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Comparison Effects of Serum Antioxidants Levels and Intensity of Inflammatory Cells in Experimental Periodontitis Treated with Low-Dose Doxycycline and Coriandrum Sativum L

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ABSTRACT

Aim: The aim of this study was to evaluate the effects of systemically-administered Coriandrum sativum L (CSL) and low dose doxycycline (LDD) on serum levels of antioxidant enzymes and intensity of inflammatory cells in rats with experimental periodontitis.

Material and Methods: Forty adult male Wistar Albino rats were divided randomly into 5 groups as follows: group 1: periodontally healthy (control); group 2: periodontitis; group 3: periodontitis+CSL (32mg/kg); group 4: periodontitis+CSL (200mg/kg); group 5: periodontitis+LDD (6 mg/kg). Serum gingival superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) levels were evaluated by ELISA. The intensity of inflammatory cells were evaluated by histopathologically.

Results: SOD levels were statistically lowest in group 1 and statistically were highest in group 2 than those of other groups. There was a statistical difference in SOD levels in paired comparisons of groups 1 and 2 with other groups. Its level did not have statistically significant among groups 3, 4 and 5. CAT levels were statistically lowest in group 2 than those of other groups, and no differences were reported among groups 1, 3, 4, and 5. GSH-Px levels did not have statistically significant among groups. Inflammatory cell infiltration was found to be statistically higher in groups 2 and 4 compared to group 1, and no statistical significance was reported among groups 1, 3, and 5.

Conclusion: CSL and LDD application groups did not show differences in terms of serum SOD, serum CAT, and intensity of inflammatory cells. Therefore, we suggest that the different dosages of CSL should be examined in the treatment of periodontitis.

Keywords: Periodontitis; Coriandrum Sativum L; antioxidant; low dose doxycycline; serum.

Düşük Doz Doksisiklin ve Kişniş ile Tedavi Edilen Deneysel Periodontitisde Serum Antioksidan Seviyelerinin ve İnflamatuar Hücre Yoğunluğunun Karşılaştırılması

ÖZ

Amaç: Bu çalışmanın amacı deneysel olarak periodontitis oluşturulmuş ratlarda sistemik olarak uygulanmış düşük doz doksisiklinin (LDD) ve kişniş bitkisinin (CSL) serumdaki antioksidan enzim seviyeleri ve inflamatuar hücre miktarı üzerine olan etkilerini değerlendirmektir.

Gereç ve Yöntemler: Çalışmanın başlangıcında 40 adet Wistar Albino erkek rat rastgele beş eşit gruba ayrıldı. Grup 1: periodontal sağlıklı; grup 2: periodontitis; grup 3: periodontitis + CSL (32 mg/kg); grup 4: periodontitis +CSL (200mg/kg); grup 5: periodontitis + doksisiklin hidroklorid (6 mg/kg). Elde edilen serum örneklerinde superoksit dismutanz (SOD), glutatyon peroksidaz (GSH-Px), katalaz (CAT) seviyeleri ELISA metodu uygulanarak analiz edildi. İnflamatuar hücre yoğunluğu histopatolojik olarak değerlendirildi.

Bulgular: Serumdaki SOD seviyesi diğer gruplar ile karşılaştırıldığında istatistiksel olarak en düşük grup 1 ve istatistiksel

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olarak en yüksek ise grup 2 de bulundu. SOD seviyesi grup 1 ve 2 nin diğer gruplarla olan ikili karşılaştırmalarında istatistiksel olarak önemli bulurken, grup 3, 4 ve 5 arasında istatistiksel olarak önemli bir fark göstermedi. CAT seviyesi diğer gruplar ile karşılaştırıldığında istatistiksel olarak en düşük grup 2'de bulunurken, grup 1, 3, 4 ve 5 arasında önemli bir farklılık göstermedi. GSH-Px seviyesi gruplar arasında istatistiksel olarak önemli farklılık göstermedi. İnflamatuar hücre yoğunluğu grup 2 ve 4 de grup 1'e göre istatistiksel olarak önemli derecede yüksek bulundu. Grup 1, 3 ve 5 arasında inflamatuar hücre yoğunluğu açısından istatistiksel olarak önemli farklılık rapor edilmedi.

Sonuç: Serum SOD, CAT ve inflamatuar hücre yoğunluğu bakımından CSL ve LDD uygulama grupları arasında farklılık gözlenmedi. Bu yüzden, periodontitis tedavisinde farklı CSL dozajlarının uygulandığı çalışmaların yapılması gerektiğini düşünmekteyiz.

Anahtar Kelimeler: Periodontitis; kişniş; antioksidan; düşük doz doksisiklin; serum.

INTRODUCTION

Periodontitis is an inflammatory disease that causes attachment loss and alveolar bone destruction caused by periodonto-pathogenic microorganisms and may result in tooth loss (1). Periodontitis is a preventable and treatable disease. The main purpose of periodontal treatment is to stop tissue destruction and to prevent the recurrence of the disease due to eliminating gingival inflammation (2). Treatment of periodontal disease is routinely based on mechanical debridement (scaling and root planning, SRP) of the tooth surface and appropriate maintenance of oral hygiene (3). However, since these treatment methods are not always sufficient for the complete elimination of periodontal microflora, various antimicrobial agents are used for host modification therapy (HMT) in addition to these treatments (4). Host modulatory therapy (HMT) is a therapy concept that aim to inhibit host-derived pro-inflammatory mediators, cytokines and proteolytic enzymes such as matrix metalloproteinases (MMPs) (5). To date, there is only subantimicrobial dose doxycycline (SDD) or low dose doxycycline (LDD) which is approved for systemically using as an HMT agent as an adjunct to the treatment of periodontal disease (6). It has been reported that tetracyclines have anticollagenase properties independent of their antibacterial effects and this effect is mostly found in doxycyclines, which is a semi-synthetic tetracycline derivative among tetracycline group drugs (7). In addition to the anti-metalloproteinase effects of these drugs, it has been reported that doxycyclines inhibit prostaglandin synthesis and the production of reactive oxygen metabolites (ROS), and they increase fibroblast activity when applied topically to tooth surfaces (7). In the presence of oxygen, phagocytic cells produce reactive oxygen species (ROS) by oxidative killing mechanisms. Production of ROS is a complementary feature of normal cellular metabolism , but these free radicals have a toxic effect on microorganisms (8). When ROS exceeds the antioxidant defense of the cells, they damage normal host cells and play a role in the pathogenesis of various diseases (8,9). In healthy organisms, the harmful effects of ROS on cells are protected by maintaining a balance between oxidants and antioxidants. The human body has a defense mechanism against ROS species with its antioxidants enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) (10). Some studies (10,11) reported that using of LDD administration decrease increased gingival and serum SOD, GSH-Px and CAT levels in rats with experimental periodontitis. Although LDD use as HMT agent as an adjunct to the treatment of periodontal disease, numerous studies have been conducted on the biological activities of plant extracts in the treatment of periodontal diseases in order to avoid the side effects caused by the systemic use of chemotherapeutics in excessive doses (3,10,12).

Polyphenols are plant metabolites. Phenolic compounds are used in antiseptics, disinfectants and mouthwashes due to their low toxicity and antibacterial properties. Polyphenols exhibit antibacterial activity against periodontal pathogens with increased antioxidant ability in oral fluids. Tannins are a family of dense protein-containing polyphenols. The use of tannins in the treatment of periodontal diseases has become widespread. Tannins precipitate microbial proteins and inhibit the growth of microorganisms (12).

Coriander (Coriandrum sativum L, CSL), a tannin-containing natural phytochemical, is an herb of the Umbelliferae Apiaceae family and it possesses significant nutritional and medicinal properties (13). The anti-microbial activity of the obtaining extracts and essential oils from leaves and seeds of CSL is the most reported biological activity of the plant. (14) In a study conducted with the application of seed extract of CSL in rats fed a high-fat diet; It has been reported that peroxidase levels, free fatty acid and glutathione levels decrease, as well as an increase in anti-oxidant enzyme activities (15). Tang et al. (13) reported that CSL has the potential to prevent diseases caused by oxidative stress. Briefly, CSL is said to have anti-inflammatory activity, analgesic effect, antibacterial activity and antioxidant activity (16,17).

To the best of our knowledge, no reports are published that have focused the effect of CSL on the serum anti-oxidant enzymes levels in the periodontitis. The authors hypothesized that CSL may exhibit antioxidant effects in the treatment of periodontal disease by scavenging radicals. The purpose of this study was to evaluate the effects of orally administered CSL on the repair process of periodontal defects in rats after experimental periodontitis induction by determination of serum SOD, GSH-Px, CAT levels and by counting the intensity of inflammatory cells in the connective tissue. These results compared with LDD, which is known as an effective adjunctive therapy in the treatment of periodontitis

MATERIAL AND METHODS

Forty adult Wistar male rats with an initial mean weight of 250 to 300 were used in this study. Each animal was inspected for the presence of clinical signs of inflammatory responses (edema, redness, bleeding, and fragility) at baseline, and all had healthy gingiva. Rats were placed in separate cages, fed a standard laboratory diet, and housed at a constant temperature (24 ±2ºC) in a 12-hour light/darkcycle. The experimental protocol of the present study is approved by the Ethics Committee on Animal Experimentation at Bülent Ecevit University, Zonguldak, Turkey (Protocol ID; 2014-15-07/05).

No sample size calculation could be performed before the study because there was no precise information available regarding CSL effects in experimental periodontitis. We, therefore, based our estimates on the pilot study, which included 5 rats in each group. The sample size was calculated based on the results of serum biochemical biomarkers levels between CSL applicated groups and their control groups. A sample size of 8 per group was required for detection of a significant difference (80% power, two-sided 5% significant level).

Study Design

The rats were randomly divided into five groups of 8 rats each: group 1= unligated control group, in which animals were fed standard chow and received no treatment; group 2= experimental periodontitis group, in which animals were fed powdered standard chow, and experimental periodontitis was induced; group 3= experimental periodontitis+32mg/kg CSL, in which animals were fed standard chow and gavaged once a day with 1ml distilled water containing CSL (32mg/kg) (16); by intragastric intubation for 14 days; group 4= experimental periodontitis+200 mg/kg CSL, in which animals were fed standard chow and gavaged once a day with 1ml distilled water containing CSL (200mg/kg) (18) by intragastric intubation for 14 days; group 5= experimental periodontitis+6 mg/kg LDD, in which animals were fed standard chow and gavaged once a day with 1ml distilled water containing LDD (6mg/kg) (10; 19; 20) by intragastric intubation for 14 days. The dosage and application form of drugs was calculated according to the method based on the literatures (10,16,18-20). Drug treatment began after periodontitis was induced.

Preparation of extracts

After the CSL seeds were purchased from spice shops, these seeds were ground into powder and dry weights of seeds were taken. The percolation method was used for extraction. Samples were shaken in 300 ml of 80% aqueous ethanol per 100 g for 24 hours and then it drained. Extraction was continued for another 24 hours with 80% aqueous ethanol by the percolation method. After 48 h, extracts were concentrated by rotary evaporator (Heidolph VV 2000) (18). The obtained extract was prepared freshly to be given to the rats by diluting with daily distilled water. The plant specimens were identified and obtained by a specialist (H. C) from the Department of Biology, Faculty of Sciences and Arts, Bülent Ecevit University, Zonguldak, Turkey.

Experimental Induction of Periodontitis

Experimental periodontitis was created in rats under systemic anesthesia by intraperitoneal administration of 75-100 mg/kg of ketamine-HCl and 10 mg/kg of xylazine HCL to the 32 rats, ligatures (3-0 silk suture) were placed around the cervix of left and right mandibulary first molars in all rats, except periodontally healthy control group (group 1). The presence of ligatures was checked periodically. On the 14th day following the placement of the sutures, the development of experimental periodontitis was observed in rats (21). All ligatures in experimental periodontitis groups were removed at 14th day following experimental periodontitis induction. Thirty-two rats with experimental periodontitis were randomly divided into 4 groups. Then, rats started receiving drugs once a daily for 14 days of by intragastric intubation

Sample Collection

At the end of 14th days of the therapy, all rats were anesthetized, and 5mL of venous blood was drained out through cardiac punctures for serum analyses. Blood samples were placed within centrifuges (Shimadzu UV160A, SNo:28006648, Kyoto, Japan) at 3000g within room temperatures for 10 minutes, enabling the collection of serum, which were then placed at −80°C before biochemical analysis.

Block biopsy samples, including the gingiva was removed from mandibulary molar regions. For histological analysis, the left mandibular molar regions were resected en block from each rat and were fixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) for 1 day.

Biochemical Analysis

The blood samples were centrifuged (Shimadzu UV160A, S.No: 28006648, Japan) at 3,000 rpm for 10 minutes and the serums were stored at - 80^{0}C. Prior to the study, the homogenates which were defrosted at room temperature were later centrifuged at +4°C for 5 minutes with 3,000 g (Sigma 3K30 , S.No: 76262, Germany). The supernatants were taken for biochemical analysis. The concentrations of SOD in the serum/homogenate were measured using commercially available SunRed Rat SOD Enzyme-linked Immunosorbent Assay (ELISA) kits (SunRed Biological Technology Co. Ltd., Cat No. 201-11-0169, Shanghai, China). The enzymatic reactions were quantified in an automatic microplate photometer. The serum SOD levels were expressed as ng/mL. The sensitivity of the SOD kit was 0,415 ng/mL and the range of the assay was 0,5-100 ng/mL.

The concentrations of CAT in the serum were measured using commercially available SunRed Rat CAT ELISA kits (SunRed Biological Technology Co.Ltd.,Cat No. 201-11-5106, Shanghai, China). The enzymatic reactions were quantified in an automatic microplate photometer. The serum CAT levels were expressed as ng/mL. The sensitivity of the CAT kit was 0,866 ng/mL and the range of the assay was 1-300 ng/mL.

The concentrations of GSH-Px in the serum were measured using commercially available SunRed Rat GSH-Px ELISA kits (SunRed Biological Technology Co.Ltd., Cat No. 201-11-5104, Shanghai, China). The serum GSH-Px levels were expressed as ng/mL. The sensitivity of GSH-Px kit was 0,735 ng/mL and the range of the assay was 0,8-200 ng/mL.

All assays were conducted according to the manufacturer's instructions. The samples, which have shown higher concentrations, were diluted and measured in duplicate.

HistologicAnalysis

The mandible with detached gingiva were fixed in 10% formalin solution. The 3 samples taken were decalcified with 8% formic acid (14 days) and embedded in paraffin. Serial paraffin sections (5µm) were taken on the mesio-distal surface of the mandibular first molar tooth. Three sections presenting the central area of each tooth were stained with hematoxylin and eosin (H&E). Histologic analyzes were evaluated under a light microscope (BX50 research microscope, Olympus, Tokyo, Japan) coupled to a digital camera (DP26 Digital Camera, Olympus, Tokyo, Japan) and analyzed with the OLYMPUS DP2-BSW application software by a calibrated examiner with no prior information of study design.

The intensity of inflammation was assessed by counting inflammatory cells in the connective tissue at magnification 400. Inflammatory cells were counted in five randomly selected high-magnification areas on H&E sections, and the intensity of inflammation was scored using a four-grade system; 0= no inflammation, 1= Less than 15 inflammatory cells per area (<15 inflammatory cells); 2= 15-50 inflammatory cells per area; and 3= > 50 or more inflammatory cells per field (19).

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 19 program (SPSS Inc., version 19.0, Chicago, IL, USA). The Shapiro–Wilk test was used to determine whether the data were normally distributed. The Kruskal – Wallis non-parametric test was used in the analysis of biochemical parameters and intensity of inflammatory cells after the normality of the data had failed. Pairwise comparisons of parameters with statistically significant differences between groups as a result of the test were performed using the Bonferroni-adjusted ($\alpha = 0.05 / 10 = 0.005$) Mann-Whitney U test. Spearman coefficient correlation test was used to identify the link between amount of serum antioxidants enzymes and intensity of inflammatory cells. The results were calculated as median (25-75th percentile) for the parameters. $p<0.05$ was considered to be statistically significant.

RESULTS

Experimental procedures were performed successfully in all groups. CSL was well tolerated by rats, no complications were observed.

Table 1. Serum levels of SOD, CAT, GSH-Px, and intensity of inflammatory cells

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	*p- value* (Kruskal Wallis/ Mann- Whitney U)
Serum SOD	2.07 (1.65-3.00)	17.64 (16.75-19.83)	10.61 (8.98-13.02)	11.84 (11.84-14.18)	12.76 (10.74-14.74)	**<0.001/ G1 vs G2 *p=0.002*** **G1 vs G3 *p=0.004*** **G1 vs G4 *p=0.002*** **G1 vs G5 *p=0.001*** **G2 vs G3 *p=0.001*** **G2 vs G4 *p=0.001*** **G2 vs G5 *p=0.001*** G3 vs G4 p=0.141 G3 vs G5 p=0.248 G4 vs G5 p=1.000
Serum CAT	39.70 (38.29-40.93)	27.24 (24.62-30.56)	40.75 (37.19-42.80)	41.98 (37.27-46.19)	43.61 (36.26-52.02)	0.007/ **G1 vs G2 *p=0.004*** G1 vs G3 p=0.366 G1 vs G4 p=0.197 G1 vs G5 p=0.126 **G2 vs G3 *p=0.003*** **G2 vs G4 *p=0.001*** **G2 vs G5 *p=0.002*** G3 vs G4 p=0.600 G3 vs G5 p=0.336 G4 vs G5 p=0.470
Serum GSH-Px	28.28 (24.70- 32.63)	28.73 (27.57- 33.53)	39.83 (25.57-32.99)	28.78 (25.66-31.40)	27.18 (25.35-32.65)	0.856/ G1 vs G2 p=0.439 G1 vs G3 p=0.699 G1 vs G4 p=1.000 G1 vs G5 p= 1.000 G2 vs G3 p=0.916 G2 vs G4 p=0.563 G2 vs G5 p=0.247 G3 vs G4 p=0.529 G3 vs G5 p=0.500 G4 vs G5 p=0.847
Inflammatory cells	1.00 (1-1.25)	2.50 (2.00-3.00)	2.00 (2.00-3.00)	3.00 (2.00-3.00)	2.00 (1.00-2.00)	**0.002/ G1 vs G2 *p=0.003*** G1 vs G3 p=0.093 **G1 vs G4 *p=0.004*** G1 vs G5 p=0.005 G2 vs G3 p=0.030 G2 vs G4 p=0.575 G2 vs G5 p=0.575 G3 vs G4 p=0.056 G3 vs G5 p=0.014 G4 vs G5 p=0.269

Group 1 (G1): periodontally healthy control, Group 2 (G2): Experimental periodontitis (no treatment), Group 3 (G3): 32mg/kg CSL administration group, Group 4 (G4): 200mg/kg administration group, Group 5 (G5): LDD (6mg/kg) administration group. Superoxide dismutase: SOD, Glutathione peroxidase: GSH-Px, Catalase: CAT. Statistically significant difference (Kruskal Wallis, Mann- Whitney U nonparametric test with Bonferroni correction, p<0.005)

Biochemical Findings

SOD levels of serum was statistically lowest in the group 1 compared group 2, 3, 4 and 5 (p= 0.002, p=0.004, p=

0.002, p=0.001, respectively), and statistically highest in the group 2 than groups 3, 4 and 5 (p= 0.001, p=0.001, p=0.001, respectively) (Table 1). No statistically differences were observed among the groups 3, 4 and 5 (p=0.141, p=0.248, p=1.000) (Table 1). The serum CAT level was statistically lower in the group 2 than group 1 (p=0.004) (Table 1). Its levels were statistically increased in the group 3, 4 and 5 compared group 2 (p= 0.003, p=0.002, p=0.001, respectively) (Table 1). There were no statistically differences among the groups 1, 3, 4 and 5 in terms of serum CAT (p=0.366, p=0.197, p=0.126, p=0.600, p=0.336, p=0.470) (Table 1). There was no statistically difference among the groups in terms of serum GSH-Px levels (p= 0.439, p=0.699, p=1.000, p=0.001, p=0.916, p=0.563, p=0.247, p=0.529, p=0.500, p=0.847) (Table 1).

Histologic Findings

Inflammatory cell infiltration was found to be statistically significantly higher in group 2 and 4 compared to group 1 (p=0.003, p=0.004, respectively) (Table 1). No statistically significance was reported between in the group 2 and the group 4 (p=0.575) (Table 1). The decrease was observed in terms of intensity of inflammatory cells in the group 3 and 5 compared to groups 2 and 4, but this decrease did not statistically significance (p=0.030, p=0.575, p=0.056, p=0.269) (Table 1). Additionally, there were no difference among the group 1, 3 and 5 (p=0.093, p=0.005, p=0.014) (Table 1).

Correlation Analysis

Correlation coefficients are presented in Table 2. A statistically significance positive correlation was discovered between the serum SOD levels and the intensity of inflammatory cells when every group was evaluated at the same time (p< 0.001).

Table 2. Spearman's rank correlation (r) among groups with respect to serum levels of SOD, CAT and GSH-Px and the intensity of inflammatory cells

Groups	SOD and I. Cells	CAT and I. Cells	GSH-Px and I. Cells
Group 1			
r	0.393	-0.131	-0.131
p	0.441	0.801	0.801
Group 2			
r	-0.293	0.488	-0.488
p	0.573	0.326	0.326
Group 3			
r	0.000	0.000	0.414
p	1.000	1.000	0.414
Group 4			
r	-0.207	-0.207	0.000
p	0.694	0.694	1.000
Group 5			
r	0.621	-0.207	0.207
p	0.188	0.694	0.694
ALL			
r	0.620	-0.132	0.258
p	**<0.001**	0.486	0.168

Group 1: periodontally healthy control, Group 2: Experimental periodontitis (no treatment), Group 3: 32mg/kg CSL administration group, Group 4: 200mg/kg administration group, Group 5: LDD (6mg/kg) administration group. Superoxide dismutase: SOD, Glutathione peroxidase: GSH-Px, Catalase: CAT, I. Cells; Intensity of inflammatory cells

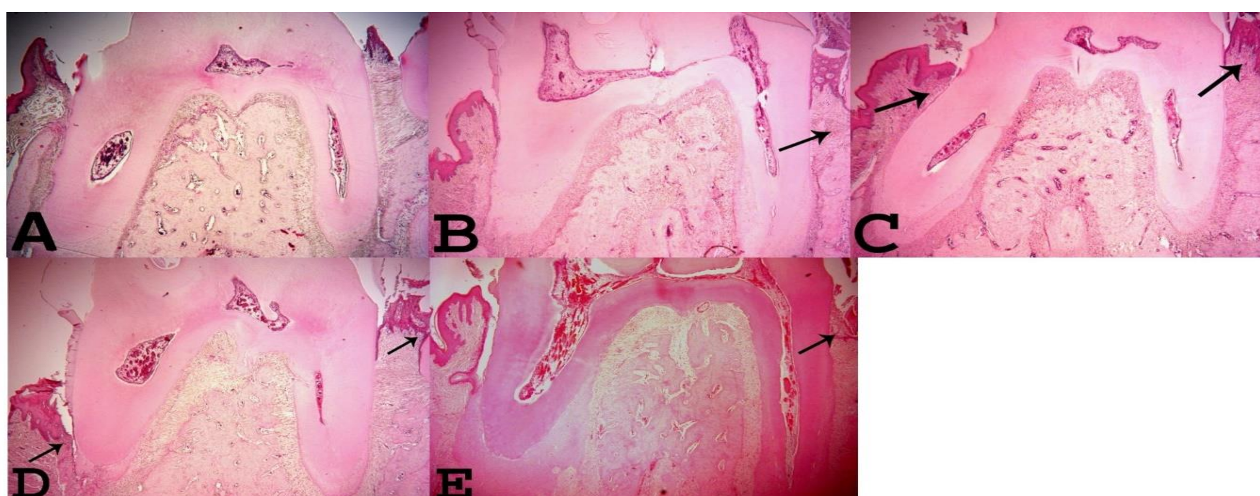

Figure 1. The intensity of inflammatory cells (arrows) for histopathologically evaluation. A) Group 1, B) Group 2, C) Group 3, D) Group 4, E) Group 5, (H&E, x4).

DISCUSSION

Many medicinal plants and their products are also used for the treatment and prevention of oral infections. Polyphenols are plant metabolites that exhibit in vitro antibacterial activity against periodontal pathogens and increase the anti-oxidant ability of oral fluids and also reduce inflammation (22). Polyphenolic classifications are phenolic acids such as hydrolyzable tannins, lignans, flavonoids. Tannins are free radical scavengers and have antioxidant activity.Tannins have been reported to inhibit inflammatory markers through oxidation of tannin and reduction of free radicals (12).

CSL extracts contain tannin, and the total phenolic content was found to be 30.77mg GAE/g according to the Folin-Ciocalteu method (22). All parts of this plant (seed, fruit, leaves) are used by people in different countries for alternative medical treatment. An in-vitro study (3), eight different gel forms of Coriandrum were obtained. When they were compared to doxycycline, some forms were good in terms of physical appearance, homogeneity and resistance, and the mucoadhesion and viscosity of the gel were high and the drug release took place in a slow time. Additionally, it was reported that this gel (F8) form significantly inhibited the development of *Porphyromonas gingivalis* (3). Due to the effects of CSL, which is reported to have analgesic, anti-inflammatory, antibacterial and antioxidant activities in the literature, (3,12,23) our study aimed to evaluate serum antioxidant levels and the intensity of inflammatory cells in the connective tissue of gingiva after systemic CSL application in rats with experimental periodontitis by comparing them with LDD application.

When we examine the rats from a periodontal point of view; It has been stated that periodontal tissues in the molar region are very similar to the human periodontium in terms of oral gingival epithelium, oral sulcular epithelium, junctional epithelium, periodontal collagen fibrils, cellular-acellular cementum and alveolar bone. The only difference is that the gingival sulcular epithelium is keratinized in rats (24). Therefore, we planned to perform our study on experimental periodontitis models in rats. Periodontal bone loss occurs quite rapidly in rats and as reported in previous studies, alveolar bone loss increases significantly 3 days after the experimental periodontitis model is created with ligature and it reaches a maximum level on the 7th day (25-27). In our study, male rats were used to keep hormonal changes constant and 3.0 silk sutures were placed in the cervical regions of the first molars to create an experimental periodontitis model. After 14 days, clinical signs such as edema, redness and bleeding in the gingival tissues and teeth mobility were observed due to experimental periodontitis formation with ligature.

When the literature was reviewed, it is found that LDD can inhibit bone resorption in rats and it can provide significant inhibition of major inflammatory mediators that play a role in periodontal tissue destruction (28-31). Therefore, we planned to determine the effects of CSL extracts on periodontal tissues by comparing them with LDD.

Consumption of plants with antioxidant properties as food strengthens the antioxidant balance of the body (13,32). Therefore, the use of antioxidants may affect the development of periodontal disease. In a study conducted with the application of seed extract of CSL in rats fed a high-fat diet; It has been reported that peroxidase levels, free fatty acid and glutathione levels decreased, as well as an increase was observed in anti-oxidant enzyme activities (15). Tang et al. (13) investigated the antioxidant activity of CSL in protection against DNA damage and cancer cell migration. They reported that CSL has the potential to prevent diseases caused by oxidative stress (13). Anti-oxidant enzymes such as SOD, GSH-Px and CAT are major components of the antioxidant system that detoxify free radicals. Especially, SOD is a free radical scavenger (18). Therefore, we aimed to evaluate the levels of serum anti-oxidant enzymes such as SOD, GSH-Px and CAT in order to determine the effect of CSL on the repair process of periodontal defects in rats with experimental periodontitis.

Aslani et al. reported that antioxidant activities of saliva and pocket fluid decrease in periodontitis (3). Oktay et al. (11) found that serum SOD, CAT, and GSH-Px levels increased in rats infected with periodontal pathogen compared to the control group and also, they determined that serum SOD, CAT and GSH-Px levels statistically decreased in doxycycline-treated group compared to the untreated group. Similarly, Yağan et al. demonstrated that gingival SOD, CAT and GSH-Px levels were significantly higher in the experimental periodontitis group and their levels significantly decreased after the LDD treatment (10). In our study, LDD and CSL were applied separately to rats with experimental periodontitis and, similar to the above studies, the serum levels of SOD were higher in the untreated experimental periodontitis group (group 2) than in the other groups. There are conflicting results regarding SOD levels in periodontal tissues. Some studies reported that SOD activities were high (33, 34), on the other-hand the other studies reported decreased SOD activity in periodontitis (35,36). The present study showed that serum SOD levels decreased after the application of CSL and LDD, and no differences were reported among the CSL and LDD application groups. Based on this result, we can say that CSL application may reflect decreased oxidative stress by causing a decrease in SOD levels. Similarly, there are conflicting results for GSH-Px levels in periodontitis patients, some of them were found increased GSH-Px levels (34), some of them were found decreased level (37) and the other studies reported no difference (38). In our study, no difference was observed among the groups in term of serum GSH-Px levels. Ellis et al. (39) found significantly lower CAT levels in than periodontitis group. Similarly, this study found the lowest serum CAT level in the untreated experimental periodontitis group than the other groups and no differences were found among the treated experimental periodontitis groups. The reason for the differences between these studies may be the evaluation of antioxidant enzyme levels in different tissues and fluids.

Bezerra et al. (26) reported that mononuclear cell influx decreased after LDD application to the experimentat periodontitis rats. The present study evaluated the intensity of inflammatory cells. Similarly to Bezerras' study, the intensity of inflammatory cells were decreased after LLD and 32mg/kg CSL applications compared to untreated experimental periodontitis group, especially in LLD group, but no significant difference was reported among

the experimental periodontitis groups. Other hand, its levels were statistically higher in the untreated experimental periodontitis group and 200mg/kg CSL group than in the non-periodontitis control group.

Fulbel et al. (23) reported that significantly better improvement clinical and microbiological parameters were observed in the treatment group with an herbal gel containing extracts of Coriandrum sativum +SRP compared to the treatment group with SRP alone. On the other hand, Yagnihini et al. (12) found that herbal gel containing extracts of *Quercus brantii* and *Coriandrum sativum* did not have considerable advantages over SRP alone as an adjunct in periodontal treatment. Since these two studies are different from our study in terms of materials and methods, it will not be possible to make a comparison with these studies. When we evaluated the results of our study, it suggest that CSL administration has some positive effects on the serum SOD levels and 32mg/kg CSL application showed a decrease in terms of intensity of inflammatory cells compared to untreated experimental periodontitis group. Therefore, 32 mg/kg CSL dose may be used as a drug to modulate host response in periodontitis, such as LDD. However, since the effective and non-toxic dose of CSL in the treatment of periodontal diseases is not yet known, two different doses, the efficacy of which were known in different studies, were applied in this study. This is the potential limitation of the our study. The present study is the first to investigate the effectiveness of CSL in an experimental periodontitis model. To understand the role of CSL in periodontitis as a treatment strategy, various dose ranges should be used and additional biomarkers (matrix metalloproteinases (MMPs), anti-inflammatory markers) should be analyzed and histological examines should be performed.

CONCLUSIONS

After the application of CSL extracts and LDD, serum SOD levels were statistically decreased in all applications groups (group 3, 4 and 5) compared to untreated experimental periodontitis groups. Serum CAT levels were statistically increased in all applicatios groups compared to untreated experimental periodontitis groups. On the other hand, the intensity of inflammatory cells were found higher in the untreated experimental periodontitis group and 200 mg/kg CSL application group compared to control group. But, no statistically differences was reported among the all applications groups in terms of serum SOD, serum CAT and intensity of inflammatory cells levels. However, the present study can not say that CSL can be applied instead of LDD as an adjunct in periodontal treatment, but we can suggest that further studies should be conducted by comparing different dosages in the treatment of periodontitis.

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