glutathione peroxidase), SOD (superoxide dismutase).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. the control group.
$^{+}p < 0.05$ $^{++}$ $p < 0.01$ $^{+++}$ $p < 0.001$ vs. periodontitis + saline group.

ligament, and gingival tissue. On another front, bromelain also plays a crucial role in regulating inflammatory reactions and cytokine activity, highlighting its potential as a potent therapeutic tool for periodontal treatment. However, to grasp the full extent of bromelain's influence on our immune responses, and to better tailor its application in the clinic, further explorations are certainly warranted.

6. Ethics approval

Experiments carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures. The experimental protocol was analyzed and accepted by the Near East University Animal Experiment Ethics Committee (number: 2019/05–79).

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Author contributions

TP, GU, AÖŞ, RBKÜ, SS, KO, ŞÇ, NBA, UA and AVÖ conceived and designed research. TP, GU, AÖŞ, RBKÜ, SS, KO, ŞÇ, NBA, and UA conducted experiments. TP, AÖŞ, SS, KO, UA and AVÖ contributed new reagents or analytical tools. TP, GU, and AÖŞ analyzed data. TP, GU, AÖŞ, and RBKÜ wrote the first draft of the manuscript. All authors read and approved the final manuscript and all data were generated in-house and that no paper mill was used.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2023.110446.

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