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Research Article

The Ability of Febuxostat on Periodontal Inflammation and Bone Loss in Rats with Periodontitis by Regulating Nuclear Factor-κB

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Abstract: We aimed to evaluate the effects of febuxostat on periodontal inflammation and bone loss in rats with periodontitis by regulating nuclear factor-κB (NF-κB). Sixty rats were randomly divided into sham (Group S), periodontitis model (Group P), febuxostat (Group F), minocycline hydrochloride (Group M), NF-κB p65 inhibitor PDTC (Group C), and febuxostat + NF-κB p65 inhibitor PDTC (Group F+C) groups (n=10). The levels of serum tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17), and IL-10 were detected by enzyme-linked immunosorbent assay. The pathological changes in periodontal tissues were detected by hematoxylin-eosin staining, and bone loss was detected using micro-CT. Western blotting was performed to measure the protein expression of NF-κB p65 in periodontal tissues. Group P had significantly higher levels of TNF-α and IL-17 and lower IL-10 levels than those of Group S (P<0.05). Group F had significantly lower levels of serum TNF-α and IL-17 and higher IL-10 levels than those of Group P (P<0.05). The protein expression of NF-κB p65 in the periodontal tissues of Group P was significantly higher than that of Group S, which was lower in Group F and M than that in Group P (P<0.05). Compared with Group P, Group C had significantly lower levels of TNF-α and IL-17, CEJ-AC and protein expression of NF-κB p65, but a higher level of IL-10 (P<0.05), and the trends were the same between Group F+C and Group F (P<0.05). Febuxostat can inhibit periodontal inflammation and reduce alveolar bone loss in rats with periodontitis, probably by inhibiting the activation of NF-κB.

Keywords: *Febuxostat, Alveolar bone loss, Periodontitis, NF-κB*

Febuksostat'ın Periodontitisli Sıçanlarda Nükleer Faktör-κB'yi Düzenleyerek Periodontal Enflamasyon ve Kemik Kaybı Üzerine Etkisi

Öz: Bu çalışmada, febuksostatın, periodontitisli sıçanlarda nükleer faktör-κB'yi (NF-κB) düzenleyerek periodontal enflamasyon ve kemik kaybı üzerine etkilerini değerlendirmeyi amaçladık. Altmış sıçan rastgele, sham (Grup S), periodontitis modeli (Grup P), febuksostat (Grup F), minosiklin hidroklorür (Grup M), NF-κB p65 inhibitörü PDTC (Grup C) ve febuksostat + NF-κB p65 inhibitörü PDTC (Grup F+C) gruplarına ayrıldı (n=10). Serum tümör nekroz faktörü-α (TNF-α), interlökin-17 (IL-17) ve IL-10 seviyeleri enzime bağlı immünosorbent yöntemi ile tespit edildi. Periodontal dokulardaki patolojik değişiklikler hematoksilen-eozin boyama ile ve kemik kaybı mikro-BT kullanılarak tespit edildi. Periodontal dokularda NF-κB p65 protein ekspresyonunu ölçmek için Western blotlama yapıldı. Grup P'nin TNF-α ve IL-17 düzeyleri, Grup S'ye göre anlamlı derecede yüksek ve IL-10 düzeyleri daha düşüktü (P<0.05). Grup F'nin serum TNF-α ve IL-17 düzeyleri, Grup P'ye göre anlamlı derecede düşük ve IL-10 düzeyleri daha yüksekti (P<0.05). Grup P'nin periodontal dokularında NF-κB p65 protein ekspresyonu, Grup S'den anlamlı derecede yüksek, Grup F ve Grup M'de ise Grup P'den daha düşüktü (P<0.05). Grup P ile karşılaştırıldığında, Grup C'de TNF-α ve IL-17 seviyeleri, CEJ-AC ve NF-κB p65 protein ekspresyonu anlamlı derecede düşükken, IL-10 seviyesi daha yüksekti (P<0.05) ve Grup F+C ile Grup F arasındaki eğilimler aynıydı (P<0.05).

Febuksostat, NF-κB aktivasyonunu inhibe ederek periodontitisli sıçanlarda periodontal enflamasyonu inhibe edebilir ve alveolar kemik kaybını azaltabilir.

Anahtar sözcükler: *Febuksostat, Alveolar kemik kaybı, Periodontitis, NF-κB*

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Introduction

Periodontitis is a chronic inflammatory disease mainly caused by microbial infection, which leads to the continuous destruction of periodontal tissues, such as gingival swelling, alveolar bone resorption, and tooth loosening, as one of the major causes of tooth loss in adults [1]. Periodontitis is closely linked to cardiovascular disease, diabetes mellitus, rheumatoid arthritis, osteoporosis, and other systemic diseases in addition to local periodontal damage [2]. Currently, periodontitis is primarily treated by removing pathogenic factors such as dental plaque and calculus on the tooth surface with instruments. However, such treatment causes discomfort to patients with dentin hypersensitivity [3]. Therefore, researchers have endeavored to overcome the problem. Local periodontal inflammation leads to metabolic syndromes such as hyperuricemia, which can raise the risk of the vascular inflammatory response [4]. Febuxostat is an oral selective inhibitor of xanthine oxidase (XO) that mediates uricogenesis as a catalytic enzyme for uric acid [5]. Currently, drugs that inhibit uricogenesis mainly function by inhibiting XO. Febuxostat can inhibit the aggregation of XO and substrates by binding the molybdopterin active site in XO [6]. However, the effect of febuxostat on serum inflammatory factors in rats with periodontitis has not been reported hitherto. Nuclear transcription factor-κB (NF-κB), as a pleiotropic eukaryotic transcription factor expressed in a variety of cells, is able to regulate cell growth, differentiation, apoptosis, and inflammation [7]. It has high expression in the case of local inflammation in various cells [8]. When gingival epithelial cells undergo pathogenic periodontal infection, NF-κB is activated, thereby inducing inflammation [9]. Furthermore, NF-κB can damage periodontal tissues by releasing multiple enzymes, aggravating the inflammatory response. Upon periodontitis, NF-κB is involved in the generation of inflammatory factors [10], but whether febuxostat can ameliorate periodontitis through NF-κB remains unclear. Therefore, the aim of this study was to explore the mechanism by which febuxostat affected periodontal inflammation and bone loss in rats with periodontitis by regulating NF-κB.

Material and Methods

Ethical Approval

This study has been approved by the animal ethics committee of Zhuji Affiliated Hospital of Wenzhou Medical University Medical University (approval No. ZAHWMU2020008).

Laboratory Animals

A total of 60 healthy SPF male SD rats (10 weeks old, 200-220 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd. [certificate No. SYXK (Hunan) 2019-0004; China]. All rats were housed in an SPF animal room at 20-24°C and humidity of 40-70%, with a 12/12 h light/dark cycle.

Reagents and Apparatus

The following reagents and apparatus were used: febuxostat (2-[3-Cyano-4-(2-methylpropoxy)phenyl]-4-methyl-5-thiazolecarboxylic acid) *(Fig. 1)* (Jiangsu Wanbang Biochemical Pharmaceutical Co., Ltd., China), NF-κB p65 antibody (Cat. No. AN365, Wuhan Amyjet Scientific Inc., China), NF-κB p65 inhibitor pyrrolidine dithiocarbamate (PDTC) (Cat. No. S1809, Beijing Biolab Technology Co., Ltd., China), ST16 low-temperature high-speed centrifuge (Xi'an Kangrui Trading Co., Ltd., China), DM-1 dissecting microscope (Zeiss, Germany), Medesy periodontal probe (Shanghai Kangqiao Dental Instruments Factory, China), and enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. RE52001, Wuhan Huamei Biological Engineering Co., Ltd., China).

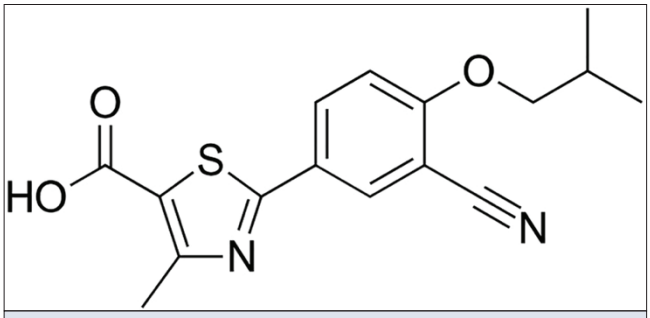

Fig 1. Chemical formula of febuxostat

Preparation of Periodontitis Model and Grouping

All rats were adaptively fed for one week, and given 2% co-trimoxazole suspension in the first week and normal drinking water in the second week. The rats were randomly divided into a sham group (Group S, n=10), a periodontitis model group (Group P, n=10), a febuxostat group (Group F, n=10), a minocycline hydrochloride group (Group M, n=10), an NF-κB p65 inhibitor PDTC group (Group C, n=10), and a febuxostat + NF-κB p65 inhibitor PDTC group (Group F+C, n=10) according to the principle of equal body weight.

After anesthesia by intraperitoneal injection using 10% chloral hydrate, the palatal gingival crevices of bilateral maxillary second molars were injected with 20 μL of sterile PBS only and applied with 100 μL of PBS for modeling in Group S. In the remaining five groups, 20 μL of *Porphyromonas gingivalis* W83 was injected into the palatal gingival crevices of bilateral maxillary second molars, the dental neck was ligated with silk ligature, and 100 μL of bacterial solution (1.5×10^9 CFU/mL) was applied at the site of ligation. The above treatment was repeated

every two days for a total of four times. Eight weeks after modeling, periodontal inflammation, attachment loss, tooth loosening and alveolar bone resorption shown in CT suggested successful modeling. The modeling failed in five rats, so five new ones were replenished. Local medication was given for each group after the ligature was removed. Febuxostat (8 mg/kg) was injected in the gingiva of Group F, 20 μL of 2% minocycline hydrochloride was injected in the gingiva of Group M, 20 μL of PDTC was injected in the gingiva of Group C, febuxostat (8 mg/kg) and 20 μL of PDTC were injected in the gingiva of Group F+C, and an equal amount of normal saline was injected in the gingiva of Group S and P. The rats were administered once daily for four consecutive weeks.

Detection of Levels of Serum Tumor Necrosis Factor-α (TNF-α), Interleukin-17 (IL-17), and IL-10 by ELISA

After drug intervention, the rats were anesthetized, and 4 mL of venous blood was drawn from the tail vein, left still, and centrifuged at 3.500 r/min for 15 min. The supernatant was harvested to detect the serum levels of TNF-α, IL-17, and IL-10.

Pathological Observation of Periodontal Tissues

After the last administration and 12-hour fasting, three rats from Group S, P, F, and M were randomly selected and sacrificed by cervical dislocation. The molar segment of the left mandible was fixed with 4% paraformaldehyde solution for 48 h. Then the tissue was decalcified with EDTA (100 g/L) for three weeks, embedded in paraffin, and sliced into 5 μm-thick sections along the median coronal section of the mandible. After hematoxylin-eosin (HE) staining, the sections were observed and photographed under a light microscope.

Detection of Bone Loss Using Micro-CT

At 12 h after the last administration, three rats randomly selected from each group were sacrificed by cervical dislocation. The maxilla, upper molar, dental tissue, and periodontal tissue were fixed with 4% paraformaldehyde for 24 h, followed by trimming. Jawbone and periodontal tissue in the molar section were preserved, and other tissues were removed. Then three-dimensional CT imaging was performed for Group S, P, F, and M, and the distances from the cemento-enamel junction to the alveolar crest (CEJ-AC) of all groups were measured. The alveolar bone resorption at the left mandibular molar was observed.

Detection of NF-κB p65 Protein Expression in Periodontal Tissues by Western Blotting

The periodontal tissues at the left mandibular molar were harvested and thoroughly ground. Then 100 mg of samples were placed into a pre-cooled 1.5 mL centrifuge tube, lysed with RIPA lysis buffer, and centrifuged in a centrifuge tube at 12.000 r/min for 10 min. The supernatant was harvested to measure the protein level, and 5% spacer gel and 8% separation gel were prepared. The protein samples were loaded, electrophoresed for 100 min, and transferred onto a PVDF membrane by wet transfer for 2 h. Then the membrane was blocked with 5% blocking buffer at room temperature for 1 h, and incubated with NF-κB p65 (1:800) and internal reference β-actin primary antibody solution at 4°C overnight. After the primary antibody was washed away, HRP-labeled secondary antibodies were added for 2-h incubation, and the membrane was washed again. Finally, the membrane was soaked in ECL solution for 2 min and imaged by a chemiluminescence system.

Statistical Analysis

GraphPad Prism 8.0 software (IBM, New York, USA) was used for statistical analysis. The measurement data were expressed as mean ± standard deviation (x ± s). The data were analyzed by one-way analysis of variance among groups and compared by the *t* test between two groups. P<0.05 was considered statistically significant.

Results

Group P had significantly higher levels of serum TNF-α and IL-17 and lower level of IL-10 than those of Group S (P<0.05). Group F had significantly lower levels of serum TNF-α and IL-17 and higher level of IL-10 than those of Group P (P<0.05). There was no significant difference in the serum levels of TNF-α, IL-17, and IL-10 between Group F and M (P>0.05) *(Table 1)*.

Table 1. *Serum levels of TNF-α, IL-17 and IL-10 (`x ± s, pg/mL)*

Group	n	TNF-α	IL-17	IL-10
S	10	100.16±5.42	312.45±15.32	286.33±10.25
P	10	312.73±14.68*	618.23±21.46*	113.28±5.31*
F	10	186.54±8.31*#	409.31±18.15*#	197.32±7.54*#
M	10	185.36±8.16*#	402.18±12.96*#	195.06±7.82*#
F		806.4	562.7	795.4
P		<0.0001	<0.0001	<0.0001

** P<0.05 vs. Group S, #P<0.05 vs. Group P*

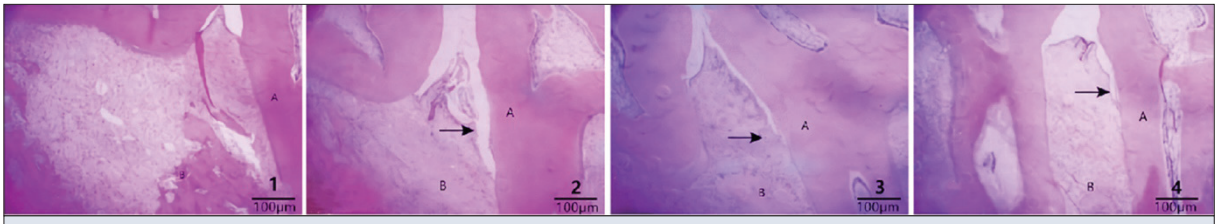

Fig 2. Pathological morphology of periodontal tissues (HE staining). 1: Group S, 2: Group P, 3: Group F, 4: Group M. A: Maxillary first molar; B: alveolar bone. Arrows indicate periodontal pockets

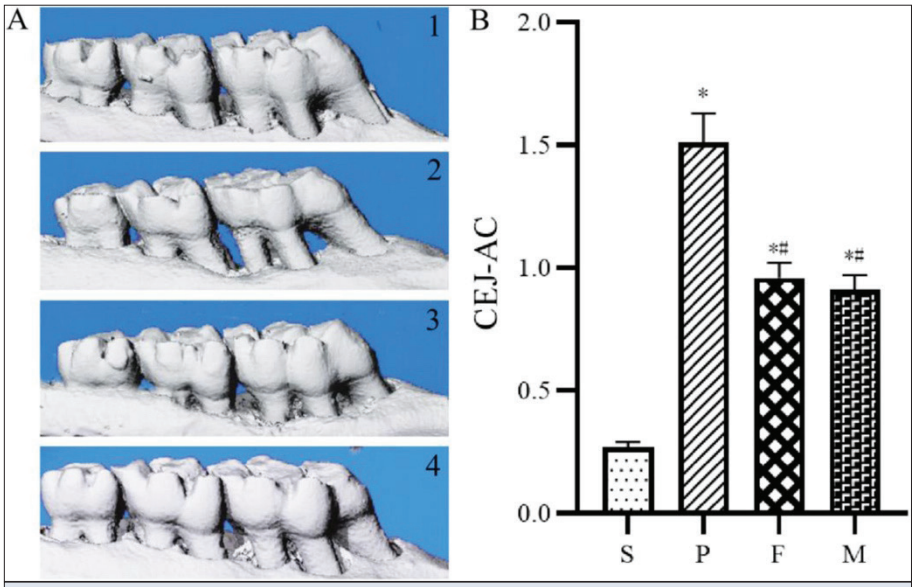

Fig 3. Bone loss. CEJ-AC significantly rose in Group P compared with that in Group S, declined in Group F and M compared with that in Group P, and had no significant difference between Group F and M. A: Micro-CT image reconstruction in each group, B: Comparison of CEJ-AC among groups. 1: Group S, 2: Group P, 3: Group F, 4: Group M. *P<0.05 *vs.* Group S, #P<0.05 *vs.* Group P. Unit for CEJ-AC: mm

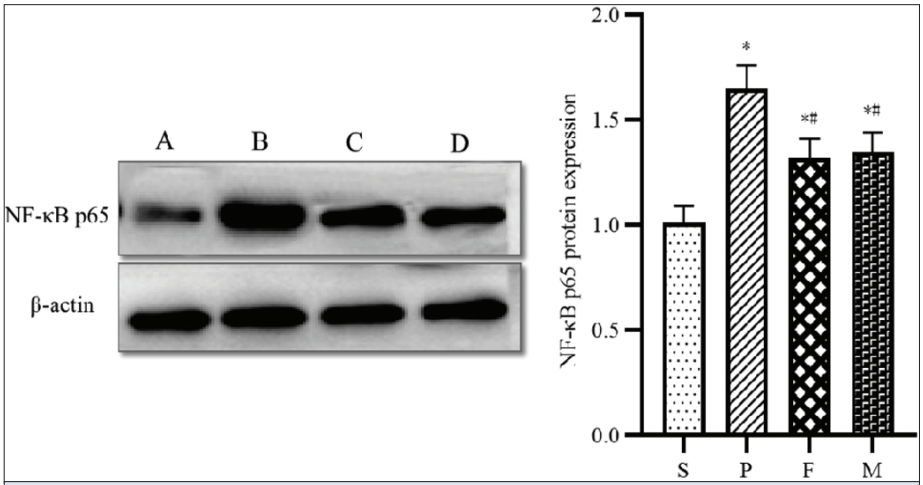

Fig 4. Protein expression of NF-κB p65 in periodontal tissues. The protein expression of NF-κB p65 in periodontal tissues was significantly higher in Group P than that in Group S, significantly lower in Group F and M than that in Group P, and had no significant difference between Group F and M. A: Group S, B: Group P, C: Group F, D: Group M. *P<0.05 *vs.* Group S, #P<0.05 *vs.* Group P

The HE staining results of Group S showed that the gingival epithelium was intact, without loss of attachment or abnormal changes in connective tissue. In Group P, the junctional epithelium underwent down-growth. The collagen fibers were degenerated, some of which were degraded. Meanwhile, there was significant alveolar bone

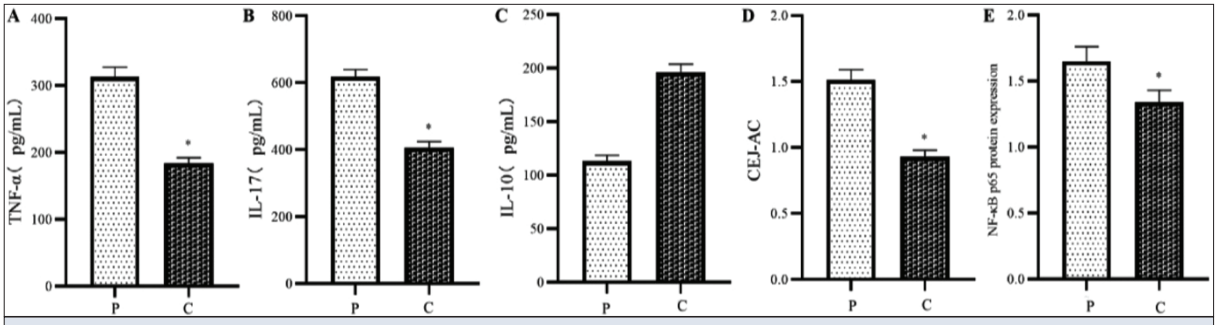

Fig 5. Effects of NF-κB p65 inhibitor PDTC on serum inflammatory factor levels, CEJ-AC and NF-κB p65 protein expression. Compared with Group P, Group C had significantly decreased levels of serum TNF-α and IL-17, CEJ-AC and protein expression of NF-κB p65, but increased level of IL-10. A: TNF-α level, B: IL-17 level, C: IL-10 level, D: CEJ-AC, E: NF-κB p65 protein expression. *P<0.05 *vs*. Group P

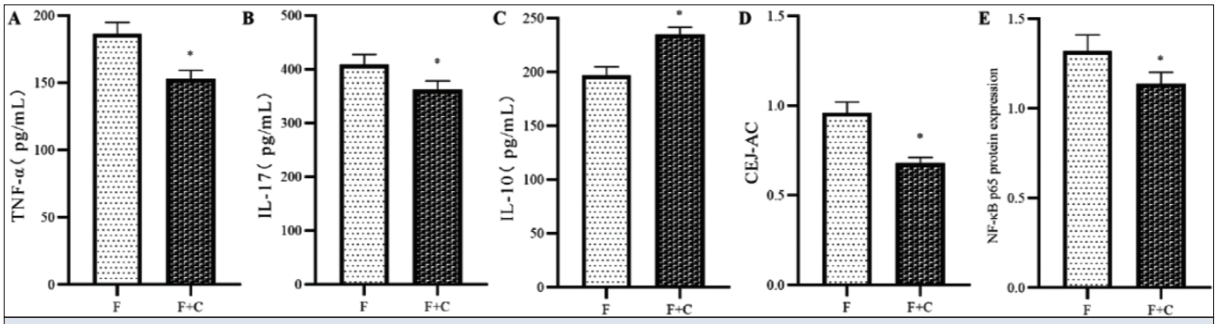

Fig 6. Effects of febuxostat combined with PDTC on serum inflammatory factor levels, CEJ-AC and NF-κB p65 protein expression. Compared with Group F, Group F+C had significantly decreased levels of serum TNF-α and IL-17, CEJ-AC and protein expression of NF-κB p65, but elevated level of IL-10. A: TNF-α level, B: IL-17 level, C: IL-10 level, D: CEJ-AC, E: NF-κB p65 protein expression. *P<0.05 *vs*. Group F

resorption. The periodontal tissues of Group F and M were markedly improved, and the periodontal pockets became shallow *(Fig. 2)*.

CEJ-AC was significantly greater in Group P than in Group S (P<0.05). Group F and M had lower CEJ-AC values than that of Group P (P<0.05), but there was no significant difference between the values of Group F and M (P>0.05) *(Fig. 3)*.

The protein expression of NF-κB p65 in periodontal tissues was significantly higher in Group P than that in Group S (P<0.05), significantly lower in Group F and M than that in Group P (P<0.05), and had no significant difference between Group F and M (P>0.05) *(Fig. 4)*.

Compared with Group P, Group C had significantly decreased levels of serum TNF-α and IL-17, CEJ-AC, and protein expression of NF-κB p65 but increased level of IL-10 (P<0.05) *(Fig. 5)*.

Compared with Group F, Group F+C had significantly decreased levels of serum TNF-α and IL-17, CEJ-AC, and protein expression of NF-κB p65 but elevated level of IL-10 (P<0.05) *(Fig. 6)*.

Discussion

Periodontitis is a destructive inflammatory disease, and its primary pathogenesis is the destruction of pathogenic bacteria to periodontal tissues and the local host response. Pathogenic bacteria, such as Gram-negative anaerobic bacteria, can induce infectious diseases in dental supporting tissues as a main cause for periodontitis [11]. Different methods have been proposed to establish periodontitis models. Currently, the rat model of periodontitis is usually established through molar ligation, inoculation of *P. gingivalis*, or the combination of them. A periodontitis model has been successfully induced in laboratory rabbits through silk-suture ligation, high-glucose water intake, and putative periodontal pathogens [12]. Until now, many kinds of animals, such as pigs, dogs, rats, and mice, have been used to successfully establish periodontitis models with the manifestations of induced periodontitis. Among them, the rat periodontitis model is most commonly used. Since rat and human molars have similar physiological structures and pathological mechanisms, their kinetics of inflammatory cytokines in the case of periodontitis also quite resemble each other [13,14]. In this study, the periodontitis model was established by gingival inoculation of *P. gingivalis* solution and silk suture ligation of the molar tooth cervix. The periodontal tissue destruction and disorderly distributed ligament fiber bundles, and considerable inflammatory cell infiltration suggested that *P. gingivalis* solution combined with silk suture ligation can cause inflammatory injury in periodontal tissue, verifying the successful establishment of the periodontitis model.

Both inflammatory response and host immunomodulatory response are of significance to remodeling after periodontal bone loss, and cytokines released by specific cell populations play crucial roles in the host response and alveolar bone resorption. TNF-α mediates various pathological processes, such as inflammatory response, alveolar bone resorption, and loss of connective tissue attachment [15]. Upon stimulus by IL-23, T cell subset Th17 can produce a variety of inflammatory chemokines and cytokines, including IL-17, which dominate the host immune response. The protein expression of IL-17 rises during the onset and progression of periodontitis [16]. The disorders of IL-17 enhance the generation of osteoclasts and promote local inflammatory response, accompanied by the increase in inflammatory mediators such as TNF-α. On the contrary, IL-10 produced by Treg cells is widely expressed in inflammatory periodontal tissues, which can alleviate bone loss by inhibiting osteoclast formation [17]. In this study, febuxostat reduced the serum levels of TNF-α and IL-17 and significantly raised the level of IL-10 in rats with periodontitis. Guo et al.[18] proved that febuxostat significantly reduced the levels of inflammatory mediators and ameliorated the endothelial function in patients with chronic periodontitis complicated by uricemia.

NF-κB regulates the inflammatory response by modulating inflammatory factors, as well as immune function- and inflammatory stimulus-related cytokines [19]. By regulating IL-6 and TNF-α, NF-κB controls the proliferation and differentiation of immune cells and mediates immune response. Suppressing its activation can inhibit the production of various inflammatory mediators [20]. Moreover, the enhanced activity of NF-κB corresponds to the enhanced synthesis of IL-6, IL-8, and TNF-α [21]. These cytokines are implicated in the regulation of immune response and osteoclasts, thereby inducing matrix degradation, bone resorption and loss of connective tissue attachment. Hiyari et al.[22] proved that the level of NF-κB in periodontal tissues significantly rose in mice with periodontitis. Minocycline hydrochloride is a semi-synthetic derivative of tetracycline which is effective in treating periodontitis [23]. In this study, febuxostat (Group F) worked similarly to minocycline hydrochloride (Group M) regarding all detected data, suggesting that febuxostat may also be effective for periodontitis treatment. Additionally, febuxostat had significantly decreased protein expression of NF-κB p65 in periodontal tissues and CEJ-AC, suggesting that it inhibited the activation of NF-κB, reduced CEJ-AC, and inhibited bone resorption by suppressing the expressions of inflammatory mediators. To further validate the postulation, the rats with periodontitis were subjected to PDTC intervention. PDTC significantly decreased the levels of serum inflammatory factors in rats with periodontitis and also CEJ-AC and NF-κB p65 protein expression. After the combined use of febuxostat and PDTC, the levels of serum inflammatory factors, CEJ-AC and NF-κB p65 protein expression all declined compared with those after the use of febuxostat alone. Collectively, febuxostat can, through regulating NF-κB, inhibit periodontal inflammation and alveolar bone loss in rats with periodontitis.

In conclusion, febuxostat can inhibit periodontal inflammation and reduce alveolar bone loss in rats with periodontitis, probably by suppressing the activation of NF-κB. Due to limited time and budget, HE staining and micro-CT image reconstruction were not performed for Group C and F+C in this study, so the results have limitations. Further experimental studies are ongoing in our group to provide a valuable experimental basis for the clinical treatment of periodontitis.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Financial Support

This study was not financially supported.

Ethical Approval

This study has been approved by the ethic committee of Zhuji Affiliated Hospital of Wenzhou Medical University, and great efforts have been made to minimize the animals' suffering.

Competing Interest

There is no conflict of interest.

Authors' Contributions

Y.Y., C.M. and X.T. designed this study and significantly revised the manuscript; Y.Z., J.Z. and C.C. performed this study and writing manuscript. The first two authors Y.Y. and C. M. contributed equally to this study. All authors have approved the submission and publication of this manuscript.

References

1. Pacheco CMF, Maltos KLM, Shehabeldin MS, Thomas LL, Zhuang Z, Yoshizawa S, Verdelis K, Gaffen SL, Garlet GP, Little SR, Sfeir C: Local sustained delivery of anti-IL-17A antibodies limits inflammatory bone loss in murine experimental periodontitis. *J Immunol,* 206, 2386-2392, 2021. DOI: 10.4049/jimmunol.2001432

2. Petain S, Kasnak G, Firatli E, Tervahartiala T, Gürsoy UK, Sorsa T: Periodontitis and peri-implantitis tissue levels of *Treponema denticola*-CTLP and its MMP-8 activating ability. *Acta Histochem,* 123:151767, 2021. DOI: 10.1016/j.acthis.2021.151767

3. Derman SH, Lantwin EM, Barbe AG, Noack MJ: Does a pretreatment with a dentine hypersensitivity mouth-rinse compensate the pain caused by professional mechanical plaque removal? A single-blind randomized controlled clinical trial. *Clin Oral Invest,* 25, 3151-3160, 2021. DOI: 10.1007/s00784-020-03643-4

4. Moro MG, Oliveira MDS, Santana MM, de Jesus FN, Feitosa K, Teixeira SA, Franco GCN, Spolidorio LC, Muscará MN, Holzhausen M: Leukotriene receptor antagonist reduces inflammation and alveolar bone loss in a rat model of experimental periodontitis. *J Periodontol,* 92, e84-e93, 2021. DOI: 10.1002/JPER.20-0718

5. Kim SC, Neogi T, Kim E, Lii J, Desai RJ: Trends in utilization of urate-lowering therapies following the US food and drug administration's boxed

warning on febuxostat. *Arthritis Rheumatol,* 73, 542-543, 2021. DOI: 10.1002/art.41550

6. Nessa N, Kobara M, Toba H, Adachi T, Yamamoto T, Kanamura N, Pezzotti G, Nakata T: Febuxostat attenuates the progression of periodontitis in rats. *Pharmacology,* 106, 294-304, 2021. DOI: 10.1159/000513034

7. Shi C, Shi R, Guo H, Shi Y, Liu X: β-amyloid-induced gonadotropin-releasing hormone decline involving Forkhead transcription factor FOXO3a and nuclear factor-κB. *Neuroreport,* 31, 923-927, 2020. DOI: 10.1097/WNR.0000000000001488

8. An L, Li Z, Shi L, Wang L, Wang Y, Jin L, Shuai X, Li J: Inflammation-targeted celastrol nanodrug attenuates collagen-induced arthritis through NF-κB and Notch1 pathways. *Nano Lett,* 20, 7728-7736, 2020. DOI: 10.1021/acs.nanolett.0c03279

9. Wang J, Wang B, Lv X, Wang L: NIK inhibitor impairs chronic periodontitis via suppressing non-canonical NF-κB and osteoclastogenesis. *Pathog Dis,* 78:ftaa045, 2020. DOI: 10.1093/femspd/ftaa045

10. Francis M, Gopinathan G, Salapatas A, Nares S, Gonzalez M, Diekwisch TGH, Luan X: SETD1 and NF-κB regulate periodontal inflammation through H3K4 trimethylation. *J Dent Res,* 99, 1486-1493, 2020. DOI: 10.1177/0022034520939029

11. Kalali N, Kadkhoda Z, Amid R, Ghourchian S, Douraghi M: Identification of oral anaerobic bacteria and the beta-lactamase resistance genes from Iranian patients with periodontitis. *Anaerobe,* 75:102515, 2022. DOI: 10.1016/j.anaerobe.2022.102515

12. Chen S, Lin G, You X, Lei L, Li Y, Lin M, Luo K, Yan F: Hyperlipidemia causes changes in inflammatory responses to periodontal pathogen challenge: implications in acute and chronic infections. *Arch Oral Biol,* 59, 1075-1084, 2014. DOI: 10.1016/j.archoralbio.2014.06.004

13. Gao J, Cai S, Wang Z, Li D, Ou M, Zhang X, Tian Z: The optimization of ligature/bone defect-induced periodontitis model in rats. *Odontology,* 110, 697-709, 2022. DOI: 10.1007/s10266-022-00715-7

14. Song JH, Yang JH, Kim MJ, Kim KY, Kim JK, Jin YB, Kim WH, Kim S, Kim KR, Park KI, Lee HJ: Anti-periodontitis effects of *Dendropanax morbiferus* H. Lév leaf extract on ligature-induced periodontitis in rats. *Molecules,* 28, 849, 2023. DOI: 10.3390/molecules28020849

15. Aleksandrowicz P, Brzezińska-Błaszczyk E, Kozłowska E, Żelechowska P, Borgonovo AE, Agier J: Analysis of IL-1β, CXCL8, and TNF-α levels in the crevicular fluid of patients with periodontitis or healthy implants. *BMC Oral Health,* 21:120, 2021. DOI: 10.1186/s12903-021-01478-3

16. Medara N, Lenzo JC, Walsh KA, Darby IB, O'Brien-Simpson NM, Reynolds EC: T helper 17 cell-related cytokines in serum and saliva during management of periodontitis. *Cytokine,* 134:155186, 2020. DOI: 10.1016/j.cyto.2020.155186

17. Cheng WC, Hughes FJ, Taams LS: The presence, function and regulation of IL-17 and Th17 cells in periodontitis. *J Clin Periodontol,* 41, 541-549, 2014. DOI: 10.1111/jcpe.12238

18. Guo ZS, Liu X, Cai YM: The efficacy of adjuvant febuxostat in the treatment of chronic periodontitis with hyperuricemia and its effect on serum inflammatory factors in patients. *China Pharmaceuti,* 27:4, 2018.

19. Bustami A, Lestari WP, Hayuningrum CF, Wuyung PE, Wibowo H, Natadisastra RM: The anti-inflammatory effect of octyl gallate through inhibition of nuclear factor-κB (NF-κB) pathway in rat endometriosis model. *J Reprod Infertil,* 21, 169-175, 2020.

20. He Y, Ruganzu JB, Zheng Q, Wu X, Jin H, Peng X, Ding B, Lin C, Ji S, Ma Y, Yang W: Silencing of LRP1 exacerbates inflammatory response via TLR4/NF-κB/MAPKs signaling pathways in APP/PS1 transgenic mice. *Mol Neurobiol,* 57, 3727-3743, 2020. DOI: 10.1007/s12035-020-01982-7

21. Phusuntornsakul P, Jitpukdeebodintra S, Pavasant P, Leethanakul C: Vibration activates the actin/NF-κB axis and upregulates IL-6 and IL-8 expression in human periodontal ligament cells. *Cell Biol Int,* 44, 661-670, 2020. DOI: 10.1002/cbin.11267

22. Hiyari S, Wong RL, Yaghsezian A, Naghibi A, Tetradis S, Camargo PM, Pirih FQ: Ligature-induced peri-implantitis and periodontitis in mice. *J Clin Periodontol,* 45, 89-99, 2018. DOI: 10.1111/jcpe.12817

23. Zhang T, Qiu Y, Song J, Zhou P, Liao H, Cheng Y, Wu X: Electrosprayed minocycline hydrochloride-loaded microsphere/SAIB hybrid depot for periodontitis treatment. *Drug Deliv,* 28, 620-633, 2021. DOI: 10.1080/10717544.2021.1902020