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Original Article

Investigation of oxidative stress in experimental periodontitis treated with myricetin

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Abstract

Aim: The aim of this study was to investigate the effects of systemically applied myricetin on oxidative stress and antioxidant status in experimental periodontitis of rats.

Materials and Methods: 24 Wistar rats were used and randomly divided into three groups. Experimental periodontitis was induced by placing a silk ligature around first molars teeth of the rats for 15 days. Following removal of the ligatures, Group 1: saline; Group 2: myricetin and Group 3: doxycycline were administered systemically. Total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), superoxide dismutase (SOD) enzyme and malondialdehyde (MDA) levels in serum; and SOD and MDA levels in gingival tissue were determined by biochemical analysis.

Results: Serum TAS increased and serum TOS and OSI values decreased in myricetin and doxycycline treated groups compared to saline group ($P<0.016$). There were no statistically significant differences in serum SOD levels between groups ($P>0.016$). Gingival tissue SOD activity was increased in the group treated with doxycycline compared with the group treated with saline ($P<0.016$). MDA levels in both serum and gingival tissue samples were decreased in myricetin and doxycycline treated groups compared to saline group ($P<0.016$).

Conclusion: The systemic administration of myricetin may be useful to diminish oxidative damage in periodontitis.

Keywords: Myricetin; oxidative stress; periodontal disease; periodontitis

INTRODUCTION

Periodontitis is a chronic destructive inflammatory disease characterized by periodontal pocket formation, attachment loss or both together with progressive periodontal ligament and alveolar bone loss (1). Periodontal tissue damage occurs due to the presence of microbial dental biofilm and host response to the bacteria and their products. During the host immune-inflammatory response, polymorphonuclear leukocytes (PMNLs) which is the primary defensive cells of host, increase and produce reactive oxygen species (ROS) (2). ROS are important molecules in defense against to periodontopathogens but also can cause periodontal tissue damage (3). When the increase in ROS production due to increased neutrophil number exceeds the antioxidant capacity, cell damage occurs including protein damage, lipid peroxidation, oxidation of enyzmes and DNA damage (4). Since, antioxidants achieve oxidative homeostasis by preventing the formation of ROS or by neutralizing the free radicals that are formed. Accordingly, the imbalance between ROS and antioxidant molecules is defined as oxidative stress that causes the periodontal breakdown and its progression (4). ROS is not only produced by the host against to microbial stimuli but also regulated by antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) to provide homeostasis in tissues (5). Depletion of these antioxidants in cell defense makes the cell vulnerable to oxidants. Numerous studies have been conducted to show that the effect of ROS induced by inflammatory conditions can be reduced by external antioxidant molecules (6,7).

Host modulation therapy is an approach that aims to reduce periodontal tissue destruction by increasing the preventive and regenerative components of the host response and/or decreasing its destructive components. As a host modulation agent, doxycycline has been shown to contribute the periodontal tissue healing with its highly anticollagenase properties and today, subantimicrobial

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dose doxycycline is recommended as an effective agent in the treatment of periodontal diseases (8,9).

In recent years, there is a growing interest in flavonoids and their biological effects. Flavonoids are low-molecular-weight polyphenolic compounds characterized by a diphenylpropane structure that form a group of plant secondary metabolites with beneficial effects including antioxidant, anti-inflammatory, antimicrobial, antiosteoporotic and antiallergic activities (10,11). Myricetin is one of the flavonoids that are abundant in numerous fruits and vegetables and has been shown to have the highest antioxidant activity among the flavonoids (11,12). Evidence shows that myricetin has anti-inflammatory, antithrombotic and cytoprotective properties as well as antioxidant properties (13-16). Grenier et al. also indicated that myricetin inhibits the expression of *Porpyromonas gingivalis* and its virulence factors (17). Myricetin is suggested to have therapeutic benefits in certain systemic inflammation such as obesity related cardiovascular diseases (18). Additionally, a recent study reported that myricetin has a therapeutic effect on the process of osteoclastogenesis in periodontal disease (19). On the other hand, no information has been found related to antioxidant effect of myricetin in inflammatory periodontal diseases. We hypothesized that myricetin can play an important role in oxidative stress mechanism on the host immune-inflammatory response in destructive periodontal disease. The purpose of this study was to examine the effect of systemic administration of myricetin on periodontium with respect to oxidative stress and antioxidant status by biochemical analysis in rats.

MATERIALS and METHODS

Animals

Twenty-four male albino Wistar rats weighing 250-300 g were used in this study. All animal care and experimental protocols were in compliance with guidelines approved by the Animal Experiments and Ethics Committee of Zonguldak Bulent Ecevit University (Protocol number: 2016-03-06/01). The rats were housed separately in plastic cages with controlled room temperature (22±1°C) and humidity (50%). They were maintained in a 12: 12-h light–dark cycle with food and water available ad libitum throughout the experiment.

Experimental design

After administration of ketamine hydrochloride and xylazine anesthetics to the rats, 3.0 sterile silk ligatures were wrapped around the cervical area of the right and left mandibular first molars to experimental periodontitis, The ligatures were kept in position for 15 days to promote the accumulation of microbial dental plaque and so inflammation (20).

Twenty-four rats were randomly divided into 3 groups; Group 1 (n=8), systemic saline was administered by oral gavage after the removal of ligature; Group 2 (n=8), systemic myricetin was administered intraperitonally after the removal of ligature; Group 3 (n=8), systemic doxycycline was administered by oral gavage after the removal of ligature. All groups had experimental periodontitis and the ligatures were removed after experimental periodontitis induction (15 days) in all groups. Then, saline, myricetin and doxycycline were administered for 15 days. Myricetin (Nanjing Zelang Medical Technology Co., Ltd, Nanjing, Jiangsu, China) was administered one time in doses of 6 mg/kg/day every day (21). Doxycycline and saline were also administered in doses of 6 mg/kg/day by oral gavage at the same time every day (22).

Sampling

All of the rats were euthanized under general anesthesia after taking 5 mL blood from the heart. Then, their mandibles were carefully removed along with the surrounding gingiva, and the gingival tissue samples were dissected from the buccal region of the mandibular right first molars. Obtained gingival tissue samples were placed into steril polypropylene tubes containing saline solution and frozen at -80°C until biochemical analysis.Taken blood samples were centrifuged at 3000 g for 10 minutes and obtained serum samples were stored at -80°C until analysis.

Biochemical analysis

On the day of analysis, gingival tissue samples thawed and weighed. Then the tissues were placed into phosphate buffered saline (PBS; 4°C, pH 7.0) and homogenized at 6000 rpm for 30 seconds, five times at 10-second intervals, using a homogenizer (T18Ultra Turrax; Ika Labortechnik, Staufen, Germany). The remaining homogenate was centrifuged at 10,000 rpm for 20 minutes at 4 °C and the supernatants were used to determine SOD and MDA levels.

In serum samples, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and the levels of superoxide dismutase (SOD) and malondialdehyde (MDA); in gingival tissue samples, SOD and MDA levels were evaluated.

TAS was determined using commercial colorimetric assay kit (OXFORD BiomedicalResearch, MI, USA). The absorbance was measured at 450 nm and the results were indicated as µmol trolox/L. The assay range was 0 to 2000 µmol trolox/L.

TOS level was measured by enzyme-linked immunosorbent assay (ELISA) method at 450 nm using commercially available kit (Sunredbio, Shangai, China). The results were indicated as µmol/L. The ratio of TOS to TAS was also calculated and OSI values were determined.

SOD activity was analysed by colorimetric assay kit (CAYMAN Chemical, AnnArbor, Michigan, USA). The absorbance was measured at 450 nm using a spectrophotometer and the results were indicated as U/mL. The assay range was 0.005 to 0.05 U/mL

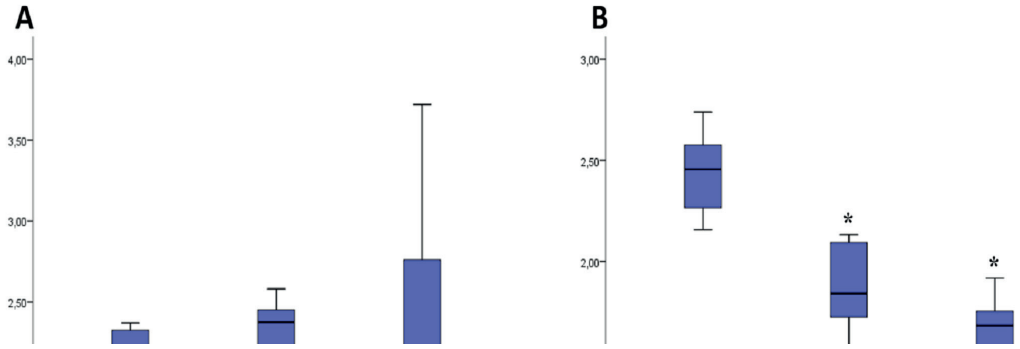
MDA analysis was performed using colorimetric assay kit (OXFORD Biomedical Research, MI, USA). The absorbance was measured at 540 nm using a spectrophotometer and the results were indicated as µmol/L. The assay range was 0 to 20,000 µmol/L. All analyses were performed according to the instructions of manufacturers.

Statistical analysis

Normality of data was tested with Shapiro-Wilk normality test. Intergroup comparisons were performed by Kruskal-Wallis nonparametric test followed by post hoc group comparisons with the Bonferroni-adjusted Mann–Whitney U-test. With Bonferroni correction, α = 0.05/ 3 = 0.016 was taken to show statistical significance. All tests were performed using SPSS software, version 19.0 (SPSS Inc., Chicago, IL, USA) and P<0.05 was considered to be statistically significant.

RESULTS

Serum TAS, TOS and OSI levels are presented in Figure 1. TAS levels were significantly higher in myricetin and doxycycline treated groups than saline group (P<0.016, for both Figure 1A). TOS levels and OSI values in serum were significantly lower in myricetin and doxycycline treated groups compared to saline group (P<0.016 for all, Figure 1B, 1C). No significant differences were found in serum TAS and TOS levels and also OSI values between myricetin and doxycycline groups (P>0.05 for all, Figures 1A-1C).

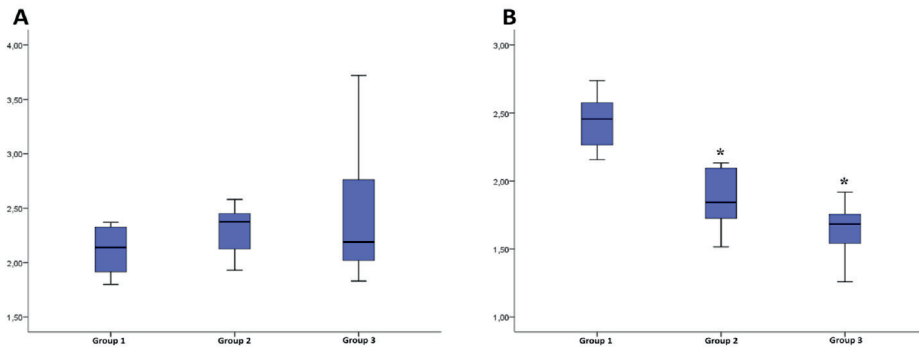


**Statistically significant difference from Group 1 (Kruskal-Wallis/Bonferroni-adjusted Mann–Whitney U) Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values*

Figure 1. Serum TAS (A) and TOS (B) levels, and OSI (C) values of the study groups

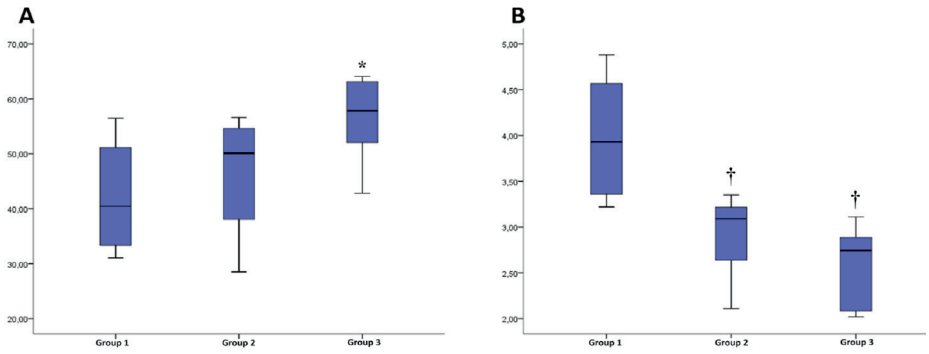
Serum SOD and MDA levels of the study groups are indicated in Figure 2. There were no significant differences in serum SOD levels among the study groups (P>0.05, Figure 2A). MDA levels in serum were found to be decreased when periodontitis induced rats were treated with myricetin and doxycycline compared to saline application (P<0.016 for both, Figure 2B). No statistically significant difference was found between myricetin and doxycycline application groups (P>0.05, Figure 2B).

SOD and MDA findings of gingival tissue are presented in Figure 3. We found that SOD activity in gingival tissues of periodontitis induced rats were increased with doxycycline administration compared to saline application (P<0.016, Figure 3A). In myricetin treatment group, SOD activity showed no statistically significant difference compared to both saline and doxycycline administration groups (P>0.05, for both Figure 3A). Similarly to serum MDA findings, gingival tissue MDA levels were decreased significantly with myricetin and doxycycline treatment compared to the saline application (P<0.016 for both, Figure 3B). However, there was no significant difference between myricetin and doxycycline treatment groups in terms of MDA (P>0.05, Figure 3B).



**Statistically significant difference from Group 1 (Kruskal-Wallis/Bonferroni-adjusted Mann–Whitney U) Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values*

Figure 2. Serum SOD (A) and MDA (B) levels of the study groups



Statistically significant difference from Group 1 and 2 (Kruskal-Wallis/Bonferroni-adjusted Mann–Whitney U)

†Statistically significant difference from Group 1 (Kruskal-Wallis/Bonferroni-adjusted Mann–Whitney U)Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values

Figure 3. Gingival tissue SOD (A) and MDA (B) levels of the study groups

DISCUSSION

Increased oxidative stress and low levels of antioxidant capacity have been shown in progressive periodontal disease as in many of chronic inflammatory diseases by numerous studies (4,23). Accordingly, host immune-response is critical for periodontal tissue destrruction and host modulation is important to reduce inflammation and tissue damage. The use of antioxidants can be needed to protect the periodontal tissues to oxidative damage. Myricetin is a flavonoid and has been reported to have the strongest antioxidant activity among flavonoids (11). Therefore we aimed to investigate the effect of myricetin on oxidative stress parameters in experimental periodontitis. To the best of our knowledge, this is the first trial examining the effect of myricetin application on periodontium by biochemical analysis of serum TAS, TOS, OSI, SOD and MDA and also of gingival tissue SOD and MDA in the experimental periodontitis model.

Rats were used to induce experimental periodontitis because of the similarity of their periodontal tissues with the human periodontal tissues. As a reliable technique for disease induction, we used ligature-induced experimental periodontitis which is a well-established model of experimental periodontitis in rats (24). Kuhr et al. reported that the highest alveolar bone loss in rats after periodontitis induction occured in 15 days (25). Thus, we removed the ligatures after 15 days of the disease induction and we observed the attachment loss and the radiographic bone loss. The dose of myricetin and doxycycline was selected to be 6 mg/kg per day (for 15 days) as recommended in the previous studies (22,26,27). These studies also reported that antioxidant and antiinflammaory effects of myricetin are detected in this dose without any adverse effect when administered intraperitoneally. It is also important to note that there is no information in the literature regarding the antioxidant effectiveness of myricetin when administered by oral gavage. Accordingly, the effect of different routes of administrations such as oral gavage, and its difference from intraperitoneal administration are unknown.

In studies evaluating TAS and TOS levels in periodontitis patients, gingival crevicular fluid and serum TOS levels were reported to be increased compared to periodontally healthy participants and TOS was found to be related to mediators of bone destruction in additon to clinical periodontal parameters (28). The decreased levels of gingival tissue and serum TAS in periodontitis patients were also reported by Konopka et al. (29). Sağlam et al. also indicated that TOS was increased while TAS was decreased in the experimental periodontitis of rats (30). Our results showed that in the presence of experimental periodontitis, both myricetin and doxycycline decreased the TOS levels and OSI values by increasing TAS capacity when compared to saline administration. In accordance with the previous studies, we suggested that decreasing TAS in periodontitis can be increased by antioxidant agents and these agents can provide the beneficial effects in favor of host.

ROS is produced continuously during normal cellular metabolism and is removed by cellular enzymatic antioxidants such as SOD. SOD is one of the primary antioxidant enzymes directly involved in the elimination of free radicals and it provides the balance by removing superoxide radicals (4). Under inflammatory conditions including periodontitis, SOD activity is reduced in relation to extreme production of ROS, as expected. Due to overproduction of ROS, antioxidant defense system collapses and oxidative damage occurs in cellular components including DNA, proteins and lipids (4). MDA is one of the indicators of oxidative stress parameter as the product of lipid peroxidation. Previos studies demonstrated higher levels of MDA in plasma, gingival crevicular fluid, saliva and gingival tissue of periodontitis patients (31,32). Accordingly, we evaluated SOD and MDA in both gingival tissue and serum samples in the present study. Serum SOD levels showed no significant differences among the groups, while a significant increase has been found in doxycycline application group compared to saline grup. SOD activity has been reported to be higher in tissues whereas less in extracellular fluids such as

plasma or serum (5). Therefore, serum and gingival tissue findings of SOD can be different in the present study.

In the earlier studies, MDA has been reported to be reduce after the administration of low dose doxycycline in experimental periodontitis (33). Another recent study also showed that high serum MDA levels were significantly decreased with low dose doxycycline application in experimental periodontitis in rats (26). Reduced MDA levels due to myricetin administration were also shown in various inflammatory systemic diseases (34-37). In consistent with these findings, we found that MDA levels were decreased in both serum and gingival tissue after myricetin and doxycycline administration compared to saline administration. Additionally, there is no published study evaluating the effect of myricetin in terms of oxidative stress in periodontal diseases to compare our findings. Nonetheless, antibacterial effect of myricetin on Porpyromonas gingivalis and also its antiinflammatory effect on bone resorption in periodontitis have been reported (17,19). Our results are in accordance that myricetin has the beneficial effects on periodontium.

A large number of studies demonstrated that doxycycline has antioxidant and antimicrobial properties in periodontal disease. Tretament with low dose doxycycline administration has been indicated to increase antioxidant capasity and to reduce oxidative stress, collagenase activity and alveolar bone loss in periodontitis (22,26,33). In clinical studies, adjunctive subantimicrobial dose doxycycline administration is recommended in periodontal treatment and it is the only FDA (Food and Drug Administration)-approved host modulating agent (8). We observed that oxidative stress was inhibited by both doxycycline and myricetin treatment in experimental periodontitis and SOD activity and TAS levels increased while TOS and MDA decreased. When compared to doxycycline, the effect of myricetin on oxidative stress and antioxidant enzyme activities were similar in the present study. Therefore, myricetin may be a potential drug to modulate host response in periodontitis.

Possible limitations of our study are the lack of further analyses and additional biomarkers related to periodontal pathogenesis to better understand the role of myricetin on periodontium. Another limitation of this study can be the use of different administration methods for myricetin. However, it should be taken into consideration that there was no information in the literature about the antioxidant efficacy of myricetin when administered by oral gavage.

CONCLUSION

According to our results, myricetin administration seems to be promoted cellular antioxidant defense and prevented tissue damage by scavenging free radicals. Myricetin may reduce gingival tissue damage by reducing oxidative stress caused by periodontitis owing to its strong antioxidant effect. To clarify the therapeutic effects of myricetin as well as to confirm these important findings need to be further analysis.

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

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Ethical approval: This study was approved by the Animal Experiments and Ethics Committee of Zonguldak Bulent Ecevit University (Protocol number: 2016-03-06/01).

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