ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Exploring the potential mechanisms of piperine against Alzheimer's disease through network pharmacology, molecular docking, and in vitro experiments

Shangzhi Wu and Kok Hong Tang*

Department of Clinical Medicine, Faculty of Pharmacy, Lincoln University College, Petaling Jaya, Selangor, Malaysia

Abstract

Introduction: Alzheimer's disease (AD) is a complex neurodegenerative disorder with limited treatment options, prompting interest in natural compounds with multi-target activity. Piperine has shown neuroprotective and anti-inflammatory potential, but its mechanisms against AD have not been fully elucidated.

Objective: This study employed a combined strategy of network pharmacology, molecular docking, and *in vitro* assays to explore the potential mechanisms by which piperine may modulate AD-related neuroinflammation.

Methods: Potential targets of piperine and AD-related targets were retrieved from multiple public databases. Common targets were analyzed via protein–protein interaction (PPI) network, Gene Ontology, and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, and binding affinities were evaluated using molecular docking. For *in vitro* validation, BV2 microglial cells were stimulated with lipopolysaccharide (LPS) and treated with piperine. Cytokine (tumor necrosis factor alpha [TNF-α], IL [interleukin]-18, IL-1β) levels were measured by ELISA, and mRNA expression of key targets was assessed by qRT-PCR.

Results: A total of 134 overlapping targets were identified between piperine and AD. Core targets, including AKT1, HSP90AA1, SRC, RELA, and MAPK1, were discerned from the PPI network. KEGG enrichment analysis implicated several key pathways, such as PI3K–Akt, apoptosis, and cAMP signaling. Molecular docking simulations predicted stable binding interactions between piperine and these core targets, with binding energies all below −5.0 kcal/mol. In cellular experiments, piperine significantly inhibited the LPS-induced release of TNF-α, IL-18, and IL-1β. At the transcriptional level, piperine downregulated the mRNA expression of *Src*, *Rela*, and *Mapk1* while upregulating the expression of *Akt1* and *Hsp90aa1* ($p < 0.05$).

Conclusion: These findings suggest that piperine alleviates neuroinflammation in a microglial model, potentially by modulating a network of targets (AKT1, HSP90AA1, SRC, RELA, and MAPK1*)* and pivotal pathways, supporting the continued assessment of piperine as a potential anti-neuroinflammatory agent relevant to AD.

Keywords: Piperine; Alzheimer's disease; Network pharmacology approach; Molecular docking; Neuroinflammation; BV2 microglial cells

***Corresponding author:**
Kok Hong Tang
(bennytang118@gmail.com)

Citation: WU S, Tang KH. Exploring the potential mechanisms of piperine against Alzheimer's disease through network pharmacology, molecular docking, and in vitro experiments. *Eurasian J Med Oncol.* 2026;10(3):026030033. doi: 10.36922/EJMO026030033

Received: January 16, 2026

Revised: April 8, 2026

Accepted: April 9, 2026

Published online: June 29, 2026

1. Introduction

Alzheimer's disease (AD) plays a central role in the global burden of dementia and cognitive decline, disproportionately affecting the elderly population. Nevertheless, the influence of contemporary lifestyles has resulted in the emergence of symptoms associated with AD in certain populations at a relatively younger age, a condition referred to as early-onset AD. As a central nervous system disorder, AD is characterized by progressive neurodegeneration, which gradually leads to cognitive decline, memory impairment, behavioral changes, and reduced ability to perform daily activities, and in some patients, neuropsychiatric symptoms.[1] Multiple studies and statistical data indicate that approximately 47 million people worldwide suffer from dementia, among whom about 37 million are AD patients.[2] Although the etiology of AD remains incompletely understood, genetic, environmental, and lifestyle-related factors are thought to contribute to its development. Its histopathological mechanisms involve multiple factors and various pathophysiological regulatory processes.[3] Despite the extensive research conducted on the pathogenesis of AD, its intricate nature remains incompletely understood. Current research on the pathogenesis of the condition primarily revolves around several theoretical frameworks, including the tau protein hypothesis, the amyloid cascade hypothesis, and explanations centered on oxidative stress and neuroinflammation.[4] The identification of optimal biological markers is essential for significantly enhancing the early detection of the risk associated with AD. Consequently, a thorough investigation into the underlying mechanisms of AD, alongside the creation of dependable predictive indicators, is vital for the formulation of effective prevention and treatment approaches.

The alkaloid piperine, which contains nitrogen, was initially extracted from black peppers by Danish researchers in 1820 and presents as a crystalline yellow solid.[5] The chemical structure of this compound is a piperine alkaloid containing conjugated fatty chains, which endows pepper with its distinctive pungent flavor. Research indicates that piperine demonstrates the ability to modulate various cellular signaling pathways, while also providing anti-inflammatory and neuroprotective effects.[6] It holds therapeutic value for Parkinson's disease, AD, stroke, cardiovascular and cerebrovascular diseases, and inflammatory disorders. *In vitro* models of AD have shown that piperine exhibits significant antioxidant properties, effectively reducing lipid peroxidation levels, improving intracellular thiol group status, and enhancing the activity of antioxidant enzymes. Research involving animal models has demonstrated that piperine improves cognitive impairments in mice that exhibit AD characteristics.[7] This enhancement is achieved through the reduction of oxidative and nitrosative stress, the restoration of neurotransmitter activity, and the suppression of neuroinflammatory processes. Emerging evidence suggests that piperine attenuates cognitive impairment through antioxidant and antiinflammatory mechanisms, with potential involvement of necroptosisrelated pathways.[8] When combined with curcumin extract or green tea extract, piperine may help improve cognitive dysfunction in elderly dogs with AD.[9] Through its dual antioxidant and cholinergic regulatory effects, piperine synergistically enhances cognitive function. It also inhibits streptozotocin-induced hippocampal synaptic toxicity and oxidative damage, with mechanisms similar to those of donepezil.[10] Both compounds effectively alleviate oxidative stress, thereby improving cognitive dysfunction. Beyond neuroprotective effects, piperine and its derivatives exhibit broad pharmacological activities, including antibacterial, antitumor, antidepressant, and antiepileptic effects.[11] Given the multifaceted and multi-target nature of piperine's mechanism of action, conventional pharmacological approaches fall short in thoroughly clarifying its involvement in AD. Consequently, there is a pressing need to establish research methodologies that can systematically evaluate its interactions with various biological targets.

Moreover, the multifactorial nature of AD presents a significant challenge for conventional single-target drug development, which often yields limited clinical efficacy. This has spurred growing interest in multi-target directed ligands and natural products capable of concurrently modulating several pathological cascades.[12,13] Piperine, with its documented polypharmacological profile, represents a promising candidate within this paradigm. However, a systematic mapping of its complex target landscape and the associated biological networks in the context of AD is still lacking. Network pharmacology, an interdisciplinary field integrating bioinformatics and pharmacological strategies, offers a powerful framework to study multidimensional drug–target–disease interactions.[14,15] By constructing interactive networks, it can identify hub targets and crucial pathways, thereby elucidating the multi-component mechanisms of natural compounds. Accordingly, this study leverages an integrative strategy—combining network pharmacology, molecular docking, and *in vitro* assays—to systematically examine the mechanistic role of piperine in alleviating AD-related neuroinflammation.[16]

2. Materials and methods

2.1. Materials

2.1.1. Reagents

The main reagents used in this study were piperine standard (purity ≥ 98%; Desite Biotech, China), lipopolysaccharide (LPS; Sigma-Aldrich, United States of America [USA]), Dulbecco's Modified Eagle Medium (DMEM)/F12 medium containing high glucose (Gibco, USA), fetal bovine serum (Gibco, USA), penicillin-streptomycin antibiotic solution (Gibco, USA), 0.25% trypsin-ethylenediaminetetraacetic acid solution (Miltenyi, Germany), Cell Counting Kit-8 (CCK-8; Dojindo, Japan), TRIzol® reagent (Thermo Fisher Scientific, USA), PrimeScript™ RT reagent kit with gDNA Eraser (RR047A, Thermo Fisher Scientific, USA), TB Green® Premix Ex Taq™ II (RR820A, Thermo Fisher Scientific, USA), mouse tumor necrosis factor alpha (TNF-α) enzyme-linked immunosorbent assay (ELISA) kit, mouse interleukin (IL)-18 ELISA kit, mouse IL-1β ELISA kit (SP13710, Wuhan Saipai Biotechnology Co., Ltd., China), and dimethyl sulfoxide (Sigma-Aldrich, USA). All other routine chemical reagents were of analytical grade.

2.1.2. Instruments

The main instruments used in this study included a CO_2 incubator (HERAcell 150i, Thermo Fisher Scientific, United States of America [USA]), an inverted phase-contrast microscope (CKX53, Olympus, Japan), micropipettes (Eppendorf, Germany), a low-speed high-temperature centrifuge (5424R, Eppendorf, Germany), a laminar flow hood (SW-CJ-2FD, Suzhou Antai Air Technology Co., Ltd., China), a NanoDrop 2000 spectrophotometer (NanoDrop 2000, Thermo Scientific, USA), a real-time quantitative polymerase chain reaction system (RT-qPCR; QuantStudio 5, Applied Biosystems, USA), and a microplate reader (Synergy H1, BioTek, USA).

2.1.3. Cell line

The BV2 mouse microglial cell line was graciously supplied by the Cell Resource Center at Guangxi University of Chinese Medicine, Nanning, China.

2.2. Network pharmacology analysis of piperine in Alzheimer's disease

2.2.1. Identification of piperine-associated targets

The SMILES representation of piperine was extracted from the HERB database (http://herb.ac.cn/), and the predicted targets of piperine were screened using the SwissTargetPrediction (https://swisstargetprediction.ch/) database with a probability threshold of ≥0.[17] To expand and corroborate the target profile, additional screenings were performed using the PharmMapper (https://www.lilab-ecust.cn/pharmmapper/) server for reverse pharmacophore matching, the Similarity Ensemble Approach (SEA; https://sea.bkslab.org/) database, the NetInfer@LMMD platform (https://lmmd.ecust.edu.cn/netinfer/), and the STITCH database (https://stitch.embl.de/) for chemical–protein interactions. The obtained target names were standardized using the UniProt database (https://www.uniprot.org/) to retrieve official gene symbols and remove duplicates.

2.2.2. Acquisition of Alzheimer's disease-related targets

Disease-associated targets were acquired from two major human disease genomics databases: GeneCards (https://www.genecards.org/) and the Online Mendelian Inheritance in Man (OMIM; https://www.omim.org/). A systematic search was performed in both repositories using the key term "Alzheimer's disease." From the GeneCards results, only targets with a Relevance score of 1 or higher were selected to ensure high confidence in their association with AD pathogenesis, and these were carried forward for further investigation.[18]

2.2.3. Protein–protein interaction network construction and analysis of overlapping targets

We cross-referenced the target sets of piperine and AD to pinpoint overlapping targets, which were then visualized using a Venn diagram tool on the MicroBioinformatics platform (https://www.bioinformatics.com.cn/). The resulting common targets were then used to construct a protein–protein interaction (PPI) network. Using the STRING database (version 11.0; https://cn.string-db.org/), we built a network for Homo sapiens, including only interactions with a confidence score of at least 0.700. To finalize the network for analysis, all proteins lacking connections (isolated nodes) under this stringent threshold were systematically removed.[19]

2.2.4. Identification of core targets

The list of common targets identified from the intersection analysis was subsequently submitted to the STRING platform to generate a TSV file containing the PPI network data. This TSV file was subsequently opened in Cytoscape (version 3.10.1; https://cytoscape.org) to proceed with visual representation and topological examination of the PPI network, utilizing the CytoHubba plugin for enhanced evaluation. Node importance was assessed based on three metrics: Degree, Betweenness, and Closeness. The intersection of results from these three dimensions constitutes the core targets. Finally, the screened core

targets are visualized and analyzed.[20]

2.2.5. Construction of the drug–target–disease network

The principal targets of piperine for AD treatment were mapped and analyzed using Cytoscape to visualize the interaction network. This facilitated the construction of a network representing "Traditional Chinese Medicine–Active Components–Target," which was then subjected to visualization for enhanced interpretation of the results.[20]

2.2.6. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses

We submitted the intersecting genes of piperine and Alzheimer's disease to the DAVID database to conduct functional annotation. Enrichment analysis covered the GO domains of biological process (BP), cellular component (CC), and molecular function (MF), with the species parameter restricted to *Homo sapiens*. The resultant data files were downloaded for subsequent visualization. GO enrichment results were graphically represented as bubble plots using the MicroBioinformatics platform. Separately, on the same platform, the pathway enrichment module was selected. After inputting the gene list, a KEGG pathway enrichment analysis was performed, generating corresponding bubble charts to illustrate the findings.[21,22] Pathway prioritization was based on statistical significance ($p \leq 0.01$) and biological relevance to the research question. Cancer-related pathways, while frequently enriched in such analyses, were excluded prior to visualization because they are broadly associated with proliferative signaling and do not specifically inform the neuroinflammatory or neuroprotective mechanisms central to AD pathogenesis. This exclusion was performed based on domain knowledge to enhance the interpretability of the results and focus on pathways directly relevant to AD.

2.2.7. Molecular docking

To translate the bioinformatic predictions into testable structural hypotheses, molecular docking simulations were conducted. The top core targets from network analysis were selected. High-resolution crystal structures of these target proteins were acquired from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/). Heteroatoms unsuitable for protein–ligand docking were eliminated, including co-crystal ligands, ATP, metal ions, and water molecules. The two-dimensional structure of piperine was obtained from PubChem and converted into a three-dimensional structure using the ChemDraw software (PerkinElmer, USA; available at https://www.perkinelmer.com/category/chemdraw). Docking of the target protein with piperine was performed using AutoDock Vina (The Scripps Research Institute, USA; available at https://autodock.scripps.edu/).[23] The docking grid box was centered on the known active site of each target protein based on literature and PDB annotation, with dimensions set to 20 × 20 × 20 Å to encompass the binding cavity. For protocol validation (performed in preliminary experiments, data not shown), the co-crystallized ligand of each target was re-docked into the same grid box, yielding root mean square deviation values below 2.0 Å, confirming the reproducibility of the docking procedure. Key protein–small molecule interactions were predicted, and protein–ligand interactions in the docked complex were studied using LigPlot+ (EMBL-EBI, United Kingdom; available at https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). Three-dimensional visualization of ligand-target binding was achieved through PyMOL software (Schrödinger, LLC, USA; available at https://pymol.org).[24,25]

2.3. *In vitro* validation studies

2.3.1. Cell culture

The mouse microglial BV2 cell line was cultured in DMEM/F12 medium containing high glucose, supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin antibiotic solution. Cells were maintained in a humidified incubator at 37 °C with 5% CO_2. Cells in the exponential growth phase were harvested for all subsequent experiments.[26] Cells were passaged every 2–3 days upon reaching 80–90% confluence, and experiments were performed using cells between passages 5 and 15 to ensure consistent responses.

2.3.2. CCK-8 assay for cell viability

To evaluate cell viability, BV2 cells in the logarithmic growth phase were plated in 96-well plates at a density of 6×10^4 cells/well and incubated overnight to allow adherence. Cells, except for the control group, received 1 μg/mL LPS treatment to induce an inflammatory response. The concentration of 1 μg/mL LPS was selected based on preliminary experiments and literature indicating that this dose induces a robust inflammatory response in BV2 cells while maintaining cell viability above 70%, thereby providing a suitable inflammatory model without excessive cytotoxicity that would confound the assessment of piperine's protective effects. Concurrently, to evaluate the protective effect, cells were co-treated with piperine at various final concentrations (10, 20, 60, 80, 120 μmol/L). A group treated with drug-free culture medium served as the control. After a 24-h intervention period, cell viability was determined using the CCK-8 assay. Because 80 μmol/L was the lowest concentration that produced a near-maximal protective effect (92.89% viability), this

concentration was selected for subsequent experiments. Although 120 μmol/L yielded slightly higher viability, 80 μmol/L was considered sufficient to achieve near-maximal protection while avoiding the use of a higher-than-necessary concentration.. Briefly, 10 μL of CCK-8 reagent was added to each well, and the plates were incubated at 37 °C for 1 h. The absorbance at 450 nm was measured with a microplate reader, and relative cell viability was determined by comparing treated wells to the control.[27]

2.3.3. ELISA for inflammatory cytokines in cell supernatants

For cytokine analysis, BV2 cells were seeded in 96-well plates (6×10^4 cells/well) and pre-cultured for 24 h. Cells were then assigned to one of three groups: control (medium only), model (1 μg/mL LPS), and piperine treatment (80 μmol/L piperine + 1 μg/mL LPS). All experimental conditions were tested in triplicate wells, with the entire experiment repeated independently on three separate occasions. After 24 h of treatment, the cell culture supernatant was carefully collected from each well. The supernatant was centrifuged at 1,000×g for 10 min at 4 °C to remove cellular debris. The clarified supernatant was then preserved at -80 °C until analysis. TNF-α, IL-18, and IL-1β levels were measured using mouse ELISA kits, with procedures performed as specified by the manufacturer. The assay procedure involved incubation of samples and standards in pre-coated wells, followed by sequential incubation with a biotin-conjugated detection antibody (as part of the respective mouse ELISA kit, Wuhan Saipai Biotechnology Co., Ltd., China) and streptavidin-horseradish peroxidase conjugate (as part of the respective mouse ELISA kit, Wuhan Saipai Biotechnology Co., Ltd., China). Absorbance at 450 nm was measured immediately after the addition of the 3,3',5,5'-tetramethylbenzidine substrate and stopping the reaction. Cytokine concentrations were calculated from standard curves generated from known concentrations.[28]

2.3.4. Quantitative real-time polymerase chain reaction

Expression of target genes was determined via RT-qPCR. Briefly, BV2 cells were plated at 4×10^5 cells per well in 6-well plates and treated according to Section 2.3.3 (control, LPS model, piperine intervention). After treatment, cells were harvested, and total RNA was extracted using TRIzol reagent. RNA quality and concentration were determined spectrophotometrically, and complementary DNA was synthesized from equal RNA amounts using a reverse transcription kit. RT-qPCR was performed using SYBR Green master mix on a real-time PCR platform. The relative mRNA levels of *Akt1*, *Hsp90aa1*, *Src*, *Rela*, and *Mapk1* were normalized to *Gapdh* and calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are provided in Table 1.[29]

2.3.5. Statistical analysis

Statistical analyses were performed using SPSS Statistics software (Version 22.0, IBM, USA). Data are presented as mean ± standard deviation from three independent biological replicates, with each replicate consisting of three technical repeats (n = 3 data points per group). The normality of data distribution was assessed using the Shapiro–Wilk test. Comparisons among multiple groups (control, model, and piperine treatment) were performed using one-way analysis of variance.[30] p-values < 0.05 were considered statistically significant, and $p < 0.01$ was considered highly significant.

3. Results

3.1. Targets associated with piperine

The targets associated with piperine were extracted from multiple public databases, including HERB, SwissTargetPrediction, PharmMapper, SEA, NetInfer@ LMMD, and STITCH. These targets were subsequently processed through the UniProt database for conversion. After deduplication, 188 candidate targets of piperine were ultimately identified.

3.2. Collection of targets related to Alzheimer's disease and overlapping drug–disease targets

From the GeneCards and OMIM databases, a comprehensive list of 5,874 targets related to AD was compiled. We screened and cross-referenced the predicted targets of piperine with AD-related targets, revealing 134 overlapping candidates (Figure 1).

3.3. Protein–protein interaction network topology analysis and screening of core targets

We built a PPI network for the candidate targets using STRING (version 11.0). To ensure the relevance and connectivity of the network, interactions with a confidence score below 0.7 were filtered out, which effectively removed isolated protein nodes. In the visualized network, each functional protein is denoted by a node, and the biological interactions between them are illustrated as connecting edges. This analysis produced a network comprising 132 nodes and 243 interaction edges (Figure 2). We computed key topological metrics, reporting an average node degree of 3.68 and a local clustering coefficient of 0.384. Analysis of the network revealed significant PPI enrichment, indicated by a PPI enrichment p-value < 1.0×10^{-16} against an expected 76 edges. Topological analysis was carried out using the CytoHubba plugin within Cytoscape. Three centrality metrics—betweenness centrality, closeness

Table 1. Primer sequences used for RT-qPCR

Gene symbol	Primer sequence (5' → 3')	Product length
Akt1	F: CTCAATGAGGTGTCTGCCATC R: GGGTGTCAGTCTCCGACAGT	150 bp
Hsp90aa1	F: GCTGAAGAGGTGCGTGTAAA R: ATCTTCACCACGTTCTCCCA	100–150 bp
Src	F: CGCAAGAGTGTGATTGAGGT R: CACGGATCTCGTAGTCGTTC	150 bp
Rela	F: ATGGCAGACGATGATCCCTAC R: TGTTGACAGTGGTATTTCTGGTG	100–150 bp
Mapk1	F: TACACCAACCTCTCGTACATCG R: TAGCCACATACTCCACACCTT	150 bp
Gapdh	F: AGGTCGGTGTGAACGGATTTG R: TGTAGACCATGTAGTTGAGGTCA	120 bp

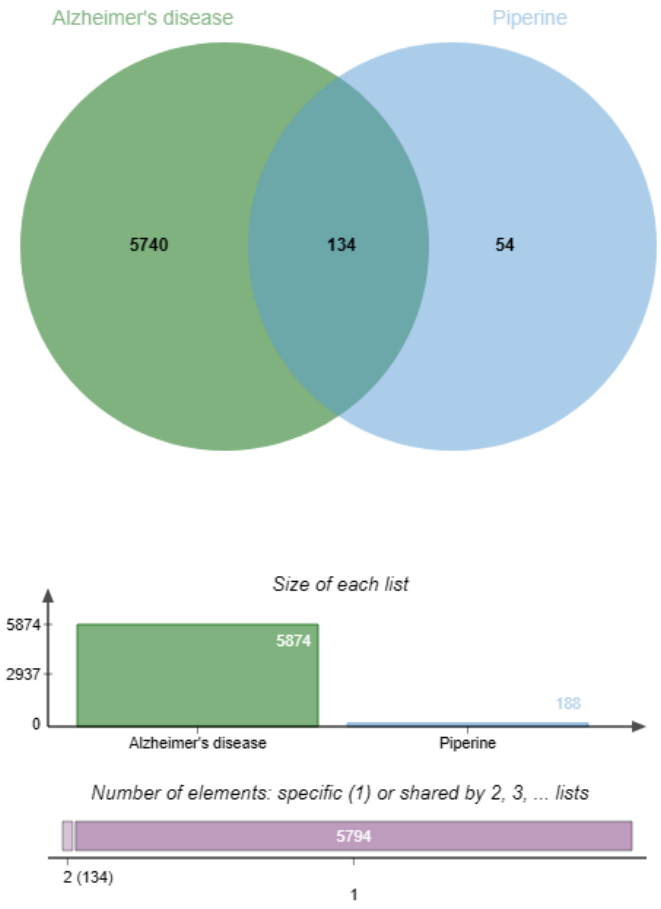

Figure 1. Acquisition of Alzheimer's disease-related targets and drug–disease common targets

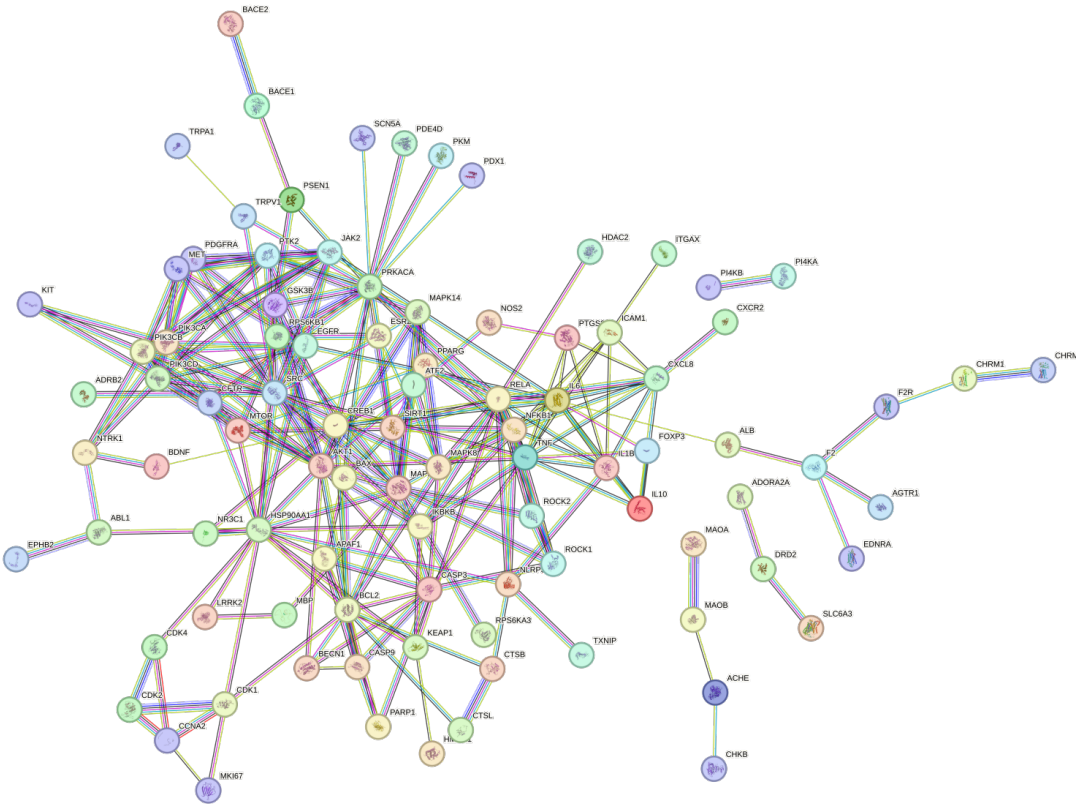

Figure 2. Potential target protein–protein interaction network

centrality, and degree centrality—were computed. Based on these results, 20 core targets were selected (Figure 3). Among these, AKT1, HSP90AA1, SRC, RELA, and MAPK1 ranked highest in degree centrality, indicating their potential as pivotal therapeutic targets.

3.4. Visualization of the network linking medicine, active constituents, and targets

The core therapeutic targets of piperine for AD, along with the compound itself, were assembled in Cytoscape to generate a compound-target-disease interaction model (Figure 4).

3.5. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis

We performed functional enrichment analysis using the DAVID bioinformatics resource to characterize the biological roles of the identified common targets. We applied a significance threshold of $p \leq 0.01$ to select significant results. The enriched terms were then ranked based on their p-values, where a lower value indicates a higher degree of statistical significance. GO annotation was performed to categorize the functional roles of the targets into three primary domains: BP, CC, and MF. The analysis revealed 2,473 significant GO terms, comprising 2,254 BP terms, 79 CC terms, and 140 MF terms. For clarity and focus, the 10 most significantly enriched terms within each of the three domains were selected and are presented visually in Figure 5. The analysis revealed distinct functional themes across the domains. In the BP domain, targets were predominantly associated with cellular responses to various stimuli, including lipopolysaccharides, molecules of bacterial origin, oxidative stress, chemical stress, and peptide hormones. For CC, the targets showed strong enrichment in specific membrane structures and compartments, including membrane rafts, membrane microdomains, and the outer membranes of organelles—particularly mitochondria. Regarding MF, the key activities involved protein kinase functions (serine, serine/threonine, and tyrosine kinases) and processes related to histone modification and kinase activity.

The KEGG enrichment analysis enables interpretation

of the roles played by PPI networks in cellular functions, while identifying key targets and their associated signaling pathways. In this study, through functional enrichment analysis, 178 significantly enriched pathways were detected. We removed disease-specific and cancer-related pathways, then visualized the 20 most significantly enriched pathways using the MicroBioinformatics platform (Figure 6). The prominently enriched pathways primarily involve the PI3K–Akt signaling pathway, apoptosis, the C-type lectin receptor signaling pathway, and the cAMP signaling pathway, among others. The 20 most significantly enriched pathways were selected for visualization based

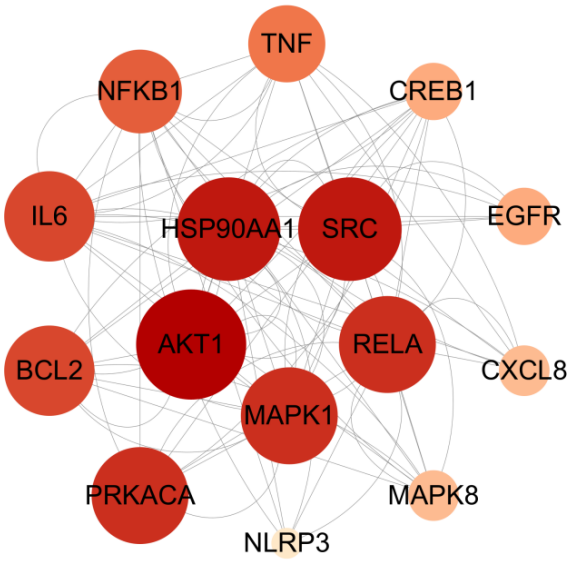

Figure 3. Piperine–Alzheimer's disease therapeutic target interaction network diagram

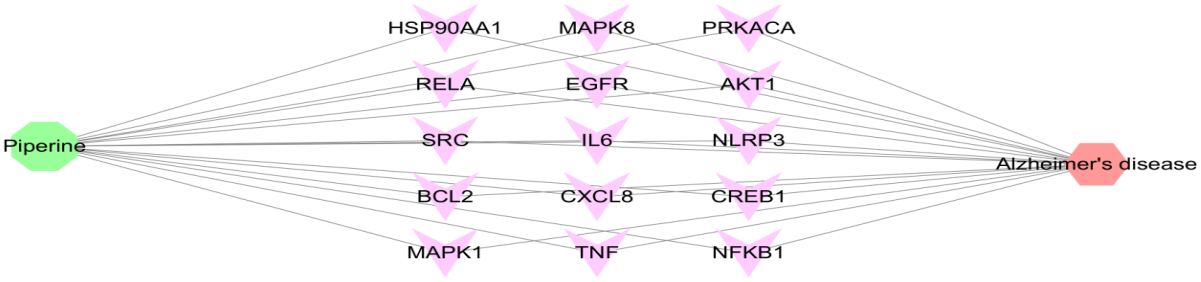

Figure 4. Integrated network mapping therapeutic targets for Alzheimer's disease

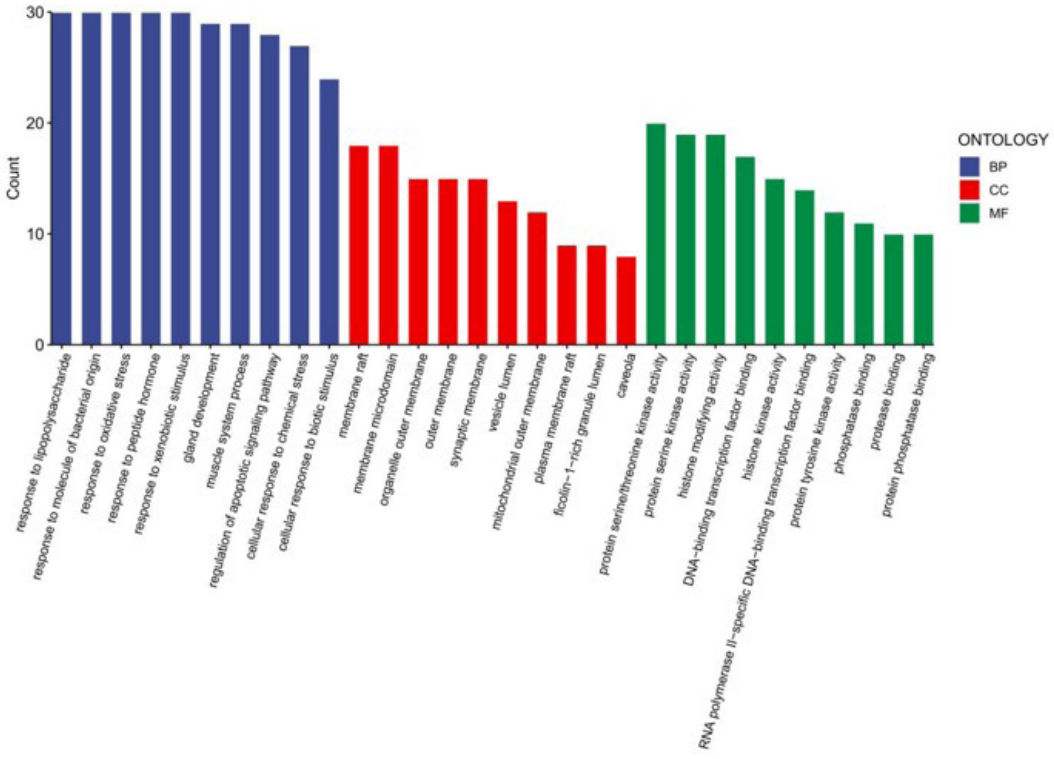

Figure 5. Gene Ontology enrichment analysis
Abbreviations: BP: Biological process; CC: Cellular component; MF: Molecular function.

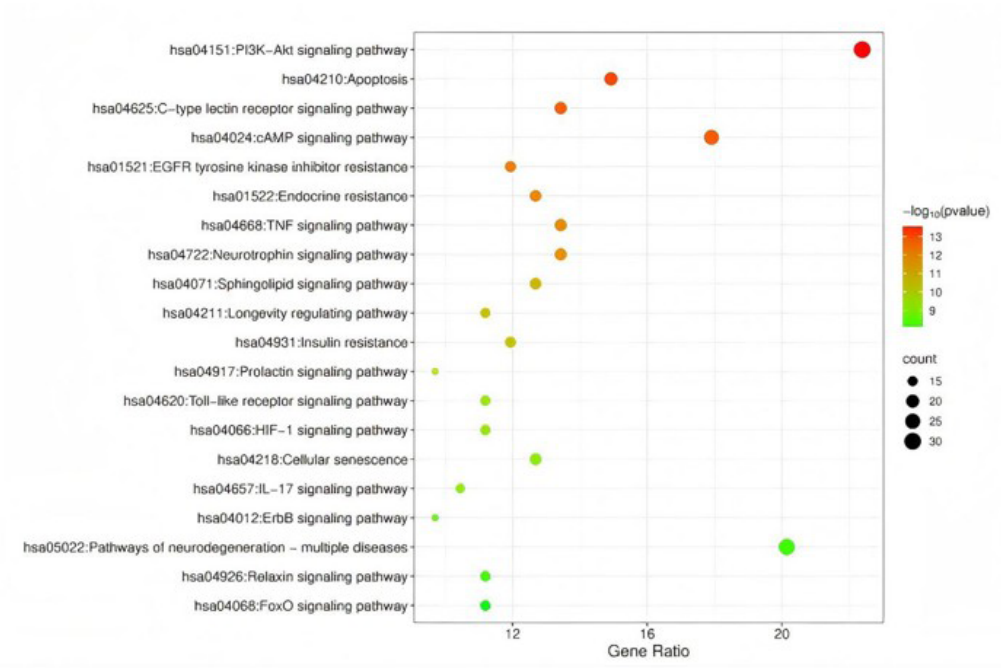

Figure 6. Kyoto Encyclopedia Genes and Genomes pathway enrichment analysis

on *p*-values after excluding cancer-related categories, which are commonly enriched across diverse gene sets and do not specifically inform AD-relevant mechanisms. The remaining pathways, including PI3K–Akt signaling, apoptosis, and cAMP signaling, are well-established in the context of neuroinflammation and neuronal survival, and their identification remained robust regardless of whether cancer pathways were included in the initial analysis.

Notably, the KEGG pathways enriched in this analysis are not isolated but exhibit significant crosstalk, potentially forming a coordinated network through which piperine exerts its effects. For example, the PI3K–Akt pathway suppresses apoptosis and modulates inflammatory responses, overlapping with the apoptosis and C-type lectin receptor signaling pathways identified separately. Furthermore, the cAMP signaling pathway is known to influence neuronal plasticity and microglial activity, potentially interacting with both PI3K–Akt and inflammatory pathways. This interconnectivity suggests that piperine's modulation of a limited set of core targets may resonate across multiple related BPs, amplifying its overall neuroprotective potential.

3.6. Molecular docking

To investigate piperine's mechanism of action, this study identified proteins with high pathway relevance and favorable molecular affinity for subsequent docking validation. Molecular docking simulations predicted potential stable interactions between piperine and the core targets AKT1, HSP90AA1, SRC, and RELA, with respective binding affinities of −6.3, −7.0, −7.1, and −6.4 kcal/mol. Additionally, molecular docking simulation revealed a relatively low binding affinity between piperine and MAPK1 (−4.2 kcal/mol), suggesting a lack of direct physical interaction between the compound and this specific kinase. We used PyMOL software to render the structural models of the docking conformations (Figure 7).

3.7. *In vitro* validation results

3.7.1. Effect of piperine on cell viability in lipopolysaccharide-stimulated BV2 microglial cells

BV2 microglial cells were treated with 1 μg/mL LPS, which significantly decreased cell viability to 72.35% of the untreated control, indicating successful induction of a moderately cytotoxic inflammatory model. Co-treatment with piperine effectively counteracted this LPS-induced damage in a concentration-dependent manner. Notably, administration of 80 μmol/L piperine markedly restored cell viability to 92.89%, demonstrating a significant protective effect compared to the LPS-only model group ($p < 0.01$; Table 2). Based on this pronounced efficacy and absence of toxicity at this dose, 80 μmol/L was chosen for

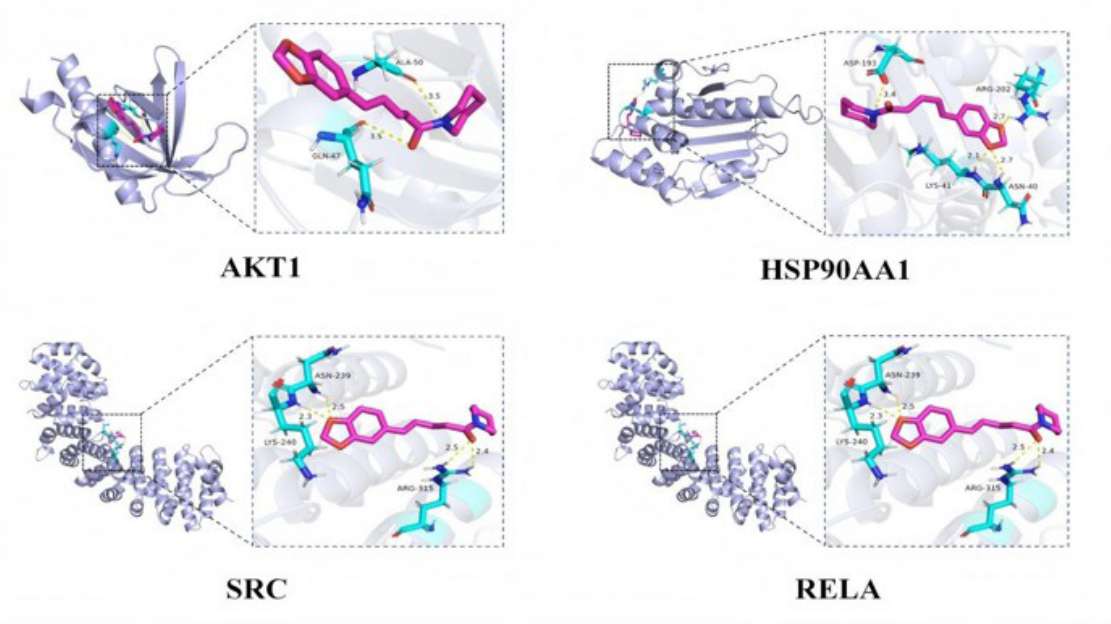

Figure 7. Predicted binding conformations of piperine with AKT1, HSP90AA1, SRC, and RELA from molecular docking simulations (MAPK1 is omitted from visualization due to weak binding affinity)

all subsequent intervention experiments.

3.7.2. Effect of piperine on lipopolysaccharide-induced inflammatory cytokine secretion

The inflammatory response was assessed by measuring key cytokine levels in the cell culture supernatant. Exposure to LPS resulted in elevated levels of TNF-α, IL-18, and IL-1β, which were significantly higher in the model group compared to the control group ($p < 0.01$). Treatment with piperine (80 μmol/L) significantly reduced levels of these pro-inflammatory cytokines, leading to markedly lower TNF-α, IL-18, and IL-1β compared with the LPS group ($p < 0.05$; Table 3). These data collectively indicate that piperine possesses significant anti-inflammatory activity in this cellular model, effectively attenuating the excessive secretion of critical inflammatory factors from activated microglia. Our findings are consistent with recent reports demonstrating that piperine suppresses LPS-induced microglial activation via inhibition of NF-κB and MAPK signaling.[31,32] Similar anti-inflammatory effects have been observed with other natural compounds targeting HSP90 in BV2 cells.[33]

3.7.3. Effects of piperine on mRNA expression of Akt1, Hsp90aa1, Src, Rela, and Mapk1 in lipopolysaccharide-induced BV2 cells

The RT-qPCR analysis revealed distinct expression patterns for the five target genes under different treatment conditions (Table 4). The model group showed a significant upregulation in the mRNA levels of *Src*, *Rela*, and *Mapk1* compared to the untreated control ($p < 0.05$). Conversely, the expression of *Akt1* and *Hsp90aa1* was significantly downregulated in the model group ($p < 0.05$). Treatment with piperine effectively reversed these LPS-induced expression alterations. Specifically, in the piperine group, the elevated expression of *Src*, *Rela*, and *Mapk1* was significantly suppressed, while the reduced levels of *Akt1* and *Hsp90aa1* were notably restored, with all changes showing statistical significance compared to the model group ($p < 0.05$).

4. Discussion

We employed a combined approach of network pharmacology and *in vitro* experiments to elucidate the molecular pathways through which piperine may act against AD. Through database screening, 188 potential targets of piperine were identified, and intersection with AD-related targets (5,874) yielded 134 common targets. PPI network topology analysis identified 20 core targets, among which AKT1, HSP90AA1, SRC, RELA, and MAPK1 exhibited the highest degree values. KEGG enrichment implicated key pathways including PI3K–Akt, apoptosis, and cAMP signaling, all of which are critically involved in neuroinflammation and neuronal survival.[34] Molecular docking predicted stable binding between piperine and these five core targets (binding energies < −5.0 kcal/mol), suggesting potential direct interactions. These findings position piperine as a multi-target modulator, with subsequent *in vitro* experiments providing initial validation.

Table 2. Effect of piperine on lipopolysaccharide-induced cytotoxicity in BV2 cells ($n = 9$)

Group	Treatment concentration (μmol/mL)	Cell viability (%; control group = 100%)	*p*-value vs. control group	*p*-value vs. model group (1 μg/mL)
Control group	0	100.00 ± 3.21	–	–
Model group	1	72.35 ± 4.12**	$p < 0.01$	–
	10	58.79 ± 3.85**	$p < 0.01$	–
	20	45.22 ± 4.03**	$p < 0.01$	–
	40	32.18 ± 3.56**	$p < 0.01$	–
Piperine group	10	78.56 ± 3.92#	–	$p < 0.05$
	20	81.33 ± 3.45#	–	$p < 0.05$
	60	85.67 ± 3.78#	–	$p < 0.05$
	80	92.89 ± 4.05##	–	$p < 0.01$
	120	95.12 ± 3.82##	–	$p < 0.01$

Notes: Values are expressed in $\bar{x} \pm$ sd. ** indicates $p < 0.01$ vs. control group; # indicates $p < 0.05$ vs. model group; ## indicates $p < 0.01$ vs. model group.

Table 3. Inflammatory cytokine levels (pg/mL) in cell supernatants (*n* = 9)

Groups	TNF-α	IL-18	IL-1β
Control group	10.57 ± 2.23	22.63 ± 3.04	35.29 ± 4.12
Model group	156.84 ± 13.72**	143.25 ± 12.68**	208.51 ± 18.93**
Piperine group	67.39 ± 6.24#	71.55 ± 7.08#	102.37 ± 9.46#

Note: ** indicates $p < 0.01$ compared to the control group; # indicates $p < 0.05$ compared to the model group.

Table 4. mRNA expression levels of *Akt1, Hsp90aa1, Src, Rela, Mapk1* in cells of each group (*n* = 9)

Groups	*Src*	*Rela*	*Mapk1*	*Akt1*	*Hsp90aa1*
control group	1.00 ± 0.08	1.00 ± 0.06	1.00 ± 0.07	1.00 ± 0.05	1.00 ± 0.098
model group	2.75 ± 0.12*	3.12 ± 0.15*	2.98 ± 0.13*	0.52 ± 0.04*	0.48 ± 0.05*
piperine group	1.82 ± 0.09#	1.95 ± 0.11#	1.78 ± 0.10#	0.85 ± 0.06#	0.80 ± 0.07#

Note: * indicates $p < 0.05$ vs. control group; # indicates $p < 0.05$ vs. model group.

From the pathological mechanism of AD, core targets such as AKT1, HSP90AA1, SRC, RELA, and MAPK1 are significantly linked to the pathological development of AD, primarily participating in neuronal survival, inflammatory responses, and cellular signaling.[35] The non-receptor tyrosine kinase SRC is commonly found within the signaling pathway networks associated with AD.[36] Intriguingly, while molecular docking indicated that piperine holds limited direct binding affinity toward MAPK1, our RT-qPCR analysis demonstrated a robust downregulation of *Mapk1* mRNA expression *in vitro*. This apparent discrepancy strongly implies that piperine modulates MAPK1 expression through an indirect upstream pathway rather than direct competitive binding. For instance, piperine may suppress the transcription of *Mapk1* by inhibiting its upstream regulators within the inflammatory cascade, such as RELA or AKT1, which exhibited excellent docking scores in this study. This phenomenon dynamically highlights the multi-target, network-regulatory characteristics typical of natural dietary compounds like piperine.[37] SRC is specifically upregulated in excitatory neurons of AD patients and co-localizes with the earliest pathological tau epitopes. Moreover, its contact frequency significantly increases during the formation of AD-related phosphorylated Tau, highlighting SRC's pivotal role in linking inflammatory stimuli with Tau pathology. RELA, an essential transcription gene within the NF-κB signaling cascade[38], functions as a pivotal immune-related hub gene during the stages of AD and mild cognitive impairment. The expression level exhibits a positive correlation with the severity of AD pathology. Continuous activation of RELA results in an overproduction of inflammatory mediators, including TNF-α and IL-18, which directly inflict damage on neurons and establish a detrimental feedback loop. Inhibiting RELA activity is a crucial approach to mitigating neuroinflammation in AD. AKT1, serving as a pivotal target, is inhibited in AD, leading to increased susceptibility to apoptosis and enhanced Tau hyperphosphorylation. Modulation of AKT1 activity may influence neuronal survival and metabolism.[39,40] Inhibition of tau hyperphosphorylation via the PI3K–Akt pathway is a common mechanism shared by several flavonoids[41], suggesting that piperine may exert similar effects. Heat shock protein HSP90AA1 is also a pivotal gene associated with AD immunity. In network pharmacology studies, it frequently co-occurs with other targets as potential drug action sites, participating in protein homeostasis regulation.[42] Our *in vitro* experiments demonstrated that piperine decreased the LPS-induced mRNA expression of *Src*, *Rela*, and *Mapk1*, supporting its role in alleviating

neuroinflammation. Conversely, it notably upregulated the mRNA expression of *Akt1* and *Hsp90aa1*, confirming a multi-target modulatory effect. This potential synergy may simultaneously dampen neuroinflammatory drivers and bolster cellular resilience, offering a mechanistic advantage over single-target approaches. Furthermore, the concurrent downregulation of pro-inflammatory mediators (*Src*, *Rela*, *Mapk1*) and upregulation of pro-survival factors (*Akt1*, *Hsp90aa1*) underscores a potential rebalancing of cellular homeostasis under inflammatory stress. This dual modulation aligns with the emerging concept of "neuroimmune resolution" in AD, where therapeutic strategies aim not merely to suppress inflammation but to actively promote its resolution and restore neural integrity. The enrichment of pathways like PI3K–Akt and cAMP signaling further supports this notion, as these cascades are critically involved in synaptic plasticity, neuronal survival, and the regulation of microglial phenotypic switching from a pro-inflammatory to a protective state. Our findings, therefore, position piperine not only as a neuroinflammation inhibitor but also as a potential modulator of the brain's endogenous repair mechanisms. This systems-level interpretation, derived from the network pharmacology approach, moves beyond a one-target-one-effect understanding and provides a holistic framework for future investigations. Validating this rebalancing effect in models encompassing neuronal-glia co-cultures or *in vivo* settings will be crucial to substantiate piperine's role in modulating the neuro-immune crosstalk that is fundamental to AD progression.

We conducted an integrative study, combining predictive analytics and preliminary experimental validation, aiming to elucidate the molecular pathways through which piperine may exert therapeutic effects against AD. GO functional enrichment analysis demonstrated that the core targets of piperine's anti-AD effects are primarily involved in BP including responses to lipopolysaccharides, bacterial-derived molecules, oxidative stress, chemical stress, and peptide hormones, and were located in CC, encompassing various structures such as membrane rafts, microdomains within membranes, the outer membranes of organelles, as well as the outer membranes of mitochondria. These targets also demonstrate MF, including activities related to nuclear receptors, protein tyrosine kinases, brain-derived neurotrophic factor receptors, protein serine kinases, protein serine/threonine kinases, histone modification, and histone kinases. KEGG pathway enrichment identified 20 key pathways, which are highly consistent with the pathological mechanisms of AD, further elucidating the "multi-pathway synergistic regulation" characteristic of piperine. The PI3K–Akt signaling cascade plays a critical role in modulating neuronal apoptosis, synaptic plasticity, and inflammatory responses, making it a highly promising regulatory axis for AD treatment.[43] Importantly, the enrichment of these pathways remained consistent after excluding cancer-related categories, supporting the robustness of the analysis and reinforcing the relevance of PI3K–Akt and apoptosis signaling to piperine's potential anti-neuroinflammatory effects. During the progression of AD, neuronal loss primarily occurs through apoptosis. Studies have demonstrated that the mechanisms of action of various compounds against AD involve the regulation of apoptosis pathways. Some compounds can protect neurons by upregulating anti-apoptotic proteins (e.g., p-Akt), thereby ameliorating AD.[44] Our results highlight novel evidence and perspectives for the development of natural compounds targeting AD.

The concentration of piperine used in our *in vitro* experiments (80 μmol/L) was selected based on the CCK-8 assay results, where it demonstrated optimal cytoprotective effects (92.89% cell viability) without significant cytotoxicity. However, we acknowledge that this concentration is relatively high compared to reported plasma concentrations of piperine in rodents following oral administration. Pharmacokinetic studies have shown that after oral administration of 20–50 mg/kg piperine in rats, peak plasma concentrations typically range from 0.5 to 10 μmol/L. Furthermore, the ability of piperine to cross the blood–brain barrier and achieve therapeutic concentrations in the brain parenchyma remains incompletely characterized. While some studies suggest that piperine can enhance the blood–brain barrier permeability of co-administered drugs, its own brain penetration is limited and may be influenced by formulation and route of administration. Therefore, the physiological relevance of the *in vitro* concentration used here should be interpreted with caution. Future studies should include pharmacokinetic assessments to determine the brain concentration of piperine achievable *in vivo* and establish whether the concentrations used *in vitro* are translationally relevant. Additionally, formulation strategies to enhance piperine's bioavailability and brain delivery should be explored.

This study offers several key contributions, including:

(i) Integration of research paradigms: We adopted a "network prediction–experimental validation" strategy, effectively bridging bioinformatic discovery with functional cell-based assays. This approach enhances the reliability of the predicted mechanisms. Functional validation using the BV2 microglia model significantly enhances the reliability and reproducibility of the prediction results.
(ii) In-depth focus on neuroinflammatory mechanisms:

The mechanism validation is anchored in microglial activation and inflammatory cytokine release, which closely aligns with current frontiers in AD research. The study directly explores targets such as RELA, providing more robust experimental support for mechanism elucidation compared to mere pathway enrichment analysis.

(iii) Clarification of core target combinations: MAPK1, RELA, SRC, AKT1, and HSP90AA1 have been collectively recognized as pivotal targets. Although existing literature independently supports the roles of these targets in AD, this study integrates them into a single target combination and directly associates them with piperine, proposing a novel multi-target interaction hypothesis that opens new perspectives for understanding the synergistic mechanisms of natural compounds.

However, we note the following limitations in the present study:

(i) Limitation of the experimental model: The functional validation is confined to an LPS-stimulated BV2 microglial model. While useful for studying neuroinflammation, this model does not encompass key AD-specific pathologies such as amyloid-β aggregation or tau hyperphosphorylation, and thus cannot directly establish efficacy against the core features of AD;

(ii) Limited direct mechanistic evidence: The identified pathways have not yet been experimentally confirmed as direct downstream mediators of the core target set, and their correlation with the anti-AD effects of piperine remains to be validated;

(iii) Insufficient depth in elucidating key target mechanisms: Although core targets have been identified as critical nodes, there is a lack of clear mechanistic evidence from small-molecule compounds directly targeting and mediating neuroprotective effects. Furthermore, the LPS-stimulated BV2 model, while useful for studying neuroinflammation, does not recapitulate key AD-specific pathologies such as amyloid-β aggregation or tau hyperphosphorylation. Therefore, the therapeutic relevance of our findings to AD should be interpreted with caution, and future studies in transgenic AD models are warranted to validate the effects of piperine on these core pathologies.

In line with current research trends, future studies could focus on the following directions:

(i) *In vivo* pharmacological and pathological validation: Utilizing classical mouse AD models, cognitive function improvement can be assessed through behavioral experiments (water maze, novel object recognition), combined with pathological detection (β-amyloid plaques, tau protein phosphorylation) to verify piperine's regulatory effects on core AD pathologies. Simultaneously, intracerebral drug concentrations should be measured to determine their blood–brain barrier permeability.

(ii) Mechanistic validation: In cell models, specific inhibitors or small interfering RNA knockdown technology could be employed to suppress core targets (e.g., AKT1, RELA), observing the reversibility of piperine's protective effects. Simultaneously, in neuronal models (e.g., β-amyloid- or tau-overexpressing cells), the impact of piperine on neuronal survival, synaptic function, and the PI3K–Akt pathway should be evaluated to supplement intrinsic neuronal protective mechanisms. In future studies, Western blot analysis should be performed to confirm the effects of piperine on the protein expression of AKT1, HSP90AA1, SRC, RELA, and MAPK1, as well as on the phosphorylation status of key signaling molecules (e.g., p-AKT, p-ERK, p-p65) to elucidate the activation state of these pathways.

(iii) Exploring compatibility and translational potential: Based on network pharmacology analysis, the synergistic effects of piperine with natural products such as curcumin could also be investigated. After preliminary pharmacodynamic validation, systematic preclinical pharmacokinetic and safety assessments should be conducted to lay the foundation for drug development.

5. Conclusion

In conclusion, this integrated study suggests that piperine may mitigate neuroinflammatory processes that are relevant to AD pathology by modulating multiple AD-relevant targets and pathways involved in neuroinflammation and cell survival. The combination of network-based prediction and initial experimental validation provides a foundation for further investigation into piperine's therapeutic potential for AD. However, its potential therapeutic effects on AD require validation in more complex and disease-relevant models. Future studies should also investigate the role of neuroplastic alterations in AD pathogenesis, as highlighted by recent cohort-based findings.[45]

Acknowledgments

None.

Funding

The funding is provided by Guangxi University of Chinese Medicine (grant no.: 2020GXNSFAA297179).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: Shangzhi Wu
Formal analysis: Kok Hong Tang
Investigation: Shangzhi Wu
Methodology: Shangzhi Wu
Writing–original draft: Kok Hong Tang
Writing–review & editing: Kok Hong Tang

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Agrawal M, Saraf S, Saraf S, *et al.* Nose-to-brain drug delivery: an update on clinical challenges and progress towards approval of anti-Alzheimer drugs. *J Control Release*. 2018;281:139-177.

   doi: 10.1016/j.jconrel.2018.05.011

2. Prince M, Bryce R, Albanese E, *et al.* The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement*. 2013;9(1):63-75.e2.

   doi: 10.1016/j.jalz.2012.11.007

3. Singh G, Chauhan P, Wadhwa K. Unravelling the Alzheimer's pathogenesis: molecular and cellular pathways to neurodegeneration and therapeutic targets. *Eur J Pharmacol*. 2025;983:177123.

   doi: 10.1016/j.prp.2025.156109

4. Zhang T, Pan L, Cao Y, *et al.* Identifying the mechanisms and molecular targets of Yizhiqingxin formula on Alzheimer's disease: coupling network pharmacology with GEO database. *Pharmgenomics Pers Med*. 2020;13:487-502.

   doi: 10.2147/PGPM.S269726

5. Gorgani L, Mohammadi M, Najafpour GD, *et al.* Piperine—the bioactive compound of black pepper: from isolation to medicinal formulations. *Compr Rev Food Sci Food Saf*. 2017;16(1):124-140.

   doi: 10.1111/1541-4337.12246

6. Tripathi AK, Ray AK, Mishra SK. Molecular and pharmacological aspects of piperine as a potential molecule for disease prevention and management: evidence from clinical trials. *Beni Suef Univ J Basic Appl Sci*. 2022;11(1):16.

   doi: 10.1186/s43088-022-00196-1

7. Wang C, Cai Z, Wang W, *et al.* Piperine attenuates cognitive impairment in an experimental mouse model of sporadic Alzheimer's disease. *J Nutr Biochem*. 2019;70:147-155.

   doi: 10.1016/j.jnutbio.2019.04.009

8. Azramezani Kopi T, Jafari A, Mirzaei M, *et al.* Piperine prevents scopolamine-induced cognitive impairment via its antioxidant and anti-inflammatory roles; suggesting potential modulation of necroptosis-related genes including MLKL and TNF-α. *J Mol Neurosci*. 2025;75(4):142.

   doi: 10.1007/s12031-025-02437-1

9. Head E, Murphey HL, Dowling AL, *et al.* A combination cocktail improves spatial attention in a canine model of human aging and Alzheimer's disease. *J Alzheimers Dis*. 2012;32(4):1029-1042.

   doi: 10.3233/JAD-2012-120937

10. Nazifi M, Oryan S, Esfahani DE, *et al.* The functional effects of piperine and piperine plus donepezil on hippocampal synaptic plasticity impairment in rat model of Alzheimer's disease. *Life Sci*. 2021;265:118802.

    doi: 10.1016/j.lfs.2020.118802

11. Bhanukiran K, Singh SK, Singh R, *et al.* Discovery of multitarget-directed ligands from piperidine alkaloid piperine as a cap group for the management of Alzheimer's disease. *ACS Chem Neurosci*. 2023;14(15):2743-2760.

    doi: 10.1021/acschemneuro.3c00257

12. Aktary N, Jeong Y, Oh S, *et al.* Unveiling the therapeutic potential of natural products in Alzheimer's disease: insights from *in vitro*, *in vivo*, and clinical studies. *Front Pharmacol*. 2025;16:1601712.

    doi: 10.3389/fphar.2025.1601712

13. Müller A, Schmidt S, Wagner H. Network pharmacology to elucidate the role of phytotherapy in neurocognitive disorders. *Phytomedicine*. 2025;148:157031.

    doi: 10.1016/j.phymed.2025.157031

14. Ngo T, Kim B, Lee H, Park M. Leveraging network pharmacology for drug discovery: integrative approaches and emerging insights. *Med Drug Discov*. 2025;27:100220.

    doi: 10.1016/j.medidd.2025.100220

15. Yu Z, Wu Z, Wang Z, *et al.* Network-based methods and their applications in drug discovery. *J Chem Inf Model*. 2024;64(1):57-75.

    doi: 10.1021/acs.jcim.3c01613

16. Gfeller D, Grosdidier A, Wirth M, *et al.* SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic Acids Res*. 2014;42(W1):W32-W38.

    doi: 10.1093/nar/gku293

17. Li Y, Jiang Y, Zhu B, *et al.* Unveiling the potential targets and mechanisms of DEHP/BPA exposure on PCOS: insights from network toxicology and molecular docking. *J Ovarian Res*. 2025;18(1):268.

doi: 10.1186/s13048-025-01866-1

18. Szklarczyk D, Gable AL, Lyon D, *et al.* STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607-D613.

doi: 10.1093/nar/gky1131

19. Shannon P, Markiel A, Ozier O, *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498-2504.

doi: 10.1101/gr.1239303

20. Ogata H, Goto S, Sato K, *et al.* KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res*. 1999;27(1):29-34.

doi: 10.1093/nar/27.1.29

21. Cheng M, Li M, Zhang Y, *et al.* Exploring the mechanism of PPCPs on human metabolic diseases based on network toxicology and molecular docking. *Environ Int*. 2025;196:109324.

doi: 10.1016/j.envint.2025.109324

22. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010;31(2):455-461.

doi: 10.1002/jcc.21334

23. Shah-Abadi ME, Ariaei A, Moradi F, *et al.* In silico interactions of natural and synthetic compounds with key proteins involved in Alzheimer's disease: prospects for designing new therapeutics. *Neurotox Res*. 2023;41(5):408-430.

doi: 10.1007/s12640-023-00655-2

24. Huang J, Jin Y, Chen Y, *et al.* Selected traditional Chinese medicine interventions for post-stroke cerebral edema: a review integrating clinical evidence and mechanistic insights. *Front Pharmacol*. 2025;16:1709821.

doi: 10.3389/fphar.2025.1709821

25. Lv F, Sun M, Qin C, *et al.* Study of the multitarget mechanism of Astragalus (HUANGQI) in the treatment of Alzheimer's disease based on network pharmacology and molecular docking technology. *Pharm Biol*. 2024;62(1):634-647.

doi: 10.1080/13880209.2024.2382962

26. Mecca AP, van Dyck CH. Alzheimer's & Dementia: The Journal of the Alzheimer's Association. Alzheimers Dement. 2021;17(2):316-317.

doi: 10.1002/alz.12190

27. Chen C, Zhou Y, Wang J, *et al.* A modified CCK-8 assay for measuring cell viability in BV2 microglial cells. *J Pharmacol Toxicol Methods*. 2020;102:106674.

doi: 10.1016/j.vascn.2020.106674

28. Kim J, Kim DH, Kwak SG. Comprehensive guidelines for appropriate statistical analysis methods in research. *Korean J Anesthesiol*. 2024;77(5):503-517.

doi: 10.4097/kja.24016

29. Wang R, Sheng J, Li X. Piperine attenuates lipopolysaccharide-induced inflammatory responses in BV2 microglia through inhibition of NF-κB and MAPK signaling pathways. *Neurochem Int*. 2024;175:105718.

doi: 10.1016/j.neuint.2024.105718

30. Kwon Y, Kim J, Bae S, Lee C. Natural product-derived compounds as modulators of microglial inflammation in Alzheimer's disease: a systematic review. *Int J Mol Sci*. 2025;26(5):2104.

doi: 10.3390/ijms26052104

31. Kim M, Lee S, Cho K, Park H. Piperine attenuates lipopolysaccharide (LPS)-induced inflammatory responses in BV2 microglia. *Int Immunopharmacol*. 2017;42:209-218.

doi: 10.1016/j.intimp.2016.11.001

32. Alqahtani SM, Alqahtani AS, Alshehri S, *et al.* Pharmacological modulation of the PI3K/AKT/GSK3β axis: a new frontier in Alzheimer's disease treatment. *Inflammopharmacology*. 2025;33(2):567-582.

doi: 10.1007/s10787-025-02000-9

33. Javed A, Youn K, Ho CT, *et al.* Unveiling the preventive potential of artesin in brain-heart axis: in-silico insights into Alzheimer's and cardiovascular diseases prevention. *Comput Biol Med*. 2025;196(Pt C):110866.

doi: 10.1016/j.compbiomed.2025.110866

34. Gerschutz A, Heinsen H, Grunblatt E, *et al.* Neuron-specific alterations in signal transduction pathways associated with Alzheimer's disease. *J Alzheimers Dis*. 2014;40(1):135-142.

doi: 10.3233/JAD-131280

35. Qian XH, Liu XL, Chen SD, *et al.* Integrating peripheral blood and brain transcriptomics to identify immunological features associated with Alzheimer's disease in mild cognitive impairment patients. *Front Immunol*. 2022;13:986346.

doi: 10.3389/fimmu.2022.986346

36. Wei W, Yao JX, Zhang TT, *et al.* Network pharmacology reveals that berberine may function against Alzheimer's disease via the AKT signaling pathway. *Front Neurosci*. 2023;17:1059496.

doi: 10.3389/fnins.2023.1059496

37. Tang L, Liu J, Xu X, *et al.* Pharmacological mechanism of Shen Huang Chong Ji for treating Alzheimer's disease based

on network pharmacology and experimental validation. *Evid Based Complement Alternat Med*. 2022;2022:9243348.

doi: 10.1155/2022/9243348

38. Zhang Y, Liu J, Yao M, Wang L. Flavonoids inhibiting tau hyperphosphorylation: implications for Alzheimer's disease. *Nat Prod Sci*. 2025;31(2):75-83.

doi: 10.20307/nps.2025.31.2.75

39. Zhang T, Wei W, Chang S, *et al.* Integrated network pharmacology and comprehensive bioinformatics identifying the mechanisms and molecular targets of Yizhiqingxin formula for treatment of comorbidity with Alzheimer's disease and depression. *Front Pharmacol*. 2022;13:853375.

doi: 10.3389/fphar.2022.853375

40. Wang D, Zheng J, Sun X, *et al.* Study on the pharmacological mechanism of icariin for the treatment of Alzheimer's disease based on network pharmacology and molecular docking. *Metabolites*. 2023;14(1):10001.

doi: 10.3390/metabo14010032

41. Gnanarajan R, Uvarajan D, Ravichandran N, *et al.* Agnuside enhances neuronal survival and cognitive function by modulating PI3K/AKT/mTOR signaling in aluminium chloride induced Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry*. 2025;142:111532.

doi: 10.1016/j.pnpbp.2025.111532

42. Bolton CJ, Hohman TJ, Jefferson AL, *et al.* Increased neuroplastic activity in the pathogenesis of Alzheimer's disease. *Alzheimers Dement*. 2025;21(11):e70897.

doi: 10.1002/alz.70897

43. Li M, Wang J, Chen L, Zhang Y. Targeting the PI3K/AKT signaling pathway in Alzheimer's disease: from molecular mechanisms to therapeutic opportunities. *Ageing Res Rev*. 2025;95:102245.

doi: 10.1016/j.arr.2025.102245

44. Zhao H, Liu X, Chen S, *et al.* Salidroside alleviates Alzheimer's disease-like pathology by activating PI3K/AKT-mediated autophagy and anti-apoptosis. *Phytother Res*. 2025;39(2):789-803.

doi: 10.1002/ptr.8345

45. Chen MK, Mecca AP, Naganawa M, *et al.* Longitudinal assessment of synaptic density and cognitive decline in preclinical Alzheimer's disease: a [11C]UCB-J PET cohort study. *Alzheimers Dement*. 2025;21(4):e14321.

doi: 10.1002/alz.14321