

Research Article

Mechanism of *Piper longum* L. in the Treatment of Cerebral Ischemic Stroke Based on Network Pharmacology and Molecular Dynamics Simulation, With Verification by Surface Plasmon Resonance

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Abstract

Objective: To investigate the potential mechanisms of *Piper longum* L. in the treatment of cerebral ischemic stroke (CIS) and conduct experimental verification. **Methods:** Network pharmacology was used to identify intersecting targets of *Piper longum* L. and CIS. A drug-active component-target-disease network and the target protein-protein interaction (PPI) network were subsequently constructed, to screen major components and key targets. The intersecting targets were subjected to comprehensive Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. Molecular docking and molecular dynamics (MD) simulations were performed to assess the binding energy and stability between the main active components and core targets. Surface plasmon resonance (SPR) was also employed to validate these binding interactions, providing further evidence for the underlying molecular mechanisms. **Results:** In total, 13 active components were identified in *Piper longum* L., and 60 intersectional targets were acquired. Among the main active components were piperine, 1,2,5,6-tetrahydrotanshinone, sesamin, N-(2,5-dimethoxyphenyl)-4-methoxybenzamide, and piperonaline. The key targets predominantly consisted of tumor necrosis factor (TNF), interleukin-6 (IL-6), prostaglandin-endoperoxide synthase 2 (PTGS2), cAMP response element-binding protein (CREB1), and interleukin-1 β (IL-1 β). These identified targets were enriched in 77 GO terms and involved in multiple signaling pathways, including the TNF and cAMP (cyclic adenosine monophosphate) pathways. The main active components exhibit low binding energies with the key targets. MD simulations further demonstrated the structural stability of the complex formed between the main component, sesamin, and the associated CREB1 target, as well as the significance of key residues ILE240 (isoleucine240), ARG241 (arginine241), and PRO244 (proline244) in the binding interaction. Finally, the SPR method provided experimental evidence of the direct binding between sesamin and CREB1. **Conclusions:** *Piper longum* L. may exert pharmacological effects on CIS by acting on targets such as CREB1, PTGS2, and TNF, and by modulating signaling pathways including cAMP and inflammation.

Keywords: *Piper longum* L.; ischemic stroke; network pharmacology; molecular docking simulation; molecular dynamics simulation; surface plasmon resonance

1. Introduction

Cerebral ischemic stroke (CIS) is primarily caused by cerebral arterial occlusion, resulting in cerebral ischemia and hypoxic injury. Following the onset of CIS, approximately 2 million neurons, 16 billion synapses, and 12 kilometers of axons are lost per minute [1]. Currently, reperfusion therapy remains the most effective treatment approach [2]. However, both thrombolysis and mechanical thrombectomy are limited by narrow therapeutic time windows and require careful patient selection. Furthermore, a substantial proportion of patients fail to achieve favorable outcomes despite successful recanalization, termed “futile recanalization” [3]. The traditional “neuroprotection” strategy aims to preserve neuronal viability after CIS. How-

ever, clinical trials targeting single molecular pathways have largely yielded negative outcomes [4]. Emerging evidence indicates that neurons, glial cells, endothelial cells, and their intricate interactions collectively contribute to secondary brain injury following CIS. Consequently, the therapeutic paradigm has shifted toward “cell protection”, which encompasses the entire neurovascular unit (NVU) and enables more comprehensive modulation of the ischemic cascade [5,6]. In 2023, the American Stroke Association emphasized the concept of neurovascular omics, which highlights the importance of integrating multiple components of the nervous system and their dynamic interplay [7].

Traditional Chinese Medicine (TCM) is recognized for its multi-component, multi-target, multi-pathway, and



multi-link characteristics in protecting brain cells during CIS, offering significant potential for therapeutic development. Tongxinluo Capsules and Butylphthalide have exhibited preliminary efficacy in large-scale randomized controlled clinical trials [8,9,10]. However, their benefits are currently limited to a subset of the patient population. *Piper longum* L., a climbing vine belonging to the Piperaceae family, has properties that target multiple pathological mechanisms of CIS such as inflammation and oxidative stress. The lignans and alkaloids derived from *Piper longum* L. show diverse pharmacological effects, including anti-apoptotic, anti-inflammatory, and neuroprotective activities [11,12], with promising results observed in pre-clinical studies on CIS [13]. However, the mechanism of *Piper longum* L. in treating CIS requires further elucidation. Network pharmacology enables the construction of multi-level interaction networks, effectively overcoming the limitations of traditional single-target approaches by screening the main active components and identifying key targets. This offers a more comprehensive and precise understanding of the complex chemical constituents, mechanisms of action, and therapeutic targets of TCM [14]. Molecular docking predicts the optimal binding conformation between ligands and target proteins, whereas molecular dynamics (MD) simulation can model the dynamic behavior of ligand-protein complexes [15]. Surface plasmon resonance (SPR) is widely regarded as the gold standard for analyzing molecular interactions, providing experimental evidence of prioritized compound-target binding. By combining these approaches, overall associations can be elucidated, followed by focused validation to ensure comprehensive and rigorous analysis.

The aim of this study was to systematically investigate the mechanisms underlying the pharmacological effects of *Piper longum* L. in CIS. This was carried out through an integrative approach combining network pharmacology, molecular docking, MD simulation, and SPR validation, thereby providing a scientific foundation for the application of TCM in the treatment of CIS.

2. Materials

2.1 Reagents

Sesamin (Catalog No. T2919) and CREB1 (cAMP response element-binding protein) recombinant protein (Catalog No. TMPH-01165) were purchased from Shanghai TOPSCIENCE BioTech Co., Ltd (Shanghai, China). The amino coupling kit (Catalog No. BR100050), sodium acetate buffer solutions (pH 4.0/4.5/5.0; Catalog No. BR100349), and 10× PBS-P+ (Catalog No. 28995084) were obtained from Cytiva (Marlborough, Massachusetts, MA, USA). DMSO (dimethyl sulfoxide) (Catalog No. D8418) was sourced from Sigma-Aldrich, USA (St. Louis, MO, USA).

2.2 Instruments

Biacore system (Model 1K, CM5 chip, Cytiva); vortex mixer (VORTEX06, IKA; Staufen, Baden-Württemberg, Germany); mini centrifuge (MiniSpin, Eppendorf; Hamburg, Hamburg, Germany).

3. Methods

3.1 Screening of Active Components From *Piper longum* L. and Prediction of Their Corresponding Targets

All bioactive components of *Piper longum* L. were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). Components meeting the pharmacokinetic criteria of oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 were selected for further analysis. The corresponding target proteins were obtained directly from TCMSP target annotations and subsequently curated using UniProt (<https://www.uniprot.org/>), with species restricted to Homo sapiens and duplicate entries removed.

3.2 CIS-Related Targets

The GeneCards database (<https://www.genecards.org/>) and the OMIM (Online Mendelian Inheritance in Man) database (<https://www.omim.org/>) were systematically searched using “ischemic stroke” as the key search term to identify potential CIS-related targets. The GeneCards database was screened with a Relevance score of ≥ 1 as the criterion. The resulting gene lists were merged, and duplicate entries were removed using Microsoft Excel (version 2501, <https://www.microsoft.com/zh-cn/microsoft-365/excel>). Subsequently, the consolidated dataset was standardized and verified through the UniProt database to ensure accurate gene nomenclature and biological relevance.

3.3 Identification of Shared Targets Between *Piper longum* L. and CIS

The target genes of *Piper longum* L. and those associated with CIS were uploaded to VENNY (version 2.1, <https://bioinfo.gp.cnb.csic.es/tools/venny/>) to identify the common intersecting targets and construct a Venn diagram.

3.4 Construction of the Drug-Active Ingredient-Target Network and PPI Network

The active ingredients and targets were imported into Cytoscape (version 3.7.2, https://cytoscape.org/release_notes_3_7_2.html) to construct the drug-active ingredient-target network. The shared targets of drugs and diseases were mapped onto PPI to help reveal functional connectivity, identify hub proteins and key modules, and prioritize biologically central targets for downstream enrichment and validation. In detail, they were uploaded to the STRING database (version 11.0, <http://string-db.org/>) to generate a PPI network. The minimum interaction score was set to 0.7, and the resulting data were exported in TSV (tab-separated

values) format. This file was then imported into Cytoscape for visualization of the PPI network. The Network Analyzer plugin in Cytoscape was utilized to screen compounds and targets where the closeness centrality, betweenness centrality, and degree are all greater than their respective means, and then the top ones were selected based on the degree value.

3.5 Gene Ontology (GO) Functional Enrichment Analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

The intersection target genes of *Piper longum* L.-CIS were uploaded to the DAVID (<https://davidbioinformatics.nih.gov/summary.jsp>). DAVID 6.8 was used for functional annotation of these shared target genes in terms of biological processes (BP), cellular components (CC), and molecular functions (MF), along with KEGG pathway enrichment analysis. A *p*-value threshold of <0.05 and a false discovery rate (FDR) value of <0.05 were applied as the significance criterion. The top 10 enriched terms in the BP, CC, and MF categories, as well as the top 20 enriched KEGG pathways, were selected for further visualization using the online bioinformatics platform (<http://www.bioinformatics.com.cn>).

3.6 Molecular Docking

Molecular docking was performed using AutoDock Vina (version 1.2.5, <https://vina.scripps.edu>). The compound structures in mol2 format were retrieved from TCMSP, while the crystal structures of target proteins were obtained from the Protein Data Bank (PDB; <http://www.rcsb.org/>). These structures were subsequently processed using ChemBio3D (version 14.0.0, <https://www.chemdraw.com.cn>) and PyMOL (version 2.6.0, <https://pymol.org>) for energy minimization, as well as for the removal of ligands and water molecules. Both ligands and proteins were hydrogenated, and Gasteiger charges were assigned using AutoDock Tools (version 1.2.5). Potential binding sites on the proteins were predicted using POCASA 1.1 (<https://g6altair.sci.hokudai.ac.jp/g6/service/pocasa>). Hydrogen bond networks and hydrophobic interactions were analyzed and visualized with PyMOL and LigPlot (version 2.2.8, <https://www.ebi.ac.uk/thornton-srv/software/LigPlus>).

3.7 Molecular Dynamics Simulations

MD simulations were performed using GROMACS software (version 2024.03, <https://www.gromacs.org>). The parameters for CREB1 were obtained from the ff14SB force field, while water molecules were modeled using the TIP3P (transferable intermolecular potential with 3 points) model. Sesamin was parameterized with the Generalized Amber Force Field (GAFF). The sesamin-CREB1 complex was solvated in a periodic dodecahedral box filled with TIP3P water molecules, and Na⁺ and Cl[−] ions were added to achieve a physiological salt concentration of 0.150 mol/L

and ensure overall system charge neutrality. Energy minimization was performed using the steepest descent algorithm for up to 50,000 steps to eliminate unfavorable atomic contacts. The system was subsequently equilibrated in two phases: a 100 ps NVT (canonical ensemble) maintained at 300 K using the V-rescale thermostat, followed by a 100 ps NPT (isothermal-isobaric ensemble) at 1 bar pressure regulated via the Parrinello-Rahman barostat. Following equilibration, a production MD simulation was carried out for 100 ns with a time step of 2 fs. Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method with a cutoff of 1.2 nm, and coordinates were recorded every 10 ps for trajectory analysis. Structural stability was evaluated through root mean square deviation (RMSD); local flexibility was assessed via root mean square fluctuation (RMSF); conformational compactness was analyzed using the radius of gyration (Rg); and hydrogen bonding patterns (including occupancy and persistence) were examined to characterize key intermolecular interactions. Binding free energy was estimated using the gmx_MMPBSA tool, decomposing contributions into van der Waals (VDWAALS), electrostatic (EEL), polar solvation (EGB), and nonpolar solvation (ESURF) energy terms [16]. Trajectory visualization and detailed analysis of binding modes, hydrogen bond networks, and hydrophobic interactions were performed using PyMOL (version 2.6).

3.8 Surface Plasmon Resonance

Sesamin and 1,2,5,6-tetrahydrotanshinone exhibited the strongest binding affinities with the five key targets. Compared with 1,2,5,6-tetrahydrotanshinone, sesamin showed a more clearly defined molecular mechanism in CIS treatment, stronger experimental validation, enhanced safety profile, and a broader therapeutic application window. In contrast to CREB1, TNF was not selected due to a recent clinical trial (anzctr.org.au, ACTRN12620001011976) that found no improvement in efficacy following treatment with etanercept, a competitive TNF- α inhibitor. MD simulation also showed favorable binding stability. The sesamin-CREB1 pair was thus prioritized for SPR. The amino coupling method was employed, wherein the CM5 sensor chip was first activated, followed by immobilization of CREB1 protein that had been diluted to 50 μ g/mL in sodium acetate buffer. The protein was immobilized under a constant flow rate of 10 μ L/min. Sesamin, at varying gradient concentrations, was subsequently applied as the analyte to facilitate molecular interaction with immobilized CREB1. Binding kinetics were analyzed using Biacore Insight software (version 2024.1, <https://cdn.cytivalifesciences.com.cn/api/public/content/digi-24236-pdf>), with sensorgrams fitted to the 1:1 Langmuir binding model to determine kinetic parameters.

4. Results

4.1 Identification of Intersection Targets for *Piper longum* L. and CIS and Their Interaction Network

A total of 13 active components of *Piper longum* L. were identified through the TCMSP database. These were associated with 74 potential therapeutic targets in the treatment of CIS, as predicted by a systematic bioinformatics approach. Additionally, 4739 and 128 CIS-associated targets were identified from the GeneCards and OMIM databases, respectively. After removing duplicate entries, a non-redundant set of 4838 CIS-related targets was compiled. Subsequent intersection of the CIS-related targets and the targets associated with *Piper longum* L. yielded 60 shared targets (Fig. 1A). The drug-active ingredient-target network was found to comprise 74 nodes and 136 edges. As shown in Fig. 1B, the top five components with the highest degree values were MOL001592 (piperine, degree = 26), MOL001601 (1,2,5,6-tetrahydrotanshinone, degree = 23), MOL001558 (sesamin, degree = 18), MOL001586 (N-(2,5-dimethoxyphenyl)-4-methoxybenzamide, degree = 14), and MOL001560 (piperonaline, degree = 11). The PPI network comprised 59 nodes (one isolated node was excluded from visualization) and 670 edges. A total of 21 core targets were identified based on closeness centrality exceeding 0.47541, betweenness centrality greater than 0.009061, and degree value higher than 10. Tumor necrosis factor (TNF, degree = 29), interleukin-6 (IL-6, degree = 28), prostaglandin-endoperoxide synthase 2 (PTGS2, degree = 27), interleukin-1 β (IL-1 β , degree = 26), and cAMP response element-binding protein (CREB1, degree = 26) were the top five nodes within the network (Fig. 1C).

4.2 GO and KEGG Enrichment Analyses

A total of 77 GO terms were significantly enriched, including 51 BP terms. The top 10 enriched BP terms were primarily associated with responses to xenobiotic stimulus, positive regulation of the MAPK (mitogen-activated protein kinase) cascade, positive regulation of DNA-templated transcription, G protein-coupled receptor signaling pathway, lipid metabolic process, positive regulation of gene expression, response to ethanol, cellular response to lipopolysaccharide, positive regulation of the ERK1 (extracellular signal-regulated kinase 1) and ERK2 cascade, and chemical synaptic transmission. In the CC category, 14 terms were identified, with the top 10 being plasma membrane, membrane, cytoplasm, postsynaptic membrane, neuron projection, presynaptic membrane, synapse, cell surface, endoplasmic reticulum membrane, and neuronal cell body. In the MF category, 12 terms were enriched, with the top 10 encompassing protein binding, identical protein binding, protein homodimerization activity, protein-containing complex binding, enzyme binding, protein heterodimerization activity, sequence-specific double-stranded DNA binding, DNA-binding transcription factor activity, flavin adenine dinucleotide binding, and

cytokine activity (Fig. 2A). A total of 61 signaling pathways associated with the therapeutic mechanisms of *Piper longum* L. in the treatment of CIS were found to be enriched, with the top 20 being TNF, neuroactive ligand-receptor interaction, lipid and atherosclerosis, cGMP-PKG (cyclic guanosine monophosphate-protein kinase G), calcium signaling, serotonergic synapse, IL-17, C-type lectin receptor, cholinergic synapse, cAMP, fluid shear stress and atherosclerosis, Toll-like receptor, AMPK (5' adenosine monophosphate-activated protein kinase), PI3K-Akt (phosphoinositide 3-kinase-protein kinase B), neurodegenerative diseases-multiple pathways, NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), Th17 (T helper 17) cell differentiation, toxoplasmosis, regulation of lipolysis in adipocytes, and metabolic pathways (Fig. 2B).

4.3 Molecular Docking Analysis of Key Targets and Major Active Ingredients

The top five components and their corresponding targets were selected for molecular docking analysis, and the interaction strengths were evaluated based on binding energies. The key targets CREB1 and TNF exhibited the strongest binding affinities with sesamin, yielding binding energies of -7.2 kcal/mol and -9.7 kcal/mol, respectively. Similarly, 1,2,5,6-tetrahydrotanshinone demonstrated the most favorable interactions with PTGS2, IL-6, and IL-1 β , with binding energies of -9.8 kcal/mol, -7.7 kcal/mol, and -7.2 kcal/mol, respectively (Fig. 3A). Molecular docking also revealed that sesamin formed hydrogen bonds with ARG-241 (arginine-241) of CREB1 at distances of 2.76 Å and 3.05 Å. Additionally, sesamin interacted with ARG-98 and ASN-112 (asparagine-112) of TNF through hydrogen bonds measuring 3.3 Å and 2.9 Å, respectively. 1,2,5,6-tetrahydrotanshinone formed hydrogen bonds with SER-125 (serine-125) of IL-1 β (3.0 Å and 2.9 Å), TYR-97 (tyrosine-97) and ASN-63 of IL-6 (3.1 Å and 3.3 Å), and TYR-385 of PTGS2 (2.9 Å). Furthermore, hydrophobic interactions between the main active components and multiple amino acid residues were observed (Fig. 3B).

4.4 Molecular Dynamics Simulation of Sesamin and CREB1

Based on the docking results, sesamin showed the strongest binding with CREB1 and was therefore selected for further MD simulation analysis. During the 100 ns simulation, the RMSD of the sesamin-CREB1 complex reached equilibrium at approximately 3.48 Å after 4 ns, indicating overall structural stability (Fig. 4A). The RMSF values remained <4 Å across most regions, except for residues 110–140 and 260–270. These displayed relatively higher fluctuations, suggesting localized stability (Fig. 4B). The radius of gyration (Rg) exhibited minimal variation throughout the simulation (average: 20.57 Å), reflecting a compact and conformationally stable complex (Fig. 4C). The time-dependent profile of hydrogen bond counts indi-

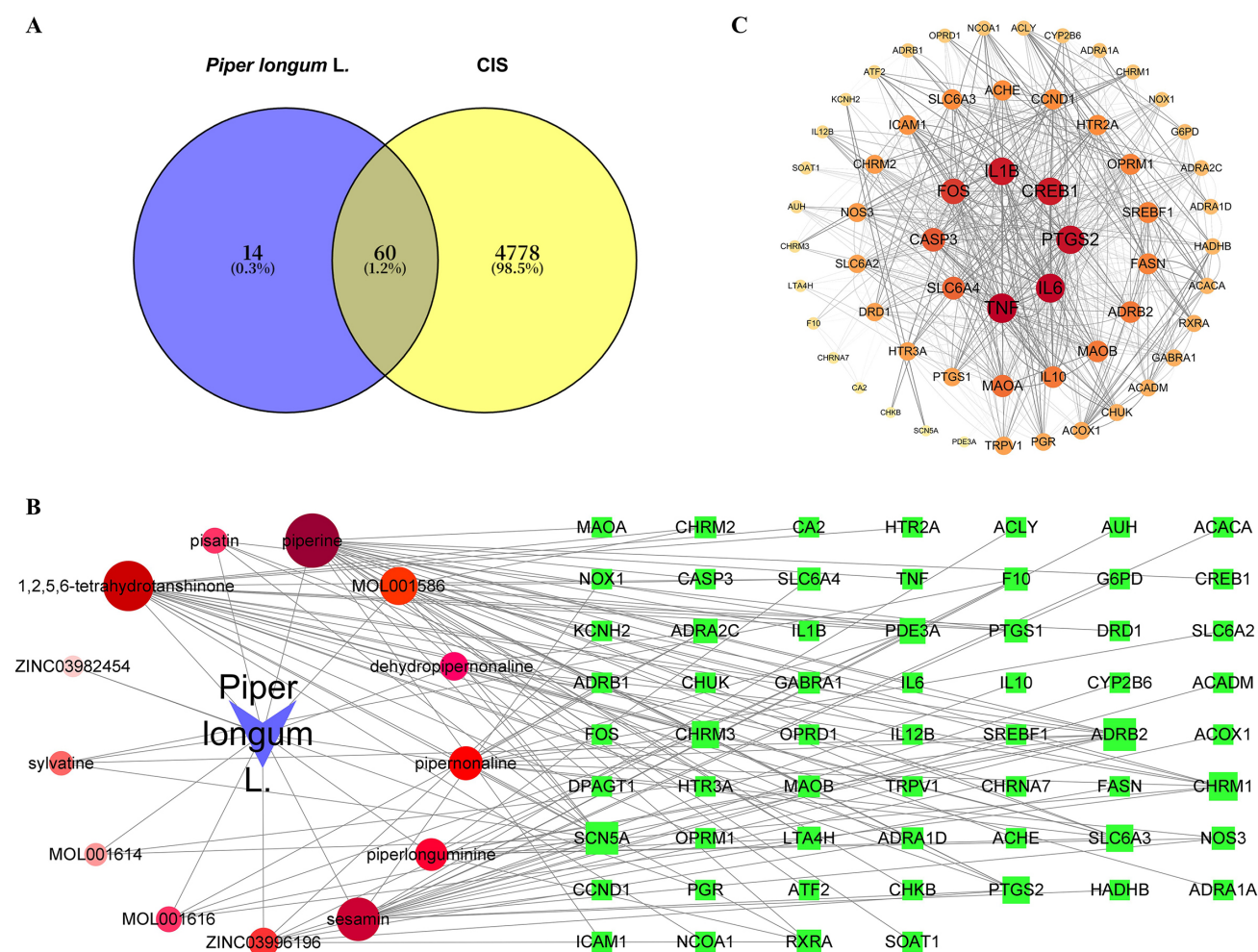


Fig. 1. Identification of the intersection targets of *Piper longum* L. and CIS, and their interaction network. (A) Venn diagram of targets of *Piper longum* L. and ischemic stroke. (B) “Drug-active ingredient-target” network drawn by Cytoscape. MOL001586 (N-(2,5-dimethoxyphenyl)-4-methoxybenzamide), MOL001614 ((E,E,E)-11-(1,3-Benzodioxol-5-yl)-N-(2-methylpropyl)-2,4,10-undecatrienenamide), MOL001616 (1-[1-oxo-9(3, 4-methylenedioxyphenyl)-2E,8E-nonadienyl] pyrrolidine). Blue, pink to red, and green nodes represent *Piper longum* L., active ingredients (OB $\geq 30\%$ and DL ≥ 0.18), and targets, respectively, while edges represent association. Node size and color intensity (pink to red) were positively correlated with degree values. (C) PPI network drawn by Cytoscape. Nodes represent potential therapeutic targets, while edges represent interaction. Color intensity and size were positively correlated with degree values, while edge thickness corresponded to the combined score. CIS, cerebral ischemic stroke; OB, oral bioavailability; DL, drug-likeness; PPI, protein-protein interaction.

cated continuous and stable binding, despite minor fluctuations (Fig. 4D). The calculated binding free energy was -40.38 kcal/mol, confirming the strong interaction between sesamin and CREB1. Energy component analysis revealed that VDWALS, EEL, and ESURF contributions were the primary driving forces for binding, whereas EGB partially offset the overall binding affinity (Fig. 4E). Residue contribution analysis identified ILE-240 (isoleucine-240), ARG-241, and PRO-244 (proline-244) as the key residues making significant contributions to the binding interface (Fig. 4F).

4.5 SPR Experiment Confirms Direct Interaction Between Sesamin and CREB1

The SPR experimental results showed that sesamin exhibits strong binding affinity toward CREB1, with the binding capacity increasing in a concentration-dependent manner. The dissociation constant (K_D) was determined to be $4.77 \mu\text{M}$, indicating a direct and specific interaction between sesamin and CREB1 (Fig. 5).

5. Discussion

This study identified 13 active compounds in *Piper longum* L., with piperine, 1,2,5,6-tetrahydrotanshinone, sesamin, and piperonaline being the principal bioactive

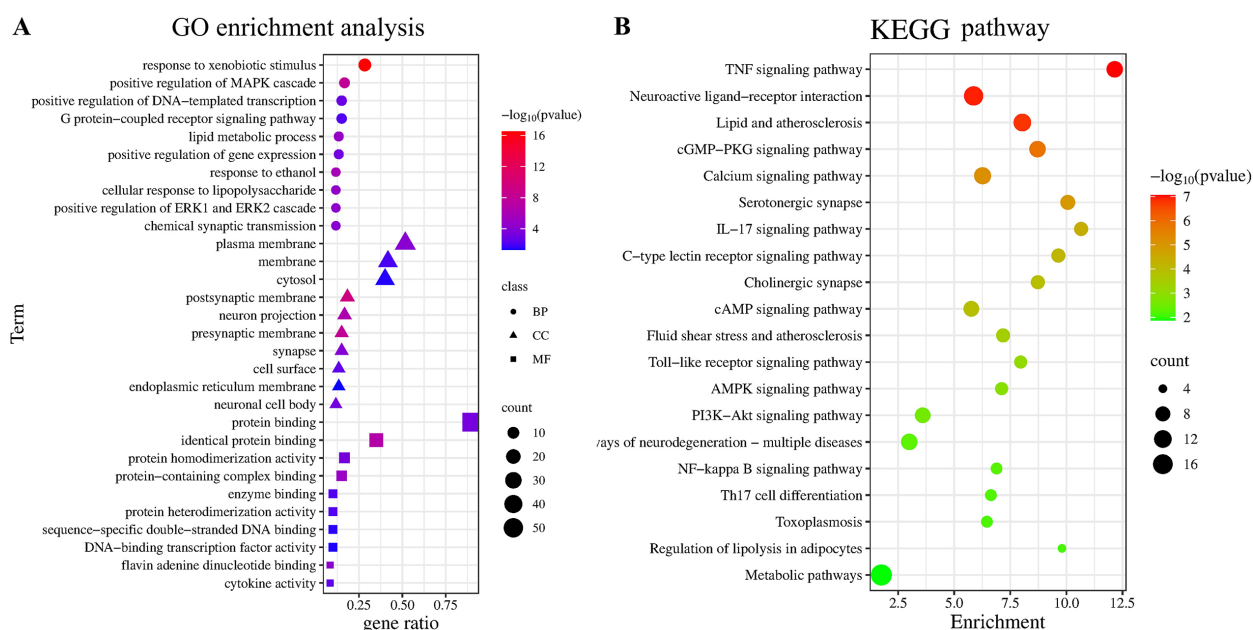


Fig. 2. GO and KEGG enrichment analysis. (A) GO function enrichment analysis (top 10). The circle, triangle, and square represent BP, CC, and MF, respectively. (B) KEGG pathway enrichment analysis (top 20). The node color represented p -value, while node size represented gene counts. $p < 0.05$, FDR < 0.05 . GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological processes; CC, cellular components; MF, molecular functions; FDR, false discovery rate.

constituents. A total of 74 potential drug targets and 4838 CIS-associated targets were identified, with 60 overlapping targets. The core targets of *Piper longum* L. in CIS treatment primarily include inflammatory mediators and transcription factors such as TNF, IL-6, PTGS2, IL-1 β , and CREB1. Strong binding affinities were observed between the top five targets and compounds in terms of the degree. SPR experiments further confirmed a direct physical interaction between sesamin and CREB1.

Notably, the compound exhibiting the highest degree value was piperine. This compound confers neuroprotective effects through multiple mechanisms, including mitigation of mitochondrial dysfunction, inhibition of apoptosis, reduction of inflammatory responses, and upregulation of CREB1 expression [17]. Furthermore, molecular docking analysis indicated a favorable binding affinity between piperine and CREB1. Sesamin exhibits anti-inflammatory and antioxidant activities and has been shown to reduce the levels of TNF- α , IL-6, and PTGS2, significantly decreasing infarct volume in experimental stroke models [18,19]. The binding energy between sesamin and these inflammatory targets was relatively low and stable interactions were observed. Moreover, piperonaline has been reported to inhibit platelet aggregation *in vitro* [20].

Secondary brain injury following ischemia involves a cascade amplification process driven by an interplay of multiple pathological factors. While PTGS2 plays a role in modulating inflammation, its function as a therapeutic target in CIS remains controversial, with some evidence suggesting that PTGS2 inhibitors may increase post-stroke

mortality [21]. KEGG pathway enrichment analysis revealed significant involvement of TNF, Toll-like receptor, and NF- κ B pathways, indicating the neuroinflammatory response after CIS may be regulated by treatment with *Piper longum* L. CREB1 is a critical transcription factor involved in cell proliferation, survival, growth, and differentiation. It exerts neuroprotective effects upon activation by promoting the expression of anti-apoptotic proteins such as BCL2 and brain-derived neurotrophic factor (BDNF), thus supporting neuronal survival and regeneration after ischemic insult [22]. The cAMP signaling pathway, which involves CREB1 activation, was also significantly enriched. Within the four functional domains of CREB1, the Q2 domain is rich in glutamine residues and plays a key role in enhancing transcriptional activity [23]. Molecular docking results indicate that sesamin interacts with the key amino acid residues ILE240, ARG241, and PRO244 of CREB1, all located within the Q2 domain. This suggests a potential mechanism by which sesamin modulates CREB1-mediated transcriptional regulation.

Several limitations of this work should be noted. Firstly, network pharmacology bias may exist due to incompleteness of the database, or uncertainty regarding target prediction. Moreover, no novel targets were identified. Another limitation is that molecular docking, MD simulation, and SPR do not prove functional modulation. Finally, although binding of the sesamin-CREB1 pair was verified by MD simulation and SPR, the other top-ranked pairs were untested.

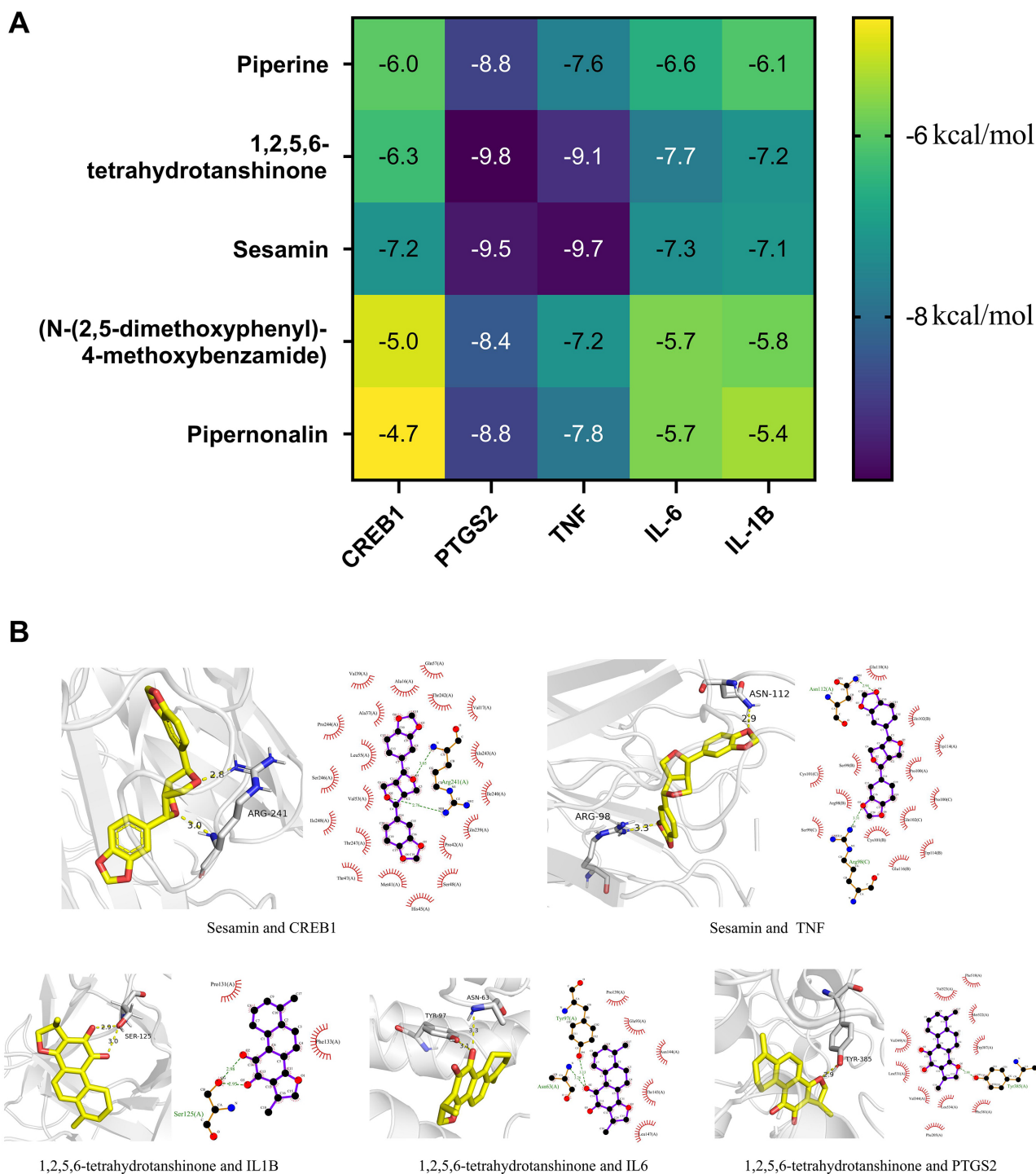


Fig. 3. Molecular docking results. (A) Heatmap analysis of CREB1, PTGS2, TNF, IL-6, and IL-1 β target binding energy. The color intensity is negatively correlated with binding energies. (B) 3D and 2D molecular docking visualization of the complex with the lowest binding energy for each target (green dashed lines and red semicircles represent hydrogen bonds and hydrophobic interactions, respectively). CREB1, cAMP response element-binding protein; PTGS2, prostaglandin-endoperoxide synthase 2; TNF, tumor necrosis factor; IL, interleukin.

Future research should aim to validate these findings using OGD/R (oxygen glucose deprivation/re-oxygenation) and MCAO/R (middle cerebral artery occlusion) experimental models. ELISA assay and Western blotting could

also be employed to measure BDNF, TNF, IL-6, IL-1 β , and CREB1 levels following treatment with *Piper longum* L. Besides sesamin-CREB1, other top-ranked pairs will also be tested by SPR. Large-scale, multicenter, high-quality

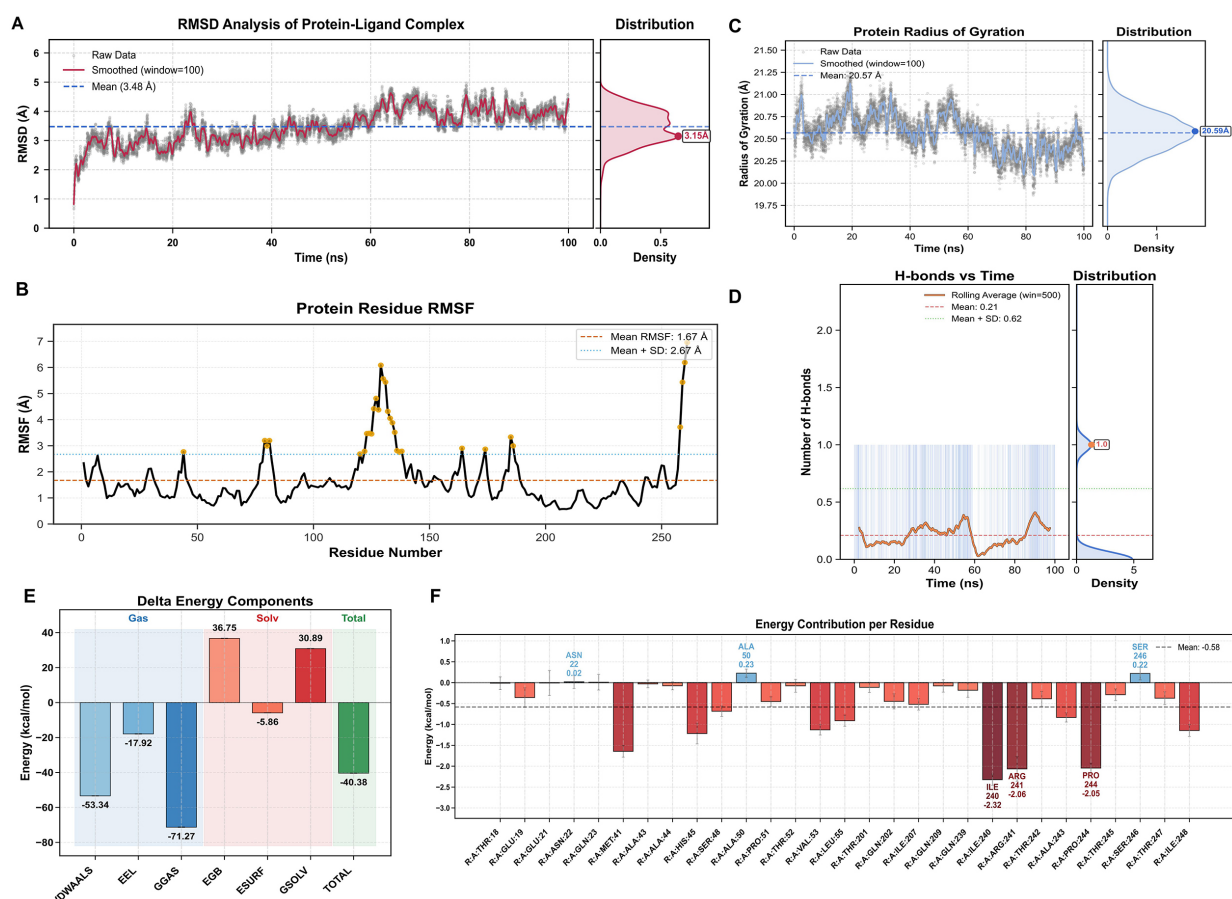


Fig. 4. Molecular dynamics simulation results for sesamin and CREB1. (A) RMSD analysis of sesamin-CREB1 complex. (B) RMSF analysis of CREB1 residue. (C) Rg analysis of CREB1. (D) Hydrogen-bond (H-bond) analysis of sesamin-CREB1 complex. (E) Analysis of delta energy components. (F) Analysis of energy contribution per residue. RMSD, root mean square deviation; RMSF, root mean square fluctuation; Rg, radius of gyration.

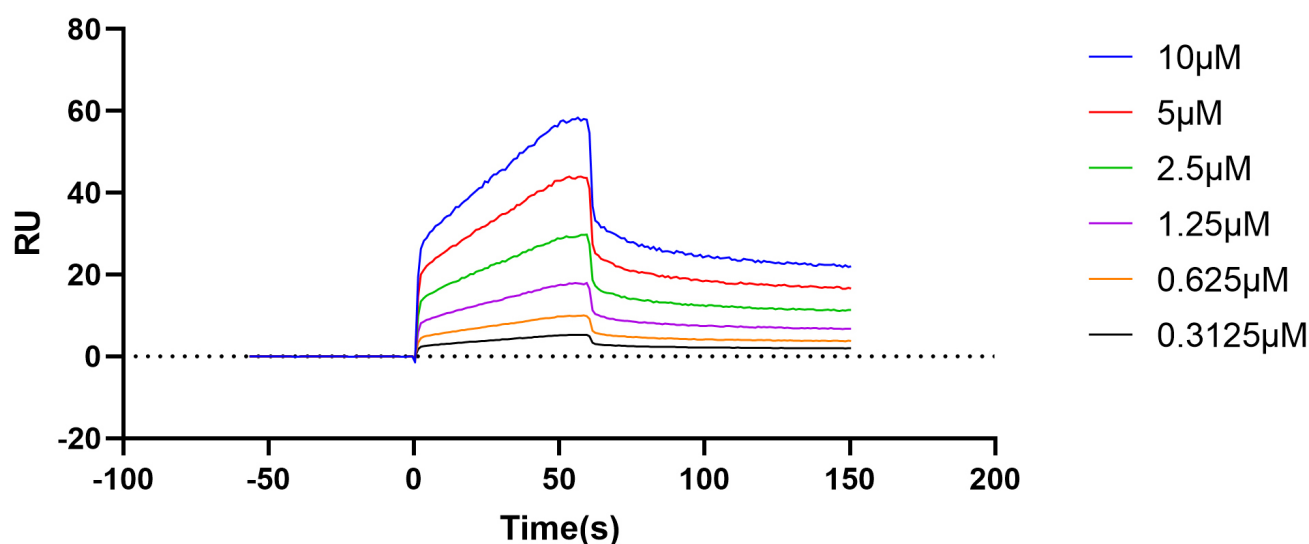


Fig. 5. SPR result for the interaction between sesamin and CREB1. SPR, surface plasmon resonance; RU, resonance units.

clinical trials are warranted to confirm the efficacy of *Piper longum* L; thereby providing robust scientific evidence for its clinical application.

6. Conclusions

This study elucidated the theoretical mechanisms underlying the pharmacological effects of *Piper longum* L.

in CIS. It may exert effects on CIS via targets including CREB1, PTGS2, and TNF, while acting via signaling such as cAMP and inflammation. These were achieved through an integrated approach combining network pharmacology, molecular docking simulations, and SPR validation.

Availability of Data and Materials

The data in this study are available from the corresponding author on reasonable request.

Author Contributions

HT, LW, CM and LS designed the study and wrote the manuscript. HT, LW, CM, LS, HL, RH, JY and ZH performed all of the experiments and analyzed data. HT and LW: manuscript drafting; CM and LS: manuscript editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

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