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Original article

Anti-inflammatory and antioxidant effects of *Myrtus communis* on ligature-induced periodontitis in rats

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Abstract

This study aimed to investigate the effects of *Myrtus communis* (MC) extract on oxidative stress, inflammation and alveolar bone loss in ligature-induced periodontitis in rats. Forty-two adult male Wistar Albino rats were randomly divided into six groups: Control (C); Periodontitis (P); Periodontitis + MC (P+GG+MC); Periodontitis + MC (P+IO+MC); Periodontitis + chlorhexidine (P+CHX); and Periodontitis + MC (P+GG+IO+MC). *Myrtus communis* extract (150 µg/mL, 0.5 mL) was administered via gastric gavage or spray. Chlorhexidine (0.3 mL) was administered once daily for 11 days via spray. Serum oxidative stress markers (TOS, TAS), inflammatory markers (TNF-α, IL-1β, IL-6, MMP-8, MMP-9), bone morphometry using micro-CT, and histopathological analyses (RANKL, ICD) were evaluated. The lowest ICD levels were observed in the P+CHX group, while RANKL activity was highest in the P group. TAS levels were significantly higher in the P+GG+MC group compared to the P, P+IO+MC, and P+GG+IO+MC groups, while TOS levels were highest in the P group. Bone quantity tended to be greater in the P+GG+MC group compared to other groups, whereas the bone ratio was higher in the P+CHX group. MMP-8 levels were significantly lower in the P+GG+MC group compared to all other groups. In conclusion, the data obtained indicate that *Myrtus communis* extract, particularly when administered systemically, may serve as an adjuvant treatment option by preventing alveolar bone loss through its antioxidant and anti-inflammatory effects.

Keywords: antioxidant, micro-CT, MMP, periodontitis, rat

Introduction

Periodontal diseases are chronic inflammatory disorders that impact the periodontal structures of the teeth. If left untreated, they progress to destruction of the periodontal ligament and alveolar bone, eventually resulting in tooth loss (Bai et al. 2024). Periodontitis has a complex pathogenesis in which inflammatory mechanisms play a central role. Dysbiotic bacterial biofilms stimulate the overproduction of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases (MMPs), leading to the gradual breakdown of connective tissues (Pavanelli et al. 2022).

Oxidative stress is a term that describes the situation which occurs due to the disruption of the oxidant-antioxidant ratio in the body and is reported to have an important role in periodontitis pathogenesis, similar to its involvement in many other diseases. Reactive oxygen species (ROS) can damage periodontal tissues through several mechanisms. These include lipid peroxidation, protein and DNA damage, and the stimulation of pro-inflammatory cytokine release (Özbek and Özkan 2020, Sczepanik et al. 2020). Therefore, maintaining the balance between oxidants and antioxidants is essential for ensuring the health of periodontal structures. Increased lipid peroxidation was reported in patients with periodontitis, underscoring the impact of oxidative stress on the progression of the disease and tissue loss (Tsai et al. 2005).

Conventional periodontal therapy generally focuses on mechanical debridement by scaling and root planing. However, these approaches are often insufficient for fully controlling the disease. Thus, there is increased interest in adjunctive therapies targeting the host response. Chlorhexidine (CHX) remains a widely used option because of its antimicrobial, bactericidal, and bacteriostatic properties. It also has low toxicity and limited bacterial resistance. Even so, it may cause tooth staining and discoloration. In addition to antimicrobial agents, host modulation therapies have been investigated for their ability to limit inflammation and bone resorption (da Costa et al. 2017, Pavanelli et al. 2022, Balli et al. 2024). Preclinical systematic reviews have identified several promising agents with anti-resorptive and anti-inflammatory effects, such as bisphosphonates, cathepsin K inhibitors, anti-receptor activator of nuclear factor kappa-B ligand (RANKL) antibodies, and a range of natural compounds (Valverde et al. 2025). Herbal medicines have also attracted particular attention for their multitarget actions, relative safety, and potential for long-term use without significant adverse effects (Giday et al. 2010).

Myrtus communis (MC) is an aromatic species of the *Myrtaceae* family, native to the Mediterranean, and its therapeutic properties have been extensively documented (Al-Snafi et al. 2024, Matsehorova and Odyntsova 2024). Phytochemical studies have shown that MC has rich bioactive compounds, with leaf extracts containing the highest total phenolic content, followed by stem and fruit extracts. In its leaves, the ethyl acetate fraction exhibits strong radical-scavenging activity which indicates its potent antioxidant activity. According to thin-layer chromatography, gallic acid, catechin and quercetin are major constituents of the plant, which together contribute to its antioxidant and anti-inflammatory effects (Kanoun et al. 2014). These antioxidative properties of MC make it a potential candidate for protection against periodontal diseases.

The purpose of this study was to evaluate the efficacy of *Myrtus communis* (MC) extract on oxidative stress markers, pro-inflammatory cytokines and alveolar bone parameters following ligature-induced periodontitis in rats, in comparison to a chlorhexidine-based treatment.

Materials and Methods

Ethical statement

This study was approved by the Afyon Kocatepe University Animal Experiments Local Ethics Committee (Protocol number: 49533702/33; Approval date: 23.03.2022). All experimental procedures were conducted in accordance with the ethical guidelines and regulations approved by the committee.

Animals

Only healthy adult male Wistar Albino rats weighing between 250-300 g were included in the study in order to standardize metabolic and physiological variability. The rats had normal activity, grooming behavior, and no visible signs of disease (e.g., tumors, lameness) during a 7-day acclimatization period. No animals were excluded from the study, and no complications were observed in the included animals.

The rats were housed in cages under standard conditions (12 h light/12 h dark intervals, 20-24°C temperature range, and 40-60% humidity range) at the Afyon Kocatepe University Experimental Animals Application and Research Center. Standard pellet feed and water were provided ad libitum daily.

A total of 42 rats were divided at random into six groups. Groups were formed using a random number generator (https://www.randomizer.org/) by an independent researcher who was not involved in group assignments. Groups were determined as follows: Control group (Group C, n=7), Periodontitis group

(Group P, n=7), P+Gastric Gavage (GG)+MC group (Group P+GG+MC, n=7), P+Intraoral (IO)+MC group (Group P+IO+MC, n=7), P+Chlorhexidine group (Group P+CHX, n=7), and P+GG+IO+MC group (Group P+GG+IO+MC, n=7).

Plant material and preparation of *Myrtus communis* extract

The *Myrtus communis* L. extract used in this study was custom-manufactured (Ars Arthro Biotechnology, Ankara, Turkey). The plant material (leaves and stems) was collected by the manufacturer in August from the foothills of the Taurus Mountains in Mersin, Turkey. The manufacturer guaranteed botanical identification and quality control. The leaves and stems were boiled in distilled water at a concentration of 6 g/L at 100°C for 15 minutes. The obtained extract was partially evaporated using a rotary evaporator and completely dried in a lyophilizer. For use in the study, solutions were prepared from the lyophilized extract at a concentration of 150 µg/mL (Saritas et al. 2022). These solutions were then sterilized in an autoclave at 120°C for one hour. The final products were stored in 50 mL Falcon tubes at +4°C until use.

Phytochemical characterization of the final MC extract was performed by the manufacturer using LC-MS analysis. According to the analysis, the presence of several phenolic compounds, including gallic acid (43.1 mg/kg plant), vanillic acid (8.4 mg/kg plant), catechin (159.7 mg/kg plant), quercetin (16.7 mg/kg plant), ellagic acid (15.2 mg/kg plant), rosmarinic acid (0.5 mg/kg plant), chlorogenic acid (1.9 mg/kg plant), apigenin-7-glucoside (10.5 mg/kg plant) and naringenin (279.1 mg/kg plant) was noted. The total phenolic content of the extract was determined as 923.5 mg/kg of plant.

Experimental periodontitis model

Anesthesia was performed intramuscularly using a combination of 13 mg/kg xylazine hydrochloride (Alfazine 2%) and 87 mg/kg ketamine hydrochloride (Alfamine 10%). To induce experimental periodontitis and promote plaque retention in the rats, 4/0 silk sutures (Dogsan Ilac Sanayi, Istanbul, Turkey) were placed subgingivally on the right, mandibular, first molars under anesthesia. The sutures were left in place for 11 days (Karakan et al. 2017).

During this period, Group P+GG+MC received 0.5 mL of MC extract once daily via gastric gavage. Group P+IO+MC was treated with 0.5 mL of MC extract administered once daily as a local spray. Group P+GG+IO+MC received 0.5 mL of MC extract both via gastric gavage and local spray once daily. In Group P+CHX, 0.3 mL of chlorhexidine was administered locally once daily. At the 12th day, 4 mL of blood was collected from all rats by cardiac puncture and transferred into anticoagulant free tubes. The rats were sacrificed with an intraperitoneal injection of sodium thiopental at a dose of 200 mg/kg, and the mandibular bones were extracted.

Micro computed tomography imaging

The mandibular blocks were analyzed using micro-CT (Bruker micro CT Skyscan 1275), and the alveolar bone structure was scanned three-dimensionally (3D) to obtain quantitative results. Following the 3D scanning, anatomical landmarks in all axes were re-evaluated. The extent of bone resorption was assessed through measurement of the distance from the cementoenamel junction to the level of the coronal crest (Fernandes et al. 2024).

Measurement of linear alveolar bones

Bone loss was evaluated in four regions located between the mandibular first and second molar teeth. Linear measurements were performed at the distobuccal and distolingual areas of the first molar, and at the mesiobuccal and mesiolingual areas of the second molar (Fernandes et al. 2024).

Volumetric analyses

Analysis was performed on the interdental region between the first and second molar teeth. A line was drawn from the peak points of the two furcation areas located mesially and distally to the target region and extended apically, through which alveolar bone loss was assessed across 100 consecutive sections. In every fifth section, a two-dimensional contour was also drawn. Subsequently, image analysis software generated three-dimensional reconstructions based on the resulting two-dimensional contouring. Using the analysis program, bone volume fraction (BVF) values were obtained, representing the remaining bone volume relative to the total bone volume (Bae et al. 2016).

Microarchitecture analysis of trabecular bone

Trabecular thickness, trabecular separation, number of trabeculae, bone mineral density and the structure model index were evaluated using image analysis software. These parameters gave information related to the amount, thickness and organization of the trabecular bone. The structure model index is essential for assessing trabecular quality. An index reveals the differential analysis of the structure based on division into triangular elements. A structure model index value of 0 indi-

cates a plate-like bone structure, a value of 3 indicates a rod-like structure, and a value of 4 reflects a sphere-like trabecular morphology (Kamiński et al. 2020).

Histopathological examinations

The obtained mandibular samples were fixed in 10% formaldehyde for 24 h. Following the fixation, the samples were decalcified in 10% formic acid for three days in a plastic container for histopathological analysis. Following decalcification, the samples were bisected mesiodistally through the center of the first molar using a scalpel. After rinsing with distilled water, the samples were dehydrated through a graded alcohol series and cleared with xylene before paraffin embedding. Serial sections with a thickness of 5 µm were obtained from the paraffin blocks, incubated in an oven at 60°C overnight, deparaffinized in xylene, and rehydrated. Tissue sections were stained with hematoxylin and eosin and subsequently examined under a light microscope (Eclipse 80i, Nikon, Tokyo, Japan) at various magnifications.

In the stained sections, inflammatory cell infiltration, as well as the numbers of osteoclasts and osteoblasts in the alveolar bone and interdental septum, were examined. Inflammatory cell infiltration was evaluated semi-quantitatively based on the following scoring system: 0 = no visible inflammatory cell infiltration, 1 = mild infiltration, 2 = moderate infiltration, and 3 = severe infiltration (Toker et al. 2008). Osteoclasts and osteoblasts were counted based on their morphological characteristics.

RANKL immonuhistochemical staining

Sections with a thickness of 3 µm were obtained from the paraffin-embedded tissues. The sections were incubated for 32 min with anti-RANKL antibody (Novus Biologicals, NB100-80849) using a fully automated slide processing system (Ventana Benchmark XT, Roche, Tucson, USA). Following the procedure, the slides, which remained in the device for approximately four hours, were rinsed with tap water. They were then passed through a series of 80%, 90%, and 100% alcohol and dried in an oven at 65°C for 1.5 h. Finally, the sections were cleared with xylene, coverslipped with Entellan, and analyzed under a light microscope. RANKL-positive areas in the bone surrounding the mandibular first molar tooth were evaluated, and the percentage of these areas was calculated relative to the total area. If the percentage of RANKL-positive cells was between 0% and 5%, the score was recorded as 0; between 5% and 25% as score 1; between 25% and 50% as score 2; between 50% and 75% as score 3; and above 75% as score 4.

Biochemical analysis

Centrifugation of blood samples was performed at 3000 rpm for 15 min and the sera were extracted. Obtained serum samples were stored at -20°C until biochemical analysis. In the serum samples, TAS (Catalog no: E1512Ra, BT LAB, China), TOS (Catalog no: E1512Ra, BT LAB, China), IL-1β (Catalog no: E0119Ra, BT LAB, China), IL-6 (Catalog no: E0135Ra, BT LAB, China), TNF-α (Catalog no: E0764Ra, BT LAB, China), MMP-8 (Catalog no: SL0488Ra, Sunlong, China), and MMP-9 (Catalog no: YLA0585RA, YL Biont, China) levels were measured using an ELISA device (MVGt Lambda Scan 200, Bio-Tek Instrument, VT) according to commercial kit procedures.

Statistical analysis

To evaluate whether the dataset followed a normal distribution, skewness and kurtosis coefficients were examined. Skewness and kurtosis coefficients ranging from -1 to +1 suggested a normal distribution of the data. Based on the results, the data were found to meet the assumption of normal distribution, and therefore parametric tests were used. For the statistical analysis of micro-CT results, one-way analysis of variance (One-Way ANOVA) was used to compare groups based on buccal distance, lingual distance, bone volume, bone ratio, inflammatory cell density, RANKL activity and biochemical parameters. Duncan's multiple range test was used for pairwise comparisons between groups. The results were expressed as mean ± standard deviation. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS version 26.0 software.

Results

Histopathological findings

Inflammatory cell density was significantly lower in the P+CHX group compared to the periodontitis group. Similarly, RANKL activity was highest in the periodontitis group and significantly reduced in treatment groups, with P+CHX showing the greatest reduction (Table 1, Fig. 1).

Micro CT findings

Bone amount was significantly greater in the P+GG+MC group compared to all other treatment groups. The P+CHX group demonstrated the highest bone ratio, while lingual distance measurements showed significant increases in periodontitis groups compared to controls (Table 2, Figs. 2 and 3).

Table 1. Comparison of histopathological findings among the rat groups.

Groups	Inflammatory Cell Density	RANKL activity
C	0.00±0.00^{c}	1.17±0.41^{b}
P	2.10±0.63^{a}	2.67±0.52^{a}
P+GG+MC	1.75±0.50^{a}	2.17±0.41^{a}
P+IO+MC	0.92±0.45^{b}	1.17±0.41^{b}
P+CHX	0.85±0.42^{b}	0.50±0.55^{c}
P+GG+IO+MC	1.00±0.63^{b}	0.83±0.41^{bc}
P	<0.01*	<0.01*

* $^{a, b, c}$ Different superscript letters in the same column indicate statistically significant differences ($p<0.05$).

Table 2. Comparison of Micro CT findings among the rat groups.

Groups	Buccal Distance	Lingual Distance	Bone Amount	Bone Rate
C	401.14±63.82	334.29±105.40^{b}	2.38±0.62^{b}	81.83±1.92^{d}
P	468.00±140.97	524.57±103.63^{a}	3.25±0.83^{ab}	85.95±2.55^{abc}
P+GG+MC	390.86±91.61	483.43±68.49^{a}	3.60±0.98^{a}	86.60±4.05^{ab}
P+IO+MC	365.14±140.09	534.86±169.40^{a}	2.33±1.29^{b}	82.91±3.31^{bcd}
P+CHX	380.57±85.34	354.86±91.61^{b}	2.39±0.68^{b}	87.18±2.64^{a}
P+GG+IO+MC	416.57±85.34	421.71±101.22^{ab}	2.33±0.62^{b}	82.40±4.29^{cd}
P	0.544	<0.01*	<0.01*	<0.01*

* $^{a, b, c}$ Different superscript letters in the same column indicate statistically significant differences ($p<0.05$).

Table 3. Comparison of biochemical findings among the rat groups.

Groups	TOS (U/mL)	TAS (U/ml)	MMP8 (pg/ml)	MMP9 (ng/mL)	IL1 (ng/mL)	IL6 (ng/L)	TNF (ng/L)
C	5.50±0.31^{b}	3.78±0.23	186.09±5.00^{a}	0.70±0.11^{b}	4.09±0.29^{b}	3.15±0.34	45.59±2.33^{b}
P	7.49±0.40^{a}	3.28±0.15	180.67±4.20^{a}	0.95±0.02^{a}	5.35±0.22^{a}	3.82±0.19	68.71±4.39^{a}
P+GG+MC	3.63±0.33^{c}	4.23±0.35	95.39±9.23^{b}	0.53±0.06^{b}	4.68±0.23^{ab}	3.42±0.17	50.27±3.23^{b}
P+ IO+MC	5.30±0.75^{b}	3.87±0.44	178.68±2.67^{a}	0.92±0.01^{a}	5.05±0.25^{a}	3.80±0.12	53.34±3.68^{b}
P+CHX	4.07±0.67^{bc}	3.59±0.11	171.27±7.22^{a}	0.88±0.04^{a}	4.84±0.19^{a}	3.67±0.28	53.74±2.84^{b}
P+GG+IO+MC +IO+MC	4.72±0.53^{bc}	3.63±0.30	167.59±6.61^{a}	0.88±0.03^{a}	4.02±0.23^{b}	3.24±0.23	46.08±2.27^{b}
P	<0.01*	0.329	<0.01*	<0.01*	<0.01*	0.242	<0.01*

* $^{a, b, c}$ Different superscript letters in the same column indicate statistically significant differences ($p<0.05$).

Biochemical findings

TOS levels were significantly elevated in the periodontitis group and dramatically reduced in the P+GG+MC group. MMP-8 levels showed remarkable reduction only in the gastric gavage group compared to all other groups (Table 3).

Discussion

In this experimental ligature-induced periodontitis model, systemic administration of *Myrtus communis* (MC) extract via gastric gavage was associated with alveolar bone preservation, modulation of oxidative stress, and reduced levels of key inflammatory mediators. Compared with intraoral administration, systemic delivery yielded greater bone volume and more significant changes in TOS, MMP-8, and RANKL levels, suggesting that systemic application may enhance bioavailability and potentiate therapeutic effects. These findings are consistent with previous experimental periodontitis studies reporting that systemic phytotherapeutics can modulate both local and systemic inflammatory responses (Karakan et al. 2017, Ballı et al. 2024).

Previous studies have reported the use of ligature placement to create dental plaque – retentive sites (Toker et al. 2009, Goes et al. 2014, Balli et al. 2024). The ligature-induced periodontitis model employed in the present study is a well-established and widely used approach for promoting periodontal inflammatory responses and progressive alveolar bone loss. In line

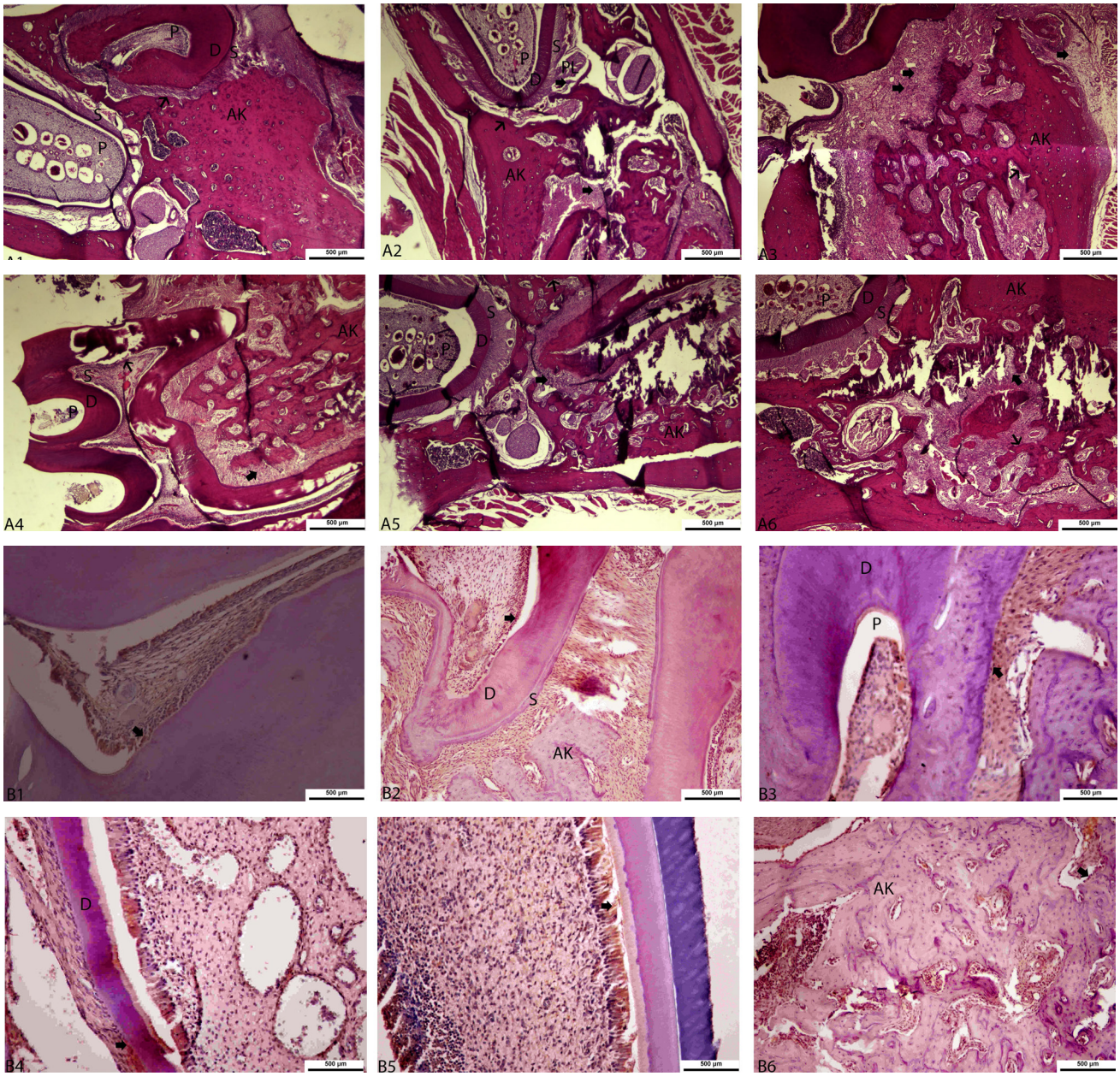

Fig. 1. Histological sections of the mandibular first-molar region in rats were stained with hematoxylin and eosin (H&E; panels A1-A6), whereas sections in panels B1-B6 were immunohistochemically labeled for receptor activator of nuclear factor kappa-B ligand (RANKL). The thick and thin arrows indicate inflammatory cell infiltration and osteoblasts, respectively, and RANKL-positive areas are shown in panels B1-B6. The groups were as follows: (1) control group (C), (2) periodontitis group (P), (3) periodontitis group treated with *Myrtus communis* by gastric gavage (P+GG+MC), (4) periodontitis group treated with *Myrtus communis* by intraoral administration (P+IO+MC), (5) periodontitis group treated with chlorhexidine (P+CHX), and (6) periodontitis group treated with *Myrtus communis* by both gastric gavage and intraoral administration (P+GG+IO+MC). Tissue labels: D – dentin; S – cementum; P – pulp; AK – alveolar bone; PL – periodontal ligament. In the group designations, P – denotes periodontitis, whereas in the tissue labels, P – denotes pulp. Original magnification: ×4; scale bar = 500 µm.

with previous reports, our findings demonstrated marked bone destruction by day 11 of the experiment. These results further corroborate earlier experimental evidence, which indicates that ligature placement reliably induces significant periodontal inflammation and alveolar bone resorption in rat models (Mustafa et al. 2021).

Chlorhexidine (CHX) is among the most widely used and best-known antimicrobial agents for treating and preventing gingival disorders. However, prolonged use can lead to adverse effects such as color changes in tooth structure and restorative composites, taste disturbances, and allergic reactions (Ahuja et al. 2011). To avoid such side effects, as well as complications such as antibiotic resistance and drug interactions associated with high-dose systemic chemotherapeutic agents, numerous studies have focused on the biological activities of plant extracts for periodontal therapy

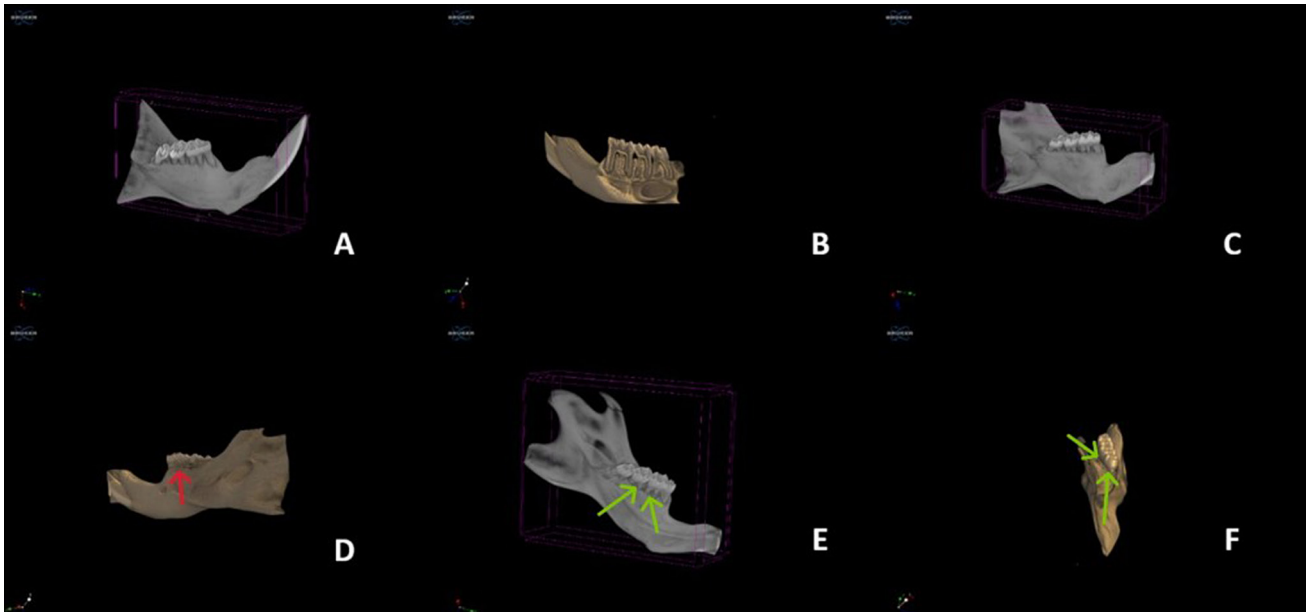

Fig. 2. Micro-CT images of rat Group C (A: Buccal, B: Lingual), Group P (C: Buccal, D: Lingual), and Group P+GG+MC (E: Buccal, F: Lingual) views. Significantly higher bone amount was observed in the P+GG+MC group than in the other treatment groups ($p<0.01$). The distance between the cemento-enamel junction and the alveolar bone crest was significantly higher in the P group than in the C group ($p<0.01$). The red arrow points out the amount of bone resorption beneath the furcation and cemento-enamel junction. The green arrows indicate the amount of bone in the apical furcation region.

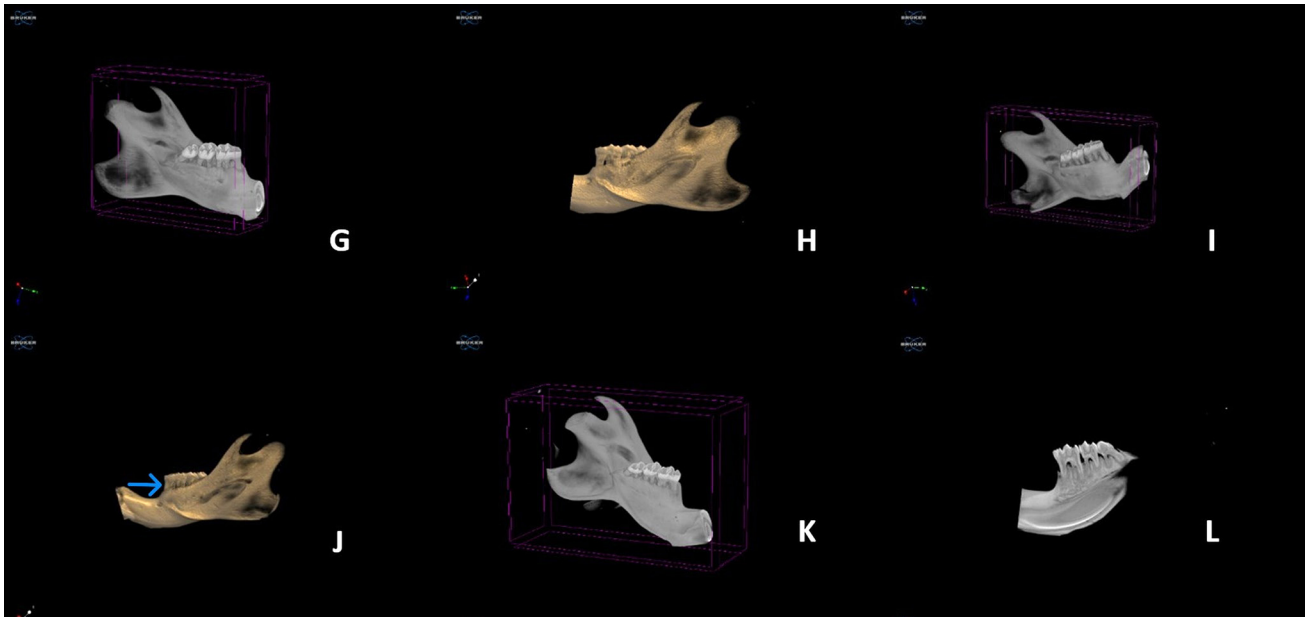

Fig. 3. Micro-CT images of rat Group P+IO+MC (G: Buccal, H: Lingual), Group P+CHX (I: Buccal, J: Lingual), and Group P+GG+IO+MC (K: Buccal, L: Lingual) views. The highest bone ratio value was found in the P+CHX group. All treatment groups showed greater buccal bone distance than the P group, but the difference was not significant. In the P+CHX group, significantly less lingual distance was detected when compared to the P group ($p<0.01$). The blue arrow shows the distance between the cemento-enamel junction and the alveolar bone crest at the lingual aspect of the image.

(Akpinar et al. 2017, Dimitriu et al. 2021, Ballı et al. 2024). Polyphenolic compounds, with their rich biological activities, high pharmacological safety, and antioxidant, anticancer, antibacterial and anti-inflammatory properties, are considered promising candidates for periodontal treatment (Petti and Scully 2009, Guimaraes et al. 2011).

Extracts derived from MC, which has a high polyphenolic content, have been shown to exert antimicrobial activity against oral pathogens (Houshmand et al. 2011, Hedayati et al. 2013). In the present study, IL-1β levels were significantly increased in the periodontitis group compared with the control group, whereas a reduction was observed in the gastric gavage group.

In the combination treatment group (GG+IO), IL-1β levels returned to similar values to those of the control group. These findings suggest that MC treatment may be useful in modulating periodontal inflammation. IL-1β and TNF-α are proinflammatory cytokines playing pivotal roles in the initiation and progression of chronic inflammation and are considered key mediators in periodontitis. NF-κB is known to regulate inflammatory mediators such as IL-1β and TNF-α in periodontitis. Therefore, the reductions observed in these inflammatory mediators following MC administration may be also associated with the modulation of inflammatory signaling pathways. In a study with puerarin, significant inhibition of NF-κB activation was associated with decreased IL-1β and TNF-α levels (Yang et al. 2015). In contrast, IL-6 levels, another cytokine evaluated in the study, did not change significantly. Since IL-6 is a late-phase acute-response protein, requiring more extended periods to return to baseline levels, the absence of significant changes within the 11-day study period may be attributable to this delayed response profile.

RANKL binds to its specific receptor RANK on pre-osteoclast surfaces, activating the NF-κB pathway and thereby playing a critical role in osteoclast differentiation and activation (Belibasakis and Bostanci 2012). Elevated RANKL levels have been reported in periodontitis cases (Bostanci et al. 2007). According to previous studies, MC was reported to influence RANKL expression and osteoclast-related inflammation (Soomro et al. 2019). In the present study, the P+CHX group exhibited both the lowest inflammatory cell infiltration and the lowest RANKL activity. In the P+GG+IO+MC group, RANKL activity was significantly reduced, though not to the same extent as with CHX. The P+IO+MC group demonstrated RANKL levels near control values with low inflammatory cell density. Although direct comparison between a systemic herbal extract and a local synthetic antiseptic (CHX) requires caution due to their different therapeutic classes, the beneficial effects of MC on bone volume and oxidative stress parameters suggest its potential as an adjunctive agent to support periodontal tissue integrity.

The preservation of alveolar bone parameters observed in the gastric gavage group indicates potential benefits of systemic MC administration in maintaining periodontal tissue integrity. The higher bone volume and increased bone ratio observed in this group may suggest a protective effect of MC against bone resorption in periodontitis. Objective bone measurements via micro-CT reduced bias from subjective evaluation, thereby increasing the accuracy of morphometric results (Yang et al. 2015, Justo et al. 2022, Frazão et al. 2024).

MMP-8, the primary collagenase responsible for type I collagen degradation in periodontal tissues, plays a critical role in periodontal ligament and alveolar bone breakdown (Zalewska et al. 2024). According to the literature, MMP enzymes degrade the collagen matrix; MMP-8 cleaves surface-bound collagen fibers, while gelatinases MMP-2 and MMP-9 degrade the resulting collagen fragments (Zhang and Kern 2009). In our study, a significant reduction in MMP-8 levels was observed only in the gastric gavage group. Moreover, decreases in MMP-8 and MMP-9 levels represent an important therapeutic outcome in reducing matrix metalloproteinases which are the key mediators of periodontal tissue destruction. Suppression of these enzymes may be an indication of inflammatory responses that are modulated by MC which potentially leads to reduced collagenolytic activity in periodontal tissues.

One of the main mechanisms of tissue damage in periodontal inflammation is increased production of reactive oxygen species (ROS) (Borges Jr et al. 2007). In the present study, significant improvements in oxidative stress parameters were detected following MC treatment. The LC-MS analysis of the final extract formulation demonstrated the presence of several phenolic compounds and a high total phenolic content, which may partially explain the antioxidant and anti-inflammatory effects observed in the present study. Literature reports indicate that MC can enhance total antioxidant capacity (TAS) and markedly reduce TOS levels through its antioxidant and anti-inflammatory effects (Saritas et al. 2022). The similar antioxidant improvement observed in the present study may be associated with phenolic constituents identified in the extract, including quercetin, rosmarinic acid, catechin, and related polyphenolic compounds. It has been reported that this compound of MC is able to exert strong anti-inflammatory activity by reducing superoxide, hydrogen peroxide and nitric oxide production in macrophages (Mansouri et al. 2001, Matsehorova and Odyntsova 2024). The reductions in oxidative stress markers observed in our study are consistent with previous studies related to periodontal tissue destruction. As with other agents known for antioxidant efficacy, MC administration achieved significant reductions in oxidative stress parameters (Akpinar et al. 2017). Therefore, enhancement of antioxidant capacity and reduction of oxidative load may reflect the beneficial effects of MC administration during periodontal inflammation.

Conclusion

This study provides a comparative assessment of the effects of systemic and local administration of MC extract on periodontal tissue responses in a ligature-induced periodontitis model. The findings suggest that, in addition to reducing oxidative stress, MC may serve as a potential adjunctive agent capable of protecting the structural stability and integrity of periodontal tissues.

Limitations of the present study include the use of an animal model exclusively, lack of long-term outcome assessment, and absence of testing for different doses/concentrations. Furthermore, the absence of detailed bioavailability and pharmacokinetic profiling limits direct translatability of the findings to clinical applications. Future studies incorporating various dosing regimens, extended follow-up, and human clinical trials are recommended to substantiate these results.

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Author Declarations

Ethics approval

All experimental procedures were approved by the Afyon Kocatepe University Local Ethics Committee for Animal Experiments (protocol no. 49533702/33; approval date: 23 March 2022).

Use of generative artificial intelligence

ChatGPT (OpenAI), a generative artificial intelligence tool, was used solely for language editing and improving the English grammar of the manuscript. All AI-assisted revisions were reviewed by the authors, who take full responsibility for the final content.

Conflict of interest

The authors declare that they have no conflict of interest.

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