Clinical Oral Investigations (2021) 25:4959–4966
https://doi.org/10.1007/s00784-021-03805-y

ORIGINAL ARTICLE

Antioxidant effect of caffeic acid phenethyl ester in experimentally induced periodontitis

Feyza Otan Özden[1] · Müge Lütfioğlu[1] · Esra Demir[2] · Birşen Bilgici[3]

Received: 10 September 2020 / Accepted: 20 January 2021 / Published online: 26 March 2021

Abstract

Objectives The aim of the present study was to evaluate the antioxidant effect of systemically administered caffeic acid phenethyl ester (CAPE) in periodontitis.

Materials and methods Forty rats were randomly divided into four groups: control, lipopolysaccharide-induced experimental periodontitis (LPS), CAPE 5: LPS+5 μmol/kg/day CAPE, and CAPE 10: LPS+10 μmol/kg/day CAPE. Following lipopolysaccharide-induced experimental periodontitis, CAPE was administered intraperitoneally for 28 days. Gingival and serumal total antioxidant status (TAS) and total oxidant status (TOS) were analyzed by enzyme-linked immunosorbent assay (ELISA).

Results Gingival tissue TAS was significantly higher with CAPE application compared with the LPS group and was highest in the CAPE 10 group ($p<0.05$). Gingival tissue TOS was highest in the LPS group, and both of the CAPE dosages decreased the gingival tissue TOS, with the highest decrease in the CAPE 10 group ($p<0.05$). The differences were not significant for serumal TAS or TOS levels ($p>0.05$).

Conclusions The effect of CAPE on increased TAS and decreased TOS levels in inflamed gingival tissue indicates the antioxidant therapeutic potential of CAPE in periodontitis.

Clinical relevance Within the limitations of this study, CAPE may be suggested as an effective host modulator agent for reducing oxidative stress in gingival tissue and might be considered as an adjunctive therapy in periodontitis.

Keywords Caffeic acid phenethyl ester · Experimental periodontitis · Total antioxidant status · Total oxidant status

Introduction

Periodontal disease is one of the most common chronic diseases and it is usually initiated by microbial dental plaque. It is now accepted that the tissue destruction in periodontal disease occurs as a result of host response to specific bacteria and their products [1], involving the release of proteolytic enzymes and reactive oxygen species (ROS) [2]. ROS production is a normal feature of cellular metabolism and induces oxidative stress, which leads to excessive DNA damage in periodontal tissues [3]. The increase in ROS formation and the reduction in antioxidant capacity increase the risk of destruction of periodontal tissues [4]. The role of antioxidants in both the prevention and treatment of such inflammatory diseases, in which tissue damage is known to be caused by oxidative stress, has received much attention from scientists, clinicians, and the general public [5]. It was aimed to increase antioxidant capacity using diet-derived exogenous antioxidant compounds against oxidative stress [6]. Due to the fact that dangerously high doses of antioxidants should be taken in order to create such a significant antioxidant effect, the search for natural herbal products that have high antioxidant properties without any side effects has intensified [7].

Caffeic acid phenethyl ester (CAPE) is a biologically active ingredient of honey bee propolis. This naturally bioactive and hydrophobic polyphenolic ester is also found in numerous plants. CAPE has important biological activities including

✉ Feyza Otan Özden
feyza_otan@yahoo.com

[1] Department of Periodontology, School of Dentistry, Ondokuz Mayıs University, Kurupelit, 55139 Samsun, Turkey

[2] Department of Periodontology, School of Dentistry, Biruni University, İstanbul, Turkey

[3] Department of Biochemistry, School of Medicine, Ondokuz Mayıs University, Samsun, Turkey

antiviral [8], anti-inflammatory [9, 10], and antioxidant activities [11], exerting its antioxidant activity by suppressing lipid peroxidation, scavenging ROS, inhibiting xanthine oxidase and nitric oxide synthase activities, and preventing the consumption of superoxide dismutase activity [12–14]. CAPE seems to act as a ROS scavenger and preventer of ROS production, consequently resulting in an antioxidant effect directly or implicitly [15].

Growing evidence suggests that certain pharmacologic agents could be of therapeutic value as host response modulators in periodontal treatment. Although CAPE has been previously characterized as having numerous potentially beneficial properties, including antioxidant, anticarcinogenic, immunomodulatory, and anti-inflammatory activities in different disease states and in vitro study designs [8–14], the effects of CAPE on the course of periodontal disease, especially in an in vivo model, have not been sufficiently investigated. A very few in vitro studies have investigated its effect on periodontopathogens and cellular responses in culture media [16, 17] and there has been one in vivo experimental periodontitis study [18], but, to the best of our knowledge, the effect of CAPE on the oxidant status of diseased periodontal tissues has not been studied before.

Therefore, the present experimental periodontitis model was used to determine the effect of CAPE application on the oxidant status of rat gingival tissues and serum.

Materials and methods

Study design

The experimental protocols were designed in accordance with ethical principles for the use of laboratory animals, as approved by the Ethics Committee for Animal Experimentation at Ondokuz Mayıs University (approval number: 2014/29). All experimental procedures were performed at the Ondokuz Mayıs University Laboratory of Experimental Animals Research Center. The biochemical analysis was carried out in the Department of Medical Biochemistry of the Faculty of Medicine at Ondokuz Mayıs University.

Forty adult (6–7 weeks old) male Wistar rats (≈125 to 150 g) were housed with food and water ad libitum at a constant room temperature (22±1 °C) under a 12-h light/dark cycle in individual cages. The rats were randomly divided into four groups of ten animals each: (1) control; (2) lipopolysaccharide-induced experimental periodontitis (LPS); (3) LPS+5 μmol/kg/day CAPE (CAPE 5); and (4) LPS+10 μmol/kg/day CAPE (CAPE 10). The control group did not receive any treatment until the end of the experiment. Gingival oxidant status was evaluated based on the total antioxidant status (TAS) and total oxidative stress (TOS) alterations in healthy and diseased conditions. Alveolar bone loss was measured morphometrically. The animals were anesthetized with ketamine–HCl (75 to 100 mg/kg intraperitoneal injection) and xylazine–HCl (5 mg/kg intraperitoneal injection) sedation on the lipopolysaccharide injection days.

Periodontitis induction and CAPE application

Experimental periodontitis was induced in the left mandibular molar teeth of all rats except the controls using an endotoxin-induction model. The method to create periodontitis was chosen so as to obtain localized, controlled, and rapid destruction [19, 20]. The buccal and lingual gingiva of the first and second left mandibular molars was injected with 10 mL of a phosphate-buffered saline (PBS) solution containing 1 mg/mL lipopolysaccharide from *Porphyromonas gingivalis* (InvivoGen, San Diego, CA, USA) using an insulin syringe equipped with a blunted edge 30-gauge needle to induce periodontitis. This injection was followed by two additional injections at 48-h intervals for 1 week. The control group received pure saline by the same route and of the same dosage and duration. Induction of periodontitis was verified by taking radiographs 24 days after the last injection. After the experimental periodontitis induction, the LPS group did not receive any treatment, while the CAPE 5 and CAPE 10 groups continued with intraperitoneal CAPE injections at the same times of the day for 28 days until the end of the experiment.

CAPE preparation and administration

The CAPE was supplied by Sigma–Aldrich (C8221, St. Louis, MO, USA), dissolved in ethanol, and subsequent dilutions were made using saline (0.9% NaCl, w/v). The rats were weighed with precision weighing scales (Precisa XB 220 A, Dietikon, Switzerland). CAPE at 5 and 10 μmol/kg/day was intraperitoneally administered once a day for 28 days based on comparable studies that revealed its anti-inflammatory and antioxidant properties at similar dosages [15, 21, 22].

Analysis of TAS, TOS, and OSI

At the end of the experiment, the animals were anesthetized and blood samples were collected from each one via cardiac puncture. Serum samples were separated by centrifugation (Shimadzu UV160A, SNo: 28006648, Japan) for 10 min at 3000×*g* and were immediately stored at −80 °C until use. The animals were sacrificed using an anesthesia overdose and neck–vertebra dislocation. Gingival tissues around the left molar teeth were excised and refrigerated at −80 °C until use. The TOS and TAS levels of serum and gingival tissues were measured using commercially available kits (Rel Assay, Mega Tıp, Gaziantep, Turkey) specific for rats according to

the manufacturer's instructions. The oxidative stress index (OSI) is defined as the ratio of the TOS to the TAS level [23].

Morphometric analysis

For the morphometric analyses, left mandibular halves were boiled for 10 min and the soft tissues were cleaned manually. Then, the jaw bones were soaked in 0.2 N NaOH solution at room temperature for 5 min to remove the remaining soft tissue debris. The alveolar bone height was measured under a stereomicroscope (Olympus SZ61, Olympus Optical Co., Japan) (×40 magnification) by recording the distance from the cementoenamel junction to the alveolar bone crest. Measurements were made at three points on the buccal and lingual sides to quantify the alveolar bone level. The mean alveolar bone loss (ABL) was calculated around each tooth [24]. Images were obtained with an Olympus C-5060 digital camera. ABL was measured by an examiner who was blinded to the study groups (E.D.).

Statistical analyses

The data were analyzed using statistical software (SPSS V.23, Chicago, IL, USA). The sample size was calculated based on the data of a previous study with a similar design to estimate the sample size [24]. The parameter used for sample size calculation was morphometric analysis of ABL in experimentally induced periodontitis by lipopolysaccharide in rats. Taking into account a type I error equal to 0.05, a confidence interval of 95%, and a power of 90%, a minimum sample size of 7 rats per group was necessary. To compensate for possible losses, three rats were added to each group. The normal distribution of data was analyzed with the Shapiro–Wilk test. One-way ANOVA was used to compare the variables showing normal distribution and a post hoc Tukey test was used for multiple group comparisons. The variables not showing normal distribution were analyzed by the Kruskal–Wallis test. When there were significant differences ($p<0.05$), post hoc two-group comparisons were assessed using the Mann–Whitney U test with Bonferroni adjustment and $p<0.008$ was considered statistically significant. Spearman correlation tests were used to identify relationships between the biochemical parameters. A p value of <0.05 was considered statistically significant. The data were presented as mean ± standard deviation (SD) and median (min–max).

Results

Alveolar bone loss

Periodontitis developed in all the experimental groups. Morphometric measurements of ABL demonstrated different bone levels among the four groups ($p<0.01$, ANOVA). The bone level measurement was given as mean ± SD. ABL was significantly higher in the LPS, CAPE 5, and CAPE 10 groups compared to the control group ($p<0.01$ for all, post hoc Tukey) (Table 1). Although the amount of bone loss was highest in the LPS group, no differences were detected between the experimental periodontitis groups depending on the CAPE application or dosages ($p<0.05$ for all, post hoc Tukey) (Table 1) (Fig. 1, Fig. 2).

TAS, TOS, and OSI values

The oxidative statuses of tissue and serum samples of the study groups are summarized in Table 1 and Fig. 3. The tissue level of TAS was significantly different among the four study groups ($p<0.001$, ANOVA). The highest tissue TAS level was detected in the CAPE 10 group, followed by the control, CAPE 5, and LPS groups in that order. The only significant difference was detected between the CAPE 10 and LPS groups ($p<0.001$, post hoc Tukey test).

The four groups differed significantly in terms of tissue TOS levels ($p<0.001$, ANOVA). The highest tissue TOS level was detected in the LPS group and the difference was significant compared to the other three groups ($p<0.001$ for each, post hoc Tukey test). The lowest tissue TOS level was detected in the control group compared to the LPS and CAPE 5 groups ($p<0.01$ for each, post hoc Tukey test) and the levels were similar between the control and the CAPE 10 groups ($p>0.05$, post hoc Tukey test).

Tissue OSI values showed statistically significant differences among the four groups ($p<0.001$, Kruskal–Wallis test). The values were highest in the LPS group and the OSI was significantly higher in the LPS group compared to the control and CAPE 10 groups ($p<0.008$, Mann–Whitney U test with Bonferroni adjustment). The tissue OSI values of the CAPE 10 and control groups were statistically similar ($p>0.008$, Mann–Whitney U test with Bonferroni adjustment).

Significant differences were not observed in serumal TAS, TOS, or OSI values ($p>0.05$) (Table 1).

Correlations

Correlations between the tissue TOS, TAS, and OSI values and the morphometric measurements of alveolar bone level were investigated for each group (Table 2). No correlation test was performed as there was no statistically significant difference between the oxidative parameters in serum. In the control group, a significantly strong negative correlation was detected between the tissue OSI and tissue TAS. With respect to the tissue OSI values, there was a strong positive correlation with the ABL, a strong negative correlation with the tissue TAS, and a moderate positive correlation with the tissue TOS in the LPS group. In the CAPE 5 group, the correlation was strongly

Table 1 The oxidative status of gingival tissue and serum samples of the study groups

	Control	LPS	CAPE 5	CAPE 10	*P* values
tTAS	0.22±0.13	0.10±0.05	0.20±0.15	0.42±0.17	0.00*
tTOS	0.02±0.00	0.063±0.02	0.041±0.00	0.024±0.01	0.00*
tOSI	0.06 (0.02–0.27)	0.63 (0.24–7.56)	0.25 (0.06–2.61)	0.09 (0.05–0.49)	0.00**
sTAS	0.33±0.03	0.29±0.004	0.35±0.05	0.37±0.05	0.06*
sTOS	0.01±0.00	0.01±0.01	0.01±0.00	0.01±0.00	0.75*
sOSI	0.03 (0.02–0.05)	0.03 (0.00–0.07)	0.02 (0.01–0.03)	0.02 (0.01–0.04)	0.37**
ABL	6.66±1.67	13.09±2.43	11.47±2.53	11.04±1.99	0.00*

LPS, lipopolysaccharide-induced experimental periodontitis; *CAPE 5*, LPS+5 μmol/kg/day of CAPE-applied group; *CAPE 10*, LPS+10 μmol/kg/day of CAPE-applied group; *tTAS*, total antioxidant status of tissue; *tTOS*, total oxidant status of tissue; *tOSI*, oxidative stress index of tissue; *sTAS*, total antioxidant status of serum; *sTOS*, total oxidant status of serum; *sOSI*, oxidative stress index of serum; *ABL*, alveolar bone loss

*One-way ANOVA, post hoc Tukey; values are given as mean ± standard deviation

**Kruskal–Wallis, followed by Mann–Whitney *U* test with Bonferroni adjustment; values are given as median (min–max)

positive with the ABL, strongly negative with the tissue TAS, and weakly positive with the tissue TOS. In the CAPE 10 group, the tissue OSI values revealed a strong positive correlation with the tissue TOS, a moderate positive correlation with the ABL, and a strong negative correlation with the tissue TAS.

Discussion

The host modulatory agents that can influence tissue response may have beneficial effects in blocking the initiation and progression of periodontal disease. The important role of oxidative stress in the pathologic mechanism of periodontal disease has been clearly proven in recent years [2–4, 25, 26]. CAPE is a promising novel extract possessing strong antioxidant, anti-inflammatory, and healing properties and has been used in folk medicine for many years [10, 11, 27]. The findings of the present study revealed that CAPE ameliorated oxidative stress and had an antioxidant effect in a dose-dependent manner in gingival tissues. To the best of our knowledge, this is the first in vivo study evaluating the host modulatory effect of CAPE on the oxidant status of inflamed periodontal tissues. In the present study, CAPE was shown to have a beneficial effect on reducing the local oxidant status of gingival tissues in an experimental periodontitis model.

Antioxidants present a strong defense function against ROS; therefore, numerous studies have attempted to examine the role of antioxidants in the treatment of periodontal diseases. It has been shown that supplemental therapies like vit

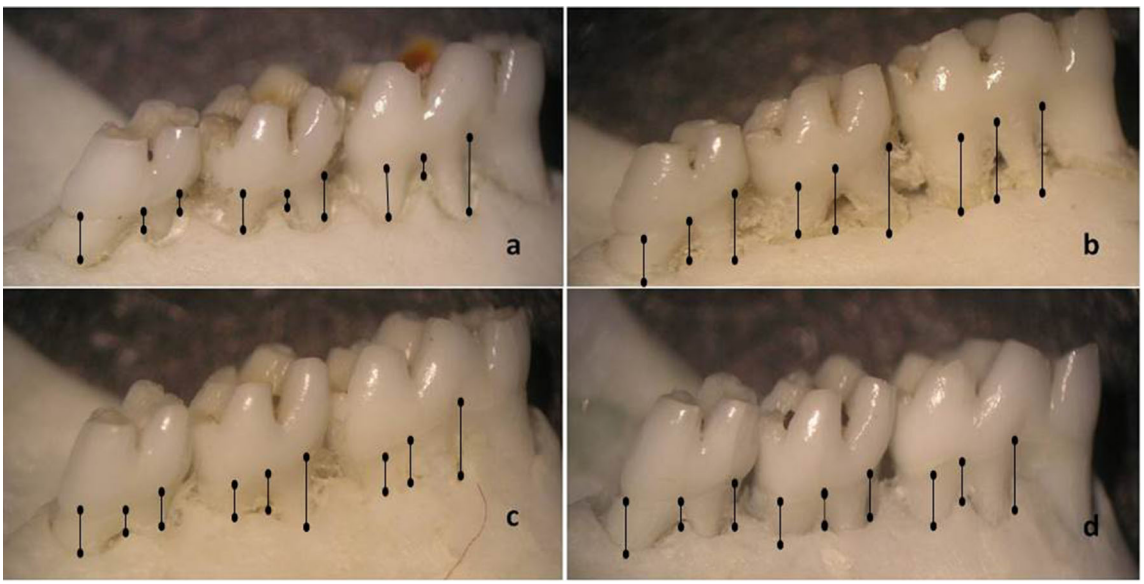

Fig. 1 Morphometric measurement revealed that alveolar bone loss was greatest in the LPS group (**b**) and lowest in the control group (**a**). Bone loss was not significantly different between the LPS (**b**), CAPE 5 (**c**), and CAPE 10 (**d**) groups

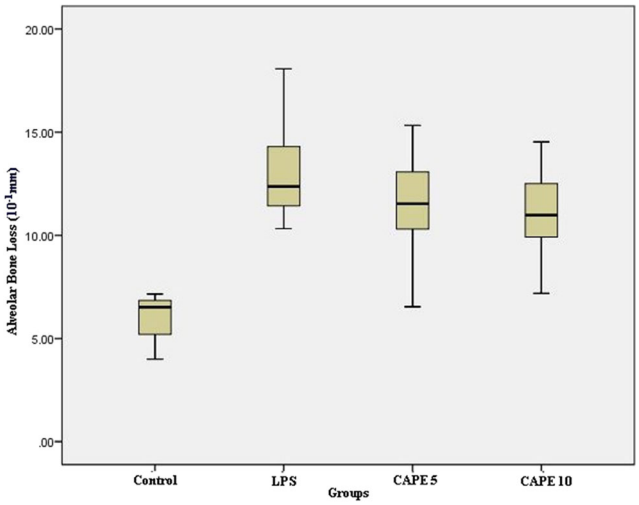

Fig. 2 Alveolar bone loss in the study groups. The groups are defined on the *x* axis. The unit for alveolar bone loss is 10^{-1} mm

Table 2 Correlations related with tissue TOS and TAS levels, OSI values, and morphometric measurements

	tOSI-ABL	tOSI-tTAS	tOSI-tTOS
Control	r=0.321 p=0.362	r=−0.988 p=0.000	r=0.224 p=0.533
LPS	r=0.909 p=0.000	r=−0.827 p=0.002	0.636 p=0.035
CAPE 5	r=0.721 p=0.019	r=−0.976 p=0.000	r=0.492 p=0.04
CAPE 10	r=0.697 p=0.025	r=−0.806 p=0.005	r=0.721 p=0.019

Values were calculated using Spearman's correlation analysis

tTAS, total antioxidant status of tissue; *tTOS*, total oxidant status of tissue; *tOSI*, oxidative stress index of tissue; *sTAS*, total antioxidant status of serum; *sTOS*, total oxidant status of serum; *sOSI*, oxidative stress index of serum; *ABL*, alveolar bone loss; *LPS*, lipopolysaccharide-induced experimental periodontitis; *CAPE 5*, LPS+5 μmol/kg/day of CAPE-applied group; *CAPE 10*, LPS+10 μmol/kg/day of CAPE-applied group

C, vit E, taurine, and lycopene improve clinical parameters, increase the activities of local and systemic antioxidants, and decrease the levels of local and systemic ROS, in comparison with the conventional periodontal treatment [28]. CAPE was reported to exert its antioxidant activity via several biological mechanisms and cellular interactions [12–14]. In a more specific manner, CAPE was shown to mediate the nuclear translocation of transcription factors such as nuclear factor-kappa B (NF-κB) and nuclear factor erythroid 2–related factor 2

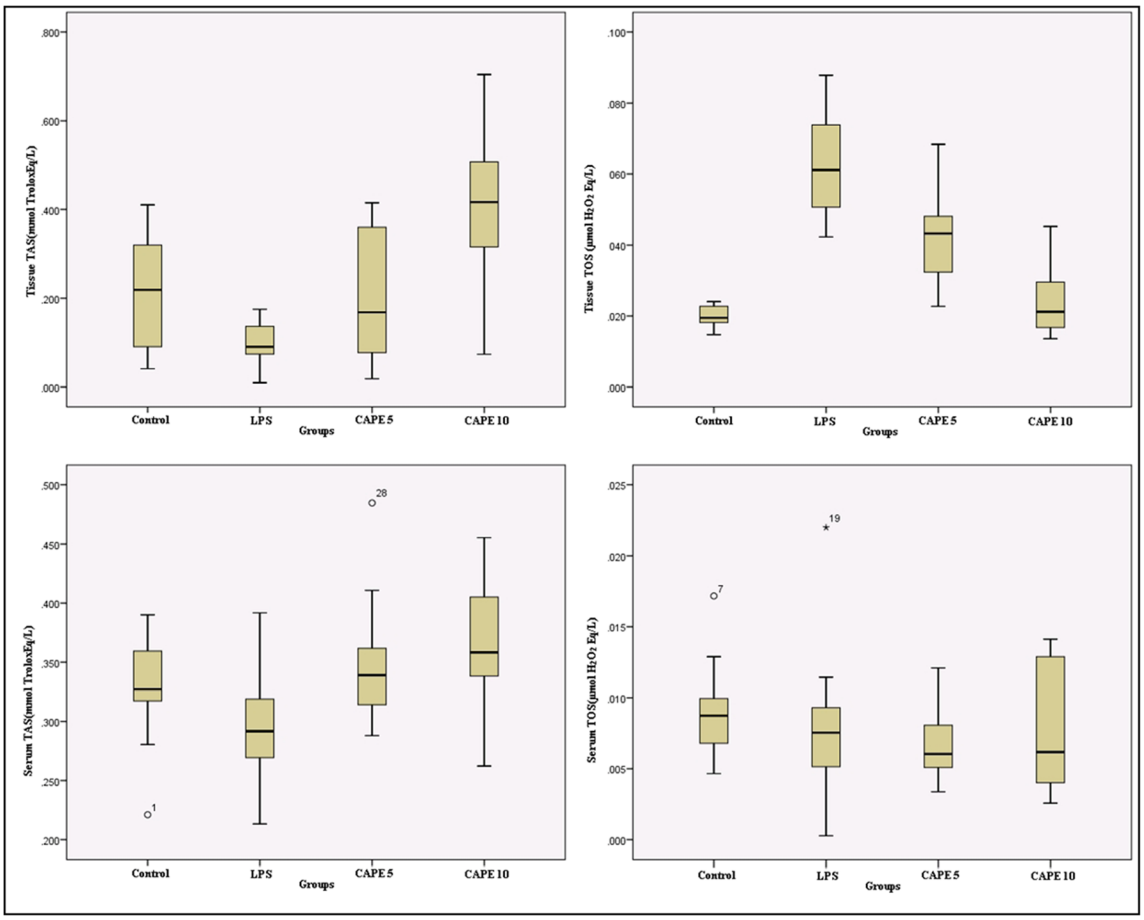

Fig. 3 The oxidative status of tissue and serum samples in the study groups. The results are expressed as mean ± SD. The groups are defined on the *x* axis. The unit for TAS is mmol TroloxEq/L and for TOS is μmol H_2O_2 Eq/L

(Nrf2) [29] and prevent ROS production. NF-κB is a multi-unit transcriptional factor that plays pivotal roles in lipopolysaccharide-induced periodontal inflammation. In periodontal diseases, bacterial lipopolysaccharide was demonstrated to activate the NF-κB signaling cascade, inducing the secretion of pro-inflammatory cytokines, and consequently increase the oxidant status of the tissue with inflammatory response [30]. CAPE suppressed the lipopolysaccharide-induced toll-like receptor-4 (TLR4)/MyD88 and nuclear factor kappa B (NF-κB) activation and attenuated the production of IL-6, IL-8, iNOS, and COX-2 in human gingival fibroblasts [17]. Nrf2 (encoded by the Nfe2l2 gene) is a transcription factor responsible for the regulation of cellular redox balance and protective antioxidant responses [31, 32] and was shown to be released from its suppressor Kelch-like ECH-associated protein 1 (Keap1) upon stimulation by CAPE [33, 34]. The antioxidant effect of CAPE in periodontal disease through the Nrf2 mechanism has been demonstrated in several studies [35, 36]. CAPE regulated Nrf2 release and initiated the transcription of heme oxygenase 1 (HO1), which in turn activated cellular defense mechanisms against oxidative stress including decreases in superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) expression [35]. From this point of view, the positive effect of CAPE on reducing oxidant status becomes prominent in inflammatory periodontal disease.

In the present study, the antioxidant effects of CAPE on lipopolysaccharide-induced experimental periodontal disease were investigated for the first time by means of tissue and serum levels of TAS and TOS and OSI values. CAPE application reduced TOS and increased TAS levels only in diseased tissues in a dose-dependent manner. An insignificant decrease was detected in morphometric measurements in the same dose-dependent manner. In our study, the decreased OSI values with CAPE application demonstrated that CAPE counterbalances the oxidant/antioxidant level in a way that reduces the oxidant status of diseased tissues. In an in vitro study, the culture supernatant of a murine macrophage cell line was incubated with different doses of CAPE and *Prevotella intermedia* lipopolysaccharide for 24 h. CAPE was shown to exert significant inhibitory effects on *P. intermedia* lipopolysaccharide–induced production of nitric oxide. In the same study, CAPE also decreased the nuclear translocation of NF-κB with lipopolysaccharide and inhibited lipopolysaccharide-induced signal transducer [16]. As mentioned above, the reduction in the oxidant status and the relative decrease in the morphometric tissue loss levels can be attributed to the suppressive effect of CAPE on nuclear translocation of the transcription signaling pathways.

The measurements of TAS, TOS, and recently added OSI in serum and gingival crevicular fluid (GCF) were reported as reliable methods and proposed as new and practical approaches to determine oxidative status in periodontal disease [23, 37, 38]. The compromised balance of antioxidant and pro-oxidant species such as decreased TAS and increased TOS in GCF, saliva, and even in serum samples of the patients with periodontal disease has been demonstrated [37–40]. Periodontitis was associated with higher values of TOS and OSI in the GCF and saliva [38, 41]. The findings of the present study are consistent with the results of previously reported studies emphasizing that periodontal disease is a pro-oxidant condition.

According to the findings presented herein, there are positive moderate correlations between morphometric measurements of ABL and tissue TOS and OSI values, and negative moderate correlations between ABL and TAS values. Moreover, there exists a positive strong correlation between tissue OSI and TOS measurements and a strong negative correlation between tissue OSI and TAS measurements. In agreement with our results, Baltacıoğlu et al. [38, 41] demonstrated significant correlations between the gingival inflammation, periodontal clinical parameters, and GCF and salivary measurements of TAS, TOS, and OSI in periodontally affected patients. In the light of the previous studies, OSI values were estimated as a disease marker of ongoing tissue deterioration and severity because an imbalance between the pro- and antioxidants leads to further oxidative damage and eventually results in the progressive destruction of periodontal tissues. OSI is a new parameter suggested to show the level of oxidative stress in disease states [23]. Based mainly on the measurement of oxidant/antioxidant imbalances, the ratio of TOS/TAS was suggested to be more advantageous and reliable in determining the oxidant status of tissue, instead of examining several anti-/pro-oxidant species separately [38]. OSI determined as a local tissue oxidant stress factor may be an early and descriptive marker of the initiative or progressive state of periodontal diseases. CAPE can be a promising natural agent for a new treatment or host modulatory strategy for inflammatory periodontal diseases by decreasing local periodontal pro-oxidant state and/or increasing local antioxidant capacity.

In untreated diabetic rats, cardiac tissue levels of malondialdehyde, superoxide dismutase, and catalase were reduced, while glutathione peroxidase activity was reduced in CAPE-treated groups. CAPE was introduced as an ameliorating agent in diabetes mellitus and had antioxidant effects [42]. In an in vitro study, the production of inducible nitric oxide synthase, an oxidative stress marker, was inhibited by CAPE in a dose-dependent manner (10-, 20-, 30-μM application) in culture supernatants of human gingival fibroblasts (inhibition increased with increasing dosage) [17]. Yiğit et al. [18] compared low-dose doxycycline (LDD) treatment with CAPE application to evaluate the latter's anti-inflammatory and antioxidant effect as a novel host modulatory agent in a ligature-induced experimental periodontitis model. The CAPE (10 μmol/kg) was administered intraperitoneally and had greater antioxidant effects than the LDD.

Serum levels of malondialdehyde decreased, while glutathione and glutathione peroxidase increased, indicating a greater antioxidant effect than LDD treatment [18]. Those results are in accordance with our results of a local reduction in TOS. However, our findings indicate a more specific and local response in gingival tissues with systemically administered CAPE.

The present study is the first study evaluating the positive antioxidant effect of different dosages of CAPE on gingival tissue levels of TAS and TOS in a dose-dependent manner in lipopolysaccharide-induced periodontitis. In the current study, serum TAS, TOS, and OSI values were also investigated. The difference between the study groups of serum TAS, TOS, and OSI values was not statistically significant. It has been stated that the systemic (intraperitoneal) administration of CAPE in several experimental designs resulted in decreases in serum TOS and OSI values and had a positive effect on oxidant status [43–45]. However, those experiments were designed using a systemic stimulant such as drug administration, intoxication, or disease stimulation to determine the effect of CAPE on general homeostasis. The stability of the serum oxidant status in our study can be explained by its being in a systemically homeostatic state without fluctuations in serumal oxidant markers. In our experimental model, *P. gingivalis* lipopolysaccharide was locally injected into the gingiva rather than being reflected in circulation and so no serumal changes were expected to appear in the same line, which is consistent with the results previously reported by Buduneli et al. [46], regardless of what was happening locally in tissues.

Apart from the limitation regarding serum measurements, different dose groups could not be included in the study, since there is no study in the literature on ideal dose adjustment for CAPE. Studies with different doses, durations, and administration methods are needed within the limits of the study. Since our study is the first study investigating the antioxidant effect of CAPE on periodontal disease, there is a need for additional studies in which the effects on all periodontal tissues are examined and other host modulatory agents are compared, and dose-dependent/nondependent and systemic effects are monitored.

Conclusions

Host modulation therapies are an area of considerable interest in periodontal therapy. In conclusion, CAPE reduced oxidative stress in inflamed gingival tissue in this experimental periodontitis model, enabling the antioxidant therapeutic potential of CAPE in periodontitis. Modulation of host response by CAPE may represent an attractive strategy for the treatment of periodontal diseases and CAPE may be valuable as an alternative host modulating agent without any known side effects.

Funding The study was financially supported by a grant from the Ondokuz Mayıs University Research Foundation (PYO.DIS.1901.15.007).

Declarations

Ethical approval This article does not contain any studies with human participants performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent For this type of study, formal consent is not required.

Conflict of interest The authors declare no competing interests.

References

1. Page RC, Kornman KS (1997) The pathogenesis of human periodontitis: an introduction. Periodontol 2000 14:9–11
2. Canakçi CF, Cicek Y, Canakçi V (2005) Reactive oxygen species and human inflammatory periodontal diseases. Biochemistry (Mosc). 70:619–628
3. Chapple IL, Matthews JB (2007) The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontol 2000 43:160–232
4. Battino M, Bullon P, Wilson M, Newman H (1999) Oxidative injury and inflammatory periodontal diseases: the challenge of antioxidants to free radicals and reactive oxygen species. Crit Rev Oral Biol Med 10:458–476
5. Niki E (2010) Assessment of antioxidant capacity of natural products. Curr Pharm Biotechnol 8:801–809
6. Bai H, Liu R, Chen HL, Zhang W, Wang X, Zhang XD, Li WL, Hai CX (2014) Enhanced antioxidant effect of caffeic acid phenethyl ester and Trolox in combination against radiation induced-oxidative stress. Chem Biol Interact 207:7–15
7. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC (1998) Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. JAMA 280:1569–1575
8. Banskota AH, Tezuka Y, Kadota S (2001) Recent progress in pharmacological research of propolis. Phytother Res 15:561–571
9. Murtaza G, Sajjad A, Mehmood Z, Shah SH, Siddiqi AR (2015) Possible molecular targets for therapeutic applications of caffeic acid phenethyl ester in inflammation and cancer. J Food Drug Anal 23:11–18
10. Murtaza G, Karim S, Akram MR, Khan SA, Azhar S, Mumtaz A, Bin Asad MH (2014) Caffeic acid phenethyl ester and therapeutic potentials. Biomed Res Int 2014:145342
11. Gocer H, Gulcin I (2011) Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. Int J Food Sci Nutr 62:821–825
12. Hosnuter M, Gurel A, Babuccu O, Armutcu F, Kargi E, Isikdemir A (2004) The effect of CAPE on lipid peroxidation and nitric oxide levels in the plasma of rats following thermal injury. Burns 30:121–125
13. Wang T, Chen L, Wu W, Long Y, Wang R (2008) Potential cytoprotection: antioxidant defence by caffeic acid phenethyl ester against free radical induced damage of lipids, DNA, and proteins. Can J Physiol Pharmacol 86:279–287
14. Tolba MF, Omar HA, Azab SS, Khalifa AE, Abdel-Naim AB, Abdel-Rahman SZ (2016) Caffeic acid phenethyl ester: a review of its antioxidant activity, protective effects against ischemia-

reperfusion injury and drug adverse reactions. Crit Rev Food Sci Nutr 56:2183–2190
15. Sud'ina GF, Mirzoeva OK, Pushkareva MA, Korshunova GA, Sumbatyan NV, Varfolomeev SD (1993) Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. FEBS Lett 329:21–24
16. Choi E-Y, Choe S-H, Hyeon J-Y, Choi J-I, Choi IS, Kim S-J (2015) Effect of caffeic acid phenethyl ester on Prevotella intermedia lipopolysaccharide-induced production of proinflammatory mediators in murine macrophages. J Periodont Res 50:737–747
17. Li L, Sun W, Wu T, Lu R, Shi B (2017) Caffeic acid phenethyl ester attenuates lipopolysaccharide-stimulated proinflammatory responses in human gingival fibroblasts via NF-κB and PI3K/Akt signaling pathway. Eur J Pharmacol 794:61–68
18. Yiğit U, Kırzıoğlu FY, Uğuz AC, Nazıroğlu M, Özmen Ö (2017) Is caffeic acid phenethyl ester more protective than doxycycline in experimental periodontitis? Arch Oral Biol 81:61–68
19. Graves DT, Kang J, Andriankaja O, Wada K, Rossa C Jr (2012) Animal models to study host-bacteria interactions involved in periodontitis. Front Oral Biol 15:117–132
20. Dumitrescu AL, Abd-El-Aleem S, Morales-Aza B, Donaldson LF (2004) A model of periodontitis in the rat: effect of lipopolysaccharide on bone resorption, osteoclast activity, and local peptidergic innervation. J Clin Periodontol. 31:596–603. https://doi.org/10.1111/j.1600-051X.2004.00528.x
21. Fitzpatrick LR, Wang J, Le T (2001) Caffeic acid phenethyl ester, an inhibitor of nuclear factor-kappaB, attenuates bacterial peptidoglycan polysaccharide-induced colitis in rats. J Pharmacol Exp Ther 299:915–920
22. Larki-Harchegani A, Hemmati AA, Arzi A, Ghafurian-Boroojerdnia M, Shabib S, Zadkarami MR, Esmaeilzadeh S (2013) Evaluation of the effects of caffeic acid phenethyl ester on prostaglandin E2 and two key cytokines involved in bleomycin-induced pulmonary fibrosis. Iran J Basic Med Sci 16:850–857
23. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103–1111
24. Gürkan A, Emingil G, Nizam N, Doğanavşargil B, Sezak M, Kütükçüler N, Atilla G (2009) Therapeutic efficacy of vasoactive intestinal peptide in Escherichia coli lipopolysaccharide-induced experimental periodontitis in rats. J Periodontol 80:1655–1664
25. Liu Z, Liu Y, Song Y, Zhang X, Wang S, Wang Z (2014) Systemic oxidative stress biomarkers in chronic periodontitis: a meta-analysis. Dis Markers 2014:931083
26. Dede FÖ, Ozden FO, Avcı B (2013) 8-Hydroxy-deoxyguanosine levels in gingival crevicular fluid and saliva in patients with chronic periodontitis after initial periodontal treatment. J Periodontol 84:821–828
27. Kuropatnicki AK, Szliszka E, Krol W (2013) Historical aspects of propolis research in modern times. Evid Based Complement Alternat Med 2013:964149
28. Muniz FW, Nogueira SB, Mendes FL, Rösing CK, Moreira MM, de Andrade GM, Carvalho Rde S (2015) The impact of antioxidant agents complimentary to periodontal therapy on oxidative stress and periodontal outcomes: a systematic review. Arch Oral Biol 60:1203–1214
29. Lee Y, Shin DH, Kim JH, Hong S, Choi D, Kim YJ, Kwak MK, Jung Y (2010) Caffeic acid phenethyl ester mediated Nrf2 activation and Ikappa B kinase inhibition are involved in NFkappaB inhibitory effect: structural analysis for NFkappaB inhibition. Eur J Pharmacol 643:21–28
30. Herath TD, Darveau RP, Seneviratne CJ, Wang CY, Wang Y, Jin L (2013) Tetra- and penta-acylated lipid A structures of Porphyromonas gingivalis lipopolysaccharide differentially activate TLR4-mediated NF-κB signal transduction cascade and immuno-inflammatory response in human gingival fibroblasts. PLoS One 8:e58496
31. Kensler TW, Wakabayashi N, Biswal S (2007) Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu Rev Pharmacol Toxicol 47:89–116
32. Mitsuishi Y, Motohashi H, Yamamoto M (2012) The Keap1-Nrf2 system in cancers: stress response and anabolic metabolism. Front Oncol 2:200
33. Balogun E, Hoque M, Gong P, Killeen E, Green CJ, Foresti R, Alam J, Motterlini R (2003) Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element. Biochem J 371:887–895
34. Zhang M, An C, Gao Y, Leak RK, Chen J, Zhang F (2013) Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. Prog Neurobiol 100:30–47
35. Keum YS (2012) Regulation of Nrf2-mediated phase II detoxification and anti-oxidant genes. Biomol Ther (Seoul) 20:144–151
36. Stähli A, Maheen CU, Strauss FJ, Eick S, Sculean A, Gruber R (2019) Caffeic acid phenethyl ester protects against oxidative stress and dampens inflammation via heme oxygenase. Int J Oral Sci 11:6
37. Akalin FA, Baltacioglu E, Alver A, Karabulut E (2007) Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. J Clin Periodontol 34:558–565
38. Baltacioglu E, Yuva P, Aydin G, Alver A, Kahraman C, Karabulut E, Akalın FA (2014) Lipid peroxidation levels and total oxidant/antioxidant status in serum and saliva from patients with chronic and aggressive periodontitis. Oxidative stress index: a new biomarker for periodontal disease? J Periodontol 85:1432–1441
39. Chapple IL, Brock GR, Milward MR, Ling N, Matthews JB (2007) Compromised GCF total antioxidant capacity in periodontitis: cause or effect? J Clin Periodontol 34:103–110
40. Ahmadi-Motamayel F, Goodarzi MT, Jamshidi Z, Kebriaei R (2017) Evaluation of salivary and serum antioxidant and oxidative stress statuses in patients with chronic periodontitis: a case-control study. Front Physiol 8:189
41. Baltacioglu E, Kehribar MA, Yuva P, Alver A, Atagün OS, Karabulut E, Akalın FA (2014) Total oxidant status and bone resorption biomarkers in serum and gingival crevicular fluid of patients with periodontitis. J Periodontol 85:317–326
42. Okutan H, Ozcelik N, Yilmaz HR, Uz E (2005) Effects of caffeic acid phenethyl ester on lipid peroxidation and antioxidant enzymes in diabetic rat heart. Clin Biochem 38:191–196
43. Ozyurt H, Irmak MK, Akyol O, Söğüt S (2001) Caffeic acid phenethyl ester changes the indices of oxidative stress in serum of rats with renal ischaemia-reperfusion injury. Cell Biochem Funct 19:259–263
44. Yılmaz A, Elbey B, Yazgan ÜC, Dönder A, Arslan N, Arslan S, Alabalık U, Aslanhan H (2016) Protective effects of caffeic acid phenethyl ester on fluoxetine-induced hepatotoxicity: an experimental study. Biomed Res Int 2016:1247191
45. Yazgan ÜC, Elbey B, Kuş S, Baykal B, Keskin I, Yılmaz A, Şahin A (2017) Effect of caffeic acid phenethyl ester on oxidant and antioxidant status of liver and serum in a rat model with acute methanol intoxication. Ir J Med Sci 186:519–523
46. Buduneli E, Buduneli N, Vardar-Sengül S, Kardeşler L, Atilla G, Lappin D, Kinane DF (2005) Systemic low-dose doxycycline and alendronate administration and serum interleukin-1beta, osteocalcin, and C-reactive protein levels in rats. J Periodontol 76:1927–1933