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Original research

Prophylactic and therapeutic effects of (6)–shogaol on alveolar bone loss in experimental periodontitis*

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Purpose
(6)-Shogaol is the most prevalent bioactive compound in ginger. The aim of this study was to examine both the prophylactic and therapeutic effects of (6)-shogaol in an experimental periodontitis model.

Materials and Methods
Thirty-five male Wistar albino rats were divided into four groups. In the healthy group (n=5), no intervention was undertaken. In the periodontitis group (n=10), periodontitis was induced by ligature placement for 14 days. In the prophylaxis group (n=10), periodontitis was induced with ligature placement for 14 days, and during this time, 20 mg/kg/day of (6)-shogaol was administered via oral gavage. In the therapeutic group (n=10), periodontitis was induced with ligature placement for 14 days, and following the removal of the ligature, 20 mg/kg/day of (6)-shogaol was administered via oral gavage for 14 days. Alveolar bone loss was histometrically measured, and malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GP), nuclear factor kappa B (NF-κB), receptor activator of nuclear factor kappa B ligand (RANKL), and osteoprotegerin (OPG) were immunohistochemically analyzed.

Results
Alveolar bone loss was significantly lower in the healthy group than in the remaining groups, as well as in the therapeutic group than in the periodontitis group ($p<0.001$). RANKL/OPG was significantly higher in the periodontitis group compared to the remaining groups and in the prophylaxis group compared to the therapeutic group ($p<0.001$). MDA was significantly lower in the healthy group than in the remaining groups ($p<0.001$). SOD was significantly lower in the periodontitis group than in the prophylaxis and therapeutic groups ($p=0.039$ and $p=0.042$, respectively). GP was significantly lower in the healthy group than in the prophylaxis and therapeutic groups ($p=0.031$ and $p=0.002$, respectively).

Conclusion
The administration of (6)-shogaol modulated the RANKL/OPG balance and antioxidant status in rats with ligature-induced periodontitis.

Keywords: *Experimental periodontitis, ginger, oxidative stress, RANKL/OPG, shogaol*

Introduction

Chronic inflammation of the periodontium begins with complex subgingival biofilms containing many periodontal pathogens. In response to these pathogens, the excessive host response causes the release of cytokines, proteinases, and osteoclastogenic factors responsible for soft and hard tissue destruction (1). For the activation of osteoclasts that initiate bone resorption, the signal is delivered by the receptor activator of nuclear factor kappa B (NF-kB) [RANK], its ligand (RANKL), and osteoprotegerin (OPG). RANK is a receptor found on the cell surfaces of osteoclasts and osteoclast precursors that stimulates the proliferation and differentiation of cells to form the osteoclast phenotype. RANK is connected to

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RANKL. On the contrary, OPG is a soluble receptor produced by many cells, such as osteoclasts, and modifies the effects of RANKL by inhibiting the RANKL/RANK interaction. The degree of bone loss depends on the RANKL/OPG ratio. This rate increases dramatically in the area of active periodontal disease and positively correlates with disease severity. RANKL and OPG can be detected in gingival tissue, gingival crevicular fluid, saliva, and serum, providing reliable knowledge concerning periodontal disease activity and alveolar bone loss (2). Reactive oxygen species (ROS) are produced as a result of the normal cellular metabolism of host defense cells against bacterial pathogens. However, when ROS are produced in large amounts, they have destructive effects. It is known that oxidative stress, which expresses an imbalance between free radical formation and the antioxidant defense mechanism, damages cellular macromolecules sensitive to oxidative damage and plays a role in the pathogenesis of many chronic degenerative diseases, such as periodontal disease. Oxidative stress is generally determined by the measurement of malondialdehyde (MDA), which is the final product of lipid peroxidation, and antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GP) (3).

Ginger is a plant that possesses anti-inflammatory, antioxidant, antibacterial, antiarthritic, antiangiogenic, antithrombotic, anticancer, hypolipidemic, and antidiabetic properties. Ginger contains many active compounds involved in various biological activities (4-6). Several studies have reported the effectiveness of compounds found in ginger in improving symptoms of chronic inflammatory diseases. Among these compounds, studies have focused on the antioxidant and anti-inflammatory effects of gingerol and shogaol, which are usually the main components of ginger. In many cases, (6)-shogaol has been reported to exhibit better biological activities than gingerol. Additionally, the anti-inflammatory or antioxidant effects of shogaol have been demonstrated in conditions such as cancer, Alzheimer's disease, Parkinson's disease, cardiovascular disease, diabetes, and allergies (7-13).

In the literature, there are no studies evaluating the effects of the systemic administration of (6)-shogaol on both the prevention and treatment of periodontitis. Therefore, this study aimed to assess both the prophylactic and therapeutic effects of (6)-shogaol on alveolar bone loss in rats with experimentally induced periodontitis. Accordingly, the null hypothesis of the study was established as 'systemic administration of (6)-shogaol would reduce the destruction of periodontal tissues in the ligature-induced periodontitis model.'

Materials and Methods

Animals

Thirty-five male Wistar albino rats (250±25 g) were obtained from a certified commercial laboratory animal facility. The rats were housed in plastic cages at 22±2 °C under a 12-hour light/dark cycle. Commercial rat pellet feed and water were provided ad libitum. All experimental procedures and applications were approved by the Animal Research Ethics Committee of the University (ethics committee report number: 17/21) and the study was conducted following the ARRIVE guidelines (14).

Experimental design

The rats were randomly assigned to one of the following four groups: Healthy group (n=5) (no ligature placement; only standard laboratory diet/water were provided). Periodontitis group (n=10) (ligature placement for 14 days; standard laboratory diet/water were provided). Prophylaxis group (n=10) [ligature placement and administration of 20 mg/kg/day (6)-shogaol for 14 days). Therapeutic group (n=10) (ligature placement for 14 days and administration of 20 mg/kg/day (6)-shogaol for 14 days after ligature removal).

Experimental induction of periodontitis and (6)-shogaol admin istration

All ligature-induced periodontitis procedures were performed under general anesthesia induced with ketamine hydrochloride (75 mg/kg) and xylazine (10 mg/kg), and ligatures were placed with 3.0 silk sutures (Ruschmed, İstanbul, Turkey) around the left mandibular first molar. All ligatures were positioned subgingivally, and lost or loose sutures were replaced. All ligature placements were performed by the same operator (D.B.). (6)-shogaol (ChemFace, Wuhan, China), dissolved in 0.1% dimethyl sulfoxide (Sigma-Aldrich D2650, Germany), was orally administered to the rats according to their weight at the same time of day.

Histomorphometric analyses and histopathological evaluation

The rats in the healthy, periodontitis, and prophylaxis groups were sacrificed on day 15, and those in the therapeutic group were sacrificed on day 29, using a CO_2 euthanasia cabinet, and their mandibles were collected. The left mesio-distal segment of each mandible was dissected, fixed in 4% buffered paraformaldehyde for 48 h, and decalcified in 8% formic acid for 14 days. The tissues were trimmed, washed, dehydrated, and embedded in paraffin wax. The paraffin-embedded tissues were sectioned at a thickness of 4–5 µm longitudinally and stained with hematoxylin and eosin. The slides were examined under light microscopy (Olympus BX51 trinocular microscope and Leica DFC450 digital camera, Germany), and digital photomicrographs were taken. The distance between the cemento-enamel junction (CEJ) and the alveolar crest (AC) was measured using digital imaging software (Leica Qwin image analysis software, Germany), and all measurements were performed at six different areas (three buccal and three lingual surfaces), and a mean value for each tooth was calculated. Histopathologically, the severity of inflammatory cell infiltration was scored as 0 if there were no cells, 1 if there were one to five cells, 2 if six to 10 cells, and 3 if more than 10 cells. All analyses were performed at 20x objective magnification.

Immunohistochemical analyses

Immunoperoxidase analyses were performed using a commercial immunoperoxidase kit [UltraVision Polyvalent (Rabbit-Mouse) HRP Kit, Labvision/Thermo, USA] to determine the expression of MDA, SOD, GP, RANKL, OPG, and NF-κB. Briefly, tissue sections were deparaffinized in xylene and hydrated through graded alcohols. To unmask antigens, the tissue sec-

tions were placed in a microwave in citrate buffer for 20 min at the highest potency. Then, endogenous peroxidase activity was inhibited using 0.1% H_2O_2 in methanol for 10 min, and non-specific labeling was blocked by pre-incubation with normal goat serum in 10 min. Thereafter, the sections were incubated with anti-MDA (Ab6463, Abcam, UK), anti-SOD (sc-101523, Santa Cruz Inc., Texas), anti-GP (sc-133160, Santa Cruz Inc., Texas), anti-RANKL (Ab-169966, Abcam, UK), anti-OPG (Ab-203061, Abcam, UK), and anti-NF-κB (sc-8414, Santa Cruz Inc., Texas) antibodies for 60 min in a humidity chamber at room temperature. The sections were then incubated with the biotinylated secondary antibody for 15 min, labeled with horseradish peroxidase for 15 min and, 3-Amino-9-ethylcarbazole (AEC) chromogen substrate solution (TA-125-HA AEC Chromogen Kit, Thermo, USA) for approximately 10 min. They were counterstained with Mayer's hematoxylin for 1 min and suspended in a water-based mounting medium. As the isotype-negative control, normal mouse serum, instead of the primary antibody, was used. The density of positive staining was measured using digital imaging software (Leica Qwin image analysis software, Germany) under a 20 × objective lens. The integrated optical density of all immunopositive staining was measured, and the mean MDA-, SOD-, GP-, RANKL-, OPG-, and NF-κB-positive areas and the total area were calculated. After calculating the proportion (% pixels) of the stained area to the whole field, the mean (in % pixels) staining area for each slide was determined.

Statistical analysis

The normality of the data distribution was examined using the Shapiro-Wilk test. In the comparisons between the groups, the variables that met the normality and variance homogeneity conditions were evaluated using the analysis of variance (ANOVA) test. When a difference was detected, the Bonferroni-corrected independent-samples t-test was used to determine the groups that caused the significant difference. The Kruskal-Wallis test was conducted to determine differences in variables that did not comply with a normal distribution, and the Bonferroni-corrected Mann-Whitney U test was used to determine the group from which the significant difference originated. Comparisons between the groups for normally distributed values that did not meet the homogeneity of variance were undertaken using the Welch-ANOVA test, which is the corrected version of the ANOVA test, and the Games-Howell binary comparison results were reported to determine the differences between the groups where the differences were significant. SPSS v. 22.0 (IBM Corp. released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used for statistical analyses. The statistical significance level was accepted as $p<0.05$.

Results

The study was completed with 35 rats. No complications or any obvious signs of systemic disorders occurred during the study period, and no animals died or were excluded.

Histopathological findings and histomorphometric analyses

Microscopic images of the tissue samples examined are shown in Figure 1. In the healthy group, cement, periodontal ligament, and alveolar bone had a normal appearance, with no inflammatory reaction or bone resorption.

Cement resorption was observed in all samples in the periodontitis group. In addition, osteolysis, characterized by osteoclastic activity at alveolar bone borders, was observed. Intense inflammatory cell infiltrations rich in lymphocytes and neutrophils were found, especially in regions with high osteoclastic activity and cement resorption. Hyperemic blood vessels and newly formed young capillaries were also found in the periodontal ligament.

In the prophylaxis group, cement resorption was observed, albeit at a lower rate than in the periodontitis group. In addition, enlarged blood vessels were observed in the periodontal ligament, similar to the periodontitis group, but there was less vascularization. While a small number of osteoclastic activities were encountered in this group, mild inflammatory cell infiltration was noted in osteoclast circles and around enlarged capillaries. However, osteoblastic activity was found in all samples. In the therapeutic group, much lower vascularization and inflammatory cell infiltration were observed compared to the periodontitis group. Similarly, there was osteoclastic activity in the alveolar bone margin in only two cases, while osteoblastic activity was more common than in the prophylaxis group. During the histopathology analysis, inflammatory cell infiltration was scored semi-quantitatively, and when compared between the groups, the number of rats with a score of 0 was one in

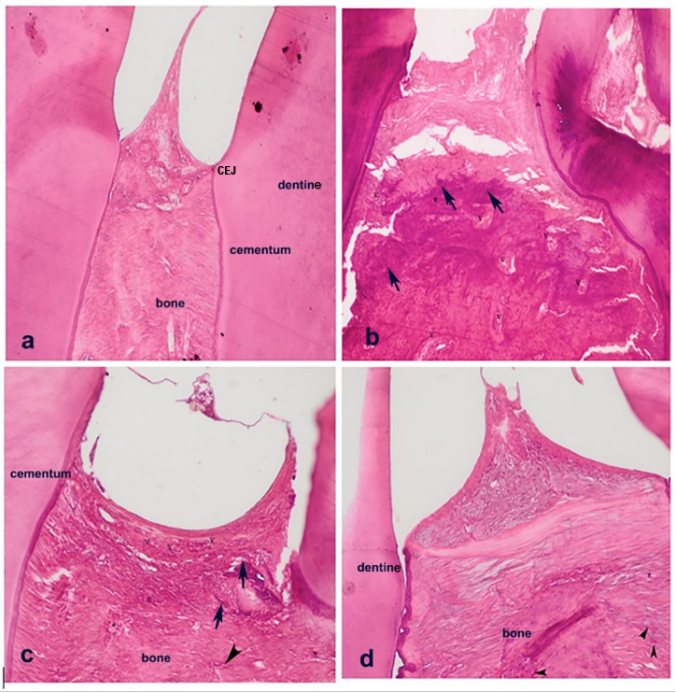

Figure 1. *Microscopic images of the tissue samples of all groups (hematoxylin and eosin staining). (a) Healthy group: Normal periodontal membrane, alveolar bone, and cement. (b) Periodontitis group: Periodontium with intense vascularization (v), hyperemia with osteoclasts (arrow) at the alveolar bone border, and moderate inflammatory cell infiltration (asterisk). (c) Prophylaxis group: Mild vascularization (v) and mild inflammatory cell infiltration (asterisk) in the periodontal ligament, osteoclasts (arrow), and osteoblasts (arrowhead) in alveolar bone. (d) Therapeutic group: Osteoblasts (arrowhead) formed at the border of the alveolar bone.*

each of the prophylaxis and therapeutic groups, while none of the rats in the periodontitis group scored 0.

The CEJ-AC distance (μm) was measured in all sections and found to be statistically significantly lower in the healthy group than in the remaining groups ($p < 0.001$ for all). It was also significantly lower in the therapeutic group than in the periodontitis group (p=0.001) (Table 1).

Immunohistochemical findings

The results of the tissue analyses for SOD, GP, and MDA are shown in Table 2 and Figure 2. The SOD level was statistically significantly lower in the periodontitis group compared to the prophylaxis and therapeutic groups (p=0.039 and p=0.042, respectively); however, there was no statistically significant difference between the periodontitis and the healthy group (p=0.931). The GP level was found to be statistically significantly lower in the healthy group compared to the prophylaxis and therapeutic groups (p=0.031 and p=0.002, respectively), while no statistically significant difference was detected between the periodontitis group and the healthy group. The MDA level was statistically significantly higher in the periodontitis group than in the healthy group (p=0.004), and it was also statistically significantly lower in the healthy group than in the prophylaxis and therapeutic groups (p=0.012 and p=0.017, respectively). The MDA did not statistically significantly differ between the periodontitis group and the prophylaxis or therapeutic group. The results of the tissue analyses for RANKL, OPG, RANKL/OPG, and NF-κB are shown in Table 3 and Figure 2. The RANKL level was observed to be statistically significantly higher in the periodontitis group compared to the healthy and therapeutic groups (p<0.001 and p<0.001, respectively). There was no statistically significant difference in the RANKL level between the periodontitis group and the prophylaxis group. The OPG level was statistically significantly lower in the periodontitis group than in the prophylaxis and therapeutic groups (p=0.002 and p<0.001, respectively). It was statistically significantly lower in the healthy group compared to the therapeutic group (p=0.049). However, no statistically significant difference was found when the periodontitis group was compared to the healthy group. The RANKL/OPG ratio was statistically significantly higher in the periodontitis group than in the healthy, prophylaxis, and therapeutic groups (p=0.005, p=0.006, and p=0.005, respectively), but it was statistically significantly higher in the prophylaxis group than in the therapeutic group (p=0.011). The NF-κB level was statistically significantly higher in the periodontitis group than in the healthy, prophylaxis, and therapeutic groups (p<0.001, p=0.017, and p=0.023, respectively).

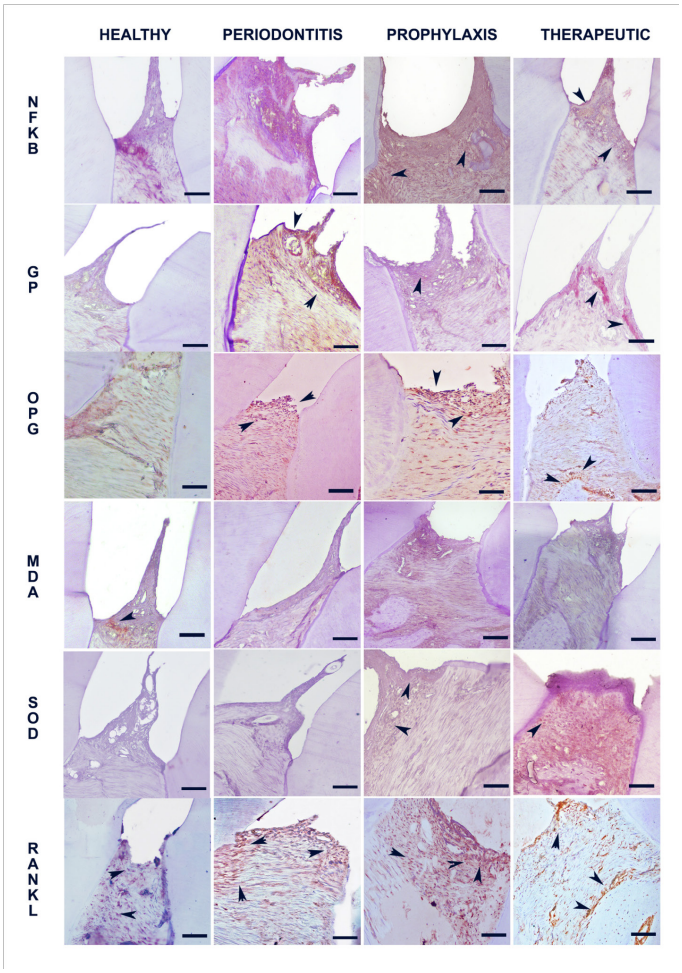

Figure 2. *Immunoperoxidase positive expression (arrow heads) of NFKB, GP, OPG, MDA, SOD, and RANKL antigens in the mesio-distal histological section of each experimental group (healthy, periodontitis, prophylaxis, and therapeutic), avidin-biotin complex immunoperoxidase test results, and Mayer's hematoxylin counterstaining. Bar = 220 μm.*

Table 1: *Comparison of the CEJ-AC distance (μm) between the groups (mean ± standard deviation)*

	Healthy Group	Periodontitis Group	Prophylaxis Group	Therapeutic Group	F	p
CEJ-AC (μm)	28.61±12.21	101.78±11.46[a]	87.23±17.4[a]	76.26±9.34[a,b]	36.586	**<0.001**

CEJ: cemento-enamel junction; AC: alveolar crest; F: ANOVA test statistic; [a]significant difference from the healthy group; [b]significant difference from the periodontitis group

Table 2: *Comparison of the SOD, GP, and MDA levels between the groups (% area)*

	Healthy Group	Periodontitis Group	Prophylaxis Group	Therapeutic Group	F, χ^2	p
SOD Median (min; max)	3.91 (2.40; 4.86)	1.54 (0.88; 4.69)	4.68[b] (2.68; 6.94)	4.44[b] (2.83; 7.92)	9.695[&]	**0.021**
GP Median (min; max)	0.73 (0.42; 2.64)	2.34 (0.99; 3.54)	2.92[a] (2.40; 5.77)	3.4[a] (2.61; 5.62)	15.611[&]	**0.001**
MDA Mean ± SD	1.14±0.54[b]	2.09±0.56	4.35±1.70[a]	5.71±2.46[a]	15.817[*]	**<0.001**

F, *: Welch-ANOVA test statistic; χ2, &: Kruskal-Wallis test statistic; SOD: superoxide dismutase; GP: glutathione peroxidase; MDA: malondialdehyde; SD: standard deviation; [a]significant difference from the healthy group; [b]significant difference from the periodontitis group

Table 3: *Comparison of RANKL, OPG, NF-κB, and RANKL/OPG between the groups (% area)*

	Healthy Group	Periodontitis Group	Prophylaxis Group	Therapeutic Group	F, χ^2	p
RANKL Median (min; max)	1.48[b] (0.82; 2.55)	11.34 (7.36; 14.59)	4.36 (1.96; 7.11)	1.64[b] (1.39; 3.40)	24.657[&]	**<0.001**
OPG Median (min; max)	2.32 (1.64; 3.40)	0.93 (0.56; 3.92)	5.24[b] (3.44; 8.24)	6.39[a,b] (3.11; 13.86)	23.270[&]	**<0.001**
RANKL/OPG Mean ± SD	0.66±0.31[b]	12.43±6.44	0.86±0.31[b]	0.38±0.24[b,c]	12.353[*]	**<0.001**
NF-Kb Median (min; max)	1.59[b] (1.16; 2.11)	6.08 (3.87; 8.72)	3.30[b] (2.46; 4.02)	3.32[b] (2.89; 4.91)	23.806[&]	**<0.001**

F, *: Welch-ANOVA test statistic; χ2, &: Kruskal Wallis test statistic; RANKL: receptor activator of nuclear factor kappa B ligand; OPG: osteoprotegerin; NF-κB: nuclear factor kappa B; SD: standard deviation; [a]significant difference from the healthy group; [b]significant difference from the periodontitis group; [c]significant difference from the prophylaxis group

Discussion

In recent years, many plants with anti-inflammatory and antioxidant properties have been utilized in the treatment of periodontal diseases. Ginger contains active ingredients such as shogaol, gingerol, paradol, and zingerone, each with various biological activities. Among these, shogaol stands out as one of its most active components (15). Studies have primarily focused on the antioxidant and anti-inflammatory effects of gingerol and shogaol, which are typically the main components of ginger. Recent research indicates that (6)-shogaol is more stable than (6)-gingerol and exhibits stronger pharmacological effects, including antioxidant, anti-inflammatory, and anticancer properties, compared to the latter (7, 16). Moreover, shogaol has demonstrated anti-inflammatory or antioxidant effects in conditions such as cancer, Alzheimer's disease, Parkinson's disease, cardiovascular disease, diabetes, and allergies (8-13). Several in vitro and in vivo studies have investigated the antioxidant and anti-inflammatory potential of (6)-shogaol. Pan *et al.* (17) demonstrated that the topical application of (6)-shogaol inhibited the activation of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 in murine macrophages in a skin model. Ahn *et al.* (18) showed that (6)-shogaol down-regulated NF-kB activation and the expression of COX-2, resulting in the inhibition of toll-like receptor-mediated signaling pathways. In another study, (6)-shogaol reduced the release of interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF) in human mast cells (19). In a study employing human primary chondrocytes, (6)-shogaol reduced the activity of cathepsin K (20). Various animal models have reported that (6)-shogaol plays a crucial role in anti-inflammatory action and allergic inflammatory reactions (12, 21). However, to the best of our knowledge, this is the first study to evaluate both the preventative and therapeutic effects of (6)-shogaol administration in an experimental periodontitis model.

According to animal studies, the placement of ligatures around the teeth, allowing for the accumulation and colonization of microorganisms, decreases tissue integrity and creates mechanical trauma in the dentogingival region (22). This leads to the ulceration of the sulcular epithelium after plaque deposition, facilitating the invasion of connective tissue and initiating periodontal tissue loss, similar to the process in humans. This model is widely accepted as the closest to natural plaque formation (23). Male animals are more frequently used in experimental studies because female animals may exhibit significant differences in phenotypes, such as gene expression and changes in the genome, due to their hormone cycles (24). Rats are the most extensively studied rodents in research on the pathogenesis of periodontal diseases and are widely employed in experimental periodontitis models due to their similarity to humans in terms of the periodontal structure of molar tooth regions, low cost, high availability, and easy feeding and breeding. In light of the literature, we used male Wistar albino rats for our experimental model. We induced experimental periodontitis over 14 days by tying ligatures around the mandibular left first molar teeth of the rats.

The RANKL/OPG ratio is an indicator of normal bone resorption and deposition regulation during bone remodeling (25). Bone tissue destruction that occurs during periodontitis is regulated by the balance between the levels of RANKL and OPG, and therefore, their ratio increases in the event of destruction (26, 27). It has been suggested that the RANKL/OPG ratio can be a good indicator of periodontitis (2). Kim *et al.* (28) investigated the preventative use of (6)-shogaol against periodontitis and showed that it had an anti-osteoclastogenic role, inhibiting RANKL-induced mitogen-activated protein kinase activation, Ca2+ signaling, ROS generation, nuclear factor-activated T cells, and cytoplasmic 1 induction in osteoclast precursors. In another study evaluating the anti-metastatic activity of (6)-shogaol, it was revealed that this compound suppressed bone resorption by decreasing RANKL expression in osteoblasts (29). In the current study, we also observed that (6)-shogaol had a significant effect on the RANKL-OPG balance. In both therapeutic and prophylaxis groups, the OPG level was significantly higher than in the periodontitis group. The RANKL level was significantly lower in the therapeutic group than in the periodontitis group. The RANKL/OPG ratio and the NF-kB level were significantly higher in the periodontitis group compared to the remaining groups. The RANKL/OPG ratio was also significantly lower in the therapeutic group than in the prophylaxis group.

In periodontal diseases, bacterial cell components and inflammatory cytokines activate polymorphonuclear leukocytes (PMNLs), which exhibit oxidative activity with ROS production. Furthermore, fibroblasts, osteoclasts, epithelial cells, and neutrophils increase ROS release, causing damage to host tissue (3, 30). Tissue destruction develops through mechanisms such as the activation of pro-inflammatory cytokines due to ROS activity, production of prostaglandin E2 (PGE2) via lipid peroxidation, and NF-κB release (31). It has also been suggested that ROS acts as an intracellular signaling device for osteoclast differentiation, inducing periodontal tissue destruction and being associated with osteoclastic bone resorption (32).

MDA is a low-molecular-weight aldehyde in volatile form resulting from lipid peroxidation, occurring as a product of prostaglandin biosynthesis during oxidative stress or through the destruction of some molecules. Tissue destruction due to ROS is measured by MDA, an indicator of lipid peroxidation (33). Antioxidants are substances that significantly delay or prevent the oxidation of an oxidizable substrate and neutralize the free radical formation that can occur due to oxidative stress (34). GP and SOD are the most important antioxidants that protect cells from oxidative damage caused by free oxygen radicals (3).

(6)-shogaol has been shown to be the strongest scavenger of superoxide and hydroxyl radicals among all ginger constituents (7). It has been demonstrated that (6)-shogaol can significantly reduce cellular oxidative stress and suppress ROS production in various cells, including PMNLs, endothelial cells, and epidermal keratinocytes (7, 11, 35, 36). In a recently published study by Qi and Han (37), an endotoxin-induced experimental periodontitis model was created, and ROS, lysosomal enzymes, lipid peroxide, and acute-phase protein levels were reported to decrease after the administration of (6)-shogaol. Moreover, antioxidant enzymes and non-enzymatic antioxidant systems increased. In 2019, Nonaka *et al.* (38) investigated the effects of (6)-shogaol on the expression of advanced glycation end-product-induced oxidative and anti-oxidative responses, IL-6, and intercellular adhesion molecules in human gingival fibroblasts and demonstrated the efficacy of this compound in the prevention and treatment of diabetes mellitus-associated periodontitis.

It has also been suggested that (6)-shogaol may be a potential agent for the treatment of cardiovascular disease via its inhibition of the production of PGE2 and different pro-inflammatory cytokines, the release of nitric oxide, the expression of iNOS, and the increase of antioxidant enzymes, such as SOD and GP (10, 39). Similarly, in our study, the SOD level was significantly higher in the prophylaxis and therapeutic groups than in the periodontitis group, and the GP level was significantly higher in the prophylaxis and therapeutic groups compared to the healthy group. These results were attributed to the antioxidant properties of (6)-shogaol.

As expected, the MDA level was significantly higher in the periodontitis group than in the healthy group. Interestingly, the MDA level was significantly lower in the healthy group than in the prophylaxis and therapeutic groups. This may be due to the psychological stress to which the rats were exposed every day due to the administration of (6)-shogaol by oral gavage, the rats' immobility during this period, and the possible irritation of their throats during gavage, all factors that can increase oxidative stress. The examination of this marker in serum samples can clarify this situation. Accordingly, the lack of serum samples and radiographic examination of periodontitis can be considered limitations of this study.

Conclusion

In this study, the administration of (6)-shogaol, an active ingredient of ginger, reduced periodontal inflammation, RANKL, and NF-kB expression and increased OPG, SOD, and GP expression in rats with experimentally induced periodontitis. It is possible to conclude that (6)-shogaol reduces alveolar bone loss by affecting the RANKL-OPG balance and antioxidant status. Further studies are needed to optimize the dosage and route of administration of (6)-shogaol and investigate its efficacy based on in vivo models.

Türkçe özet: *Deneysel periodontitiste (6)-shogaol'ün alveoler kemik kaybı üzerindeki profilaktik ve terapötik etkileri Amaç: (6)-shogaol, zencefildeki en yaygın biyoaktif bileşiktir. Bu çalışmanın amacı, deneysel bir periodontitis modelinde (6)-shogaol'ün hem profilaktik hem de terapötik etkilerini incelemektir. Gereç ve Yöntem: Otuz beş adet erkek Wistar albino rat dört gruba ayrıldı. Sağlıklı gruba (n=5) herhangi bir müdahale yapılmadı. Periodontitis grubunda (n=10) 14 gün boyunca ligatür yerleştirilmesi ile periodontitis oluşturuldu. Profilaksi grubunda (n=10) 14 gün ligatür yerleştirilmesi ile periodontitis oluşturuldu ve bu süre içinde oral gavaj ile 20 mg/kg/gün (6)-shogaol verildi. Terapötik grubunda (n=10) 14 gün ligatür yerleştirilmesi ile periodontitis oluşturuldu ve ligatürün çıkarılmasını takiben 14 gün 20 mg/kg/gün (6)-shogaol oral gavaj ile uygulandı. Alveolar kemik kaybı histometrik olarak ölçüldü ve malondialdehit (MDA), süperoksit dismutaz (SOD), glutatyon peroksidaz (GP), nükleer faktör kappa B (NF-κB), nükleer faktör kappa B ligandının reseptör aktivatörü (RANKL) ve osteoprotegerin (OPG) immünohistokimyasal olarak analiz edildi. Bulgular: Alveoler kemik kaybı, sağlıklı grupta diğer gruplara göre ve terapötik grupta ise periodontitis grubuna göre anlamlı olarak daha düşüktü (P<0.001). RANKL/OPG periodontitis grubunda diğer gruplara göre ve profilaksi grubunda terapötik gruba göre anlamlı olarak daha yüksekti (P<0.001). MDA, sağlıklı grupta diğer gruplara göre anlamlı olarak düşüktü (P<0.001). SOD, periodontitis grubunda profilaksi ve terapötik gruplara göre anlamlı olarak daha düşüktü (sırasıyla P = 0.039 ve P = 0.042). GP, sağlıklı grupta profilaksi ve terapötik gruplara göre anlamlı olarak daha düşüktü (sırasıyla P = 0.031 ve P = 0.002). Sonuç: (6)-shogaol uygulaması, ligatür indüklü periodontitisli ratlarda RANKL/OPG dengesini ve antioksidan durumunu modüle etti. Anahtar Kelimeler: Deneysel periodontitis, zencefil, oksidatif stres, RANKL/OPG, shogaol*

Ethics Committee Approval: All experimental procedures and applications were approved by the Animal Research Ethics Committee of the University (number: 17/21)

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Conflict of Interest: The authors declare that they have no conflict of interest.

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