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Original Article

Effects of alpha-lipoic acid and its combined use with vitamin C on periodontal tissues and markers of oxidative stress in rats with experimental periodontitis

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ABSTRACT

Objective: The potential impacts of antioxidants and their combinations upon inflammatory periodontal diseases are a popular research area. This study aimed to investigate the therapeutic effects of alpha-lipoic acid (ALA) and its combined use with Vitamin C (VitC) on periodontal tissues in terms of oxidative stress and alveolar bone loss in rats with experimental periodontitis. **Methods:** Thirty-six male rats were divided into four groups: control (C), experimental periodontitis (PED), periodontitis treated with ALA (P-ALA), and periodontitis treated with ALA and VitC (P-ALA/VitC). For periodontitis induction, rats' first mandibular molars were ligatured submarginally for 5 weeks. After ligature removal, intragastric treatments with ALA of VitC (50 mg/kg each) were given for 15 consecutive days. Following euthanizing of the animals, mandibular tissues were obtained for biochemical and histologic analysis. **Results:** Malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) levels were lower in both P-ALA and P-ALA/VitC groups compared to the PED group. While 8-OHdG levels were statistically higher in the P-ALA/VitC group compared to the ALA group, MDA and reduced glutathione (GSH) levels were statistically similar between these groups. Also, GSH levels were statistically higher in the P-ALA and P-ALA/VitC groups compared to the PED group. Additionally, histologic analysis showed that treatment with ALA and ALA/VitC combination inhibited periodontal tissue destruction. **Conclusions:** This study revealed that ALA regulated oxidant and antioxidant parameters in periodontally diseased tissues and restricted periodontal tissue destruction. However, VitC did not provide any additional supportive therapeutic effect to ALA.

KEY WORDS: Alpha-lipoic acid, periodontitis, vitamin C, antioxidant

INTRODUCTION

Periodontitis, which is the most common inflammatory disease among people, leads to destruction of tooth-supporting tissues, as well as early tooth loss [1]. It is characterized by a shift in the microbial ecology of sub-gingival plaque and the progressive, host-mediated destruction of the tooth-supporting structure [2]. In this process, bacterial pathogens in sub-gingival plaque stimulate host cells to release pro-inflammatory cytokines. These cytokines recruit polymorphonucleocytes (PMN) to the infection site. PMNs constitute the first line of cellular host defense against bacteria in the gingival sulcus and play an important role in the pathogenesis of periodontal diseases by producing proteolytic enzymes and reactive oxygen species (ROS) [3, 4]. PMN-derived ROS production primarily kills bacteria, but excessive production and extracellular release of these reactive substances result in destruction of the surrounding periodontal tissues [5].

Free radicals and ROS are continuously generated in the body during mitochondrial oxidative metabolism and by the NADPH-oxidase enzyme complex on the inner lipid membrane of inflammatory and other cell types. They are essential in many normal biologic processes, such as controlling gene expression, cellular signal transduction and maintaining vascular health, but at higher concentrations they can cause tissue damage [6]. This damage occurs via various mechanisms, including lipid peroxidation, protein damage, DNA damage, enzyme oxidation and the stimulation of pro-inflammatory cytokines released by different cells, especially monocytes and macrophages [7].

Overproduction of ROS is observed at chronic inflammation sites [8]. Control, modulation and removal of such reactive substances by antioxidants are essential for maintaining health [9, 10]. There is a dynamic balance between ROS activity and antioxidant defenses. Specifically, when there is an imbalance in favor of ROS production or against antioxidant defense, oxidative stress occurs [7].

Recently, interest in the use of antioxidant substances in the treatment of periodontitis has increased markedly [11-14]. Alpha-lipoic acid (ALA) is a powerful antioxidant that is able to scavenge a number of free radicals, such as superoxide radicals, singlet oxygen, hydroxyl radicals and hypochlorous acid. It

occurs with a chelate of Fe^{2+} and Fe^{3+} in both hydrophilic and lipophilic environments [15, 16].

Vitamin C (VitC), an important water-soluble vitamin, is another substance which was studied in relation to periodontal disease [12]. Some of these studies have suggested that there is a negative association between plasma VitC levels and the severity of periodontitis [17,18] and gingival bleeding [19]. However, it is unclear how VitC intake affects gingival oxidative stress in inflammatory periodontal lesions. Some researchers have proposed that VitC exerts antioxidant effects by increasing glutathione [20] and decreasing oxidative DNA damage [21] in mononuclear cells.

The present study was designed to evaluate the therapeutic effects of ALA in terms of oxidant and antioxidant parameters in periodontally diseased tissues, as well as its effects on alveolar bone destruction. Additionally, we aimed to evaluate whether VitC in combination with ALA can provide any supportive effects to that treatment. Although there have been a limited number of studies on the use of ALA and VitC on oxidative damage, to our knowledge, this is the first study investigating the possible effects of systemic administration of ALA and ALA plus VitC combination on malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG) and reduced glutathione (GSH) levels in experimental periodontitis in rats.

MATERIALS AND METHODS

Animal model and procedure

Thirty-six male Wistar albino rats weighing about 220-250 g were housed in an air conditioned room (23-25°) with a 12/12-h light-dark cycle. The animals were given standard rat pellets and tap water *ad libitum*. They were randomly divided into four groups: control (C, n = 9), experimental periodontitis (PED, n = 9), experimental periodontitis treated with ALA (P-ALA, n = 9), and experimental periodontitis treated with ALA and VitC combination (P-ALA/VitC, n = 9). All the procedures were performed in accordance with the protocol approved by the Ethical Committee of the Faculty of Veterinary Sciences at Ataturk University. After anesthesia, by intramuscular injection of xylazine HCI (4 mg/kg) combined with ketamine HCI (40 mg/kg), periodontitis induction was performed by placing the ligatures around the cervix of the rats' right and left first mandibular molars submarginally for 5 weeks according to previous experimental periodontitis studies [11-14]. After ligature removal, the PED group was given a single intragastric dose of 1 ml saline, while the P-ALA and P-ALA/VitC groups were treated with intra-gastric doses of 50 mg/kg ALA or ALA + 50 mg/kg VitC, respectively, for 15 days. The doses of ALA and VitC were determined by previous animal studies [11, 12].

Tissue preparation

At the end of the study, all rats were anesthetized again, blood samples were collected from their hearts under anesthesia and then the rats were euthanized by intracardiac sodium pentothal (60 mg/kg) injection. After the animals were euthanized, their left mandible tissues were carefully removed, along with the surrounding gingiva for biochemical analysis. According to standardized procedures [22], these were washed with physiological saline solution and stored at -80°C until analyzed. All tissues were maintained at +4°C throughout preparation. For all assays, a portion of the mandible gingiva mucosal tissue (1/9, w/v) was homogenized in 0.9% NaCl solution using an OMNI International TH homogenizer (Warrenton, USA). The tissue homogenates were centrifuged for 15 min at 15,000g, and then the clear supernatants were removed for analysis.

Biochemical analysis

Measurement of 8-hydroxydeoxyguanosine levels

Since the assessment of DNA-associated 8-OHdG provides an authentic quantification of oxidative DNA damage, samples from all the groups were extracted using a tissue DNA extraction kit (Vivantis Technologies, Selangor Darul Ehsan, Malaysia). Next, isolated DNA from the groups was denatured at 95°C for 5 min and immediately cooled on ice. The DNA samples were then treated with nuclease P1 (Sigma Aldrich, St. Louis, MO, USA) for 2 h at 37°C, followed by treatment with 10 U of alkaline phosphatase in reaction buffer for 1 h at 37°C. 8-OHdG was quantified by competitive Elisa kit (Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacturer's instructions, and the results were estimated according to standard graphic formulation (μ-Quant, BioTek Instruments, Winooski, VT, USA).

Measurement of glutathione and malondialdehyde levels

To measure MDA levels, the method described by Ohkawa *et al* [23] was used. The content of gingival tissue reduced glutathione was estimated using 5,50-dithiobis-(2-nitrobenzoic acid) by the method of Sedlak and Lindsay [24].

Histopathologic analysis

After the animals were euthanized, their right mandible tissues were carefully removed and fixed in 10% neutral formaldehyde for 72 h. Then, the mandibular tissues were decalcified with 5% nitric acid (HNO3) solution at room temperature for 5 days. The acid solution was changed every 48 h, and decalcification of the tissues was tested with a needle each day. After decalcification was completed, the tissues were neutralized with sodium bicarbonate for 3 h and then subjected to histological processing. The tissues were rinsed in tap water for 6 h, and then dehydrated in ascending alcohols series, cleaned with xylene and embedded in paraffin. The paraffin blocks were sectioned into 5-mm thicknesses in the bucco-lingual direction, and 24 sections were obtained for each block. The sections of all the groups were stained with Crossman's modification of Mallory's triple stain, for histologic analysis.

The histopathologic analysis was performed in a blinded manner. The specimens were visualized and analyzed using light microscopy. In the histometric analysis, mean attachment distances between the cementoenamel junction-periodontal ligament (CEJ-PL) and cementoenamel junction-alveolar bone crest (CEJ-BC) were measured for the buccal and lingual sides of all the mandible samples. All distance measurements were performed as described by previous studies [13, 25] and the mean of these histometric values were calculated.

Statistical analysis

All data were tested with ANOVA and Duncan's post hoc test using SPSS (version 17.0, Chicago, IL, USA). All data were expressed as mean averages ± standard deviation (SD), of which $P < 0.05$ was considered significant.

RESULTS

Biochemical results

Compared to the control group, it was shown that MDA and 8-OHdG levels increased, but GSH levels decreased in PED, P-ALA and P-ALA/VitC groups ($P < 0.05$). Decreases in MDA and 8-OHdG levels and increases in GSH levels were observed after the ALA treatment ($P < 0.05$). It was also determined that MDA and GSH levels did not differ significantly between P-ALA and P-ALA/VitC groups ($P > 0.05$), while 8-OHdG levels differed significantly ($P < 0.05$). The entire biochemical outcome is presented in Table 1.

Histologic results

Frequencies of PMN and mononuclear cell infiltration were determined to be higher at various levels in the experimental groups compared to the control group. Inflammatory cell infiltration as well as bone and collagen destructions were determined to be much more severe in the PED group compared to P-ALA and P-ALA/VitC groups. There was no significant difference in terms of cell infiltration and connective tissue destruction between P-ALA and P-ALA/VitC samples (Figure 1).

In buccal and lingual histometrical analyses, it was determined that mean of the buccal and lingual CEJ-PL and CEJ-BC distances increased in experimental groups compared to the control group, and the most significant increase was seen in the PED group. In addition, it was observed that the degree of attachment and bone loss decreased significantly in P-ALA and P-ALA/VitC groups ($P < 0.05$ compared to PED group), whereas there was no significant difference between both of treatment groups. The measurements regarding histological attachment and alveolar bone losses are presented in Table 2.

Table 1. Comparison of *8-hydroxydeoxyguanosine (8-OHdG),* glutathione (GSH) and malondialdehyde (MDA) levels

Groups	8-OHdG (ng/ml)	GSH (nmol/mg)	MDA(nmol/gr)
Control	1.28 ± 0.23^{a}	29.42 ± 2.33^{a}	18.43 ± 3.94^{a}
PED	3.11 ± 0.3^{b}	19.52 ± 3.73^{b}	44.25 ± 6.27^{b}
P-ALA	2.26 ± 0.29^{c}	24.53 ± 4.23^{c}	33.45 ± 3.34^{c}
P-ALA/VitC	2.82 ± 0.27^{b}	26.22 ± 3.35^{c}	30.26 ± 5.19^{c}

The results are expressed as mean ± SD. Different superscript letters $^{(abc)}$ in the same column indicate significant differences between groups ($P < 0.05$, ANOVA and Duncan's post hoc test).

Table 2. Estimation of the average of the buccal and lingual histometric attachment levels of cement enamel junction-periodontal ligament (CEJ-PL) and CEJ-alveolar bone crest (BC) distances (estimation distances are shown in Figure 1b)

Groups	CEJ-PL (*a*, µm)	CEJ-BC (*a+b*, µm)
Control	74.82 ± 13.48^{a}	127.22 ± 11.91^{a}
PED	244.54 ± 15.52^{b}	356.28 ± 23.84^{b}
P-ALA	150.73 ± 19.82^{c}	250.52 ± 19.51^{c}
P-ALA/VitC	140.94 ± 21.29^{c}	276.12 ± 22.39^{c}

The results are expressed as means ± SD. Different superscript letters (^{abc}) in the same column reflect significant differences ($P < 0.05$; ANOVA followed by Duncan's post hoc test).

DISCUSSION

Studies of periodontal therapy in humans are quite limited because of ethical considerations and the risks involved in the experimental design. This limitation has led to the development of experimental animal models and the particular ligature-induced periodontitis model in rats is widely used [11-14]. In this model, submarginal ligature placement causes both mechanical trauma and plaque accumulation around the marginal gingiva. Thus, intense host-plaque interaction induces chemotaxis and activation of the inflammatory cells, as well as destruction of the tooth-supporting tissues similar to what has been observed in human periodontitis cases.

VitC exhibits both antioxidant [26] and co-antioxidant [27] properties. VitC intake may reduce gingival oxidative stress by decreasing the production of pro-inflammatory cytokines and nitric oxide in periodontal lesions [12]. ALA, a naturally occurring antioxidant, is water- and fat-soluble; therefore, it is distributed in both cellular membranes. This antioxidant, which is reduced to dihydrolipoic acid (DHLA) in various tissues, plays a protective role against oxidative stress-mediated tissue destruction [16, 28, 29]. ALA and DHLA display antioxidant effects such as scavenging of free radicals, chelation of metal ions, and regeneration of antioxidants, including vitamins E and C and GSH [15, 16]. ALA has been thoroughly investigated as a therapeutic agent for several pathological conditions, including hypertension, insulin resistance, and diabetic peripheral neuropathy [30].

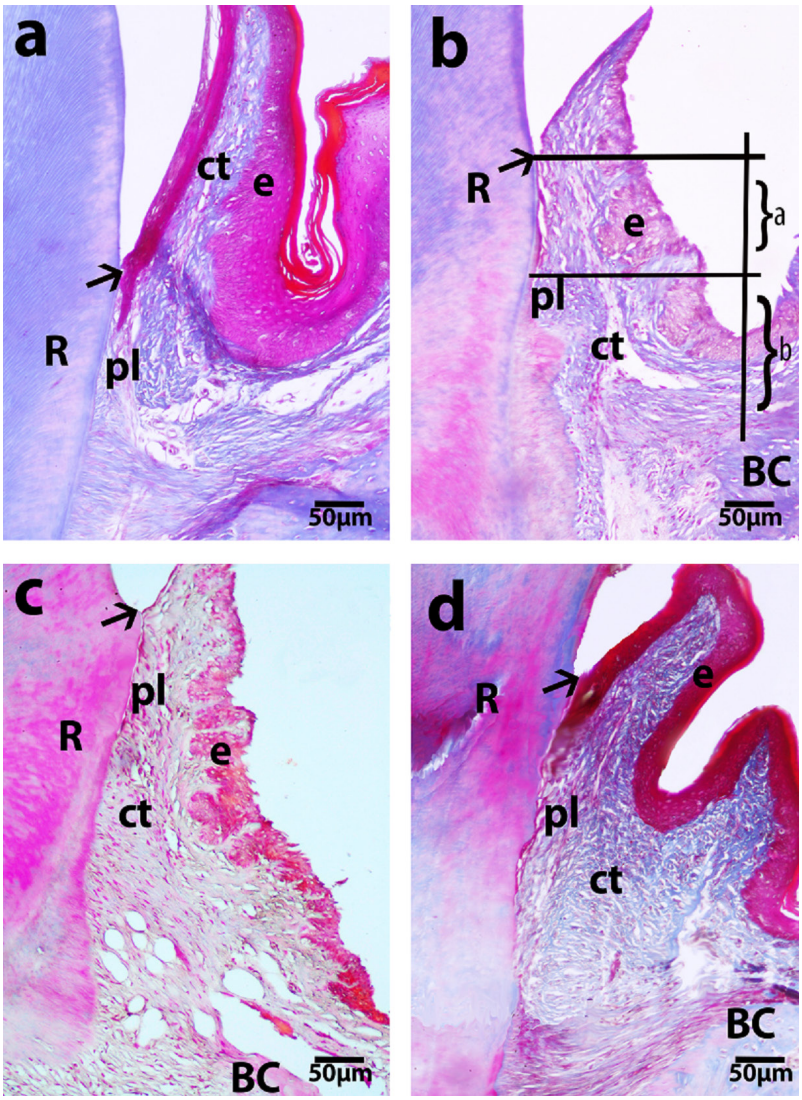

Figure 1. Illustration of gingivo-mucosal tissues from all groups in the bucco-lingual sections of mandibular first molars; **(a)** control group, **(b)** PED group, **(c)** P-ALA group, and **(d)** P-ALA/VitC group: ***a*** is the distance between the cement-enamel junction and the periodontal ligament, ***b*** is the distance between the bone crest and the periodontal ligament, and ***a+b*** is the distance between the bone crest and the cement-enamel junction in the first molar of the buccal side. **R**, root of tooth; **arrow**, cemento-enamel junction; **pl**, periodontal ligament; **ct**, connective tissue; **BC**, alveolar bone crest distances; **e**, and epithelium (Crossman's modified Mallory's triple staining)

In addition to their antioxidant effects, both ALA [28, 31, 32] and VitC [12] inhibit osteoclastic activity. Therefore, this study hypothesized that an ALA and VitC combination might support the positive effects of ALA, and constitutes a potential therapeutic strategy in the treatment of periodontal diseases. Many studies on both human [17, 18, 33] and rats [12, 34] have investigated the effects of VitC on periodontal diseases; therefore, we did not use VitC alone in any of the groups. In the present study, ALA and VitC doses of 50 mg/kg were used, in accordance with previous studies [32, 35].

The present study revealed that systemic ALA treatment ameliorated biochemical and histopathological parameters significantly in an experimental periodontitis model. Treatment with ALA restricted lipid and DNA damage and promoted cellular antioxidant capacity. Additionally, contrary to our expectations, it was shown that combining VitC with ALA did not provide any additional value to the latter's therapeutic effect on diseased periodontal tissues.

Previous studies evaluated the therapeutic effects of ALA in periodontitis pathogenesis experimentally, utilizing similar methodology to the present study design. Akman *et al* [11] evaluated the therapeutic effects of ALA comprehensively in an experimental periodontitis model and showed that ALA treatment inhibited inflammatory alveolar bone resorption; this study also revealed that ALA treatment significantly reduced myeloperoxidase (MPO) levels, an indicator of inflammation activity in tissue samples. Similarly, Kara *et al* [36] also showed that ALA treatment reduced inflammatory cell accumulation and connective tissue destruction in gingival tissue samples. Our study is consistent with these two studies with regard to inhibition of inflammatory cell (PMNL and MNL) accumulation and connective tissue destruction in periodontal tissues and alveolar bone loss after ALA treatment. Our study also showed that ALA inhibited periodontal tissue destruction by limiting oxidative stress.

Degradation of phospholipids, especially in the polyunsaturated fatty acids' structure, is an important oxidative stress indicator, and MDA is one of the lipid peroxidation end products. An increase in ROS levels

results in higher MDA levels, so it has been emphasized that MDA can be an important oxidative stress marker [37]. ROS causes damage in the DNA of the cells, as well as lipids. 8-OHdG is a major indicator in the interpretation of DNA damage [38]. Our study is consistent with the experimental periodontitis studies that show higher MDA [13, 39] and 8-OHdG [12, 36] levels in gingival samples. To our knowledge, this study is the first that evaluates the effects of ALA in periodontal tissue in terms of lipid peroxidation levels and oxidative DNA damage. In their above-mentioned study, Kara *et al* [36] also evaluated the effects of ALA on 8-OHdG levels in gingival tissues immunohistochemically and showed that ALA treatment resulted in lower 8-OHdG immunopositive cells, but it was not statistically significant. The present results showed that ALA treatment significantly decreased MDA and 8-OHdG levels. These favorable effects can be associated with the powerful neutralizing effects on ROS and lipophilic structure of ALA, which provide it a higher penetration to cell membrane and cell core.

Excessive ROS production that results in oxidative stress is inhibited by various antioxidant defense mechanisms [40]. For instance, GSH is a non-enzymatic antioxidant and it has been considered a primary intracellular antioxidant; it plays important role in the inhibition of cellular oxidative damage [41]. The results of this study showed that the lower GSH levels found in the PED group compared to the control group are consistent with earlier experimental periodontitis studies [12, 13]. Lower GSH levels can be seen as a reflective of decreased cellular antioxidant capacity, which makes cellular structures more vulnerable to oxidative damage; so decrease in GSH levels can be an important result of significantly higher 8-OHdG levels. The present results also showed that ALA treatment resulted in significantly higher GSH levels, so it can be related to its direct or indirect antioxidant effects.

On the other hand, ALA and VitC combination did not reflect an additive benefit on periodontal tissues to each other in the study; only statistically insignificant differences were presnet MDA and GSH levels as well as alveolar bone measures among P-ALA and P-ALA/VitC groups. In contrast to our findings, Akman *et al* [11] showed that the combination of VitC and ALA significantly restricted hard and soft periodontal tissue destruction as compared to ALA treatment alone; in that study it was pointed out that VitC was useful in collagen repairing.

Many studies which clearly revealed that both ALA [42] and Vit C [21, 34] decreased oxidative DNA damages suggest that combination of these products might protect DNA against ROS attacks more effective. In contrast, the present findings showed that gingival tissue 8-OHdG levels were significantly higher in P-ALA/VitC group compared to P-ALA group. Decrease in the DNA protection efficacy of ALA when combined with VitC might be related to biochemical interactions between these antioxidants at nuclear or perinuclear areas. In this context, further studies that evaluate the effects of ALA and VitC combination on periodontal tissues, especially focusing on their molecular interactions, are necessary.

In conclusion, this study revealed that ALA treatment can be a supportive modality in the restriction of oxidative damage occurring around periodontal tissues and alveolar bone loss. However, VitC did not provide any additive contribution to the beneficial effects of ALA.

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REFERENCES

1. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005; 366: 1809-20.
2. Bascones-Martinez A, Munoz-Corcuera M, Noronha S, Mota P, Bascones-Ilundain C, Campo-Trapero J. Host defence mechanisms against bacterial aggression in periodontal disease: basic mechanisms. Med Oral Patol Oral Cir Bucal 2009; 14: 680-5.
3. Graves DT. The potential role of chemokines and inflammatory cytokines in periodontal disease progression. Clin Infect Dis 1999; 28: 482-90.
4. Page RC, Kornman KS. The pathogenesis of human periodontitis: an introduction. Periodontol 2000 1997; 14: 9-11.
5. Guentsch AP, Preshaw M, Bremer-Streck S, Klinger G, Glockmann E, Sigusch BW. Lipid peroxidation and antioxidant activity in saliva of periodontitis patients: effect of smoking and periodontal treatment. Clin Oral Investig 2008; 12: 345-52.
6. Genestra M. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Cell Signal 2007; 19: 1807-19.
7. Chapple IL, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontol 2000 2007; 43: 160-232.
8. Halliwell B. Oral inflammation and reactive species: a missed opportunity? Oral Dis 2000; 6: 136-7.
9. Halliwell B. Mechanisms involved in the generation of free radicals. Pathol Biol (Paris) 1996; 44: 6-13.
10. Halliwell B. How to characterize an antioxidant: an update. Biochem Soc Symp 1995; 61: 73-101.
11. Akman S, Canakci V, Kara A, Tozoglu U, Arabaci T, Dagsuyu IM. Therapeutic effects of alpha lipoic acid and vitamin C on alveolar bone resorption after experimental periodontitis in rats: a biochemical, histochemical, and stereologic study. J Periodontol 2013; 84: 666-74.
12. Tomofuji T, Ekuni D, Sanbe T, Irie K, Azuma T, Maruyama T, Tamaki N, Murakami J, Kokeguchi S, Yamamoto T. Effects of vitamin C intake on gingival oxidative stress in rat periodontitis. Free Radic Biol Med 2009; 46: 163-8.
13. Kara A, Akman S, Ozkanlar S, Tozoglu U, Kalkan Y, Canakci CF, Tozoglu S. Immune modulatory and antioxidant effects of melatonin in experimental periodontitis in rats. Free Radic Biol Med 2013; 55: 21-6.
14. Carvalho Rde S, de Souza CM, Neves JC, Holanda-Pinto SA, Pinto LM, Brito GA, de Andrade GM. Vitamin E does not prevent bone loss and induced anxiety in rats with ligature-induced periodontitis. Arch Oral Biol 2013; 58: 50-8.
15. Goraca A, Huk-Kolega H, Piechota A, Kleniewska P, Ciejka E, Skibska B. Lipoic acid - biological activity and therapeutic potential. Pharmacol Rep 2011; 63: 849-58.
16. Abdel-Zaher AO, Mostafa MG, Farghaly HS, Hamdy MM, Abdel-Hady RH. Role of oxidative stress and inducible nitric oxide synthase in morphine-induced tolerance and dependence in mice. Effect of alpha-lipoic acid. Behav Brain Res 2013; 247: 17-26.
17. Amarasena N, Ogawa H, Yoshihara A, Hanada N, Miyazaki H. Serum vitamin C-periodontal relationship in community-dwelling elderly Japanese. J Clin Periodontol 2005; 32: 93-7.

18. Amaliya, Timmerman MF, Abbas F, Loos BG, Van der Weijden GA, Van Winkelhoff AJ, Winkel EG, Van der Velden U. Java project on periodontal diseases: the relationship between vitamin C and the severity of periodontitis. J Clin Periodontol 2007; 34: 299-304.
19. Staudte H, Sigusch BW, Glockmann E. Grapefruit consumption improves vitamin C status in periodontitis patients. Br Dent J 2005; 199: 213-7.
20. Lenton KJ, Sane AT, Therriault H, Cantin AM, Payette H, Wagner JR. Vitamin C augments lymphocyte glutathione in subjects with ascorbate deficiency. Am J Clin Nutr 2003; 77: 189-95.
21. Panayiotidis M, Collins AR. Ex vivo assessment of lymphocyte antioxidant status using the comet assay. Free Radic Res 1997; 27: 533-7.
22. Cuzzocrea S, Zingarelli B, Hake P, Salzman AL, Szabo C. Antiinflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. Free Radic Biol Med 1998; 24: 450-9.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8.
24. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968; 25: 192-205.
25. Susin C, Rosing CK. Effect of variable moderate chronic stress on ligature-induced periodontal disease in Wistar rats. Acta Odontol Scand 2003; 61: 273-7.
26. Halliwell B. The wanderings of a free radical. Free Radic Biol Med 2009; 46: 531-42.
27. Vinson J, Hsu C, Possanza C, Drack A, Pane D, Davis R, Klock C, Graser K, Wang X. Lipid peroxidation and diabetic complications: effect of antioxidant vitamins C and E. Adv Exp Med Biol 1994; 366: 430-2.
28. Ha H, Lee JH, Kim HN, Kim HM, Kwak HB, Lee S, Kim HH, Lee ZH. Alpha-Lipoic acid inhibits inflammatory bone resorption by suppressing prostaglandin E2 synthesis. J Immunol 2006; 176: 111-7.
29. Zhao RR, Xu F, Xu XC, Tan GJ, Liu LM, Wu N, Zhang WZ, Liu JX. Effects of alpha-lipoic acid on spatial learning and memory, oxidative stress, and central cholinergic system in a rat model of vascular dementia. Neurosci Lett 2015; 587: 113-9.
30. Park S, Karunakaran U, Jeoung NH, Jeon JH, Lee IK. Physiological effect and therapeutic application of alpha lipoic acid. Curr Med Chem 2014; 21: 3636-45.
31. Koh JM, Lee YS, Byun CH, Chang EJ, Kim H, Kim YH, Kim HH, Kim GS. Alpha-lipoic acid suppresses osteoclastogenesis despite increasing the receptor activator of nuclear factor kappaB ligand/osteoprotegerin ratio in human bone marrow stromal cells. J Endocrinol 2005; 185: 401-13.
32. Sanbe T, Tomofuji T, Ekuni D, Azuma T, Tamaki N, Yamamoto T. Oral administration of vitamin C prevents alveolar bone resorption induced by high dietary cholesterol in rats. J Periodontol 2007; 78: 2165-70.
33. Kuzmanova D, Jansen ID, Schoenmaker T, Nazmi K, Teeuw WJ, Bizzarro S, Loos BG, van der Velden U. Vitamin C in plasma and leucocytes in relation to periodontitis. J Clin Periodontol 2012; 39: 905-12.
34. Ekuni D, Tomofuji T, Sanbe T, Irie K, Azuma T, Maruyama T, Tamaki N, Murakami J, Kokeguchi S, Yamamoto T. Vitamin C intake attenuates the degree of experimental atherosclerosis induced by periodontitis in the rat by decreasing oxidative stress. Arch Oral Biol 2009; 54: 495-502.
35. Maritim AC, Sanders RA, Watkins JB 3rd. Effects of alpha-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. J Nutr Biochem 2003; 14: 288-94.
36. Kara A, Akman S, Demirci T, Arabaci T. Influence of alpha-lipoic acid on epithelial apoptosis in the experimental periodontitis. Turk J Med Sci 2013; 43: 747-55.
37. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 2005; 15: 316-28.
38. Irie K, Tomofuji T, Tamaki N, Sanbe T, Ekuni D, Azuma T, Maruyama T, Yamamoto T. Effects of ethanol consumption on periodontal inflammation in rats. J Dent Res 2008; 87: 456-60.
39. Yagan A, Kesim S, Liman N. Effect of low-dose doxycycline on serum oxidative status, gingival antioxidant levels, and alveolar bone loss in experimental periodontitis in rats. J Periodontol 2014; 85: 478-89.
40. Nordberg J, Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 2001; 31: 1287-312.
41. Grant MM, Brock GR, Matthews JB, Chapple IL. Crevicular fluid glutathione levels in periodontitis and the effect of non-surgical therapy. J Clin Periodontol 2010; 37: 17-23.
42. Sun LQ, Chen YY, Wang X, Li XJ, Xue B, Qu L, Zhang TT, Mu YM, Lu JM. The protective effect of alpha lipoic acid on Schwann cells exposed to constant or intermittent high glucose. Biochem Pharmacol 2012; 84:961-73

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