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ORIGINAL RESEARCH

Isoliquiritigenin Alleviates Periodontitis by Suppressing Inflammation via NF-κB Signaling Pathway of Immune Cell

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Background: Periodontitis, a chronic infectious disease, presents significant treatment challenges due to antibiotic resistance and high recurrence rates, necessitating novel therapeutics.

Methods: Network pharmacology identified ISL's potential targets in periodontitis, focusing on NF-κB signaling pathway. Toxicity was assessed via MTT assay and long-term toxicity studies in vivo. Anti-inflammatory effects were evaluated using enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC), while antibacterial activity was tested in vivo. Micro-computed Tomography (Micro-CT) and hematoxylin and eosin (H&E) staining analyzed periodontal tissue recovery. Western blotting measured NF-κB-p65 and IκB phosphorylation. Molecular docking and dynamics simulations explored ISL's targets.

Results: ISL exhibited low toxicity and reduced IL-6, IL-1β, and TNF-α levels in vitro and in vivo. It demonstrated strong antibacterial effects and mitigated alveolar bone loss. Phosphorylation of NF-κB-p65 and IκB decreased in immune cells, with IKKB (−8.4 docking score) identified as a stable target.

Conclusion: ISL effectively treats periodontitis by combining antibacterial and anti-inflammatory actions, targeting IKKB to suppress NF-κB signaling pathway. This study highlights ISL's therapeutic potential and provides a foundation for developing periodontitis treatments.

Keywords: periodontitis, isoliquiritigenin, network pharmacology, NF-κB signaling pathway

Introduction

Periodontitis, a chronic inflammatory disease characterized by the progressive destruction of the tooth-supporting apparatus, is a significant global health burden.[1] If left untreated, this leads to the destruction of periodontal tissues, ultimately resulting in tooth loss. Periodontitis is the sixth most prevalent disease worldwide and affects half of the global population.[2] Approximately 796 million individuals have severe periodontitis, which significantly impairs their quality of life.[3] Notably, there are a strong association between periodontitis and overall health status, with increased risk factors for diabetes, cardiovascular disease, sleep disorders, and even COVID-19.[4–6] Currently, mechanical debridement, supplemented with antibiotic therapy, is the primary treatment strategy for periodontitis. However, due to the intricate anatomical structure of periodontal tissue, eradicating pathogenic bacteria proves challenging.[7] Although antibacterial agents such as metronidazole and tetracycline could enhance clinical efficacy, they may also foster the development of local oral microbial resistance and disrupt normal oral microbiota.[8] Furthermore, the high recurrence rate of periodontitis suggests that more effective and targeted therapeutic strategies are required.[9] Consequently, it is important to explore novel therapeutic strategies, particularly those involving natural antibacterial agents.

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Isoliquiritigenin (ISL) is a flavonoid compound that is predominantly extracted from the roots of *Glycyrrhiza uralensis* and has been used in traditional Chinese medicine (TCM) for numerous centuries, owing to its extensive range of therapeutic properties.[10] In recent years, ISL has garnered significant attention owing to its diverse pharmacological activities. It has been reported to have anti-cancer, anti-inflammatory, antioxidant and antibacterial properties,[11–13] which makes ISL an ideal candidate for treating various diseases related to inflammation and oxidative stress. Recent studies have shown that ISL can inhibit the growth of *Porphyromonas gingivalis* (*P. gingivalis*), the main pathogen responsible for periodontitis, and inhibit the production of inflammatory mediators in periodontal tissues.[14] In addition, ISL promoted the regeneration of periodontal tissue, further supporting its potential as a periodontitis treatment agent.[15] However, there are no systematic and comprehensive studies on the mechanism of action of ISL in the treatment of periodontitis. More comprehensive research is needed to elucidate the mechanism underlying the effects of ISL on periodontitis and to evaluate its efficacy and safety in clinical practice.

Traditional ways to predict and assess the therapeutic effects and safety of drugs are tedious owing to their immense expense and time.[16] Network pharmacology is an emerging field that combines systems biology with polypharmacology to dissect intricate interactions between drugs and biological systems. It provides a comprehensive view of drug-target-disease networks, facilitating the identification of pivotal targets and pathways in disease pathogenesis.[17–19] In addition, by combining molecular docking, it is possible to predict the targeted site of a drug for a potential protein.[20,21]

In this study, we employed network pharmacology analysis to identify the key targets and pathways involved in the treatment of periodontitis with ISL. We assessed the safety and efficacy of ISL both in vitro and in vivo while also exploring the detailed pharmacological mechanisms underlying its effects in periodontitis. Our findings enhance the understanding of the multi-target pharmacology and delineate the mechanism of ISL in combating periodontitis, setting the stage for the development of more effective treatment strategies for this condition.

Materials and Method

Search for Targets of ISL and Periodontitis

The molecular structures of ISL were obtained from PubChem, and the corresponding targets of action were obtained from literature sources, Comparative Toxicogenomics Database (CTD), SuperPred, Phammapper, and TargetNet databases.[22–26] In terms of therapeutic targets for periodontitis, the initial exploration involved downloading GSE10334 from the GEO database. This dataset contained transcriptomic data from 183 periodontitis samples and 64 healthy samples.[27] The study utilized legally obtained public data. According to Article 32 of China's Ethical Review Measures (2023), the research qualified for exemption from ethical approval. Differential expression analysis was conducted using the limma package with a screening threshold set to |log FC|> [mean (|log FC|) + 2sd (|log FC|)] and a *P*-value of <0.05.[28] We then probed potential targets of periodontitis using the Disgenet, GeneCards, CTD, and OMIM databases. Subsequently, an intersection between the targets of ISL and periodontitis was conducted to identify the potential targets of ISL in the treatment of periodontitis.

Enrichment Analyses of Targets

To investigate the therapeutic role of ISL and the unique biological processes involved in the pathogenesis of periodontitis, we performed enrichment analysis using the cluster profiler package. Disease ontology (DO) enrichment analysis was conducted to compare genes with disease ontology, thereby determining the enrichment of related diseases in a given gene set and revealing the potential therapeutic value of drugs for diseases.[29] Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed to categorize biological functions and analyze whether there was a specific metabolic or signaling pathway enrichment in the gene set, thereby revealing the roles of genes in specific biological processes.[30] Gene ontology (GO) enrichment categorizes genes into those related to biological processes, cellular components, or molecular functions.[31] Gene Set Enrichment Analysis (GSEA) was used to statistically analyze the enrichment of genes in a given expression dataset, in order to identify differences in gene expression and regulatory mechanisms under different conditions, providing important clues for studying specific biological states.[32]

Protein–Protein Interaction Analysis

The overlapping targets of ISL and periodontitis were analyzed using the STRING dataset with species restricted to *Homo sapiens*, and their confidence values were >0.7. This network visualizes the relationships between a disease and drug-related targets through nodes and degree values.

Molecular Docking and Molecular Dynamics Simulation

Molecular docking can be used to identify the contacts between the targeted site and active compounds. The 3D structure of ISL was searched using PubChem (https://pubchem.ncbi.nlm.nih.gov/) and transformed into the PDB format. In addition, it was necessary to attach the hydrogen, binding polar hydrogen, and calculate the charge between the ligands and protein receptors using AutodockTools 1.5.7. IKKB was downloaded from the PDB database. Both were converted into the pdbqt format, and molecular docking was performed using AutoDock Vina 1.1.2. Finally, the mocking model was visualized using PyMol and LigPlot+ 2.4.2.[33,34] MD simulations were performed using the Gromacs software. The simulation process was initiated according to the instructions of pdb2gmx and gmx editconf to switch to the topology file and simulated box. Next, energy minimization and MD simulations were performed using the gmx grompp and gmx mdrun. The docked complexes were predicted based on an investigation lasting for 100 ns. RMSD was calculated using gmx rms to evaluate the binding stability between the protein and molecular.[35]

Cell Culture and Chemical Agents

Raw264.7 (ATCC, T1B-71) were cultured in DMEM high sugar medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were cultured at 37°C in a humidified incubator with 5% CO_2. ISL (Macklin Biochemical Technology Co., Ltd, Shanghai) was dissolved in ethanol absolute and stored at –20°C. LPS (5 μg/mL; Sigma, USA) was used to induce inflammation in macrophages.

Western Blotting

Cells treated with LPS and ISL were cultured and lysed in radioimmunoprecipitation assay (RIPA) buffer (Thermo Fisher Scientific, USA) with a phosphatase and protease inhibitor cocktail. The lysate was centrifugated at 12,000 rpm and 4°C for 15 min, and the supernatant was transferred to a new tube. The protein concentration of the lysate was determined using the BCA Protein Assay Kit (NCM Biotech, China) according to the manufacturer's instructions. The sample was denatured by 5 × SDS-PAGE Loading Buffer at 98°C for 15 min. Subsequently, proteins of different weights were separated by 10% SDS-PAGE and transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore Corporation, Billerica, USA), which was then blocked with 5% BSA for 1 h at room temperature (RT). Then, the membrane was incubated with a primary antibody at 4°C overnight and secondary antibody (1:10,000) (SA00001-1) at RT for 1 h. IκB (1:1000) (10268-1-AP), p-IκB (1:1000) (82349-1-RR), NF-κB-p65 (1:1000) (10745-1-AP), p-NF-κB-p65 (1:1000) (82335-1-RR), and GAPDH (1:10000) (60004-1-Ig) antibodies were purchased from Proteintech. The membrane was then washed with TBST three times for 5 min at least after primary and secondary antibodies. Blots were visualized using an ECL reagent (NCM Biotech, China) and Developer (GelView 600Pro II, BLT, China).

Enzyme-Linked Immunosorbent Assay

A total of 1×10^5 cells were seeded in 12-well plates and incubated for 24 h, and LPS was used to promote inflammation. The cells were treated with ISL (10 and 20 μM) and minocycline hydrochloride ointment (5μM) for 12 h. The supernatant was collected and the levels of IL-1β, IL-6, and TNF-α were determined using an ELISA kit according to the manufacturer's instructions (Protein Tech, China). For assessment of inflammatory response in the animal model, blood samples were collected and centrifuged to obtain serum. Rat serum samples were then analyzed according to the manufacturer's protocols (MULTI SCIENCES, China).

In vitro and in vivo Viability Assay

The toxicity of ISL was assessed using the MTT assay and Animal Toxicology Studies. A total 1×10^3 cells were seeded in 96-well plates. After 24 h, cells were treated with ISL with different concentration (0, 2.5, 5, 10, 20, 40 μM) for 24 h. Next, cells were treated with 0.5 mg/mL MTT for 4 h to form insoluble dark blue formazan. After incubation, formazan was dissolved in DMSO, and the OD at 560 nm was measured using a microplate reader (Bio-Rad, USA). For animal toxicology studies, 2.5 mg and 5 mg ISL were administered to healthy rat via gavage once daily for 2 weeks. Liver Function Tests (LFTs), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Total Bilirubin (TBIL), Direct Bilirubin (DBIL), Albumin (ALB), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (γ-GT), Total Bile Acids (TBA) and histopathological examination of different organs were performed for the toxicity assessment.

Antibacterial Testing of ISL in a Periodontitis Rat Model

For the in vivo antibacterial evaluation of ISL, a suspension consisting of 1 mL of *P. gingivalis* suspension (1×10^6 CFUs/mL) mixed with 0.02 g of carboxymethyl cellulose (CMC) powder was applied and inoculated onto the tooth surfaces and gingival tissues of the experimental rat model. The rats were randomly allocated into five groups (n = 6 per group): (1) health, (2) experimental periodontitis (untreated), (3) experimental periodontitis treated with minocycline hydrochloride ointment (20% w/v, containing 10 mg) (positive control), (4) experimental periodontitis treated with Pluronic F127 (25% w/v) containing 2.5 mg ISL (PF127-ISL-2.5), and (5) experimental periodontitis treated with Pluronic F127 (50% w/v) containing 5 mg ISL (PF127-ISL-5). Gingival crevicular fluid (GCF) was collected from the experimental rat model and diluted in PBS. Subsequently, 10 μL of the diluted solution was plated onto blood agar solid plates. After overnight incubation at 37°C in anaerobic incubator, the number of colonies in each group was counted to assess bacterial load.

Animal Model Construction of Periodontitis and Drug Treatment

Male SD rats (180 ± 20 g, male) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. All experimental procedures were approved by the Animal Welfare and Biomedical Ethics Committee of Ji Nan University (Approval No. IACUC-20250217-10) and conducted in strict accordance with the 3R principles—Replacement, Reduction, and Refinement. Rats were randomly divided into five groups (n = 6): (1) healthy control, (2) experimental periodontitis, (3) experimental periodontitis treated with minocycline hydrochloride ointment (20% w/v, containing 10 mg) (positive control), (4) experimental periodontitis treated with mixed Pluronic F127 (25% w/v, containing 2.5 mg ISL) (PF127-ISL-2.5), and (5) experimental periodontitis treated with mixed Pluronic F127 (50% w/v, containing 5 mg ISL) (PF127-ISL-5). A periodontal inflammation model was constructed using the classical silk thread ligation method.[36] Briefly, a sterile wire rope was tied to the maxillary second molar. After 3 weeks, the treated mice were completely covered with 50 μL PF127-ISL-2.5, and 50 μL PF127-ISL-5 three times and minocycline hydrochloride ointment per week for 2 weeks. At the end of the experiment, the rats were sacrificed, and serum and bilateral maxillae were collected.

Histological Analysis and Immunohistochemistry

For histological analysis, the alveolus tissues were fixed in 4% paraformaldehyde, dehydrated through ethanol, embedded in paraffin, and sectioned at 5 μm thickness. Hematoxylin and eosin staining were performed and histological images of were captured to assess the structural features of periodontal tissues using Pannoramic MIDI (3DHISTECH). The sections after deparaffinization with xylene and rehydration underwent heat-induced epitope retrieval in citrate buffer (pH 6.0) at 95°C for 20 minutes, followed by treatment with 3% hydrogen peroxide in methanol for 15 minutes to quench endogenous peroxidase activity. Next, it was blocked with 5% bovine serum albumin (BSA) in PBS for 1 hour at room ttemperature, and then were incubated at 4°C overnight with IL-1β (1:100) (ab315084), IL-6 (1:100) (ab9324), and TNF-α (1:100) (ab307164) in a humidified chamber. After PBS washes, sections were treated with HRP-conjugated secondary antibody (ab6734) (1:500) for 1 hour at room temperature. Diaminobenzidine (DAB) substrate was applied for 5 minutes,

followed by counterstaining with Mayer's hematoxylin. Stained sections were visualized and photographed under a Pannoramic MIDI (3DHISTECH).

Micro-Computed Tomography Analysis

The left/right maxillae were fixed with 4% neutral paraformaldehy (PFA) for 24 h and stored in 70% ethanol at 4°C for further analysis. The images were visualized using a micro-CT scanner (μCT40, Scanco Medical AG, Bassersdorf, Switzerland) with the following key parameters: source voltage of 55 kV, current of 145 μA, and resolution of 15.0 μm. Finally, the alveolar bone loss was assessed according to linear distance from cementoenamel junction (CEJ) to alveolar bone crest (ABC) referring to Li et al by Skyscan Data viewer software.[37] The parameters, including mean bone volume (BV), bone volume/tissue volume (BV/TV), and trabecular thickness (Tb.Th) were determined using CT-Analyzer software.

Statistical Analysis

All statistical analyses were performed using R software (version 4.3.0) and GraphPad Prism 5. Group differences were analyzed using a one-way analysis of variance (ANOVA). All data are presented as mean ±SEM. Statistical significance was set $P < 0.05$.

Result

Potential Targets of ISL and Periodontitis

A total of 470 potential targets for ISL were obtained from the literature, CTD, Superpred, Phammapper and TargetNet databases (Figure 1A). First, DO enrichment of the relevant targets indicated that ISL plays an important role in various oral diseases, including oral cavity cancer, stomatitis, periodontal disease, and periodontitis (Figure 1B). KEGG enrichment revealed that ISL could exert pharmacological effects through pathways such as the AGE-RAGE signaling pathway in diabetic complications, the toll-like receptor, and the NF-κB signaling pathway (Figure 1C). In addition, the regulation of inflammatory and protein serine/threonine kinase activity was enriched in the GO enrichment analysis (Figure 1D), which also illustrated that ISL was strongly linked to inflammatory reactions.

Furthermore, analysis of the GSE10334 data yielded 655 upregulated and 353 downregulated genes in periodontitis, as shown in Figure 2A and B. GSEA enrichment analysis showed that multiple inflammatory pathways were activated in periodontitis, supporting a strong inflammatory response in periodontitis (Figure 2C and D).

Obtainment of Coincident Targets by PPI Networks Analysis

A total of 2002 potential therapeutic targets were identified by combining the treatment targets for periodontitis retrieved from the Disgenet, GeneCard, CTD, and OMIM databases (Figure 3A). There were 139 overlapping targets that determined the intersection of the 470 ISL-related targets and 2002 periodontitis-related targets presented by Venny (Figure 3B). PPI network analysis predicted the relationships between various disease-related targets. The PPI network showed 120 nodes and 142 edges, where the size and color of the nodes varied based on their degree values (Figure 3C). In addition, the top 30 proteins were enriched (Figure 3D), which are particularly important in inflammation because of the enriched nodes, including IL-6, IL-10, NFKBIA, and LTR4. KEGG analysis showed that the MAPK, PI3K-Akt, and NF-κB signaling pathways were crucial targets (Figure 3E). Regulation of the immune response and kinase activity were enriched in GO analysis (Figure 3F). Thus, inflammation may be a potential target in ISL treating periodontitis treatment.

ISL is Non-Toxic for Raw264.7 Cells and Rats

It is well known that Raw264.7 cells exhibit a crucial role in inflammation, such as periodontitis, so drug treatment targeting Raw264.7 cell is necessary. To confirm the cytotoxicity of ISL on Raw264.7 cells, an MTT assay was performed. The results showed no impact for Raw264.7. After treatment with 2.5, 5, 10, and 20 μM ISL, more than 95% of cells were viable. However, high concentrations of ISL (40 μM) slightly inhibited cell proliferation with only 10% mortality of Raw264.7 cells (Figure 4A), which was negligible. Besides, the H&E staining and blood routine test

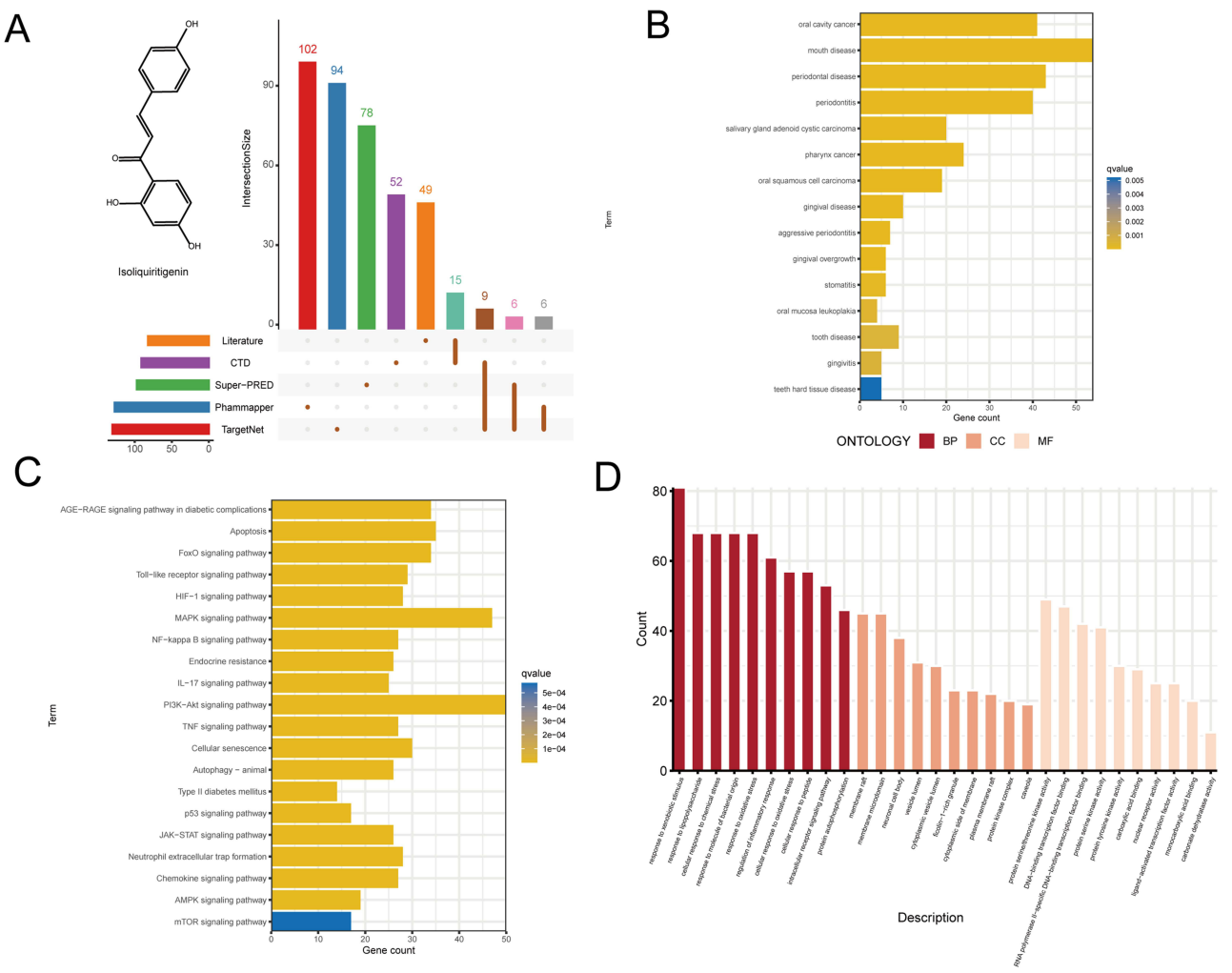

Figure 1 The target and signaling pathway prediction for ISL. (**A**) Structural and related targets of ISL (**B**) DO (disease ontology) of ISL (**C**) KEGG (Kyoto Encyclopedia of Genes and Genomes) of ISL (**D**) GO (gene ontology) enrichment of ISL.

parameters of rat also showed ISL was non-toxic (Supplemental Figure 1A and B). Thus, ISL is safe and expected to be an effective drug for the treatment of periodontitis.

Inflammatory Respond is Reduced with ISL Treatment in LPS-Induced Immune Cell Model and Ligature-Induced Periodontitis Rat Model

Accumulating evidence has shown that the systemic inflammatory response is triggered by proinflammatory cytokines including interleukin 1 (IL-1), tumor necrosis factor-alpha (TNF-α) and IL-6 and so on.[38] In our LPS-induced immune cell model, we found a significant increase of IL-6, IL-1β and TNF-α by ELISA in LPS-induced immune cell model, while IL-6, IL-1β and TNF-α were reduced with 20 μM ISL treatment (Figure 4B). In the rat model of inflammation, IL-6, IL-1β, and TNF-α levels in the serum and periodontal tissues were decreased after ISL treatment (Figure 5A and B). In addition, the increasing CEJ-ABC distance in the periodontitis group compared to that in the control group confirmed that periodontitis modeling was completed successfully (Figure 6A and B), and the images showed that distance at the central sites on the alveoli of the maxillary first and second molars decreased with ISL treatment, which illustrated alleviation of alveolar bone loss after ISL treatment (Figure 6A and B). In addition, the retained bone volume (BV), trabecular thickness (Tb.Th), and trabecular bone number (Tb.N) were increased with ISL treatment compared to those in the periodontitis group (Figure 6B). H&E analysis also showed

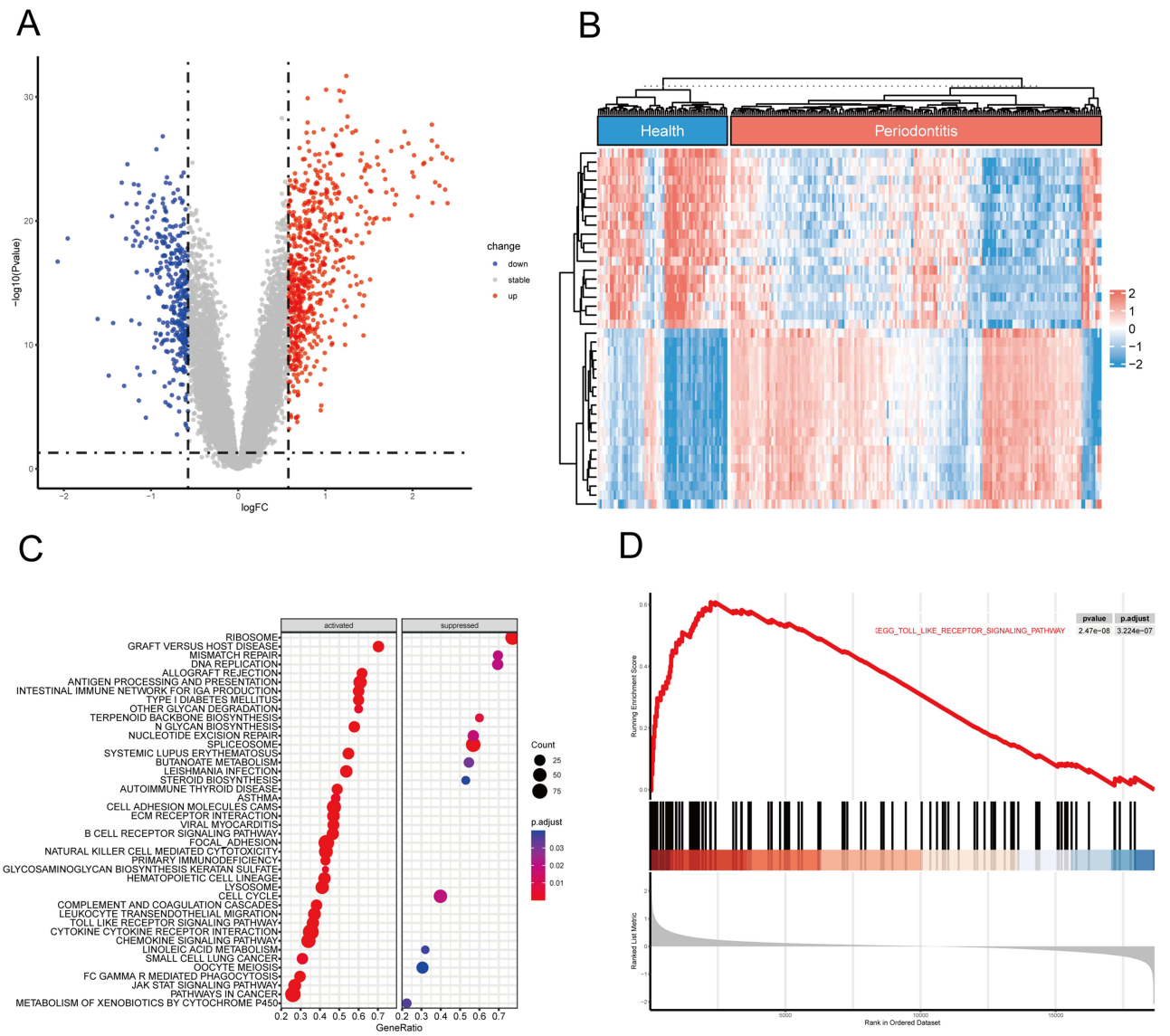

Figure 2 The targets and signaling pathway prediction for periodontitis. (**A**) Volcano plot of periodontitis vs healthy (**B**) Heatmap of periodontitis vs healthy (**C**) GSEA enrichment of periodontitis (**D**) Toll-like signaling receptor signaling pathway in periodontitis.

that ISL exhibited markedly reduced inflammatory cell density, preserved tissue architecture, and minimal vascular congestion (Figure 6C). Thus, ISL effectively prevents the destruction of periodontal tissues caused by a strong inflammatory response of immune cells.

ISL Has an Excellent Antibacterial Effect in vivo

In the bacterial infectious rat model, we observed that ISL exhibits significant antibacterial activity against *P. gingivalis*. In the periodontitis rat model, the bacterial load was notably higher compared to the healthy control group. Remarkably, ISL demonstrated a concentration-dependent inhibition of *P. gingivalis* growth. Specifically, the treatment of experimental periodontitis with a mixed formulation of Pluronic F127 (50% w/v) containing 5 mg ISL (PF127-ISL-5) showed pronounced therapeutic efficacy, exhibiting effects comparable to those of the positive control (Figure 7A and B).

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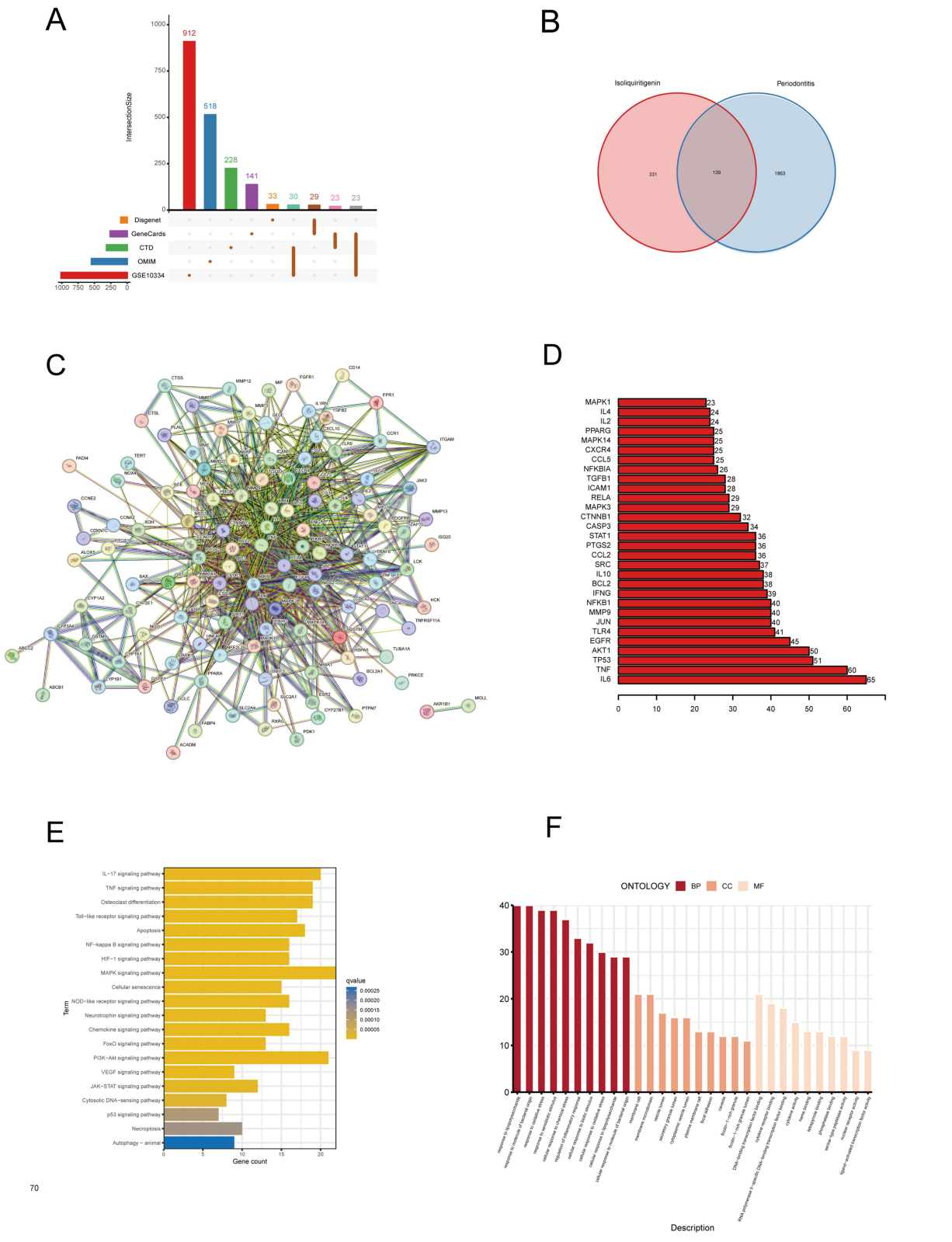

Figure 3 The network analysis for ISL and periodontitis. (**A**) Structural and related targets. (**B**) Venn diagrams (**C**) PPI (protein–protein interaction) Networks periodontitis. (**D**) Ranking of protein in PPI (**E**) KEGG enrichment of ISL and periodontitis (**F**) GO enrichment of ISL and periodontitis.

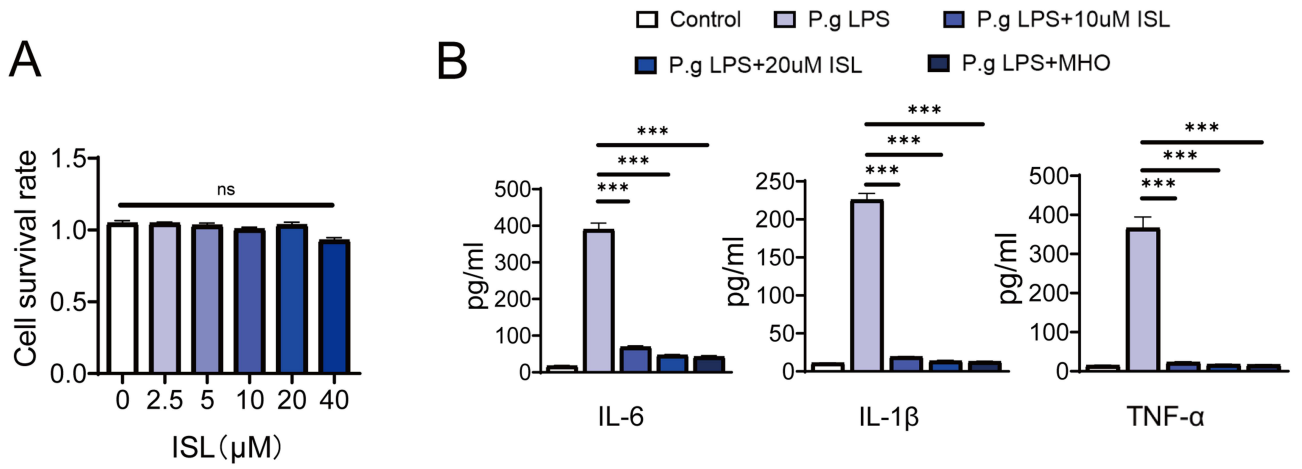

Figure 4 ISL is non-toxic and exhibits anti-inflammatory effects on immune cells. (**A**) The MTT assay to confirm the cytotoxicity of different concentration ISL in Raw264.7 cells (**B**) Levels of inflammatory cytokines (IL-6, IL-1β, and TNF-α) in Raw264.7 cells treated with different concentrations of ISL. MHO: Minocycline hydrochloride ointment (5 μM). Significance was determined by ANOVA and indicated as ***$P < 0.001$.
Abbreviation: ns, not significant.

ISL Inhibits the Inflammation by Downregulating NF-κB Signaling Pathway of Immune Cell

ISL inhibits innate immune responses in vivo.[39] The potential mechanisms underlying ISL in periodontitis require further investigation. The NF-κB signaling pathway is a potential target of ISL and periodontitis, which also is associated with inflammatory.[40] Thus, we performed WB to detect the phosphorylation levels of the key factors NF-κB-p65 and IκB. As expected, our results suggested that the phosphorylation of NF-κB-p65 and IκB was significantly upregulated in LPS-induced Raw264.7 cells, and was reduced after ISL treatment (Figure 8). These results showed that ISL downregulated the NF-κB signaling pathway to inhibit the LPS-induced inflammatory response in immune cells.

IKKB is Potential Target of ISL

To further investigate how ISL inhibits the NF-κB signaling pathway, molecular docking was performed to predict the potential target and binding mode(s).[41] In our study, the crucial component of IκB kinase (IKK), IKKB, was found to be a potential target of ISL by molecular docking (Figure 9) with a high score (–8.4) by PLIP. To clarify the protein-ligand stability and movement between the docked complex of IKKB and ISL, we performed a 100 ns MD simulation using Gromacs.[35] The results showed the ligand was stable after 25 ns by RMSD (root mean square deviation) though fluctuating within 0.3 nm, which was within the acceptable range. The RMSD value illustrates the stable bonding between IKKB and ISL (Figure 10A). In addition, the H-bond represents a stable state for IKKB and ISL (Figure 10B). In summary, ISL was anticipated to inhibit the NF-κB signaling pathway and decrease the inflammatory reaction by targeting IKKB in immune cells.

Discussion

In the present study, our findings demonstrate that ISL significantly inhibits bacterial proliferation and attenuates immune cell-mediated inflammatory responses by targeting the NF-κB signaling pathway (Figure 11). These experimental results are consistent with our network pharmacology predictions, further supporting ISL's potential as a promising therapeutic candidate for periodontitis through its regulatory effects on the NF-κB signaling pathway. The dual mechanisms of ISL in exerting both anti-inflammatory and antibacterial activities highlight its critical role in periodontal therapy and provide substantial implications for developing novel treatment strategies for periodontitis.

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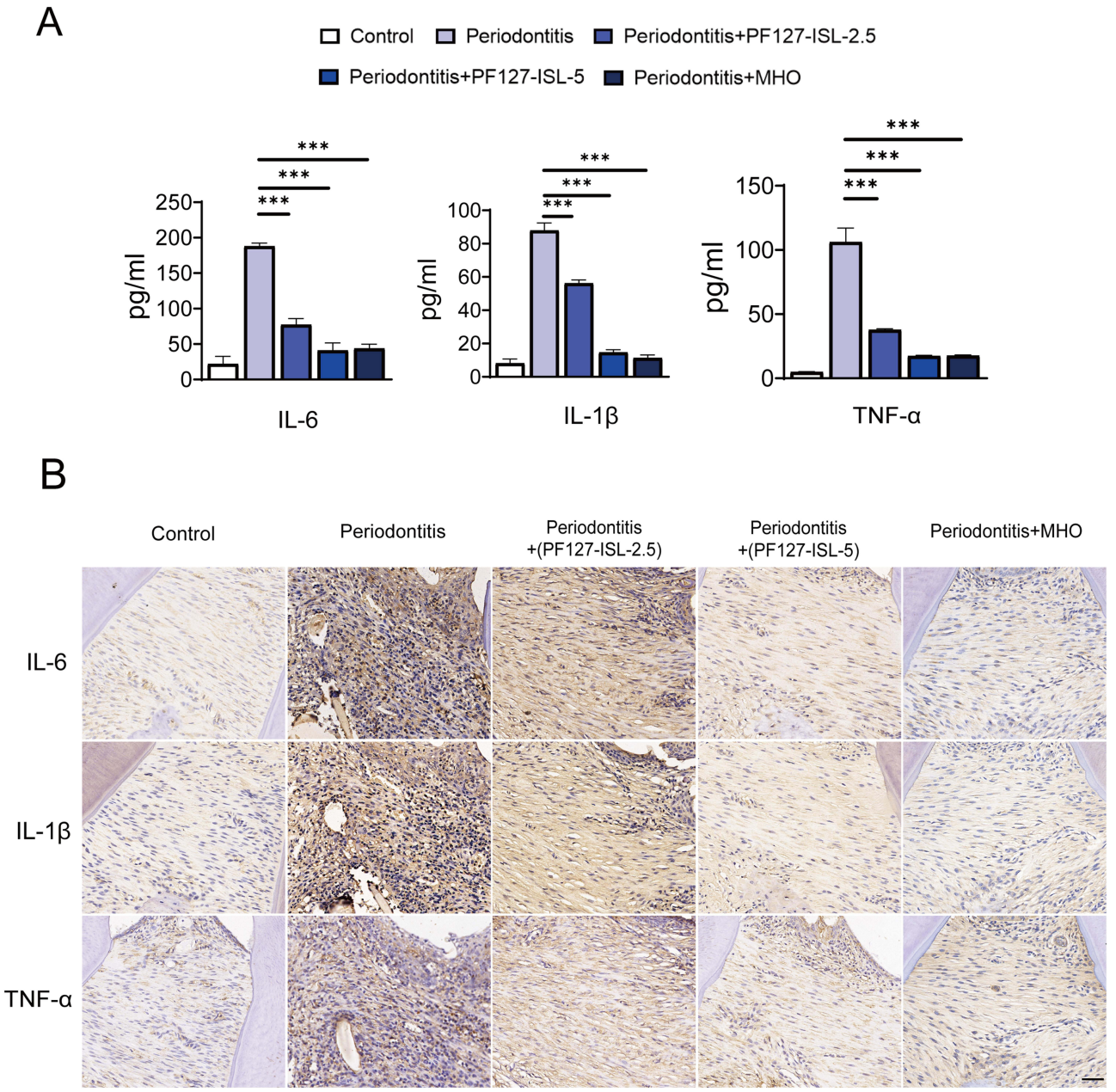

Figure 5 ISL reduces inflammation in a periodontitis animal model. (**A**) Serum levels of inflammatory cytokines (IL-6, IL-1β, and TNF-α) in rats treated with different concentrations of ISL (**B**) Expression levels of IL-6, IL-1β, and TNF-α in periodontal tissues of rats treated with different concentrations of ISL by IHC. Scale bars: 50 μm, MHO: Minocycline hydrochloride ointment (20% w/v, containing 10 mg). Significance was determined by ANOVA and indicated as ***$P < 0.001$.

Network pharmacology, as an effective tool, has been widely utilized to identify the targets of drugs for various diseases.[42] An analysis combining network pharmacology with molecular docking revealed some key targets of *Baihe Zhimu* decoction under postpartum depression (PPD) treatment.[43] Muhammad Sadaqat et al revealed multi-pathway and multi-gene regulatory molecular actions of *Bacopa monnieri* in liver cancer through advanced network pharmacology, finding potential therapeutic targets, including HSP90AA1 and JUN.[44] In addition, Cheng et al identified that *Buyang Huanwu Decoction* (BYHWD) plays a critical role in regulating apoptosis and further validated its anti-apoptotic effects

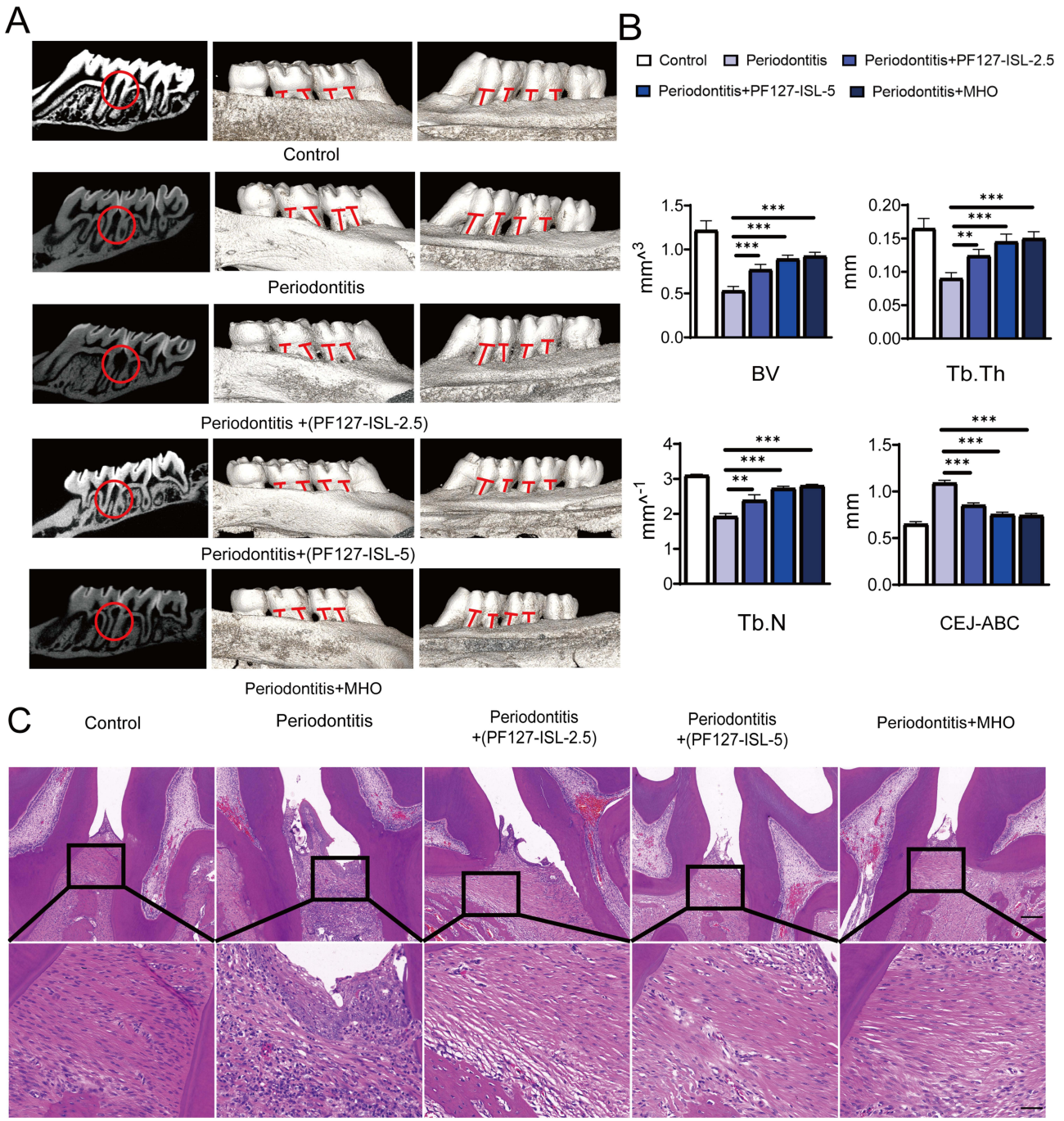

Figure 6 Micro-CT and histological assessment of ISL effects on periodontal tissues. (**A**) Representative micro-CT images demonstrating the impact of varying ISL concentrations on maxillary molars, showing the region from cementoenamel junction (CEJ) to alveolar bone crest (ABC) (**B**) Quantitative analysis of periodontal parameters: bone volume (BV), trabecular thickness (Tb.Th), and trabecular number (Tb.N) and CEJ-ABC distance following treatment with different ISL concentrations (**C**) H&E staining of periodontal tissues treated with varying ISL concentrations. Scale bars: 200 μm (top) and 50 μm (bottom), MHO: Minocycline hydrochloride ointment (20% w/v, containing 10 mg). Significance was determined by ANOVA and indicated as $^{**}P < 0.01$, $^{***}P < 0.001$.

through network pharmacology analysis.[45] In our current study, this approach has proven effective in identifying critical therapeutic agents, ISL, and their molecular pathways and targets for periodontitis treatment. These representative studies collectively highlight the crucial role of network pharmacology in disease treatment research. Given its high efficiency and accuracy in target identification and drug discovery, the broader application of this approach across different diseases should be strongly encouraged in pharmacological research.

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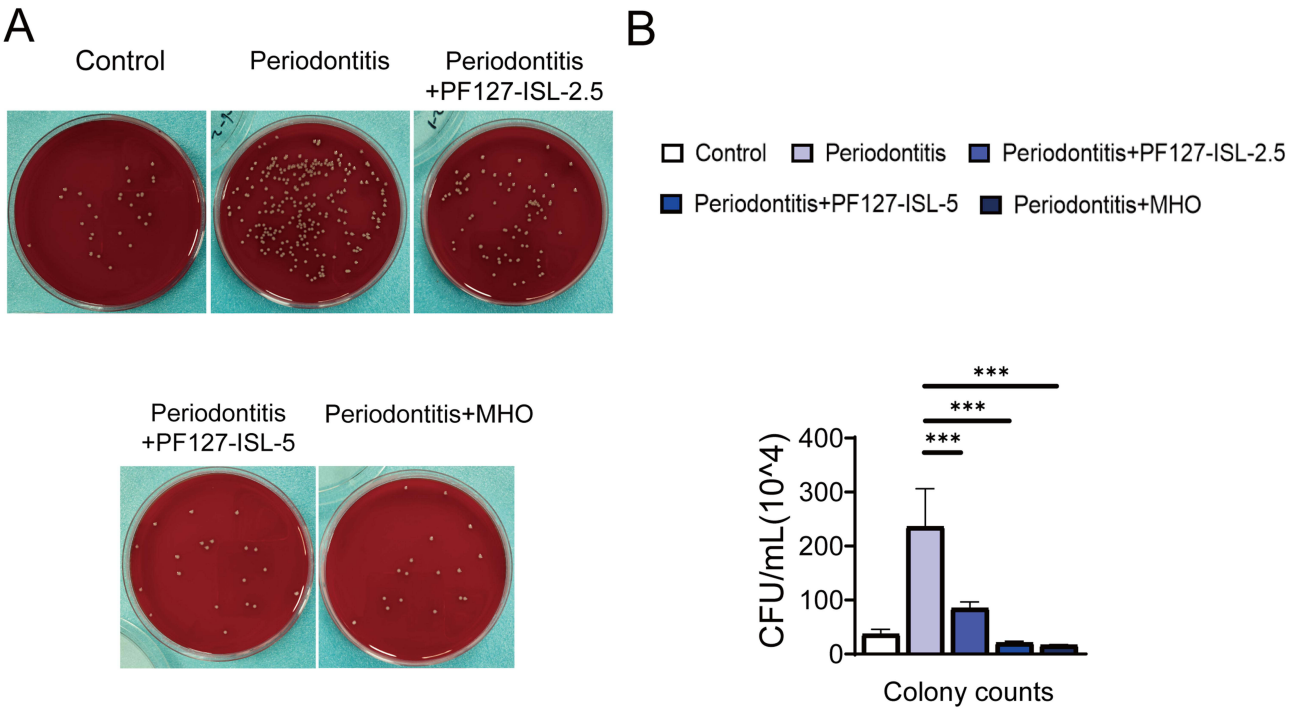

Figure 7 The ISL exhibits an excellent antibacterial effect in vivo. (**A**) Bacterial colony counts in rat periodontal tissues following treatment with varying concentrations of ISL. (**B**) Quantitative analysis of colony counts in rat periodontal tissues treated with different ISL concentrations. MHO: Minocycline hydrochloride ointment (20% w/v, containing 10 mg). Significance was determined by ANOVA and indicated as ***$P < 0.001$.

Periodontitis is one of the most common diseases that severely impairs oral health and reduces quality of life.[46] Reports have shown that periodontitis is linked to a number of systemic diseases such as increased morbidity and mortality due to diabetes, obesity and osteoporosis.[47] Thus, there is an urgent need to identify appropriate treatments for this condition. ISL is a bioactive ingredient in plants that has been used for disease treatment.[48] Numerous studies have reported its anticancer and anti-inflammatory effects in various diseases.[48] We found that ISL exhibits the anti-inflammatory in periodontitis treatment with significantly reduced immune factors including IL-6, IL-1β, and TNF-α in LPS-induced Raw264.7 and periodontitis rat model. The NF-κB signaling pathway being revealed by GO analysis is crucial for the ISL treatment of periodontitis, which has been consistently demonstrated in periodontitis.[49] The results showed that NF-κB signaling pathway was inhibited with ISL treatment. More importantly, the detailed mechanism refers to the reduced p-NF-κB-p65 and p-IκB in LPS-reduced Raw264.7 with ISL treatment. Thus, ISL could inhibit the NF-κB signaling pathway by reducing p-NF-κB-p65 and p-IκB, resulting in the suppression of inflammatory processes with reducing the expression of IL-6, IL-1β, and TNF-α. However, the targeted protein of ISL is still uncovered. Molecular docking and MD simulation analyses indicated that IKKB is a potential target of ISL, making it a promising candidate for rational drug design. However, the specific binding sites on IKKB remain to be elucidated.

Oral pathogens, the main murderers of periodontitis, exert detrimental effects by triggering severe inflammatory responses. These responses contribute to the destruction of periodontal tissues, exacerbating the progression of the disease.[50] The current clinical treatment for periodontitis involves in antimicrobial agents. However, antibiotic resistance of oral pathogens has rendered many antibiotics ineffective. The reports have demonstrated that ISL exhibits broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, which could disrupt bacterial cell membranes, inhibit biofilm formation, and interfere with bacterial metabolic pathways.[51–53] *P. gingivalis*, a prevalent periodontal pathogen, is capable of producing various virulence factors, resulted in the strong inflammatory.[54] We found that the growth of *P. gingivalis* was inhibited by ISL in vivo. Thus, ISL also exhibits good antimicrobial activity in periodontitis treatment.

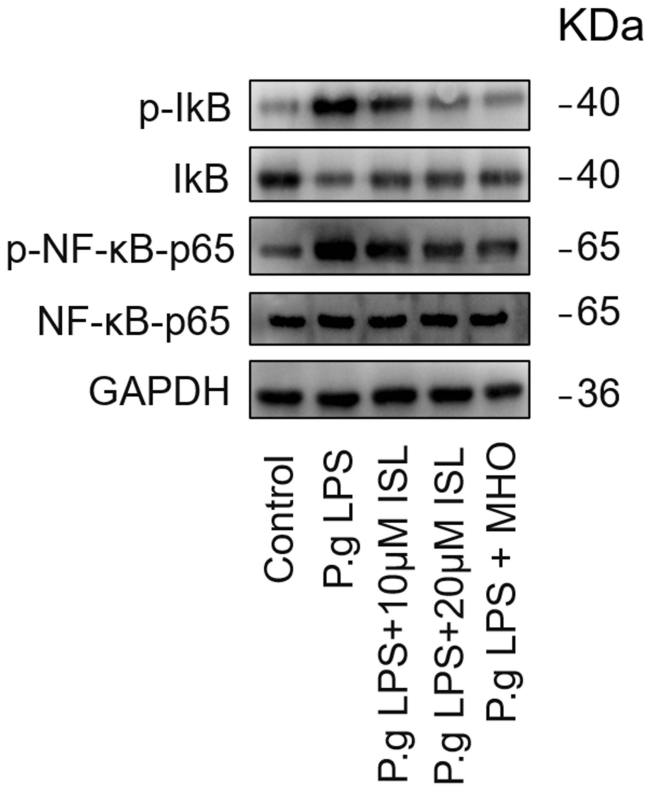

Figure 8 ISL inhibits NF-κB signaling pathway in LPS-induced cell by decreasing the phosphorylation of IκB and NF-κB-p65. MHO: Minocycline hydrochloride ointment (5 μM).

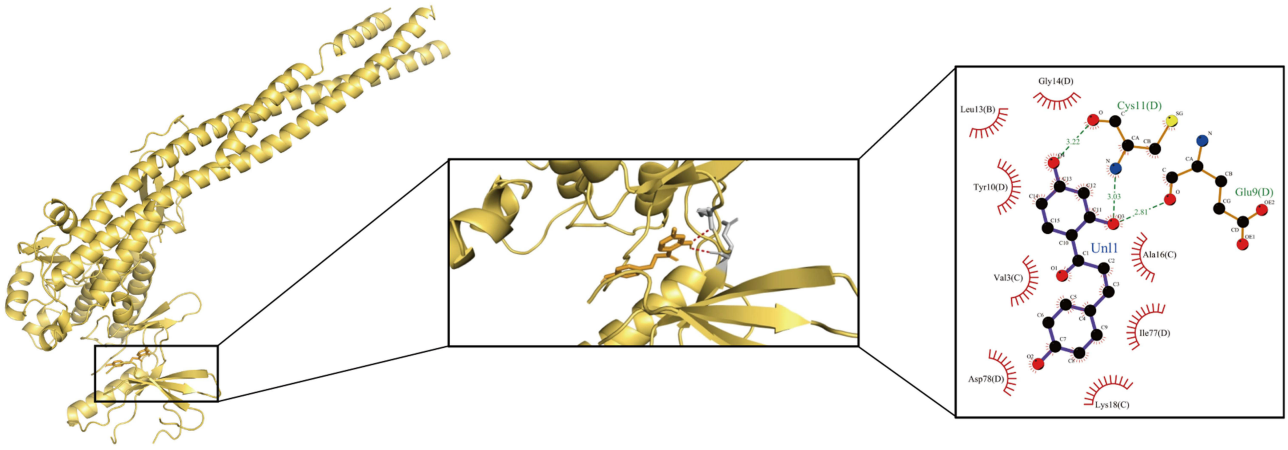

Figure 9 Molecular docking of ISL and IKKB. The ISL was combined with different amino acid of IKKB.

Isoliquiritigenin (ISL) is a promising natural compound with dual anti-inflammatory and antimicrobial activities. Its ability to modulate inflammatory pathways and inhibit microbial growth makes it a potential therapeutic agent for inflammatory diseases and infections. Our research emphasizes the significance of network pharmacology in disease treatment. Additionally, we found that ISL exhibits anti-inflammatory properties and effective antimicrobial activity in the treatment of periodontitis while also uncovering its anti-inflammatory mechanisms. One limitation of this study is the lack of clinical trials, which may restrict the generalizability of our findings. Future research could address this by applying ISL in patients with periodontitis.

https://doi.org/10.2147/JIR.S505554

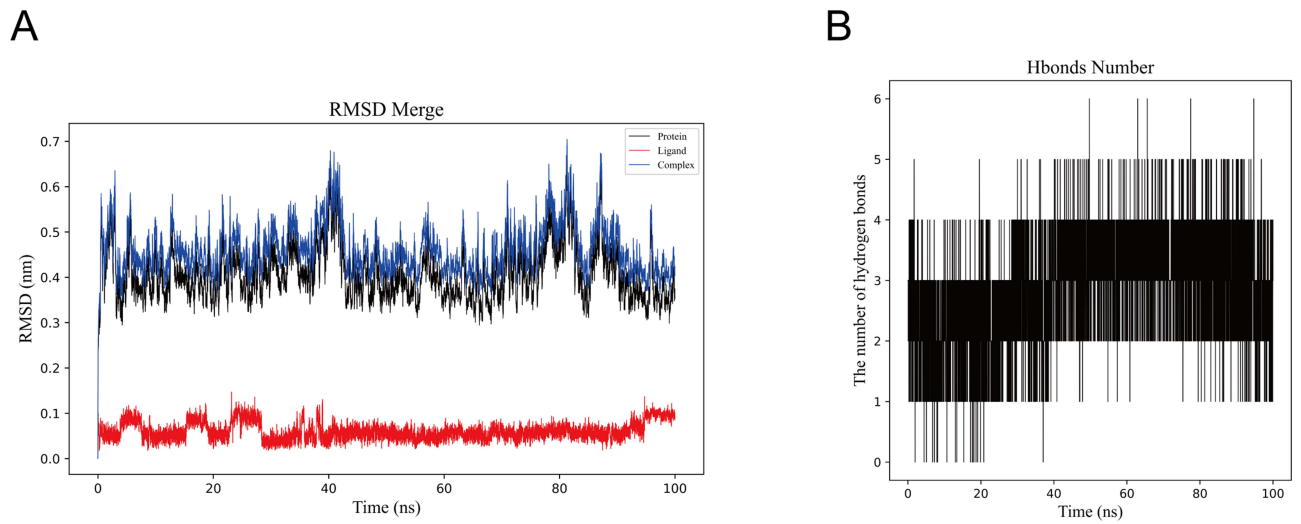

Figure 10 MD stimulation between ISL and IKKB for 100 ns. (**A**) RMSD of ISL with the active site of target proteins IKKB (**B**) H-bond between ISL and IKKB for 100 ns.

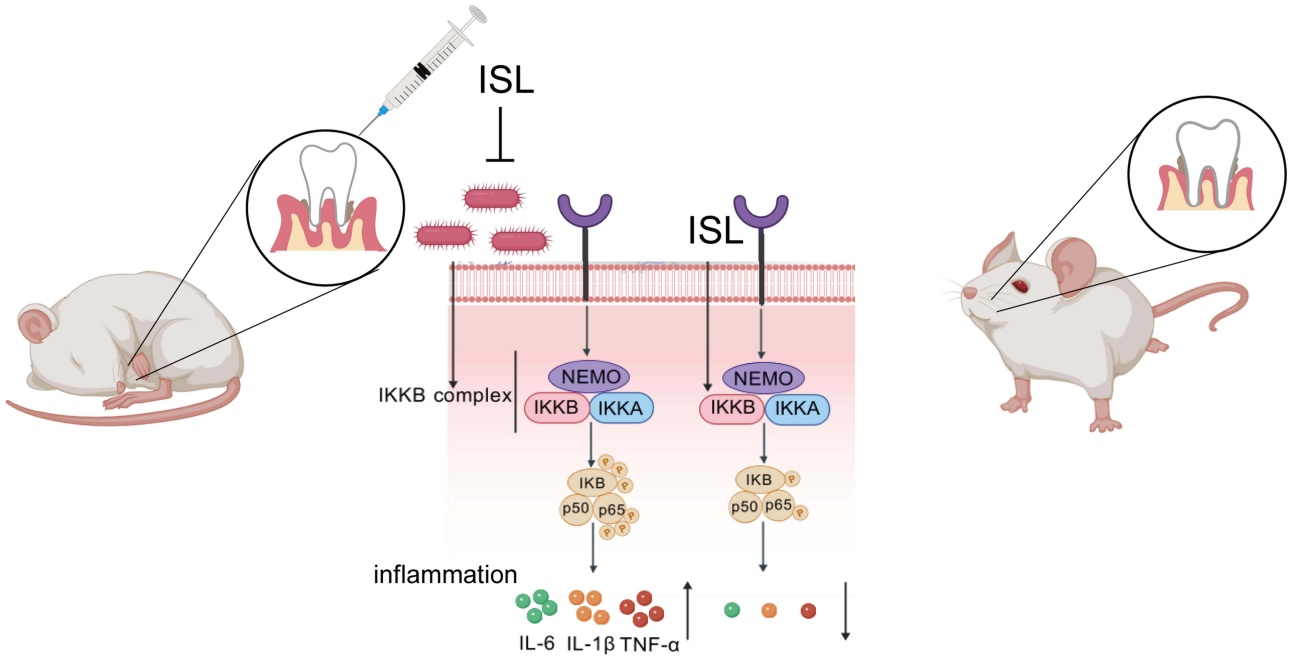

Figure 11 Sketch diagram describes pharmacology mechanism of isoliquiritigenin on periodontitis. ISL has a great potential in curing periodontitis through its dual mechanisms of antibacterial activity and anti-inflammatory effects on immune cells. NF-κB signaling pathway is great important, which is inhibited by ISL targeting to protein, IKKB, in immune cell of periodontitis.

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

The authors report no conflicts of interest in this work.

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