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# Investigation of the mechanism of baicalein in the treatment of periodontitis based on network pharmacology, molecular docking and experimental validation

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## Abstract

**Purpose** To verify the effect and mechanism of baicalein in the treatment of periodontitis through network pharmacology, molecular docking and in vitro experiments.

**Methods** Firstly, multiple databases were used to predict targets of baicalein and periodontitis. And the screened key target genes of baicalein for treating periodontitis were subjected to GO and KEGG analysis; then these targets were analyzed by molecular docking techniques. In vitro experiments including CCK-8, RT-qPCR, ELISA and Immunofluorescence were conducted to validate the efficacy of baicalein in treating periodontitis.

**Results** Seventeen key targets were screened from the databases, GO and KEGG analysis of these targets revealed that baicalein may exert therapeutic effects through regulating TNF, PI3K-Akt, HIF-1 and other signaling pathways. Molecular docking analysis showed that baicalein has good binding potential to several targets. In vitro cellular assays showed that baicalein inhibited the expression of TNF-α, MMP-9, IL-6 and MCP1 in *P.g*-LPS-induced macrophages at both the mRNA and protein level. And the immunofluorescence intensity of iNOS, a marker of M1 type macrophages, which mainly secretes inflammatory factors, was significantly reduced.

**Conclusion** Baicalein has the characteristics and advantages of "multicomponent, multitarget, and multipathway" in the treatment of periodontitis. In vitro cellular assays further confirmed the inhibitory effect of baicalein on the secretion of inflammatory factors of macrophages in periodontitis models, providing a theoretical basis for further study of the material basis and molecular mechanism of baicalein in the treatment of periodontal diseases.

**Keywords** Baicalein, Periodontitis, Network pharmacology, Molecular docking, Macrophage

## Background

Periodontitis is a chronic multifactorial inflammatory disease associated with plaque accumulation and characterized by progressive destruction of tooth-supporting tissues, including the periodontium and alveolar bone. The disease involves a complex dynamic interaction between specific bacterial pathogens, a destructive host immune response, and environmental factors (e.g. smoking) [1]. Moreover, periodontitis is epidemiologically

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associated with a variety of chronic diseases, such as cardiovascular disease, metabolic syndrome, rheumatoid arthritis, Alzheimer's disease, and non-alcoholic fatty liver disease [2–6]. Therefore, the threat posed by periodontitis to human health should not be underestimated, and research on its prevention and treatment will be one of the priorities of future life science research. Given the significant impact that periodontitis has on both oral and systemic health, prevention and treatment strategies must be developed and refined.

Traditional Chinese Medicine (TCM) emphasizes a holistic approach to maintaining balance in the body's functions. For thousands of years, TCM has recognized that changes in the gums are not isolated from the rest of the body and that various internal dysfunctions may have an impact on oral health [7]. Baicalein, a flavonoid compound extracted from the roots of the Chinese herb *Scutellaria baicalensis*, is a major component of the herb with demonstrated anti-inflammatory, antibacterial, antiviral, and antitumor activities [8, 9]. Existing research indicates that baicalein is effective in treating periodontitis.

Bi and colleagues [10] found that in a rat model of periodontitis, the levels of serum inflammatory factors IL-1β, IL-6, and TNF-α in the baicalin treated group were significantly reduced, and the number of osteoclasts in periodontal tissue was reduced. This suggests that baicalin can reduce the secretion of inflammatory factors in periodontal tissue and the whole body, and reduce the absorption of alveolar bone. Lee and colleagues [11] found that baicalein promotes odontoblast differentiation and induces vessel formation in human dental pulp cells through the BMP and Wnt/β-catenin signaling pathways, thus facilitating tooth pulp repair and regeneration. Chen and colleagues [12] discovered that 10 μM baicalein can significantly enhance osteogenic differentiation of human periodontal ligament cells through the activation of the Wnt/β-catenin pathway, a crucial factor in periodontal tissue repair and regeneration.

However, there is currently a lack of systematic and comprehensive studies on the mechanism of action of baicalein in treating periodontitis, which limits the understanding of its multi-target pharmacological effects. Network pharmacology is a newly emerging discipline based on systems biology, polypharmacology, computational biology, and network analysis, which can help to reveal the multi-component, multi-target, and multi-signaling pathway mechanisms of traditional Chinese medicines and their active components [13]. Molecular docking is a technique that examines the interactions between small molecules and large molecules (such as target proteins) and displays their binding energies [14]. In this study, we employed network pharmacology, molecular docking, and validation cellular experiments to provide theoretical and experimental evidence for the use of baicalein in treating periodontitis.

## Data and methods

### Baicalein compound information collection and target prediction

Baicalein compound information, absorption, distribution, metabolism and excretion (ADME) screening criteria including bioavailability (ob), drug similarity (dl) and blood–brain barrier (bbb) data were obtained from the TCMSP (https://old.tcmsp-e.com/tcmsp.php) [15] database, a systematic pharmacology database and analysis platform for Chinese medicine.

Two methods were used to predict the target site information of baicalein. In the TCMSP database (https://tcmspw.com/), the corresponding targets were obtained by searching the "baicalein" MOLID; the SwissTargetPrediction database (http://www.Swisstargetprediction.ch/) [16] was used to predict small molecule targets by identifying the structural formula of baicalein molecules and to screen for targets with probability > 0 (set the gene origin species as "Human"). Target names were corrected with the UniProt database (https://www.uniprot.org/) [17] (set the gene source species as "Human").

### Periodontitis target prediction

Based on the GeneCards database (https://www.genecards.org/) [18] and Drugbank database (https://www.drugbank.com/) [19], the disease targets related to periodontitis were predicted by entering the keyword "Periodontitis", and targets with relevance score > 2 from the GeneCards database were screened, and the results of each database were aggregated and de-weighted to obtain periodontitis-related targets. The target names were corrected with the UniProt database (https://www.uniprot.org/) (set the gene origin species as "Human").

### Construction and analysis of PPI networks for the intersection of baicalein and periodontitis targets

Based on the collected target information, the jvenn online software (http://jvenn.toulouse.inra.fr/app/example.html) [20] was used to map the Venn diagram of baicalein and periodontitis to obtain the common targets. The drug-disease intersection targets were imported into the STRING database (http://string-db.org/cgi/ input.pl) [21], and the gene source was set as "*Homo sapiens*" with a confidence level ≥ 0.4 to construct a protein–protein interaction network (PPI). Then import the PPI data into Cytoscape 3.9.1 [22] and filter the core targets with the MOCDE plug-in [23] (set degree cutoff = 2, node cutoff = 0.2, k-core = 2, and max. depth = 100).

#### GO and KEGG enrichment analyses

The core intersection targets were imported into the Metascape Gene Function Analysis database (https://metascape.org/) [24] for GO functional annotation and KEGG pathway enrichment analysis, The species was restricted to *Homo sapiens*, and the statistical significance threshold of the enrichment analysis was set at $P < 0.05$. The results were visualized using the platform (http://www.bioinformatics.com.cn).

#### Construction of the "drug-pathway-target" network

The above baicalein, target prediction results and pathway analysis were imported into Cytoscape 3.9.1 software to construct a "drug-pathway-target" network.

#### Ligand and protein preparation

The three-dimensional (3D) structure of the baicalein molecule was download from the TCMSP database (https://tcmspw.com/) in mol2 format. And AutodockTools 1.5.6 software [25] was used to hydrogenate, dehydrate and calculate the charge of baicalein.

The PDB database (https://www.rcsb.org/) [26] was used to obtain the 3D structure of the 17 core proteins in pdb format. And proteins pre-preparation includes assigning bond orders, the addition of formal charges as well as hydrogen atoms, and missing chain residues added. The water molecules beyond 5A$^{o}$ distance from the hetero atom were removed, and a possible ionization state was generated. Finally, after pre-preparation, proteins were energy-minimized [27].

#### Molecular docking validation

The treated compounds were docked to the targets by Autodock 1.5.6 software with the docking parameters set to default, and the binding activity between the active ingredients and the targets was evaluated by the Binding Energy, and PyMOL software [28] was used to visualize the results of baicalein and protein target docking.

#### Cells, drugs, reagents

THP-1 cell line was purchased from Wuhan Procell Life Science & Technology Co.Ltd.

Fetal bovine serum (FBS) (Shanghai ProPen Biotechnology Co.Ltd.); RPMI 1640 (Shanghai Thermo Fisher Scientific Co.Ltd); CCK8 (Shanghai Shenger Biotechnology Co.Ltd.); phorbol 12-myristate 13-acetate (PMA), baicalein (purity ≥ 99%) (MedChemexpress Biotechnology Co.Ltd.); dimethyl sulfoxide (DMSO), mercaptoethanol, anti-fluorescence quenching blocker containing DAPI, goat serum (Beijing Solarbio Technology Co.Ltd); *P.gingivalis*-lipopolysaccharide (*P.g*-LPS) (Sigma-Aldrich Co.Ltd); iNOS rabbit anti-human antibody (Wuhan proteintech Biotechnology Co.Ltd.); AlexaFluor488-labeled goat anti-rabbit antibody (Shanghai Po Wan Biotechnology Co.Ltd.); Trizol (Shanghai Beyotime Biotechnology Co.Ltd); TNF-α, MMP-9, IL-6, MCP1 and GAPDH upstream and downstream primers (Shanghai Sangon Biotechnology Co.Ltd.); RT-qPCR kit Code No.RR820A (Beijing Takara Biotechnology Co.Ltd); TNF-α (Code No.RK00030), MMP-9 (Code No.RK00217), IL-6 (Code No.RK00004) and MCP1 (Code No.RK00052) ELISA kits (Wuhan ABclonal Biotechnology Co.Ltd.)

#### Cell culture and treatment

THP-1 cells were incubated in RPMI 1640 medium containing 10% FBS, 1% penicillin/streptomycin and 0.05 mM mercaptoethanol at 37 ℃ in a humidified 5% $CO_2$ atmosphere. Dissolve baicalein in DMSO, and pass 0.22 μM filter, make 50 mM solution and then dissolve it in the culture medium containing 2% FBS to reach the final concentration of 10, 1, 0.1 μM. Inoculate THP1 cells at a density of $2 \times 10^5$ cells/mL in a 6-well plate, and induce cell adhesion growth with a culture medium containing 100 ng/mL PMA for 48 h to differentiate into macrophages. The cells were pre-treating with culture medium containing baicalein of different concentrations for 6 h, and then were followed by treatment with *P.g*-LPS (1 μg/mL) for 6 h. The blank control group was incubated with equal amount of medium only, and the positive control groups, namely LPS group and baicalein group, were incubated with 1 μg/mL of *P.g*-LPS and 1 μM of baicalein for 6 h respectively.

#### Cell Counting Kit-8 (CCK8) assay for cell viability

The experiment was divided into 7 groups, namely the blank group, LPS (1 μg/mL) group and baicalein (1000, 100, 10, 1, 0.1 μM concentration) groups. Inoculate THP1 cells at a density of $0.8 \times 10^5$ cells/mL in a 96-well plate, and induce cell adhesion growth with a culture medium containing 100 ng/mL PMA for 48 h to differentiate into macrophages. Each group was incubated with the corresponding baicalein concentration or *P.g*-LPS medium for 24 h. The blank group was incubated with equal amount of medium only. After 24 h of incubation, replace the old culture medium with the new medium containing 10% CCK8 solution to incubated for 2 h further. The absorbance OD value at 450 nm was measured with an enzyme marker, and then plot the cell viability curve to select the appropriate baicalein concentration for subsequent experiments.

### RT-qPCR method to detect the TNF-α, MMP-9, IL-6 and MCP1 mRNA expression

Total RNA was extracted from each group of cells in 2.9 using Trizol. cDNA was synthesized by reverse transcription. Fluorescence qPCR using the SYBR Green dye method, and GAPDH was used as the internal reference gene for correction analysis. The results were analyzed by the relative quantitative $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Table 1.

### ELISA method to detect the TNF-α, MMP-9, IL-6 and MCP1 in the cell supernatant

Collect the cell supernatant in 2.9, centrifuge at 3000 rpm for 20 min, and assay the concentration of TNF-α, MMP-9, IL-6 and MCP1 in the supernatant according to the instructions of the ELISA kit.

### Immunofluorescence assay

Place crawlers and THP1 cells at a density of $2\times10^5$ cells/mL in a 24-well plate respectively, and treat them as described in 2.9. The cells were fixed with 4% paraformaldehyde for 20 min and then were permeabilized in buffer containing 0.1% TritonX-100 in PBS for 5 min at 37 ℃. Nonspecific binding sites were blocked in 5% goat serum for 30 min at 37 ℃. The iNOS antibody (1:200) was incubated at 4 °C overnight, then the secondary antibody (1:200) was incubated for 1 h at 37 °C. After blocking the slices with a DAPI-containing anti-fluorescence quencher, the fluorescence intensity was detected and the fluorescence images were captured by laser confocal microscopy. Five to ten high-magnification fields were randomly selected for each group and the average fluorescence intensity was calculated using ImageJ software.

### Statistical analysis

IBM SPSS Statistics 26.0 statistical software was used for data analysis, and GraphPad Prism 9.0 software was used for data plotting. The results of the above in vitro experiments were repeated three times. All results were expressed as the mean ± the standard deviation (means ± SD). Multiple group comparisons were performed by one-way ANOVA with Tukey and TamhaneT2 (M)'s post-hoc test to identify differences between specific groups. $P<0.05$ was considered statistically significant.

## Results

### Collection of baicalein targets

There were totally 37 targets corresponding to baicalein retrieved by TCMSP, meanwhile 102 ones were obtained based on SwissTargetPrediction. 127 potential targets were obtained after de-duplication.

### Collection of periodontitis targets

Using "periodontitis" as the search term, 2661 relevant disease targets were retrieved from the GeneCards database, 356 ones left with a relevance score > 2. Similarly, Drugbank retrieved 34 related disease targets. 378 periodontitis-related targets were obtained after two databases were combined and de-duplicated.

### Construction and analysis of PPI networks

A total of 26 common targets were obtained by taking the intersection of 127 baicalein-related targets and 378 periodontitis-related targets (Fig. 1). The PPI network which includes 26 nodes and 194 edges was constructed on the STRING platform. 17 core targets were identified as VEGFA, HIF1A, ESR1, MMP2, MMP9, FOS, EGFR, MAPK3, etc. The visualization sees Fig. 2 and Table 2.

### GO and KEGG pathway enrichment analysis

#### *GO analysis*

Four hundred twelve GO analysis items were obtained ($P<0.05$), including 371 biological processes (BP),

**Table 1** qRT-PCR primers used in this study

| Target gene | Sequences | Size of amplified products (bp) |
|---|---|---|
| GAPDH | Forward: 5′-CAGGAGGCATTGCTGATGAT-3′<br>Reverse: 5′-GAAGGCTGGGGCTCATTT-3′ | 138 |
| TNF-α | Forward: 5′-CTCATCTACTCCCAGGTCCTCTTC-3′<br>Reverse: 5′-CGATGCGGCTGATGGTGTG-3′ | 82 |
| MMP-9 | Forward: 5′-CCCTGGTCCTGGTGCTCCTG-3′<br>Reverse: 5′-CTGCCTGTCGGTGAGATTGGTTC-3′ | 113 |
| IL-6 | Forward: 5′-GACAGCCACTCACCTCTTCAGAAC-3′ | 121 |
| MCP1 | Reverse: 5′-GCCTCTTTGCTGCTTTCACACATG-3′<br>Forward: 5′-CAGCAGCAAGTGTCCCAAAGAAG-3′<br>Reverse: 5′-TGCTTGTCCAGGTGGTCCATG-3′ | 114 |

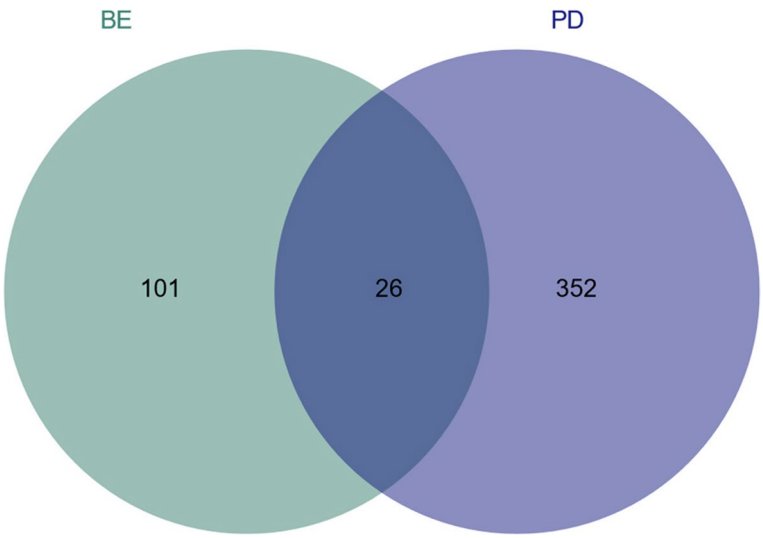


**Fig. 1** Venn diagram of Baicalein(BE) and Periodontitis(PD) targets

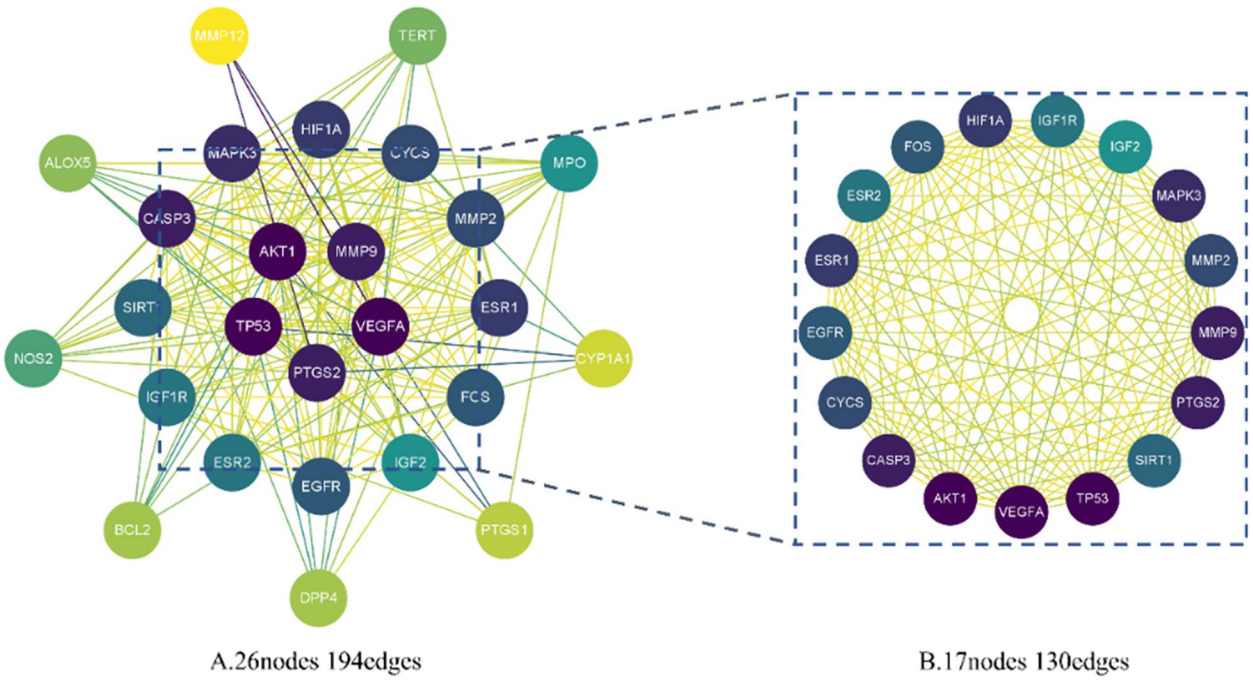


**Fig. 2** The core target PPI network. As shown in the figure, in this network, the darker the circle color can be considered as more important

13 cell composition (CC), and 28 molecular function (MF). Each of the first 10 items were selected based on the *P* value for visual analysis. The BP of baicalein against periodontitis were mainly involved in processes like enzyme-linked receptor protein signaling pathway, response to oxidative stress, and positive regulation of cell migration. The CC were enriched in the membrane raft, membrane micro domain, caveola, nuclear envelope and plasma membrane raft. The MF were focused on kinase binding, RNA polymerase II-specific DNA-binding transcription factor binding, DNA-binding transcription factor binding, protein kinase binding, signaling receptor regulator activity and ubiquitin protein ligase binding (Fig. 3).

**Table 2** Core target information table

| Target | MCODE Score | Betweenness Centrality | Closeness Centrality |
|---|---|---|---|
| AKT1 | 13.76666667 | 0.056172318 | 0.925925926 |
| SIRT1 | 13.76666667 | 0.003068783 | 0.735294118 |
| MAPK3 | 13.76666667 | 0.018545334 | 0.833333333 |
| TP53 | 13.76666667 | 0.056172318 | 0.925925926 |
| EGFR | 13.76666667 | 0.004647066 | 0.757575758 |
| FOS | 13.76666667 | 0.004098244 | 0.757575758 |
| CASP3 | 13.76666667 | 0.026902477 | 0.862068966 |
| PTGS2 | 13.76666667 | 0.060687951 | 0.862068966 |
| MMP9 | 13.76666667 | 0.0429329 | 0.862068966 |
| ESR1 | 13.76666667 | 0.022710558 | 0.806451613 |
| HIF1A | 13.76666667 | 0.014751563 | 0.806451613 |
| VEGFA | 13.76666667 | 0.070108826 | 0.925925926 |
| MMP2 | 12.87619048 | 0.014281987 | 0.78125 |
| IGF1R | 12.87619048 | 0.001096681 | 0.714285714 |
| CYCS | 12.87619048 | 0.019734247 | 0.78125 |
| ESR2 | 12.87619048 | 0.007071429 | 0.714285714 |
| IGF2 | 12 | 0.002857143 | 0.675675676 |

#### *KEGG*

There were totally 103 related pathways enriched, mainly the MAPK, HIF-1, TNF, IL-17, PI3K-Akt, VEGF and so on (Fig. 4).

#### "Baicalein-pathway-target" network

The "baicalein-pathway-target" network analysis is shown in Fig. 5. Comprehensive information can be obtained and the complex relationships that manage cellular activities can be revealed. According to the analysis, the pathways with the highest enrichment level included MAPK, HIF-1, and TNF signaling pathways, and six proteins participated in the first 20 pathways at a high frequency, indicating that they played an important role in the enrichment pathway. The protein with six higher degree values is AKT1, EGFR, VEGFA, FOS, CASP3 and MMP9. These proteins have also shown higher importance in previous PPI analysis results, so they may be crucial in the treatment of periodontitis with baicalein.

#### Molecular docking analysis

A total of 17 core targets were simulated for molecular docking using AutoDock software (Table 3), and the target proteins bind to baicalein through hydrogen bonding energy intermolecular forces. In Table 3, the estimated minimum binding energy of baicalein with 17 core targets were all $\leq 0$, and the binding energy of baicalein with MMP9, MMP2, ESR1, EGFR, FOS, HIF1A, SIRT1 were all less than -5.0 kcal/mol, showing good binding force. PyMOL software was used to visualize the molecular docking results (Fig. 6).

#### Effect of baicalein and LPS on THP1 cell viability

THP1 cells were exposed to different concentrations of baicalein (1000, 100, 10, 1, 0.1 μM) or LPS (1 μg/mL). The treatment with 1000, 100 μM baicalein affected the viability of cells, while the treatment with 1 μg/mL LPS or 10, 1, 0.1 μM baicalein had no significant effect on the cells (Fig. 7). Therefore, we adopted 10, 1 and 0.1 μM baicalein as high, middle and low drug concentrations in the subsequent experiments to test the effect of baicalein on THP1 cells.

#### Effect of baicalein on the inflammatory factors' level in macrophages

Compared with the LPS group, the RT-qPCR results showed the mRNA expression of TNF-α, MMP-9, IL-6 and MCP1 were reduced after pretreatment with 10, 1, and 0.1 μM baicalein, and there was no significant dose relationship between the drug concentration and the decreased level (Fig. 8). ELISA results showed TNF-α, MMP-9, IL-6 and MCP1 were also reduced while compared with the LPS group (Fig. 9). The results indicated that baicalein pretreatment significantly inhibited the secretion of inflammatory factors in macrophages.

#### Effect of baicalein on iNOS expression in LPS-activated macrophages

iNOS is an M1-type macrophage marker and also a downstream product of the stimulation of inflammatory cytokines such as TNF-α and IL-6. Immunofluorescence results showed 1 μM baicalein treatment basically did not increase the fluorescence intensity of iNOS, and the 1 μg/ml LPS group increased the fluorescence intensity of iNOS by 28.21% compared to the blank control. In contrast, pretreatment with 10, 1 and 0.1 μM baicalein all resulted in a significant decrease in the fluorescence intensity of iNOS (Fig. 10).

## Discussion

In TCM, periodontitis belongs to the category of "Tooth Declaration" and "Tooth Exclusion". According to the "Miscellaneous Works of Ming Medicine", "Zhi Zhi Fang" and "Pu Ji Fang", TCM uses dialectical thinking to classify it into kidney-yin deficiency type, qi-blood deficiency type and stomach-fire-up steam type, the last one is a more common type [29, 30]. To this type of periodontitis, TCM often treated with periodontal defeat drink and stomach-clearing soup, both of which are based on raw gypsum and scutellaria and often achieve better results in clinical treatment [29, 31, 32]. *Scutellaria baicalensis*, as

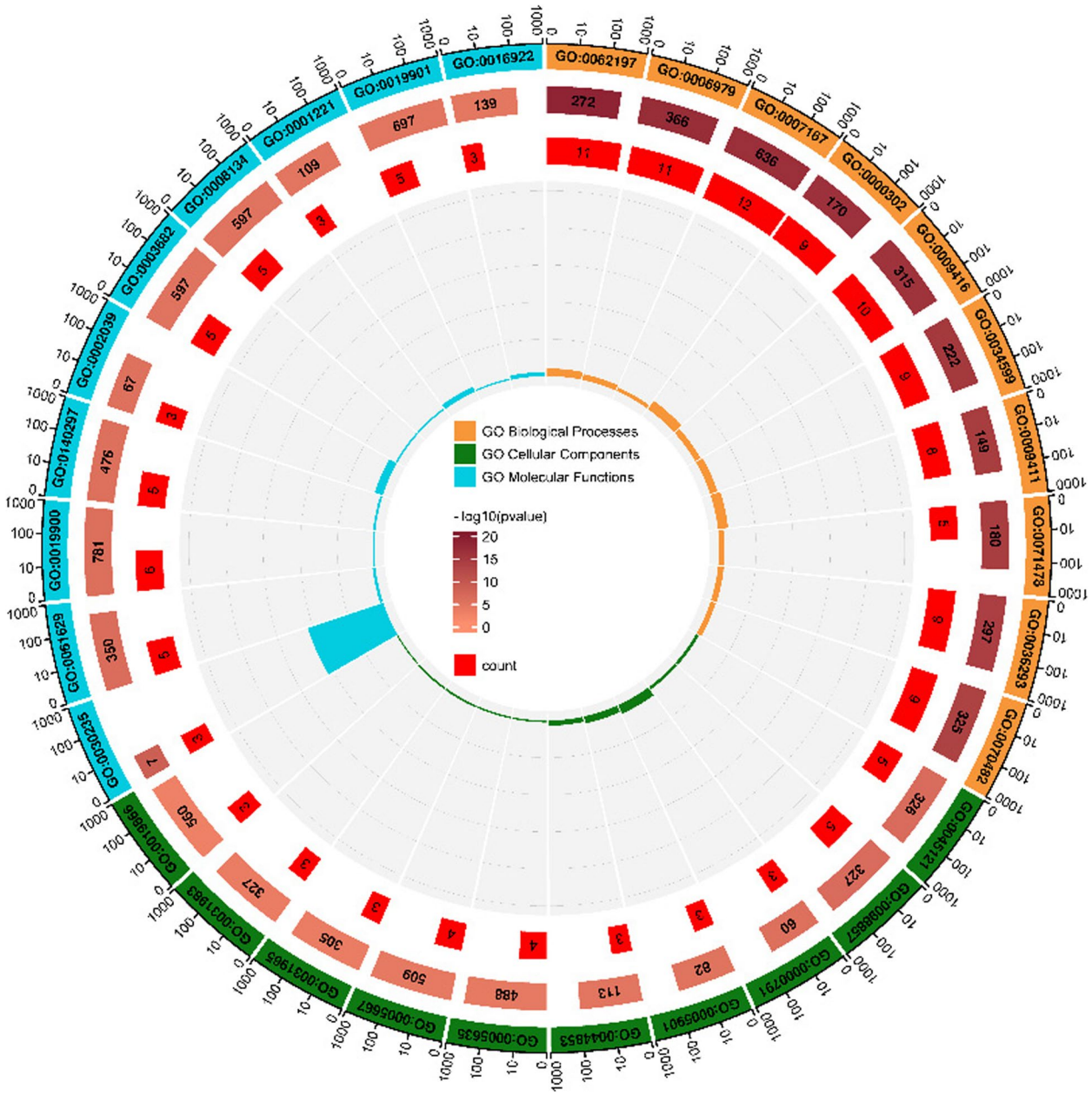


**Fig. 3** The GO function enrichment of potential targets of baicalein against periodontitis

the main ingredient of the two soups mentioned above, is good at "clearing the fire in the upper energizer" and can clear the fire in the heart, lung and stomach, and is now also used in oral inflammatory diseases such as oral ulcer and periodontitis [33]. So far, *Scutellaria baicalensis* has been developed into a variety of products for oral diseases, such as the compound *Scutellaria baicalensis* rinse, Shuanghuanglian toothpaste and Shuanghuang tonic preparations [34–37]. Modern medical research has shown that baicalein, one of the main components of *Scutellaria baicalensis*, has anti-tumor, anti-bacterial, anti-inflammatory, anti-oxidant and anti-apoptosis effects [38].

Using the topological analysis of the PPI network and the results of GO analysis and KEGG pathways, we hypothesized that the mechanism of baicalein's treatment for periodontitis might be mainly related to the MAPK, HIF-1, TNF, PI3K-Akt, VEGF and FoxO signaling pathway. It involves many targets such as VEGFA, HIF1A, ESR1, ESR2, MMP2, MMP9, FOS, EGFR, MAPK3, and

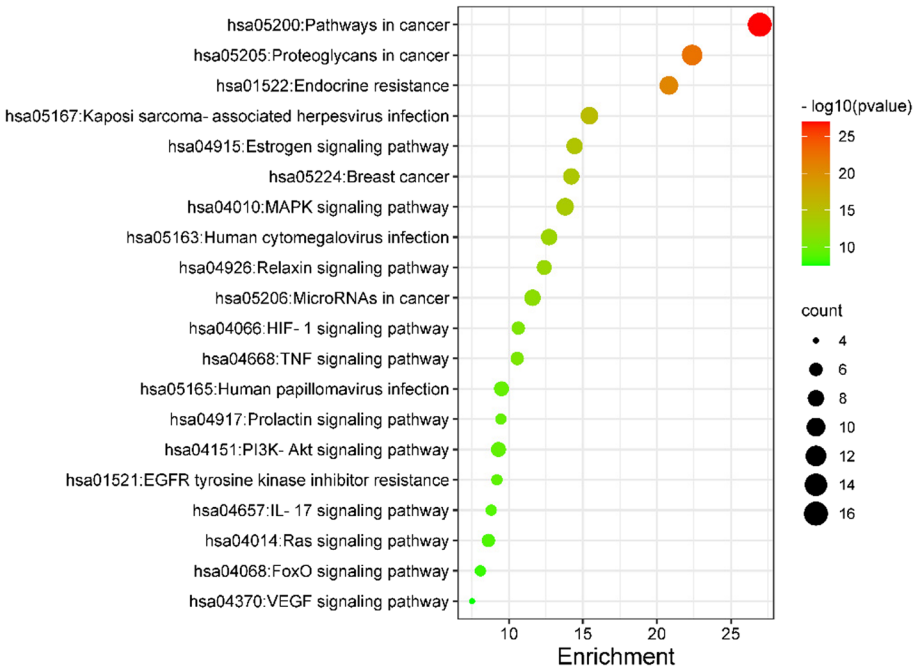


**Fig. 4** The bubble chart of first 20 signals pathways of baicalein against periodontitis

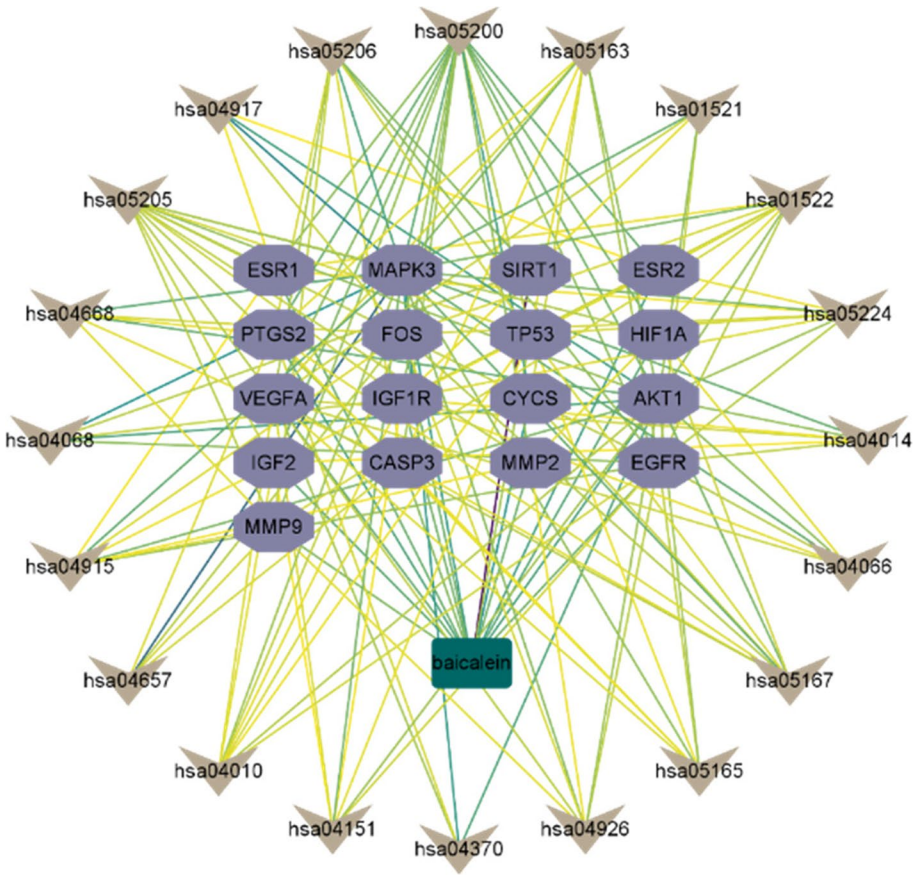


**Fig. 5** Drug-target-pathway network diagram. Rectangle is baicalein; octagons are targets; V are signaling pathways

**Table 3** Docking results of core target proteins and baicalein

| Ligand | Target | | | Minimum binding energy (kcal/mol) |
|---|---|---|---|---|
| | Protein name | Gene name | Protein PDB ID | |
| Baicalein | Matrix metalloproteinase-9 | MMP9 | 6ESM | -6.48 |
| | Estrogen receptor 1 | ESR1 | 6CHZ | -6.11 |
| | Epidermal growth factor receptor | EGFR | 1K37 | -5.68 |
| | Protein c-Fos | FOS | 6YQP | -5.44 |
| | Matrix Metallopeptidase-2 | MMP2 | 3AYU | -5.38 |
| | NAD-dependent histone deacetylase sirtuin-1 | SIRT1 | 3RIY | -5.38 |
| | Hypoxia-inducible factor 1-alpha | HIF1A | 6GMR | -5.18 |
| | TP53-binding protein 1 | TP53 | 3D06 | -4.88 |
| | Insulin-like growth factor II | IGF2 | 6GX6 | -4.85 |
| | Estrogen receptor beta | ESR2 | 2QTU | -4.77 |
| | Caspase-3 | CASP3 | 5IBP | -4.75 |
| | Vascular endothelial growth factor A | VEGFA | 5DN2 | -4.71 |
| | RAC-alpha serine/threonine-protein kinase | AKT1 | 5KCV | -4.4 |
| | Prostaglandin G/H synthase 2 | PTGS2 | 5IKV | -3.91 |
| | Cytochrome c | CYCS | 5TY3 | -3.91 |
| | Insulin-like growth factor 1 receptor | IGF1R | 5U1M | -3.85 |
| | Mitogen-activated protein kinase 3 | MAPK3 | 6GES | -2.78 |

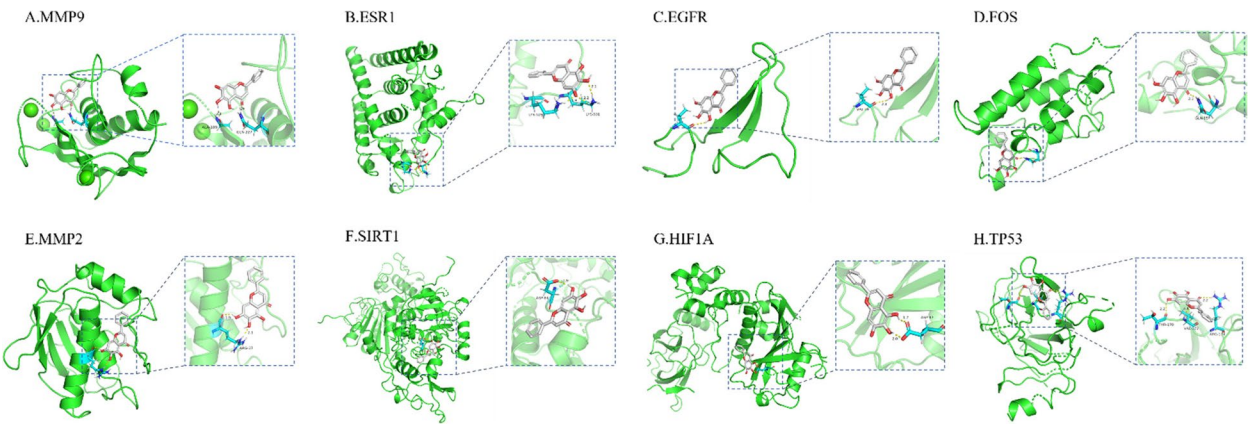


**Fig. 6** Molecular docking pattern diagram of baicalein with some main targets against periodontitis. Target in (A-H) is MMP9, ESR1, EGFR, FOS, MMP2, SIRT1, HIF1A, TP53 respectively. In these figures, the whiten compound was baicalein, and the lake blue represented the amino acid residue that produces hydrogen bonding with baicalein

other cellular biological responses like enzyme-linked receptor protein signaling pathway, response to oxidative stress, and positive regulation of cell migration, kinase binding, RNA polymerase II-specific DNA-binding transcription factor binding and DNA-binding transcription factor binding.

In molecular docking, a binding energy < 0 indicates the ligand molecule can spontaneously bind to the receptor protein, and a binding energy ≤ -5.0 kcal/mol indicates good binding activity between ligand molecule and receptor proteins [39]. It was observed that MMP9 had shown the highest binding energy value of − 6.48 kcal/mol, and the minimum binding energy of baicalein to 7 core targets were ≤ -5.0 kcal/mol, and to most core targets were ≤ -4.0 kcal/mol (Table 3). we also found out that the target proteins and baicalein were bound by hydrogen bonding energy inter molecular force, indicating that baicalein has high binding activity to target proteins

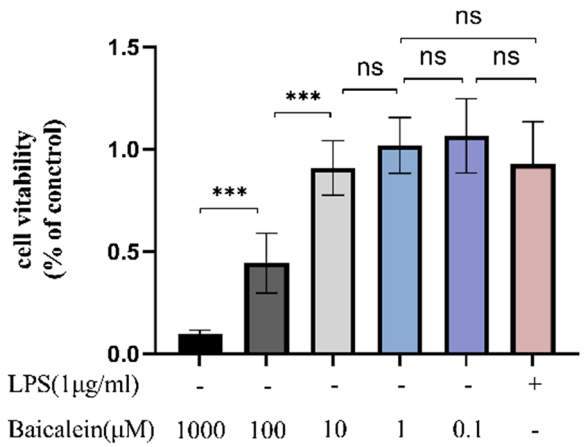


**Fig. 7** Effect of baicalein and LPS on cell viabilities of THP-1 macrophages cells. *$p < .05$; **$p < .01$; ***$p < .001$

such as MMP9, MMP2, ESR1, EGFR, FOS, HIF1A, TP53, which may be the key component of the treatment for periodontitis (Fig. 6).

MMPs are a group of enzymes that degrade the extracellular matrix. MMP-2 and MMP-9 are type IV collagenases, which mainly degrade type IV collagen, gelatin and elastin, and the levels are significantly increased in the gingival tissue and gingival sulcus fluid of patients with periodontitis [40]. The two play a synergistic role in the development of periodontitis and MMP9 is often used as an indicator of periodontal status [41, 42].

EGFR is an epidermal growth factor receptor, and studies have shown that EGFR promotes IL-1β expression and inhibits Smad3 phosphorylation, a mediator of TGF-β1 signaling, leading to inflammation of periodontal tissue and resorption of alveolar bone [43]. TP53 is a cellular tumor antigen. Liu and colleagues [44] have confirmed that in a *P.g*-LPS induced cellular inflammation model, the enhanced TP53 activity was involved in periodontal inflammatory response by increasing the secretion of IL-1β, IL-6 and TNF-α after leading to cellular redox imbalance and mitochondrial dysfunction.

HIF1A is a hypoxia-inducible factor closely related to chronic inflammation and is an important transcriptional regulator of cells under hypoxic conditions, promoting the glycolytic process by inducing the expression of lactate dehydrogenase and pyruvate dehydrogenase kinases [45]. Studies have shown that HIF1A

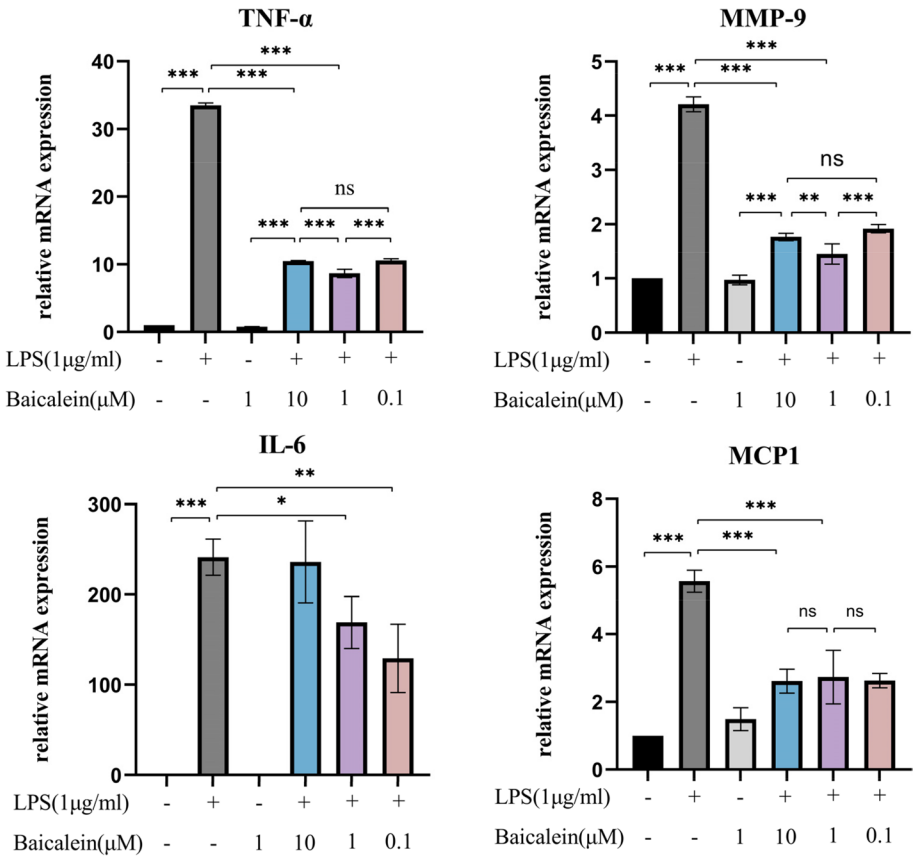


**Fig. 8** Detection of TNF-α, MMP-9, IL-6 and MCP1 mRNA expression in each group by qRT-PCR. *$p < .05$; **$p < .01$; ***$p < .001$

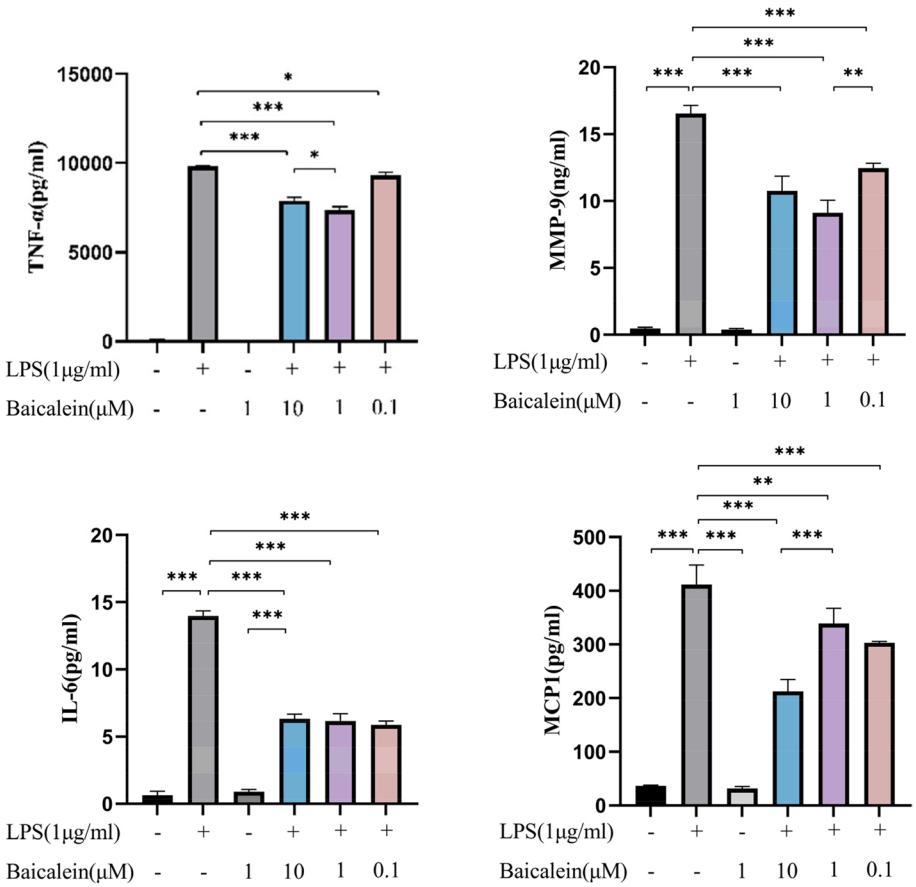


**Fig. 9** Detection of TNF-α, MMP-9, IL-6 and MCP1expression in the supernatant of each group by ELISA. *$p < .05$; **$p < .01$; ***$p < .001$

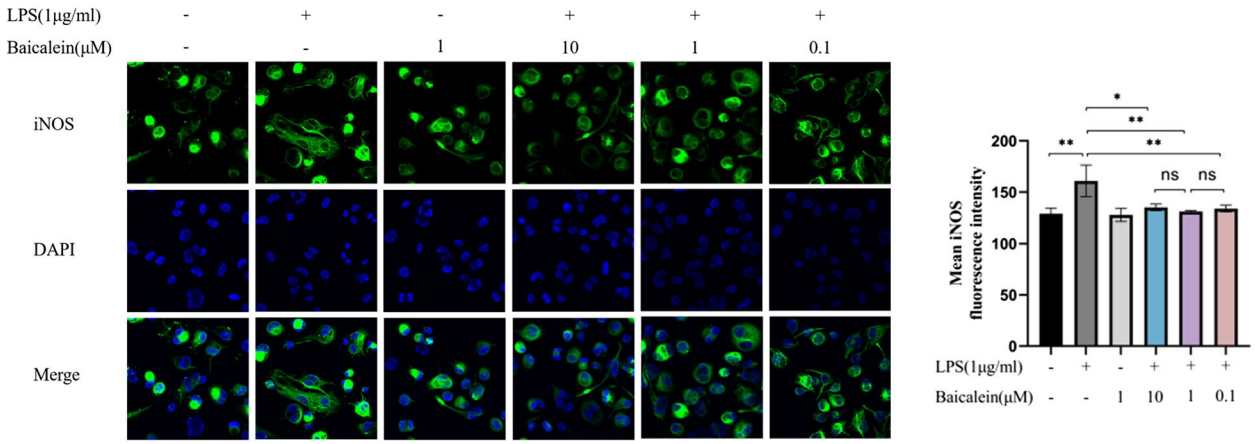


**Fig. 10** iNOS production in each group. *$p < .05$; **$p < .01$

downregulates the expression of TNF-α, IL-6, CD86 and the M1/M2 type macrophage ratio, inhibits osteoclast formation, prevents bone resorption and protects periodontal tissue [46, 47]. FOS is a member of the AP-1 family of transcription factors, which is activated in osteoclast precursors and is required for osteoclast differentiation [48]. Studies have shown that mechanical forces induce the upregulation of FOS in periodontal cells, leading to the resorption of alveolar bone [49].

EGFR, FOS, IGF1R, IGF2, MAPK3, TP53, and VEGFA are commonly found in the MAPK signaling pathway. The MAPK signaling pathway family is complex and mainly includes ERK1/2, p38, JNK, and ERK5. It plays an important role in the immune inflammatory and anti-inflammatory response of periodontal tissues, and in the destruction and formation of alveolar bone. Activation of the p38/MAPK signaling pathway has been proved to increase the expression of protein c-Fos in osteoblasts, activate NFATC1, and promote osteoblasts differentiation [50]. Through the ERK1/2 and JNK signaling pathways, IL-1β activates AP-1 to induce MMP-9 expression in osteoblasts and may enhance collagen degradation via MMP-13 or MMP-9 [51], causing periodontal tissue destruction.

EGFR, IGF1R, MAPK3 and VEGFA are commonly found in the PI3K-AKT and HIF-1 signaling pathways. The PI3K/AKT signaling pathway involved in many biological processes, such as cellular inflammation, apoptosis and glucose metabolism [52, 53].Research has found that by activating the PI3K/AKT/Nrf2 signaling pathway, Qianghuo alcohol can inhibit the synthesis of inflammatory mediators such as IL-1β, IL-32, and IL-8 by human gingival fibroblasts under lipopolysaccharide stimulation. At the same time, it can upregulate the expression of antioxidant proteins such as heme oxygenase 1 (HO-1), catalase, and glutathione reductase, inhibit oxidative stress levels, and alleviate periodontal inflammation [54]. Zhao and colleagues [55] found that activating the PI3K/AKT/mTOR signaling pathway via GPR30 could promote the proliferation and osteogenic differentiation in periodontal ligament cells. Park and colleagues [56] proved that schisandrin could induce HO-1 expression in RAW 264.7 cells through activating of signaling pathways such as PI3K/AKT and ERK, downregulate TNF-α and IL-1β, and stimulate the anti-inflammatory effects of macrophages. HIF-1 signaling pathway mainly plays a role in hypoxia-related physiological conditions and pathological processes, including pro-angiogenesis, apoptosis, and inflammation [57, 58]. Studies have found that $CoCl_2$ can promote the expression of IL-1β and MMP-8 through HIF-1 pathway, triggering cellular autophagy after inducing cytotoxicity of periodontal ligament cell [59]. Activation of HIF-1α can also induce the production of MCP-1 and activation of nuclear factor kappa B (NF-κB) in human macrophages, promote the expression of IL-1β, and cause macrophage inflammation and autophagy processes [60].

AKT1, CASP3, FOS, MMP9, MAPK3, and PTGS2 are enriched in the TNF signaling pathway, This pathway mainly involved in immune function and inflammatory response, and is associated with the PI3K/AKT and MAPK signaling pathways. TNF-α, a key protein in the TNF signaling pathway, could upregulate Blimp1 expression by inhibiting PI3K/AKT signaling pathway, promote osteoclastogenesis, and lead to bone resorption [61]. Through NF-kB and p38/MAPK signaling pathways, TNF-α significantly promoted the production of MMP-3 in cementoblasts, which may involve in the degradation or remodeling of periodontal tissue.

Combined with the results of molecular docking, we can speculate that baicalein may act on key signaling pathways like the TNF, PI3K-AKT, HIF-1 and MAPK through core targets such as MMP9, TNF-α, FOS, MAPK3 and AKT1 to inhibit the expression of pro-inflammatory factors and local inflammatory responses, to reduce apoptosis and collagen degradation, and to promote the proliferation and differentiation of osteoblasts for the treatment of periodontal disease.

Macrophages, as an important component of the host defense system, play an important role in tissue destruction and bone resorption. They are also closely associated with the development of periodontitis. As resident cells or monocyte-derived cells recruited after inflammation, phagocytic capacity of macrophages is a key factor in the development of acquired immunity [62]. Macrophages have a high degree of cellular plasticity and are able to respond to different environmental signals. Upon activation, macrophages can differentiate into M1 (classical) or M2 (alternative) phenotype. M1 type has a pro-inflammatory effect manifested by increased secretion of inflammatory cytokines such as TNF-α, MMP-9, IL-6 and MCP1. iNOS is an M1 type macrophage marker. Under the stimulation of inflammatory cytokines such as TNF-α, IL-6 and IL-1β, the body can express iNOS, which can catalyze the synthesis of a large amount of nitric oxide. NO exacerbates autoimmune tissue damage by stimulating the inflammatory response of macrophages and promoting inflammation or cytotoxicity in peripheral cells [63].

In this study, we used *P.g*-LPS induced macrophages to construct periodontitis model in vitro, and observed the effect of baicalein on periodontitis cell model. Immunofluorescence results showed baicalein downregulated the expression of iNOS, which indicated the reduction of M1 macrophages. RT-qPCR and ELISA results showed the expression of MMP-9, TNF-α, IL-6 and MCP1 were all significantly reduced both at the mRNA level and the protein level. Through cellular experiments, we could find that 10, 1, and 0.1 μM baicalein inhibited the expression of pro-inflammatory factors and the polarization toward M1 type without affecting macrophage viability, and significantly reduced *P.g*-LPS induced cellular inflammation.

## Conclusion

In this study, through an in-depth analysis of the pharmacological mechanism of baicalein by combining network pharmacology with molecular docking, we found that baicalein acts on multiple targets and proteins of multiple signaling pathways, intervenes inflammation and delays the progression of periodontitis. In vitro cellular assays further confirmed the inhibitory effect of baicalein on the secretion of inflammatory cytokines in LPS-activated THP-1 cells. In the follow-up study, we plan to design in vivo animal pharmacological experiments to make an in-depth observation on the mechanism of baicalein in treating periodontitis and to provide more references for its clinical application and development.

### Abbreviations

| | |
|---|---|
| BE | Baicalein |
| PD | Periodontitis |
| TCM | Traditional Chinese Medicine |
| iNOS | Nitric oxide synthase |
| *P.g*-LPS | *P.gingivalis*-Lipopolysaccharide |
| TNF-α | Tumor necrosis factor-α |
| IL-6 | Interleukin-6 |
| IL-8 | Interleukin-8 |
| IL-1β | Interleukin-1β |
| FBS | Fetal bovine serum |
| PMA | Phorbol 12-myristate 13-acetate |
| DMSO | Dimethyl sulfoxide |
| PPI | Protein–protein interaction |
| BP | Biological processes |
| CC | Cell composition |
| MF | Molecular function |
| GO | Gene ontology |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| MMP9 | Matrix metalloproteinase-9 |
| ESR1 | Estrogen receptor 1 |
| EGFR | Epidermal growth factor receptor |
| FOS | Protein c-Fos |
| MMP2 | Matrix Metallopeptidase-2 |
| SIRT1 | NAD-dependent histone deacetylase sirtuin-1 |
| HIF1A | Hypoxia-inducible factor 1-alpha |
| TP53 | TP53-binding protein 1 |
| IGF2 | Insulin-like growth factor II |
| ESR2 | Estrogen receptor beta |
| CASP3 | Caspase-3 |
| VEGFA | Vascular endothelial growth factor A |
| AKT1 | Threonine-protein kinase |
| PTGS2 | Prostaglandin G/H synthase 2 |
| CYCS | Cytochrome c |
| IGF1R | Insulin-like growth factor 1 receptor |
| MAPK3 | Mitogen-activated protein kinase 3 |
| NFATC1 | Nuclear factor of activated T-cells |
| CD86 | T-lymphocyte activation antigen CD86 |
| Blimp1 | B lymphocyte induced maturation protein-1 |

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### Authors' contributions

YL, ZM and FC conceived and designed the project; ZD, YL and MS acquired the data; YM, MB analyzed and interpreted the data; and YL wrote the manuscript. All authors contributed to the article and approved the submitted version.

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### Availability of data and materials

The datasets analyzed during the current study are available in https://www.rcsb.org/structure/6ESM, https://www.rcsb.org/structure/6CHZ, https://www.rcsb.org/structure/1K37, https://www.rcsb.org/structure/6YQP, https://www.rcsb.org/structure/3AYU, https://www.rcsb.org/structure/3RIY, https://www.rcsb.org/structure/6GMR, https://www.rcsb.org/structure/3D06, https://www.rcsb.org/structure/6GX6, https://www.rcsb.org/structure/2QTU, https://www.rcsb.org/structure/5IBP, https://www.rcsb.org/structure/5DN2, https://www.rcsb.org/structure/5KCV, https://www.rcsb.org/structure/5IKV, https://www.rcsb.org/structure/5TY3, https://www.rcsb.org/structure/5U1M, https://www.rcsb.org/structure/6GES, https://tcmsp-e.com/molecule.php?qn=2714, https://go.drugbank.com/indications/DBCOND0010286#targets, https://www.uniprot.org/uniprotkb?facets=model_organism%3A9606&query=reviewed%3Atrue and some websites only provide analysis services, the data can't store in its website forever, the data need to be downloaded to study further, and this part of data are available from the corresponding author upon request.

### Data availability

Data is provided within the manuscript or supplementary information files.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

All authors consent to publication of the present manuscript.

### Competing interests

The authors declare no competing interests.

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