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ORIGINAL RESEARCH

Effects of Baicalin on Gout Based on Network Pharmacology, Molecular Docking, and in vitro Experiments

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Purpose: Baicalin is a flavonoid of Scutellaria baicalensis Georgi. It possesses antipyretic, analgesic, and anti-inflammatory effects. It has great potential to treat gout. A network pharmacology approach, molecular docking and experimental validation were applied to investigate the pharmacological mechanisms of baicalin in treating gout.

Methods: The potential targets of baicalin were retrieved from the TCMSP, PharmMapper, STITCH, and Swiss Target Prediction databases. The gout-related targets were retrieved from the DrugBank, TTD, and Genecards databases. Then, the potential targets and signaling pathways were acquired via protein–protein interaction (PPI), as well as the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Subsequently, the key targets were selected to dock with baicalin based on molecular docking. Finally, in vitro experiments were conducted to further validate the predictions.

Results: A total of 318 potential targets of baicalin and 752 gout-related targets were screened. TNF, VEGFA, MMP9, PTGS2, and TLR4 might be the hub therapeutic target genes. The TLR4/NF-κB signaling pathway might be the foremost pathway in baicalin against gout. Moreover, molecular docking showed that baicalin combined well with TNF, VEGFA, MMP9, COX-2, and TLR4, respectively. The results of cell experiments suggested that baicalin could reduce the levels of inflammatory cytokines by inhibiting the TLR4/NF-κB signaling pathway in MSU-stimulated THP-1 cells and regulate the expression of these hub targets.

Conclusion: These results revealed that baicalin possesses "multitarget, multipathway, multilevel" regulatory effects. From a therapeutic standpoint, baicalin may be a promising anti-inflammatory agent for alleviating gout.

Keywords: baicalin, gout, network pharmacology, molecular docking, in vitro experiments

Introduction

Gout is a purine metabolism disease that causes MSU (Monosodium Urate) crystals in the joint and its surroundings.[1] Gout attacks can cause severe pain and swelling in the joints. If gout were not treated properly, it can cause joint damage, kidney disease, and aggravate cardiovascular disease. With the development of society, the incidence of gout has been increasing year by year and threatens people's health seriously.[2,3] Previous studies have shown that the deposition of MSU crystals in the joints could trigger an acute inflammatory response.[4] Inflammatory cytokines such as TNF-α (Tumor Necrosis Factor-α), IL-1β (Interleukin-1β), and IL-6 are released in large quantities, causing severe pain and systemic reactions.[5] NSAIDs (Nonsteroidal Anti-inflammatory Drugs) colchicine, or other symptomatic treatments are often applied in the clinic.[6] However, there is a high incidence of adverse reactions such as gastrointestinal reactions, liver and kidney damage, and allergic reactions.[7–9] Recently, some studies suggested that natural compounds are expected to be formulated as therapeutic drugs for the treatment of gout.

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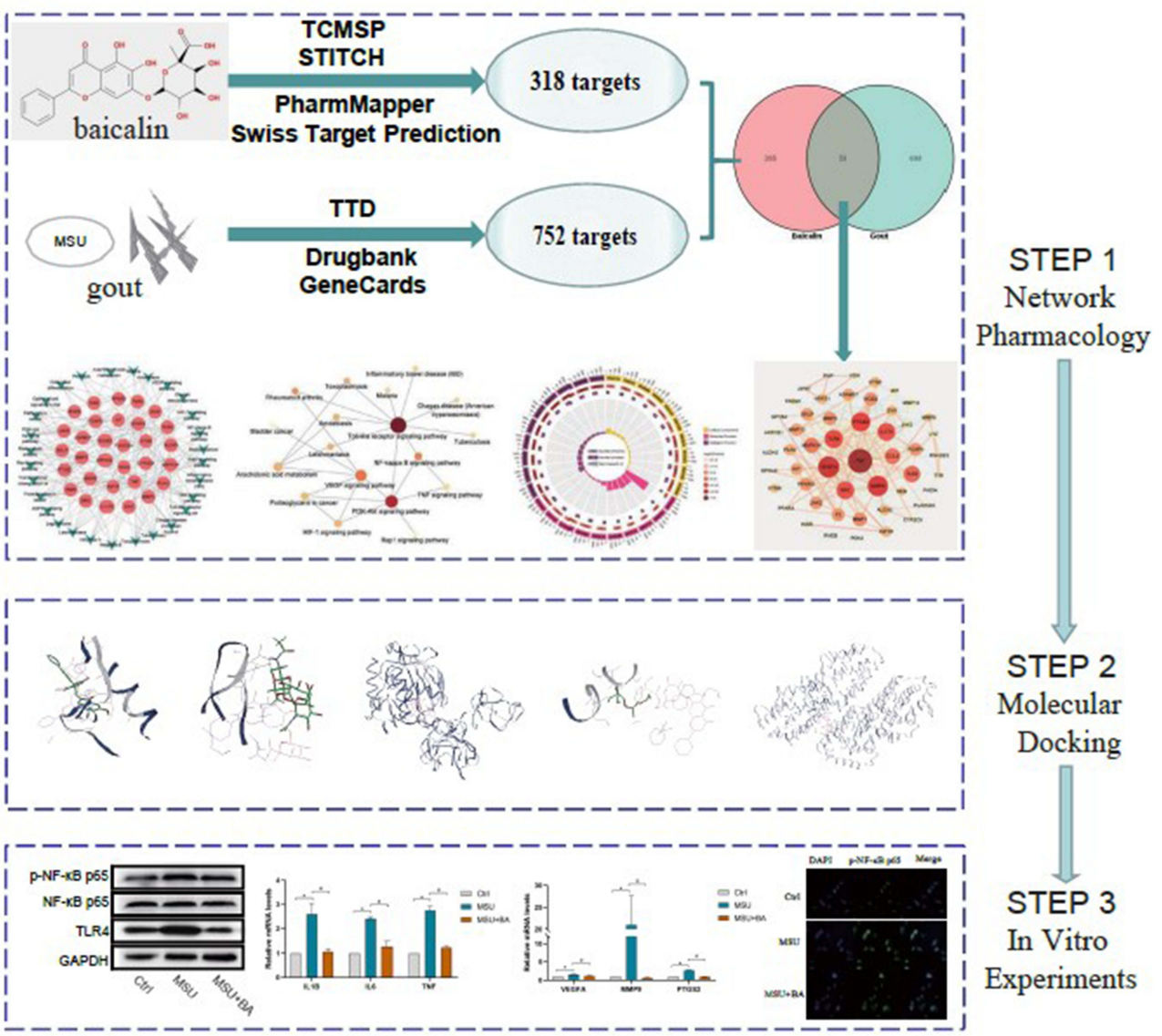
Graphical Abstract



Baicalin is an effective component of Scutellaria baicalensis Georgi with a remarkable anti-inflammatory effect.[10] Wang YZ et al have shown that baicalin exhibited in vitro and in vivo efficacy against gouty arthritis.[11] Another study indicated that baicalin suppressed MSU crystal-induced necrosis and IL-1β release in human THP-1 derived macrophages.[12] These studies show that baicalin is a very potential drug for the treatment of gout. However, the mechanism of baicalin in the treatment of gout needs to be further explored.

From the perspective of the biological network of the system, network pharmacology determines the targets of the natural compounds and uses network analysis to establish the relationship between natural compounds and targets.[13] Network pharmacology clarifies the potential relationship between NPCs (Natural Phytochemical Compounds) and diseases and reveals the pharmacological mechanism of drugs. Molecular docking is a method for drug design based on the characteristics of receptors and the interaction between receptors and drug molecules.[14] A theoretical simulation method that mainly studies the interactions between molecules (such as ligands and receptors) predicts their binding modes and affinities.[15]

Therefore, this study combined network pharmacology to explore the potential targets and mechanisms of baicalin in gout treatment. The predicting results were confirmed by molecular docking and in vitro experiments. The overall process of the study is shown in graphical abstract.

Materials and Methods

Collection of the Potential Targets of Baicalin

Basic information, such as mol2 format structure file, OB (Oral Bioavailability), a DL (Drug Like index), and potential targets of baicalin were obtained from the traditional Chinese medicine system pharmacology database (TCMSP: https://tcmspw.com/tcmsp.php).[16] In the form of ADME (absorption, distribution, metabolism, and excretion) processes, OB and DL are the two most important parameters to measure the pharmacokinetic process of drugs in vivo.[15] Then, the mol2 format structure file was uploaded to the PharmMapper online service platform (http://www.lilab-ecust.cn/pharmmapper/) to predict the potential targets.[17] In addition, the 2D structure of baicalin was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/),[18] and then imported to the STITCH (http://stitch.embl.de/)[19] and Swiss Target Prediction (http://www.swisstargetprediction.ch/) databases[20] to predict the potential targets. The target names were converted into official gene names through the UniProt database (https://www.uniprot.org/).[21] The species was set to "Homo sapiens".

Collection of Gout-Related Targets

Gout-related targets were retrieved from TTD (http://db.idrblab.net/ttd/),[22] Drugbank (https://www.drugbank.ca/)[23] and GeneCards[24] (https://www.genecards.org/) (Relevance score ≥1.0). The target names were converted into official gene names through the UniProt database (https://www.uniprot.org/). The species was set to "Homo sapiens". The targets collected from the above databases were merged and deduplicated.

Construction of PPI Network

Baicalin and gout-related targets were uploaded to OmicShare Tools[25] (http://www.omicsshare.com/tools/index.php/) to draw a Venn diagram and obtain the common genes. The STRING database (https://string-db.org)[26] was used to draw the PPI (protein–protein interactions) network. With the help of Cytoscape 3.7.2 software, topology analysis of the PPI network was carried out.[27] Network topology eigenvalues, such as degree and betweenness centrality, were obtained. The hub genes were screened according to the parameters.[28]

Bioinformatic Annotation

The Ensembl ID of the common genes was obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/). After that, the GO enrichment analysis was carried out via OmicShare Tools (http://www.omicsshare.com/tools/index.php/).[29] KEGG of the common genes. The threshold value of $p < 0.05$ was set. The first 10 of BP (Biological Process) items, CC (Cellular Composition) items, MF (Molecular Function) items, and the first 20 KEGG items were imported into OmicShare Tools for realize the visualization. Concurrently, the topology analysis of the relationship network of the top 20 pathways was performed by Cytoscape 3.7.2 software.

Target-Pathway Network Construction

The top 20 enriched signaling pathways and their enriched targets were imported into Cytoscape 3.7.2. The "target-pathway" network diagram was drawn.

Molecular Docking

The PDB (Protein Data Bank) (https://www.rcsb.org/) database[30] was used to retrieve the 3D structure files of the hub targets as the receptors. The 3D structure files and the mol2 format of baicalin were uploaded to iGEMDOCK software,[31] respectively. The files after molecular docking were visualized.

Experiment Verification

Cell Culture, Differentiation and Intervention

THP-1 cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. THP-1 cells cultured in RPMI-1640 medium (Gibco-BRL, China) containing 10% FBS (Fetal Bovine Serum) (Gibco-16000-044, China), 100 U/mL penicillin and 100 μg/mL streptomycin (Solarbio, China). THP-1 cells in the logarithmic growth phase were treated with 100 ng/mL PMA (Sigma-Aldrich, USA) to induce their differentiation into M0 macrophages. The MSU group was stimulated with 200 ug/mL MSU (Sigma-Aldrich, USA) to simulate the inflammatory response of macrophages in gout. In the baicalin group, MSU-stimulated macrophages were given 10 uM/mL baicalin (MedChemexpress, USA). The cells were cultured in a 37°C, 5% CO2 cell incubator.

RNA Extraction and Quantitative Real-Time PCR (RT-qPCR)

TLR4, NF-κB/RELA, IL-1β, IL-6, TNF, VEGFA, MMP9 and PTGS2 mRNA were measured by RT-qPCR. After the intervention of each group, Trizol (Solarbio, China) was used to extract total RNA, which was reverse transcribed into cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Germany). RT-qPCR was performed using a BioEasy Master Mix SYBR Green Kit (Bioer Technology, China). The sequences of the primers used for RT-qPCR analysis are given in Table 1. The relative expression of the target mRNA was calculated by the formula 2-ΔΔCT.

Western Blot

After intervention, the culture medium in each group of culture dishes was aspirated and discarded. RIPA cell lysate was added, and cell proteins were extracted. Then, the protein concentrations were measured with the BCA Protein Assay Kit (CWBIOtech, China). Equivalent amount of protein from each group was separated by SurePAGETM gel (GenScript, China) and transferred to PVDF (polyvinylidene difluoride) membranes (Millipore, Billerica, MA). Subsequently, the PVDF membranes were incubated overnight with different primary antibodies against TLR4 (1: 1000 dilution, Abcam, UK), NF-κB p65 (1: 1000 dilution, Santa Cruz Biotechnology, USA), p-NF-κB p65 (1: 1000 dilution, Santa Cruz Biotechnology, USA), and GAPDH (1: 5000 dilution, Proteintech, USA) at 4°C, respectively. Following 3 washes, the membranes were incubated for 1 h with secondary horseradish peroxidase-conjugated antibody at room temperature. Immunolabelled protein bands were detected with ECL substrate (Engreen Biosystem, China). Semiquantitative analysis was performed by ImageJ software. Target protein levels were normalized against the GAPDH level.

Table 1 Index Primer Sequences

Gene Name	Primer Sequences (5'–3')	
TLR4	Forward	TGAGCAGTCGTGCTGGTATC
	Reverse	CAGGGCTTTTCTGAGTCGTC
RELA	Forward	AAGAAGCGGGACCTGGAGCA
	Reverse	GGGCACGATTGTCAAAGATGG
IL1B	Forward	CAAAGGCGGCCAGGATATAA
	Reverse	CTAGGGATTGAGTCCACATTCAG
IL6	Forward	AACTCCTTCTCCACAAGCGCC
	Reverse	CCGTCGAGGATGTACCGAAT
TNF	Forward	AGGCAGTCAGATCATCTTCTCG
	Reverse	TATCTCTCAGCTCCACGCCAT
VEGFA	Forward	AGGGCAGAATCATCACGAAGT
	Reverse	AGGGTCTCGATTGGATGGCA
MMP9	Forward	CCTGGAGACCTGAGAACCAA
	Reverse	AGTGTAACCATAGCGGTACAGG
PTGS2	Forward	CCCTTGGGTGTCAAAGGTAA
	Reverse	GCCCTCGCTTATGATCTGTC
GAPDH	Forward	GGAGCGAGATCCCTCCAAAAT
	Reverse	GGCTGTTGTCATACTTCTCATGG

Immunofluorescence

THP-1 cells were placed in 24-well plates and adhesion was induced. After treatment, the cells were fixed with 4% paraformaldehyde for 10 min and rinsed with cold PBS for 3 times. Then, 0.5% Triton X-100 (Solarbio, China) was added for 10 min and rinsed again. After blocking with 10% fetal bovine serum at 37°C for 1 hour, the p-NF-κB p65 primary antibody (1:200 dilution, Cell Signaling Technology, USA) was incubated overnight at 4°C. The next day the cells were washed again with cold PBS 3 times. The cells were incubated with the same type of secondary antibody for 30 minutes in the dark, stained with DAPI for 10 minutes, and then blocked. Images were detected with a fluorescence microscope.

Statistical Analysis

GraphPad Prism 8 software (San Diego, CA) was used to perform statistical analysis. Data were expressed as the mean ± standard deviation. P values were calculated by analysis of one-way ANOVA followed by Tukey post hoc tests as appropriate. Significance is determined at $p < 0.05$.

Results

Information and Potential Targets of Baicalin and Gout

The molecular ID of baicalin was MOL002776, OB is 40.12%, and DL is 0.75. Canonical SMILES:C1=CC=C(C=C1)C2=CC(=O)C3=C(C(=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O, which was obtained from the PubChem database. The molecular structure of baicalin is shown in Figure 1. There were 300 targets predicted from the PharmMapper online server, 2 from the TCMSP database, 10 from the STITCH database, and 18 from the Swiss Target Prediction database. After eliminating duplicates, a total of 318 targets of baicalin were collected. Baicalin has the characteristic of multi-targets regulation. A total of 752 gout-related targets were collected after eliminating duplicates. A total of 114 gout-related targets were obtained from the Drugbank database, 18 from the TTD database, and 655 from the GeneCards database. The occurrence and development of gout are closely related to many targets.

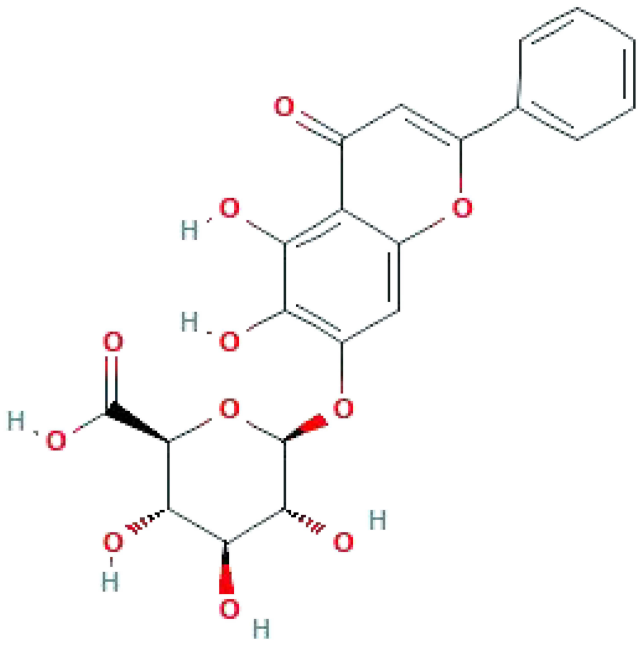


Figure 1 Molecular structure of baicalin.

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Constructing PPI Network and Screening of Hub Targets

In this study, 53 common targets were obtained for baicalin and gout for further analysis (Figure 2A). Cytoscape 3.7.2 was used to draw the PPI network diagram of common targets (Figure 2B). There were 51 nodes and 319 connections in the PPI network. The larger the node is, the darker the color, and the thicker the connection to other nodes, the more important the node is.

According to the main parameters of a topological analysis, TNF, VEGFA, MMP9, PTGS2, and TLR4, etc. might be considered the key targets. Baicalin may exert the therapeutic effect on gout by regulating the hub targets primarily. The main topological parameters of the top 20 targets are shown in Table 2.

Bioinformatic Annotation

GO and KEGG enrichment analyses were performed to identify the functional characteristics of the common genes. A total of 2799 GO items were enriched via OmicShare tools, including 2459 BP items, 97 CC items, and 243 MF items ($p < 0.05$). According to the p value from small to large, the top 10 items are shown through the enrichment circle graph. In BP items, regulation of response to external stimulus, inflammatory response, cell activation, etc. were the main enrichment items; CC items were mainly enriched in the extracellular region part, extracellular space, extracellular region, etc.; MF items were mainly concentrated in catalytic activity, identical protein binding, transmembrane receptor protein tyrosine kinase activity,

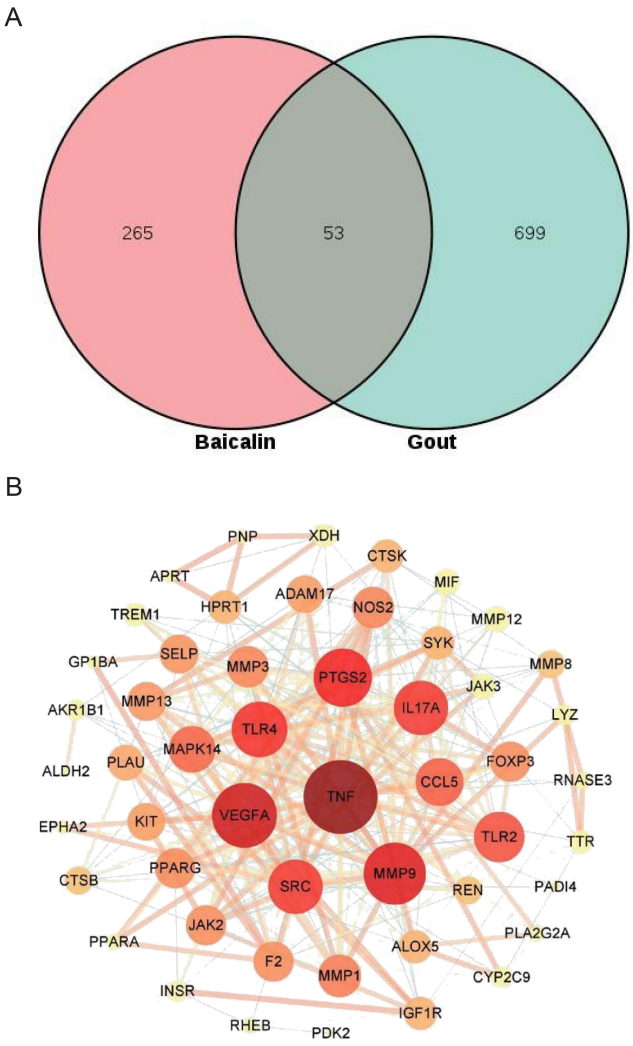

Figure 2 Common targets of baicalin and gout and PPI network analysis. (**A**) venn diagram of baicalin and gout targets; (**B**) PPI network of baicalin in treating potential targets of gout).

Table 2 Topological Parameters of the Top 20 Genes in the PPI Network

Targets	Degree	Betweenness	Closeness	Targets	Degree	Betweenness	Closeness
TNF	41	550.632	0.833	*MMP1*	17	8.533	0.568
VEGFA	34	259.646	0.746	*MMP3*	16	6.747	0.562
MMP9	32	141.742	0.694	*NOS2*	16	23.677	0.562
PTGS2	29	146.076	0.676	*PPARG*	15	14.127	0.556
TLR4	27	95.624	0.667	*FOXP3*	15	10.523	0.562
IL17A	26	70.147	0.658	*JAK2*	15	18.357	0.562
TLR2	23	42.345	0.633	*MMP13*	14	4.516	0.543
SRC	26	223.165	0.649	*SELP*	14	32.233	0.556
MAPK14	20	24.952	0.588	*ADAM17*	13	6.822	0.549
CCL5	21	48.303	0.602	*KIT*	13	39.022	0.562

etc. (Figure 3A). The KEGG pathway was enriched to obtain 29 items ($p < 0.05$). The top 20 significantly enriched KEGG terms are shown in Figure 3B. The relationship between the top 20 enriched pathways was analysed by OmicShare tools and visualized by Cytoscape 3.7.2 (Figure 3C). Among them, 18 signaling pathways have connections with each other. According to the degree, the TLR4/NF-κB signaling pathway might be a significant pathway of baicalin in treating gout.

Target-Pathway Network

The first 20 KEGG pathways and their enriched targets were imported into Cytoscape 3.7.2 to draw the "target-pathway" network, which connected these potential targets, and corresponding pathways. The network involves 29 signaling pathway nodes, 31 target nodes and 165 edges. (Figure 3D) As seen in the figure, TNF, TLR4, PTGS2, etc. can be involved in mediating toll-like receptor signaling, NF-kappa B signaling pathway and other pathways. It reflects the multi-target and multi-pathway action characteristics of baicalin.

Molecular Docking

Molecular docking was carried out between TNF, VEGFA, MMP9, COX-2 (PTGS2), TLR4 and baicalin. The molecular structures of these targets can be found in the supplementary materials. The larger the absolute value of the docking affinity, the greater the binding capability between the active sites of targets and compounds. Baicalin could well combine with the hub targets, respectively. The molecular docking structures and energy are shown in Figure 4A-E and Table 3.

Baicalin Inhibits the Expression of Multiple Inflammatory Cytokines in THP-1 Cells

We first used RT-qPCR to examine the expression of inflammatory cytokines to evaluate the anti-inflammatory effect of baicalin. Compared with the control group, MSU could significantly upregulated the expression of IL-1β, IL-6, and TNF in THP-1 cells (*$p < 0.05$ compared with the control group). In comparison to the MSU group, baicalin greatly suppressed the expression of IL-1β, IL-6, and TNF (#$p < 0.05$ compared with the model group). (Figure 5A) These data suggested that baicalin could inhibit the MSU-induced inflammatory response in THP-1 cells.

Baicalin Regulates the TLR4/NF-κB Signaling Pathway in THP-1 Cells

Based on the KEGG enrichment and molecular docking results, we speculated that baicalin might attenuate MSU-induced inflammation by regulating the TLR4/NF-κB signaling pathway. To verify this hypothesis, we used RT-qPCR and Western blot to detect the expression of key proteins of this signaling pathway. After stimulation with MSU crystal, the relative mRNA levels of TLR4 and RELA, and the relative protein levels of TLR4, NF-κB p65, and p-NF-κB p65 in THP-1 cells were significantly increased ($p < 0.05$). Baicalin significantly decreased their levels ($p < 0.05$). (Figure 5B and C) In addition, the immunofluorescence results were consistent with the Western blot results. BA treatment blocked the MSU-activated NF-κB signaling pathway, as evidenced by the reduced fluorescence intensity of p-NF-κB p65 in the nucleus (Figure 5D). The data above indicated that baicalin could inhibit the TLR4/NF-κB signaling pathway activated by MSU in THP-1 cells.

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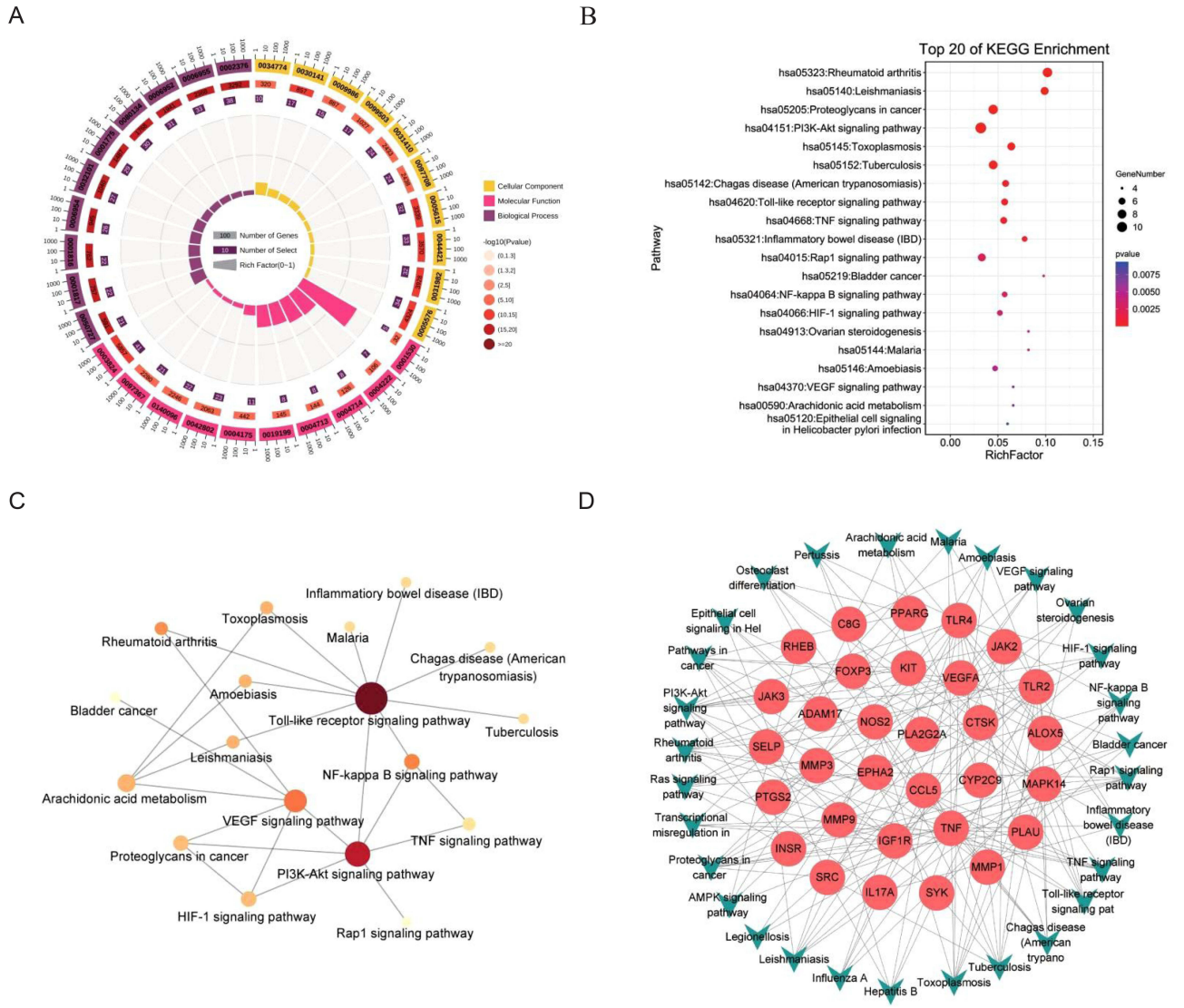

Figure 3 GO and KEGG enrichment analysis of potential targets from the baicalin for treatment of gout. (**A**) GO enrichment circle diagram. From outside to inside, the first circle is the ID of the item, different colors represent different classifications; At the second circle, the number and p value of the classification in the total gene; The longer the bars, the more genes, and the smaller the p value, the redder the color; The third circle are the number of scene genes; The fourth circle is the Richfactor value of each category; (**B**) bubble diagram of the top 20 significant KEGG enrichment terms. The larger size of a bubble indicates the larger number of genes in the item, and the redder color of a bubble stands for the lower the p value; (**C**) KEGG enriched pathway network diagram; The node represents the signaling pathway, and the line represents the crosstalk relationship between signaling pathways. (**D**) The enriched pathway-targets network diagram. Circular nodes represent targets, irregular nodes represent signaling pathways. The line represents the enrichment relationship between target and pathway).

Baicalin Inhibits the Expression of the Hub Targets in THP-1 Cells

At last, the expression of other hub targets (VEGFA, MMP9, and PTGS2) were further detected by RT-qPCR. The results showed VEGFA, MMP9, and PTGS2 mRNA levels were significantly up-regulated after MSU stimulation ($p < 0.05$), while MSU+BA group significantly decreased ($p < 0.05$). (Figure 6) The results showed that the hub targets screened above may play biological roles in gout and that baicalin has a regulatory effect on them.

Discussion

With the improvement of modern medicine, the understanding of the pathogenesis of gout has gradually deepened. However, gout treatment still faces challenges. Thus, new drugs for gout therapy should be developed. A study results showed that the extracts from Scutellaria baicalensis Georgi could alleviate MSU crystal-induced inflammation.[32] The flavonoids, baicalin and its aglycone, baicalein 2 are found in Scutellaria baicalensis Georgi. The antioxidant and anti-

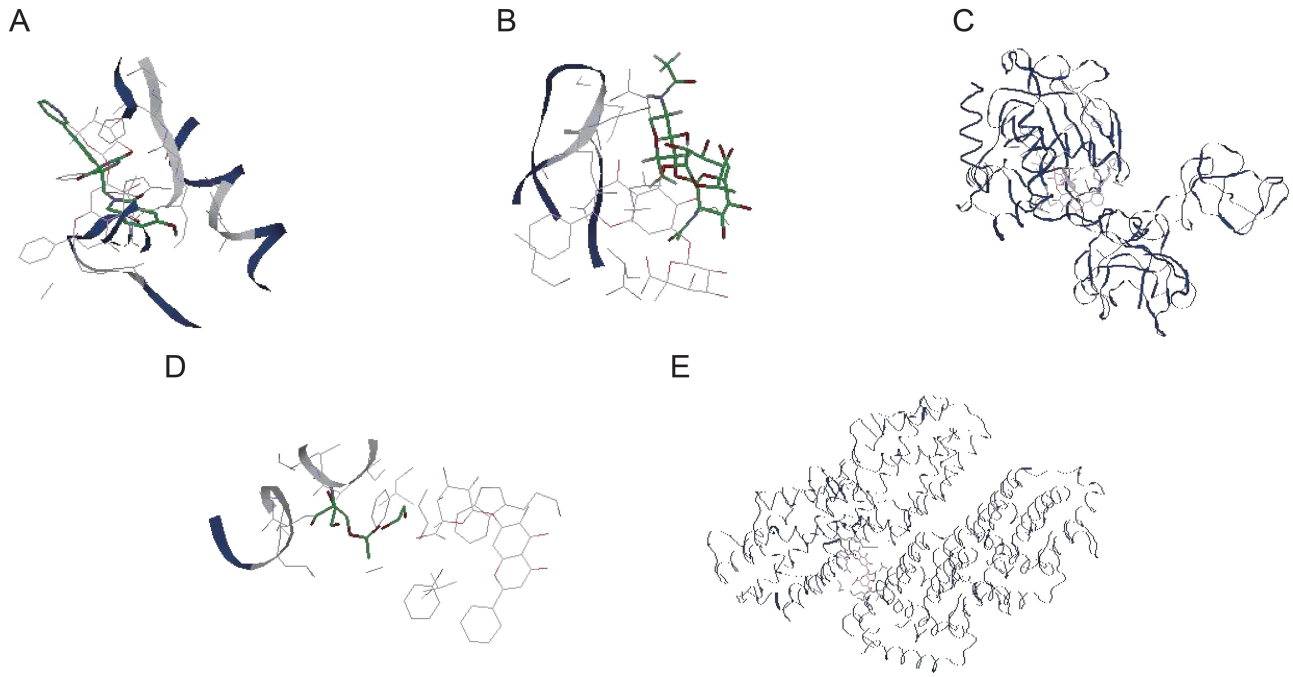

Figure 4 Schematic diagram of docking results. (**A**). Baicalin-TNF; (**B**) Baicalin-VEGFA; (**C**) Baicalin-MMP9; (**D**) Baicalin-COX2; (**E**) Baicalin-TLR4).

inflammatory effects of baicalin have been demonstrated in inflammatory disorder models including gout.[33] In ADME processes, NPCs with OB ≥ 30% and DL ≥ 0.18 were considered good pharmacokinetically active components.[34] The OB of baicalin is 40.12%, and the DL is 0.75. This indicates that baicalin has good pharmacological activity. A Venn diagram showed that there were 53 common genes between baicalin and gout. This indicates that baicalin might alleviate gout by regulating the 53 common targets. To further explore the mechanism of baicalin in the treatment of gout, bioinformatics analysis was carried out.

PPI topology analysis showed that there was a close and complex relationship between the 53 common genes. TNF, VEGFA, MMP9, PTGS2, and TLR4 might be the most important targets of baicalin in gout treatment. TNF-α is a proinflammatory factor mainly secreted by macrophages and is widely involved in inflammatory responses.[35] Zhou M et al found that the expression level of TNF-α was significantly increased in the local inflammatory response in an MSU crystal-induced rat gout model.[36] MMP9 belongs to the MMP family, which degrades the extracellular matrix. MSU crystals could upregulate the expression of MMP9 in macrophages. This can reflect the joint pathological status of patients with gouty arthritis.[37] COX-2 (Cyclooxygenase-2), also known as PTGS2 (prostaglandin endooxidase reductase 2), is a key enzyme that catalyzes the conversion of arachidonic acid into prostaglandins and plays an important biological role in inflammatory diseases.[38] A systematic review summarized that selective COX-2 inhibitors may, next to their pain killing properties, act in a chondroprotective manner in vivo.[39] MSU crystals were injected into the ankles of rats to replicate the gout disease model, and increased expression of COX-2 was observed.[40] This suggests that COX-2 might mediate pain attacks and cartilage injuries in gout. TLR4 is a key regulator of innate and adaptive immune

Table 3 Molecular Docking Energy Scores of Baicalin and Target Proteins

Targets	Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
TNF	3lea	−121.370	−84.602	−35.908	−0.860	20.091
VEGFA	5o4e	−107.172	−54.697	−49.853	−2.623	14.758
MMP9	1l6j	−107.643	−79.090	−27.198	−1.355	17.121
COX-2	5f19	−83.365	−67.812	−15.553	0	14.182
TLR4	5uc8	−123.851	−86.888	−37.317	0.354	22.333

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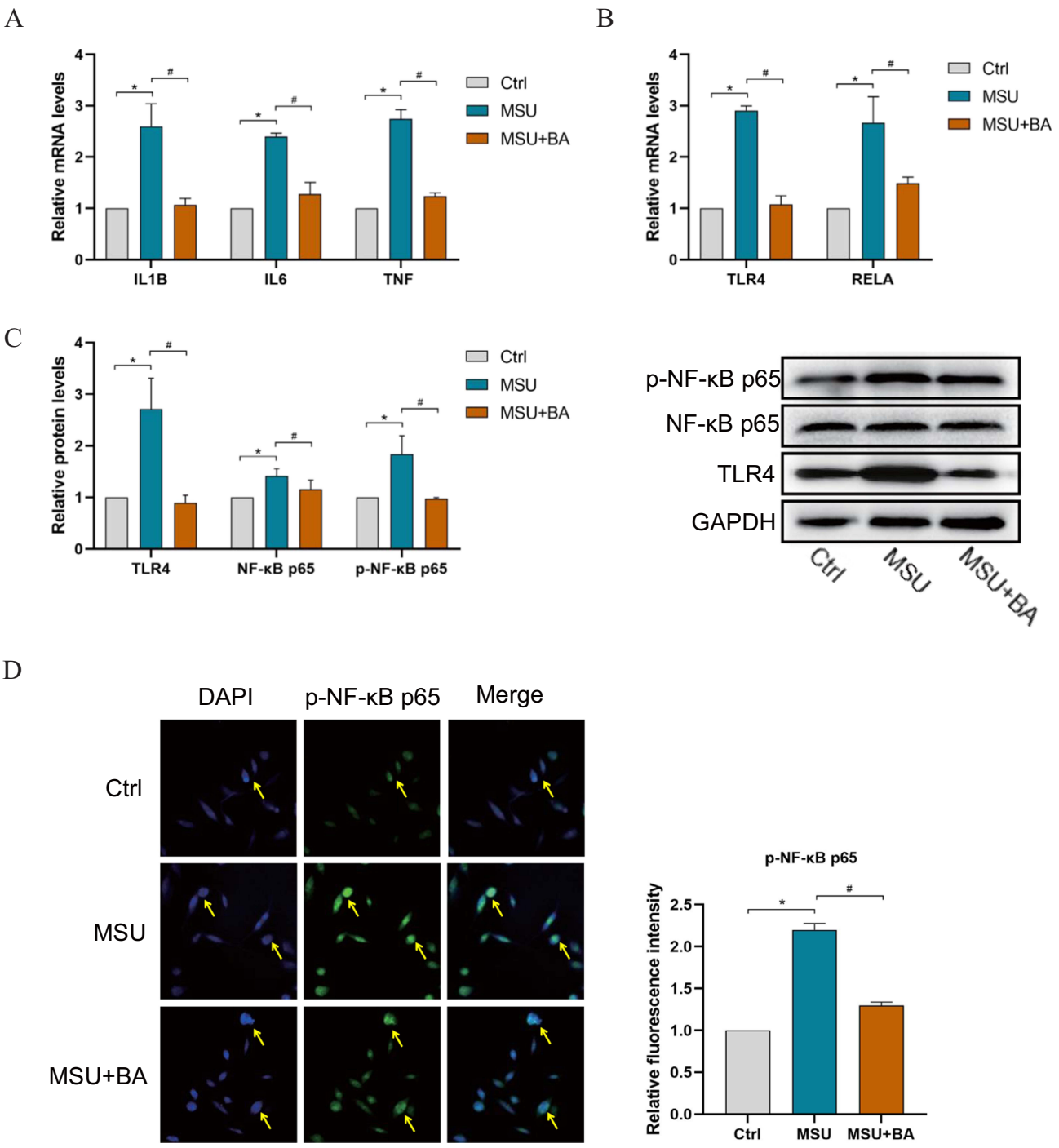

Figure 5 Anti-inflammatory effects of baicalin on MSU-induced inflammation in THP-1 cells (**A**) RT-qPCR results for baicalin regulatory effect of reducing the mRNA level of IL-1β, IL-6, and TNF; (**B**) RT-qPCR results for baicalin regulatory effect of reducing the mRNA level of TLR4 and RELA; (**C**) Baicalin inhibits the expression of the TLR4/NF-κB by Western blot; (**D**) Effect of baicalin on the nuclear translocation of NF-κB p65. *Compared with the control group, $p<0.05$; # Compared with the MSU group, $p<0.05$. Positive cells were indicated by yellow arrows).

responses and plays a crucial role in amplifying inflammatory responses. A study suggested that intra-articular injection of MSU in mice increased IL-1β release mediated by TLR4 activation, causing severe pain and an inflammatory response.[41] VEGFA plays an important biological role in angiogenesis and endothelial cell growth. A genome-wide association analysis identified VEGFA associated with serum urate concentrations, which could cause gout.[42] It is speculated that baicalin not only relieves gout inflammation but also has a beneficial effect on the repair of gout

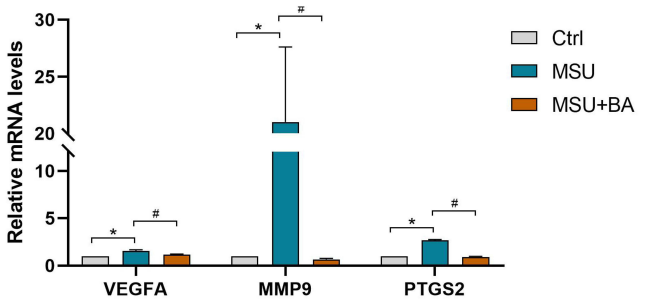

Figure 6 Regulatory effects of baicalin on the expression of hub targets in MSU-stimulated THP-1 cells (RT-qPCR results for baicalin regulatory effect of reducing the mRNA level of VEGFA, MMP9, and PTGS2. *Compared with the control group, $p<0.05$; # Compared with the MSU group, $p<0.05$).

pathological damage. Previous studies have shown that MSU crystal can activate inflammatory signaling pathways, resulting in the massive release of inflammatory cytokines.[43,44] Subsequently, the release of inflammatory cytokines induced vasodilation, promoted the rapid accumulation of neutrophils and upregulated neuron-mediated inflammatory pain sensitivity, causing vascular endothelial injury.[45–47] These results are consistent with the results of the PPI analysis.

The GO and KEGG analyses demonstrated that baicalin might regulate a variety of biological processes and signaling pathways in gout. Further analysis illustrated that multiple target genes could be found in any enriched signaling pathway, and a gene could be enriched in different signaling pathways. Interestingly, most of the signaling pathways interacted with each other. Among these enriched main signaling pathway networks, the TLR4 signaling pathway might play the most critical role. NF-κB is a transcription factor downstream of TLR4. When stimulated by inflammatory mediators, NF-κB is activated by phosphorylation and enters the nucleus to play a role in transcriptional regulation.[48] The TLR4/NF-κB signaling pathway was activated in gout mice, and promoted the inflammatory response by releasing extensive inflammatory factors such as IL-1β, IL-6, and TNF-α.[44] And acute gouty arthritis could be alleviated in rats by inhibiting inflammation via the TLR4/NF-κB pathway.[49] It was also confirmed by the in vitro experiment of this study. It has been proven that inflammation often induces severe local tissue hypoxia. Cells sense and respond to hypoxia through the activity of the transcription factor hypoxia-inducible factor (HIF) and its regulatory hydroxylases.[50] In the MSU crystal-induced peritonitis mouse model, the peritoneal macrophages showed significantly increased mRNA levels of HIF-1α.[51]

Another study indicated that NF-κB, which plays a central role in the inflammatory response, was involved in the increased expression of HIF-1α in AKI. Mechanistically, NF-κB directly bound to the HIF-1α promoter and enhanced its transcription.[52] Furthermore, LPS-induced NF-κB activation could be inhibited via the PI3K/Akt pathway.[53] The pathogenesis of gout involves multiple-signal transduction pathways, and there is a relationship between them.

Molecular docking results indicated that baicalin could stably bind to the hub targets, thereby exerting a pharmacological effect on relieving gout, and it is a good idea for baicalin to treat gout through the predicted targets. And the results of in vitro experiments also confirmed that baicalin could inhibit the increased expression of these hub targets induced by MSU crystal. This study provides evidence that the prediction results based on network pharmacology are reliable.

Our previous study has confirmed that targets such as IL-1 β, NFKBIA, IL-6, TNF - α, and MMP9 are important targets for mediating gouty joint inflammation. And Its anti-inflammatory mechanism in treating gout involves the NF-κB p65 signaling pathway.[54] We further verified the prediction results of network pharmacology through in vitro experiments. THP-1 cells were induced by MSU crystal to simulate the inflammatory response in gout. Baicalin inhibited the expression of TLR4, RELA, IL-1β, IL-6, and TNF mRNA, reduced the protein levels of TLR4, NF-κB p65, and p-NF-κB p65, and inhibited p65 activation. It is suggested that baicalin could inhibit the TLR4/NF-kB signaling pathway to play an anti-inflammatory role.

However, the present study has several limitations. First, the database is limited and it is impossible to predict all potential targets of baicalin. Second, although we have performed molecular docking, it still cannot meet the needs of some specific in vivo biological processes and high-precision calculations. Third, neither network pharmacology,

molecular docking nor in vitro experiments can predict the in vivo metabolism and efficacy of baicalin. Therefore, it is highly necessary to conduct further in vivo experiments and clinical trials.

Conclusion

Based on the network pharmacology, molecular docking analysis, and experimental validation, the potential mechanism of baicalin in gout treatment involves the regulation of various signaling pathways and targets. The anti-inflammatory effects of baicalin in gout might inhibit the release of inflammatory cytokines including IL-1β, IL-6, and TNF-α and regulate the TLR4/NF-κB signaling pathways. Moreover, baicalin could regulate the expression of COX-2, VEGFA, MMP9, etc., which may be beneficial for reducing gout symptoms and facilitating tissue recovery. These issues need further research to be explored. Overall, the obtained results suggested that baicalin might be used as a promising therapeutic agent and provide additional evidence for the promotion of the wide use of baicalin in the clinic for the treatment of gout.

Data Sharing Statement

All drug compounds and related disease targets data were available from public databases.

Ethical Approval

The study was approved by the Ethics Committee of First Teaching Hospital of Tianjin University of Traditional Chinese Medicine (TYLL2021[Z]017).

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.

References

1. Narang RK, Dalbeth N. Pathophysiology of gout. *Semin Nephrol*. 2020;40(6):550–563. doi:10.1016/j.semnephrol.2020.12.001
2. Singh JA, Gaffo A. Gout epidemiology and comorbidities. *Semin Arthritis Rheumatism*. 2020;50(3s):S11–s16. doi:10.1016/j.semarthrit.2020.04.008
3. Richette P, Doherty M, Pascual E, et al. 2018 updated European league against Rheumatism evidence-based recommendations for the diagnosis of gout. *Ann Rheumatic Dis*. 2020;79(1):31–38. doi:10.1136/annrheumdis-2019-215315
4. Lan Z, Chen L, Feng J, et al. Mechanosensitive TRPV4 is required for crystal-induced inflammation. *Ann Rheumatic Dis*. 2021;80(12):1604–1614. doi:10.1136/annrheumdis-2021-220295
5. Chen B, Li H, Ou G, Ren L, Yang X, Zeng M. Curcumin attenuates MSU crystal-induced inflammation by inhibiting the degradation of IκBα and blocking mitochondrial damage. *Arthritis Res Therapy*. 2019;21(1):193. doi:10.1186/s13075-019-1974-z
6. Dalbeth N, Choi HK, Joosten LAB, et al. Gout. *Nat Rev Dis Primers*. 2019;5(1):69. doi:10.1038/s41572-019-0115-y
7. Ly J, Gow P, Dalbeth N. Colchicine prescribing and safety monitoring in patients with gout. *N Z Med J*. 2007;120(1265):U2808.
8. Paydas S. Management of adverse effects/toxicity of ibrutinib. *Crit Rev Oncol Hematol*. 2019;136:56–63. doi:10.1016/j.critrevonc.2019.02.001
9. Moore N, Duong M, Gulmez SE, Blin P, Droz C. Pharmacoepidemiology of non-steroidal anti-inflammatory drugs. *Therapie*. 2019;74(2):271–277. doi:10.1016/j.therap.2018.11.002

10. Fu YJ, Xu B, Huang SW, et al. Baicalin prevents LPS-induced activation of TLR4/NF-κB p65 pathway and inflammation in mice via inhibiting the expression of CD14. *Acta Pharmacol Sin*. 2021;42(1):88–96. doi:10.1038/s41401-020-0411-9
11. Wang Y, Liu M, Li J, et al. Preparing a novel baicalin-loaded microemulsion-based gel for transdermal delivery and testing its anti-gout effect. *Saudi Pharma J Official Public Saudi Pharm Society*. 2024;32(6):102100. doi:10.1016/j.jsps.2024.102100
12. Zhong CS, Zeng B, Qiu JH, et al. Gout-associated monosodium urate crystal-induced necrosis is independent of NLRP3 activity but can be suppressed by combined inhibitors for multiple signaling pathways. *Acta Pharmacol Sin*. 2022; 43(5): 1324–1336. doi:10.1038/s41401-021-00749-7
13. Wang X, Wang ZY, Zheng JH, Li S. TCM network pharmacology: a new trend towards combining computational, experimental and clinical approaches. *Chin J Nat Med*. 2021;19(1):1–11. doi:10.1016/S1875-5364(21)60001-8
14. Pinzi L, Rastelli G. Molecular docking: shifting paradigms in drug discovery. *Int J mol Sci*. 2019;20(18):18. doi:10.3390/ijms20184331
15. Zeng YJ, Wu M, Zhang H, et al. Effects of qinghuang powder on acute myeloid leukemia based on network pharmacology, molecular docking, and in vitro experiments. *Evidence-Based Complementary Alternat Med*. 2021;2021:6195174. doi:10.1155/2021/6195174
16. Ru J, Li P, Wang J, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminf*. 2014;6(1):13. doi:10.1186/1758-2946-6-13
17. Wang X, Shen Y, Wang S, et al. PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. *Nucleic Acids Res*. 2017;45(W1):W356–w360. doi:10.1093/nar/gkx374
18. Wang Y, Bryant SH, Cheng T, et al. PubChem BioAssay: 2017 update. *Nucleic Acids Res*. 2017;45(D1):D955–d963. doi:10.1093/nar/gkw1118
19. Kuhn M, Szklarczyk D, Pletscher-Frankild S, et al. STITCH 4: integration of protein-chemical interactions with user data. *Nucleic Acids Res*. 2014;42(Database issue):D401–7. doi:10.1093/nar/gkt1207
20. Yang Y, Zhou X, Jia G, et al. Network pharmacology based research into the effect and potential mechanism of Portulaca oleracea L. polysaccharide against ulcerative colitis. *Comput Biol Med*. 2023;161:106999. doi:10.1016/j.compbiomed.2023.106999
21. Bateman A, Martin M-J, Orchard S. UniProt: the Universal Protein Knowledgebase in 2023. *Nucleic Acids Res*. 2023;51(D1):D523–d531. doi:10.1093/nar/gkac1052
22. Zhou Y, Zhang Y, Zhao D, et al. TTD: therapeutic target database describing target druggability information. *Nucleic Acids Res*. 2024;52(D1): D1465–d1477. doi:10.1093/nar/gkad751
23. Wishart DS, Feunang YD, Guo AC, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res*. 2018;46(D1): D1074–d1082. doi:10.1093/nar/gkx1037
24. Rebhan M, Chalifa-Caspi V, Prilusky J, Lancet D. GeneCards: integrating information about genes, proteins and diseases. *Trends Genetics: TIG*. 1997;13(4):163. doi:10.1016/S0168-9525(97)01103-7
25. Mu H, Chen J, Huang W, et al. OmicShare tools: a zero-code interactive online platform for biological data analysis and visualization. *iMeta*. 2024;3(5):e228. doi:10.1002/imt2.228
26. Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*. 2015;43(Database issue):D447–52. doi:10.1093/nar/gku1003
27. Kang P, Wu Z, Zhong Y, et al. A network pharmacology and molecular docking strategy to explore potential targets and mechanisms underlying the effect of curcumin on osteonecrosis of the femoral head in systemic lupus erythematosus. *Biomed Res Int*. 2021;2021(1):5538643. doi:10.1155/2021/5538643
28. Li H, Ma X, Tang Y, Wang D, Zhang Z, Liu Z. Network-based analysis of virulence factors for uncovering Aeromonas veronii pathogenesis. *BMC Microbiol*. 2021;21(1):188. doi:10.1186/s12866-021-02261-8
29. Wang S, Su W, Zhong C, et al. An eight-CircRNA assessment model for predicting biochemical recurrence in prostate cancer. *Front Cell Dev Biol*. 2020;8:599494. doi:10.3389/fcell.2020.599494
30. Berman HM, Westbrook J, Feng Z, et al. The protein data bank. *Nucleic Acids Res*. 2000;28(1):235–242. doi:10.1093/nar/28.1.235
31. Guo W, Xu B, Wang X, Zheng B, Du J, Liu S. The analysis of the anti-tumor mechanism of ursolic acid using connectively map approach in breast cancer cells line MCF-7. *Cancer Manag Res*. 2020;12:3469–3476. doi:10.2147/CMAR.S241957
32. Jung SM, Schumacher HR, Kim H, Kim M, Lee SH, Pessler F. Reduction of urate crystal-induced inflammation by root extracts from traditional oriental medicinal plants: elevation of prostaglandin D2 levels. *Arthritis Res Therapy*. 2007;9(4):R64. doi:10.1186/ar2222
33. Dinda B, Dinda S, DasSharma S, Banik R, Chakraborty A, Dinda M. Therapeutic potentials of baicalin and its aglycone, baicalein against inflammatory disorders. *Eur J Med Chem*. 2017;131:68–80. doi:10.1016/j.ejmech.2017.03.004
34. Zhang Q, Li X, Li J, et al. Mechanism of anti-inflammatory and antibacterial effects of qingxiaowuwei decoction based on network pharmacology, molecular docking and in vitro experiments. *Front Pharmacol*. 2021;12:678685.
35. Wang Y, Zhang H, Chen Q, et al. TNF-α/HMGB1 inflammation signalling pathway regulates pyroptosis during liver failure and acute kidney injury. *Cell Prolif*. 2020;53(6):e12829. doi:10.1111/cpr.12829
36. Zhou M, Ze K, Hua L, et al. Cyr61 promotes inflammation of a gouty arthritis model in rats. *Mediators Inflamm*. 2020;2020:8298615. doi:10.1155/2020/8298615
37. Hsieh MS, Ho HC, Chou DT, et al. Expression of matrix metalloproteinase-9 (gelatinase B) in gouty arthritis and stimulation of MMP-9 by urate crystals in macrophages. *J Cell Biochem*. 2003;89(4):791–799. doi:10.1002/jcb.10530
38. Zhang Z, Li L, Huang G, et al. Embelia Laeta aqueous extract suppresses acute inflammation via decreasing COX-2/iNOS expression and inhibiting NF-κB pathway. *J Ethnopharmacol*. 2021;281:114575. doi:10.1016/j.jep.2021.114575
39. Timur UT, Caron MMJ, Jeuken RM, et al. Chondroprotective actions of selective COX-2 inhibitors in vivo: a systematic review. *Int J mol Sci*. 2020;21(18):18. doi:10.3390/ijms21186962
40. Liu Y, Tang H, Liu X, et al. Frontline science: reprogramming COX-2, 5-LOX, and CYP4A-mediated arachidonic acid metabolism in macrophages by salidroside alleviates gouty arthritis. *J Leukoc Biol*. 2019;105(1):11–24. doi:10.1002/JLB.3HI0518-193R
41. Rossato MF, Hoffmeister C, Trevisan G, et al. Monosodium urate crystal interleukin-1β release is dependent on Toll-like receptor 4 and transient receptor potential V1 activation. *Rheumatology*. 2020;59(1):233–242. doi:10.1093/rheumatology/kez384
42. Köttgen A, Albrecht E, Teumer A, et al. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat Genet*. 2013;45(2):145–154. doi:10.1038/ng.2500

43. Yan CY, Ouyang SH, Wang X, et al. Celastrol ameliorates Propionibacterium acnes/LPS-induced liver damage and MSU-induced gouty arthritis via inhibiting K63 deubiquitination of NLRP3. *Phytomed: Internat J Phytotherapy and Phytopharmacol*. 2021;80:153398. doi:10.1016/j.phymed.2020.153398
44. Huang Q, Gao W, Mu H, et al. HSP60 regulates monosodium urate crystal-induced inflammation by activating the TLR4-NF-κB-MyD88 signaling pathway and disrupting mitochondrial function. *Oxid Med Cell Longev*. 2020;2020:8706898. doi:10.1155/2020/8706898
45. Chen CJ, Shi Y, Hearn A, et al. MyD88-dependent IL-1 receptor signaling is essential for gouty inflammation stimulated by monosodium urate crystals. *J Clin Invest*. 2006;116(8):2262–2271. doi:10.1172/JCI28075
46. Dumusc A, So A. Interleukin-1 as a therapeutic target in gout. *Current Opin Rheumatol*. 2015;27(2):156–163. doi:10.1097/BOR.0000000000000143
47. Zhao Z, Zhao Y, Zhang Y, et al. Gout-induced endothelial impairment: the role of SREBP2 transactivation of YAP. *FASEB J*. 2021;35(6):e21613. doi:10.1096/fj.202100337R
48. Bagaev AV, Garaeva AY, Lebedeva ES, Pichugin AV, Ataullakhanov RI, Ataullakhanov FI. Elevated pre-activation basal level of nuclear NF-κB in native macrophages accelerates LPS-induced translocation of cytosolic NF-κB into the cell nucleus. *Sci Rep*. 2019;9(1):4563. doi:10.1038/s41598-018-36052-5
49. Sun X, Li P, Qu X, Liu W. Isovitexin alleviates acute gouty arthritis in rats by inhibiting inflammation via the TLR4/MyD88/NF-κB pathway. *Pharm Biol*. 2021;59(1):1326–1333. doi:10.1080/13880209.2021.1979595
50. Watts ER, Walmsley SR. Inflammation and hypoxia: HIF and PHD isoform selectivity. *Trends Mol Med*. 2019;25(1):33–46. doi:10.1016/j.molmed.2018.10.006
51. Mian W, Zhang M, Ma Y, et al. Chaetocin attenuates gout in mice through inhibiting HIF-1α and NLRP3 inflammasome-dependent IL-1β secretion in macrophages. *Arch Biochem Biophys*. 2019;670:94–103. doi:10.1016/j.abb.2019.06.010
52. Li ZL, Ji JL, Wen Y, et al. HIF-1α is transcriptionally regulated by NF-κB in acute kidney injury. *Am J Physiol Renal Physiol*. 2021;321(2):F225–f235. doi:10.1152/ajprenal.00119.2021
53. Li M, Ye J, Zhao G, et al. Gas6 attenuates lipopolysaccharide-induced TNF-α expression and apoptosis in H9C2 cells through NF-κB and MAPK inhibition via the Axl/PI3K/Akt pathway. *Int J Mol Med*. 2019;44(3):982–994. doi:10.3892/ijmm.2019.4275
54. Fan YH, Liu W, Jin Y, et al. To Investigate the Mechanism of Qinpi Tongfeng Formula in Treating Acute Gouty Arthritis by UHPLC-Q-Orbitrap-MS, Network Pharmacology and Experimental Validation. *J Inflamm Res*. 2024;17:3475–3498. doi:10.2147/JIR.S454098