

Icariin, an Up-and-Coming Bioactive Compound Against Neurological Diseases: Network Pharmacology-Based Study and Literature Review

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Abstract: Icariin is a biologically active substance in *Epimedium herba* that is used for the treatment of neurologic disorders. However, a comprehensive analysis of the molecular mechanisms of icariin is lacking. In this review, we present a brief history of the use of icariin for medicinal purposes; describe the active chemical components of *Epimedium herba*; and examine the evidence from experimental studies that have uncovered molecular targets of icariin in different diseases. We also constructed a protein-protein interaction network and carried out Gene Ontology and Kyoto Encyclopedia of Genes and Genomes functional enrichment analyses to predict the therapeutic actions of icariin in nervous system diseases including Alzheimer disease, Parkinson disease, ischemic stroke, depressive disorder, multiple sclerosis, glioblastoma, and hereditary spastic paraplegias. The results of our analyses can guide future studies on the application of icariin to the treatment of neurologic disorders.

Keywords: *Epimedium herba*, icariin, network pharmacology, literature review, nervous system diseases

Introduction

Neurologic disorders are the leading cause of disability and the second leading cause of death worldwide.¹ Degenerative, inflammatory, infectious, vascular, and neoplastic disorders of the central nervous system (CNS) are among the most severe diseases in humans; and cerebrovascular diseases such as stroke are a considerable burden and challenge for individuals and for society. Unhealthy lifestyle habits including alcoholism, smoking, and a high-fat diet, as well as other factors such as aging, psychosocial stress, and environmental pollution contribute to the occurrence and development of stroke.² Dementia accounts for 10% of neurologic disorders.³ Alzheimer disease (AD), the most common form of dementia, is a major strain on healthcare systems because of its chronic and debilitating nature.⁴ Different medications are used to treat neurologic diseases such as stroke and AD. For example, alteplase, nimodipine, and aspirin are conventional therapeutics for cerebrovascular diseases.^{5,6} Alteplase, a recombinant tissue plasminogen activator, has demonstrated benefits in the treatment of severe ischemic stroke; however, it can also delay reperfusion and increases the risk of intracranial hemorrhage.⁷ Rivastigmine is often used to alleviate the symptoms of AD but its side effects such as nausea, diarrhea, vomiting, and dizziness limit its clinical use.⁸

Recently, active compounds of herbal medicines have attracted interest in the medical community because of their pharmacologic activity combined with low toxicity. For example, the sesquiterpene lactone artemisinin is a natural product used to treat malaria.⁹ Herbal medicines have also shown benefits in the treatment of nervous system diseases. As an ancient natural aphrodisiac, *Epimedii herba* is commonly used in Chinese and Korean traditional medicine to treat nocturnal emissions, impotence, limb weakness, muscle contracture, lethargy, and headache.^{10,11} Natural products typically act via multiple targets and pathways.¹² Icariin, the main chemical component of *Epimedii herba* is transported in the circulation and can penetrate the blood–brain barrier (BBB) to exert effects on the CNS.^{13–17} However, the pharmacologic properties of icariin and the molecular basis for its effects on nervous system diseases are not fully understood.

Network pharmacology is a relative novel approach for systematically and comprehensively investigating the mechanism of action of drugs^{18,19} that can be applied to natural products including icariin.²⁰ The computational power of this approach can also facilitate the design of studies to experimentally validate the role of icariin in various diseases in a more efficient manner than by trial and error.²¹ In this review, we present a brief history of the use of icariin for medicinal purposes; describe the active chemical components of *Epimedii herba*; and examine experimental evidence from studies that have uncovered the molecular targets of icariin in different CNS diseases. We also carried out functional enrichment analyses to predict the mechanisms of action icariin in the treatment of these diseases. The results of these analyses, and the evidence from the literature presented herein, can guide future studies on the application of icariin to the treatment of neurologic disorders.

History

Epimedii herba

Epimedii herba is the dried leaf of epimedium—an herbaceous plant belonging to the Berberidaceae family²²—and is commonly referred to as horny goat weed, Xian-Ling-Pi, Gang-Qian, and San-Zhi-Jiu-Ye-Cao. *Epimedii herba* is widely distributed across eastern, southern, and central Asia and Europe²³ and has an over 2000-year history of clinical application in countries such as China, South Korea, and Japan. *Epimedii herba* was initially documented in Sheng Nong Ben Cao Jing, the oldest classical text on medicinal

plants in China. Ben Cao Gang Mu, another important compendium of traditional Chinese medicine, describes *Epimedii herba* as pungent, cold, and nontoxic. Since 1963, *Epimedii herba* has been officially listed in the pharmacopeia of the People's Republic of China as a treatment for chronic conditions such as hemiparesis and erectile dysfunction.^{9,24} The medicinal benefits of *Epimedii herba* include strengthening the body, improving fertility, and relieving stress and fatigue. Practitioners of traditional Chinese medicine use *Epimedii herba* as a remedy for kidney-Yang deficiency syndrome, which is characterized by soreness or a cold sensation in the loins and knee, impotence, seminal emission, female sterility, difficulty in urination, or general edema. According to the Chromatographic Fingerprint Analysis of Herbal Medicines, *Epimedii herba* can be used to treat neurasthenia, climacteric hypertension, chronic bronchitis, viral myocarditis, and leucopenia.²⁵ More recently, the therapeutic effects of *Epimedii herba* on the reproductive²⁶ and skeletal²⁷ system have been experimentally validated. *Epimedii herba* also has demonstrated neuroplasticity-promoting¹⁰ and antioxidant²⁸ effects.

Icariin

Icariin is a well-characterized component of *Epimedii herba* with multiple potent biological activities. Icariin was first isolated and identified in 1990 from the oral liquid of Luohan Jindan by column partition chromatography–thin layer chromatography–ultraviolet spectroscopy.²⁹ A high-speed countercurrent chromatography technique was later used to extract icariin from epimedium with >98% purity.³⁰ Microwave pretreatment of raw materials significantly improved the efficiency of icariin extraction.³¹ Icariin was reported to function as a regulator of adaptive immunity in 1995,³² and subsequent studies have revealed antihepatotoxic³³ and antioxidant³⁴ activities as well as the ability to stimulate corticosterone production³⁵ and promote neurite outgrowth.³⁶ Icariin has pharmacologic effects on the immune,³⁷ skeletal³⁸ and reproductive³⁹ systems. Over the past few years, an increasing number of studies have focused on the protective effects of icariin in the nervous system diseases.

Active Chemical Compounds of *Epimedii herba*

Flavonoids

A total of 53 flavonoids have been identified in *Epimedii herba* including flavones, biflavonoids, flavanones, and

flavonoid glycosides, such as baohuoside I, 5,7,4'-trihydroxy-8,3'-diprenylflavone, ginkgetin, robinetin, apigenin, luteolin, hyperin, and icariin. Some flavonoids in *Epimedium herba* have demonstrated medicinal benefits.⁴⁰ Baohuoside I was shown to alleviate cognitive dysfunction and exert antiosteoporotic, and antitumor effects.⁴¹ Ginkgetin is a natural biflavonoid with anti-inflammatory, antiviral, and antitumor properties.⁴² Icariin, a flavonoid glycoside, is the main bioactive ingredient of *Epimedium herba* and is a promising therapeutic agent for the treatment of various disorders because of its antiapoptotic, anti-inflammatory, and antioxidant activities.^{43,44}

Terpenoids

Fifteen terpenoids have been isolated from *Epimedium herba* that are classified as monoterpenes, sesquiterpenes, and triterpenes. Terpenoids exert beneficial effects on bone metabolism and have anti-inflammatory, neuroprotective, and antioxidant functions.^{45,46} Oleanolic acid (OA), a pentacyclic triterpenoid, has pharmacologic effects in osteoporosis and neurodegenerative disease. For example, OA was shown to induce the differentiation of bone marrow-derived mesenchymal stem cells into osteoblasts by regulating the cell cycle and metabolism⁴⁷ or by inhibiting Notch signaling,⁴⁸ and regulated calcium balance by promoting calcium entry across the brush border membrane.⁴⁹ OA was also found to exert a neuroprotective effect by activating nuclear factor erythroid 2-related factor (Nrf)2 and inhibiting the expression of nitric oxide synthase in the hypoxic brain.⁵⁰ Given its modulatory effects on endogenous antioxidants and mitochondrial function, OA is considered as a promising agent for the treatment of cerebral ischemia.⁵¹

Other Compounds

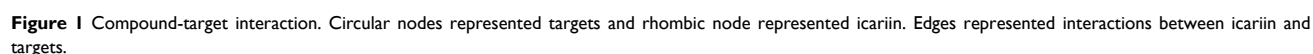
Several phytochemicals, such as steroids, acids, lignans, alkaloids, and anthraquinones have been isolated from *Epimedium herba*. Treatment with the steroid sitosterol significantly reduced the immobility time of rats in the forced swim and tail suspension tests, indicating that sitosterol has an antidepressant effect that may involve increasing 5-hydroxytryptamine and norepinephrine levels in the CNS.⁵² Lauric acid can stimulate the production of ketone bodies by astrocytes, which exert a neuroprotective effect on adjacent neurons.⁵³ Emodin is a naturally occurring anthraquinone derivative that was reported to confer neuroprotection in AD and epilepsy and alleviate ischemia-reperfusion brain injury and glutamate-induced neuronal damage.⁵⁴ The alkaloid magnoflorine increased the latency index of mice in

the passive avoidance test by inhibiting acetylcholinesterase, suggesting a role in improving short-term memory. Thus, magnoflorine is a promising candidate drug for the treatment of diseases associated with memory deficits such as dementia or AD.⁵⁵ In a rat model of global brain ischemia-reperfusion p-tyrosol prevented neuronal loss in the hippocampus.⁵⁶ Isoliquiritigenin suppressed the inflammatory response after traumatic brain injury by inhibiting the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/glycogen synthase kinase-3 beta (GSK-3 β)/nuclear factor kappa B (NF- κ B) signaling axis.⁵⁷ In summary, the evidence to date indicates that compounds found in *Epimedium herba* can improve neurologic function and mitigate nervous system damage although the molecular mechanisms underlying these effects are not well understood.

Network Pharmacology Analysis of Icariin

Screening of Potential Targets

In order to elucidate the molecular basis for the pharmacologic effects of icariin based on the existing evidence, we constructed a database of molecular targets in neurologic diseases including AD (disease ID: C0002395, n=1981), Parkinson disease (PD; disease ID: C0030567, n=1063), ischemic stroke (disease ID: C0948008, n=393), depressive disorder (disease ID: C0011581, n=740), multiple sclerosis (MS; disease ID: C0026769, n=1105), glioblastoma (disease ID: C0017636, n=1936), and spastic paraplegias (disease ID: C0037772, n=312) using the DisGeNET database v6.0 (<https://www.disgenet.org/>).^{58,59} In a previous report,⁵⁹ 219 targets of icariin were predicted using PharmMapper (<http://lilab-ecust.cn/pharmmapper/>), Drug Repositioning and Adverse Reaction via Chemical-Protein Interactome (<https://cpi.bio-x.cn/drar/>), TargetNet (<http://targetnet.scbdd.com/>), and ChemMapper (<http://lilab-ecust.cn/chemmapper/>) after eliminating duplicates (Figure 1). The intersection of each disease with icariin identified 72 targets in AD, 33 in PD, 15 in ischemic stroke, 32 in depressive disorder, 29 in MS, 73 in glioblastoma and 2 in spastic paraplegia (Supplementary Table S1); all of these were standardized according to their gene names by searching the UniProtKB (<https://www.UniProt.org/>) database with "Homo sapiens" as the species.⁶⁰ Compound-target interactions were determined using Cytoscape v3.7.2 software (<https://cytoscape.org/>) and are shown in Figure 1.



As proteins involved in biochemical processes form macromolecular complexes to execute biological functions, exploring the interaction of icariin with different proteins and their networks is important for characterizing its pharmacologic activities. We constructed a PPI network as described in our previous work.¹⁹ All 219 putative targets of icariin were entered into the Search Tool for the Retrieval of Interacting Genes/Proteins (v

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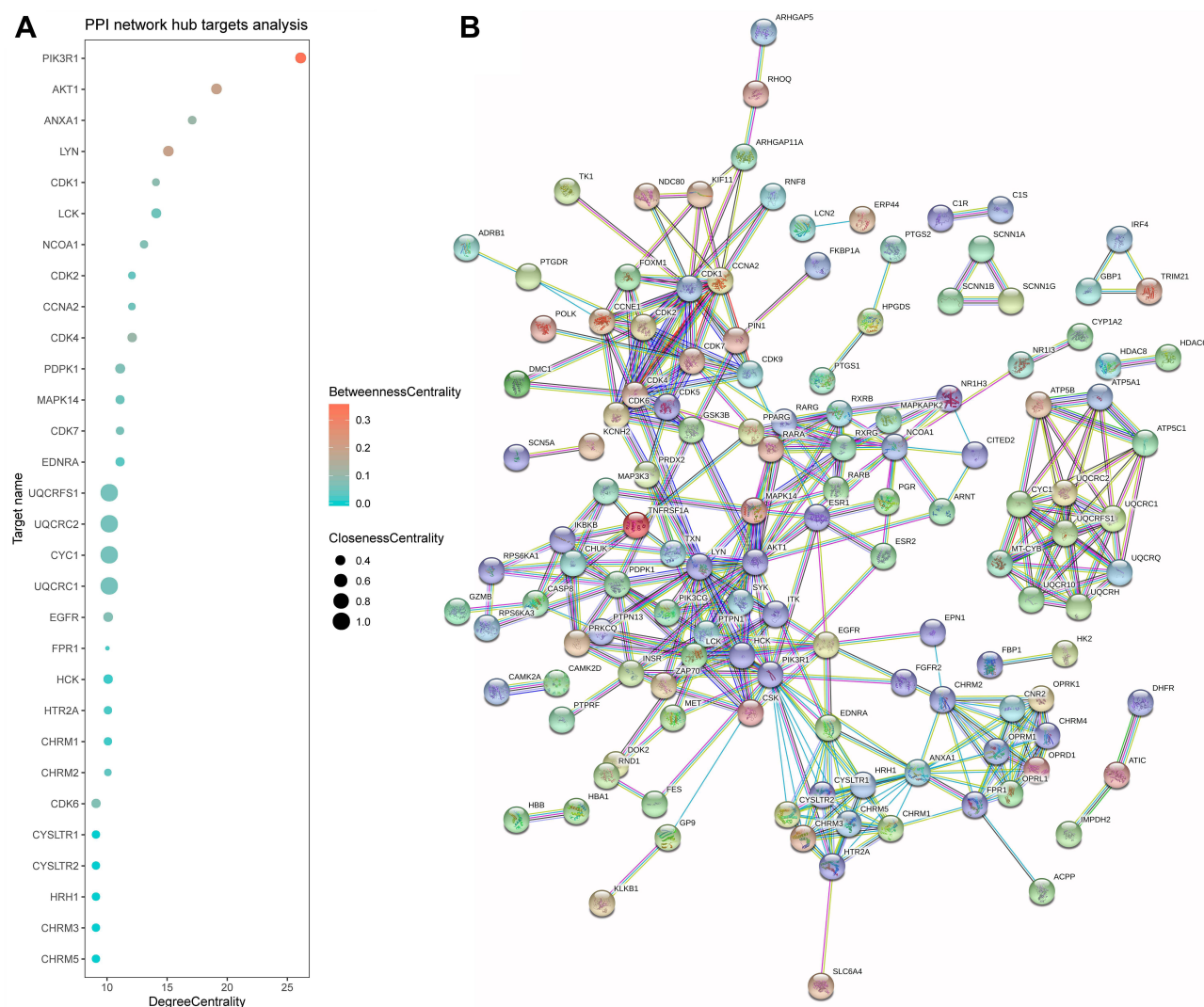


Figure 2 The PPI network for icariin and hub target analysis. **(A)** The centrality of targets was evaluated according to the degree centrality, betweenness centrality, and closeness centrality, which exhibited variation in terms of the x-axis values, the colors of nodes, and the sizes of nodes. **(B)** The nodes indicated proteins, and edges represented protein-protein associations. The cyan edge indicated that the interaction was from a curated database, and the purple edge was experimentally determined. The green, red, and blue edges represented the interactions that were judged from the gene neighborhood, fusions, and cooccurrence, respectively.

average betweenness centrality was 0.435. Based on a critical degree value ≥ 9 , 30 targets were selected as hubs in the network (Figure 2A). The value of hub proteins was assessed based on degree, betweenness, and closeness centrality. The role of these targets in different diseases is discussed in detail below.

Gene Ontology (GO) Analysis

GO is an international standardized system for classifying gene function in 3 categories—namely, cellular component, molecular function, and biological process.⁶² The biological process category best reflects changes in the biological functions of the body. We first mapped all 219 targets of icariin to the GO biological process ontology in

the GO database (<http://www.geneontology.org/>; Figure 3, and Supplementary Table S3). Gene numbers were calculated for every GO term, and the significance of enrichment relative to the background genome was evaluated with the hypergeometric test and subjected to false discovery rate (FDR) correction. $FDR \leq 0.05$ was set as the threshold for significant enrichment. The data were collected using the ClueGO and CluePedia Cytoscape plug-ins.^{63–65}

The effects of icariin on biological processes were mainly related to the following functional groups: purine-containing compound metabolic process, cellular response to oxygen-containing compound, cellular response to reactive oxygen species (ROS), adenylate

cyclase-inhibiting G protein-coupled receptor signaling pathway, regulation of organ growth, response to drug, and peptidyl-serine phosphorylation (Figure 3C). We observed that terms related to the regulation of nervous system function showed a higher level of enrichment; these included sensory perception of pain, opioid receptor pathway, opioid receptor activity, serotonin receptor signaling pathway, and G protein-coupled serotonin receptor signaling pathway. Additionally, there were several biological processes related to lipid metabolism, response to ROS, apoptosis-related signaling, regulation of immune cells, and cell cycle (Figure 3A and B). These results indicate that icariin acts on genes that are involved in nervous system function.

To clarify the effects of icariin on angiogenesis, we identified significantly enriched GO terms related to this process;⁶⁶ the terms were depicted in visual form using R v3.6.1 software (<http://www.r-project.org>) with the clusterProfiler and ggplot2 packages (Figure 4).

Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis

KEGG pathway analysis (<http://www.genome.jp/kegg/>) can provide additional insight into the biological functions of genes.⁶⁷ The KEGG pathway analysis of icariin targets revealed significant enrichment of metabolic or signal transduction pathways; the calculated p-values were subjected to FDR correction based on a threshold value of $p \leq 0.05$, and the data were collected using the ClueGO and CluePedia plugins.^{68,69} Target genes that were common to both the disease and icariin were also subjected to KEGG pathway analysis. No results were obtained for ischemic stroke and MS because of the limited number of common targets and stringent criterion for significance. The KEGG pathway enrichment results of AD and glioblastoma are shown in Figures 5 and 6, respectively, with a filtering cutoff of ≥ 40 ; results that are not shown in the figures can be found in [Supplementary Table S2](#). The clusterProfiler package of R software available on Bioconductor (<https://www.bioconductor.org/>) was used to generate KEGG annotation graphs (Figure 6A).^{70,71}

Therapeutic Mechanisms of Icariin in Nervous System Diseases: Literature Review and Network Pharmacology Analysis

Potential Targets of Icariin in Neurologic Diseases

Some targets of icariin were found to play a key role in the pathogenesis or treatment of nervous system diseases. For example, icariin may regulate GSK-3 β , a constitutively active serine/threonine-protein kinase that has been linked to the pathophysiology of AD, PD, and mood disorders (eg, depressive disorder),^{72–74} and was identified as a hub in the PPI network of icariin (Figure 7). Abnormal activation of GSK-3 β has been demonstrated to accelerate the AD pathology process in AD patients.⁷⁵ Amyloid (A) β and amyloid precursor protein (APP) as well as hyperphosphorylated tau protein are involved in AD pathogenesis.⁷⁶ GSK-3 β regulates A β production by interfering with APP cleavage,⁷⁷ while GSK-3 β inhibition decreased β -secretase (BACE1)-mediated cleavage of APP via a mechanism involving nuclear factor (NF)- κ B signaling, thereby alleviating A β pathology.⁷⁸ GSK-3 β controls numerous signaling pathways in the brain that promote tau hyperphosphorylation and neuronal degeneration, and interfere with normal synaptic plasticity.⁷⁹ Interestingly, AD inclusions have also been observed in the PD brain.⁷² In PD patients, hyperphosphorylated tau tends to aggregate in the substantia nigra in addition to other brain regions.^{80,81} Increased GSK-3 β activity was correlated with the presence of hyperphosphorylated tau aggregates, suggesting that GSK-3 β is responsible for tau phosphorylation in PD as in AD.⁷² GSK-3 β stabilization is the gold standard for pharmacologic treatment of mood disorders.⁸² The decreased phosphorylation of GSK-3 β in platelets of patients treated for depression supports the notion that GSK-3 β contributes to the pathophysiology of depressive disorder.⁷⁴ In fact, inhibiting GSK-3 β activity was shown to impact the efficacy of antidepressant therapy.^{73,74} GSK-3 β also plays a critical role in glioblastoma tumorigenesis through phosphorylation of lysine demethylase (KDM)1A.⁸³ Inflammation is also regulated by GSK-3 β in neurodegenerative disorders: GSK-3 β

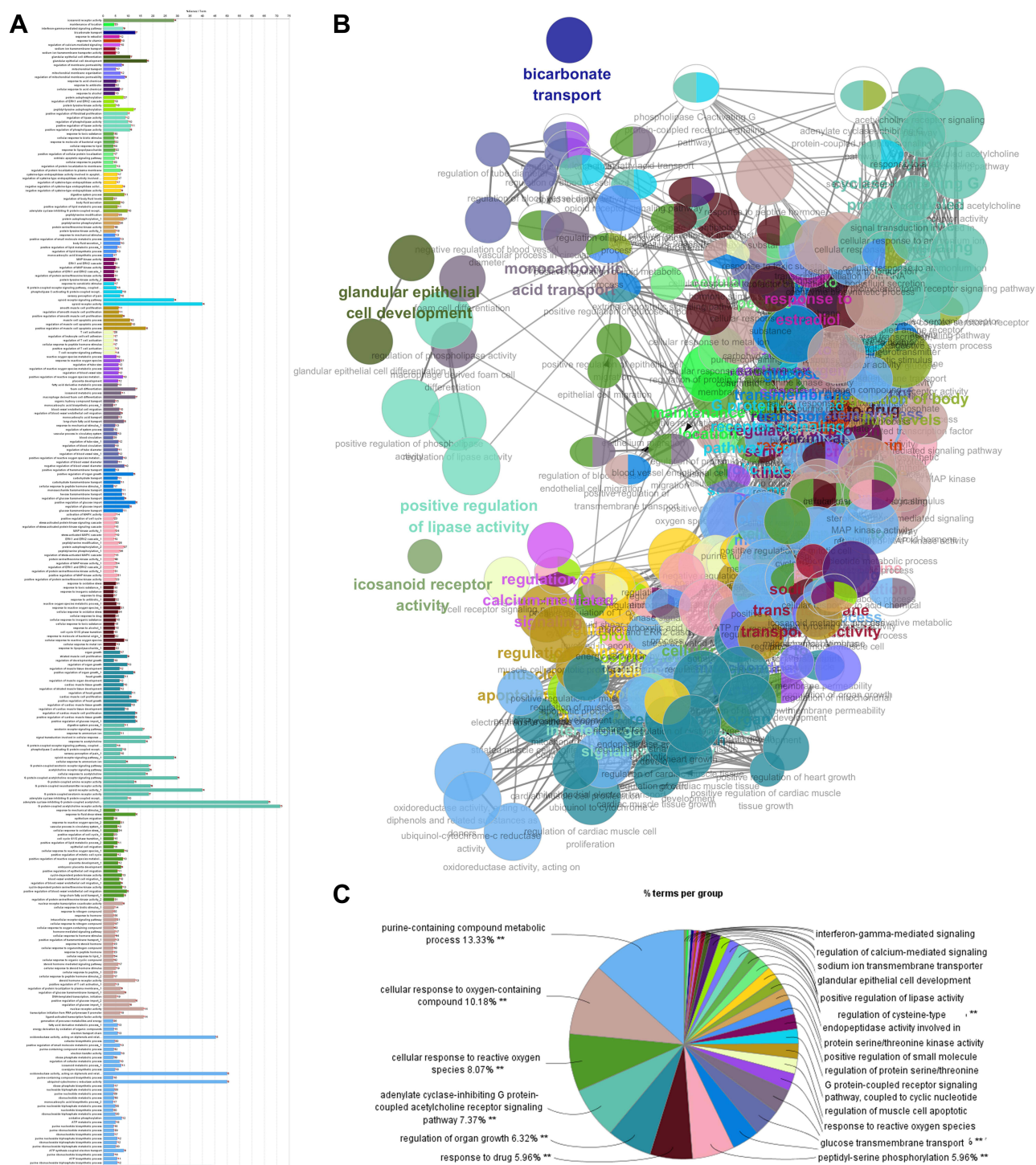


Figure 3 The target genes of icariin are mapped for the biological process terms during GO enrichment analysis by utilizing Cytoscape equipped with the ClueGO and CluePedia plugins. **(A)** The bars show the percentage of genes in GO terms. **(B)** Each node represents a GO term, and its size represents the significance. An edge indicates the existence of common genes; a finer line indicates a smaller overlap. **(C)** Different functional groups of GO terms were reflected by different node colors and are shown in the pie chart. **Means $p < 0.01$.

overexpression stimulated the production of specific cytokines in the brain and created a proinflammatory environment that was detrimental to immature neurons.^{84–86} As icariin inhibits GSK-3 β in part through activation of the

PI3K/AKT signaling pathway,⁷⁶ we speculate that icariin may improve AD, PD, depressive disorder, and glioblastoma by alleviating A β pathology as well as neuroinflammation via inhibition of GSK-3 β .

Enrichment of GO Terms that Associated with Angiogenesis

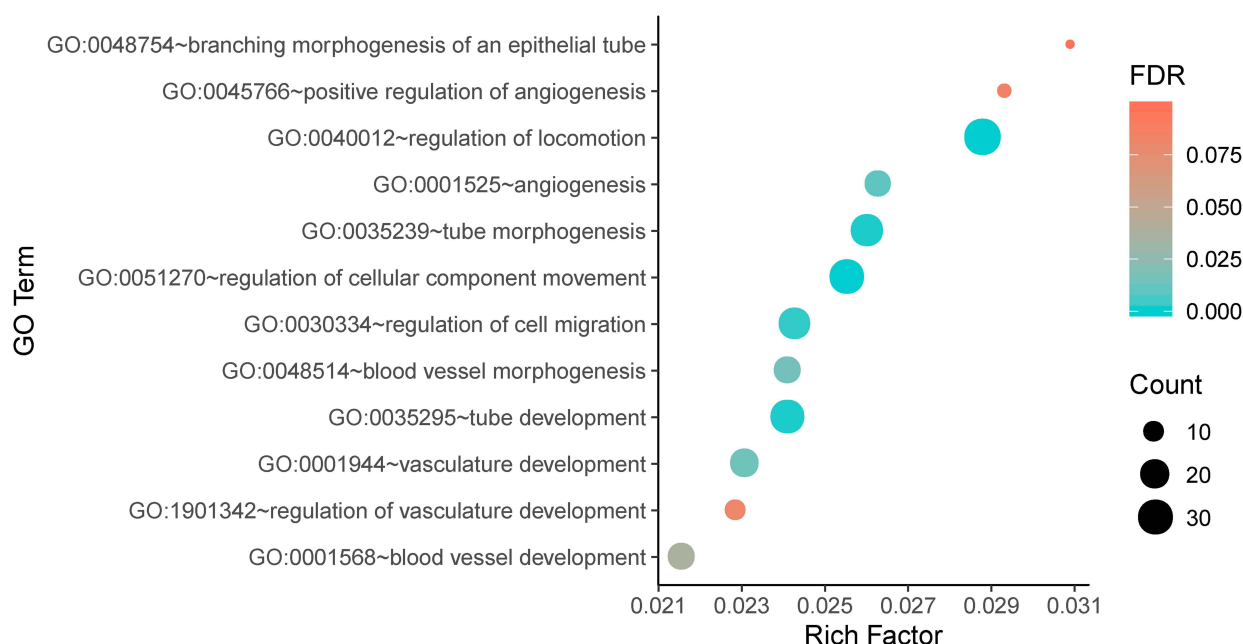


Figure 4 The bubble diagram shows significantly enriched biological process terms that pertain to angiogenesis processes. The biological process terms were shown on the y-axis, the rich factor of the annotated genes was presented on the x-axis, the size of term represented the gene number, and the color indicated the FDR value.

AD

AD is an irreversible neurodegenerative disease that is characterized by progressive deterioration of cognitive function and memory. Various factors have been implicated in the etiology of AD, such as abnormal protein aggregation, oxidative stress, dysregulation of calcium homeostasis, neuron and synapse degeneration, and neuroinflammation.^{76,87} Icarin may alleviate AD symptoms by regulating A β production, tau phosphorylation, oxidative stress, and calcium homeostasis.⁷⁶

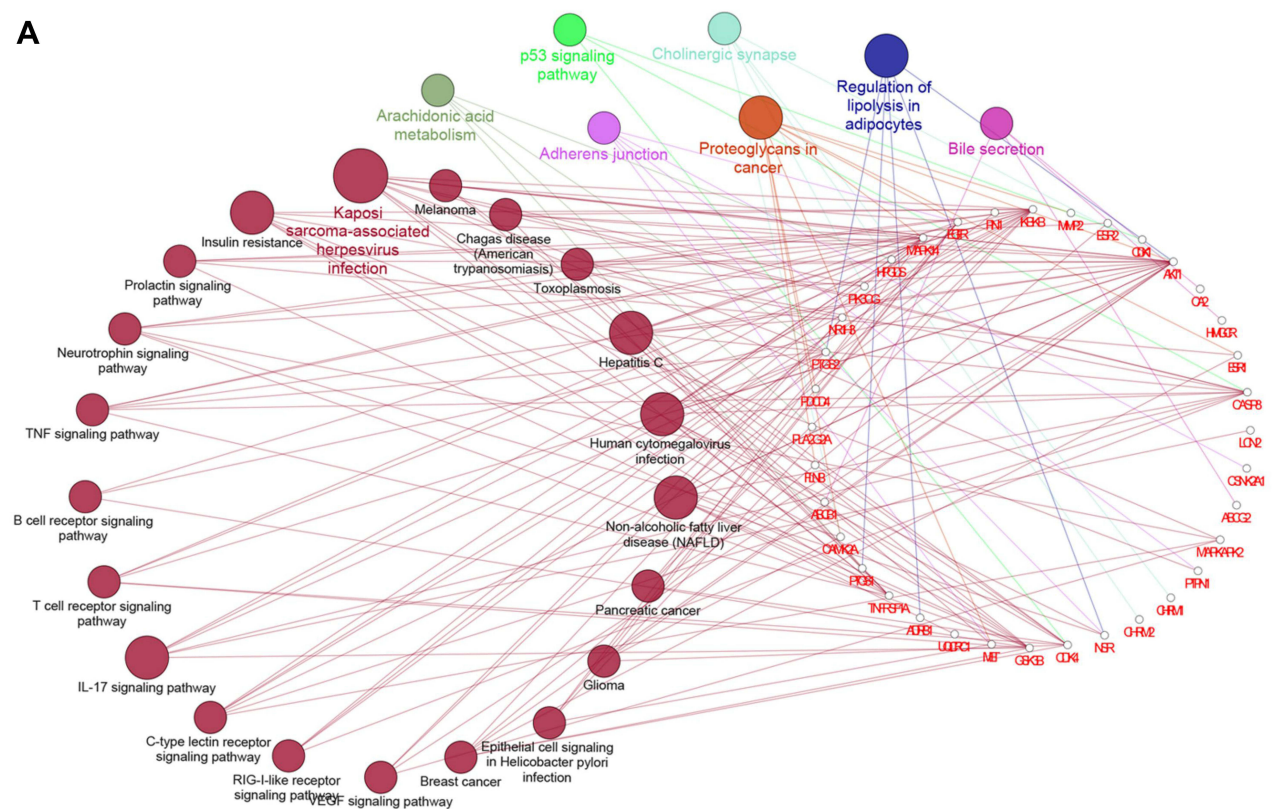
The pathogenesis of AD is associated with accumulation of A β and hyperphosphorylated tau in the brain. A β is released from APP by BACE1, membrane-bound proteases, and γ -secretase.^{88,89} Icarin reduces A β burden and deposition by inhibiting the expression of both APP and BACE1.⁸⁹ Icarin counters the negative effect of A β on synaptic plasticity via modulation of the brain-derived neurotrophic factor (BDNF)/tropomyosin receptor kinase B/AKT pathway.⁹⁰ Hyperphosphorylation alters the net charge on tau protein and the conformation of the microtubule-binding region, leading to the detachment of tau from microtubules and its accumulation in neurons and aggregation as neurofibrillary

tangles.⁹¹ Icarin mitigates AD symptoms by reducing A β and hyperphosphorylated tau levels.

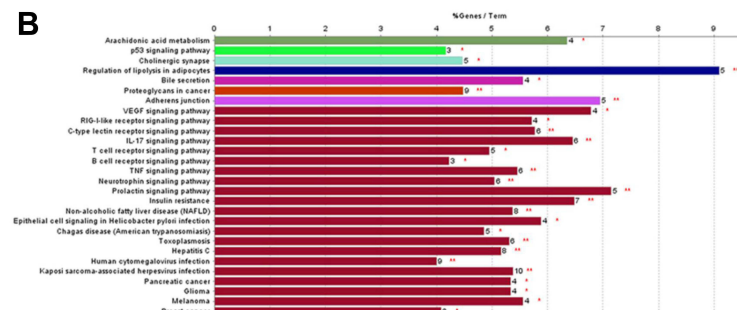
Oxidative stress is also known to contribute to the pathogenesis of AD.⁹² Icarin was shown to counteract H₂O₂-induced neurotoxicity by suppressing ROS production, and increasing the expression levels of the antioxidant enzymes catalase and peroxiredoxin (PRDX)1 via upregulation of sirtuin (SIRT)1.⁹³ In primary microglia, icaric acid attenuated lipopolysaccharide (LPS)-induced oxidative stress and reduced ROS levels in a dose-dependent manner.⁹⁴ It protected against learning and memory deficits induced by increased superoxide dismutase activity and decreased malondialdehyde level⁹⁵ and abrogated the iron overload-induced Fenton reaction and oxidative stress, thereby reducing lipid peroxidation and stimulating the activities of superoxide dismutase and glutathione peroxidase.⁹⁶ Thus, icaric acid alleviates AD symptoms by decreasing oxidative stress.

Calcium dysregulation is implicated in the progression of AD; the failure of neurons to maintain Ca²⁺ homeostasis is a common feature of aging-linked neurodegenerative pathologies.⁸⁷ Icarin was found to attenuate

A



B



C

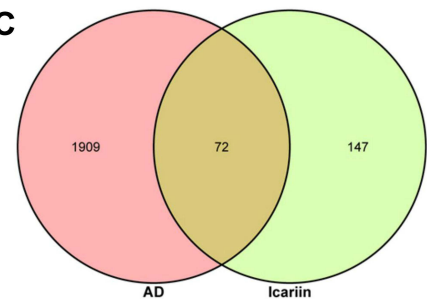


Figure 5 KEGG enrichment analysis and pathway mapping. (A) The functionally grouped network of enriched categories was generated for the target genes using ClueGO and CluePedia. Pathway terms were represented as nodes, the node colour represented the grouping information, and the node size represented the significance of the enrichment of the term. (B) The bars showed the percentage of genes in pathway terms. * means $p < 0.05$, ** means $p < 0.01$. (C) The Venn diagram showed the intersection of the targets of AD and icariin.

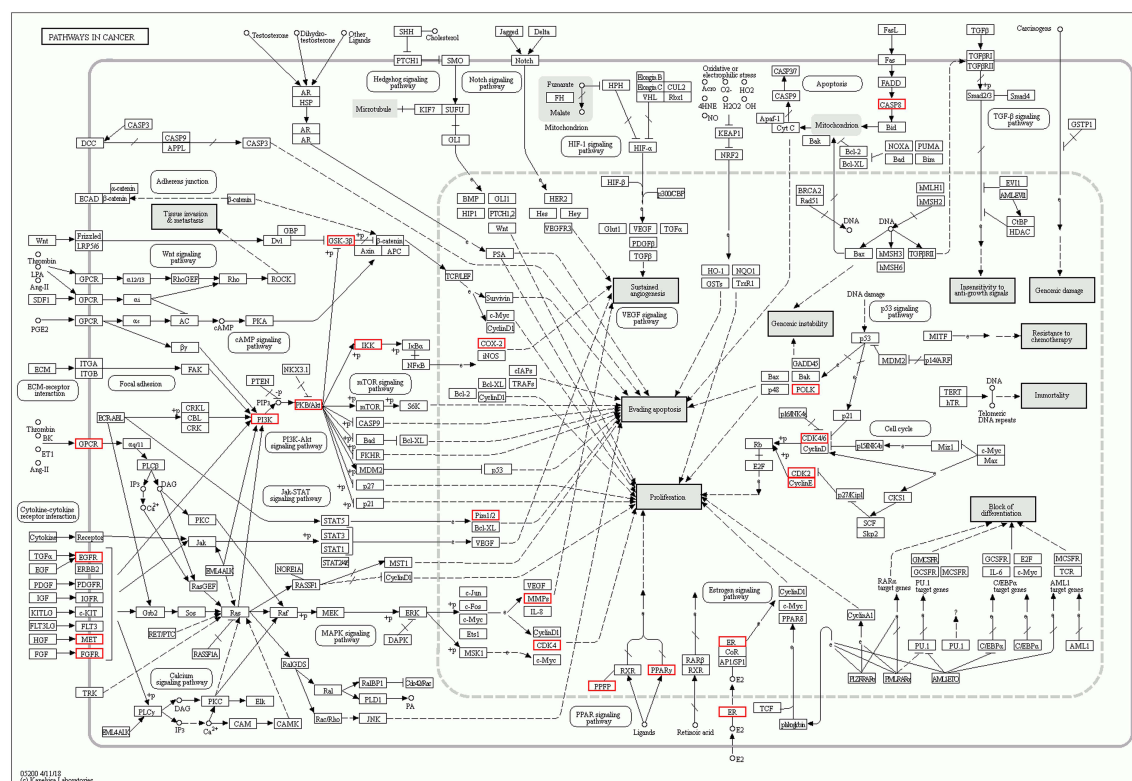
neuronal damage in a concentration-dependent manner by preventing an increase in intracellular calcium concentration.⁹⁵

We identified 72 target genes common to icariin and AD (Figure 5C). The results of the KEGG pathway enrichment analysis indicated that icariin may mitigate AD by modulating the tumor necrosis factor (TNF) signaling pathway via TNF receptor (TNFR) superfamily member (TNFRSF)1A, along with mitogen-activated protein kinase (MAPK)14, and GSK-3 β (Figure 5A and B). TNF, was linked to synaptic dysfunction in the cognitive decline

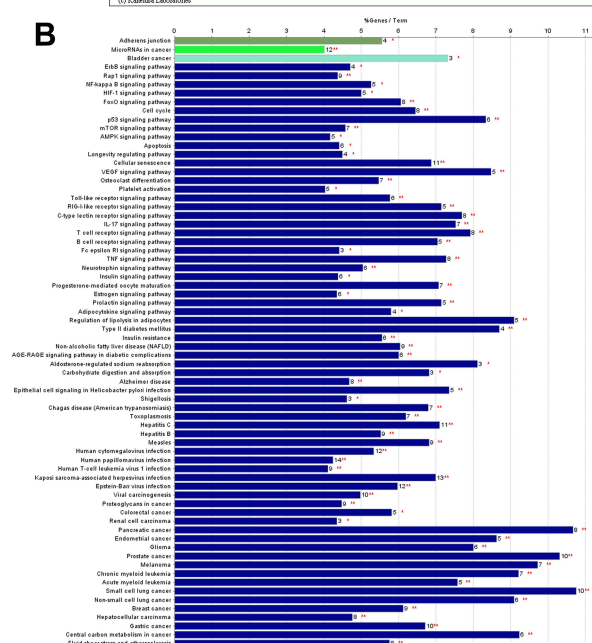
associated with AD.⁹⁷ TNFR1, was shown to mediate A β -induced neuronal cell death in AD and participated in amyloidogenesis by regulating BACE1, the enzyme that processes APP.⁹⁶ As icariin is known to block the secretion of TNF- α ,⁹⁸ we speculated that icariin inhibits the TNF signaling pathway and TNFRSF1A to prevent AD progression.

The autophagy-lysosome system plays an important role in the pathogenesis of AD.⁹⁹ Genetic deficiency of MAPK14 stimulated autophagy, leading to reduced amyloid pathology via enhanced autophagic-lysosomal

A



B



C

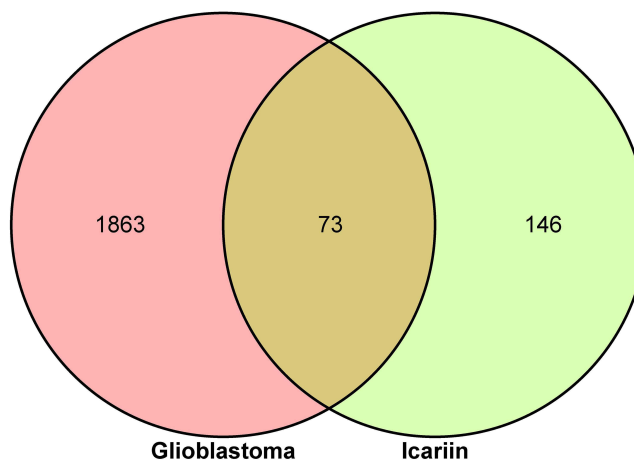
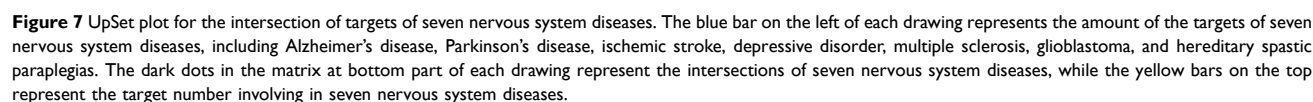


Figure 6 KEGG enrichment analysis and pathway annotation. (A) KEGG pathway map: hsa05200, Pathway in cancers, Homo sapiens (human). Red boxes marked the proteins or pathways targeted by icariin. (B) The bars showed the percentage of genes in pathway terms during KEGG enrichment analysis. *Means $p < 0.05$, **Means $p < 0.01$. (C) The Venn diagram showed the intersection of the targets of glioblastoma and icariin.

degradation of BACE1.⁹⁹ Thus, the suppression of MAPK14 activity is a potential therapeutic strategy to mitigate neurodegeneration in AD. Icariin was reported to inhibit the protein expression of MAPK14.¹⁰⁰ These

findings and the result of the KEGG pathway enrichment analysis suggest that icariin exerts a neuroprotective effect in AD by modulating the autophagy-lysosomal system through MAPK14.



PD—which is characterized by motor symptoms of tremor, bradykinesia, and postural instability—is the second most common neurodegenerative disease after AD.^{101,102} The pathologic changes in PD are generally caused by the loss of dopaminergic neurons and depletion of dopamine pools.¹⁰³

Icariin may reduce PD symptoms through multiple mechanisms. Neuroinflammation is a major pathologic event in PD.¹⁷ Nrf2 is a key regulatory factor in endogenous defense systems; activation of Nrf2 signaling inhibits the production of proinflammatory factors and reduces inflammation.^{17,104} Microglia are brain-resident immune cells that release various proinflammatory and cytotoxic

factors that can damage dopaminergic neurons, ultimately leading to PD.¹⁰⁵ Icarin was found to mitigate the proinflammatory response of microglia by suppressing NF- κ B signaling, thereby protecting dopaminergic neurons from damage.¹⁴

The KEGG pathway enrichment analysis revealed multiple modules shared between icariin and PD (Supplementary Table S2). Based on the results of the network pharmacology analysis, the therapeutic effects of icariin in PD are likely associated with RAC- α serine/threonine-protein kinase (AKT1), MAPK14, prostaglandin endoperoxide synthase (PTGS2) (also known as cyclooxygenase [COX]2), and GSK-3 β . PD is related to dysfunction of the nigrostriatal dopaminergic system.¹⁰⁶ The PI3K/AKT pathway modulates the antiapoptotic protein B cell lymphoma (Bcl)-2 in dopaminergic neurons¹⁰⁷ activated AKT promotes the transcription and posttranscriptional activation of Bcl-2. As AKT activation was reported to be correlated with the protective effect of icariin on the nigrostriatal system,¹⁰⁷ icariin may target AKT/Bcl-2 activity in PD. As mentioned earlier, neuroinflammation contributes to the development and progression of PD.¹⁰⁷ The p38 MAPK signaling pathway plays a key role in microglia activation and response that result in neuroinflammation and the degeneration of dopaminergic neurons.¹⁰⁸ The phosphorylation and activation of MAPK 14 lead to upregulation of PTGS2, a critical enzyme in PD pathogenesis,^{109,110} this induces an inflammatory response that induces dopaminergic neuron degeneration via activation of caspase 3.¹¹¹ PTGS2 overexpression also stimulates the proliferation of reactive glia, which increases collagen degradation.^{109,111} As indicated by the results of our network pharmacology analysis, icariin inhibits MAPK14 activation and PTGS2 expression^{14,112} and thus has the potential to suppress neuroinflammation and improve PD symptoms.

Ischemic Stroke

Ischemic stroke is associated with high disability and mortality and is among the leading causes of death worldwide.¹¹³ Acute brain injury in stroke is caused by a transient restriction of the blood supply in the brain, followed by perfusion and concomitant reoxygenation.¹¹⁴ Icarin may exert therapeutic effects by influencing the ischemia/reperfusion process.⁹³

ROS generation is the initial step in brain damage after cerebral ischemia/reperfusion.¹¹⁵ ROS induce oxidative stress by activating several signaling pathways and react

with and cause damage to lipids, proteins, and DNA.^{116,117} Nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP) are essential for maintaining the intracellular balance between the generation of ROS (which ensures a reductive environment for cellular activities) and neutralization (which maintains energy homeostasis).^{118,119} Icarin may protect against ischemia-related brain injury by reducing oxidative stress caused by NADPH and the NADH-induced increase in ROS levels.¹²⁰ Icarin was shown to inhibit NADPH oxidase (Nox)2^{120,121} by decreasing the half-life of the protein, thereby suppressing ROS production to alleviate cerebrovascular smooth muscle cell hyperplasia and remodeling after ischemic stroke.¹²⁰ Poststroke angiogenesis is discussed in greater detail below. The transcriptional coactivator peroxisome proliferator-activated receptor (PPAR)- γ coactivator (PGC)-1 α , which regulates the expression of genes involved in energy metabolism, is a key target of Nox.¹²² PGC-1 α is directly regulated by SIRT1 through phosphorylation and deacetylation,¹²³ Meanwhile, SIRT1 is upregulated by icariin,⁹³ which stimulates the transcriptional activity of PGC-1 α in neuron metabolism and mitigates mitochondrial dysfunction by inducing the upregulation of SIRT1 deacetylase.¹¹⁶ Additionally, icariin reduced ROS levels and brain edema following middle cerebral artery occlusion by inhibiting lactate dehydrogenase release, thereby decreasing the level of malondialdehyde and enhancing superoxide dismutase activity.^{95,124}

Inflammation contributes to the pathophysiology of AD, cerebral injury, cardiomyopathies, atherosclerosis, and stroke.^{125–128} The expression of inflammatory mediators including interleukin (IL)-1 β and transforming growth factor (TGF)- β 1 is upregulated during ischemia.¹²⁹ NF- κ B is an important transcription factor in the inflammatory response,¹³⁰ PPAR α and PPAR γ suppress inflammation by inhibiting the NF- κ B pathway that mediates the transcription of inflammatory mediators.^{131,132} In one study, icariin not only decreased the expression of IL-1 β and TGF- β 1 in a dose-dependent manner, but also acted as an agonist of PPAR α and PPAR γ and blocked NF- κ B activation to counter the effects of ischemic stroke¹³³ including neurologic dysfunction and infarction.¹³³ Additionally, energy failure during ischemic stroke triggers glutamate release,¹³⁴ glutamate excitotoxicity and acidosis cause brain injury through membrane receptor-based mechanisms and the resultant Ca²⁺ toxicity.¹³⁵ As a calcium channel blocker, icariin may abrogate this effect.^{136,137}

The results of the network pharmacology analysis suggested that icariin has therapeutic potential for the treatment of ischemic stroke ([Supplementary Table S1](#)). Disruption of the BBB plays a key role in the progression and exacerbation of brain injury following stroke.¹³⁸ Matrix metalloproteinase (MMP)-2 mediates the degradation of tight junction proteins such as occludin and claudin-5.¹³⁹ Icariin protects the BBB by reducing MMP-2 protein level,^{140,141} a mechanism that may be relevant to the treatment of ischemic stroke. Cyclin-dependent kinase (CDK)5, is known to promote ischemic injury and stroke-induced neuronal death and potentiates the excitotoxicity caused by ischemia.¹⁴² Icariin inhibits the expression of CDK2 and CDK4;¹⁴³ in our analyses, CDK5 was identified as a target of icariin, which warrants further exploration in the context of stroke treatment.

Depressive Disorder

Stress can influence the occurrence and development of depressive disorder and alter neuroendocrine function by stimulating the hypothalamic–pituitary–adrenal (HPA) axis, leading to the release of corticotropin-releasing factor (CRF) from the hypothalamus, which in turn promotes the release of adrenocorticotropin hormone and glucocorticoids such as cortisol.³⁵ In a chronic unpredictable stress model of depression, icariin exerted an antidepressant effect by decreasing serum CRF and cortisol levels and inhibiting brain monoamine oxidase A and B activities, which increased monoamine neurotransmitter levels in brain.⁴⁴ Icariin was also shown to increase glucocorticoid receptor and serotonin1A receptor (HTR1A) expression in the hippocampus and prefrontal cortex, thereby reversing chronic mild stress,¹⁴⁴ and partly reversed the chronic mild stress-induced increases in serum and glucocorticoid-inducible kinase (SGK) 1 and FK506-binding protein (FKBP) 5 levels in the hippocampus and prefrontal cortex.¹⁴⁴ Additionally, icariin reversed corticosterone-induced decreases in glucose, amino acid, and lipid metabolism.³⁵ Thus, icariin may relieve depressive disorder by reversing metabolic disturbance in the brain.

Proinflammatory cytokine levels are elevated in the brain and blood of patients with depression and aggravate depressive symptoms by increasing oxidative stress and inhibiting BDNF signaling.⁴⁴ Icariin reversed the corticosterone-induced decrease in BDNF level in the hippocampus and reduced the immobility time of rats in the forced swim test;³⁵ reduced the levels of proinflammatory factors in the hippocampus of rats under stress by inhibiting the

Nod-like receptor protein (NLRP) 3 inflammasome and caspase 1 signaling axis,¹⁴⁵ and decreased the expression of high mobility group box (HMGB) 1 protein in the hippocampus and facilitated its translocation to the nucleus via activation of Toll-like receptor (TLR)4/X-box-binding protein (XBP) 1/NF- κ B signaling.¹⁴⁶

The hyperactivation of microglia is thought to play an essential role in the pathogenesis of depressive disorder. By blocking p21-activated kinase (PAK)1/I κ B kinase (IKK)/NF- κ B and c-Jun N-terminal kinase (JNK)/p38 signaling, icariin inhibited the release and expression of proinflammatory factors such as nitric oxide, prostaglandin E-2, PTGS2, TNF- α , IL-1 β , and IL-6 in LPS-activated microglia.¹⁴⁷ In an in vivo study of rats treated with LPS, icariin alleviated memory and spatial learning impairments by increasing the expression of TNF- α , IL-1 β , and PTGS2 in the brain.¹⁴⁸ These findings provide insight into the molecular mechanisms underlying the therapeutic effects of icariin in disorders associated with neuroinflammation.

Icariin may also exert antidepressant effects through the regulation of metabolism and neurotransmission. For example, icariin decreased the levels of metabotropic glutamate receptor (mGluR) 1 and mGluR 5 and increased that of excitatory amino acid transporter (EAAT) 2 in prenatal restraint stress-induced depressive disorder,¹⁴⁹ and reversed the corticosterone-induced decreases in glucose, amino acid, and lipid metabolism.⁴⁴

The antidepressant function of icariin was supported by the results of the KEGG pathway enrichment analysis of target genes common to icariin and depressive disorder, which were mainly associated with the estrogen, MAPK14, AKT1, estrogen receptor alpha and beta (ESR1 and ESR2, respectively), and GSK-3 β signaling pathways ([Supplementary Table S2](#)). Neuronal death in the hippocampus induced by corticosterone is associated with depression;¹⁵⁰ moreover, the activation of MAPK14 plays a critical role in corticosterone-induced apoptosis whereas PI3K/AKT signaling is linked to neuronal survival.¹⁵¹ The neuroprotective effect of icariin in depression may involve blocking MAPK14 and activating the PI3K/AKT pathway.^{112,152} ESR-signaling mediates susceptibility to depression and may impact the response to antidepressants.¹⁵³ ESR1 plays an important role in depression, especially in women, and specific gene variants have been linked to severe depressive symptoms;^{154,155} meanwhile, ESR1 overexpression in the nucleus accumbens enhanced stress resilience in both

sexes.¹⁵⁶ ESR2 may promote desensitization of HTR1A signaling and thus contribute to the antidepressant response, suggesting that it could be a therapeutic target in the treatment of depressive disorder.¹⁵⁴ Icariin activates the ESR1 signaling pathway; our network pharmacology analysis revealed that its antidepressant effect may be related to the regulation of estrogen signaling.^{155,157} On the other hand, the decreased GSK-3 β phosphorylation detected in the platelets of patients treated for depression implies that GSK-3 β hyperactivation contributes to the pathophysiology of depressive disorder; this is underscored by the observation that blocking GSK-3 β activity enhanced the effects of antidepressant therapy.^{75,158} Icariin was been reported to inhibit GSK-3 β in part by activating the PI3K/AKT pathway,⁷⁶ which may be the mechanism by which it alleviates depressive symptoms.

MS

MS is an autoimmune inflammatory disease that affects the CNS, with clinical manifestations such as muscle weakness, sensory deficits, cognitive impairment, and fatigue.^{159,160} MS is thought to be caused by axon demyelination is induced by T cells¹⁶¹ and has 2 stages: early inflammation, which is responsible for relapsing–remitting disease, and delayed neurodegeneration resulting from non–relapsing progression.^{162,163} Icariin can potentially mitigate the symptoms of MS by suppressing immune cells: in experimental autoimmune encephalomyelitis (EAE), a widely used animal MS model, icariin decreased the pool of type 1 helper T cells (Th1) and Th17 cells and inhibited T cell proliferation and differentiation.¹⁶⁴

Hyperactivation of the HPA axis has been linked to the pathogenesis of MS.^{165,166} An elevated corticosterone level was associated with increased inflammation in an EAE model and plaque remyelination in MS patients in clinical trials.¹⁶⁶ In EAE, icariin was shown to modulate the HPA axis and ESR2, reduced corticosterone level, and induce the upregulation of glucocorticoid receptors in cerebral white matter.¹⁶⁷ In another study, icariin improved EAE symptoms by decreasing corticosterone level and inhibiting inflammation and apoptosis.¹⁶⁷ The repair of damaged myelin sheath and axons occurs during remission in the relapsing–remitting stage of MS;¹⁶⁸ during this period, icariin accelerated myelin restoration and axon repair by stimulating neurotrophic factor production and oligodendrogenesis in the cuprizone-induced MS model.¹⁶⁹

The network pharmacologic analysis identified annexin (ANX)A1 and histamine H1 receptor (H1R) as potential mediators of the therapeutic effects of icariin in MS ([Supplementary Table S1](#)). ANXA1 is an endogenous regulator of glucocorticoids that exerts anti-inflammatory effects by controlling leukocyte migration, macrophage phagocytosis, and neutrophil apoptosis.¹⁷⁰ In a clinical trial, ANXA1 level was inversely associated with the risk of MS, and patients with lower expression of ANXA1 had more severe disabilities that was possibly due to loss of the anti-inflammatory activity of ANXA1.¹⁷¹ H1R is upregulated in chronic MS lesions;¹⁷² it was reported that H1R and histamine signaling increased EAE susceptibility by altering antigen-specific T cell effector responses, immune function, and BBB permeability.¹⁷³ As pharmacologic targeting of H1R was reported to ameliorate EAE,¹⁷⁴ we speculate that icariin inhibits T cell effector responses by suppressing H1R in endothelial cells. Thus, icariin may improve the symptoms of MS by targeting ANXA1 and H1R.

Glioblastoma

Icariin plays an antitumor role in many cancers including hepatocellular carcinoma, small cell lung cancer, melanoma, gastric cancer, breast cancer, and colorectal cancer.^{180–183} Glioblastoma multiforme (GBM) is among the most invasive, fatal, and treatment-refractory solid tumors¹⁸⁴ and is characterized by uncontrolled proliferation, angiogenesis, and evasion of apoptosis. GBM metastasis and invasion are associated with reduced survival and poor prognosis.^{185–188} In our network pharmacology analysis, icariin modules were closely related to evasion of apoptosis, enhanced angiogenesis, and cell proliferation in GBM; and the results of the KEGG pathway enrichment analysis of target genes shared by icariin and glioblastoma indicated that icariin regulates the cell cycle, cellular senescence, and apoptosis, thereby contributing to the initiation, development, and progression of tumors including glioblastoma ([Figure 6B](#)).

Icariin suppresses the growth of human tumor cells by interfering with multiple signaling pathways that are critical to tumor cell growth, invasion, and apoptosis. Icariin was shown to exert antitumor effects by inducing apoptosis via the mitochondrial-mediated signaling pathway and by downregulating NF- κ B, which was accompanied by decreases in Bcl-2 and Bcl-2 extra-large (Bcl-xL) levels.^{176,189,190} It was also reported to inhibit the growth of cancer cells by inducing cell cycle arrest at the G2/M

and G0/G1 phases in gallbladder carcinoma and colorectal cancer cells, respectively. Inhibiting angiogenesis can prevent tumor invasion and metastasis.¹⁹¹ Icariin exhibited antiangiogenic effects in xenograft models of tumors including hepatocellular carcinoma and renal cell carcinoma,¹⁹² and suppressed tumor cell migration and invasion by regulating the Rac1-dependent vasodilator-stimulated phosphoprotein (VASP) pathway.¹⁹³ Icariin also enhanced the effects of antineoplastic drugs and radiation therapy; in one study, the combination of icariin and gemcitabine resulted in an enhanced antitumor effect compared to either drug alone,¹⁹⁴ highlighting its therapeutic potential for cancer treatment.

We found that 73 target genes were shared by icariin and glioblastoma (Figure 6C). The results of the KEGG pathway enrichment analysis indicated that the antitumor functions of icariin may involve vascular endothelial growth factor (VEGF), proviral integration site for Moloney murine leukemia virus (PIM)1, CDK2, CDK4, PTGS2, epidermal growth factor receptor (EGFR), and MMP-2, among other factors (Figure 6A and B). Evasion of apoptosis is essential for tumor initiation and development; thus, inducing apoptosis is a common anticancer strategy.¹⁹⁸ PIM1, which is overexpressed in various human cancers, modulates signaling events that promote cell growth and survival. By phosphorylating apoptosis signal-regulating kinase (ASK)1 at Ser83, PIM1 inhibited ASK1-mediated p38 kinase phosphorylation and enhanced the survival of H1299 human lung cancer cell.¹⁹⁶ As icariin was shown to repress PIM1 expression and induce apoptosis in acute promyelocytic leukemia cell lines,¹⁹⁷ we speculate that it can inhibit GBM progression by promoting apoptosis.

Over proliferation of tumor cells is associated with worse prognosis in GBM.¹⁹⁸ Cyclin E2 mediates the transition from G0/G1 to S phase via phosphorylation of the retinoblastoma (Rb) and binding of CDK2.^{199,200} PIM1-mediated phosphorylation of the tyrosine phosphatase cell division cycle (CDC)25A activates CDK2/cyclin E to stimulate cell proliferation by promoting cell cycle progression from G1 to S phase.^{199,200} CDK4 also plays important roles in the regulation of G0/G1 phase and G1/S phase transition and was reported to be dysregulated in GBM. Icariin was shown to inhibit the expression of PIM1, cyclin E, CDK2, and CDK4.^{197,201} Based on these findings and our KEGG pathway enrichment analysis results, we propose that icariin inhibits

GBM by blocking tumor cell proliferation, possibly through negative regulation of PIM1, CDK2, and CDK4.

Angiogenesis contributes to tumor development and progression.¹⁸⁶ As mentioned above, icariin has demonstrated antiangiogenic effect in some diseases. PTGS2 is highly expressed in diverse human cancers involving angiogenesis. Amplification of the EGFR gene is the most common genetic alteration in primary GBM, and high EGFR expression is observed in many primary human tumors.²⁰² Hyperactivated EGFR can phosphorylate specificity protein (SP) via the p38 MAPK signaling pathway, leading to upregulation of PTGS2 and enhanced the secretion of VEGF, which stimulates angiogenesis.^{175,177} The inhibition of EGFR signaling and suppression of VEGF and PTGS2 by icariin also provide indirect evidence for its antiangiogenic activity.^{203,204}

Diffuse cell invasion is a hallmark of GBM that contributes to its lethality. Upregulation of MMP-2 was found to be correlated with higher glioma malignancy, whereas its downregulation reduced tumor invasion.¹⁷⁸ EGFR has been shown to induce the upregulation of MMPs in the extracellular matrix, resulting in the degradation of basal membrane components and invasion of cancer cells into surrounding tissue and blood vessels. Icariin was reported to inhibit the expression of EGFR and MMP-2,¹⁷⁹ which could prevent diffuse cell invasion in GBM based on the results of the network pharmacology analysis.

Spastic Paraplegia

Hereditary spastic paraplegias are a genetically heterogeneous group of neurodegenerative disorders characterized by length-dependent corticospinal tract and dorsal column degeneration.²⁰⁵ Patients present the core clinical features of bilateral lower limb spasticity, hyperreflexia, and extensor plantar responses.²⁰⁶ Hereditary spastic paraplegias can emerge in infancy, childhood, adolescence, or adulthood and are usually associated with autosomal dominant or recessive or X-linked modes of inheritance.²⁰⁷ There is currently no direct evidence that icariin has therapeutic effects in spastic paraplegia; however, a possibly link through aldehyde dehydrogenase 18 family member A1 (ALDH18A1) and EGFR was suggested by our network pharmacology analysis. ALDH18A1 mutations cause dominant (*SPG9A*) or recessive (*SPG9B*) spastic paraplegia. Troyer syndrome, a hereditary spastic paraplegia, is an autosomal recessive disease characterized by pathogenic mutations in the *SPG20* gene that result in degradation of

the protein and a loss-of-function phenotype that includes length-dependent axonopathy of corticospinal motor neurons.²⁰⁸ Methylation-induced *SPG20* silencing was reported to activate the EGFR/MAPK signaling pathway.²⁰⁹ Spartin, a multifunctional protein encoded by *SPG20*, is involved in the endocytic trafficking of EGFR,²⁰⁹ as icariin inhibits EGFR signaling, icariin may alleviate hereditary spastic paraplegia by interfering with the function of spartin.

Conclusion

In this review, we outlined the traditional uses and chemical components of *Epimedium herba* and summarized the pharmacologic studies that have investigated its main active component icariin, which shows promising therapeutic effects in AD, PD, ischemic stroke, depressive disorder, MS, angiogenesis, and glioblastoma. We also used a network pharmacology approach to identify targets of icariin and performed a functional enrichment analysis to elucidate the molecular basis for the effects of icariin in these diseases. Our results along with current evidence from the literature provide a basis for future studies on the mechanisms and applications of icariin to the treatment of CNS diseases.

Abbreviations

A β , amyloid beta; AD, Alzheimer disease; AKT, protein kinase B; AKT1, RAC-alpha serine/threonine-protein kinase; ALDH18A1, aldehyde dehydrogenase 18 family member A1; ANXA1, annexin A1; APP, amyloid precursor protein; ASK1, apoptosis signal-regulating kinase 1; BACE1, β -secretase; BBB, blood-brain barrier; Bcl-2, B cell lymphoma 2; BDNF, brain-derived neurotrophic factor; CDK, cyclin-dependent kinase; CNS, central nervous system; CRF, corticotropin-releasing factor; EAE, experimental autoimmune encephalomyelitis; EGFR, epidermal growth factor receptor; ESR1, estrogen receptor alpha; ESR2, estrogen receptor beta; FDR, false discovery rate; GBM, glioblastoma multiforme; GO, Gene Ontology; GSK-3 β , glycogen synthase kinase 3 beta; H1R, histamine H1 receptor; HPA, hypothalamic-pituitary-adrenal; HTR1A, serotonin1A receptor; IL, interleukin; KEGG, Kyoto Encyclopedia of Genes and Genomes; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; mGluR, metabotropic glutamate receptor; MMP-2, matrix metalloproteinase 2; MS, multiple sclerosis; NADP, nicotinamide adenine dinucleotide phosphate; NF- κ B, nuclear factor kappa B; NOX2, NADPH oxidase 2; Nrf2, nuclear factor erythroid 2-related factor 2; OA, oleanolic acid; PD,

Parkinson disease; PGC-1 α , proliferator-activated receptor-gamma coactivator 1 alpha; PI3K, phosphoinositide 3-kinase; PIM1, proviral integration site for Moloney murine leukemia virus 1; PPAR, peroxisome proliferator-activated receptor; PPI, protein-protein interaction; PTGS, prostaglandin endoperoxide synthase 2; ROS, reactive oxygen species; SIRT1, sirtuin 1; SPG9A, spastic paraplegia 9A, autosomal dominant; SPG9B, spastic paraplegia 9B, autosomal recessive; TGF, transforming growth factor; Th1/17 cells, type 1/17 helper T cells; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TNFRSF1A, tumor necrosis factor receptor superfamily member 1A; VEGF, vascular endothelial growth factor.

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Author Contributions

All authors listed on an article meet all of the following criteria. "All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work."

Disclosure

The authors report no conflicts of interest in this work.

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