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ORIGINAL RESEARCH

Hylotelephium mingjinianum Extract Attenuates Oxidative Stress and Inflammation in Experimental Periodontitis

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Background: *Hylotelephium mingjinianum* is a traditional Chinese herbal medicine with anti-inflammatory, analgesic and antibacterial effects. However, its role in the treatment of Periodontal disease has not been elaborated in detail.

Purpose: This study aimed to elucidate the dual-target anti-inflammatory and antioxidant mechanisms of *Hylotelephium mingjinianum* extract (HME) in periodontitis treatment, focusing on its modulation of the Nrf2/NF-κB crosstalk.

Methods: The periodontitis animal model of SD rats was established by ligation combined with Porphyromonas gingivalis (Pg) stimulation. The rats were locally administered HME (0.25%, 0.5%, and 1%, 0.5 mL/twice/day) for 14 days. Inflammatory responses of alveolar bone, expression of osteogenic related biomarkers, and activation of Nrf2/NF-κB signaling pathway were detected. In addition, LPS induced human periodontal ligament cells (HPDLs) to measure the effect of HME on cell viability, inflammatory response, Nrf2/NF- κB pathway and oxidative stress.

Results: HME administration demonstrated significant efficacy in a ligature-induced periodontitis rat model: serum pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-18, GM-CSF, and ICAM1) decreased by 24.9–50.6% at high HME concentrations, while Th2-related factors IL-4/IL-13 returned to baseline levels. Histopathological analysis revealed that HME maintained gingival epithelial integrity and suppressed osteoclast activity in a dose-dependent manner by downregulating RANKL. Mechanistic studies indicated that HME attenuated NF-κB activation by reducing nuclear p65 protein (44.1%) and enhanced the Nrf2-mediated antioxidant response, normalizing oxidative stress markers (MDA decreased by 55.3%; SOD restored to 142.3 U/mg). In vitro experiments confirmed HME's cytocompatibility at concentrations below 200μg/mL and its resistance to LPS stimulation, reducing ROS overaccumulation by 16.46% through modulation of the Nrf2/NF-κB axis.

Conclusion: HME inhibits the progression of periodontitis in rats by downregulating the expression of inflammatory factors, alleviating oxidative stress, and repairing the Nrf2/NF-κB signaling pathway.

Keywords: periodontitis, inflammatory factors, RANKL, Nrf2, NF-κB signaling pathway, ROS

Introduction

Periodontitis, a chronic inflammatory disease of periodontal supportive tissues, is predominantly initiated by local microbial biofilm dysbiosis and represents the leading cause of adult tooth loss worldwide.[1,2] Despite advancements in conventional therapies such as scaling/root planing and systemic antibiotics, these approaches exhibit limited long-term efficacy (38.6% recurrence rate at 3-year follow-up) and fail to address oxidative stress-mediated tissue destruction, while raising concerns about antibiotic resistance development.[3] Its pathogenesis involves progressive destruction of periodontal ligament and alveolar bone, driven by sustained inflammatory responses to microbial challenge within periodontal pockets.[4,5] Epidemiological data reveal that mild periodontitis affects 62% of the global population, with severe forms impacting 23.6%, establishing it as the seventh most prevalent disease entity.[5] *Sedum* is a traditional

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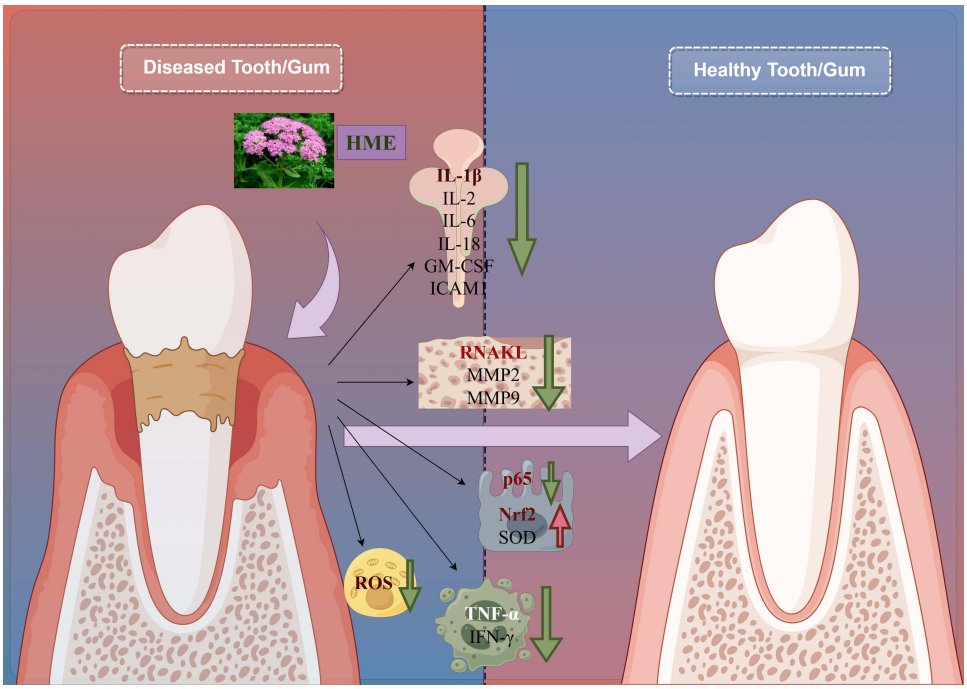
Graphical Abstract



Chinese herbal medicine, mainly composed of flavonoids, which have good anti-inflammatory, analgesic, and antibacterial activities.[6–8] Because of its application in folk medicine, people are increasingly interested in Sedum. *Hylotelephium mingjinianum* (Fu) H.Ohba (also known as *Hylotelephium purpureum* or *Sedum mingjinianum Fu* which has been checked with http://www.worldfloraonline.org on April 24th 2023) is a unique plant species in China, distributed in Anhui, Zhejiang, Guangxi, and other places.[9] It grows mostly in damp and shady areas by mountain streams. In a previous study, we explored the isolation, preparation, and identification of *Hylotelephium mingjinianum*, as well as the duration and effectiveness of *Hylotelephium purpureum* in treating experimental periodontitis. The findings revealed that *Hylotelephium mingjinianum* exhibited a notable inhibition rate of 32.7% on mouse edema, concurrently altering the permeability of mouse peritoneal capillaries. Additionally, it displayed strong antibacterial and bactericidal properties, along with notable analgesic effects. Based on these findings, we propose that *Hylotelephium purpureum* has significant potential for application in animal models. Its active components likely contribute positively to managing periodontitis.[10] However, more research is required to understand the molecular mechanisms through which this plant exerts its anti-inflammatory effects. Previous studies have shown that oxidative stress caused by reactive oxygen species in periodontal tissue plays an important role in the occurrence and development of periodontitis.[11] During periodontitis, immune cells can release reactive oxygen species (ROS) and some inflammatory cytokines, thereby eliminating periodontal pathogens.[12] These molecules can regulate the interaction between periodontal pathogens and host immune responses, and are important factors in causing local tissue damage and pathogenesis.[13,14] Nuclear factor E2 related factor 2 (Nrf2) is a fundamental transcriptional regulatory factor that primarily encodes and expresses various important cellular protective factors, including detoxifying enzymes, antioxidant proteins, efflux transporters, anti-apoptotic proteins, anti-inflammatory factors, and other stress response factors.[15,16] As an important cellular defense mechanism, its activation can improve oxidative damage. Downregulation of Nrf2 expression and subsequent inhibition of antioxidant production may lead to worsening of periodontitis inflammation.[15,17] Therefore, detecting the expression level of Nrf2 in the local periodontal microenvironment is of great significance for exploring the role of oxidative stress in periodontal disease.

Based on the aforementioned theoretical foundation, this study systematically evaluates the therapeutic effects of HME on experimental periodontitis in rats through two main research dimensions: 1) quantitative assessment of inflammatory cytokine regulation; 2) exploration of the Nrf2-mediated oxidative stress regulatory mechanism. The aim is to elucidate the therapeutic potential of HME against periodontitis and its molecular mechanisms and providing new perspectives for optimizing periodontal treatment strategies.

Materials and Methods

Plant Material

With permission from the local government, fresh grass of *Hylotelephium mingjinianum* were collected from the Changbai mountain, Jilin province, China. The voucher sample was deposited at Herbarium of the Faculty of Sciences of Jilin University, Jilin, China.

Preparation of HME

The preparation and characterization of HME are provided by Zhiduo Niu, an experimental technician from the Preparation Room of Jilin Provincial Academy of Traditional Chinese Medicine (Changchun, China), with batch number: 20120725 (Wang et al, 2016). After separation and identification, the contents of quercetin and kaempferol in the total flavonoids of the extract of *Hylotelephium purpureum* were more than 50%.

Animal Models

This study selected 60 Wistar rats aged 5–10 weeks (average weight 200g±20g) from the Animal Experiment Center of Jilin University. The control group (Group 1) neither underwent ligation nor bacterial inoculation but received the same treatment process and an equal volume of normal saline was injected. Groups 2–6 underwent periodontal ligation treatment as described below.

Inclusion/Exclusion Criteria: The inclusion criteria required confirmation of intact periodontal structures, while the exclusion criteria included: 1) existing oral inflammatory lesions; 2) systemic metabolic disorders; 3) body weight deviating from the group mean by >15%.

Periodontitis Induction: After a 14-day environmental adaptation (22±1°C, 55% humidity, 12-hour light/dark cycle), 10 rats were randomly selected as the normal control group, and the remaining 50 rats underwent periodontitis modeling.

Under anesthesia with pentobarbital sodium (100mg/mL, SIGMA-P3761), the second maxillary molars of the rats were ligated at the cervical region using 4–0 sterile silk containing Porphyromonas gingivalis (Pg, ATCC 33277), with the ends of the silk embedded in the gingival sulcus. Postoperatively, every 2 days, 2 μL of Pg bacterial solution (1×10^9 CFU/mL) and 15 μL of LPS (1 mg/mL, Sigma) in saline were injected into the gingival sulcus.

Model Validation: After 4 weeks, individuals that did not meet the following criteria were eliminated: (1) silk falling off; (2) no significant gingival redness or swelling; (3) accidental death. A total of 8 rats were eliminated, leaving 42 modeling rats. Two modeling rats and two normal control rats were randomly euthanized, and gingival tissues were collected for HE staining. The pathological criteria were: the modeling group exhibited ≥3 levels of inflammatory cell infiltration (neutrophil/lymphocyte aggregation) and alveolar bone resorption (resorption depth >20% compared to the control group), confirming successful modeling.

Grouping Design: The remaining 40 modeling rats were randomly divided into 5 groups (n=8) using a random number table: (1) control group; (2) periodontitis group (ligation + PBS); (3) low-dose HME group (ligation + 0.25% HME); (4) medium-dose HME group (ligation + 0.5% HME); (5) high-dose HME group (ligation + 1.0% HME); (6) positive control group (SXYTND, produced by Hubei Wushi Pharmaceutical Co., Ltd., China; Approval No. Z42020010).

Dosing Scheme: Each group received 200 μL of the corresponding drug applied to the ligation site twice daily (8-hour interval) for 14 days.

Assessment Method: Each rat was assessed for gingival index (GI, Löe-Silness standard) and sulcus bleeding index (SBI, Muhlemann-Mazor standard) before and after treatment by two trained physicians using a blind method. After four

weeks, each rat was injected intraperitoneally with 400 μL of pentobarbital sodium (100mg/mL, SIGMA-P3761) for euthanasia,[18] and the maxillary molars and periodontal tissue samples were collected for subsequent immunohistochemical analysis. All procedures complied with the GB/T 35892–2018 standard and were approved by the Animal Ethics Committee of Jilin University (Approval No. KT201902015).

HE Staining and Immunohistochemistry Staining

Rat periodontal tissue was fixed with 4% Paraformaldehyde and embedded in paraffin for H&E staining (Yuanye Biotechnology, Shanghai, China). Immunohistochemical staining was performed using anti-RANKL (1:100, Proteintech, Shanghai, China), anti-p65 (1:200, Proteintech) and anti-Nrf2 (1:100, Proteintech). The NIS-ELEMNT BR image analysis system measured the average optical density value of the protein expression, and counted the differences in the expression of various proteins in each group.

Determination of Markers of Oxidative Stress

Serum malondialdehyde (MDA, A003-1-2, Nanjing Jiancheng Bioengineering Institute, China) levels and superoxide dismutase (SOD, A001-2-1, Nanjing Jiancheng Bioengineering Institute, China) activity were measured using commercial assay kits. All procedures strictly followed the manufacturer's instructions. Use a microplate reader to determine the OD value of samples (BioTek, USA).[19]

ELISA

Serum were collected from the rats. Levels of IFN-γ were detected with ELISA kits (R&D Systems ValukineTM) according to manufacturer's instructions. Repeat the experiment three times.

Serum Factor Detection

The serum of rats in each group was extracted and the expression of serum inflammatory factors was detected. As previously described, the rat multiple cytokine kit (Minneapolis, USA) was used to analyze the production of cytokines.[19]

Cell Culture

Human periodontal ligament cells (HPDLs) were purchased from Biotechnology Co., Ltd. The cells were cultured in DMEM medium containing 10% fetal bovine serum, 100 U/mL penicillin and 100 μ g/mL streptomycin. Raw264.7 were cultured in 1640 Medium (Gibco Life Technologies) supplemented with 10% FBS and antibiotics.

CCK8

Cells were seeded in 96 well plates. After cell adhesion, HME and LPS (sigma Aldrich) were added for 24 hours. Added 10 μL CCK-8 to each well. Two hours later, use Bio Rad Microplate Reader (Bio Rad, USA) to measure the OD value.

Immunofluorescent Staining

Cells were fixed with 4% paraformaldehyde for 1 day. After adding 0.5% Triton X-100 permeating cells, PBS washed the sample three times, and then blocked it with 10% FBS to eliminate the nonspecific fluorescence. The primary antibody used anti p65 (1:50), anti RANKL (1:50) and anti NRF2 (1:5), incubated at 4 ° C overnight and washed the sample with PBS for three times, added the secondary antibody labeled with Alexa Fluor 488 (green) (Proteintech), and incubated at 37 ° C for 1 hour. Leica TCS SP5 confocal fluorescence microscope was used to take fluorescence images of stained cells (Leica Microsystems, Buffalo Grove, USA).

ROS Detection

Using the fluorescent probe DCFH-DA (Sigma-Aldrich) to detect reactive oxygen species (ROS) generation, cells were seeded at a density of 1×10^6/well in a 6-well plate. After adherence, LPS and HME treatments were added separately. The ROS levels in the cells were measured using a reactive oxygen species detection kit (Biyuntian, Catalog No. S0033S). After 24 hours, the culture medium was discarded, and 10 μmol/L DCFH-DA probe (in the dark) was loaded

according to the reagent instructions and incubated at 37°C for 1 hour. The cells were washed three times with PBS to remove uninternalized probes, and after digesting and collecting the cells with trypsin, the DCF fluorescence signal was detected and quantified using a flow cytometer (Beckman, USA) at an excitation wavelength of 488 nm and an emission wavelength of 525 nm.

Western Blot Analysis

The Western blotting process is described previously (Zhao et al, 2020). Antibodies for immunoblotting, including anti-p65 (10,745), NRF2 (16396) and GAPDH (10494), were purchased from Proteintech. Antibody binding was detected by infrared imaging system (Odyssey).

Statistical Analysis

The experimental data results are expressed as mean ± standard deviation. Using StatView software for statistical analysis (SAS Institute). Use Student's *t*-test to analyze the differences between the two groups. Use one-way ANOVA to analyze the differences between two or more groups. When $p<0.05$, the difference is considered significant.

Result

HME Can Effectively Treat Experimental Periodontitis in Rats

As shown in Figure 1, the periodontal squamous epithelial cells in the normal group were continuous, and there was no inflammatory reaction in the epithelium or subepithelium. In the model group, there was a defect in the gingival squamous epithelium, forming a gingival ulcer (Figure 1A and B). There were more inflammatory cells and granulomas. IFN-γ in the serum of rats was detected using ELISA. The serum levels of IFN-γ in the model group was higher than those in control group (Figure 1C). These results further verified the established periodontitis model.

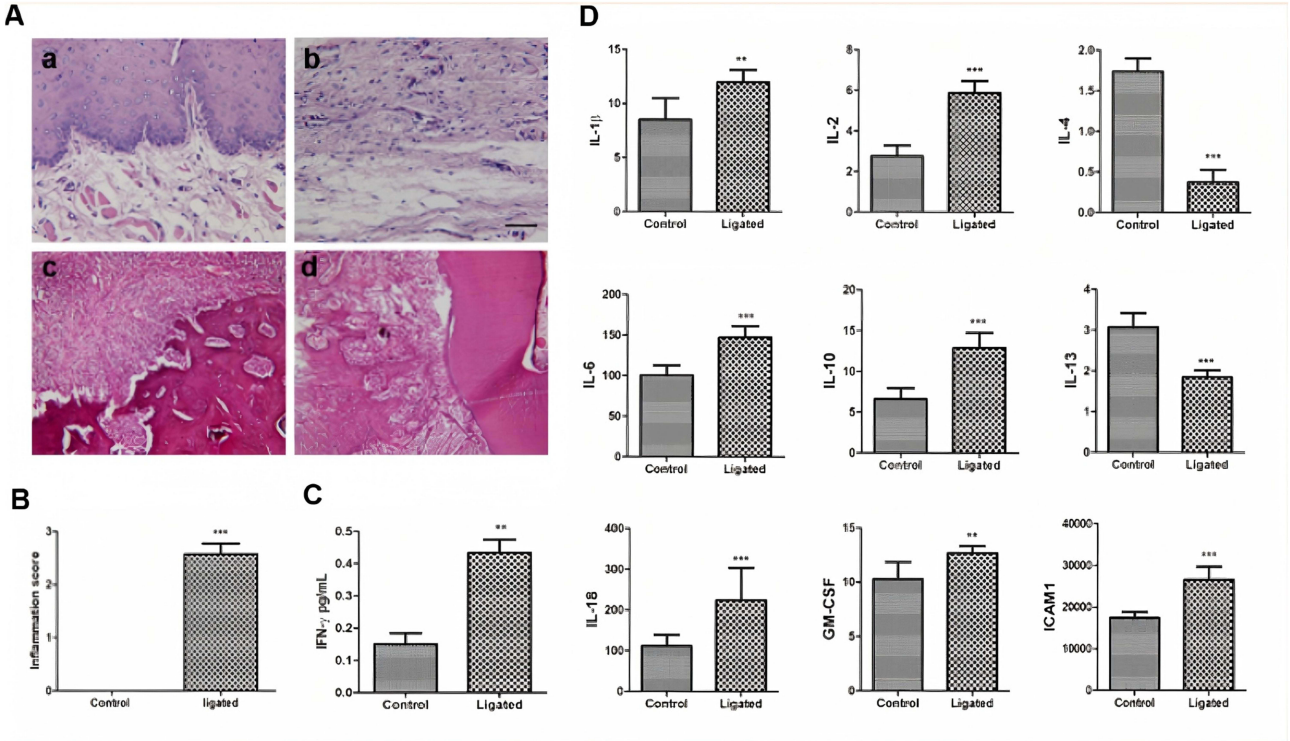


Figure 1 Establishment of periodontitis model in rats. (**A**) Representative images of gingival tissues H&E staining at a 200× magnification. (a) Gingival tissue in control group; (b) Gingival tissue in model group; (c) Alveolar bone forms absorptive lacunae; (d) Osteoclast proliferation. (**B**) Inflammation score. (**C**) The concentration of IFN-γ in the serum of rats. (**D**) Cytokine concentrations were measured using the Luminex assay. Bars represent the SD. ** $p<0.01$ and *** $p<0.001$.

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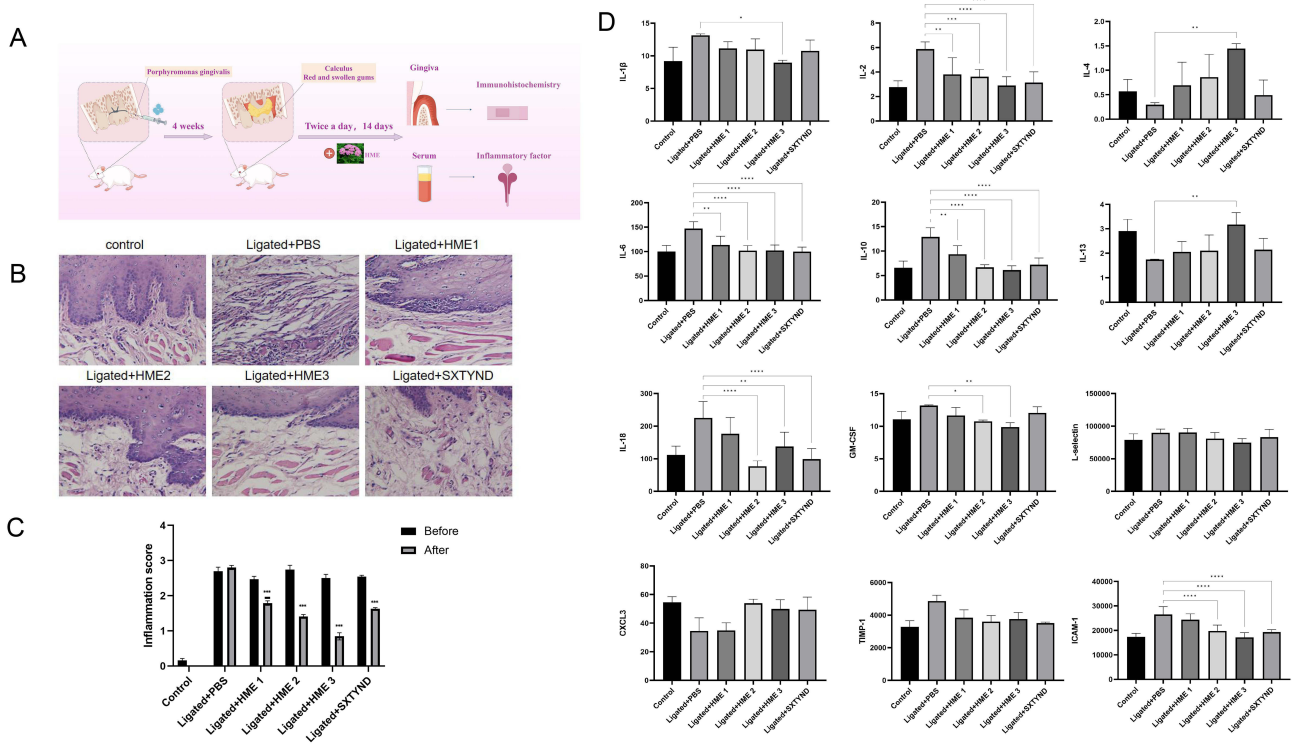

Figure 2 Evaluation of therapeutic effect of HME on rat periodontitis model. (**A**) Animal experiment process. (**B**) Representative images of gingival tissues H&E staining at a 200× magnification. (**C**) Inflammation score. (**D**) Cytokine level analysis in the rat model. Bars represent the SD. *p <0.05, **p <0.01, ***p <0.001 and ****p<0.0001.

We found that treatment with ligation resulted increased serum levels of IL-1β, IL-2, IL-6, IL-10, IL-18, GM-CSF and ICAM1 compared to normal rats. Treatment with ligation attenuated the expression of IL-4 and IL-13 (Figure 1D).

Next, we investigated the effects of HME on a rat model of periodontitis. H&E staining results showed that, HME can protect the gingival epithelium and reduce the inflammatory reaction in the upper and subcutaneous stroma. Slight edema and inflammatory cells were found in low-dose group, slight tissue edema was found in the middle dose group, and gingival tissue returned to normal in the high-dose group (Figure 2). Changes in serum inflammatory factors were also analyzed. The results are shown in Figure 2D. HME can alleviate the secretion of inflammatory factors IL-1B, IL-2, IL-6, IL-18, GM-CSF and ICAM1 to different degrees. The therapeutic effect increased with concentration; The high-dose group can increase IL-4 and IL-13. But IL-10 levels did not change after treatment. These results indicate that HME can effectively reduce the inflammatory response in rats with periodontitis.

HME Can Repair Alveolar Bone Loss Caused by Periodontitis

Alveolar bone resorption is an important pathological change associated with periodontitis. As shown in the immunohistochemically images in Figure 3A, the expression level of RANKL protein in inflammatory cells of the periodontal disease group increased compared with the control group. After HME treatment, the RANKL protein level decreased compared to the model group.

MMPs are involved in osteoblast/osteoblast differentiation, bone formation, osteoid dissolution during bone resorption, and recruitment and migration of osteoclast. Therefore, we detected the expression level of matrix metalloproteinase in periodontal tissue. Immunohistochemical results showed that MMP2 and MMP9 were less expressed in the gingival epithelium of the control group, but higher in the interstitial cells of the periodontitis model group. After HME treatment, the expression intensity decreased (supplement Figure 1).

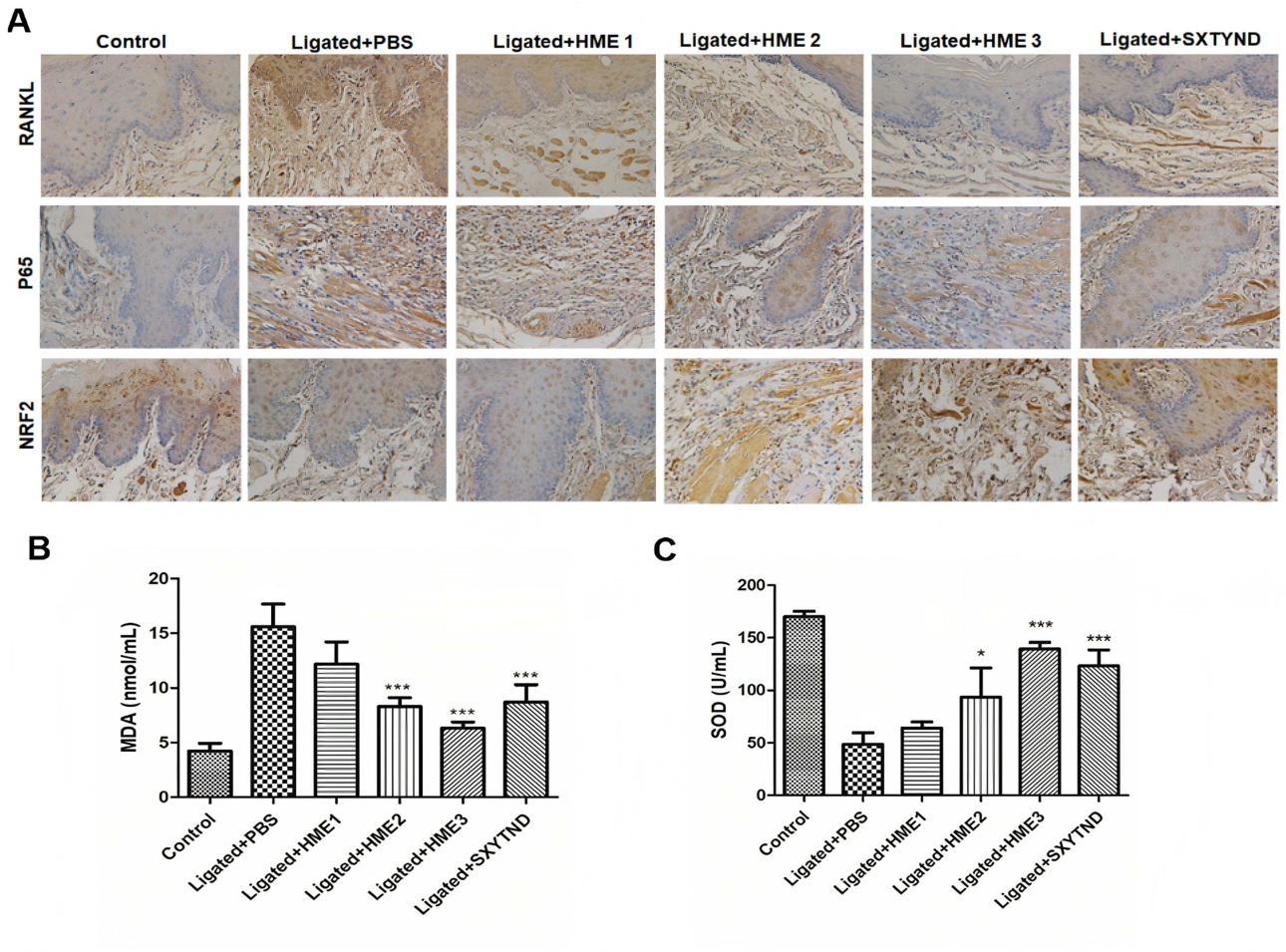

Figure 3 Effect of HME on alveolar bone resorption and oxidative stress. (**A**) Representative figures from anti-RANKL, P65 and NRF2 immunohistochemical assays of gingival tissues at a 400×magnification. (**B**) The activity of MDA in gingival tissues. (**C**) The concentration of SOD in gingival tissues. * p <0.05 and *** p <0.001.

HME Restored Nrf2/NF-κB Pathway and Remitted the Oxidative Stress State in vivo

NF-κB signaling pathway plays a key regulatory role in bone development and remodeling processes by regulating RANKL/RANK expression, osteogenic differentiation signaling pathway, and bone matrix formation. Nrf2 is an important effector of oxidative stress. The decrease in p65 activity in the NF-κB pathway is usually leads to a change in Nrf2 expression. Therefore, we detected p65 and Nrf2 in gingival tissue. The expression of p65 protein in the cell nucleus in periodontitis group was higher than control group, but this increase was reduced in the periodontitis group treated with HME (Figure 3A). In contrast, the expression of Nrf2 in the periodontal disease group was lower than that in the control group, but increased in the HME treatment group.

To further explore the effect of HME on oxidative stress, we detected the levels of malondialdehyde (MDA) and superoxide dismutase (SOD) in rat gingiva. As shown in Figure 3, we found that the MDA concentration in the periodontitis group was higher than that in the control group. The moderate and high concentrations of HME can effectively reduce the expression level of MDA (Figure 3B, $P< 0.01$). Meanwhile, the SOD activity in the gingival tissue of the model group decreased compared to the control group. After high concentration HME treatment, SOD activity was effectively restored and almost returned to the normal level. (Figure 3C, $P< 0.01$). These results indicating that HME can alleviate the oxidative stress of periodontal tissue in experimental periodontitis rats.

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Effects of HME on Cell Viability

As shown in Figure 4, HME will not affect the viability of Raw264.7 and HPDL when the concentration are less than or equal to 200 μg/mL. Therefore, HME (50, 100 and 200 μg/mL) has no significant cytotoxicity. We selected these three concentrations to investigate the function of HME in vitro.

Subsequently, we used FACS to detect the role of HME in cell cycle and apoptosis. As shown in Figure 4B and C, after adding 0–200 μg/mL HME, the proportion of the G1 phase of HPDLs did not change, and only the highest concentration of S phase cells decreased from 26.6% to 6.9%. Therefore, we believe that when the HME concentration is less than 200μg/mL, it has no effect on the cell cycle of HPDLs.

Similarly, low concentrations of HME had no effect on cell apoptosis. After adding different concentrations of HME, the cell activity was higher than 90% (Figure 4D). Therefore, we believe that at concentrations under 200μg/mL, HME has no obvious biological toxicity in vitro.

Effect of HME on LPS-Induced Inflammatory Factors

Evaluate the changes in cytokine expression levels in each group of culture media using ELISA. LPS stimulation increased the generation of TNF-α and IFN-γ compared to the control group. HME processing dramatically inhibited LPS-induced TNF-α and IFN-γ production (Figure 5).

Effects of HME on Nrf2/NF-κB Signaling Pathway in vitro

As shown in Figure 6C, the immunofluorescence results showed that after LPS stimulation, the expression level of P65 protein in HPDLs cells increased and the cell location changed, which was obviously aggregated in the nucleus. HME treatment can improve this change, reduce the intensity of protein expression, and reduce nuclear aggregation. The Western blot results are similar. LPS stimulation of HPDLS can cause an increase in the expression of P65 protein. However, in the group pretreated with high concentrations of HME, the expression of p65 remained close to normal levels under LPS stimulation (Figure 6D).

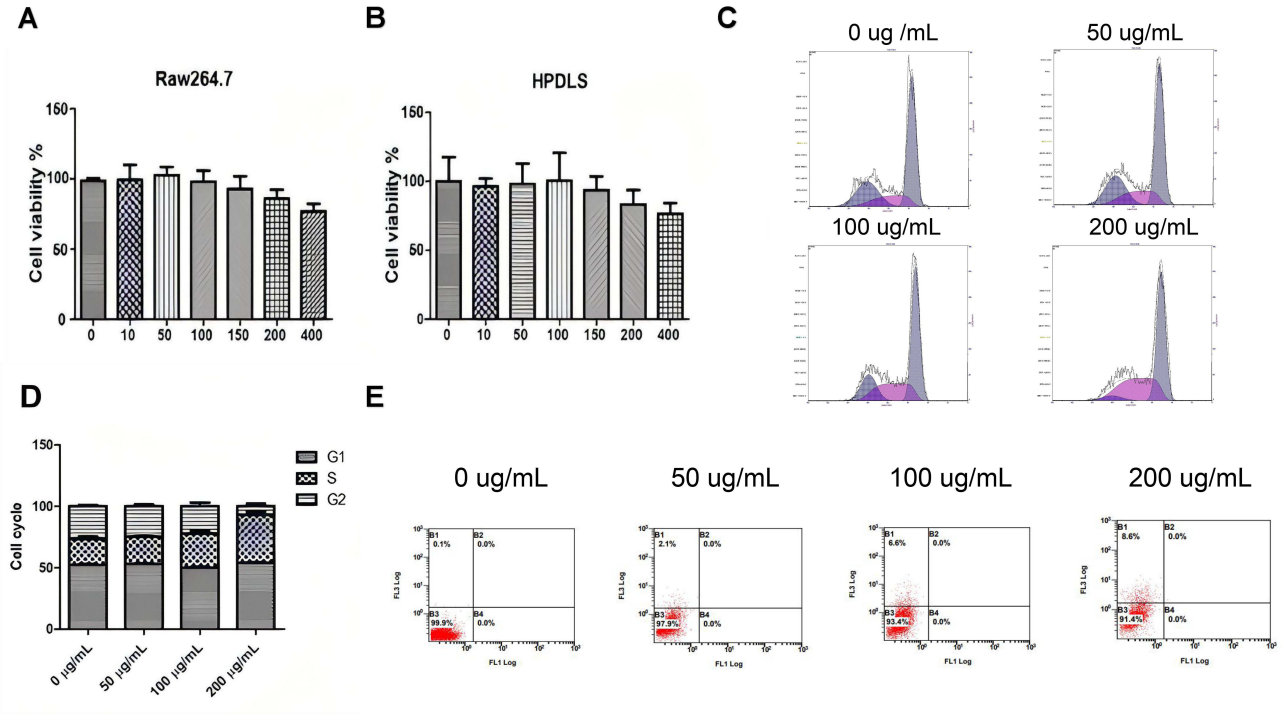

Figure 4 Effects of HME on cell viability. (**A** and **B**). Cytotoxicity of HME. Raw264.7 and HPDLS cells were seeded in 100 μL of culture medium with various concentrations of HME for 24 h and evaluated by CCK8 assays. (**C** and **D**). Cell cycle analysis by flow cytometry. After HPDLS was treated with different concentrations of HME for 24h, the cell cycle was analyzed with FACS. (**E**) Apoptosis analysis of HPDLS after treated with different concentrations of HME. Cells were analyzed by flow cytometry followed by Annexin V-FITC/PI staining.

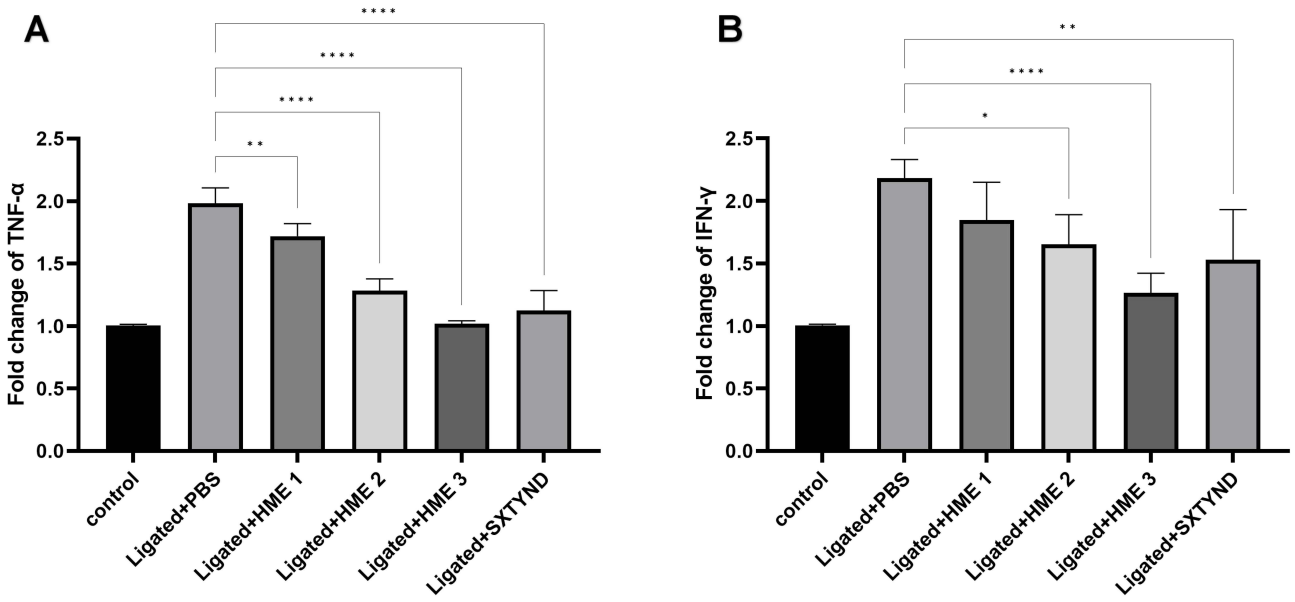

Figure 5 Analysis of inflammatory factor production. HPDLs were treated with HME (50, 100, 200μg/mL) and stimulated with LPS (100ng/mL) for 24 h. ELISA tests were conducted to evaluate the changes in the expression levels of TNF-α (**A**) and IFN-γ (**B**), vs the LPS group, *p <0.05, **p<0.01 and ****p <0.0001. HME1, 50 μg/mL. HME2, 100 μg/mL. HME3, 200μg/mL.

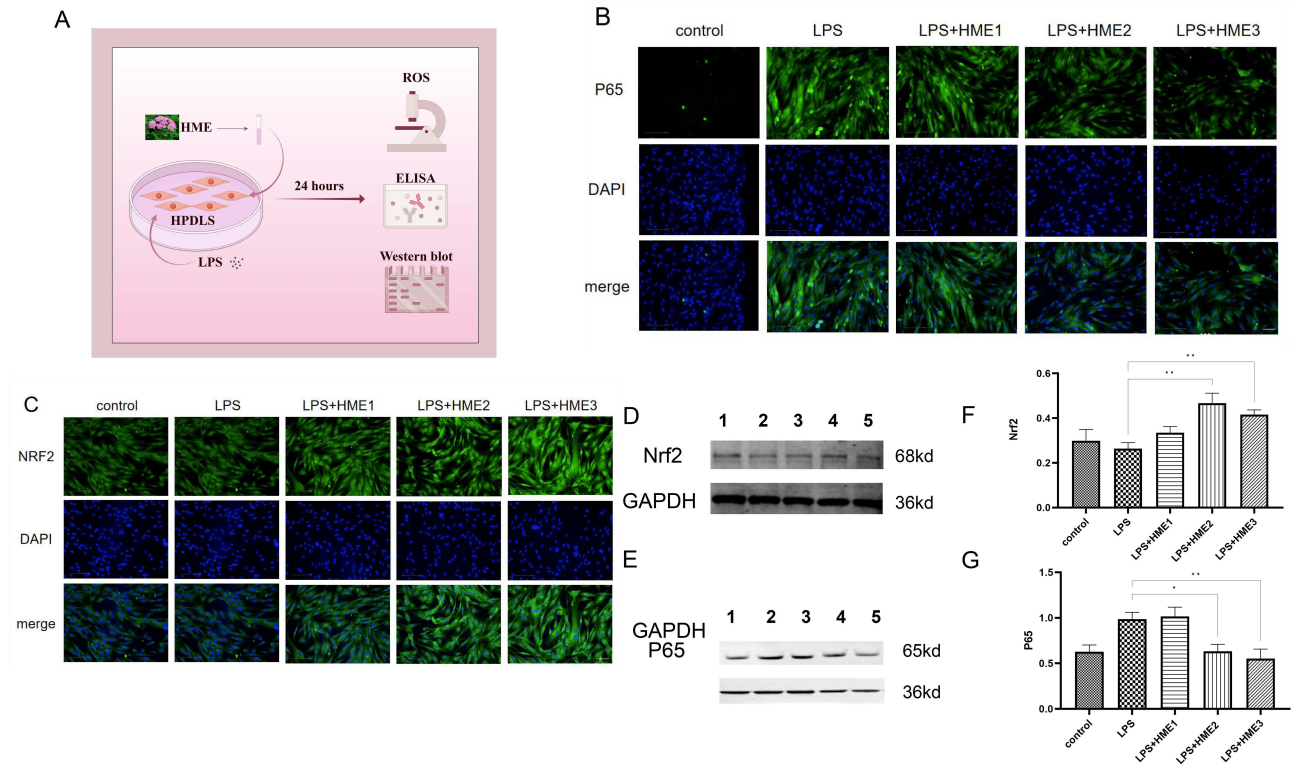

Figure 6 Effect of HME on the NF-κB pathway and Nrf2 expression. (**A**) Cell experiment schematic diagram. (**B** and **C**) Immunofluorescence assays of P65 and NRF2 expression. Scale bar, 50 μm. (**D** and **E**) The P65 and NRF2 proteins were analyzed by Western blotting. GAPDH was used as a protein loading control. 1–5: control, LPS, LPS+HME1, LPS+HME2, LPS+HME3. (**F**) Quantitative grayscale analysis of NRF2. (**G**) Quantitative grayscale analysis of P65 proteins, *p <0.05 and **p<0.01.

Contrary to the above results, both immunofluorescence images and immunoblotting results showed a decrease in NRF2 expression and fluorescence intensity in HPDLs cells after LPS stimulation. After HME treatment, NRF2 expression and fluorescence intensity increased significantly (Figure 6B and D). These results indicate that HME can promote the recovery of the Nrf2/NF-κB signaling pathway.

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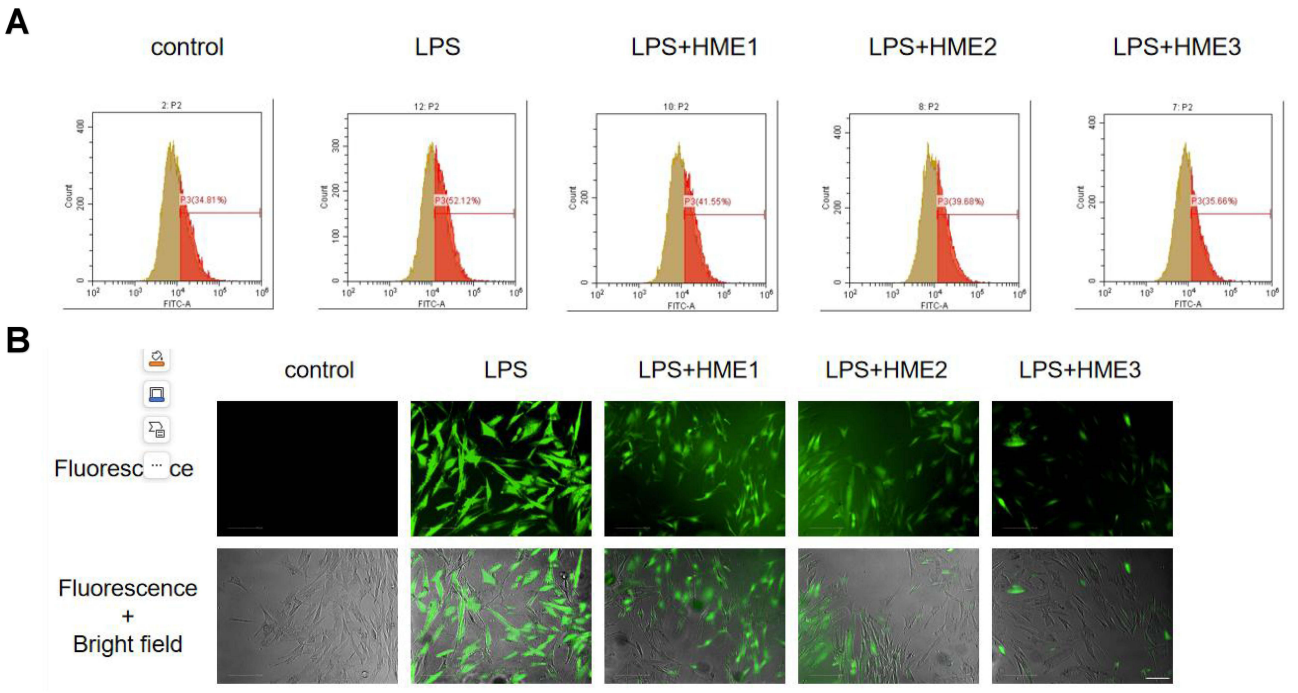

Figure 7 Intracellular ROS assay of HPDLS. (**A**) Cells were determined by the 2′,7′-dichlorofluorescein diacetate (DCFDA) using FACS. (**B**) Intracellular ROS fluorescence image of HPDLS. Scale bar, 50 μm.

Effects of HME on Oxidative Stress

To investigate the role of HME in oxidative stress, we measured changes in intracellular ROS levels. Flow cytometry and fluorescence microscope images showed that the ROS of HPDL inflammatory model cells was increased under LPS induction. HME administration can effectively reduce the expression of ROS (Figure 7).

Discussion

In previous studies, we explored the biological toxicity of HME. We conducted acute toxicity tests on animals and found that HME did not exhibit significant biological toxicity. To further study the mechanism of HME in the treatment of periodontitis, we used HPDLs and Raw 264.7 to detect the cytotoxicity of HME in vitro. It was found that at an HME concentration of 400 μg/mL, after 48 h of treatment, the cell viability was still greater than 80%, indicating that HME had no obvious cytotoxicity. Histological analysis demonstrated characteristic periodontitis features in model rats, including inflammatory cell infiltration, alveolar bone resorption lacunae, and osteoclast proliferation (Figure 1A), accompanied by significantly elevated inflammation scores (**p <0.01) and increased serum concentrations of IFN-γ and other pro-inflammatory cytokines (*p <0.001, Figure 1B–D), collectively validating the successful establishment of the experimental periodontitis model. Notably, the HME used in this study was derived from wild plants in the Changbai Mountain region of China, which is an endemic species in China and is also distributed in Anhui, Zhejiang, Guangxi, and other areas. Compared with traditional Chinese medicinal materials, HME exhibits sustainability and geographical indication characteristics: Changbai Mountain, as an ecological reserve, has a high density of HME and strong regenerative capacity, meeting the demand for large-scale collection while avoiding the risk of resource depletion. Bhattarai et al studied the effect of resveratrol on the activity of gingival fibroblasts and found that when the concentration was lower than 150 μ M it did not cause significant cytotoxicity; Feng Qi et al studied the effect of isorhamnetin on the cell viability of human gingival fibroblast cells (HGFs) and found that isorhamnetin ((10, 20, 40 μM) had no obvious toxicity to HGFs.[20] Li et al found that paeonol can reduce ligation-induced periodontitis in rats and has no cytotoxicity at concentrations below 100 μM.[21] Glycyrrhiza uralensis and its active components inhibit neuronal apoptosis through antioxidation and anti-inflammation.[22] These results indicate that Chinese herbal medicine and its active extracts have the

characteristic of low biological toxicity, so it is of great significance to fully study their anti-inflammatory and other biological activities.

HME regulates the "oxidative stress-inflammation-bone resorption" pattern through "multi-target synergistic effects". Compared to other Chinese herbal medicines that only target a single pathway, the innovative mechanism of HME is reflected in: (1) Reducing the expression of inflammatory factors: HME significantly decreases the production of multiple pro-inflammatory factors; (2) Inhibiting the NF-κB inflammatory signaling pathway; (3) Clearing reactive oxygen species (ROS) and alleviating oxidative damage; (4) Activating the antioxidant pathway (Nrf2 pathway). Periodontal disease is a chronic inflammatory disease caused by bacteria such as *Porphyromonas gingivalis, Forsythia suspensa* and *Treponema denticola*. LPS is the main component of the Gram-negative bacterial extracellular membrane, which can promote the occurrence and development of periodontal disease. LPS and a variety of inflammatory factors cause alveolar bone metabolic imbalance, inhibit osteoblast activity and induce osteoclast activation and proliferation. Pro-inflammatory factors such as IL-1 β, IL-8 and IFN-γ can increase in the early stages of inflammation and promote the occurrence of inflammation, while Th2 cells produce anti-inflammatory cytokines such as IL-4, IL-10, and IL-13, which have immunosuppressive effects and can limit inflammation. In the process of periodontitis, LPS and other factors activate immune responses such as T lymphocytes, macrophages and neutrophils, and regulate proinflammatory cytokines such as IL-1β,[23] IL-6[24] and TNF-α,[25] while inhibiting the expression of anti-inflammatory factors such as IL-4 and IL-13. In our experiment, compared with the control group, the expression of the proinflammatory factor IL-1B/ 2/6/18/GM-CSF/ ICAM1 increased in the model group. In the HME treatment group, the expression levels of these factors decreased. On the contrary, the expression level of anti-inflammatory factor IL-4/13 in the model group was lower than control. After HME treatment, its expression level returned to nearly normal levels.

ROS-mediated oxidative stress may be a key driving factor in periodontal inflammation-related bone loss. Excessive ROS generation can promote osteoclast differentiation by activating the NF-κB pathway, which is highly consistent with our observed results. RANKL is a key cytokine regulating the formation, activation and survival of osteoclasts in bone metabolism. It was found that RANKL played a key role in bone resorption of periodontitis (McDonald et al, 2021). When comparing the RANKL content in gingival crevicular fluid of healthy people and patients with periodontal disease, it was found that the RANKL content in gingival crevicular fluid of patients with periodontal disease was higher than that of healthy people, and the secretion of RANKL tended to increase with the deepening of periodontal disease (Francisconi et al, 2018). Gu found that BV reduced P. gingivalis-induced inflammatory bone loss-related periodontitis and RANKL-induced osteoclast differentiation and activation (Gu et al, 2019). In this study, we found that the expression level of RANKL in periodontal tissue of rat periodontitis model was higher than the normal rats. The use of HME therapy can reduce the expression level of RANKL. This result suggests that HME may have a good regulatory effect on bone resorption in periodontitis.

Based on the above mechanisms, HME possesses unique advantages in treating chronic inflammatory diseases: HME not only reduces RANKL expression to inhibit osteoclast activation but also directly protects the bone matrix by upregulating SOD activity and downregulating MMP-9, demonstrating bidirectional effects and broader clinical application prospects. Recent research suggests that the direct and indirect participation of oxidative stress is also a major factor leading to the destruction of periodontal tissue. ROS are product of oxidative stress in the body. Previous study found a significant decrease of total antioxidant capacity. Meanwhile, the levels of MDA, nitric oxide, TOS, and 8-hydroxydeoxyguanosine significantly increased.[13] This result further strengthens the relationship between oxidative stress and periodontal inflammation. By testing saliva samples from patients with chronic periodontitis, it was found that the levels of total TAS, TOS, 8-OHdG, MDA and OSI in saliva were higher than those in healthy controls, and there was a correlation with an internal self-inflammatory disease familial Mediterranean fever (FMF).[26] When detecting the levels of oxidative stress markers and bone resorption in periapical periodontitis caused by Epstein Barr virus infection, the researchers found that the levels of bone resorption regulators and oxidative stress biomarkers in patients with periapical periodontitis were higher than those in healthy dental pulp tissue.[27] Our research findings also support the fact that inflammation in periodontal tissue increases oxidative stress, bone resorption, and ROS levels in both in vivo and in vitro models. The administration of HME can reduce the expression level of ROS and RANKL. These result suggested that HME can reduce oxidative stress and bone resorption levels in periodontitis models.

Nrf2, as a key transcription factor regulating antioxidant stress, plays an important role in inducing the body's antioxidant response. Researchers have found that Nrf2 plays a defensive role in immune response and inflammation. The downregulation of Nrf2 expression in polymorphonuclear neutrophils in patients with chronic periodontitis can inhibit osteoclast production, regulate intracellular ROS[28] and inhibit periodontal ligament cell apoptosis,[29] thus showing the protective effect of antioxidation.[15] In recent years, researchers have found that Chinese herbal medicine and its active ingredients can affect the progression of periodontitis through the Nrf2 antioxidant pathway. Paeonol, an extract of the cortex of peony, is a natural phenolic compound with anti-inflammatory and antioxidant activities. Li et al found that paeonol treatment in a rat periodontitis model reduced the expression of NF-κB, increased the expression of osteoprotegerin, inhibit the formation of osteoclasts, and enhance the activity of Nrf2.[21] Zhao et al synthesized the monocarbonyl analogue of curcumin and found that it can prevent and treat periodontitis by causing nuclear translocation of Nrf2 and inducing HO-1 to reduce the levels of MDA and ROS.[30] Our study suggests that LPS stimulation can increase the expression of p65 in HPDLs cells and downregulate the expression of Nrf2. Meanwhile, HME treatment markedly alleviated these changes. The above results indicate that Nrf2 may be a potential target for HME treatment of periodontitis. In future research, we will use Nrf2 activators and inhibitors or establish Nrf2 knockout mouse models to conduct more in-depth research on HME.

Conclusion

Our study investigated the therapeutic effects of HME and its potential mechanisms of action using a rat periodontitis model and a lipopolysaccharide-induced inflammatory model of human periodontal ligament cells. We found that HME effectively alleviates inflammatory responses, mitigates oxidative stress, and suppresses alveolar bone resorption through multi-target synergistic effects. Mechanistically, HME activates the Nrf2-mediated antioxidant pathway while inhibiting NF-κB activation and downregulating RANKL expression, thereby suppressing bone loss in periodontitis. The efficacy and safety demonstrated by HME in preclinical studies, coupled with its mode of action, strongly suggest its potential as a candidate drug for treating periodontitis. Subsequent research will focus on elucidating its molecular targets and optimizing treatment protocols to facilitate clinical translation.

Abbreviation

GM-CSF, granulocyte-macrophage colony-stimulating factor; HME, *Hylotelephium mingjinianum* extract; HPDLs, human periodontal ligament cells; ICAM-1, intercellular cell adhesion molecule-1; MDA, malondialdehyde; MMP2, metalloprotease-2; MMP9, metalloprotease-9; NF-κB, nuclear factor kappa-B; Nrf2, nuclear factor E2 related factor 2; Pg, Porphyromonas gingivalis; RANKL, nuclear factor-kappa B ligand; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha.

Data Sharing Statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Ethical Statement

This study involving animals was approved by the Animal Experimental Ethical Inspection of Jilin University (Approval Number: KT201902015). All animal husbandry and experimental procedures were conducted in strict accordance with the regulations governing the management and use of laboratory animals.

Hylotelephium purpureum is a perennial wild herbaceous plant of the Crassulaceae family, growing in mountainous forest understories and grasslands at forest edges. It is distributed in northeastern China, Inner Mongolia, and other regions, and is a non-protected wild plant. The *Hylotelephium purpureum* used in this experiment was prepared by the Jilin Academy of Traditional Chinese Medicine in China, batch number 20120725. Additionally, we ensured that our research adhered to ethical guidelines and regulations throughout the study, emphasizing our commitment to responsible research practices.

Summary

A study finds that *Hylotelephium mingjinianum* extract effectively reduces inflammation and oxidative stress in experimental periodontitis.

Author Agreement Statement

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that they are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. She is responsible for communicating with the other authors about progress, submissions revisions and final approval of proofs.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

There are no conflicts of interest in this work to declare.

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