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Preventive effects of *Capparis Spinose* extract on experimental periodontitis in rats: a histopathological and biochemical study

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ABSTRACT

This study aimed to assess the effectiveness of Capparis Spinose (CS) in preventing the initiation and progression of experimental periodontitis and to evaluate the effect of its on systemic oxidative stress in rats by experimental periodontitis model. Twenty-four male rats were equally divided into; Ligatured (L), non-ligatured (NL), and Ligatured with CS (11 days/day per 20 mg/kg) (LC) groups. Experimental periodontitis was induced with the silk suture technic. Alveolar bone loss was examined, and total antioxidant capacity(TAOC), total oxidant status(TOS), and oxidative stress index(OSI) were analyzed in rat serum. Although; alveolar bone loss showed statistically significant lower values in the LC group compared to L ($p < 0.05$), not NL. In the LC group, osteoclast and osteoblast numbers were statistically significant compared to L, but there were no statistical differences between LC and NL. Serum TAOC levels were significantly lower in group L compared to others and also LC group showed significant differences from NL. TOS and OSI levels were significantly higher in group L than in other groups. Within the limitation of the present study, it can be said that the destruction via local inflammation that may occur after the experimental periodontitis can be prevented by using CS.

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Introduction

Periodontal disease is an infectious and inflammatory condition that develops after a specific and intricate interaction between pathogenic bacteria and the host tissue with accompanying signs of inflammation, such as loss of connective tissue, teeth, and supportive tissues. Microbial biofilm is one of the primary factors in the etiology of periodontal diseases. The primary source of tissue destruction is the gram-negative anaerobic and/or facultative bacteria in the dental biofilm and the enzymes that are secreted by the host defense system in the struggle against these bacteria (1).

The main objective in the treatment of periodontal diseases is primarily the removal of etiological factors. Accordingly, the first step in the treatment of periodontal diseases is non-surgical periodontal treatment (2). Non-surgical periodontal treatment involves removing bacterial biofilm and bacterial by-products from the environment and eliminating the roughness and irregularities on the root surface. After that, positive changes in the periodontal pocket depth occurred, and it was providing a suitable environment for the patient to perform an effective plaque control (3). Although non-surgical periodontal treatments are the golden standard in the treatment of periodontal diseases, host modulation, anti-inflammatory therapy (such as resolvins and host modulator drugs, etc.) or sub-antimicrobial antibiotics have emerged in the treatment or prevention of periodontal diseases in the light of developing technologies and new research (4). The underlying logic behind such treatments is the suppression of direct or indirect destruction mechanisms in periodontal diseases or increasing the efficiency of periodontal treatments (5).

In this context, synthetic materials are used, as well as alternative medicinal products that are known for their effectiveness in promoting bone regeneration (6). One of them is Capparis spinose (CS). *Capparis* is one of the most abundant two genera of the *Capparidaceae* family and is represented by 350 species in the world. 65 mg *flavonoid glycoside* and 40 mg *quercetin* can be obtained from 10 g CS. *Quercetin*, one of the flavonoids contained in CS, has been shown to have a positive effect on new bone formation in current research (7). The anti-inflammatory properties of *CS* and *C. decidua* extracts have been proven in several studies. It has also been shown that *C. decidua* has antipyretic properties, but neither has analgesic properties (8,9). New studies have also shown *Capparis zeylanica's* analgesic and antipyretic properties (10,11)as well as *C. spinosa's* anti-allergic properties (12).

The antioxidant mechanism functions by counteracting the harmful effects of free radicals within cells. Free radicals serve as intermediates in the activation of osteoclasts, which are essential for bone resorption. These radicals can stimulate the differentiation of osteoclast precursors and enhance osteoclast activity, resulting in bone resorption. Capparis spinosa (CS), with its antioxidant properties, has the potential to significantly contribute to expediting bone regeneration and shortening the time required for consoli-

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dation(13).

In light of this information, this animal study intends to investigate the histopathologic effect of CS on the experimental periodontitis lesion, based on the hypothesis that a specific dose of CS may stop the bone destruction process.

Materials and Methods

The study was approved by the Cumhuriyet University Faculty of Medicine Animal Ethics Committee (B.30.2.CUM.0.01.00.00-50/101). Twenty-four male Wistar rats with average body weight (275-300 g) were randomly and equally divided into three groups: non-ligatured (NL), Ligatured (L), and Ligatured with C.Spinosa (11 days/day per 20 mg/kg) (LC). Rats were kept at room temperature was maintained at about 25 ° C in 12 light / dark cycles.

Experimental periodontitis Model in Rats

Rats were treated with general anesthesia using Ketamine (40 mg/kg) before producing experimental periodontitis. After anesthesia, 4/0 silk sutures were performed by a single clinician (H.O.) in the subgingival position around the mandibular first molar at the right. Animals of all groups were kept in single cages by maintaining their daily food (ad libitum) and water requirements except the test group (LC). Rats in the LC group were administered 20 mg of CS daily at the dose stated (H.O.). After 11th day of the experiment, the rats were sacrificed. Moreover, all mandible of rats were cleaned from the muscles and soft tissues. The jaws were divided into two parts by the middle. The examined side of the mandible was stored under appropriate conditions for histopathological examination.

Preparation and administration of Capparis Spinosa extract

The reconstituted extract was prepared by boiling method according to traditional methods used in Gaziantep, Turkey. Accordingly, 1 g (dried and powdered) fruit (Cappari) with 100 ml of distilled water was boiled for 10 minutes and cooled for 15 minutes. The existing extracts were filtered by using filters (Millipore 0.2 mm, France). The filtrate was freeze-dried, and the powder was dissolved in 1.5 cc of water and administered via gastric feeding to the rats.

Measurement alveolar bone loss

Rat jaws examined were stained with aqueous methylene blue (Merck, Rahway, NJ; 1%) to determine cemento-enamel junction (CEJ) by a single examiner (H.O.). Alveolar bone loss was determined as the distance from CEJ to alveolar bone crest, this distance was measured at three points on the buccal and lingual sides by using a stereomicroscope (Leica Microsystems, Wetzlar, Germany) average measure used for each specimen.

Biochemical analysis of serum oxidative stress markers analyses

Total antioxidant capacity(TAOC) and total oxidant status TOS levels in serum were analyzed by using an automated colorimetric method using a commercially available kit (Rel Assay Diagnostics, Gaziantep, Turkey) according to the manufacturer's instructions and Erel's technique (14). TAOC and TOS levels were expressed in mmol Trolox Equiv/L, and in µmol H2O2 Equiv/L, respectively. OSI levels were the ratio of TOS to TAOC, it examined by the formula in:

OSI =[TOS (µmol H2O2 Equiv./L) / TAOC (mmolTroloxEquiv./L)](15).

Histopathologic evaluation

Histopathological evaluations were performed by a single person (D. Ş. İ.) who did not have a command of clinical data and treatment regimens. The right mandible obtained from the animals was fixed in a 10% neutral buffered formalin. Afterward, it was decalcified in a 10% formic acid solution until the bone tissue softened. After the specimens were dehydrated with alcohol and put in paraffin blocks, approximately 6-micron cross-sections were taken from the blocks and stained with hematoxylin-eosin and examined with a light microscope (Eclipse E600, Nikon, Tokyo, JAPAN).

The scoring for Inflammatory cell infiltration (ICI) of periodontium was configured to yield the value of [0] if they were not visible; the value of [1] if they were slightly visible and the value of [2] if they were intensely visible.

The presence of osteoblastic ("surface-forming") activity is determined by the visibility of active bone formation surfaces delimited by osteoid and cuboidal osteoblasts. The visibility of osteoblastic activity was interpreted as indicating its presence. Osteoclasts were calculated according to their morphology.

Statistical analysis

All data except ICI and osteoblastic activity were analyzed by a software program (SPSS v24.0, SPSS Inc., Chicago, IL, USA). The rates of ICI and osteoblastic activity were not compared by x^2 test between groups because of a low number of samples. TAOC, TOS, OSI levels and osteoclast, osteoblast counts and alveolar bone loss were analyzed using the Kruskal Wallis test. $p < 0.05$ was considered statistically significant. The results were presented as the standard deviation mean of the average value.

Results

All rats showed no signs of disease during the experimental period and no critical changes in body weight (290-330 g) occurred. The presence of silk suture around the mandibular first molar tooth caused a clear alveolar loss of bone in the periodontal tissue. In addition, suturing has remained in existence until the end of the study.

In terms of alveolar bone loss; Although L group showed a statistically significant lower value compared to NL ($P<0.05$), the LC group was less than the NL group, but the differences were not statistically significant ($p >0.05$) (Table 1 and Figure 1). For ICI value; while L showed no

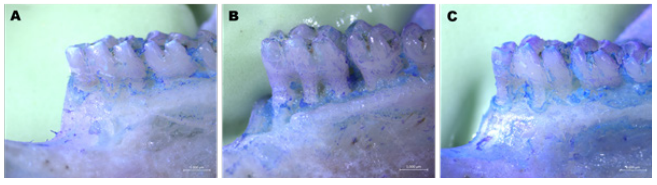

Figure 1. Representative photographs of the alveolar bone loss in the mandibular first molar tooth in the NL (A), L (B), LC (C) groups.

Table 1. Alveolar bone loss, osteoclast and osteoblast numbers of groups.

	NL	L	LC	P value
Alveolar Bone Loss mm	0,58±0,07	1,86±0,14*	0,48±0,05†	* 0,033 † 0,000
Osteoclast Number	10,38±1,92	32,63±7,11*	9,5±1,6†	* 0,006 † 0,001
Osteoblast Number	62,75±7,04	31,88±5,43*	71,75±8,13†	* 0,019 † 0,000

* significant difference from NL ($p < 0.05$). † significant difference from L ($p < 0.05$).

Table 2. Total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) of groups.

	NL	L	LC	P value
TAS (mmol trolox equiv./l)	1,03±0,16	0,35±0,09*	0,79±0,16†	* 0,000 † 0,027
TOS (lmol h2o2 equiv./l)	0,28±0,12	3,17±1,72*	1,55±2,05	* 0,000
OSI	0,28±0,14	8,79±4,5*	2,26±3,47	* 0,000

* significant difference from NL ($p < 0.05$). † significant difference from L ($p < 0.05$).

score 0, NL showed no score 2, and also except for one specimen all specimens showed a score of 1 (4 samples) and a score of 2 (3 samples) in LC. (Figure 2)

The histopathological examination has shown that particularly in the LC group, the structure of de-novo bone formation has been seen (Figure 3). Although the ligature placed in the LC, the count of osteoclast was similar to the NL group (Table 1), only L showed statistically significant differences from NL. Table 2 shows the count of osteoclast and osteoblast. L groups showed a statistically significant increased number of osteoclasts compared to NL ($P<0.05$) (Table 1). Regarding the osteoblastic activity score, the count of score 2 was higher in the LC group compared to others (Figure 4).

In terms of TAOC, all groups showed statistically significant differences; while L showed the lowest value, NL showed the highest ($p < 0.05$). There were no differences between NL and LC groups in terms of TOS and OSI ($p > 0.05$), only L showed a statistically significant increased value compared to NL and LC ($p< 0.05$) (Table 2).

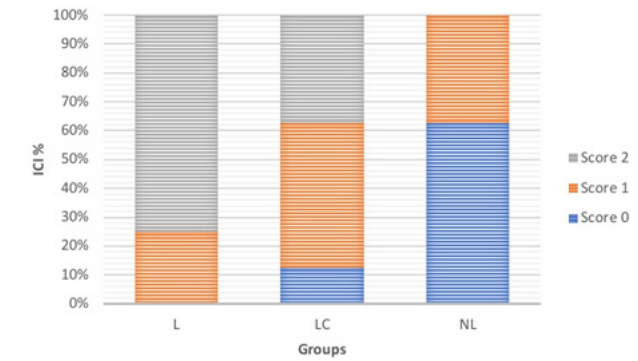

Figure 2. Distribution graphic of ICI score in groups.

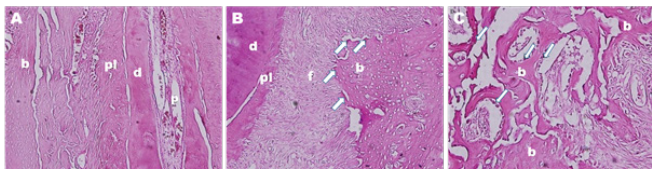

Figure 3. Histopathology in mandibular first molar tooth in all groups. A) Normal mandibula, showing alveolar bone (b), periodontal ligament (pl), pulpa (p), and dentin (d). B) Mandibula after 11 days of periodontitis; mainly alveolar bone with numerous osteoclasts ,and fibrozis (f) (arrows). C) Mandibula after 11 days of periodontitis treated with C. Spinosa (20 mg/kg), showing induced in osteoblast number, respectively. (Hematoxylin and eosin; original magnification: A, ·25; B, ·25; C, ·50).

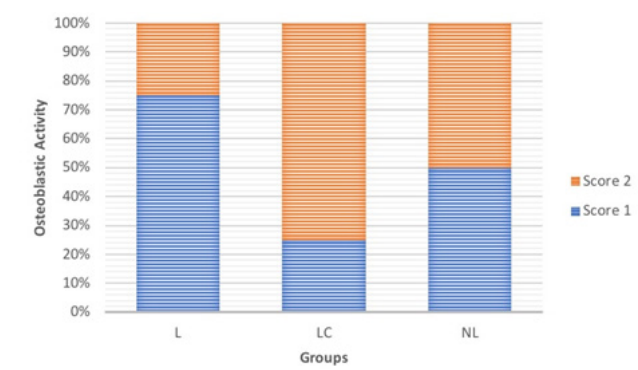

Figure 4. Distribution graphic of Osteoblastic activity score in groups.

Discussion

In the present study, the effect of Capparis Spinose on experimental periodontitis was investigated. According to the data obtained from the study, CS decreases alveolar bone loss. Regarding bone-related changes, it has been shown that bone loss is suppressed, osteoclastic activity decreased, and osteoblastic activity increases. In this context, the data from our experimental study support the idea that CS acts as an anti-inflammatory agent in preventing alveolar bone loss due to periodontal inflammation.

In many previous experimental studies, ligatures were used in rats to create periodontitis due to the formation of a plaque retention area (16). Tissue destruction in the periodontitis model with the ligatures follows an acute process, unlike chronic periodontitis (17). Furthermore, the process of creating experimental periodontitis with ligatures should not exceed 15 days, as following the placement of the ligatures, there may appear migration in the teeth in the occlusal, buccal and distal directions. Otherwise, destruction severity can be significantly reduced (18).

In our study, experimental periodontitis was obtained

by attaching ligatures to the mandibular molar teeth of rats and allowing them to stay there for 11 days. Previous studies also report the most intense bone loss occurring on days 7-11(19). This current study has also found that the use of ligatures in rats increases alveolar bone destruction around the tooth for 11 days.

The positive effect of antioxidants on new bone formation is already known. The *C.spinosa* agent used in our study, which originates from a family of antioxidants, is made up of different active chemical components. Its buds have glucosinolates and polyphenols such as lipids, alkaloids, and glucocapperin, as well as flavonoids with antioxidant properties. The most important of these are flavonoids. Plants contain higher amounts of it when compared to other components. Flavonoids have very different biological effects. Some of these are anti-inflammatory, antiallergic, antiviral, and anticarcinogenic. Flavonoids have antioxidant and metal-binding properties (8-10,19,20). The antioxidant effect of flavonoids was documented about forty years ago. Flavonoids are polyphenols, and they acquire their antioxidant properties through a separation of the hydrogen atom from the hydroxyl substituents. One of the flavonoids contained in CS, *quercetin* has been shown to promote bone production (8,11,21). The review of the literature published so far has revealed that there is no experimental or clinical study published until today on the effect of *C. Spinosa* on bone healing. Therefore, the application program was tried for the first time in this study. Because there are only a limited number of experimental studies regarding the dose to be administered.

There are many different results in the studies evaluating the TOS/TOS and OSI levels of periodontitis and healthy sites. Sezer et al. showed no statistically significant differences between the healthy side and the periodontitis side (15). Another experimental study to evaluate the oxidative stress markers in the rat by Kose et al. showed that; experimental periodontitis affects the serum TAOC, TOS and OSI levels (13) and also a new meta-analysis related oxidative stress markers in patients with periodontitis, emphasized that the markers are high in patients with periodontitis (22). According to the results of the present study, the oxidative stress markers are reduced by the application of *C.spinosa*, and It can be said that this antioxidative effect is an essential feature for clinical studies in the future, as reported in other reports on other medicinal plants (23-32)

In a study conducted by Eddauks et al. showed the hypoglycemic effects of CS on the diabetics rats (21). Streptozocin, 20 mg/kg of CSE, was orally administered to the rats for 14 days, and it was found that blood glucose returned to normal without a change to the baseline insulin values. Another study by Germano et al. (9) showed, the antioxidant properties of *C.spinosa* originated from its phenol content, and the antioxidant effect was maintained by the exclusion of glucosinolates. In a study conducted by Feng et al. (8) using an extract obtained from the leaves of CS, it was reported that the CS doses of 240 mg/kg and 600 mg/kg did not have a toxic effect.

It is important to acknowledge some limitations of the present study. Firstly, the study was conducted on animal models, specifically rats, which may not fully reflect the complexities of human periodontal disease. Therefore, caution should be exercised when extrapolating the findings to human clinical scenarios. Furthermore, although the antioxidant properties of CS have been demonstrated, the specific mechanisms underlying its effects on alveolar bone loss and bone regeneration are not fully elucidated. Future research should aim to explore these mechanisms in more detail. Moreover, the study focused on the application of CS without investigating optimal dosages or potential side effects.

In the present study, the active effect of applying CS to lesions on periodontal destruction was investigated in rats in which experimental periodontitis was induced moreover, osteoblastic activity in CS-administered LC group was higher than that in the control and experimental periodontitis groups, and osteoclastic activity was low. In this context, it can be suggested that the destruction that may occur after periodontitis can be reduced through the utilization of CS. However, Therefore, further research is needed to determine the appropriate dosage and safety profile of CS in clinical settings. Finally, while the present study indicates potential benefits of CS in preventing alveolar bone loss, additional large-scale animal studies and well-designed clinical trials involving human subjects are warranted to validate these findings and establish the clinical efficacy of CS in periodontal disease management.

Conflict of interest

The authors declared that there are no conflict of interest. This study was funded by self-funding of authors.

Author contribution

HO interpreted the rats regarding the experimental Periodontitis and applied the experimental design. ZDŞİ performed the histological examination. HG maintained biochemical analysis and he was a major contributor in writing the manuscript. All authors read and approved the final manuscript. This study was self-funded.

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