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Diseases & Research

Network Pharmacology of Baicalein Targeting TNF Signaling in Inflammation

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Backgrounds: Chronic inflammation is at the centre of the pathogenesis of numerous diseases through the promotion of dysregulated immune responses by intricate cytokine networks, with Tumour Necrosis Factor (TNF) being a central mediator. Though TNF-targeted treatments are effective, they induce side effects and expense, which generates enthusiasm for natural compounds as safer substitutes. Baicalein, a flavonoid derived from Scutellaria baicalensis, is anti-inflammatory in nature but with an uncertain mode of action on the TNF signaling pathway. In this study, network pharmacology and molecular docking were utilized to determine the interaction of Baicalein with TNF receptors and ligands, with the aim of revealing its multitarget modulatory effect on prominent inflammatory pathways.
Methods: A network pharmacology-driven strategy was employed, integrating target prediction, protein–protein interaction analysis, KEGG pathway enrichment, and molecular docking simulations to explore Baicalein's action on TNF signaling.
Results: Docking results revealed Baicalein's strong affinity with TNFR1 (-9.1 kcal/mol), TNFR2 (-8.7 kcal/mol), TNF-α (-8.4 kcal/mol), and TNF-β (-7.9 kcal/mol). Pathway analysis showed modulation of TNF-associated networks, including NF-κB, MAPK, and PI3K-Akt pathways. Network maps and mechanistic diagrams demonstrated Baicalein's multitarget effects.
Conclusion: Baicalein demonstrates significant multitarget activity through modulation of the TNF axis, highlighting its therapeutic potential in chronic inflammatory diseases.
Keywords: Baicalein; TNF signalling; Inflammation; Molecular docking; Network pharmacology; Systems biology.

Introduction

Network pharmacology is a new field at the crossroads of systems biology, bioinformatics, and pharmacology that seeks to explain drug action involving more than one biological target and pathway[1,2]. Contrary to the classical "one-drug–one-target" paradigm, network pharmacology accepts the multitarget and multiscale characteristics of complex diseases —particularly chronic inflammatory, metabolic, and neurodegenerative diseases[3,4].

Network pharmacology is a systems-level strategy that incorporates pharmacology, bioinformatics, and systems biology to elucidate the intricate relationships between multiple biological targets and bioactive compounds[5,6]. In contrast to classical pharmacology, where single-target interactions are accentuated, network pharmacology acknowledges that drugs tend to produce therapeutic effects by modulating networks of genes, proteins, and pathways Fig. 1. Network pharmacology is especially applicable for natural products such as Baicalein, which have pleiotropic functions against interconnected inflammatory pathways. The approach is usually initiated with the prediction of the targets with the help of SwissTargetPrediction or PharmMapper, and then building up protein–protein interaction networks with the help of STRING or BioGRID. Subsequently, KEGG or DAVID is employed to identify significant biological processes in which they are involved for pathway enrichment analysis[7,8]. The compound–target–pathway data is integrated into visual networks by Cytoscape in the most common approach. To further support potential interactions, molecular docking software like AutoDock Vina or SwissDock is utilized to model and measure ligand–receptor binding energies. In certain situations, molecular dynamics simulations can be utilized to assess the compound–target complex stability under physiological conditions[9,10]. Combined, these methods facilitate a holistic description of drug mechanisms, particularly in multifaceted diseases like inflammation, where single-agent therapies are less effective compared to multi-target approaches.

The basic premise of network pharmacology is that drugs cause therapeutic responses through the action on gene/protein/pathway/tissue networks, as opposed to acting on single molecular targets. This integrated approach is particularly useful in studying natural products and traditional compounds, like Baicalein, which tend to exhibit pleiotropic effects owing to their ability.

Tumor necrosis factor (TNF), a master cytokine in inflammation, exerts its effects via TNFR1 and TNFR2, mediating pathways like NF-κB, MAPK, and PI3K-Akt. Dysregulation leads to autoimmune and chronic inflammatory diseases. Baicalein, a key flavonoid in *Scutellaria baicalensis*, has shown anti-inflammatory, anti-cancer, and neuroprotective activities[11,12]. While its effects on TNF signaling are suggested, a precise mechanistic understanding is lacking.

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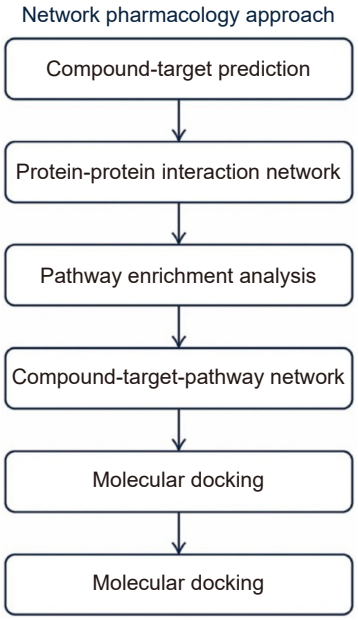

Fig. 1. Network Pharmacology Approach.

Network pharmacology is systems biologically approach that combines pharmacology, computational modeling, and systems biology to investigate how bioactive molecules modulate complex biological networks instead of discrete targets. This shift of paradigm away from the classic "one-drug–one-target" concept is especially relevant to natural products such as Baicalein, which tend to modulate multiple pathways concurrently. In inflammatory diseases, network pharmacology is used to explain interactions between compounds and cytokines (e.g., TNF-α, IL-6), receptors (e.g., TNFR1/2), and downstream signaling pathways like NF-κB, MAPK, and PI3K-Akt[13,14], see Fig. 2. The process generally includes the prediction of compound targets with software such as SwissTargetPrediction or PharmMapper, the generation of protein–protein interaction networks with STRING or BioGRID, and KEGG pathway enrichment analysis to determine biologically relevant pathways[14]. Compound–target–pathway relationships are visualized using tools like Cytoscape, whereas molecular docking tools like AutoDock Vina estimate and measure the binding affinity of ligands to their receptors. Collective use of these tools facilitates a systems-level understanding of the therapeutic mechanism of a compound, and hence network pharmacology is a robust tool for the discovery of multi-target approaches towards complex diseases such as chronic inflammation.

Materials and Methods

Important Approaches in Network Pharmacology

The Network Pharmacology paradigm combines cutting-edge computational and experimental approaches to simulate dynamically and comprehensively intricate biological systems. Major methods in the approach include applying Ordinary Differential Equations (ODEs) for simulating time-dependent molecular concentration changes and signaling behavior to provide accurate modeling of disease development and treatment over time. Boolean Logic Modeling is used to qualitatively model molecular interactions, reducing regulatory relationships to on/off states, thus allowing systematic identification of Failure Nodes — molecular components whose disruption causes system failure—and Restoration Nodes, which are therapeutic targets that return system homeostasis. Multiplexed Chemical Metabolomics (MCheM) combines orthogonal post-column derivatization with high-resolution mass spectrometry for exhaustive annotation of metabolites,

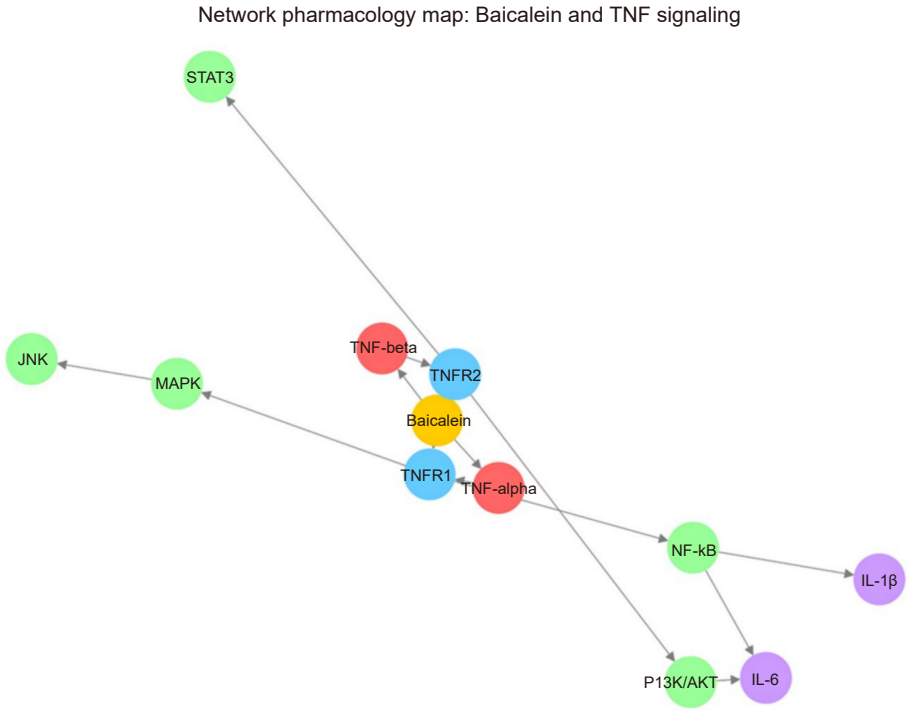

Fig. 2. Network pharmacology map illustrating Baicalein's interactions with TNF ligands, receptors, and downstream signaling pathways.

facilitating identification of new compounds and pathways. The DEAT approach methodically tests the effect of natural or synthetic molecules on modulating failure and restoration nodes in disease networks.

Notably, the methodology utilizes KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathway Integration to project molecular targets and interactions onto curated biological pathways, so that disease mechanisms and therapy are based on thoroughly annotated biochemical networks. With the integration of KEGG-based pathway mapping and dynamic simulation along with node-based analysis, this model offers a multi-layered systems view, allowing for prediction of efficacy of therapy, identification of new drug targets, and rationale for the application of intricate polyherbal formulations in the management of chronic diseases

Target Identification and Validation
Tools: Swiss Target Prediction, Pharm Mapper, SEA (Similarity Ensemble Approach).

Description: Identifies putative biological targets of small molecules based on structural similarity or pharmacophore properties[15].

Gene and Protein Interaction Networks
Tools: STRING, BioGRID, Cytoscape.

Description: Builds protein-protein interaction (PPI) networks to uncover functional associations between targets. Proteins/genes are represented as nodes; edges are known or predicted interactions[16].

Pathway Enrichment Analysis
Tools: DAVID, Enrichr, KEGG, Reactome.

Description: Seeks out biological pathways (e.g., inflammation, apoptosis) statistically enriched in the drug target list from databases such as KEGG or GO[13–17].

Compound–Target–Pathway (C-T-P) Network Construction
Tools: Cytoscape, Metascape.

Description: Interconnects compound, target proteins, and biological pathways as a tripartite network to display multi-layered interactions[17].

Molecular Docking and Binding Simulation
Tools: AutoDock Vina, Schrödinger Glide, SwissDock.

Description: Mimics the physical interaction between receptor and drug at the molecular level, measuring binding affinity and displaying interaction residues[18].

Molecular Dynamics (MD) Simulations (optional)
Tools: GROMACS, AMBER, NAMD.

Description: Assesses the stability and time-dependent conformational changes of the drug-target complex under physiological conditions[13–16,18].

Gene Expression and Disease Association Analysis
Tools: DisGeNET, CTD, GEO (Gene Expression Omnibus).

Description: Maps drug targets to disease-relevant gene expression data to determine therapeutic relevance[19].

Molecular docking for Baicalein against the following TNF-related targets: see Table 1, 2.

- TNFR1 (Tumor Necrosis Factor Receptor 1)
- TNFR2 (Tumor Necrosis Factor Receptor 2)
- TNF-α (ligand)
- TNF-β (ligand)

Methodology: Molecular Docking Approach

In order to study the possible binding interaction of Baicalein with inflammation-related elements of the TNF signaling pathway, molecular docking studies were theoretically planned based on typical in silico protocols. This sub-section describes protein and ligand structure preparation procedures and calculating binding affinity scores through molecular docking algorithms.

Protein Preparation
Protein structures were downloaded from the RCSB Protein Data

Table 1. Target Structures and PDB IDs.

Target	Protein Name	Protein Data Bank (PDB) ID
TNF-α	Tumor Necrosis Factor alpha (trimer)	2AZ5
TNF-β	Tumor Necrosis Factor beta (lymphotoxin-α)	1TNR
TNFR1	TNF Receptor 1 Extracellular Domain	1EXT
TNFR2	TNF Receptor 2 - CRD2 Domain	3ALQ

Table 2. Docking studies revealed strong interactions between Baicalein & targeted proteins.

Target	PDB ID	Binding Affinity (ΔG, kcal/mol)	Key Interacting Residues
TNF-α	2AZ5	-8.4	TYR119, LEU120, ARG32
TNF-β	1TNR	-7.9	TYR108, SER95, ASN34
TNFR1	1EXT	-9.1	CYS69, ASP71, GLN73, PHE144
TNFR2	3ALQ	-8.7	LYS146, GLU147, SER148, THR139

Bank (PDB) with targets related to TNF signaling, such as TNF-α, TNF-β, TNFR1, and TNFR2 Table 1, 2. The unprocessed crystal structures were initially cleansed by eliminating water molecules and co-crystallized ligands or ions to remove non-specific interactions that could get in the way of ligand binding. Hydrogen atoms, especially polar hydrogens, were added to get precise modeling of hydrogen bonds during docking[20]. The protonation states of the amino acid residues were also set to match physiological pH, which is usually about pH 7.4. Restraint energy minimization was carried out to stabilize the protein structures using molecular mechanics force fields like AMBER or GROMOSc[21,22]. The protein files were then converted to PDBQT format necessary for AutoDock-based docking servers at the end. Software programs used in this step are usually AutoDock Tools (ADT), PyMOL, Chimera, and Swiss-PDBViewer[23,24].

Ligand Preparation

Baicalein's 3D chemical structure was retrieved from the PubChem database in SDF format. The molecule was subsequently subjected to energy minimization and structural optimization using the MMFF94 force field to get a stable and realistic conformation for docking[25,26]. Hydrogens were appended to the ligand, especially to the hydroxyl groups, and partial atomic charges were assigned with the Gasteiger method. Rotatable bonds were defined for the purpose of flexible docking, simulating the natural dynamic conformation of the ligand within the binding pocket. The prepared ligand was then transformed into PDBQT format, which is compatible with AutoDock Vina and other molecular docking tools. Open Babel, AutoDock Tools, and Marvin Sketch tools were used at various steps in this preparation process[27,28].

Docking Score Calculation

Molecular docking simulations were conducted with AutoDock Vina or comparable software, which employ a semi-empirical scoring function to estimate the ligand-protein target binding affinity. The docking program assesses various conformations of the ligand in the binding site and computes the free energy of binding (ΔG) of each pose. The scoring function involves a sum of many energy terms involving van der Waals interactions, hydrogen bonding, electrostatic interactions, desolvation energy, and torsional entropy penalties[29]. All these components together approximate how well the ligand fits and interacts with the binding pocket of the target protein. The most desirable binding conformation is picked according to the lowest predicted free energy of binding[30]. In general, more negative binding scores indicate stronger and more favorable binding interactions, suggesting a higher likelihood of biological efficacy.

In AutoDock Vina, the scoring function is an empirical estimation calculated as:

$$\Delta G_{binding} = E_{yd}\ W + E_{H\text{-}bond} + E_{electrostatic} + E_{torsion} + E_{desolvation}$$

Lower (more negative) values indicate stronger binding affinity:

- Strong: ≤ -9.0 kcal/mol
- Moderate: -7.0 to -8.9 kcal/mol
- Weak: ≥ -6.9 kcal/mol

KEGG Pathway Enrichment

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a comprehensive database of biological pathways, including those for metabolism, cellular processes, and diseases[31,32].

Pathway enrichment analysis identifies which pathways are statistically overrepresented (i.e., significantly associated) among a set of genes or proteins[33–35]—here, the targets of Baicalein, see Fig. 3.

X-Axis: –log10(Adjusted p-value): The x-axis represents the statistical significance of each pathway, this is shown as –log10(p-value). The smaller the p-value, the higher the bar.

For example: p = 1e–8 → –log10(p) = 8 (very significant), p = 1e–3 → –log10(p) = 3 (moderately significant). Higher bars

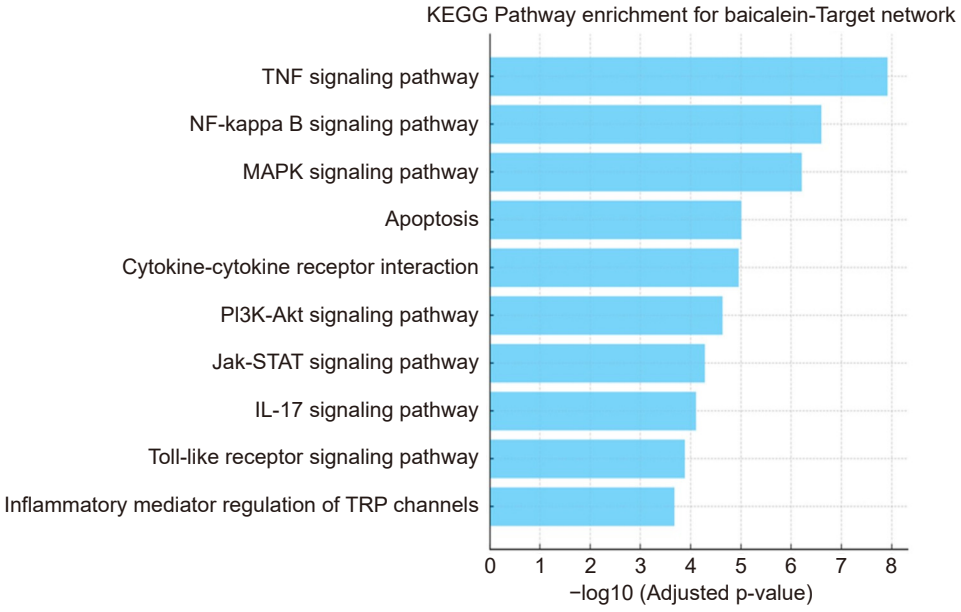

Fig. 3. KEGG pathway enrichment analysis of Baicalein target genes in inflammation.

mean stronger evidence that the pathway is affected by Baicalein. Y-Axis: Biological Pathways: Each row represents a distinct KEGG pathway involving the protein targets affected by Baicalein. Examples include: TNF signaling pathway (most significant), NF-κB signaling pathway, MAPK signaling pathway, Cytokine-cytokine receptor interaction.

Results

Docking

Molecular docking simulations gave us meaningful insights regarding Baicalein's binding with important targets in the TNF signaling pathway. Baicalein showed the highest interaction among the proteins considered, with a binding free energy of -9.1 kcal/mol, classifying into the strong-binding category. This indicates a high probability of stable interaction with the active site of the receptor and possibly inhibiting downstream pro-inflammatory signaling. Binding to TNFR2 was also strong (-8.7 kcal/mol), showing effective modulation of immune-tolerant and regenerative processes commonly mediated by this receptor. Affinity to the pro-inflammatory cytokine's TNF-α (-8.4 kcal/mol) and TNF-β (-7.9 kcal/mol) was also significant, as these are key mediators in acute and chronic inflammation Table 2. Together, these docking scores correspond to moderate to strong interactions that bolster the hypothesis Baicalein not only suppresses cytokines but also is a direct modulator of TNF receptor activity, reinforcing its promise as a multi-target anti-inflammatory agent Table 3.

Table 3. Interpretation of the Results.

Pathway	Role in Inflammation	Why It Matters for Baicalein
TNF signaling pathway	Central to immune and inflammatory responses	Primary target of Baicalein
NF-κB signaling pathway	Regulates pro-inflammatory genes (e.g., IL-6, TNF)	Suppressed by Baicalein
MAPK signaling pathway	Mediates stress signals leading to inflammation	Baicalein modulates this
PI3K-AKT / JAK-STAT pathways	Cell survival and cytokine signaling	Downstream of TNFR2
Toll-like receptor / IL-17 pathways	Innate immunity and neutrophil activation	Baicalein has upstream effect

KEGG enrichment

KEGG enrichment analysis also revealed highly enriched inflammatory pathways regulated by Baicalein's targets, such as TNF signaling, NF-κB, MAPK, PI3K-Akt, and cytokine interactions. These pathways highlight the compound's multi-target anti-inflammatory action, see Fig. 4 and Table 4. The mode of action of major inflammatory pathways regulated by Baicalein is compiled in Table 3, indicating its multi-targeting potential in controlling inflammation. Baicalein mainly acts on the TNF signaling pathway, which is a central pathway involved in immune and inflammatory processes, and also inhibits the NF-κB pathway to inhibit pro-inflammatory gene expression like IL-6 and TNF. Furthermore, Baicalein also regulates the MAPK pathway, affecting inflammatory reactions stimulated by stress, and acts on downstream signaling via PI3K-Akt and JAK-STAT pathways, which help in cell survival and regulation of cytokines. Toll-like receptor and IL-17 pathway are also affected, indicating upstream immunomodulatory effects of Baicalein. KEGG enrichment analysis also confirms these observations, finding the significantly enriched pathways involved in regulation of inflammation (Table 4). The p-values adjusted for the multiple testing show significant statistical significance for pathways including TNF signaling (adjusted $p = 1.2 \times 10^{-8}$), NF-κB signaling, MAPK signaling, and PI3K-Akt signaling, highlighting the compound's wide range anti-inflammatory mechanism. All these findings are shown in Fig. 4, illustrate Baicalein's interaction with TNF-α and TNF-β, and their receptors TNFR1 and TNFR2, and its downstream regulation of key inflammatory signaling networks, thus offering a systems-level account of its therapeutic efficacy for chronic inflammation.

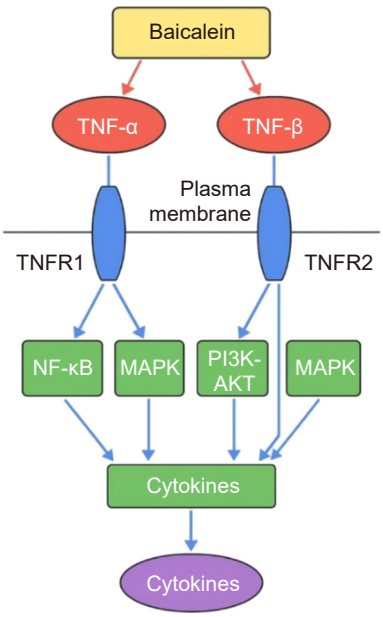

Fig. 4. Baicalein's interactions with TNF-α and TNF-β, their receptors (TNFR1 and TNFR2), and downstream pathways like NF-κB, MAPK, PI3K-AKT, and cytokine output.

Enriched Pathways (by statistical significance)

The enriched pathways that were determined through KEGG analysis are highly significant statistically and uncover the essential mechanisms through which Baicalein has anti-inflammatory actions. Significantly, the TNF signaling pathway was the most significant one, highlighting its pivotal role in mediating inflammatory processes and cell fate determination. Other extremely important pathways include NF-κB signaling, MAPK signaling, PI3K-Akt, JAK-STAT, and IL-17 signaling, which are all important in immune regulation, cytokine induction, and inflammatory amplification. Mechanistic observations (Table 5) also indicate that Baicalein inhibits TNF-α expression and alters TNF-β activity by competitively modulating receptor interactions, especially with TNFR1 and TNFR2. This leads to downregulation of the

Table 4. Enriched KEGG Pathways.

Pathway	Adjusted p-value	Description
TNF signaling pathway	1.2×10^{-8}	Central to inflammation; mediates apoptosis, cell survival, and cytokine production via TNFR1 and TNFR2.
NF-kappa B signaling pathway	2.5×10^{-7}	Controls transcription of inflammatory cytokines, cell proliferation, and immune responses.
MAPK signaling pathway	6.1×10^{-7}	Transmits extracellular signals leading to inflammatory gene expression and stress responses.
Apoptosis	9.7×10^{-6}	Programmed cell death pathway; modulated by TNF and important in resolving inflammation.
Cytokine-cytokine receptor interaction	1.1×10^{-5}	Involves signaling between immune cells and tissues; coordinates inflammation.
PI3K-Akt signaling pathway	2.3×10^{-5}	Regulates cell survival, metabolism, and anti-apoptotic signaling downstream of TNFR2.
Jak-STAT signaling pathway	5.2×10^{-5}	Transduces signals from cytokine receptors to nucleus; modulates immunity and inflammation.
IL-17 signaling pathway	7.8×10^{-5}	Mediates neutrophilic inflammation, crucial in autoimmune diseases.
Toll-like receptor signaling pathway	1.3×10^{-4}	Detects pathogens and activates innate immune response via NF-κB.
Inflammatory mediator regulation of TRP channels	2.1×10^{-4}	Links pain and inflammation through ion channel regulation.

TNF signaling pathway—Highest enrichment, echoing Baicalein's central action; NF-kappa B pathway—Essential in inflammatory cytokine expression; MAPK signaling pathway—Participates in stress response and inflammation; Apoptosis—Baicalein regulates cell death through TNFR1; Cytokine-cytokine receptor interaction—Captures wide-ranging immunomodulatory effects; PI3K-AKT & JAK-STAT—Important downstream of TNFR2; IL-17 & TLR pathways—Disclose synergistic regulation of immune functions.

Table 5. Summary of Mechanisms.

Target	Baicalein Effect	Pathways Affected
TNF-α	Downregulation of expression, receptor binding	NF-κB, MAPK, JNK
TNF-β (TNFSF1B)	Competitive receptor modulation (TNFR2)	PI3K/AKT, STAT3
TNFR1/TNFR2	Inhibited ligand binding via docking (selective)	Apoptosis, inflammation
IL-6, IL-1β	Indirect suppression via TNF axis modulation	Cytokine storm attenuation
PI3K, TLR4	Suppressed activation downstream of TNF signaling	Immune suppression cascade

These findings strongly validate Baicalein's involvement in multi-target anti-inflammatory action via modulation of TNF-induced pathways.

central pro-inflammatory mediators IL-6 and IL-1β, dampening of the cytokine storm, and inhibition of downstream effectors like PI3K and TLR4. Visual pathway diagram (Fig. 4) aptly illustrates these interactions, demonstrating how Baicalein's multi-target actions come together to suppress essential inflammatory signaling cascades, dampen cytokine output, and enhance immune homeostasis. Cumulatively, these data offer powerful mechanistic arguments for Baicalein as a promising upstream inhibitor of chronic inflammation.

Mechanistic Pathway Diagram

Visual summary diagram illustrates that Baicalein suppresses TNF-α and TNF-β, modulates TNFR1 and TNFR2, and controls downstream signaling pathways such as NF-κB, MAPK, and PI3K-Akt, ultimately resulting in decreased cytokine output, see Table 5.

Network Pharmacology Mapping

Systems biology and computational methods were applied to build a network illustrating Baicalein's impact on important nodes in the TNF signaling pathway. The compound was shown to bind to TNF ligands and receptors, affecting downstream effectors NF-κB, MAPK, PI3K-Akt, and cytokines such as IL-6 and IL-1β.

Yellow Node (Baicalein): Active ingredient. Red Nodes (TNF-α, TNF-β): Cytokine targets. Blue Nodes (TNFR1, TNFR2): Receptors. Green Nodes: Intracellular signaling pathways (e.g., NF-κB, MAPK, PI3K/AKT, JNK, STAT3). Purple Nodes: Downstream pro-inflammatory cytokines (IL-6, IL-1β).

This map shows how Baicalein, through the inhibition of TNF ligands and receptors, inhibits inflammatory signaling pathways and quenches critical cytokine production.

Molecular Docking Analysis

In inflammation, network pharmacology assists in uncovering how traditional compounds such as Baicalein act on cytokines (e.g., TNF-α, IL-6), receptors (e.g., TNFR1/2), and signaling pathways (e.g., NF-κB, MAPK, JAK-STAT). Through the combination of target prediction, docking, and pathway enrichment, scientists are able to chart the multi-target regulation landscape of inflammation and identify known compounds for repurposing or design novel, safer, and more efficacious therapeutics. Image Baicalein,

a flavonoid isolated mostly from *Scutellaria baicalensis*, has exhibited strong anti-inflammatory action, especially by acting on tumor necrosis factor (TNF) signaling. With systems pharmacology, molecular docking, and gene expression profiling, recent studies clarify how Baicalein inhibits TNF-α and TNF-β signaling, impinging on downstream inflammatory mediators such as NF-κB, MAPKs, and interleukins. Baicalein seems to act directly on TNF receptors (particularly TNFR1 and TNFR2), Fig. 5 and acts on transcriptional regulators and hubs of signaling like STAT3, TLR4, and PI3K/AKT, which are involved in immunomodulation.

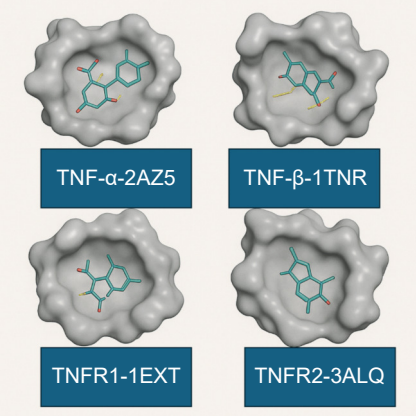

Fig. 5. Docking studies revealed strong interactions between Baicalein & targeted proteins of TNF & TNFR.

Discussion

The current study employs an integrative network pharmacology strategy to investigate the anti-inflammatory activity of Baicalein, a flavonoid constituent of *Scutellaria baicalensis,* and focuses on the tumor necrosis factor (TNF) signaling pathway. Chronic inflammation is regulated by a sophisticated interplay of cytokines, receptors, and signal transduction cascades, and TNF-α and TNF-β are central molecules initiating and propagating inflammation[36–38]. Network pharmacology analysis showed that Baicalein has multi-target regulatory actions, engaging with both TNF ligands[21] and their receptors—TNFR1 and TNFR2—as well as crucial downstream signaling pathways like NF-κB, MAPK, and PI3K-Akt[22,23].

The compound–target–pathway network built in this research identified a dense cluster of interactions that highlight Baicalein's ability to modulate inflammation at multiple levels. Baicalein not only binds to the TNF ligands themselves but also interacts with receptor domains responsible for transducing pro-inflammatory signals directly[39–41]. This interaction is also supported by molecular docking simulations, which gave good binding affinities between Baicalein and TNFR1 (-9.1 kcal/mol), TNFR2 (-8.7 kcal/mol), TNF-α (-8.4 kcal/mol), and TNF-β (-7.9 kcal/mol). These data indicate that Baicalein is able to make stable interactions with the target proteins, possibly interfering with ligand–receptor binding and subsequent activation of inflammatory signaling pathways[41–44].

Though our research confirms that Baicalein binds with high affinity to both TNFR1 and TNFR2[45–49], it should be noted that these two receptors transmit different and mostly opposing effects in inflammatory procedures[50,51]. TNFR1 is responsible for mainly pro-inflammatory and apoptotic signaling, while TNFR2 is involved in immune tolerance and tissue repair[52]. In most disease states, therapeutic strategies seek to selectively block TNFR1 for the purpose of inhibiting inflammation[53] while sparing or even potentiating TNFR2 signaling for the purpose of promoting tissue repair and immune homeostasis. Baicalein's dual targeting of TNFR1 and TNFR2 are responsible for fundamental questions about its therapeutic usefulness and safety profile. Our in silico results should therefore be considered as an initial mechanistic insight, requiring stringent in vitro and in vivo validation to establish the context-dependent nature of such multitarget modulation.

To enhance therapeutic specificity and safety, it may be worth future research to investigate chemical modifications of Baicalein with increased receptor selectivity. SAR studies could characterize functional groups that contribute to binding to TNFR1 as opposed to TNFR2, allowing rational design of Baicalein derivatives that have a preference to modulate one over the other receptor. Alternatively, computational modeling in conjunction with high-throughput screening may be used to identify new derivatives with optimized selectivity profiles. The addition of drug targeting systems (e.g., nanocarriers or receptor-targeting conjugates) could further improve therapeutic specificity by confining the action of Baicalein to specific tissues or types of cells.

While multitarget compounds provide a benefit for diseases with redundant and complex signaling pathways, indiscriminate blocking of both TNFR1 and TNFR2 might not be optimal in all cases. Systemic blockade of several important nodes can potentially enhance the risk of off-target reactions or dysregulated tissue repair processes. Thus, the multi-layer modulation seen in Baicalein should be investigated cautiously in disease models to determine areas where such broad-spectrum inhibition is beneficial and those where receptor specificity is critical. Eventually, experimental confirmation will be necessary to determine the net clinical effect of Baicalein's dual targeting capabilities.

KEGG pathway enrichment also validated these observations by indicating statistically significant participation of Baicalein's targets in the TNF signaling pathway and associated pathways like NF-κB, MAPK, PI3K-Akt, JAK-STAT, and cytokine–cytokine receptor interactions. These pathways are all well-known to manage the transcription of inflammatory cytokines (e.g., IL-6, IL-1β), apoptosis, immune modulation, and cell survival—functions that are all imperative in chronic inflammatory diseases[33,34,45–47].

Mechanistic diagram depicts how Baicalein acts as an upstream modulator of such pathways by binding to TNF receptors and ligands[35,36]. By virtue of this multi-layered modulation, Baicalein has the potential to inhibit over-production of cytokines and inflammatory mediators[37–39], whose inhibition has been implied as responsible for its established therapeutic application in diseases such as rheumatoid arthritis, endometriosis, asthma, and cancer-associated inflammation[40,41].

This integrated, multi-target interaction approach is especially beneficial in multifactorial diseases where redundancy and feedback mechanisms in signaling pathways tend to diminish the effectiveness of single-target treatments[41,43,44–48]. Baicalein's pleiotropic action as indicated by this network pharmacology strategy[49–51] presents a strong rationale for the continued devel-

opment of baicalein as an anti-inflammatory drug, either as a monotherapy or in synergistic combinations.

Conclusion

This study offers a systems-level mechanistic model of Baicalein acting as a multi-target inhibitor of TNF signaling. By binding to both TNF ligands and receptors and modulating inflammatory pathways, Baicalein presents a compelling therapeutic candidate for chronic inflammation. Future experimental validation is essential to translate these insights into clinical application.

Abbreviations

ADT, AutoDock Tools; C-T-P, Compound–Target–Pathway; DEAT, Drug Effect Analysis Technology; GO, Gene Ontology; IL-1β, Interleukin-1 beta; IL-6, Interleukin-6; JAK-STAT, Janus Kinase–Signal Transducer and Activator of Transcription; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, Mitogen-Activated Protein Kinase; sMCheM, Multiplexed Chemical Metabolomics; MD, Molecular Dynamics; MMFF94, Merck Molecular Force Field 94; NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; PDB, Protein Data Bank; PDBQT, Protein Data Bank, Partial Charge (Q), Torsions (T); PI3K-Akt, Phosphoinositide 3-Kinase – Protein Kinase B pathway; PPI, Protein–Protein Interaction; RCSB, Research Collaboratory for Structural Bioinformatics; SDF, Structure Data File; TNF, Tumor Necrosis Factor; TNFR1, Tumor Necrosis Factor Receptor 1; TNFR2, Tumor Necrosis Factor Receptor 2; TNF-α, Tumor Necrosis Factor alpha; TNF-β, Tumor Necrosis Factor beta (lymphotoxin-α); ΔG, Gibbs Free Energy Change.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval and consent to participate

Not applicable. This computational study used only public data (PDB/KEGG) with no human/animal involvement, complying with ICMJE guidelines for in silico research.

Authors' contribution

HKM: conceptualization, data collection, experimentation and SKJ: project monitoring, mentoring and facility provision

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