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Review

Icariin against neuroinflammation: emerging evidence for gut-brain axis modulation involving short-chain fatty acids, vagal signaling, and extracellular vesicles

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ABSTRACT

Neuroinflammation is a central pathological process in a wide range of central nervous system disorders, yet therapeutic strategies directed solely at single inflammatory targets have shown limited efficacy. Gut–brain axis dysfunction may contribute to neuroinflammatory progression through interconnected metabolic, immune, neural, and vesicle-associated routes. Icariin, a major flavonoid glycoside from *Epimedii Folium*, has attracted attention because of its anti-inflammatory, neuroprotective, and microbiota-modulating properties. In this review, we summarize current evidence for the anti-neuroinflammatory effects of icariin and explicitly stratify the evidence into three levels: direct icariin-related findings, icariin-related findings without causal gut–brain validation, and mechanisms inferred from broader gut–brain axis biology. Available studies provide relatively stronger support for intestinal barrier protection, microbiota remodeling, peripheral cytokine suppression, and central inflammatory pathway regulation. In contrast, short-chain fatty acid (SCFA)-associated signaling, vagal neuroimmune communication, and extracellular vesicle (EV)-mediated cargo transfer remain plausible but incompletely validated candidate routes. We further compare icariin with its major microbial metabolites, especially icariside II and icaritin, because oral icariin may act partly through metabolite-mediated exposure rather than through robust brain penetration of the parent compound. Major limitations include insufficient causal perturbation, incomplete organ-specific pharmacokinetic data, inter-individual microbiota variability, and uncertain therapeutic time windows. Overall, icariin emerges as a promising multi-target natural product for anti-neuroinflammatory research, but the causal hierarchy and translational relevance of proposed gut–brain pathways require direct validation.

1. Introduction

Neuroinflammation is a common pathological feature of many central nervous system disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and traumatic brain injury (TBI) [1-4]. Sustained activation of microglia and persistent release of inflammatory mediators such as TNF-α and IL-1β contribute to neuronal damage and progressive neurological dysfunction [5-7]. Although suppression of neuroinflammation is considered a promising therapeutic strategy, clinical translation remains difficult because many anti-inflammatory agents show limited brain exposure and because inflammatory responses in the CNS are sustained by interacting immune, metabolic, and glial networks rather than by a single dominant target [8, 9]. In addition, growing evidence indicates that gut microbiota imbalance and intestinal barrier dysfunction can amplify systemic inflammation and further aggravate neuroinflammatory processes [10-13].

Against this background, the gut–brain axis has emerged as an important framework for understanding neuroinflammation beyond the brain itself [14-16]. Current evidence suggests that gut-derived signals may influence central inflammatory states through several interconnected routes. Among them, microbiota-derived metabolites, especially short-chain fatty acids (SCFAs), can affect blood–brain barrier function and microglial activity; vagal pathways can relay peripheral immune and metabolic information to the brainstem and engage anti-inflammatory reflexes; and circulating extracellular vesicles (EVs) may participate in long-distance inter-organ communication by transporting bioactive cargo across biological barriers [3, 17-23]. Together, these routes provide a conceptual basis for multi-organ intervention in neuroinflammation [16, 24-26].

Icariin, a major bioactive flavonoid glycoside from Epimedii Folium, has been widely studied for its anti-inflammatory and neuroprotective activities [1, 27, 28]. Recent studies indicate that its

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actions may not be restricted to direct CNS effects. Instead, icariin appears to influence several peripheral processes relevant to gut–brain communication, including gut microbial remodeling [1, 18, 29], intestinal barrier preservation [21-23], and suppression of peripheral inflammatory signaling [19, 20, 29, 30]. In this review, we summarize current evidence for the anti-neuroinflammatory effects of icariin from a gut–brain axis perspective, focusing on three candidate routes—SCFA-associated metabolic signaling, vagal neuroimmune communication, and EV-mediated inter-organ transfer—while also discussing current evidence gaps and translational challenges. To avoid overclaiming, each proposed mechanism is interpreted using three evidence tiers: Tier 1, direct evidence from icariin or icariin metabolite studies; Tier 2, icariin-related pharmacological evidence in which gut–brain causality has not been proven; and Tier 3, mechanistic rationale extrapolated from broader gut–brain axis literature. Particular attention is given to distinguishing direct icariin-related findings from indirect evidence derived from related flavonoids or general microbiota–gut–brain biology.

2. Molecular characteristics and bioactivity profile of icariin

2.1. Chemical structure and bioavailability characteristics

Icariin is one of the predominant flavonoids found in *Epimedii Folium*. It is a prenylated flavonoid with a characteristic structure based on an 8-prenylkaempferol core, to which two sugar moieties, rhamnose and glucose, are attached. This glycosylated structure allows icariin to undergo stepwise deglycosylation. Removal of the rhamnose moiety yields icariside I, whereas removal of the glucose moiety produces icariside II [31]. Further deglycosylation generates the aglycone icaritin, which may subsequently be metabolized through demethylation to form desmethylicaritin [31].

Several studies have reported low oral bioavailability of icariin across different animal models and experimental settings. Early research estimated the oral bioavailability of icariin to be only 0.12% [32]. More recent studies have reported values generally ranging from approximately 0.1% to 1.8% in rats, suggesting limited systemic exposure after oral administration [33]. Other studies have described somewhat higher, but still relatively low, bioavailability values of around 12% [23, 33-35]. Consistent with these findings, clinical studies have shown that blood concentrations of icariin are very low or undetectable at the tested doses, further supporting the limited bioavailability of oral formulations [36].

The poor oral bioavailability of icariin is likely attributable to a combination of physicochemical and biological factors that limit its absorption and systemic exposure. Its unfavorable physicochemical properties appear to play an important role [37, 38]. In particular, icariin has poor aqueous solubility, limited membrane permeability, and a relatively slow dissolution rate in biological fluids [35]. These features are consistent with its classification as a Biopharmaceutics Classification System (BCS) Class IV compound, a category generally associated with challenges in oral drug delivery [33]. In addition, metabolic processes may further influence its oral absorption. Icariin can be hydrolyzed by lactase-phlorizin hydrolase in the small intestine and by microbiota-derived β-glucosidases, leading to the formation of metabolites that may be more readily absorbed [13, 39].

2.2. Intestinal metabolism and systemic exposure characteristics

After oral administration, icariin appears to undergo substantial metabolism in the gastrointestinal tract before reaching the systemic circulation. Studies have shown that icariin can be converted in the intestine into several metabolites, including icariside I, icariside II, and icaritin [32]. Human studies suggest that this process may be relatively rapid and complex; for example, available evidence indicates that intestinal microbiota can convert icariin into icariside II within approximately 1 hour [40, 41]. Animal studies further support the involvement of gut microbiota in this conversion. After oral administration, approximately 91.2% of icariin has been reported to be transformed into icariside II, whereas only about 0.4% undergoes this conversion after intravenous administration [42]. This difference indirectly suggests that intestinal microbiota may contribute substantially to the metabolism of orally administered icariin [13, 43].

Icariin metabolism is generally considered to proceed mainly through a stepwise deglycosylation pathway in the gastrointestinal tract. Removal of the glucose residue can generate icariside II, whereas removal of the rhamnose residue forms icariside I [31, 44]. Further deglycosylation produces the aglycone icaritin, which may subsequently undergo demethylation to form desmethylicaritin [13, 45]. This transformation is thought to involve intestinal hydrolysis, potentially mediated by small-intestinal lactase-phlorizin hydrolase and microbiota-derived β-glucosidases. These processes may facilitate the formation of metabolites with greater absorptive potential than the parent compound [13, 39].

Among these metabolites, icariside II appears to exhibit more favorable systemic exposure than icariin. Reported maximum plasma concentration and area under the curve values for icariside II are approximately 3.8-fold and 13-fold higher than those of icariin, respectively, suggesting that icariside II may be an important contributor to systemic exposure after oral administration [42, 46]. Consistent with this interpretation, urinary excretion data show that less than 0.425% of icariin is recovered unchanged, supporting the possibility that orally administered icariin is largely metabolized and eliminated mainly in the form of metabolites rather than as the intact parent compound [13, 45]. In addition, hepatic first-pass glucuronidation of the related compound icaritin may further influence oral bioavailability and systemic exposure, highlighting the broader relevance of metabolic pathways within this class of compounds [47].

Compared with the parent compound, microbial metabolites should be considered pharmacologically distinct entities rather than interchangeable forms of icariin. Icariside II has fewer sugar residues and shows higher reported systemic exposure than icariin, whereas icaritin, as an aglycone, is more lipophilic and has broader reported pharmacological activity in inflammation, mitochondrial regulation, and tumor biology. However, the extent to which these metabolites reach the brain, engage CNS targets, or mediate gut–brain anti-neuroinflammatory effects after oral icariin remains incompletely quantified. Therefore, effects observed after oral icariin administration should be interpreted as potentially involving parent-compound exposure, metabolite-mediated activity, and indirect peripheral regulation.

Comprehensive metabolic profiling has identified up to 11 icariin metabolites in rat feces, with major metabolites accounting for more than 90% of the total metabolite pool [45]. These metabolic pathways include hydrolysis, demethylation, oxidation, and conjugation reactions. *In vitro* studies using intestinal flora have also suggested differences in metabolic rates among related compounds, with the reported order being icariin > epimedin B > epimedin A > epimedin C > baohuoside I [48]. The proposed gut-liver biotransformation route of icariin after oral administration is summarized in Fig. 1.

2.3. Differences in individual gut microbiota and organ exposure

The metabolism of icariin by gut microbiota appears to involve several bacterial strains with different metabolic capacities. Studies have shown that *Streptococcus sp.* MRG-ICA-B and *Enterococcus sp.* MRG-ICA-E can mainly convert icariin into icariside II

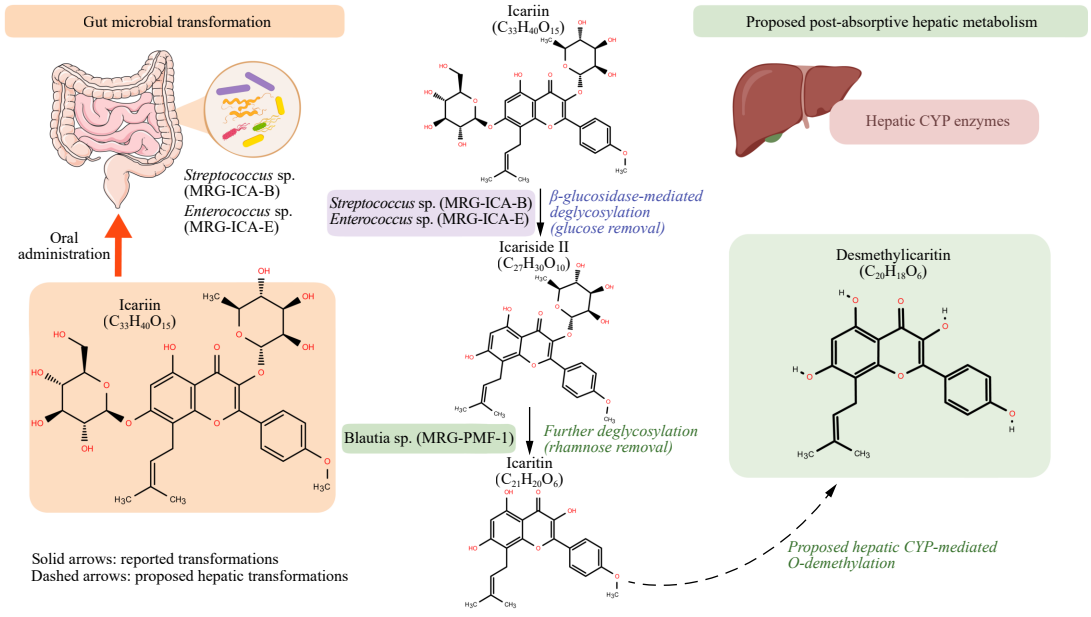


Fig. 1 Gut microbial transformation and proposed post-absorptive hepatic metabolism of icariin after oral administration. Following oral administration, icariin undergoes stepwise deglycosylation in the gastrointestinal tract. *Streptococcus* sp. MRG-ICA-B and *Enterococcus* sp. MRG-ICA-E have been reported to convert icariin to icariside II through β-glucosidase-mediated removal of glucose, whereas *Blautia* sp. MRG-PMF-1 may contribute to further deglycosylation of icariside II to icaritin. After absorption, icaritin is proposed to undergo hepatic cytochrome P450 (CYP)-mediated O-demethylation to form desmethylicaritin. Black solid arrows indicate reported metabolic transformations; the black dashed arrow indicates a proposed hepatic transformation. This scheme summarizes current biotransformation evidence and does not establish a causal gut–brain mechanism.

under anaerobic conditions, suggesting that these strains may participate in the early deglycosylation steps of icariin metabolism [13, 31, 41]. In comparison, *Blautia sp.* MRG-PMF-1 may contribute to further deglycosylation and demethylation, leading to the formation of metabolites such as icaritin and desmethylicaritin, which have been reported to show estrogen-like activity in some experimental settings [31, 41]. Fungi may also be involved in the metabolism of this type of flavonoid glycoside. For example, *Paecilomyces terricola* M2 has been reported to efficiently transform icariin into icariside II [49].

Following oral administration, icariin exhibits organ-specific distribution patterns in rats, and these patterns may differ between sexes. In male rats, tissue exposure has been reported in the order liver > lung > spleen > heart > kidney > brain > testicle, suggesting relatively greater distribution in the liver and lung. In female rats, the reported order is liver > uterus > heart > ovary > lung > kidney > spleen > brain, with relatively higher accumulation in the uterus and ovary [45]. Overall tissue exposure appears to be higher in males than in females, whereas female reproductive organs show relatively greater accumulation. These findings suggest a possible sex-related difference in tissue distribution, although further studies may be needed to clarify its biological significance [13, 45]. In both sexes, brain exposure remains low, which may indicate limited penetration of icariin across the blood–brain barrier [45].

The route of administration also appears to influence icariin metabolism and systemic exposure. After oral administration, icariin is largely converted to icariside II, which has been identified as a predominant form in rat plasma, whereas intravenous administration results in much lower conversion. This difference is consistent with the involvement of intestinal microbiota in the metabolism of orally administered icariin [13, 42]. Regardless of the administration route, fecal excretion appears to be the main elimination pathway for icariin and/or its metabolites [45].

2.4. Blood-brain barrier penetration and CNS bioactivity

Icariin may have some capacity to reach the central nervous system; however, the available evidence is not sufficient to describe its blood-brain barrier (BBB) permeability as efficient or unequivocal. Several preclinical studies and reviews suggest that icariin can be detected in brain tissue or exert pharmacological effects within the brain, including attenuation of neuroinflammation, antioxidant activity, protection of dopaminergic neurons, improvement of learning and memory, and regulation of brain-related signaling pathways [50-53]. For example, An et al. [54] detected icariin-related exposure in rat plasma and brain tissue after administration, while Li et al. , [55] Guo et al., [56] and Li et al. [57] provided indirect support for its potential CNS activity through evidence of cognitive improvement, neuroprotection, and inflammatory modulation. Nevertheless, many of these conclusions are derived mainly from animal models or functional outcomes rather than from direct quantitative evidence demonstrating robust BBB transport of intact icariin. This cautious interpretation is further supported by pharmacokinetic and tissue-distribution studies showing low oral bioavailability, rapid systemic clearance, and limited brain exposure of icariin. Xu et al. [45] reported a low brain AUC for icariin, suggesting inefficient BBB penetration, while Zeng et al. [58] and Fehsel [59] also noted its low brain accumulation, rapid elimination, and insufficient BBB transport. Structurally, the methoxy group of icariin may contribute to lipophilicity, whereas its glycosidic chains increase hydrophilicity, thereby restricting passive diffusion across the BBB [60, 61]. Therefore, intact icariin may cross the BBB only to a limited extent under specific experimental conditions, rather than freely or efficiently entering the brain.

The extensive intestinal metabolism of icariin should also be considered when interpreting its CNS-related bioactivity after oral administration. Icariin can be metabolized in the intestine into compounds such as baohuoside I, icariside II, and icaritin [39], and some of these less glycosylated metabolites may possess better membrane permeability and brain exposure potential than the parent compound [40, 62]. Selected studies suggest that less glycosylated metabolites may have greater membrane permeability than icariin; however, direct head-to-head brain pharmacokinetic evidence remains limited [40, 62, 63]. In terms of bioactivity, icariin

and its microbial metabolites exhibit overlapping but not necessarily identical neuroprotective and anti-inflammatory effects. Icariin has been reported to inhibit NF-κB-related inflammatory signaling, reduce microglia-mediated inflammatory responses, and protect dopaminergic neurons, whereas icariside II may suppress the expression of inflammatory mediators such as IL-1β, TNF-α, COX-2, and iNOS and improve Aβ-induced cognitive impairment [64, 65]. Icaritin has also shown potential in inhibiting BACE1, modulating amyloid precursor protein processing, and exerting neuroprotective effects [66, 67]. Thus, the neuroprotective and anti-neuroinflammatory effects attributed to orally administered icariin may not depend entirely on substantial BBB penetration by the intact parent compound. Instead, they may reflect the combined contribution of limited parent-compound brain exposure, metabolite-mediated CNS activity, and indirect regulation of peripheral inflammatory or BBB-related pathways. Delivery strategies such as liposomes, intranasal administration, and cyclodextrin inclusion complexes have shown potential for improving CNS exposure [68, 69]. However, further direct pharmacokinetic, brain-distribution, and translational studies comparing icariin with its major microbial metabolites are still needed to clarify their relative BBB permeability, CNS target engagement, and clinical relevance. The comparative pharmacokinetic characteristics, BBB-related evidence, and neuroprotective activities of icariin and its major microbial metabolites are summarized in Table 1.

2.5. Pharmacological potential and translational prospects

As summarized in Table 1, icariin and its major metabolites differ in glycosylation status, systemic exposure, membrane permeability, and reported biological activity. These differences indicate that the metabolites should be regarded as pharmacologically distinct entities rather than interchangeable forms of the parent compound. In oncology-related studies, icariin and its derivatives have been reported to modulate apoptosis, cell-cycle progression, tumor-associated inflammatory responses, mitochondrial quality control, and signaling pathways such as PI3K/Akt, JAK/STAT3, MAPK, and PINK1/Parkin-related mitophagy [70, 71]. In models of neurodegenerative diseases, their potential benefits appear to be associated with attenuation of oxidative stress, regulation of autophagy, improvement of brain insulin signaling, and protection of dopaminergic neurons [13]. However, these activities should not be generalized across all metabolites or interpreted as proof that intact icariin itself is the dominant CNS-active species after oral administration. Direct side-by-side comparisons of icariin, icariside I, icariside II, icaritin, and desmethylicaritin are needed to define their relative BBB permeability, target engagement, and anti-neuroinflammatory bioactivity.

From a translational perspective, the clinical development of icariin and its metabolites is still constrained by several pharmacokinetic and formulation-related limitations, particularly poor aqueous solubility, low oral bioavailability, rapid systemic clearance, and insufficiently defined dose–safety relationships. To address these challenges, recent studies have explored advanced delivery strategies, including nanostructured lipid carriers, cyclodextrin inclusion complexes, intranasal thermosensitive gels, micelles, liposomes, and other nanoformulation platforms, which may improve solubility, tissue targeting, and therapeutic exposure [68, 72, 73]. Clinical evidence for icaritin remains limited and is concentrated in oncology. Recent literature includes review and experimental studies, whereas direct human outcome data are currently represented mainly by a real-world study in hepatocellular carcinoma. These findings do not establish efficacy in neurological or gut–brain axis disorders [74-76]. Nevertheless, these clinical data do not directly establish efficacy for neuroinflammation or gut–brain axis indications. Further progress will require standardized extracts or well-characterized active compounds, optimized dosing regimens, systematic pharmacokinetic and toxicological evaluation, and rigorously designed clinical trials with disease-relevant inflammatory, microbiome, and neurological endpoints [77, 78].

3. Key regulatory nodes of bidirectional communication in the gut-brain axis

3.1. Gut microbiota-immune-neuroendocrine triangular network

The gut microbiota forms a complex bidirectional communication network with the central nervous system through the microbiota-gut-brain axis, wherein the immune and neuroendocrine systems serve as key regulatory components. Emerging evidence suggests that alterations in the composition of the gut microbiota can modulate neuroinflammatory processes *via* Toll-like receptor (TLR) signaling pathways [79, 80]. In models of AD, gut dysbiosis exacerbates neuroinflammation by promoting beta-amyloid deposition and the formation of neurofibrillary tangles [81, 82]. Furthermore, following TBI, changes in the gut microbiota contribute to neuronal damage and cognitive impairment by regulating the activation states of microglia and astrocytes [83]. This communication primarily relies on three mechanisms: 1) Microbial metabolites such as SCFAs directly influence the central nervous system; 2) Modulation of intestinal barrier integrity affects systemic inflammation; 3) The activation of TLR-dependent signaling pathways regulates neuroinflammatory responses [26]. Treatment with icariin has been shown to significantly alter the gut microbial structure in APP/PS1 transgenic mice, while concurrently ameliorating aberrant protein expression in the hippocampus and alleviating spatial memory deficits [1]. This modulation may involve microbial metabolites, including short-chain fatty acids, as well as microbiota-immune interactions that influence neuroendocrine and inflammatory states [84]. Furthermore, icariin has been associated with modulation of TLR-related inflammatory signaling, which may be relevant to gut–brain axis regulation, although pathway specificity remains insufficiently resolved [26]. These findings underscore the complex role of the gut microbiota in neuroinflammation and provide insights into potential therapeutic strategies targeting the microbiota-gut–brain axis.

Table 1 Pharmacokinetic and neuropharmacological comparison of icariin and its major microbial metabolites.

Compound	Systemic exposure	BBB/CNS evidence	Main bioactivity	Key limitation	Refs
Icariin	Low	Limited brain exposure	Peripheral and central anti-inflammatory findings	Parent contribution unclear	36, 42, 45
Icariside II (baohuoside I)	Higher than icariin	Improved membrane permeability suggested; direct brain PK remains unavailable	Anti-inflammatory and neuroprotective	No head-to-head brain PK	41, 42, 63-65
Icaritin	Incompletely quantified after oral icariin	More lipophilic than icariin; direct comparative brain PK remains unavailable	Anti-inflammatory and amyloid-related activity	Exposure after oral icariin unclear	5, 7, 47, 62
Icariside I and desmethylicaritin	Sparse data	Unknown	Limited or context-specific findings	No comparative brain PK or target-engagement data	41, 44, 48

Note: BBB/CNS evidence refers to direct brain pharmacokinetic data where available; otherwise, the assessment is based on physicochemical characteristics or indirect pharmacological findings.

3.2. SCFA-associated signaling in gut–brain communication

SCFAs, principal metabolites derived from microbial fermentation of dietary fiber, are important signaling molecules in gut–brain communication and neuroimmune regulation [17]. Broader gut–brain axis studies indicate that acetate, propionate, and butyrate can affect BBB integrity, glial activity, HDAC activity, GPCR signaling, and cytokine profiles [85-91]. Some icariin studies suggest microbial remodeling or enrichment of taxa associated with SCFA production, but most have not simultaneously quantified SCFA flux, manipulated SCFA receptors or HDAC signaling, and measured central inflammatory outcomes [92, 93]. Therefore, the SCFA route is best classified as Tier 3 evidence for icariin-specific gut–brain signaling: biologically plausible and supported by indirect data, but not yet causally validated. Future studies should combine fecal, plasma, and brain SCFA measurements with antibiotic depletion, FMT, SCFA rescue, receptor blockade, and microglial readouts to determine whether SCFAs are necessary or sufficient for icariin-related anti-neuroinflammatory effects.

3.3. Neuroanatomical basis of vagal signaling

The vagus nerve is a major neural route connecting the gut and the brain and is well positioned to transmit gut-derived immune and metabolic information to the nucleus tractus solitarius and downstream neuroimmune circuits [25]. Gut microbiota and microbial metabolites can activate receptors on vagal afferent fibers, while vagal efferents can engage cholinergic anti-inflammatory reflexes that regulate peripheral cytokine release [94-100]. In neuroinflammatory and hypertensive models, vagal activity or vagus nerve stimulation has been associated with suppression of pro-inflammatory cytokines and reduced CNS inflammatory drive [83, 98]. However, direct evidence demonstrating that icariin exerts anti-neuroinflammatory effects through a defined vagal mechanism is lacking. Because icariin can improve gut microbial and inflammatory states in selected models, downstream vagal signaling remains a testable hypothesis rather than an established pathway [93, 101]. Vagotomy, pharmacological blockade, vagus nerve stimulation, neural tracing, and electrophysiological recording in icariin-treated models are needed to move this route from Tier 3 rationale to causal evidence. These peripheral modules and candidate gut–brain communication routes—including SCFA-associated metabolic signaling, vagal neuroimmune signaling, and EV-mediated inter-organ communication—are summarized in Fig. 2.

4. Peripheral actions of icariin relevant to gut–brain communication

4.1. Regulation of intestinal barrier function and reconstruction of immune homeostasis

Icariin enhances intestinal barrier function through various mechanisms, including the regulation of tight junction proteins, the enhancement of intestinal cell function, and the reduction of inflammatory responses. At the molecular level, this compound significantly upregulates the expression of tight junction proteins such as occludin-1, zonula occludens, and claudin-1, while also enhancing the activity of Paneth cells and Goblet cells [102]. These changes are consistent with improved mucosal barrier integrity and reduced vulnerability to inflammatory injury. Studies have confirmed that icariin treatment reduces the expression of miR-122a in the Caco-2 cell model, thereby attenuating TNF-α-induced occludin disruption and epithelial barrier damage, which provides direct support for a barrier-protective role in this experimental context [103]. Under inflammatory conditions, icariin mitigates the disruption of tight junction structures induced by the pro-inflammatory factor TNF-α through the inhibition of the NF-κB signaling pathway [104]. Animal experiments have shown that icariin intervention significantly improves morphological damage to intestinal villi, reduces inflammatory cell infiltration [101], and enhances the activity of antioxidant enzymes such as SOD, GPx, and CAT [105], supporting a combined antioxidant and barrier-protective effect in intestinal inflammatory settings.

4.2. Reshaping of microbial metabolite profiles

Icariin exhibits multidimensional characteristics in regulating gut microbiota. Current evidence suggests that icariin may help ameliorate gut microbial dysbiosis associated with disease or aging, as reflected by increased bacterial diversity, partial restoration of a youth-like microbial profile, and enrichment of beneficial or metabolically active bacteria. For example, in Alzheimer's disease and aging models, icariin treatment increased bacterial diversity and was accompanied by increased abundance of *Akkermansia*, reduced abundance of *Alistipes*, and enrichment of short-chain fatty acid-producing bacteria such as *Akkermansia muciniphila* and *Eubacterium ramulus* [1, 29, 102, 106-108]. In ulcerative colitis and inflammatory bowel disease models, icariin or icariin-derived compounds also improved gut microbial structure, restored impaired intestinal barrier function, and helped normalize commensal microbiota and their metabolites, effects that may be partly related to microbiota-mediated immune regulation [109, 110]. Functionally, icariin-associated microbial remodeling has been linked to a reduced Firmicutes/Bacteroidetes ratio, increased abundance of probiotics, improved levels of short-chain fatty acids and indole-derived metabolites, decreased lipopolysaccharide (LPS) production and translocation, and enhanced intestinal barrier integrity and metabolic pathway regulation [44, 101, 111-115].

4.3. Regulation of enterogenic inflammatory factor levels

Icariin has shown inhibitory effects on multiple inflammatory mediators in several peripheral inflammatory models. Experimental data indicate its capacity to significantly reduce the levels of pro-inflammatory factors such as IL-1β, IL-6, IL-12, and TNF-α in both plasma and colon tissues [102, 103], while concurrently enhancing the expression of the anti-inflammatory factor IL-10 [103]. This bidirectional regulatory effect is especially significant in gut–brain axis research, as the systemic dissemination of gut-derived inflammatory factors is a major contributor to neuroinflammation [116, 117]. Mechanistic studies reveal that icariin obstructs the amplification of the inflammatory cascade by inhibiting the activities of STAT1 and STAT3 [109], as well as the TLR4/NF-κB signaling pathway [110, 118]. Notably, the inhibition of inflammatory factors by this compound exhibits a dose-dependent characteristic [104], thereby supporting future dose-response studies, although clinically relevant dosing remains unresolved.

5. Targeted regulatory network of central nervous system inflammation

Current evidence for icariin-related central anti-inflammatory regulation is stronger than the evidence linking these effects to specific gut–brain axis communication routes. Beyond its peripheral effects, icariin has been reported to modulate central inflammatory programs associated with microglial activation, inflammatory transcription, and neuroimmune phenotypic switching. Current evidence mainly converges on SIRT1-related regulation, NF-κB-associated pathways, and microglial polarization dynamics.

5.1. SIRT1-mediated epigenetic regulatory mechanisms

SIRT1, a nicotinamide adenine dinucleotide (NAD+)-depend-

ent class III HDAC, is a member of the sirtuin family and plays a pivotal role in regulating inflammation in the central nervous system [119]. Studies have demonstrated that SIRT1 activation significantly inhibits the expression of pro-inflammatory mediators, such as inducible nitric oxide synthase, cyclooxygenase-2, and interleukin-1β in microglia, while promoting the polarization of M2-type microglia [120]. At the molecular level, SIRT1 suppresses the transcriptional activity of nuclear factor kappa B (ac-NF-κB) through deacetylation [121], and this epigenetic regulation can impede the cascade amplification of neuroinflammation. Notably, the SIRT1 inhibitor EX527 can completely block FGF21-induced M2-type microglial polarization [122], supporting an important role for this pathway in neuroinflammatory regulation. In models of age-related cognitive dysfunction, the reduction in SIRT1 expression is closely associated with the downregulation of DNA methyltransferase 1 (DNMT1) and the upregulation of ac-NF-κB [123], suggesting age-dependent characteristics of its epigenetic regulatory network.

5.2. Regulation of RAGE-related inflammatory signaling

The binding of the RAGE receptor to advanced glycation end products (AGEs) activates the NF-κB signaling pathway, thereby forming a RAGE-NF-κB positive feedback loop [124]. In spinal cord injury models, the inhibition of SEMA3C-mediated RAGE signaling significantly reduces the production of pro-inflammatory cytokines and reverses the pro-inflammatory polarization of microglia and macrophages [125]. Furthermore, the activation of this signaling axis promotes the transcellular transport of low-density lipoprotein, accelerating the process of atherosclerosis [124]. Available studies suggest that icariin may interfere with components of the AGE/RAGE-NF-κB axis, although the exact molecular steps and disease-context specificity require further validation [124, 126]. In cases of cerebral ischemia-reperfusion injury, targeted inhibition of the RAGE/NF-κB pathway effectively alleviates neuroinflammation and protects axonal integrity [9], thereby identifying the AGE/RAGE-NF-κB axis as a potentially relevant target within a broader multi-organ inflammatory framework.

5.3. Dynamic regulation of microglial polarization states

Although the M1/M2 framework remains useful for conceptual discussion, microglial phenotypes *in vivo* are likely more heterogeneous and context-dependent. Nevertheless, the M1/M2

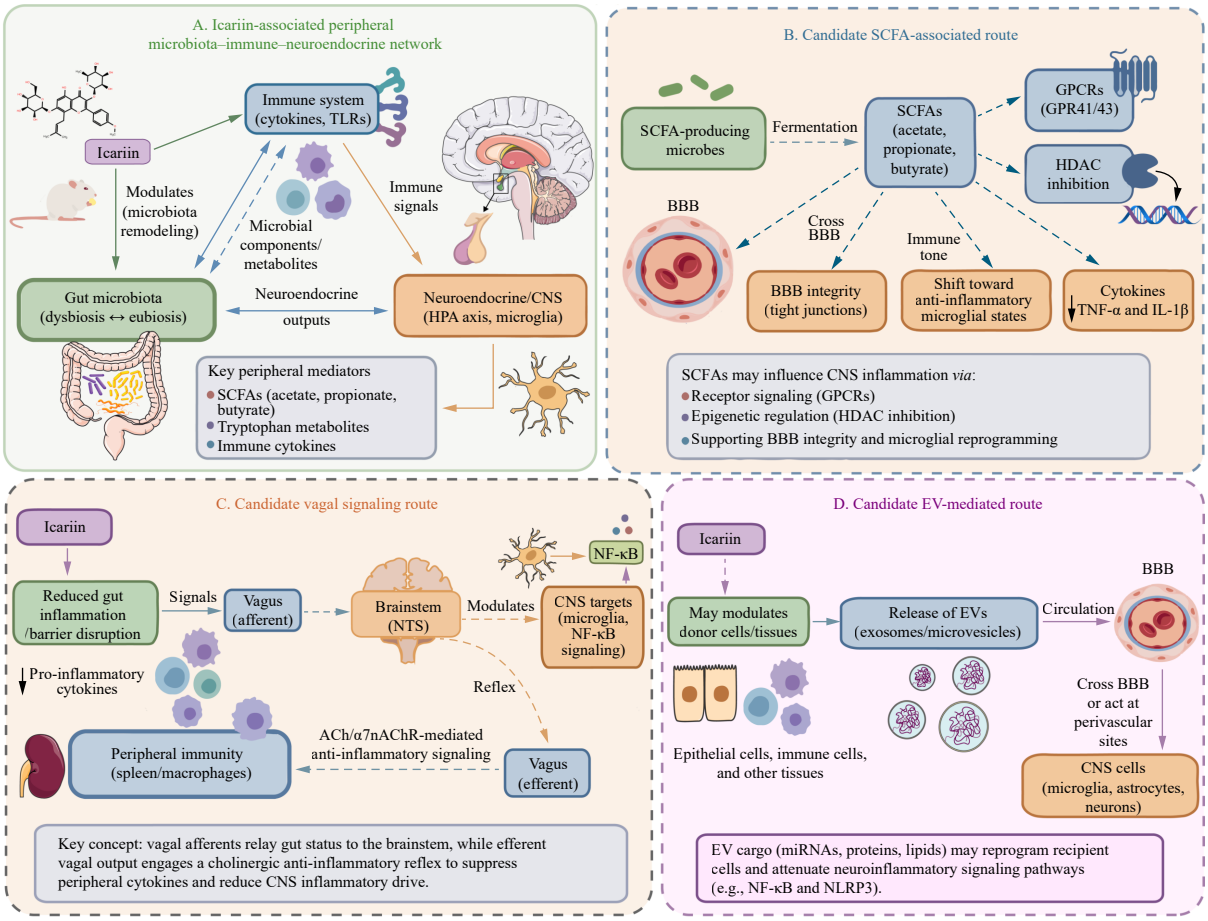


Fig. 2 Evidence-stratified peripheral network and candidate gut–brain routes involved in icariin-related anti-neuroinflammatory regulation. (A) Icariin-associated peripheral microbiota–immune–neuroendocrine network. Icariin may remodel the gut microbiota, improve intestinal inflammatory and barrier states, and alter peripheral mediators, including short-chain fatty acids (SCFAs), tryptophan metabolites, and immune cytokines. These peripheral effects are relatively better supported, although their causal mediation of central outcomes has not been established. (B) Candidate SCFA-associated route. Icariin-associated microbial remodeling may influence the production of acetate, propionate, and butyrate, which could regulate G-protein-coupled receptor signaling, histone deacetylase activity, blood–brain barrier integrity, cytokine production, and microglial inflammatory states. Direct SCFA-dependent mediation of icariin-related central effects remains unverified. (C) Candidate vagal signaling route. Improvement of intestinal barrier and inflammatory states may alter vagal afferent signaling to the nucleus tractus solitarius and engage an efferent cholinergic anti-inflammatory reflex; however, an icariin-specific microbiota–vagus–brain pathway has not been causally demonstrated. (D) Candidate extracellular-vesicle-mediated route. Icariin may influence extracellular-vesicle release or cargo composition in peripheral donor cells and tissues, and these vesicles may interact with or cross biological barriers to modulate microglia, astrocytes, or neurons. Endogenous EV-mediated gut–brain signaling should be distinguished from exosome-based drug-delivery systems and remains a hypothesis requiring direct validation in icariin-treated models. Solid arrows indicate experimentally supported or relatively direct icariin-associated relationships, whereas dashed arrows indicate proposed gut–brain links requiring causal validation.

phenotypic switch of microglia is a critical aspect of neuroinflammation regulation. The SIRT1 activator SRT2104 promotes the transformation of microglia from the pro-inflammatory M1 type to the anti-inflammatory M2 type [127]. This polarization switch is directly associated with the inhibition of NF-κB signaling and the enhancement of SIRT1 expression [121]. At the molecular level, the suppression of the HPK1-JNK-NF-κB signaling pathway and the blockade of the TLR2-NF-κB signaling pathway are common mechanisms regulating microglial polarization [128, 129]. Notably, SIRT1 maintains the polarization homeostasis of microglia [120], and its activation can simultaneously reduce the expression of M1 markers while increasing the characteristic factors of M2 [127]. This bidirectional regulatory characteristic makes SIRT1 an ideal therapeutic target. In depression models, SIRT1-dependent regulation of microglial polarization correlates significantly with behavioral improvement [130], revealing the molecular link between neuroinflammation and psychiatric disorders. The integrated central anti-neuroinflammatory mechanisms potentially associated with icariin intervention are illustrated in Fig. 3.

6. Molecular bridges of multi-organ synergy

6.1. Bioactive substances carried by circulating EVs

6.1.1. General biological rationale for EV-mediated gut–brain communication

Extracellular vesicles (EVs), particularly exosomes, are increasingly regarded as potential mediators of gut–brain axis communication, while gut microbiota-derived EVs (GMEVs) are thought to contribute to bidirectional signaling between the gut and the central nervous system [131, 132]. These nano-sized vesicles can carry various proteins, small RNAs, metabolites, and other bioactive compounds, some of which may influence distant organs, including the brain [133]. Existing evidence suggests that GMEVs can cross or interact with the intestinal epithelial barrier and, under certain conditions, may reach the brain by crossing the blood–brain barrier or through indirect peripheral pathways [131, 134]. Metabolomic studies have detected neuro-related compounds within these vesicles, such as arachidonyl-dopamine, gabapentin, glutamate, and N-acylethanolamines, while *in vivo* studies have shown that they can be transported across intestinal and endothelial barriers and distributed to multiple organs, including the brain [134]. In addition, the detection of EVs in brain regions such as the subventricular zone, a neural stem cell niche, suggests that extracellular signals from distant sources, potentially including gut-derived vesicles, may enter specific brain regions through cerebrospinal fluid or vascular routes, supporting further investigation of microbiome–EV pathways as potential targets for brain health [135, 136].

6.1.2. Icariin-related EV evidence outside the gut–brain axis

Current evidence suggests that icariin may influence EV- or exosome-related intercellular communication, although a complete icariin-regulated gut–brain EV pathway has not been directly established. Based on general EV biology and non-gut–brain icariin-related studies, icariin may theoretically influence EV biogenesis or cargo loading through redox-sensitive pathways; however, this possibility remains to be directly tested in

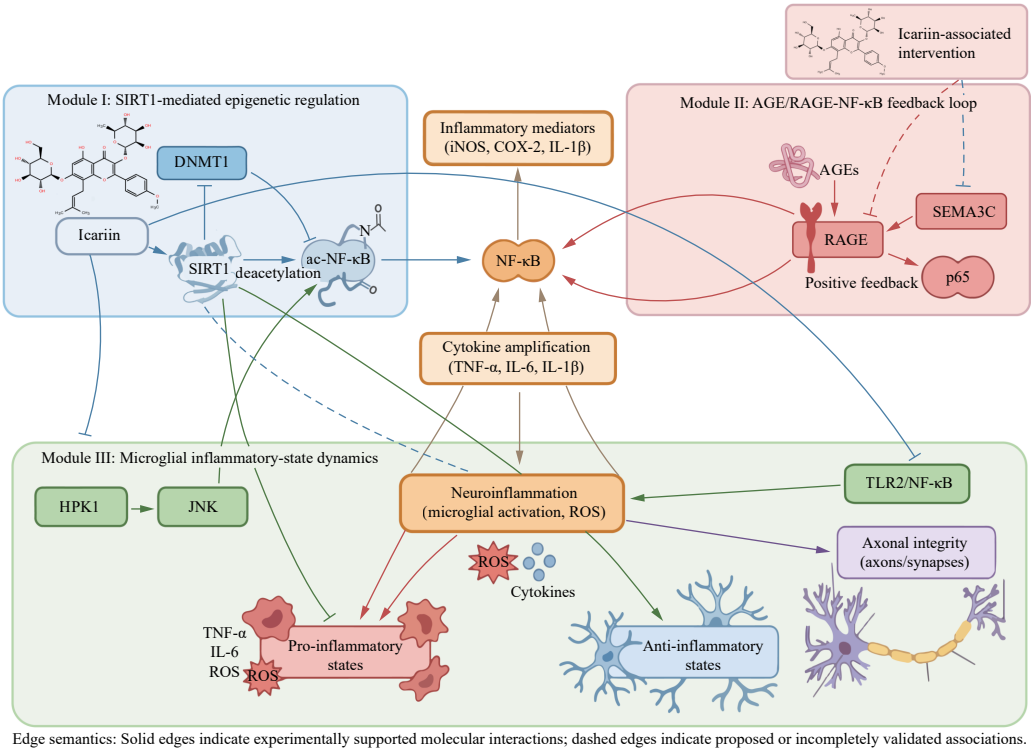


Fig. 3 Central anti-neuroinflammatory regulatory modules potentially associated with icariin intervention. The figure summarizes three interacting central regulatory modules associated with icariin-related neuroinflammatory modulation. Module I depicts SIRT1-mediated epigenetic regulation, in which icariin-related activation of SIRT1 may promote deacetylation of acetylated NF-κB and suppress NF-κB-dependent expression of inflammatory mediators, including inducible nitric oxide synthase, cyclooxygenase-2, and interleukin-1β. DNMT1-related regulation is shown as an additional context-dependent epigenetic component whose relationship with icariin requires further clarification. Module II depicts the AGE/RAGE/NF-κB positive-feedback loop, through which AGE-induced RAGE activation may sustain NF-κB signaling and cytokine amplification; icariin may interfere with selected components of this axis, although direct target engagement and disease-context specificity remain incompletely defined. Module III illustrates microglial inflammatory-state dynamics, including suppression of pro-inflammatory signaling and transition toward anti-inflammatory states through pathways involving JNK, HPK1, TLR2, SIRT1, and NF-κB. These central regulatory modules are better supported than the proposed upstream SCFA-, vagal-, or EV-mediated gut–brain routes, but they should not be interpreted as a single linear pathway or as evidence of causal gut-to-brain transmission. Solid edges indicate reported or experimentally supported molecular relationships, dashed edges indicate proposed or incompletely validated icariin-related associations, arrowheads indicate activation or positive regulation, and T-shaped ends indicate inhibition.

gut–brain axis models [137]. In non-gut–brain models, icariin has been associated with changes in exosomal cargo, including increased circulating exosomal miR-145 in liver fibrosis and carcinogenesis models, and altered osteoclast-derived exosomal miR-331-3p during bone remodeling [138, 139]. In Alzheimer's disease models, icariin may exert neuroprotective effects partly through gut microbiota and lipid metabolism regulation, providing an indirect link to gut–brain signaling [1]. In addition, exosome-based delivery systems, including icariin-loaded serum-derived or adipose-derived stem cell exosomes, have been explored to improve delivery efficiency and modulate inflammatory responses [13, 140]. Overall, the EV route should be regarded as a Tier 3 candidate mechanism for endogenous gut–brain signaling, whereas exosome-based drug delivery should be discussed separately from naturally generated gut-derived EV communication.

6.1.3. Future validation strategies

Exosome-mediated gut–brain communication is increasingly viewed as a potential framework for understanding how intestinal signals may influence neural function [141]. Microfluidic gut–brain axis chip models, which co-culture intestinal epithelial cells with brain endothelial or neuronal cells, enable assessment of barrier integrity through TEER measurements and allow observation of fluorescently labeled exosome transport under controlled conditions [142, 143]. More recently, real-time tracking approaches such as CD63-GFP labeling and nanophotonic biosensing have made it possible to monitor exosome dynamics, although further validation is still needed to clarify underlying mechanisms [144, 145]. Overall, advances in organ-on-chip systems and exosome detection methods may help bridge *in vitro* and *in vivo* studies, while offering a cautious but useful basis for exploring icariin-related regulation of the gut–brain axis.

6.2. Metabolic reprogramming across peripheral and central contexts: current clues and limitations

Icariin has been associated with metabolic changes across multiple organs in preclinical studies. At the intestinal level, icariin or icariin-related interventions have been associated with changes in SCFA-related metabolic profiles and tryptophan metabolism in selected preclinical models [44, 110]. In the central nervous system, these metabolic changes correspond to the upregulation of the glutamate transporters GLT-1 and GLAST in the hippocampal region, thereby maintaining glutamate homeostasis in the brain [5, 146, 147]. Separate studies have reported that icariin can simultaneously regulate the AGE/RAGE metabolic axis in the liver and peripheral blood by inhibiting the accumulation of advanced glycation end products (AGEs), which alleviates systemic inflammatory responses and improves blood–brain barrier function [9, 124, 148]. Notably, SIRT1 activation has been broadly associated with barrier protection, metabolic regulation, and anti-inflammatory effects in experimental models of the gut, liver, and brain; however, its consistent modulation by icariin across these organs has not been directly established [102, 149, 150]. These findings were reported in separate experimental settings and therefore provide cross-context clues rather than direct evidence of coordinated inter-organ regulation.

6.3. Cascade effects of epigenetic modifications

Icariin may participate in multi-organ regulatory responses involving epigenetic mechanisms. At the spinal cord level, it reverses the paclitaxel-induced downregulation of SIRT1 and the abnormal acetylation of histone H4. These observations suggest that peripheral and central epigenetic changes may be functionally linked, although direct transmission across organ systems has not been clearly demonstrated [151]. Separate studies have linked icariin to hippocampal FKBP5/SGK1 expression and have implicated microbiota-related epigenetic regulation in other contexts; a direct mechanistic connection has not been established [120, 152]. Furthermore, icariin activates the non-classical NF-κB pathway through the CEBP-β/G-CSF axis, inducing long-term epigenetic memory of M2 polarization in microglia after retinal ischemia [153]. The major signaling pathways, evidence categories, and current limitations underlying icariin-related anti-neuroinflammatory effects within the gut-brain axis framework are summarized in Table 2.

7. Controversies and challenges in existing research

7.1. Dose-response heterogeneity and reporting limitations across organ systems

The anti-neuroinflammatory effects of icariin appear to show a dose-dependent pattern, although the effective dose range may vary across organ systems and disease models. Current evidence suggests that the central nervous system, particularly the brain and spinal cord, may be relatively responsive to icariin, with commonly effective doses ranging from 10–100 $mg \cdot kg^{-1} \cdot d^{-1}$ [57, 102]. Systemic administration, including oral or intraperitoneal routes, has been associated with reduced inflammatory responses in neurological disorders such as Alzheimer's disease, epilepsy, and cerebral ischemia, especially after relatively prolonged treatment. These effects are reflected by decreased levels of inflammatory mediators such as TNF-α and IL-1β and by modulation of neuroinflammation-related signaling pathways [154]. Potential mechanisms may involve promoting microglial polarization toward the M2 phenotype [6], inhibiting the JNK/cJUN cascade [9], regulating the AGE/RAGE pathway [9], and activating antioxidant pathways such as SIRT-1, Nrf-2, and HO-1 [26].

In an Alzheimer's disease model [155], oral icariin at 40 $mg \cdot kg^{-1} \cdot d^{-1}$ reduced Aβ deposition and Tau hyperphosphorylation and improved cognitive performance, with dose-related effects observed within 10–40 $mg \cdot kg^{-1}$. In aged mice, intraperitoneal administration of 100 $mg \cdot kg^{-1} \cdot d^{-1}$ for 15 days decreased oxidative stress markers in the brain and improved learning and memory [102]. Similarly, in spinal cord injury models, 50–100 $mg \cdot kg^{-1}$ icariin inhibited astrocyte proliferation and supported motor function recovery [156]. These findings suggest that icariin's effects in the CNS may be partly mediated through regulation of microglia and astrocytes, including suppression of iNOS and COX-2 expression [5]. Limited BBB penetration or metabolite-mediated CNS exposure may contribute to the observed CNS effects, although the relationship between dose, tissue exposure, and efficacy remains to be further clarified [27].

Outside the CNS, icariin has also shown antioxidant effects in organs such as the liver and kidneys, where 100 $mg \cdot kg^{-1} \cdot d^{-1}$ reduced MDA levels and increased SOD activity [102]. However, these effects may reflect broader antioxidant protection rather than specific anti-neuroinflammatory activity. In cochlear explants, icariin inhibited gentamicin-induced injury and inflammation, although the effective dose range has not been clearly defined [157]. In an optic nerve ischemia model, local delivery using PLGA-icariin reduced inflammatory death of retinal ganglion cells, suggesting that targeted administration may lower the required systemic dose [153]. Overall, organ-specific responses may be influenced by tissue distribution, blood-brain barrier permeability, absorption efficiency, and local metabolic characteristics. Moreover, combination therapy may further complicate the dose-effect relationship; for example, icariin combined with FPS-ZM1 enhanced anti-neuroinflammatory effects, although the optimal dosing ratio remains uncertain [9].

Table 2 Mechanistic pathways and evidence strength for icariin-related anti-neuroinflammatory effects.

Signaling axis/pathway	Principal effects/readouts	Evidence tier	Key limitation and priority test	Refs
Barrier–LPS–TLR4/NF-κB/STAT axis	↑ ZO-1/occludin/claudin-1; ↓ LPS, NF-κB/STAT1/3, and cytokines	Tier 1 (supported event); Tier 2 (unvalidated gut-to-brain mediation)	Time-course analysis plus LPS/cytokine blockade.	15, 26, 101-105, 116-118, 158
Microbiota–SCFA–HDAC/GPCR axis	Changes in acetate, propionate, and butyrate; GPCR/HDAC signaling; BBB, cytokine, and microglial responses.	Tier 3 (candidate icariin-specific gut–brain mechanism)	Measure fecal, plasma, and brain SCFAs and use antibiotics/FMT, receptor antagonists, and SCFA rescue experiments.	1, 17, 85, 86, 88, 89, 102, 112, 113
Microbiota–tryptophan/kynurenine axis	Changes in indole and kynurenine metabolites and corresponding inflammatory or neuroactive signaling.	Tier 2 (unvalidated gut-to-brain mediation); Tier 3 (candidate icariin-specific gut–brain mechanism)	Icariin-specific causality remains unresolved; targeted metabolomics should be combined with enzyme or receptor perturbation.	1, 44, 113, 147, 176
Vagus nerve/cholinergic anti-inflammatory reflex	Vagal afferent–NTS signaling; α7nAChR-related efferent reflex; reduced peripheral cytokines and CNS inflammatory drive.	Tier 3 (candidate icariin-specific gut–brain mechanism)	Vagotomy or pharmacological blockade, VNS, neural tracing, and electrophysiology are required in icariin-treated models.	79, 83, 94, 95, 98, 100
SIRT1/acetyl-NF-κB axis	↑ SIRT1; ↓ acetylated NF-κB, iNOS, COX-2, and IL-1β; promotion of anti-inflammatory glial states.	Tier 1 (supported event); Tier 2 (unvalidated gut-to-brain mediation)	Better supported as a central mechanism than as a gut-derived pathway; direct CNS effects should be separated from peripheral mechanisms.	57, 119-123, 127, 130, 151
AGE/RAGE/NF-κB axis	Reduced inflammatory amplification and improved vascular or neural injury-related outcomes in selected models.	Tier 2 (unvalidated gut-to-brain mediation)	Direct molecular steps and disease specificity remain uncertain; target engagement requires further validation.	9, 124, 125, 178
Microglial inflammatory-state and polarization signaling	↓ Pro-inflammatory markers and transition toward anti-inflammatory microglial phenotypes.	Tier 1 (supported event)	M1/M2 terminology is reductive; single-cell, spatial, and region-specific analyses are needed.	6, 7, 50, 120, 127-130, 156
IGF-1R/astrocytic inflammatory signaling	Regulation of astrocytic inflammatory responses and IGF-1R-related signaling.	Tier 2 (unvalidated gut-to-brain mediation)	Central relevance is plausible, but its placement within a causal gut–brain route remains uncertain.	5
EV cargo signaling	EV release, biodistribution, target-cell uptake, cargo transfer, and recipient-cell inflammatory responses.	Tier 3 (candidate icariin-specific gut–brain mechanism)	Track tissue origin, inhibit EV release or transfer, sequence cargo, and distinguish naturally generated EVs from delivery vehicles.	131, 132, 134, 137-140, 142-145

Evidence tiers: Tier 1, direct icariin or metabolite evidence for the listed event; Tier 2, icariin-associated evidence without causal gut-to-brain validation; Tier 3, mechanism inferred mainly from broader gut–brain axis literature.

7.2. The regulatory challenge of individualized impact on microbiota composition

Personalized regulation of gut microbiota by icariin may be influenced by several interrelated factors, including baseline microbiota heterogeneity, microbial biotransformation capacity, host inflammatory/barrier status, and external modifiers such as diet, metal ions, or co-treatment.

First, baseline microbiota heterogeneity may affect the predictability of icariin-induced microbial changes. In aged mouse models, icariin restored some beneficial bacteria and suppressed senescence-associated microbial alterations, but the specific taxa affected varied among individuals [102]. Similarly, in AD model mice, icariin combined with saponins modulated gut microbiota and hippocampal protein expression, although the relationship between microbiota changes and disease improvement remains insufficiently standardized [1, 29]. These findings suggest that individual baseline microbial profiles should be considered when evaluating or optimizing icariin-based interventions.

Second, microbial biotransformation capacity may partly determine icariin bioavailability and efficacy. Icariin has been reported to reduce LPS production by affecting ATP synthesis in *Catenibacterium*, thereby contributing to activation of the hepatic PPARα/CD36 axis and lipid metabolism regulation; however, the abundance of this bacterium differs among individuals [101]. In addition, gut microbes such as *Streptococcus* sp. and *Blautia* sp. can convert icariin into active metabolites, including icariside II, but this conversion capacity may vary substantially across microbiota profiles [41, 49]. Therefore, individual differences in microbial metabolism may influence the generation of active compounds and the subsequent host response.

Third, host inflammatory and intestinal barrier baseline status may further shape the regulatory outcomes of icariin. Icariin has been shown to enhance intestinal barrier function by upregulating tight junction proteins such as ZO-1, Occludin, and Claudin-1 in rat models [103], and to alleviate inflammation and support mucosal repair in colitis models [110, 158]. However, the extent to which microbiota remodeling translates into barrier repair may depend on the host's initial inflammatory state, barrier integrity, and broader metabolic or immune phenotype. For example, in aging models, FMT experiments suggest that icariin-related effects may depend partly on donor microbiota composition [102], while in PCOS with insulin resistance, microbiota dysbiosis appears to interact with host metabolic status [159].

Finally, external modifiers, including diet, metal ions, and co-treatment, may add further variability to icariin-mediated microbiota regulation. Transition metal ions such as iron, copper, zinc, and manganese can independently influence gut microbiota structure, and icariin may also interact with metal ion balance, although potential synergistic or antagonistic effects remain unclear [160]. Because metal ion-dependent enzymes participate in microbial metabolism, individual trace element status may modify the apparent effects of icariin on microbiota function [160]. In addition, dietary background and combined interventions may influence microbial composition and metabolic activity, suggesting that these external factors should be considered when interpreting icariin-related microbiota responses.

7.3. The temporal window effect of peripheral and central actions

Icariin-mediated regulation of the gut–brain axis may involve temporal asynchrony between peripheral and central responses. Peripheral changes, such as improvement of intestinal barrier function, can appear relatively early in animal models [103], whereas attenuation of central neuroinflammation may require a longer intervention period [161]. This suggests that peripheral modulation may precede, and potentially contribute to, later central effects. Different mechanisms may also operate on distinct time scales: acute anti-inflammatory responses, vagal signaling, circulating extracellular vesicles, and longer-term metabolic reprogramming may not occur simultaneously [16, 162]. However, current evidence remains insufficient to define a clear time-effect sequence, as most studies lack systematic time-course analyses and causal perturbation designs. Therefore, future studies should better distinguish early peripheral events from subsequent central changes and clarify whether these temporal associations reflect

causal gut–brain interactions.The major controversies, evidence gaps, and recommended validation strategies for future icariin studies are summarized in Table 3.

8. Future research directions and technical pathways

8.1. Multi-omics–integrated systems pharmacology

Multi-omics strategies provide an important framework for investigating how icariin may regulate neuroinflammation *via* the gut–brain axis. Integrative analyses combining gut microbiota profiling, metabolomics, and network pharmacology have suggested potential mechanisms in Alzheimer's disease [1]. In particular, associations between microbial metabolites (e.g., SCFAs) and central inflammatory responses merit further investigation [161]. 16S rRNA sequencing indicates that icariin can modulate gut microbiota composition in APP/PS1 mice, while metabolomic data suggest involvement of the tryptophan–kynurenine pathway [29]. Network pharmacology combined with experimental validation has identified candidate targets, including TLRs signaling and IGF-1 receptor pathways [1, 8]. However, the detailed regulatory network remains unclear. Future integration of spatiotemporal omics may help establish a "component–target–pathway–phenotype" framework to better characterize its cross-organ effects [163].

8.2. Organ-on-a-chip models of gut–brain axis

Organoid and organ-on-a-chip technologies offer new tools to model gut–brain axis interactions and explore the multi-organ effects of icariin [164]. Integrated systems incorporating intestinal organoids, blood–brain barrier models, and brain organoids may enable dynamic assessment of gut-derived mediators and neuroactive substances under icariin intervention [25, 164].

These platforms may also facilitate investigation of intestinal barrier function, neuroinflammation, and gut–brain signaling pathways, including vagal and neurotransmitter-related mechanisms (e.g., 5-HT, CCK) [16, 25]. Coupling electrophysiological analysis with single-cell sequencing could further clarify temporal relationships between intestinal immune responses and microglial activation [7]. Microfluidic systems additionally allow real-time monitoring of signaling molecules such as SCFAs [101]. Collectively, these approaches may support reconstruction of the "microbiota–epithelium–immune–neural" network *in vitro* [165, 166].

8.3. Precision microbiota intervention and combination strategies

Given the heterogeneity of gut microbiota, precision inter-

Table 3 Key translational challenges and validation priorities for icariin-related gut–brain mechanisms.

Translational issue	Current evidence gap	Priority validation strategy	Core evaluation endpoints	Refs
Dose translation, formulation, and long-term use	Differences in extraction, purity, formulation, route, dose, and treatment duration limit comparison across studies. Long-term safety and clinically relevant exposure ranges remain insufficiently characterized.	Standardize chemical composition and formulation, establish exposure–response relationships, and conduct repeated-dose toxicology and interaction studies before efficacy-oriented clinical trials.	Safety; PK	30, 35, 36, 69, 153, 154
Parent compound versus active metabolites	Most studies attribute biological effects to icariin without simultaneously quantifying icariin, icariside II, icaritin, and other relevant metabolites. The relative contributions of the parent compound and its metabolites therefore remain unclear.	Conduct comparative pharmacokinetic and pharmacodynamic studies with simultaneous quantification of the parent compound and major metabolites in plasma, intestinal contents, liver, cerebrospinal fluid, and brain tissue.	PK; MB; NO	36, 41, 42, 45, 67
Microbiota dependence of treatment effects	Changes in microbial composition and host phenotypes are frequently reported in parallel, but direct microbiota-mediated causality is insufficiently demonstrated.	Combine antibiotic depletion, germ-free models, fecal microbiota transplantation, defined microbial consortia, and metabolite-rescue experiments to determine whether treatment effects are microbiota dependent and transferable.	MB; NO	1, 102, 113, 167, 168
SCFA-mediated signaling	Current evidence mainly supports associations among microbial remodeling, SCFA-related changes, barrier regulation, and neuroinflammation. Direct involvement of specific SCFAs, receptors, or HDAC-dependent pathways remains uncertain.	Quantify individual SCFAs and apply receptor antagonists, genetic models, isotope tracing, and SCFA supplementation or depletion to test pathway-specific causality.	MB; NO	1, 88, 102, 112, 113
Vagus-dependent communication	Vagal signaling is biologically plausible, but direct evidence that icariin or its metabolites act through a microbiota–vagus–brain pathway is lacking.	Use selective vagotomy, chemogenetic or optogenetic modulation, electrophysiological recording, and cholinergic receptor blockade to distinguish vagal signaling from circulating immune or metabolic routes.	MB; NO	79, 95, 100
EV-mediated signaling	Gut microbial and host-derived extracellular vesicles are emerging mediators of gut–brain communication, but their involvement in icariin treatment has not been directly established. Evidence from exosome-based drug delivery should not be interpreted as evidence of endogenous vesicle-mediated signaling.	Isolate and characterize microbial and host-derived extracellular vesicles, trace their biodistribution, identify functional cargo, and perform vesicle depletion, transfer, or uptake-blocking experiments.	MB; NO	131, 134, 139, 142, 144, 145
Blood–brain barrier exposure and central target engagement	Neuroprotective effects do not necessarily demonstrate sufficient brain exposure. BBB protection, BBB penetration, and direct central target engagement are often conflated.	Integrate unbound brain-to-plasma ratios, cerebrospinal-fluid measurements, brain microdialysis, transporter studies, imaging, and central target-engagement assays.	PK; MB; NO	35, 45, 54, 63, 69
Cross-organ signaling through the gut, liver, and brain	SIRT1, NF-κB, NLRP3, RAGE, and related pathways have been implicated across different organs and experimental models, but consistent cross-organ modulation by icariin has not been directly demonstrated.	Perform matched temporal analyses in the gut, liver, circulation, and brain, supported by tissue-specific inhibition or genetic intervention, to distinguish parallel pharmacological effects from an integrated signaling axis.	MB; NO	9, 101, 102, 109, 151
Clinical translation	Clinical evidence for icariin in neurological or gut–brain axis disorders is lacking. Human data are limited, brain-targeted formulations remain preclinical, and microbiota-directed trials show inconsistent, non-icariin-specific outcomes.	Conduct stepwise trials using standardized formulations to assess safety, pharmacokinetics, central target engagement, mechanistic biomarkers, microbiota features, and validated neurological outcomes.	Safety; PK; MB; NO	36, 68, 76, 183-187

Note: Safety: safety and tolerability; PK:parent/metabolite pharmacokinetics; MB: mechanism-based biomarkers; NO: validated neurological outcomes.

vention based on metagenomics is likely to be important. FMT studies suggest that microbial composition can influence neuroinflammation in Alzheimer's disease models [167], implying potential synergy between icariin and beneficial microbes such as butyrate-producing bacteria. FMT has also been associated with modulation of glial activation and inflammasome signaling in depression, possibly *via* serotonin-related pathways [168], and shows potential in Parkinson's disease models [169]. Emerging synthetic biology approaches may enable the development of engineered strains responsive to icariin, facilitating sustained production of metabolites such as SCFAs and modulation of the microbiota–tryptophan axis [161, 170-172].

8.4. Artificial intelligence-assisted multi-scale network modeling

Artificial intelligence may contribute to the analysis of multi-level regulatory effects of icariin. Current evidence suggests that icariin may influence immune cell migration, microglial activation, and neuroinflammatory signaling *via* the gut–brain axis [8, 166], with potential temporal variability. Integration of single-cell transcriptomics, metabolic, and electrophysiological data using AI-based approaches could support the construction of predictive models across molecular and organ scales [16, 173]. The BDNF–TrkB pathway may represent an important target associated with neuroprotective and antidepressant-like effects [174]. AI-assisted modeling may also aid in optimizing dosing strategies, although further validation is needed.

9. Summary and implications for translational medicine

9.1. Integrated mechanisms of the multi-organ regulatory network of Icariin

Icariin appears to exert its pharmacological effects through a multi-target and multi-pathway regulatory pattern, which may contribute to coordinated modulation across the intestine, metabolism, immune system, and central nervous system. At the intestinal level, icariin has been reported to regulate gut microbiota composition, including an increase in the abundance of SCFA-producing bacteria [102, 103]. It may also help maintain intestinal barrier integrity and influence inflammation-related signaling pathways, such as IL-17, NF-κB, and MAPK/ERK [101, 175]. These effects represent relatively well-supported peripheral modules, but they should not be interpreted as proof that each downstream gut–brain route is causally required.

At the metabolic level, icariin is associated with changes in lipid metabolic reprogramming and the tryptophan-kynurenine metabolic axis [101, 148, 176], which may further affect peripheral-central immune communication. Within the central nervous system, available evidence suggests that icariin can attenuate microglial activation, reduce the expression of pro-inflammatory mediators including TNF-α, IL-1β, and IL-6, regulate the AGE/RAGE-NF-κB signaling axis, and promote SIRT1-related epigenetic modulation [7, 9, 127]. Together, these effects may form an interconnected regulatory network involved in the modulation of neuroinflammation.

In addition, extracellular vesicles and their cargoes may represent candidate mediators of gut–brain communication, although icariin-specific EV-mediated gut–brain signaling remains to be experimentally confirmed [17, 163]. Thus, the systemic actions of icariin provide a useful framework for hypothesis generation in complex neuroinflammatory disorders, but future studies should explicitly separate direct CNS effects, metabolite-mediated effects, peripheral anti-inflammatory effects, and causal gut–brain communication routes [16, 25]. Evidence level: Tier 2—icariin-associated evidence is available, but causal gut-to-brain mediation has not been established.

9.2. Paradigm innovation in the modernization research of traditional Chinese medicine

The pharmacological actions of icariin reflect the potential value of integrating traditional Chinese medicine concepts with modern biomedical approaches. As an active compound derived from traditional Chinese medicine, icariin may regulate the gut microbiota-immune-neuroendocrine network through multi-target and multi-pathway mechanisms, thereby showing features consistent with holistic regulation [29, 30]. Experimental evidence also suggests that icariin, when combined with other traditional Chinese medicine components such as notoginsenoside, may exert synergistic effects and improve cognitive performance in animal models of Alzheimer's disease [29, 163]. These effects are likely related, at least in part, to the modulation of neuroinflammation through the gut–brain axis.

This strategy preserves the holistic therapeutic perspective of traditional Chinese medicine while incorporating modern insights into microbiome-host interactions, providing a potentially valuable direction for theoretical development and translational application [166]. From a methodological perspective, studies integrating network pharmacology, molecular docking, and experimental validation have adopted a multi-dimensional "component-target-pathway" framework [173, 177, 178]. Through GO and KEGG pathway enrichment analyses, these studies have helped identify possible molecular mechanisms by which icariin may affect key signaling pathways, including TNF, PI3K-Akt, and FoxO [164, 179].

Such research approaches help bridge traditional Chinese medicine theory and modern systems biology, offering a practical technical route for the modernization of Chinese medicine research [178, 180]. Moreover, quality control strategies combining HPLC fingerprinting with multi-component quantitative analysis may improve the reproducibility and standardization of related studies [181]. Future investigations should further clarify the compatibility rules and regulatory networks of Chinese herbal formulas targeting neuroinflammation through the gut–brain axis, thereby supporting the development of more evidence-based therapeutic strategies [27, 30].

9.3. Translational prospects of icariin in neuroinflammatory and gut–brain axis disorders

The pharmacological profile of icariin suggests that it may modulate gut–brain axis-related processes at multiple levels, with preclinical studies indicating potential effects on gut microbiota homeostasis, neuroinflammation, and cognitive impairment [22, 167, 182]. The potential mechanisms underlying the anti-neuroinflammatory effects of icariin include modulation of gut microbial composition and intestine-derived inflammatory signaling [30, 161], remodeling of the host metabolic environment toward a less inflammatory state [17, 163], and enhancement of neuronal resistance to cellular stress through epigenetic regulation [7, 9]. These findings provide preliminary mechanistic support for investigating icariin in the early or preclinical stages of neurodegenerative diseases. However, most available evidence remains preclinical, and neither causal icariin-mediated gut-to-brain signaling nor clinical neurological efficacy has been established.

To date, no definitive clinical trial has demonstrated that icariin is an effective treatment for neuroinflammatory, neurological, or gut–brain axis disorders. Available human evidence for icaritin, the aglycone metabolite of icariin, remains largely confined to oncology. A recent multicenter real-world study reported the clinical effectiveness and tolerability of icaritin in patients with unresectable advanced hepatocellular carcinoma [183], while a multicenter cohort study suggested that icaritin com-

bined with transarterial chemoembolization may improve clinical outcomes in patients with advanced hepatocellular carcinoma and macrovascular invasion [184]. Although these studies support the clinical feasibility of developing an Epimedium-derived compound, their findings cannot be directly extrapolated to neurological or gut–brain axis-related indications because of differences in disease biology, treatment context, dosing regimens, and outcome measures.

Recent formulation studies have sought to overcome the poor aqueous solubility, limited oral bioavailability, rapid systemic elimination, and insufficient brain exposure of icariin. Wang et al. [68] developed an intranasal cyclodextrin-based thermosensitive formulation that enhanced nose-to-brain delivery and increased icariin distribution in relevant brain regions in a preclinical model of Parkinson's disease. Another study developed targeted pH-sensitive nanoliposomes that improved icariin delivery to pathological brain regions and attenuated neuroinflammation, oxidative stress, neuronal injury, and cognitive impairment in an experimental epilepsy model (Wang et al., 2025) [185]. These findings provide encouraging preclinical evidence for brain-targeted delivery but do not establish neurological efficacy, long-term safety, or clinically relevant target engagement in humans.

The clinical translation of icariin for neurological and gut–brain axis disorders will therefore require a multidimensional evaluation framework integrating standardized and chemically characterized formulations, dose–response relationships, therapeutic windows, matched pharmacokinetic profiles of icariin and its active metabolites, brain exposure or central target-engagement biomarkers, systemic inflammatory indices, intestinal-barrier measurements, microbiome composition, microbial metabolites, and validated disease-specific neurological outcomes [57, 177]. The clinical evaluation framework proposed by Zhang et al. [186] further emphasizes the need to link microbiome alterations with mechanistic biomarkers and clinically meaningful neurological endpoints rather than relying solely on changes in microbial composition.

Clinical findings from microbiota-directed interventions also remain heterogeneous. In a randomized clinical trial, Scheperjans et al. [187] found that fecal microbiota transplantation induced sustained and donor-dependent alterations in the gut microbiota of patients with Parkinson's disease but did not produce clinically meaningful symptomatic improvement compared with placebo. This negative result highlights the importance of adequate placebo control, sufficient follow-up, careful participant and donor stratification, prespecified mechanistic analyses, and cautious interpretation of microbiome changes.

Future studies should further evaluate gut-targeted and brain-targeted delivery systems to improve local exposure, metabolic conversion, barrier penetration, and tissue-specific pharmacological effects [188]. AI-assisted multiscale modeling may help prioritize candidate mechanisms, therapeutic combinations, and biomarkers; however, computational predictions should be validated through causal perturbation experiments before being used to support translational conclusions [189, 190]. Collectively, oncology studies, brain-targeted formulation studies, and microbiota-directed clinical trials provide complementary evidence for clinical feasibility and trial design but should not be interpreted as direct proof of therapeutic effectiveness in neurological or gut–brain axis disorders. Evidence level: Tier 2—icariin-associated mechanistic and preclinical evidence is available, but causal gut-to-brain mediation and clinical neurological efficacy have not been established.

Overall, these findings support further investigation of icariin as a potential multilevel modulator of neuroinflammatory and gut–brain axis-related processes, while its clinical relevance remains to be established through rigorously designed translational studies.

Declaration of competing interest

These authors have no conflict of interest to declare.

References

1 Liu Y, Li H, Wang X, et al. Anti-Alzheimers molecular mechanism of icariin: insights from gut microbiota, metabolomics, and network pharmacology. *J Transl Med*. 2023;21(1):277. https://doi.org/10.1186/s12967-023-04137-z.
2 Sabry HA, Zahra MM. Icariin attenuates dopaminergic neural loss in haloperidol-induced Parkinsonism in rats *via* GSK-3β and tyrosine hydroxylase regulation mechanism. *J Chem Neuroanat*. 2024;136:102385. https://doi.org/10.1016/j.jchemneu.2023.102385.
3 Morais LH, Schreiber HLth, Mazmanian SK. The gut microbiota-brain axis in behaviour and brain disorders. *Nat Rev Microbiol*. 2021;19(4):241-255. https://doi.org/10.1038/s41579-020-00460-0.
4 Roy S, Saha P, Bose D, et al. Periodic heat waves-induced neuronal etiology in the elderly is mediated by gut-liver-brain axis: a transcriptome profiling approach. *Sci Rep*. 2024;14(1):10555. https://doi.org/10.1038/s41598-024-60664-9.
5 Zhang WD, Li N, Du ZR, et al. IGF-1 receptor is involved in the regulatory effects of icariin and icaritin in astrocytes under basal conditions and after an inflammatory challenge. *Eur J Pharmacol*. 2021;906:174269. https://doi.org/10.1016/j.ejphar.2021.174269.
6 Wang J, Liu Y, Wu Y, et al. Anti-inflammatory effects of icariin in the acute and chronic phases of the mouse pilocarpine model of epilepsy. *Eur J Pharmacol*. 2023;960:176141. https://doi.org/10.1016/j.ejphar.2023.176141.
7 Yu Z, Su G, Zhang L, et al. Icaritin inhibits neuroinflammation in a rat cerebral ischemia model by regulating microglial polarization through the GPER-ERK-NF-κB signaling pathway. *Mol Med*. 2022;28(1):142. https://doi.org/10.1186/s10020-022-00573-7.
8 Rahman Z, Padhy HP, Dandekar MP. Cell-free supernatant of *Lactobacillus rhamnosus* and *Bifidobacterium breve* ameliorates ischemic stroke-generated neurological deficits in rats. *Probiotics Antimicrob Proteins*. 2025;17(5):3083-3099. https://doi.org/10.1007/s12602-024-10256-w.
9 Wang X, Chen W, Yuan P, et al. RAGE acted as a new anti-inflammatory target for Icariin's treatment against vascular dementia based on network pharmacology-directed verification. *J Biomol Struct Dyn*. 2024;42(19):10189-10209. https://doi.org/10.1080/07391102.2023.2256409.
10 Borsom EM, Keefe CR, Hirsch AH, et al. A longitudinal study of the unique gut microbiota of 3xTg-AD mice modeling key AD pathologies. *Alzheimers Dement*. 2021;17(S3):e054633. https://doi.org/10.1002/alz.054633.
11 Busayli AM, Xu W, Raffah GA, et al. Gut microbiome, neuroinflammation, and fetal alcohol spectrum disorders: insights from rodent models. *Biology (Basel)*. 2025;14(6):593. https://doi.org/10.3390/biology14060593.
12 Wanyi Z, Jiao Y, Wen H, et al. Bidirectional communication of the gut-brain axis: new findings in Parkinson's disease and inflammatory bowel disease. *Front Neurol*. 2024;15:1407241. https://doi.org/10.3389/fneur.2024.1407241.
13 Angeloni C, Barbalace MC, Hrelia S. Icariin and its metabolites as potential protective phytochemicals against Alzheimer's disease. *Front Pharmacol*. 2019;10:00271. https://doi.org/10.3389/fphar.2019.00271.
14 Lin YC, Pan YJ, Chang SM, et al. Transcranial ultrasound stimulation ameliorates dextran sulphate sodium-induced colitis and behavioural disorders by suppressing the inflammatory response in the brain. *Brain Commun*. 2025;7(2):fcaf119. https://doi.org/10.1093/braincomms/fcaf119.
15 Li T, Chen H, Xu B, et al. Deciphering the interplay between LPS/TLR4 pathways, neurotransmitter, and deltamethrin-induced depressive-like behavior: perspectives from the gut-brain axis. *Pestic Biochem Physiol*. 2023;197:105697. https://doi.org/10.1016/j.pestbp.2023.105697.
16 Liu T, Wu H, Wei J. Beyond the brain: exploring the multi-organ axes in Parkinson's disease pathogenesis. *J Adv Res*. 2026;80:451-474. https://doi.org/10.1016/j.jare.2025.05.034.
17 Gao H, Song R, Li Y, et al. Effects of oat fiber intervention on cognitive behavior in LDLR$^{(-/-)}$ mice modeling atherosclerosis by targeting the microbiome-gut-brain axis. *J Agric Food Chem*. 2020;68(49):14480-14491. https://doi.org/10.1021/acs.jafc.0c05677.
18 Khezri MR, Ghasemnejad-Berenji M. Icariin: a potential neuroprotective agent in Alzheimer's disease and Parkinson's disease. *Neurochem Res*. 2022;47(10):2954-2962. https://doi.org/10.1007/s11064-022-03667-0.
19 Ye T, Yuan S, Kong Y, et al. Effect of probiotic fungi against cognitive impairment in mice *via* regulation of the fungal microbiota-gut-brain axis. *J Agric Food Chem*. 2022;70(29):9026-9038. https://doi.org/10.1021/acs.jafc.2c03142.
20 Richards EM, Li J, Stevens BR, et al. Gut microbiome and neuroinflammation in hypertension. *Circ Res*. 2022;130(3):401-417. https://doi.org/10.1161/circresaha.121.319816.
21 Zhu J, Fu Y, Olovo CV, et al. The influence of gut microbiota on the gut-brain-kidney axis and its implications for chronic kidney disease. *Front Microbiol*. 2025;16:1535356. https://doi.org/10.3389/fmicb.2025.1535356.
22 Xu TC, Lv Y, Liu QY, et al. Long-term atorvastatin improves cognitive decline by regulating gut function in naturally ageing rats. *Immun Ageing*. 2022;19(1):52. https://doi.org/10.1186/s12979-022-00311-x.
23 Jin J, Wang H, Hua X, et al. An outline for the pharmacological effect of icariin in the nervous system. *Eur J Pharmacol*. 2019;842:20-32. https://doi.org/10.1016/j.ejphar.2018.10.006.

24 Jeong DY, Ryu MS, Yang HJ, et al. γ-PGA-rich chungkookjang, short-term fermented soybeans: prevents memory impairment by modulating brain insulin sensitivity, neuro-inflammation, and the gut-microbiome-brain axis. *Foods*. 2021;10(2):221. https://doi.org/10.3390/foods10020221.

25 Tran VTA, Lee LP, Cho H. Neuroinflammation in neurodegeneration *via* microbial infections. *Front Immunol*. 2022;13:907804. https://doi.org/10.3389/fimmu.2022.907804.

26 Yao WH, Tao RP, Wang K, et al. Icariin attenuates vascular endothelial dysfunction by inhibiting inflammation through GPER/Sirt1/HMGB1 signaling pathway in type 1 diabetic rats. *Chin J Nat Med*. 2024;22(4):293-306. https://doi.org/10.1016/S1875-5364(24)60618-7.

27 Sharma S, Mehan S, Khan Z, et al. Exploring the neuroprotective potential of Icariin through modulation of neural pathways in the treatment of neurological diseases. *Curr Mol Med*. 2025;25(8):962-979. https://doi.org/10.2174/0115665240317650240924041923.

28 Mythri RB, Aishwarya MRB. Biopolymers as promising vehicles for drug delivery to the brain. *Drug Metab Rev*. 2024;56(1):46-61. https://doi.org/10.1080/03602532.2023.2281855.

29 Zhang T, Dong K, Xiao L, et al. Effects of co-administration of icariin and panax notoginseng saponins on intestinal microbiota and hippocampal protein expression in a mouse model of Alzheimer's disease. *Neuropsychiatr Dis Treat*. 2020;16:2169-2179. https://doi.org/10.2147/ndt.S253972.

30 Zhu T, Zhang F, Li H, et al. Long-term icariin treatment ameliorates cognitive deficits *via* CD4(+) T cell-mediated immuno-inflammatory responses in APP/PS1 mice. *Clin Interv Aging*. 2019;14:817-826. https://doi.org/10.2147/cia.S208068.

31 Ionescu VS, Popa A, Alexandru A, et al. Dietary phytoestrogens and their metabolites as epigenetic modulators with impact on human health. *Antioxidants (Basel)*. 2021;10(12):1893. https://doi.org/10.3390/antiox10121893.

32 Cao YF, He RR, Cao J, et al. Drug-drug interactions potential of icariin and its intestinal metabolites via inhibition of intestinal UDP-glucuronosyltransferases. *Evid Based Complement Alternat Med*. 2012;2012:395912. https://doi.org/10.1155/2012/395912.

33 Hu W, Si Y, Xie X, et al. Research progress on icariin promoting bone injury repair and regeneration. *Pharmaceuticals (Basel)*. 2025;18(8):1174. https://doi.org/10.3390/ph18081174.

34 Ma M, Fan AY, Liu Z, et al. Baohuoside I inhibits osteoclastogenesis and protects against ovariectomy-induced bone loss. *Front Pharmacol*. 2022;13:874952. https://doi.org/10.3389/fphar.2022.874952.

35 Szabó R, Rácz CP, Dulf FV. Bioavailability improvement strategies for icariin and its derivates: A Review. *Int J Mol Sci*. 2022;23(14):7519. https://doi.org/10.3390/ijms23147519.

36 Brown ES, Bice C, Putnam WC, et al. Human safety and pharmacokinetics study of orally administered icariin: randomized, double-blind, placebo-controlled trial. *Nat Prod Commun*. 2019;14(6):1934578X19856789. https://doi.org/10.1177/1934578x19856789.

37 Mensah A, Chen Y, Asinyo BK, et al. Bioactive icariin/β-CD-IC/bacterial cellulose with enhanced biomedical potential. *Nanomaterials*. 2021;11(2):387. https://doi.org/10.3390/nano11020387.

38 Asfour HZ, Alhakamy NA, Fahmy UA, et al. Zein-stabilized nanospheres as nanocarriers for boosting the aphrodisiac activity of icariin: response surface optimization and in vivo assessment. *Pharmaceutics*. 2022;14(6):1279. https://doi.org/10.3390/pharmaceutics14061279.

39 Chen Y, Wang J, Jia X, et al. Role of intestinal hydrolase in the absorption of prenylated flavonoids present in yinyanghuo. *Molecules*. 2011;16(2):1336-1348. https://doi.org/10.3390/molecules16021336.

40 Seong SH, Kim SH, Ryu JH, et al. Effects of icariin and its metabolites on GPCR regulation and MK-801-induced schizophrenia-like behaviors in mice. *Molecules*. 2023;28(21):7300. https://doi.org/10.3390/molecules28217300.

41 Wu H, Kim M, Han J. Icariin metabolism by human intestinal microflora. *Molecules*. 2016;21(9):1158. https://doi.org/10.3390/molecules21091158.

42 Cheng T, Zhang Y, Zhang T, et al. Comparative pharmacokinetics study of icariin and icariside II in rats. *Molecules*. 2015;20(12):21274-21286. https://doi.org/10.3390/molecules201219763.

43 Li N, Yuan M, Che J. Development and validation of UPLC-MS/MS method for icariin and its metabolites in mouse urine. *Front Pharmacol*. 2024;15:1389754. https://doi.org/10.3389/fphar.2024.1389754.

44 Chen G, Cao Z, Shi Z, et al. Microbiome analysis combined with targeted metabolomics reveal immunological anti-tumor activity of icariside I in a melanoma mouse model. *Biomed Pharmacother*. 2021;140:111542. https://doi.org/10.1016/j.biopha.2021.111542.

45 Xu S, Yu J, Zhan J, et al. Pharmacokinetics, tissue distribution, and metabolism study of icariin in rat. *Biomed Res Int*. 2017;2017:4684962. https://doi.org/10.1155/2017/4684962.

46 Ouyang X, Ma Q, Zhou C, et al. Natural bioactive products in the regulation of bone metabolism and regeneration. *Front Pharmacol*. 2025;16:1683279. https://doi.org/10.3389/fphar.2025.1683279.

47 Zhang B, Chen X, Zhang R, et al. Metabolite profiling, pharmacokinetics, and in vitro glucuronidation of icaritin in rats by ultra-performance liquid chromatography coupled with mass spectrometry. *J Anal Methods Chem*. 2017;2017(1):1073607. https://doi.org/10.1155/2017/1073607.

48 Zhou J, Chen Y, Wang Y, et al. A comparative study on the metabolism of epimedium koreanum nakai-prenylated flavonoids in rats by an intestinal enzyme (lactase phlorizin hydrolase) and intestinal flora. *Molecules*. 2014;19(1):177-203. https://doi.org/10.3390/molecules19010177.

49 Wang G, Yan F, Wang Y, et al. Visual sensing of β-glucosidase from intestinal fungus in the generation of cytotoxic icarisid II. *Front Chem*. 2022;10:919624. https://doi.org/10.3389/fchem.2022.919624.

50 Zhang B, Wang G, He J, et al. Icariin attenuates neuroinflammation and exerts dopamine neuroprotection via an Nrf2-dependent manner. *J Neuroinflammation*. 2019;16(1):92. https://doi.org/10.1186/s12974-019-1472-x.

51 Wang M, Yang X, Zhou Q, et al. Neuroprotective mechanism of icariin on hypoxic ischemic brain damage in neonatal mice. *Oxid Med Cell Longev*. 2022;2022:1330928. https://doi.org/10.1155/2022/1330928.

52 Li W, Liu E, Zhou Y, et al. Therapeutic potential of natural products in ischemic stroke: targeting angiogenesis. *Front Pharmacol*. 2025;16:1579172. https://doi.org/10.3389/fphar.2025.1579172.

53 Niu P, Zhang Y, Wang B, et al. Modulation of neuroinflammation by natural plant compounds: a promising approach for ischemic stroke. *Front Pharmacol*. 2025;16:1603417. https://doi.org/10.3389/fphar.2025.1603417.

54 An HM, Li MN, Yang H, et al. A validated UHPLC-MS/MS method for pharmacokinetic and brain distribution studies of twenty constituents in rat after oral administration of Jia-Wei-Qi-Fu-Yin. *J Pharm Biomed Anal*. 2021;202:114140. https://doi.org/10.1016/j.jpba.2021.114140.

55 Li XA, HO YS, Chen L, et al. The protective effects of icariin against the homocysteine-induced neurotoxicity in the primary embryonic cultures of rat cortical neurons. *Molecules*. 2016;21(11):1557. https://doi.org/10.3390/molecules21111557.

56 Guo Y, Cai Y, Zhang X. Icariin ameliorates the cognitive function in an epilepsy neonatal rat model by blocking the GluR2/ERK I/II pathway. *Folia Neuropathol*. 2020;58(3):245-252. https://doi.org/10.5114/fn.2020.100067.

57 Li LR, Sethi G, Zhang X, et al. The neuroprotective effects of icariin on ageing, various neurological, neuropsychiatric disorders, and brain injury induced by radiation exposure. *Aging (Albany NY)*. 2022;14(3):1562-1588. https://doi.org/10.18632/aging.203893.

58 Zeng NX, Li HZ, Wang HZ, et al. Exploration of the mechanism by which icariin modulates hippocampal neurogenesis in a rat model of depression. *Neural Regen Res*. 2022;17(3):632-642. https://doi.org/10.4103/1673-5374.320993.

59 Fehsel K. Why is iron deficiency/anemia linked to Alzheimer's disease and its comorbidities, and how is it prevented? *Biomedicines*. 2023;11(9):2421. https://doi.org/10.3390/biomedicines11092421.

60 Skapetze L, Owino S, Lo EH, et al. Rhythms in barriers and fluids: Circadian clock regulation in the aging neurovascular unit. *Neurobiol Dis*. 2023;181:106120. https://doi.org/10.1016/j.nbd.2023.106120.

61 Pan P, Zhao T, Zhang J, et al. Pharmacological mechanisms and therapeutic prospects of plant metabolites from traditional chinese medicine in regulating programmed cell death in Alzheimer's disease. *Front Pharmacol*. 2025;16:1605214. https://doi.org/10.3389/fphar.2025.1605214.

62 Feng F, Li Y, Huang N, et al. Icaritin, an inhibitor of beta-site amyloid cleaving enzyme-1, inhibits secretion of amyloid precursor protein in APP-PS1-HEK293 cells by impeding the amyloidogenic pathway. *PeerJ*. 2019;7:e8219. https://doi.org/10.7717/peerj.8219.

63 Liu MB, Wang W, Gao JM, et al. Icariside II attenuates cerebral ischemia/reperfusion-induced blood-brain barrier dysfunction in rats via regulating the balance of MMP9/TIMP1. *Acta Pharmacol Sin*. 2020;41(12):1547-1556. https://doi.org/10.1038/s41401-020-0409-3.

64 Zhou J, Deng Y, Li F, et al. Icariside II attenuates lipopolysaccharide-induced neuroinflammation through inhibiting TLR4/MyD88/NF-κB pathway in rats. *Biomed Pharmacother*. 2019;111:315-324. https://doi.org/10.1016/j.biopha.2018.10.201.

65 Zheng Y, Deng Y, Gao JM, et al. Icariside II inhibits lipopolysaccharide-induced inflammation and amyloid production in rat astrocytes by regulating IKK/IκB/NF-κB/BACE1 signaling pathway. *Acta Pharmacol Sin*. 2020;41(2):154-162. https://doi.org/10.1038/s41401-019-0300-2.

66 Xie Q, Li H, Lu D, et al. Neuroprotective effect for cerebral ischemia by natural products: a review. *Front Pharmacol*. 2021;12:607412. https://doi.org/10.3389/fphar.2021.607412.

67 Liu W, Li X, Li N, et al. UPLC-MS/MS method for icariin and metabolites in whole blood of C57 mice: development, validation, and pharmacokinetics study. *Front Pharmacol*. 2023;14:1195525. https://doi.org/10.3389/fphar.2023.1195525.

68 Wang YQ, Wang LM, Han LF, et al. A promising strategy of brain targeted delivery for the treatment of Parkinson's disease: cyclodextrin supramolecular inclusion complex based thermosensitive gel. *J Pharm Anal*. 2025;15(5):101102. https://doi.org/10.1016/j.jpha.2024.101102.

69 Wang J, Kong L, Guo RB, et al. Multifunctional icariin and tanshinone IIA co-delivery liposomes with potential application for Alzheimer's disease. *Drug Deliv*. 2022;29(1):1648-1662. https://doi.org/10.1080/10717544.2022.2072543.

70 Chen M, Wu J, Luo Q, et al. The anticancer properties of herba epimedii and its main bioactive componentsicariin and icariside II. *Nutrients*. 2016;8(9):563. https://doi.org/10.3390/nu8090563.

71 Luo P, An Y, He J, et al. Icaritin with autophagy/mitophagy inhibitors synergistically enhances anticancer efficacy and apoptotic effects through PINK1/Parkin-mediated mitophagy in hepatocellular carcinoma. *Cancer Lett*. 2024;587:216621. https://doi.org/10.1016/j.canlet.2024.216621.

72 Tan HL, Chan KG, Pusparajah P, et al. Anti-cancer properties of the naturally occurring aphrodisiacs: icariin and its derivatives. *Front Pharmacol*. 2016;7:00191. https://doi.org/10.3389/fphar.2016.00191.

73 Kamal NH, Heikal LA, Helmy MW, et al. Bromelain-functionalized omega-3 nanocarriers for targeted icariin delivery: a multifunctional shield for cardiac repair in doxorubicin-induced injury. *J Mater Sci Mater Med*. 2025;36(1):115. https://doi.org/10.1007/s10856-025-06915-1.

74 Liu FY, Ding DN, Wang YR, et al. Icariin as a potential anticancer agent: a review of its biological effects on various cancers. *Front Pharmacol*. 2023;14:1216363. https://doi.org/10.3389/fphar.2023.1216363.

75 Luo P, Chen J, An Y, et al. Icaritin ameliorates cisplatin-induced

mitochondrial metabolic dysfunction-associated nephrotoxicity and synergistically potentiates its antitumor efficacy. *Adv Sci (Weinh)*. 2026;13(7):e06712. https://doi.org/10.1002/advs.202506712.
76 He J, Zhuang L, Lei Y, et al. Clinical efficacy and safety of icaritin in patients with hepatocellular carcinoma: a real-world study. *Front Pharmacol*. 2026;17:1677794. https://doi.org/10.3389/fphar.2026.1677794.
77 Mi J, Yang Y, Zhu X, et al. Osteogenic action of the natural plant material icariin and its potential use in biomedical tissue engineering. *Am J Transl Res*. 2025;17(8):5814-5828. https://doi.org/10.62347/ltgz9375.
78 Goyal K, Afzal M, Babu MA, et al. Icariin as a therapeutic agent in breast cancer: modulating apoptosis and suppressing proliferation. *J Complement Integr Med*. 2025;22(3):417-426. https://doi.org/10.1515/jcim-2024-0405.
79 Ma X, Kim JK, Shin YJ, et al. Lipopolysaccharide-producing Veillonella infantium and Escherichia fergusonii cause vagus nerve-mediated cognitive impairment in mice. *Brain Behav Immun*. 2024;118:136-148. https://doi.org/10.1016/j.bbi.2024.02.031.
80 Zhao Z, Ning J, Bao XQ, et al. Fecal microbiota transplantation protects rotenone-induced Parkinson's disease mice via suppressing inflammation mediated by the lipopolysaccharide-TLR4 signaling pathway through the microbiota-gut-brain axis. *Microbiome*. 2021;9(1):226. https://doi.org/10.1186/s40168-021-01107-9.
81 Luo YX, Yang LL, Yao XQ. Gut microbiota-host lipid crosstalk in Alzheimer's disease: implications for disease progression and therapeutics. *Mol Neurodegener*. 2024;19(1):35. https://doi.org/10.1186/s13024-024-00720-0.
82 Ding Y, Yu B, Zhou S, et al. Improvement of solubility and pharmacokinetic profile of hepatoprotector icariin through complexation with HP-γ-cyclodextrin. *Front Pharmacol*. 2023;14:1138686. https://doi.org/10.3389/fphar.2023.1138686.
83 Oyovwi MO, Udi OA. The gut-brain axis and neuroinflammation in traumatic brain injury. *Mol Neurobiol*. 2025;62(4):4576-4590. https://doi.org/10.1007/s12035-024-04585-8.
84 Xie P, Ma Z, Yang X, et al. The regulatory effects and mechanisms of plant food-derived bioactive components on gut barrier function and intestinal homeostasis: a comprehensive review. *Crit Rev Food Sci Nutr*. 2025;65(31):7810-7833. https://doi.org/10.1080/10408398.2025.2481983.
85 Taherkhani S, Ahmadi P, Nasiraie LR, et al. Flavonoids and the gut microbiome: a powerful duo for brain health. *Crit Rev Food Sci Nutr*. 2025;65(28):6029-6044. https://doi.org/10.1080/10408398.2024.2435593.
86 Baky MH, Elshahed M, Wessjohann L, et al. Interactions between dietary flavonoids and the gut microbiome: a comprehensive review. *Br J Nutr*. 2022;128(4):577-591. https://doi.org/10.1017/s0007114521003627.
87 Lin CH, Chen CC, Chiang HL, et al. Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. *J Neuroinflammation*. 2019;16(1):129. https://doi.org/10.1186/s12974-019-1528-y.
88 Wei H, Yu C, Zhang C, et al. Butyrate ameliorates chronic alcoholic central nervous damage by suppressing microglia-mediated neuroinflammation and modulating the microbiome-gut-brain axis. *Biomed Pharmacother*. 2023;160:114308. https://doi.org/10.1016/j.biopha.2023.114308.
89 Skawratananond S, McCrea GE, Lie P, et al. The synergistic interplay between vitamin A, dietary fiber, and the microbiota-gut-brain axis: a potential mechanism for preventing Alzheimer's disease. *Am J Physiol Gastrointest Liver Physiol*. 2025;329(3):G484-g499. https://doi.org/10.1152/ajpgi.00097.2025.
90 Holcomb M, Marshall AG, Flinn H, et al. Probiotic treatment induces sex-dependent neuroprotection and gut microbiome shifts after traumatic brain injury. *J Neuroinflammation*. 2025;22(1):114. https://doi.org/10.1186/s12974-025-03419-1.
91 Mao Z, Hui H, Zhao X, et al. Protective effects of dioscin against Parkinson's disease via regulating bile acid metabolism through remodeling gut microbiome/GLP-1 signaling. *J Pharm Anal*. 2023;13(10):1153-1167. https://doi.org/10.1016/j.jpha.2023.06.007.
92 Salinas E, Reyes-Pavón D, Cortes-Perez NG, et al. Bioactive compounds in food as a current therapeutic approach to maintain a healthy intestinal epithelium. *Microorganisms*. 2021;9(8):1634. https://doi.org/10.3390/microorganisms9081634.
93 Tang W, Chen D, Yu B, et al. Capsulized faecal microbiota transplantation ameliorates post-weaning diarrhoea by modulating the gut microbiota in piglets. *Vet Res*. 2020;51(1):55. https://doi.org/10.1186/s13567-020-00779-9.
94 Wang X, Chen Z, Geng B, et al. The bidirectional signal communication of microbiota-gut-brain axis in hypertension. *Int J Hypertens*. 2021;2021:8174789. https://doi.org/10.1155/2021/8174789.
95 Faraji N, Payami B, Ebadpour N, et al. Vagus nerve stimulation and gut microbiota interactions: a novel therapeutic avenue for neuropsychiatric disorders. *Neurosci Biobehav Rev*. 2025;169:105990. https://doi.org/10.1016/j.neubiorev.2024.105990.
96 Vilela C, Araújo B, Soares-Guedes C, et al. From the gut to the brain: is microbiota a new paradigm in Parkinson's disease treatment? *Cells*. 2024;13(9):770. https://doi.org/10.3390/cells13090770.
97 Naqvi S, Asar TO, Kumar V, et al. A cross-talk between gut microbiome, salt and hypertension. *Biomed Pharmacother*. 2021;134:111156. https://doi.org/10.1016/j.biopha.2020.111156.
98 Claudino Dos Santos JC, Oliveira LF, Noleto FM, et al. Gut-microbiome-brain axis: the crosstalk between the vagus nerve, alpha-synuclein and the brain in Parkinson's disease. *Neural Regen Res*. 2023;18(12):2611-2614. https://doi.org/10.4103/1673-5374.373673.
99 Bostick JW, Schonhoff AM, Mazmanian SK. Gut microbiome-mediated regulation of neuroinflammation. *Curr Opin Immunol*. 2022;76:102177. https://doi.org/10.1016/j.coi.2022.102177.
100 Zhang Y, Xie B, Chen X, et al. A key role of gut microbiota-vagus nerve/spleen axis in sleep deprivation-mediated aggravation of systemic inflammation after LPS administration. *Life Sci*. 2021;265:118736. https://doi.org/10.1016/j.lfs.2020.118736.
101 Wei Y, Mao J, Tang W, et al. Targeting Catenibacterium mitsuokai with icariin modulates gut microbiota and improves hepatic lipid metabolism in intrauterine growth restriction. *ISME J*. 2025;19(1):wraf141. https://doi.org/10.1093/ismejo/wraf141.
102 Li X, Khan I, Xia W, et al. Icariin enhances youth-like features by attenuating the declined gut microbiota in the aged mice. *Pharmacol Res*. 2021;168:105587. https://doi.org/10.1016/j.phrs.2021.105587.
103 Li Y, Liu J, Pongkorpsakol P, et al. Relief effects of icariin on inflammation-induced decrease of tight junctions in intestinal epithelial cells. *Front Pharmacol*. 2022;13:903762. https://doi.org/10.3389/fphar.2022.903762.
104 Soh H, Park S, Lee HJ, et al. P104 icariin attenuates intestinal inflammation through inhibition of the nuclear factor-κB signaling. *J Crohns Colitis*. 2022;16(Supplement_1):i199-i200. https://doi.org/10.1093/ecco-jcc/jjab232.232.
105 Xiong W, Ma H, Zhang Z, et al. The protective effect of icariin and phosphorylated icariin against LPS-induced intestinal epithelial cells injury. *Biomed Pharmacother*. 2019;118:109246. https://doi.org/10.1016/j.biopha.2019.109246.
106 Chen Y, Hamidu S, Yang X, et al. Dietary supplements and natural products: an update on their clinical effectiveness and molecular mechanisms of action during accelerated biological aging. *Front Genet*. 2022;13:880421. https://doi.org/10.3389/fgene.2022.880421.
107 Li X, Zhang Y, Duan L. Microbiome-kidney metabolism provides insight into the mechanism of icariin alleviates aging in older female mice. *Innov Aging*. 2024;8(Supplement_1):878-879. https://doi.org/10.1093/geroni/igae098.2840.
108 Zhang Y, Silang Q, Wang Y, et al. Integrated gut microbiota, metabolomics, and network pharmacology to investigate the anti-Alzheimer's mechanism of tripterygium glycoside. *Neuropsychiatr Dis Treat*. 2025;21:1911-1933. https://doi.org/10.2147/ndt.S537129.
109 Li C, Tang Y, Ye Y, et al. Potential of natural flavonols and flavanones in the treatment of ulcerative colitis. *Front Pharmacol*. 2023;14:1120616. https://doi.org/10.3389/fphar.2023.1120616.
110 Zhang C, Cao Z, Lei H, et al. Discovery of a novel small molecule with efficacy in protecting against inflammation in vitro and in vivo by enhancing macrophages activation. *Biomed Pharmacother*. 2023;165:115273. https://doi.org/10.1016/j.biopha.2023.115273.
111 Liang J, Li S, Dai Y, et al. Integrating transcriptomics and gut microbiota analysis reveals the anti-Osteoporotic mechanisms of wine and oil co-processed epimedium. *Faseb j*. 2026;40(2):e71319. https://doi.org/10.1096/fj.202501534RR.
112 Wang S, Wang S, Wang X, et al. Effects of icariin on modulating gut microbiota and regulating metabolite alterations to prevent bone loss in ovariectomized rat model. *Front Endocrinol (Lausanne)*. 2022;13:874849. https://doi.org/10.3389/fendo.2022.874849.
113 Dong Y, Wang L, Yang M, et al. Effect of icariin on depressive behaviour in rat pups. Evidences for its mechanism of action by integrating network pharmacology, metabolomics and gut microbiota composition. *Phytomedicine*. 2024;126:155422. https://doi.org/10.1016/j.phymed.2024.155422.
114 Song W, Ren C, Dong Y, et al. Icariin ameliorates exercise-induced acute muscle injury and inflammation by regulating the expression of NF-κB and shaping gut microbiota in mice. *Int J Vitam Nutr Res*. 2025;95(6):39821. https://doi.org/10.31083/ijvnr39821.
115 Ji J, Phang HC, Gu X, et al. A review on colorectal cancer and the role of traditional chinese herbal medicine as complementary therapy. *Progress In Microbes & Molecular Biology*. 2024;7(1). https://doi.org/10.36877/pmmb.a0000451.
116 Bano N, Khan S, Ahamad S, et al. Microglia and gut microbiota: A double-edged sword in Alzheimer's disease. *Ageing Res Rev*. 2024;101:102515. https://doi.org/10.1016/j.arr.2024.102515.
117 O'Riordan KJ, Moloney GM, Keane L, et al. The gut microbiota-immune-brain axis: therapeutic implications. *Cell Rep Med*. 2025;6(3):101982. https://doi.org/10.1016/j.xcrm.2025.101982.
118 Cui D, Li M, Liu M, et al. Synergistic effect and mechanism of monoacylglycerol lipase inhibitor and icaritin in the treatment of ulcerative colitis. *Int Immunopharmacol*. 2024;142(Pt A):113155. https://doi.org/10.1016/j.intimp.2024.113155.
119 Jiao F, Gong Z. The beneficial roles of SIRT1 in neuroinflammation-related diseases. *Oxid Med Cell Longev*. 2020;2020:6782872. https://doi.org/10.1155/2020/6782872.
120 Wu LH, Huang BR, Lai SW, et al. SIRT1 activation by minocycline on regulation of microglial polarization homeostasis. *Aging (Albany NY)*. 2020;12(18):17990-18007. https://doi.org/10.18632/aging.103542.
121 Song Y, Wu Z, Zhao P. The protective effects of activating Sirt1/NF-κB pathway for neurological disorders. *Rev Neurosci*. 2022;33(4):427-438. https://doi.org/10.1515/revneuro-2021-0118.
122 Yang C, Wang W, Deng P, et al. Fibroblast growth factor 21 modulates microglial polarization that attenuates neurodegeneration in mice and cellular models of Parkinson's disease. *Front Aging Neurosci*. 2021;13:778527. https://doi.org/10.3389/fnagi.2021.778527.
123 Shi J, Zou X, Jiang K, et al. SIRT1 mediates improvement of cardiac surgery-induced postoperative cognitive dysfunction via the TLR4/NF-κB pathway. *World J Biol Psychia*. 2020;21(10):757-765. https://doi.org/10.1080/15622975.2019.1656820.
124 Shu M, Cheng W, Jia X, et al. AGEs promote atherosclerosis by increasing LDL transcytosis across endothelial cells via RAGE/NF-κB/Caveolin-1 pathway. *Mol Med*. 2023;29(1):113. https://doi.org/10.1186/s10020-023-00715-5.
125 Shen J, Gong L, Sun Y, et al. Semaphorin3C identified as mediator of

neuroinflammation and microglia polarization after spinal cord injury. *iScience*. 2024;27(5):109649. https://doi.org/10.1016/j.isci.2024.109649.
126 Guangtao F, Zhenkang W, Zhantao D, et al. Icariin alleviates wear particle-induced periprosthetic osteolysis via down-regulation of the estrogen receptor α-mediated NF-κB signaling pathway in macrophages. *Front Pharmacol*. 2021;12:746391. https://doi.org/10.3389/fphar.2021.746391.
127 Fu CY, Zhong CR, Yang YT, et al. Sirt1 activator SRT2104 protects against oxygen-glucose deprivation/reoxygenation-induced injury via regulating microglia polarization by modulating Sirt1/NF-κB pathway. *Brain Res*. 2021;1753:147236. https://doi.org/10.1016/j.brainres.2020.147236.
128 Li D, Yin J, Zhang Y, et al. Panax notoginseng saponins regulate the polarization of microglia by inhibiting the hematopoietic progenitor kinase 1 signaling pathway. *Neuroreport*. 2025;36(13):737-745. https://doi.org/10.1097/wnr.0000000000002196.
129 Sun Y, Zhang C, Lei T, et al. HIV1 gp120 activates microglia via TLR2-nf-κb signaling to up-regulate inflammatory cytokine expression and induce neuropathic pain. *Neuropharmacology*. 2024;260:110136. https://doi.org/10.1016/j.neuropharm.2024.110136.
130 Duan CM, Zhang JR, Wan TF, et al. SRT2104 attenuates chronic unpredictable mild stress-induced depressive-like behaviors and imbalance between microglial M1 and M2 phenotypes in the mice. *Behav Brain Res*. 2020;378:112296. https://doi.org/10.1016/j.bbr.2019.112296.
131 Sun B, Sawant H, Borthakur A, et al. Emerging therapeutic role of gut microbial extracellular vesicles in neurological disorders. *Front Neurosci*. 2023;17:1241418. https://doi.org/10.3389/fnins.2023.1241418.
132 Li Y, Zhou C, Liu H, et al. Emerging roles of extracelluar vesicles derived from bacteria, mammalian or plant cells in the pathogenesis and clinical application of neurodegenerative diseases. *Biomolecules*. 2024;14(3):312. https://doi.org/10.3390/biom14030312.
133 Abildinova GZ, Benberin VV, Vochshenkova TA, et al. The gut-brain-metabolic axis: exploring the role of microbiota in insulin resistance and cognitive function. *Front Microbiol*. 2024;15:1463958. https://doi.org/10.3389/fmicb.2024.1463958.
134 Mottawea W, Yousuf B, Sultan S, et al. Multi-level analysis of gut microbiome extracellular vesicles-host interaction reveals a connection to gut-brain axis signaling. *Microbiol Spectr*. 2025;13(2):e0136824. https://doi.org/10.1128/spectrum.01368-24.
135 Velikic G, Maric DM, Maric DL, et al. Harnessing the stem cell niche in regenerative medicine: innovative avenue to combat neurodegenerative diseases. *Int J Mol Sci*. 2024;25(2):993. https://doi.org/10.3390/ijms25020993.
136 Bátiz LF, Castro MA, Burgos PV, et al. Exosomes as novel regulators of adult neurogenic niches. *Front Cell Neurosci*. 2015;9:501. https://doi.org/10.3389/fncel.2015.00501.
137 Park MN, Kim M, Lee S, et al. Targeting redox signaling through exosomal microRNA: insights into tumor microenvironment and precision oncology. *Antioxidants (Basel)*. 2025;14(5):501. https://doi.org/10.3390/antiox14050501.
138 Wang Y, Meng X, Wang S, et al. Investigating the molecular mechanism of icariin in inhibiting liver cirrhosis carcinogenesis by regulating miR-145 based on the ROS-NLRP3 pathway. *Pak J Pharm Sci*. 2026;39(4):970-978. https://doi.org/10.36721/pjps.2026.39.4.Reg.15414.1.
139 Yan Q, Song C, Liu H, et al. Adipose-derived stem cell exosomes loaded with icariin attenuated M1 polarization of macrophages via inhibiting the TLR4/Myd88/NF-κB signaling pathway. *Int Immunopharmacol*. 2024;137:112448. https://doi.org/10.1016/j.intimp.2024.112448.
140 Mo C, Zhao J, Liang J, et al. Exosomes: A novel insight into traditional Chinese medicine. *Front Pharmacol*. 2022;13:844782. https://doi.org/10.3389/fphar.2022.844782.
141 Belelli D, Lambert JJ, Wan MLY, et al. From bugs to brain: unravelling the GABA signalling networks in the brain-gut-microbiome axis. *Brain*. 2025;148(5):1479-1506. https://doi.org/10.1093/brain/awae413.
142 Kim MH, van Noort D, Sung JH, et al. Organ-on-a-chip for studying gut-brain interaction mediated by extracellular vesicles in the gut microenvironment. *Int J Mol Sci*. 2021;22(24):13513. https://doi.org/10.3390/ijms222413513.
143 Guo Y, Chen X, Gong P, et al. The gut-organ-axis concept: advances the application of gut-on-chip technology. *Int J Mol Sci*. 2023;24(4):4089. https://doi.org/10.3390/ijms24044089.
144 Seo GM, Lee H, Kang YJ, et al. Development of in vitro model of exosome transport in microfluidic gut-brain axis-on-a-chip. *Lab Chip*. 2024;24(19):4581-4593. https://doi.org/10.1039/d4lc00490f.
145 Lee H, Seo GM, Yoo H, et al. Random metastructures for nanoscale visualization of single-exosome dynamics in a gut-brain-axis-on-a-chip. *ACS Nano*. 2025;19(33):30349-30360. https://doi.org/10.1021/acsnano.5c09164.
146 Tsai SF, Hsu PL, Chen YW, et al. High-fat diet induces depression-like phenotype via astrocyte-mediated hyperactivation of ventral hippocampal glutamatergic afferents to the nucleus accumbens. *Mol Psychiatry*. 2022;27(11):4372-4384. https://doi.org/10.1038/s41380-022-01787-1.
147 Sun P, Wang M, Li Z, et al. Eucommiae cortex polysaccharides mitigate obesogenic diet-induced cognitive and social dysfunction via modulation of gut microbiota and tryptophan metabolism. *Theranostics*. 2022;12(8):3637-3655. https://doi.org/10.7150/thno.72756.
148 Choi J, Choi H, Chung J. Icariin supplementation suppresses the markers of ferroptosis and attenuates the progression of nonalcoholic steatohepatitis in mice fed a methionine choline-deficient diet. *Int J Mol Sci*. 2023;24(15):12510. https://doi.org/10.3390/ijms241512510.
149 Lin W, Jin Y, Hu X, et al. AMPK/PGC-1α/GLUT4-mediated effect of icariin on hyperlipidemia-induced non-alcoholic fatty liver disease and lipid metabolism disorder in mice. *Biochemistry (Mosc)*. 2021;86(11):1407-1417. https://doi.org/10.1134/s0006297921110055.
150 Wang W, Liu F, Xu C, et al. Lactobacillus plantarum 69-2 combined with galacto-oligosaccharides alleviates d-galactose-induced aging by regulating the AMPK/SIRT1 signaling pathway and gut microbiota in mice. *J Agric Food Chem*. 2021;69(9):2745-2757. https://doi.org/10.1021/acs.jafc.0c06730.
151 Gui Y, Zhang J, Chen L, et al. Icariin, a flavonoid with anti-cancer effects, alleviated paclitaxel-induced neuropathic pain in a SIRT1-dependent manner. *Mol Pain*. 2018;14:1744806918768970. https://doi.org/10.1177/1744806918768970.
152 Wei K, Xu Y, Zhao Z, et al. Icariin alters the expression of glucocorticoid receptor, FKBP5 and SGK1 in rat brains following exposure to chronic mild stress. *Int J Mol Med*. 2016;38(1):337-344. https://doi.org/10.3892/ijmm.2016.2591.
153 Desai TD, Wen YT, Daddam JR, et al. Long term therapeutic effects of icariin-loaded PLGA microspheres in an experimental model of optic nerve ischemia via modulation of CEBP-β/G-CSF/noncanonical NF-κB axis. *Bioeng Transl Med*. 2022;7(2):e10289. https://doi.org/10.1002/btm2.10289.
154 Guo X, Qin Y, Feng Z, et al. Investigating the anti-inflammatory effects of icariin: A combined meta-analysis and machine learning study. *Heliyon*. 2024;10(15):e35307. https://doi.org/10.1016/j.heliyon.2024.e35307.
155 Yu X, Chen J, Lu F, et al. Study on the ameliorative effect of icariin mediating NO/cGMP/PKGI signalling pathway in Alzheimer's disease. *Altern Ther Health Med*. 2024;6:AT10549.
156 Ma R, Zhou Y, Huang W, et al. Icariin maintaining TMEM119-positive microglial population improves hippocampus-associated memory in senescent mice in relation to R-3-hydroxybutyric acid metabolism. *J Ethnopharmacol*. 2025;340:119287. https://doi.org/10.1016/j.jep.2024.119287.
157 Hu Y, Ma X. Icariin treatment protects against gentamicin-induced ototoxicity via activation of the AMPK-SIRT3 pathway. *Front Pharmacol*. 2021;12:620741. https://doi.org/10.3389/fphar.2021.620741.
158 Zhang H, Zhuo S, Song D, et al. Icariin inhibits intestinal inflammation of DSS-induced colitis mice through modulating intestinal flora abundance and modulating p-p65/p65 molecule. *Turk J Gastroenterol*. 2021;32(4):382-392. https://doi.org/10.5152/tjg.2021.20282.
159 Mei Y, Li W, Wang B, et al. Gut microbiota: an emerging target connecting polycystic ovarian syndrome and insulin resistance. *Front Cell Infect Microbiol*. 2025;15:1508893. https://doi.org/10.3389/fcimb.2025.1508893.
160 Li CY, Li XY, Shen L, et al. Regulatory effects of transition metals supplementation/deficiency on the gut microbiota. *Appl Microbiol Biotechnol*. 2021;105(3):1007-1015. https://doi.org/10.1007/s00253-021-11096-2.
161 Yan F, Liu J, Chen MX, et al. Icariin ameliorates memory deficits through regulating brain insulin signaling and glucose transporters in 3 × Tg-AD mice. *Neural Regen Res*. 2023;18(1):183-188. https://doi.org/10.4103/1673-5374.344840.
162 Celorrio M, Friess SH. Gut-brain axis in traumatic brain injury: impact on neuroinflammation. *Neural Regen Res*. 2022;17(5):1007-1008. https://doi.org/10.4103/1673-5374.324839.
163 You M, Yuan P, Li L, et al. HIF-1 signalling pathway was identified as a potential new pathway for icariin's treatment against Alzheimer's disease based on preclinical evidence and bioinformatics. *Front Pharmacol*. 2022;13:1066819. https://doi.org/10.3389/fphar.2022.1066819.
164 Li T, Yang Y, Yang F, et al. Organoids and organoids-on-chip in traditional chinese medicine research: applications, advantages, and future prospects. *Cell Biol Int*. 2025;49(10):1233-1244. https://doi.org/10.1002/cbin.70067.
165 Zhou Q, Chen T, Wang X, et al. Role of gut microbiota in neuroinflammation: a focus on perioperative neurocognitive disorders. *Front Cell Infect Microbiol*. 2025;15:1582909. https://doi.org/10.3389/fcimb.2025.1582909.
166 Song WS, Hung TH, Liu SH, et al. Neuroprotection by abdominal ultrasound in lipopolysaccharide-induced systemic inflammation. *Int J Mol Sci*. 2023;24(11):9329. https://doi.org/10.3390/ijms24119329.
167 Cope EK, Borsom EM, Bolyen EK, et al. Influence of fecal microbiota transplantation on gut microbiota composition and neuroinflammation of 3xTg-AD mice. *Alzheimers Dement*. 2021;17(S3):e054648. https://doi.org/10.1002/alz.054648.
168 Rao J, Qiao Y, Xie R, et al. Fecal microbiota transplantation ameliorates stress-induced depression-like behaviors associated with the inhibition of glial and NLRP3 inflammasome in rat brain. *J Psychiatr Res*. 2021;137:147-157. https://doi.org/10.1016/j.jpsychires.2021.02.057.
169 Jia X, Chen Q, Zhang Y, et al. Multidirectional associations between the gut microbiota and Parkinson's disease, updated information from the perspectives of humoral pathway, cellular immune pathway and neuronal pathway. *Front Cell Infect Microbiol*. 2023;13:1296713. https://doi.org/10.3389/fcimb.2023.1296713.
170 Yu Y, Wang W, Zhang F. The next generation fecal microbiota transplantation: to transplant bacteria or virome. *Adv Sci (Weinh)*. 2023;10(35):e2301097. https://doi.org/10.1002/advs.202301097.
171 Kelly BJ, Kwon JH, Woodworth MH. Escape velocity-the launch of microbiome therapies. *J Infect Dis*. 2024;230(1):2-4. https://doi.org/10.1093/infdis/jiae099.
172 Sahle Z, Engidaye G, Shenkute Gebreyes D, et al. Fecal microbiota transplantation and next-generation therapies: A review on targeting dysbiosis in metabolic disorders and beyond. *SAGE Open Med*. 2024;12:20503121241257486. https://doi.org/10.1177/20503121241257486.
173 Xie C, Tang H, Liu G, et al. Molecular mechanism of Epimedium in the treatment of vascular dementia based on network pharmacology and molecular docking. *Front Aging Neurosci*. 2022;14:940166. https://doi.org/10.3389/fnagi.2022.940166.
174 Di X, Wan M, Bai YN, et al. Exploring the mechanism of icariin in the treatment of depression through BDNF-TrkB pathway based on network

pharmacology. *Naunyn Schmiedebergs Arch Pharmacol*. 2024;397(1):463-478. https://doi.org/10.1007/s00210-023-02615-1.
175 Xu H, You M, Xiang X, et al. Molecular mechanism of epimedium extract against ischemic stroke based on network pharmacology and experimental validation. *Oxid Med Cell Longev*. 2022;2022:3858314. https://doi.org/10.1155/2022/3858314.
176 Chen G, Huang J, Lei H, et al. Icariside I - a novel inhibitor of the kynurenine-AhR pathway with potential for cancer therapy by blocking tumor immune escape. *Biomed Pharmacother*. 2022;153:113387. https://doi.org/10.1016/j.biopha.2022.113387.
177 Gao X, Li S, Cong C, et al. A network pharmacology approach to estimate potential targets of the active ingredients of epimedium for alleviating mild cognitive impairment and treating Alzheimer's disease. *Evid Based Complement Alternat Med*. 2021;2021:2302680. https://doi.org/10.1155/2021/2302680.
178 Yuan P, Chen W, Wang X, et al. RAGE: a potential target for epimedium's anti-neuroinflammation role in vascular dementia-insights from network pharmacology and molecular simulation. *J Biomol Struct Dyn*. 2024;42(20):10856-10875. https://doi.org/10.1080/07391102.2023.2259480.
179 Wu K, Han L, Zhao Y, et al. Deciphering the molecular mechanism underlying the effects of epimedium on osteoporosis through system bioinformatic approach. *Medicine (Baltimore)*. 2022;101(32):e29844. https://doi.org/10.1097/md.0000000000029844.
180 Cui J, Lin L, Hao F, et al. Comprehensive review of the traditional uses and the potential benefits of epimedium folium. *Front Pharmacol*. 2024;15:1415265. https://doi.org/10.3389/fphar.2024.1415265.
181 Xu N, Zhou G, Li X, et al. Geographical classification of Epimedium based on HPLC fingerprint analysis combined with multi-ingredients quantitative analysis. *Biomed Chromatogr*. 2017;31(5). https://doi.org/10.1002/bmc.3871.
182 Yang X, Zheng M, Hao S, et al. Curdlan prevents the cognitive deficits induced by a high-fat diet in mice via the gut-brain axis. *Front Neurosci*. 2020;14:384. https://doi.org/10.3389/fnins.2020.00384.
183 Zhang D, Xie Q, Zhang Z, et al. Real-world evidence on the efficacy of icaritin for unresectable advanced hepatocellular carcinoma: a multicenter retrospective study. *Int J Cancer*. 2026;159(10):2525-2539. https://doi.org/10.1002/ijc.70590.
184 Lu H, Xu T, Li J, et al. Icaritin plus TACE improves survival in advanced HCC with macrovascular invasion: a multicenter cohort study. *Front Immunol*. 2026;17:1684486. https://doi.org/10.3389/fimmu.2026.1684486.
185 Wang J, Du Y, Su G, et al. Dual-functional pH-sensitive nanoliposomes modified with CD47 mimicry peptide enhance icariin delivery to attenuate neuroinflammation and oxidative stress in epilepsy. *Mater Today Bio*. 2025;35:102528. https://doi.org/10.1016/j.mtbio.2025.102528.
186 Zhang R, Feng R, Wang J, et al. Gut microbiota modulation via repeated donor fecal transplantation improves motor and gastrointestinal symptoms in drug-naïve Parkinson's disease: a randomized phase 2 trial. *Signal Transduct Target Ther*. 2026;11(1):94. https://doi.org/10.1038/s41392-026-02604-9.
187 Scheperjans F, Levo R, Bosch B, et al. Fecal microbiota transplantation for treatment of Parkinson disease: a randomized clinical trial. *JAMA Neurol*. 2024;81(9):925-938. https://doi.org/10.1001/jamaneurol.2024.2305.
188 Liu XR, Li M, Hao QQ, et al. Unraveling cysteinyl leukotrienes and their receptors in inflammation through the brain-gut-lung axis. *Virulence*. 2025;16(1):2502555. https://doi.org/10.1080/21505594.2025.2502555.
189 Lim LWK. A micro review on the role of recently emerged Artificial Intelligence (AI) tools and algorithms in microbiome-gut-brain-axis associated disease therapy via psychobiotics. *Precision Medication*. 2025;2(3):100039. https://doi.org/10.1016/j.prmedi.2025.100039.
190 Andafa TW, Imoh EC, Adekanmbi SA. Artificial intelligence applied to the brain-gut axis in irritable bowel syndrome: advancing toward clinical translation. *Cureus*. 2026;18(5):e109142. https://doi.org/10.7759/cureus.109142.