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Investigation of the preventive effect of methylsulfonylmethane on alveolar bone loss and oxidative stress in a rat model of periodontitis

Melek Beder[1*], Hatice Yemenoglu[1], Semih Alperen Bostan[1], Oğuz Kose[1], Sibel Mataracı Karakas[2], Tolga Mercantepe[3], Adnan Yılmaz[2] and Levent Tumkaya[4]

Abstract

Background To investigate the preventive efficacy of methylsulfonylmethane (MSM) on alveolar bone destruction in rats with periodontitis.

Methods Twenty-four male Sprague-Dawley rats were randomly divided into three groups: control, experimental periodontitis (Ep), and Ep-MSM. Periodontitis was induced by placing 4.0 silk sutures in the subparamarginal position on the necks of the mandibular first molars and applying the suture for 5 weeks. The Ep-MSM group was given 500 mg/body weight/day MSM intraperitoneally for 35 days. At the end of the study, bilateral mandibular samples were taken. Periodontal bone loss was measured through histologic sections. Histomorphometric and immunohistochemical (receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG)) evaluations were performed on right mandibular tissue samples, and biochemical (interleukin (IL)-1 beta (β)/IL-10, malondialdehyde (MDA), glutathione (GSH), oxidative stress index (OSI)) evaluations were performed on left mandibular tissue samples.

Results No significant difference was found between the groups in IL-1β and IL-1β/IL-10 values ($p > 0.05$). A significant decrease in IL-10 levels was observed in the Ep-MSM and Ep groups compared with the control group ($p < 0.05$). MDA levels significantly increased in the Ep and Ep-MSM groups compared with the control group, and GSH levels significantly decreased in the Ep group compared with the other groups ($p < 0.05$). OSI values were significantly higher only in the Ep group ($p < 0.05$). RANKL levels showed a significant increase in the Ep group compared with the other groups. OPG levels were significantly increased only in the Ep-MSM group ($p < 0.05$).

Conclusions The results of this study may suggest that MSM has preventive effects on alveolar bone loss and oxidative stress.

Keywords Alveolar bone loss, Methylsulfonylmethane, Oxidative stress, Periodontitis

*Correspondence:
Melek Beder
melek.beder@erdogan.edu.tr
[1]Department of Periodontology, Faculty of Dentistry, Recep Tayyip Erdoğan University, Rize 53020, Turkey
[2]Department of Biochemistry, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey
[3]Department of Histology and Embryology, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey
[4]Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

Background

Periodontitis is a chronic inflammatory disease associated with inflammation of the supporting tissues of the teeth, which results in destruction of periodontal tissues if left untreated [1]. The host response, along with microbial dental plaques (MDP), play an important role in the development of periodontal diseases [2, 3]. As a result of the immune-inflammatory events that develop as a result of the two-way interaction between the host response and bacteria, overproduction of pro-inflammatory cytokines such as IL-1β, IL-6, tumor necrosis factor-alpha (TNF-α), prostaglandin E_2 (PGE_2), and matrix metalloproteinases (MMP) by the host tissues leads to destruction of periodontal tissues [4]. IL-10, an anti-inflammatory cytokine, has been reported to inhibit collagenase activity, prevent MMP activity [5], and suppress RANKL release in periodontal diseases [6].

Osteoclast activation is controlled by the RANK/RANKL/OPG signaling pathway [7]. OPG inhibits osteoclastogenesis by inhibiting the RANK/RANKL interaction [8]. A close relationship has been shown between alveolar bone resorption and increased RANKL levels in the presence of periodontitis [9, 10]. Pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α have been reported to up-regulate RANKL-mediated osteoclastogenesis and increase the production and release of reactive oxygen species (ROS) [11]. Increased ROS production and oxidative stress have been reported to play an important role in osteoclast formation through the up-regulation of RANKL [12, 13]. Oxidative stress occurs because of the disruption of the balance between free radicals and the protective antioxidant system [14]. Oxidative stress levels in tissues or body fluids can be determined using different methods. Some of these are the measurement of MDA, GSH, total oxidant status (TOS), total antioxidant status (TAS), and OSI levels [15, 16].

With the understanding of the effect of the host response on tissue destruction, many studies have been conducted to develop host modulatory agents [17, 18]. Current studies on the treatment and prevention of periodontal diseases are investigating strategies that address the balance between the microbiota and the host response [19, 20]. MSM is an organic sulfur compound with anti-inflammatory, anti-tumoral, antioxidant, and bone regeneration effects [21–23]. It has been proposed as an effective chemotherapeutic agent for the treatment of breast cancers, metastatic melanoma, and other metastatic cancers. Additionally, it has been employed in the treatment of certain diseases, both as a therapeutic intervention and for symptom alleviation [24].

MSM has been shown to be an inducer of differentiation of mesenchymal stem cells into osteoblasts and osteogenesis [25]. When bone morphogenic protein (BMP2) was used together with MSM, the mineralization process was found to be more advanced than in cells treated with BMP2 alone [26, 27]. Another study found that MSM increased bone formation markers (osteocalcin, N-terminal propeptide), decreased bone resorption markers (acid phosphatase, type 1 collagen, C-terminal telopeptide), and increased mandibular trabecular bone density through the ability to induce osteoblast formation [28]. Another animal study showed that MSM significantly reduced tissue pro-inflammatory cytokine levels in ethanol-induced cerebral injury [29]. Abdel-Rafei et al. [30] reported an increase in IL-10 levels with MSM application. In rat studies, in addition to studies reporting a decrease in MDA levels and an increase in GSH levels with MSM treatment [31, 32], it has also been reported that there is no significant difference in MDA and GSH levels with MSM treatment [33].

Considering all this information, this study was planned based on the hypothesis that MSM had antioxidant, anti-inflammatory, and anti-resorptive effects and that systemic MSM administration might limit periodontal tissue destruction. The aim of the study was to investigate the preventive effects of MSM on periodontal tissue destruction and its effects on RANKL, OPG, IL-1β/IL-10, MDA, GSH, and OSI levels biochemically, histologically, and immunohistochemically.

Methods

Ethical approval, animal housing and study groups

The experimental protocol of this study was approved by the Recep Tayyip Erdoğan University, Faculty of Medicine, Animal Research Ethics Committee (approval number: 2023/24). Experimental studies were conducted using 24 male Sprague Dawley rats (age 3–4 months, weighing 250–300 g) provided by the center at the Recep Tayyip Erdoğan University Experimental Animal Application and Research Center. All animals were treated humanely as specified in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health and reported according to ARRIVE guidelines 2.0 for preclinical studies [34]. The rats were housed under standard laboratory conditions (12-hour light, 12-hour dark cycle, temperature: 21 ± 2 °C; relative humidity: 58%) and fed with standard rat chow and water during the study. The standard feed contains fat (3.5%), carbohydrates (7.5%), protein (23%), vitamins-minerals (1–2%) and trace elements such as iron, selenium, cobalt, zinc manganese (3%). Sample size was calculated using the TOS parameter as the primary outcome variable [35]. The sample size calculated using the G*Power statistical package program (ver.3.1.9.7)* with an effect size of 0.76, a power of 80%, and a type-1 error (α) of 0.05 was determined as at least 7 rats for each group. In the current study, considering possible losses (rat disease or death)

that may occur during the study, the sample size was determined to include 8 rats in each group. The post-hoc power value recalculated according to this sample size was 85% and the effect size was 0.76.(G*Power statistical package program (ver.3.1.9.7)*. Animals were divided into groups by simple randomization using the coin toss method into three groups: control ($n=8$), Ep ($n=8$) and Ep-MSM ($n=8$) (Fig. 1).

Induction of periodontitis and MSM administration

After a 7-day adaptation period, rats in the Ep and Ep-MSM groups were given general anesthesia through injections of xylazine hydrochloride (10 mg/kg body weight) (Rompun, Bayer, Istanbul, Turkey) and ketamine hydrochloride (40 mg/kg body weight) (Ketalar, Pfizer, Istanbul, Turkey). A 4.0 sterile silk suture was placed in the cervical region of both the right and left lower 1st molar teeth of the rats in a sub-paramarginal [36, 37]. Because of its structure and position, the suture is designed to lead to plaque accumulation, inflammatory changes and eventually periodontitis. The ligatures were checked daily by two operators (MB, HY) to avoid the observer bias. There was no loosening, detachment or displacement of the sutures during the experimental process. Experimental periodontitis was induced by keeping it in place for 5 weeks [38, 39].

MSM (Bereket Kimya, Istanbul, Turkey) was measured and weighed at 2.14 g per day. It was placed in a 10 mL volumetric flask and the final volume was made up to 10 mL with distilled water. This mixture was vortexed and made ready. Similar to previous studies, 500 mg/body weight MSM was administered intraperitoneally at 12:00 for 5 weeks starting from the first day of ligature placement as a single daily dosage [31]. Saline was applied to the Ep and control groups. The weights of the rats were measured before starting the experiment and periodically throughout the experiment, and the MSM dose was adjusted using a scale according to the changing weights of the animals.

Collection and preparation of samples

At the end of week 5, rats in all groups were euthanized through decapitation (TM, MB). Mandibular tissues including the first molars and surrounding tissues were removed together. Right mandible samples were transferred to containers containing 10% neutral formaldehyde solution for histomorphometric and immunohistochemical analyses. Left mandible samples were washed with cold saline, quickly wiped with filter paper, and frozen at -80 °C for use in biochemical analyses. Both histologic and biochemical examinations were performed by two expert researchers who were unaware of the study group assignment (histology: TM, LT; biochemistry: AY, SMK).

Histological analyses

Histomorphometric measurements

Tissue samples removed from the area between the mesial of the first molar and the distal of the second molar in the right mandibular jaw bones were fixed for 24–36 h using 10% neutral formalin solution to be evaluated in histopathologic analyses. Histologic tissue tracking and paraffin embedding (Leica EG 1150 H, Lecia Biosystems, Germany) procedures were performed as described in detail in previous studies [38, 40]. Then, serial sections were taken in the mesio-distal direction using a rotary microtome at a thickness of 5 micrometers (μm) using the long axis of the tooth as a guide (Leica RM2255, Leica Biosystems, Germany). The sections were stained with Harris's hematoxylin (Merck KGAa,

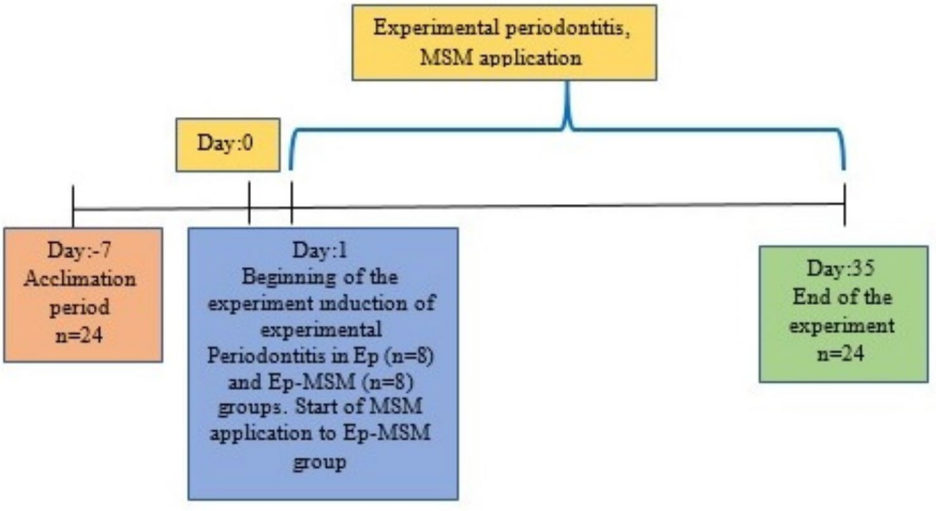

Fig. 1 Time schedule of experimental period

Germany) and eosin G (Merck KGAa, Germany) using an automatic staining machine (Leica ST5020, Germany). Alveolar bone loss, the distance between cementoenamel junction (CEJ) and bone crest (BC), and the distance between furcal bone crest (FBC) and furcation roof (FR) were measured quantitatively in µm units in stained sections. The measurements were performed on three different serial sections that were not adjacent to each other using the Arbitrary probe of the CellSens (Imaging Software, Olympus Corp., Japan) computer program attached to a light microscope (Olympus BX51, Olympus Corp., Japan) [31].

Immunohistochemical analysis

2–3 µm thick serial sections were taken from paraffin blocks of jaw bone tissue using a rotary microtome (Leica RM2255, Leica Biosystems, Germany) and placed on positively charged slides. During the deparaffinization phase, the sections were incubated with antibodies according to the manufacturer's catalogs for RANKL primary antibody (ab239607, Abcam, United Kingdom) and OPG primary antibody (ab203061, Abcam, United Kingdom) using the Leica Bond Max (Leica Biosystems, Australia) immunohistochemical/in-situ hybridization (IHC/ISH) platform and the staining procedures were continued.

Semi-quantitative analyses

The number of cells showing RANKL and OPG positivity was evaluated in sections containing tissues removed from the area between the mesial of the first molar and the distal of the second molar in the right mandibular jaw bones stained with RANKL and OPG. In the semi-quantitative evaluation, the scoring of osteocytes showing RANKL and OPG positivity was analyzed as shown in Table 1 [41, 42]. Twenty different fields from each section were evaluated by two blinded histopathologists (TM, LT).

Biochemical analyses

Mandibular tissue analysis

The obtained gingival tissue samples were washed with cold phosphate buffered saline (PBS) (pH 7.4). Then, cold phosphate buffer with a volume twice the tissue weight was added to the tissue. The mixture was homogenized with a homogenizer at 30 Hz for 5 min (min). Finally, the tissue homogenates were centrifuged at 12.000 rpm at 4℃ for 15 min and the tissue MDA, GSH, TOS, TAS, IL-1β, and IL-10 levels were examined. All tissue results are expressed as g/tissue.

Table 1 Method of measuring ımmunopositive cells by semi-quantitative methods

Scor	Finding (Positive cell rate)
0	None
1	Mild (less than 5%)
2	Moderate (between 6% and 25%)
3	Severe (between 26% and 50%)
4	More severe (between 51% and 75%)

Determination of MDA and GSH levels

Two hundred microliters of supernatants were pipetted and 50 µL of 8.1% SDS, 375 µL of 20% acetic acid (pH = 3.5), and 375 µL of 0.8% thiocarboxylic acid (TBA) were added. The mixture was vortexed and then incubated in a boiling water bath for 1 h. After incubation, it was cooled in ice water for 5 min and centrifuged at 750 *g* for 10 min. The pink complex product formed was read at 532 nm in a spectrophotometer and the concentrations were calculated [43]. Ellman's method was used to determine the GSH level [44]. One hundred microliters of 3 M disodium hydrogen phosphate (Na_2HPO_4) and 25 µL of Ellman's reagent were added to the 25 µL pipetted blank, standard and supernatants, then the yellow color formed after gentle shaking was read at 412 nm in the spectrophotometer and the concentrations were calculated. The results were given as mmol/g tissue.

Determination of TOS and TAS levels and calculation of OSI

TOS and TAS levels of the supernatants were determined using commercially available kits (Mega Tıp Industry and Trade Limited Company, Turkey), both using a DxC700 Beckman Coulter autoanalyzer. Results are expressed as µmol hydrogen peroxide (H_2O_2) equivalents/g protein and µmol Trolox equivalents/g protein, respectively [45]. The percentage ratio of TOS to TAS was used as the OSI = [(TOS (µmol H_2O_2 equivalents/L) / (TAS (µmol Trolox equivalents/L)] [46].

Determination of inflammatory and anti-inflammatory cytokine levels

IL-1β (BT Lab kit, Cat. No E0119Ra) and IL-10 (BT Lab kit, Cat. No: E0108Ra) levels of supernatant mandibular tissue samples were determined using a commercial rat-specific enzyme-linked immunosorbent assay (ELISA) using Bioassay Technology Laboratory (BT LAB) commercial kits. The results are expressed as pg/mL.

Statistical analysis

The sample size and power of the study were calculated using the G*Power statistical package program (ver.3.1.9.7). The sample size was established considering three groups and TOS levels were used as the primary outcome variable [35]. For the analysis of biochemical measurements in the study, Shapiro-Wilk test were used to determine whether the continuous measurements were normally distributed; parametric tests were applied because the measurements were normally distributed. Descriptive statistics for the variables in the study are

Table 2 Comparison of alveolar bone losses between groups

	Control Mean ± SD	Ep Mean ± SD	Ep-MSM Mean ± SD
CEJ-BC (µm)	603.34 ± 93.56	1222.24 ± 76.41[a]	817.59 ± 78.67[a, b]
FR-FBC (µm)	285.60 ± 27.95	908.69 ± 261.58[a]	487.16 ± 20.64[a, b]

[a]p=0.001 compared to Control group, [b]p=0.001 compared to Ep group, One-Way ANOVA-Tukey HSD test

SD; standard deviation, µm; micrometer, (Ep; experimental periodontitis, MSM; methylsulfonylmethane, CEJ; cementoenamel junction, BC; bone crest, FR; furcation roof, FBC; furcal bone crest)

expressed as mean, standard deviation, number (n), and percentage (%). One-way analysis of variance (ANOVA) was used to compare continuous measurements according to groups. Following the variance analysis, the Duncan test was used to determine different groups. Pearson's correlation coefficients were calculated to determine the relationship between measurements. For the analysis of histologic data, the Shapiro-Wilk test, Q-Q plot, Skewness-Kurtosis values, and Levene's test were used to evaluate whether the data were normally distributed. One-way ANOVA and Tukey's HSD test were applied in the comparison of alveolar bone losses between groups because the data were normally distributed. Data are given as mean and standard deviation. In the analysis of immunohistochemical findings, Kruskall-Wallis test, Duchan test and Tamhane T2 test were performed for the comparison between groups since the data were not normally distributed. Non-parametric data are expressed as median, minimum, and maximum. In the calculations, the level of statistical significance was accepted as $p < 0.05$. The SPSS (IBM SPSS for Windows, ver.26) statistical package program was used for analysis.

Results

Histologic results

Histomorphometric results

CEJ-BC and FR-FBC measurements of the study groups were significantly higher in the periodontitis group compared with the control group ($p = 0.001$). Although MSM treatment provided a significant decrease in both measurement findings, bone loss levels were still significantly higher in the Ep-MSM group compared with the control group ($p = 0.001$) (Table 2; Fig. 2).

Immunohistochemical results

RANKL activity was observed to be significantly increased in the rats in the periodontitis group compared with the control group, whereas it was significantly decreased in the MSM group ($p = 0.001$). OPG activity was observed to be significantly increased in the rats in the MSM group compared with both the periodontitis and control groups ($p = 0.001$) (Fig. 3; Table 3). No significant difference was found in OPG activity between the periodontitis and control groups ($p > 0.05$).

Biochemical results

Tissue pro-inflammatory and anti-inflammatory cytokine levels

Biochemical findings regarding pro-inflammatory and anti-inflammatory cytokine levels in the study are given in Fig. 4. No significant difference was found between the groups in terms of IL-1β values ($p > 0.05$). The IL-10 level was found to be statistically significantly higher in the control group compared with the Ep and Ep-MSM groups ($p < 0.05$), but was higher in the Ep-MSM group compared to the Ep group, although not significantly ($p > 0.05$). The IL-1β/IL-10 ratio showed a slight increase in the periodontitis group compared with the MSM and control groups, which was not statistically significant ($p > 0.05$) (Fig. 4).

Tissue oxidative stress levels

Biochemical findings showing the level of oxidative stress in the study are given in Fig. 5. Significant differences were found between the groups in terms of MDA levels ($p < 0.05$). Accordingly, the highest MDA value was observed in the periodontitis group, and the lowest value was seen in the control group. GSH levels were significantly lower in the periodontitis group compared with the MSM and control groups ($p < 0.05$), but there was no significant difference between the MSM and control groups ($p > 0.05$). No significant differences were found in terms of TOS and TAS values ($p > 0.05$). The highest TOS value was observed in the periodontitis group, and the highest TAS value was seen in the MSM group. Although OSI was significantly higher in the periodontitis group than in the other groups ($p < 0.05$), there was

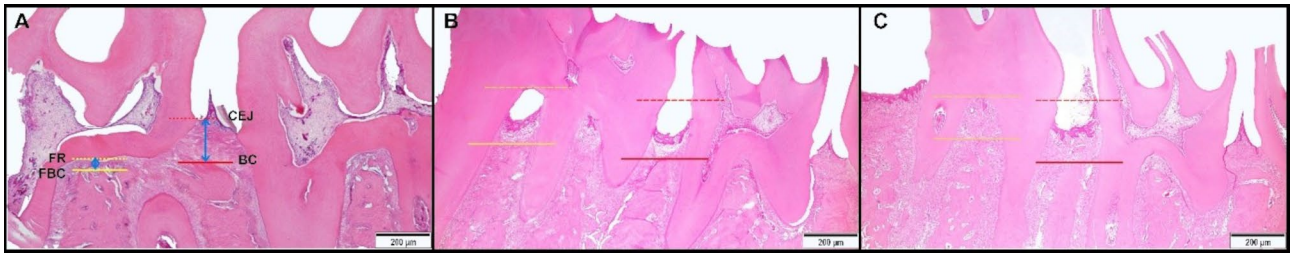

Fig. 2 Evaluation of alveolar bone loss in histological sections. **A**; Control, **B**; Ep, **C**; Ep-MSM groups are represented. (Ep; Experimental periodontitis, MSM; Methylsulfonylmethane, CEJ; cementoenamel junction, BC; bone crest, FR; furcation roof, FBC; furcal bone crest)

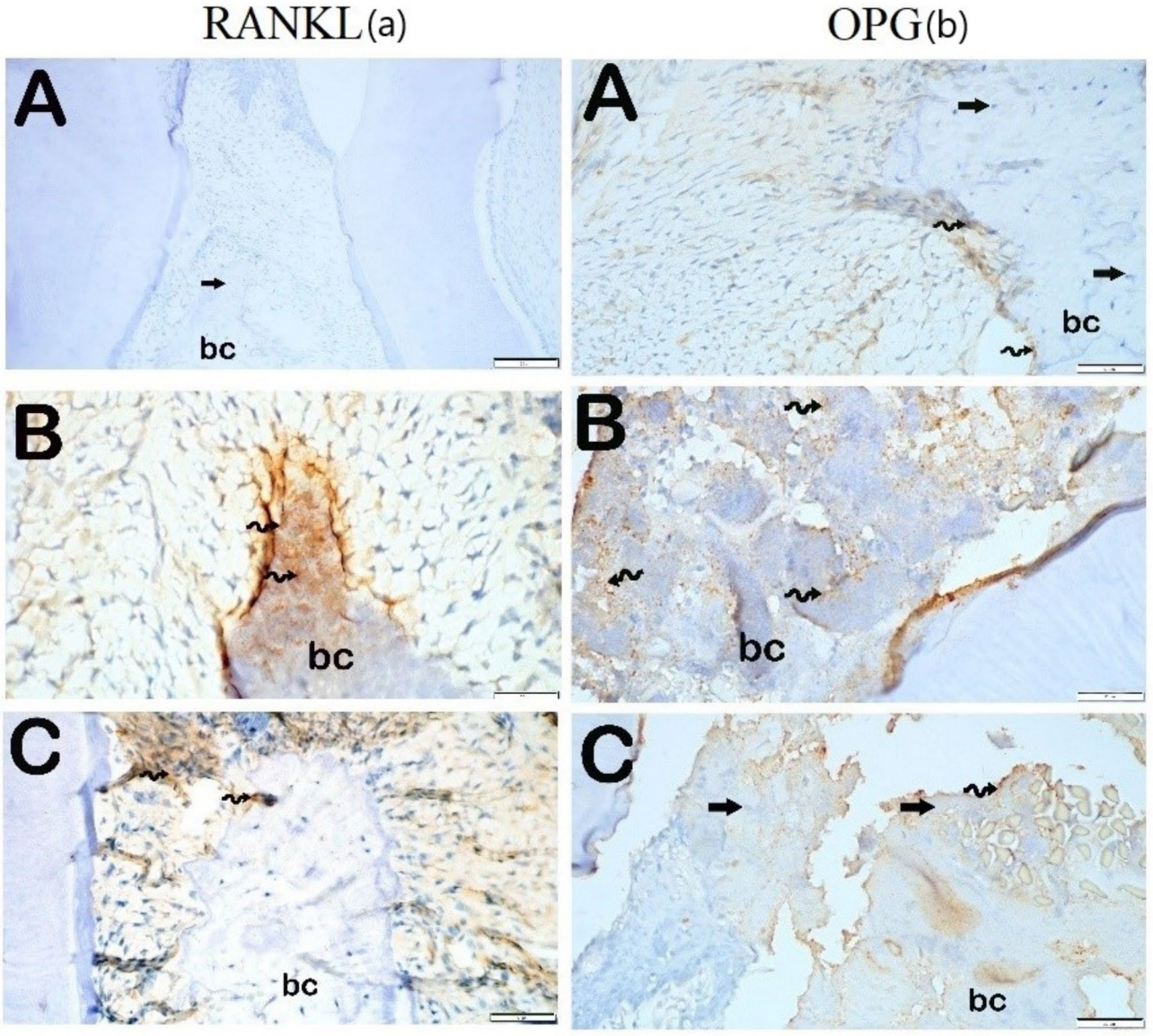

Fig. 3 Representative light microscope image of mandible tissue incubated with RANKL and OPG primary antibodies. **a**) Representative light microscopy image of mandible tissue incubated with RANKL primary antibody. **b**) Representative light microscopy image of mandible tissue incubated with OPG primary antibody. (**A**) control, (**B**) Ep, (**C**) Ep-MSM (Ep; Experimental periodontitis, MSM; methylsulfonylmethane, bc; bone crest, RANKL; receptor factor of nuclear factor kappa B ligand, OPG; osteoprotegerin. RANKL, OPG positive cells (tailed arrow), RANKL, OPG negative cells (arrow). **a**) Control group; In sections of normal mandible tissue, cells were observed to be immunonegative for RANKL primary antibody (RANKL positivity score 1(0–1)) Ep Group; In sections of periodontitis group, cells were observed to be intensely RANKL immunopositive (RANKL positivity score 3(2–3)). Ep-MSM group; In the sections of the periodontitis group to which MSM was applied, it was observed that the number of cells showing intense RANKL positivity decreased (RANKL positivity score 1(1–2)). **b**) Control group; In the sections of the normal structured mandible tissue, it was observed that the cells were immunopositive for OPG primary antibody (OPG positivity score 1(1–1)). Ep Group; In the sections of the periodontitis group, it was observed that the cells were immunopositive for OPG primary antibody (OPG positivity score 1(1–1)). Ep-MSM group; It was observed that the sections of the periodontitis group to which MSM was applied were immunopositive for intense OPG primary antibody (OPG positivity score 2(1–2))

Table 3 Comparison of RANKL and OPG activities between groups

	Control median (%25-%75)	Ep median (%25-%75)	Ep-MSM median (%25-%75)
RANKL	1(0–1)	3(2–3)[a]	1(1–2)[b, c]
OPG	1(1–1)	1(1–1)	2(1–2)[a, b]

[a]p=0.001 Compared to control group, [b]p=0.001 Compared to Ep group, [c]p=0.002 Compared to control group, Kruskal-Wallis/Tamhane T2 test, Ep; experimental periodontitis, MSM; methylsulfonylmethane, RANKL; receptor factor of nuclear factor kappa B ligand, OPG; osteoprotegerin

no significant difference between the control and MSM groups ($p > 0.05$) (Fig. 5).

Discussion

To our knowledge, this is the first study to evaluate the preventive efficacy of MSM on alveolar bone loss in periodontitis in a rat model. Our findings showed that systemic MSM application could significantly limit ligature-related periodontal bone loss and oxidative stress. In addition, a significant increase in IL-10 levels was detected.

There is no current consensus on the dose and duration of MSM administration. In a study, it was reported that oral administration of 1.5 g MSM to rats for 90 days did not cause any side effects and mortality [47]. Aljohani et al. [28], in an experimental study investigating the effects of MSM on mandibular bone density in aged mice, administered 100 mg/kg MSM to mice for 13 weeks. Amirshahrokhi and Niapour [29], in a rat study investigating the protective effect of MSM on ethanol-induced brain injury, administered 200 mg/kg/day and 400 mg/kg/day doses of MSM to different groups for 12 days and found that the healing effects of MSM were greater when 400 mg/kg/day was administered. Miao et al. [48] investigated the effect of MSM on mycoplasma-induced inflammatory damage in chicken trachea and reported that the optimum concentration of MSM administration

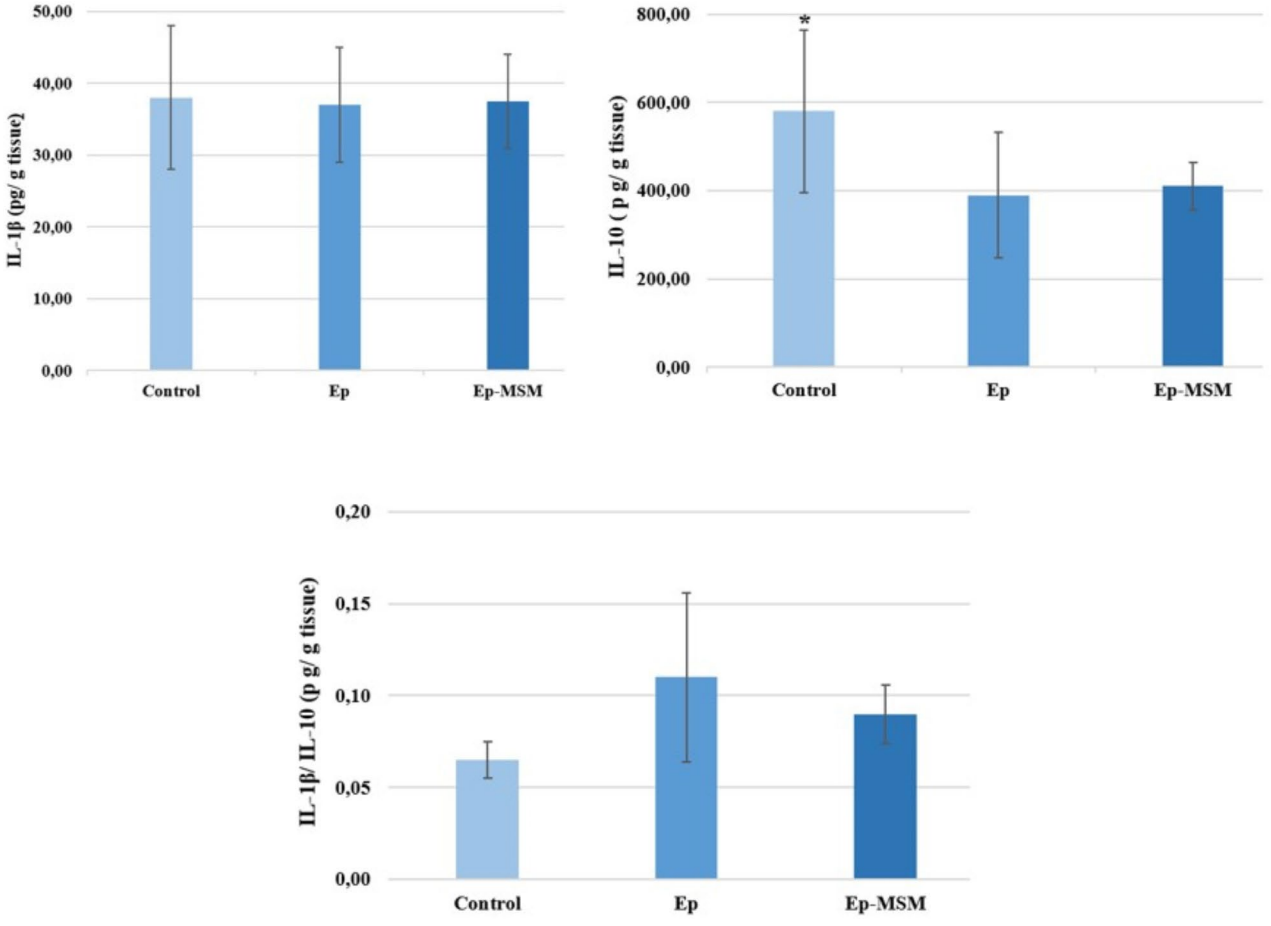

Fig. 4 Comparison of inflammatory parameters of mandibular tissue between groups. Ep, experimental periodontitis; MSM, methylsulfonylmethane; IL-1β, interleukin-1β; IL-10, interleukin 10; Results are expressed as mean ± SD. Symbols (*) indicate significant differences between groups. One-way ANOVA and Tukey test were performed ($p < 0.05$)

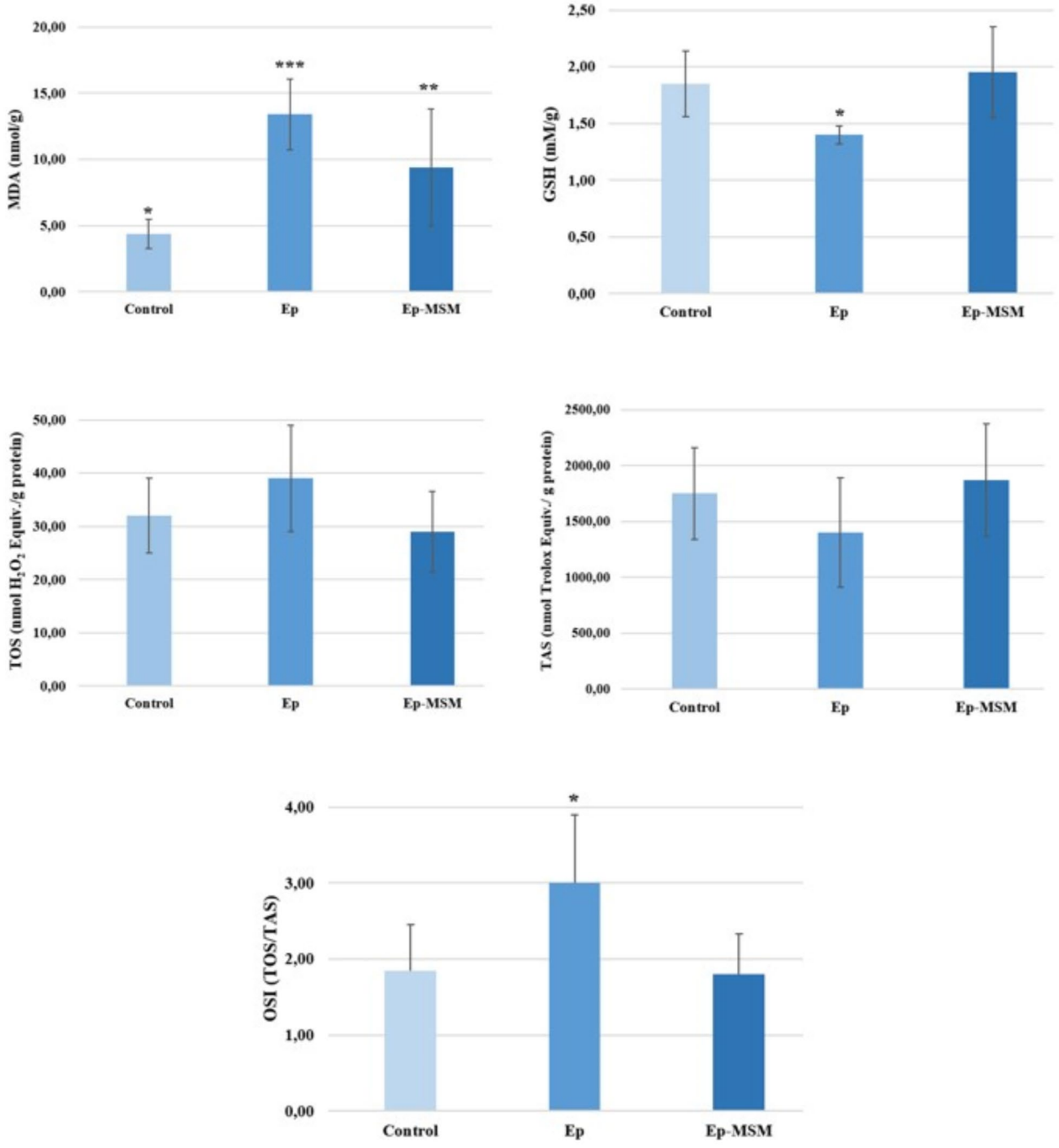

Fig. 5 Comparison of oxidative stress parameters of mandibular tissue between groups. Ep, experimental periodontitis; MSM, methylsulfonylmethane; MDA, malondialdehyde; GSH, glutathione; TOS, total oxidant level; TAS, total antioxidant level; OSI, oxidative stress index. Results are expressed as mean ± SD. Symbols (*) indicate significant differences between groups. One-way ANOVA and Tukey test were performed ($p < 0.05$)

was 500 mg/kg. As a result of the literature review, in the present study, 500 mg/kg intra-peritoneal MSM administration was performed to evaluate the preventive and limiting effects of MSM administration in an experimental periodontitis model in rats. There are models in which ligature is applied for different durations to induce experimental periodontitis [36, 38, 49]. Since prolonged ligature application may cause severe mobility and nutritional problems in the teeth of rats, in the present study, 5-week experimental periodontitis model application was preferred considering our previous studies [38, 39, 50] and MSM was applied for 5 weeks.

The increasing evidence for the role of immune-inflammatory response in the pathogenesis of periodontal diseases provides the basis for studies investigating the efficacy of various host modulation agents in the treatment of these diseases [51, 52]. Due to the central role of pro-inflammatory mediators and oxidative stress in the pathogenesis of periodontitis, these mechanisms are specifically investigated in a large part of the studies. In this context, in our study, we investigated the periodontal protective efficacy of MSM based on these main mechanisms and their effects on the RANK/RANKL/OPG system.

In the present study, the level of periodontal bone loss was analyzed by measuring the distance between the CEJ-BC and the FR-FBC on histologic sections taken in the mesio-distal direction. This study demonstrated for the first time that MSM significantly limited alveolar bone loss in rats with periodontitis. There was no related study with which we could directly compare our histomorphometric findings. However, there were studies whose findings we could indirectly compare. Joung et al. [26] found that the use of BMP2 together with MSM improved the mineralization process compared with cells treated with BMP2 alone, and Aljohani et al. [28] found study that MSM increased mandibular trabecular bone density in an animal with its ability to induce osteoblast formation.

There is much current evidence for the central role of the RANKL/RANK/OPG system in periodontal bone loss [53–55]. In our study, RANKL and OPG were evaluated using immunohistochemical methods to assess alveolar bone destruction. According to our findings, it was observed that RANKL activity was significantly increased in the periodontitis group compared with the control group, and a significant decrease was observed in the MSM group compared with the periodontitis group. In the literature, similar to our findings, it was observed that there was a statistically significant increase in RANKL activity in rats with periodontitis compared with control groups [38, 56, 57]. In addition, Taubman et al. [58] reported that osteoclastogenesis in periodontal disease could be improved by inhibiting RANKL activity. When looking at the studies examining the effect of MSM use on RANKL activity, Amirshahrokhi et al. [29] reported a significant decrease in RANKL activity as the administered MSM concentration increased in a rat study examining ethanol-induced cerebral damage. In a study by Ateş et al. [42] examining the effects of mineral trioxide aggregate (MTA) and MSM on pulp tissue, it was found that there was a significant decrease in RANKL activity in the treatment group compared with the diseased pulpitis group. The findings of our study support the findings of the above studies.

In the literature, a statistically significant decrease in OPG activity was observed in rats with periodontitis compared with control groups [38, 56, 57]. In our study, OPG activity was significantly higher in rats with periodontitis administered MSM compared with rats in both the periodontitis and control groups and there was no significant difference between the periodontitis and control groups.

Many studies have proven that cytokines play a key role in each stage of the periodontal disease mechanism through the immune response to MDP [59–61]. IL-1β is a pro-inflammatory cytokine and affects the periodontal destruction process, including bone resorption [61]. In animal studies, it was reported that IL-1β levels were statistically significantly higher in the periodontitis group compared with the control group [62–64]. In our study, unlike these studies, no significant difference was found between the groups in terms of IL-1β levels. We think that the reason for this may be the low number of samples and the different sensitivity levels of the kits used.

When looking at the studies examining the effect of MSM on IL-1β levels, it has been reported that there is a significant decrease in IL-1β levels with MSM administration [29, 30]. Unlike these studies, in the study conducted by Üçüncü et al. [33] in which the authors created formalin-induced cartilage damage in the knee joints of rats, the cartilage protective effects of some anti-inflammatory agents were examined and it was reported that there was no statistically significant difference in IL-1β levels between the group that was administered the MSM + glucosamine hydrochloride + chondroidin sulfate combination and the group that was not. In our study, no significant difference was observed in IL-1β levels in the MSM group compared with the other groups. We think that this situation may be associated with the IL-1 genotype differences that can be seen in rats and the inadequacy of the MSM dose used in affecting the gingival tissue.

IL-10 is an anti-inflammatory cytokine and acts by preventing the activation of osteoclasts and controlling the amount of bone produced by osteoblasts in preserving the existing bone amount [5]. It has been reported that there is a statistically significant decrease in IL-10 levels in rats with periodontitis compared with healthy controls [65, 66]. According to our findings, in line with these studies, IL-10 levels were significantly lower in the periodontitis group compared with the control group. In their study examining the regulatory effects of MSM against neurodegenerative changes in rats, Abdel-Rafei et al. [30] found that there was a significant increase in IL-10 levels in the MSM-treated group compared with the untreated and control groups. In our study, IL-10 levels were slightly higher, although not significantly, in the MSM group compared with the periodontitis group, and

it was significantly higher in the control group compared with the other groups.

Using IL-1β/IL-10 ratios instead of just IL-1β and IL-10 is a more valid approach in examining the total pro-inflammatory effect in tissues [67]. In their rat study examining the relationship between stress and melatonin, Bostan et al. [38] found that the IL-1β/IL-10 ratio was significantly higher in rats with periodontitis than in the control group. In our study, unlike their study, no significant difference was found between the groups in terms of the IL-1β/IL-10 ratio. We think that this may be due to the different sensitivity levels of the kits.

In our study, MDA, GSH, TAS, TOS, and OSI parameters were used to evaluate the oxidative status in the gingival tissue. MDA is one of the most frequently used oxidative stress markers in determining the level of oxidative damage in the cell membrane. GSH is known as the basic intracellular antioxidant and has an important role in preventing cellular oxidative damage [68]. Current literature has shown that TAS and TOS measurements reveal oxidative and antioxidative statuses more clearly [45]. OSI is a highly reliable parameter suggested by Erel to reflect the level of oxidative stress more practically and clearly [45].

According to our results, there were significant differences in MDA levels between the groups and the highest MDA level was observed in the periodontitis group, and the lowest value was observed in the control group. In the literature, many studies have reported that, similar to our findings, MDA levels in rats with periodontitis were statistically significantly higher than in the control group [39, 64, 69, 70]. By contrast, Atalay et al. [62] reported that there was no statistically significant difference in MDA levels in the periodontitis group compared with the control group in their study examining the antioxidant effects of pistacia eurycarpa salt. Amirshahrokhi and Bohlooli [31] found that MSM treatment significantly reduced MDA levels compared with the untreated diseased group and increased them compared with the healthy control group in their study examining the effects of MSM on acute lung and liver injury. In a study investigating the effect of MSM on acetaminophen-induced hepatotoxicity in rats, it was reported that MDA levels decreased in the MSM-treated group compared with the untreated group [32]. In a study examining the effects of MSM on ethanol-induced cerebral damage in mice, Amirshahrokhi and Niapour [29] reported that MSM treatment significantly decreased MDA levels compared with the ethanol-damaged group and significantly increased MDA levels compared with the control group.

According to our findings, GSH levels were significantly lower in the periodontitis group compared with the MSM group and the control group, but there was no significant difference between the MSM group and the control group. Similar to our study, França et al. [71] found that GSH levels were significantly lower in the periodontitis group compared with the control group in the study in which they evaluated the kidney parameters of rats. Unlike our findings, there are studies that found no significant difference in GSH levels in rats with periodontitis compared with the control group [40, 63, 68].

Amirshahrokhi and Bohlooli [31] conducted a study on rats in which they examined the effects of MSM on acute lung and liver injury and they found that GSH levels increased in the MSM group compared with the untreated diseased group, and decreased compared with the healthy control group. Bohlooli et al. [32] investigated the effect of MSM on acetaminophen-induced hepatotoxicity in rats, reporting that there was an increase in GSH levels in the MSM group compared with the untreated group and a decrease compared with the healthy control group. Laham [72] examined the healing effects of MSM against glycerol-induced acute renal failure in rats and observed an increase in GSH levels in the MSM-treated group compared with the glycerol-only group, and a decrease compared with the control group. In our study, an increase in GSH levels was observed in the MSM-treated group compared with the periodontitis group, but no significant difference was observed compared with the control group. In contrast to our results, Üçüncü et al. [33], who created formalin-induced cartilage damage in the knee joints of rats, found no statistically significant difference in GSH levels in the MSM + glucosamine hydrochloride + chondroidin sulfate combination group compared with the diseased group without treatment. It may be thought that this situation is related to the antioxidant adaptive response given depending on the applied MSM dose and the application period.

Although there was no significant difference between the groups in terms of TOS values in our study, it was slightly higher in the periodontitis group compared with the control and MSM-treated groups. In the literature, unlike our findings, the TOS values in rats with periodontitis were significantly higher than the control group [38, 62, 73, 74]. In the current study, no significant difference was observed between the groups in TAS values. Similar to our findings, there are studies that found no significant difference in TAS values between rats with periodontitis and the control groups [38, 39, 64], as well as studies reporting that TAS levels were significantly lower in rats with periodontitis compared with the control groups [62, 73, 74].

In our study, the OSI value was significantly higher in the periodontitis group than in the control and treatment groups. Similar to our findings, the OSI value was found to be statistically significantly higher in the periodontitis group than in control groups [62–64, 73, 74]. Bostan et al. [38], examined the relationship between stress and

melatonin in a rat model and found the OSI value higher in the periodontitis group than in the control group, although not statistically significant.

There are some limitations of this study. First, the sample size was limited. Second, there was no clear information in the literature about the optimal dose that should be applied to rats to evaluate the mechanism of action of MSM. Third, is that ligature-induced periodontitis causes acute inflammation in rats, but periodontitis shows a chronic course in humans in terms of pro-inflammatory and anti-inflammatory cytokine activities and oxidant/antioxidant balance. Another limitation is that although the inclusion of only male rats is adequate for standardization, it may not be accurate to generalize the findings to both sexes. In our study, histopathological and IHC analyses were used to examine tissue damage at the cellular level. In addition, our study needs to be supported by further studies including Western blot and ELISA analyses. Due to the limited amount of tissue examined, several potential molecules that may play a role in the mechanism of action of MSM could not be evaluated. Further and more comprehensive animal studies based on this pilot study may determine the need for human studies aimed at establishing the periodontal protective efficacy of MSM.

Conclusions

The present study showed that MSM had a positive effect on anti-inflammatory cytokine levels and oxidative stress parameters, prevented RANKL-induced osteoclastogenesis, and improved new bone activity and bone formation. In conclusion, our findings suggest that MSM administration may reduce alveolar bone destruction resulting from periodontitis and be an immune-modulatory agent that can be used in host modulation therapy.

Abbreviations

BMP2	Bone morphogenic protein
BC	Bone crest
CEJ	Cementoenamel junction
ELISA	Enzyme-linked immunosorbent assay
Ep	Experimental periodontitis
FBC	Furcal bone crest
FR	Furcation roof
GSH	Glutathione
IL	Interleukin
MDA	Malondialdehyde
MDP	Microbial dental plaques
MMP	Matrix metalloproteinases
MSM	Methylsulfonylmethane
PGE_2	Prostaglandin E_2
RANKL	Receptor nuclear factor kappa B ligand
OPG	Osteoprotegerin
OSI	Oxidative stress index
TAS	Total antioxidant status
TNF-α	Tumor necrosis factor-alpha
TOS	Total oxidant status

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Author contributions

Melek Beder: study design, experimental procedures, sacrification of rats, writing. Hatice Yemenoglu: study design, experimental procedures, writing. Semih Alperen Bostan: study design, experimental procedures. Oğuz Kose: study design, experimental procedures. Sibel Mataracı Karakaş: sacrification of rats, biochemical procedures. Tolga Mercantepe: sacrification of rats, histological procedures. Levent Tumkaya: histological procedures. Adnan Yılmaz: biochemical procedures. All authors read and approved the final manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval and consent to participate

All experimental procedures included in this study were approved by the Recep Tayyip Erdoğan University, Faculty of Medicine, Animal Research Ethics Committee (approval number: 2023/24). We confirm that all methods were carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines 2.0 for reporting of animal experiments.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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