Putri, D, Y., et al. International Journal of Biomedical Science and Travel Medicine (IJBSTM)
Volume 2, Number 1, 2025
https://doi.org/10.22225/ijbstm.2.1.2025.14-18

International Journal of Biomedical Science and Travel Medicine (IJBSTM)

Systematic Review **Open Access**

Neuroprotective Mechanism of Icariin against Cerebral Ischemic-Reperfusion Injury: A Systematic Review

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Abstract

Background: Ischemic stroke has become one of the most life-threatening diseases with high disability and mortality rates. New treatment strategies with neuroprotective functions are urgently needed to treat it. Icariin is a flavonol glycoside that has antioxidant capacity, promote neurite outgrowth and modulate the immune system. This systematic review was conducted to assess and evaluate the efficacy, safety and feasibility of icariin in the treatment of ischemic stroke.

Methods: This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta -Analyses (PRISMA) 2020. We included predefined inclusion criteria, including: original articles published in the last 10 years and in English. Abstracts, preprints, reviews, articles not published in English and inaccessible full-text articles are excluded. Data extracted from PubMed and ScienceDirect databases using keywords "ischemic stroke“ OR ‘”cerebral ischemic-reperfusion injury“ AND "'icariin". The JBI critical appraisal tool was used to assess the quality of the data.

Results: From the data obtained, a total of 7 research data were found eligible to review. The results showed that icariin had positive effects in reducing the levels of pro-inflammatory cytokines such as interleukin-1beta (IL-1beta), IL-6, and tumour necrosis factor-α (TNF-α) as well as maintaining brain cell viability. In addition, icariin either given alone or in combination improves post-ischemic neurological function and reduces infarct volume.

Conclusion: Icariin has demonstrated neuroprotective effects necessary for neuroprotection and neurovascular recovery. In vitro and in vivo studies using Icariin alone or in combination with other modalities have also shown enhanced protection.

Keywords: Icariin; neuroprotective mechanism; cerebral ischemic-reperfusion injury

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Introduction

Ischemic stroke is a sudden medical condition due to reduced perfusion to brain tissue, accounting for 80-85% of strokes and leading to high disability and mortality rates(Sedova et al., 2021; Xie et al., 2020).According to the American Heart Association 2020 statistics, approximately 795,000 people experience a new stroke or recurrent stroke each year(Virani et al., 2020). Ischemic stroke is a condition in which there is a sudden interruption of brain tissue perfusion due to blood vessel occlusion caused by thrombus or atherosclerosis(Tadi & Lui, 2025; Wu et al., 2021; Xie et al., 2020). Decreased oxygen levels in the brain, known as hypoxia, stimulate tissue response signals associated with reperfusion injury(Tasoulis & Douzinas, 2016). These include glutamate excitation due to excessive calcium influx, oxido-nitrosative stress, danger-associated molecular patterns (DAMPS) (Agalave & Svensson, 2014), matrix protein accumulation(Amruta et al., 2020; Kawakita et al., 2019) and proinflammatory production(Jian et al.,

2019; Nakamura & Shichita, 2019), which are triggered after the clot is removed or dissolved(Jian et al., 2019).

Following an ischemic stroke, cerebral blood flow immediately decreases, which restricts the availability of glucose and oxygen in neurons, resulting in neuronal dysfunction and serious damage to neurons and brain tissue(Andrabi et al., 2020; Mo et al., 2020). Reperfusion generates large amounts of reactive oxygen species (ROS) through various mechanisms, exacerbating the damage and being the main cause of I/R injury(Zhou et al., 2024).

Currently, recombinant tissue plasminogen activator (rtPA) is the only thrombolysis drug approved by the FDA for ischemic stroke, which dissolves blood clots and restores blood flow in the brainc(Hui et al., 2025; Wu et al., 2021). However, strict prescription rules and the potential risk of bleeding are limitations that need to be overcome. Therefore, new treatment strategies with neuroprotective functions are urgently needed to address ischemic stroke(Wu et al., 2021).

Icariin is a flavonol glycoside, the main active constituent of the herbal extract of Epimedium - a Chinese herb of the Berberidaceae family with a variety of potent biological activities(Mo et al., 2020; Wu et al., 2021; Xu et al., 2021). Studies demonstrated icariin has hepatoprotective(Wang et al., 2021), antioxidant capacity(Liu et al., 2018), stimulate corticosterone production(Gong et al., 2016), promote neurite outgrowth(Chen et al., 2024), and modulate the immune system(Li et al., 2015). In recent years, research has increasingly focused on the protective effects of icariin in nervous system disorders(Wang et al., 2021). Considering the urgency to develop a treatment for ischaemic stroke and the limited number of studies on icariin in ischaemic stroke, we conducted a systematic review to assess and evaluate the efficacy, safety and feasibility of icariin in the treatment of ischaemic stroke.

Methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020(Page et al., 2021). A PICO (Population, Intervention, Comparison, and Outcome) approach was used to develop the research questions, which included (1) population used in in vitro and or in vivo studies; (2) intervention: icariin administration; (3) comparison: control; and (4) outcome: oxidative stress levels, proinflammatory biomarkers, and neuroprotective effect.

We included predefined inclusion criteria, including: original full-text articles related to neuroprotective studies of icariin against cerebral ischemic-reperfusion injury published in the last 10 years and in english. We excluded abstracts, preprints, reviews, articles not published in english and inaccessible full-text articles.

Two researchers (DYP and SU) independently extracted articles from PubMed and ScienceDirect databases using the MeSH keywords "ischemic stroke“ OR ‘”cerebral ischemic-reperfusion injury“ AND "'Icariin". YY assisted as a verifier in sorting the articles. Study quality was evaluated by NH and HK using JBI critical appraisal tool, designed specifically for the evaluation of quasi-experimental research(Barker et al., 2024). Baseline data included author name, year of publication, sample source, methods, and outcomes.

Results

The PRISMA 2020 flowchart was used in the study selection process, as shown in **Figure 1**. PubMed (n=8) and ScienceDirect (n=7) provided 15 data for the last 10 years. A total of 2 data were excluded due to duplication. Title/abstract screening excluded 6 data. For eligibility assessment, 7 research data were declared eligible for further review(Chen et al., 2024; Dai et al., 2021; Liu et al., 2018; Mo et al., 2020; Wu et al., 2021; Xiong et al., 2016; Zhou et al., 2024). The results of the JBI critical appraisal indicated that most of the reviewed studies were of high quality, as shown in **Table 1**. 1 study had a single parameter and there was no continuity of known parameters. However, overall the results shown were significantly beneficial in ischaemic conditions.

The baseline characteristics of the reviewed studies can be seen in **Table 2**. All icariin-related studies were conducted within the last 10 years and located in China. The methods used in the reviewed studies showed that 2 studies applied the oxygen-glucose deprivation model to the cells used (primary microglia and neuroblastoma N2A cell lines)(Chen et al., 2024; Mo et al., 2020), 5 studies applied the MCAO model to male Sprague-Dawley (Liu et al., 2018; Mo et al., 2020; Xiong et al., 2016; Zhou et al., 2024) and ICR mice(Wu et al., 2021), and 1 study applied a combination of MCAO and hypothermic condition(Dai et al., 2021).

Icariin showed favourable results from all reviewed studies. Icariin showed significant results in reducing the levels of pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumour necrosis factor-α (TNF-α)(Mo et al., 2020; Xiong et al., 2016) by maintaining brain cell viability(Chen et al., 2024; Mo et al., 2020). Icariin has also been shown to have antioxidant capacity, capable of downregulating cleaved caspase 3, Bax, and upregulating Bcl2. In addition, icariin at doses of 10 and 30 mg/kgBB twice daily significantly improved neurological function and reduced infarct volume(Wu et al., 2021; Xiong et al., 2016; Zhou et al., 2024).

Discussion

Ischemic stroke has become one of the most life-threatening diseases in human life, attracting intensive attention from scientists(Gu et al., 2022). Studies have shown that the inflammatory cascade through the pathological progression of cerebral ischemia-reperfusion (I/R) is one of the main causes of neuronal death(Su et al., 2017). Inflammation that occurs after injury due to brain ischemia and reperfusion mainly involves the activation of microglia, which release proinflammatory mediators, matrix metalloproteins, and reactive oxygen species (ROS), leading to further damage and inflammation.

The effects of icariin on OGD-induced N2a cells were observed in vitro. Cell viability was dramatically decreased, followed by increased apoptosis. However, icariin administration significantly increased neuronal viability and suppressed neuronal death in a dose-dependent manner compared to the OGD group ($P<0.05$). The rate of apoptosis was also significantly reduced with icariin pretreatment ($P<0.05$)(Chen et al., 2024; Mo et al., 2020).

The study by Mo *et al.* and Xiong *et al.* examined the inhibitory effects of icariin on inflammatory responses induced by OGD/R protein levels and showed that ICA administration significantly decreased the levels of all three proinflammatory mediators in the icariin + OGD/R group in a dose-dependent manner(Mo et al., 2020; Xiong et al., 2016). The increase in proinflammatory proteins, especially in the IRE1α/XBP1 pathway suggests increased endoplasmic reticulum (ER) stress, leading to decreased cell viability and increased apoptosis. However, these effects were suppressed by icariin, suggesting that icariin protects neurons through the IRE1α/XBP1 pathway(Mo et al., 2020).

The MCAO model for I/R brain injury is the most frequently used experimental model in vivo, which successfully mimics the pathological evolution of stroke in humans(Boyko et al., 2010). From 4 studies reviewed, the MCAO rat model was applied to observe the effects of icariin on ischemic brain injury and focused on underlying molecular mechanisms. ICA was shown to exert neuroprotective effects, significantly improve neurological deficits, and reduce infarct volume at doses of 10 and 30 mg/kg administered twice daily for 3 consecutive days(Xiong et al., 2016).

In addition, MSC transplantation and icariin therapy together can improve neurological function and memory after ischemic brain injury. The combination of the two resulted in therapeutic effects and significant improvements in spatial and motor memory functions in rats, and a reduction in infarct volume was also reported with the use of the combination. Furthermore, treatment with the combination of icariin and MSCs also increased the production of brain-derived neurotrophic factor (BDNF) in the frontal cortex and ischemic hippocampus(Liu et al., 2018; Wu et al., 2021; Xiong et al., 2016; Zhou et al., 2024).

The combination of therapeutic hypothermia and icariin has a better protective effect on ischemic stroke. Mild hypothermia, icariin, and JSH-23 can reduce cerebral infarct volume, neurological deficits, and brain cell death in rats (Dai et al., 2021). They also decreased the expression of apoptotic/inflammatory factors such as TNF-α, IL-6, C-caspase 3, and Bax, and increased the expression of Bcl-2. Icariin has a synergistic effect with mild hypothermia in reducing infarcts and neurological deficits. Icariin also helped inhibit the activation of the NF-κB pathway through the PPARs/Nrf2/NF-κB and JAK2/STAT3/NF-κB pathways(Xiong et al., 2016). In addition, icariin enhanced the effect of mild hypothermia on reducing brain temperature. These results suggest that Icariin may enhance neuroprotection in stroke by mild hypothermia through regulation of apoptotic factors, inflammation, and related signaling pathways(Chen et al., 2024; Dai et al., 2021; Wu et al., 2021).

Conclusion

Icariin has shown at the molecular level to address the underlying pathomechanism of ischemic stroke and to have neuroprotective effects necessary for neuroprotection, neurovascular recovery. In vitro and in vivo studies using icariin alone or in combination with other modalities have also shown enhanced protection. Further studies in clinical trials may help to evaluate the efficacy of icariin in more complex conditions.

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Appendix

Table 1. JBI critical appraisal tool of quasi experimental study (n=7)

Appraisal checklist	Yes (%)	No (%)	Unclear (%)	Not applicable (%)
Does the study clearly distinguish between the 'cause' and 'effect' (i.e., is it obvious which variable precedes the other)?	7 (100)	(0)	(0)	(0)
Did the study include a control group?	7 (100)	0 (0)	(0)	(0)
Did the study include participants in any comparisons that were comparable?	7 (100)	0 (0)	(0)	(0)
Did the participants in any comparisons receive comparable treatment or care, aside from the exposure or intervention being studied?	7 (100)	(0)	(0)	(0)
Did the study include multiple outcome measurements, both before and after the intervention or exposure?	6 (85.71)	1 (14.29)	(0)	(0)
Did the study use the same method to measure the outcomes for all participants in the comparisons?	7 (100)	(0)	(0)	(0)
Did the study ensure that outcomes were measured consistently and accurately?	6 (85.71)	1 (14.29)	(0)	(0)
Did the study ensure complete follow-up, and if there were gaps, were the differences between groups in follow-up thoroughly addressed and analyzed?	7 (100)	(0)	(0)	(0)
Did the study employ the correct statistical methods for analysis?	7 (100)	(0)	(0)	(0)

Source: obtained from Barker *et al*, 2024(Barker et al., 2024)

Table 2. Baseline characteristic of included studies

No	Author's name	Year	Study design	Type of samples	Method	Results
1	Zhen-tao Mo(Mo et al., 2020)	2020	In vitro and in vivo study (China)	primary microglia and cortical neurons isolated from the brains of neonatal Sprague-Dawley rats.	Microglia cells were treated with ICA before being subjected to oxygen-glucose deprivation/ reperfusion (OGD/R). The levels of IL-1β, IL-6, TNF-α were measured using ELISA. Cortical neurons were assessed for apoptosis using TUNEL staining and neuronal survival using a CCK-8 assay. Western blotting was used to analyze protein levels of IRE1α, XBP1u, XBP1s, and cleaved caspase-3. Additionally, quantitative real-time PCR (qRT-PCR) was utilized to measure mRNA expression levels of the same proteins.	Icariin (ICA) administration effectively suppressed the inflammatory response, as shown by decreased secretion of IL-1β, IL-6, and TNF-α. ICA also had a protective effect against neuronal apoptosis induced by ischemic conditions and improved their viability and it inhibition of IRE1/XBP1 signaling pathway associated with stress and apoptosis after ischemic injury and decreased expression of cleaved caspase-3.
2	Dandan Liu(Liu et al., 2018)	2018	In vivo study (China)	The mesenchymal stem cells (MSCs) isolated from the femurs and tibias of Sprague-Dawley (SD) rats, as well as the SD rats themselves that were used for the in vivo experiments.	Adult male Sprague-Dawley rats were divided into five groups: control, MCAO/R, MSC, ICA, and ICA and MSC combination. After 28 days, the brain tissues of the rats were analyzed to measure the infarct area and lesion volume. In addition, the number of micro-blood vessels in the perifocal area was counted. To follow MSCs and their differentiation in the brain, MSCs were injected with BrdU and brain sections were stained by immunofluorescence. DCX gene expression and protein levels of BDNF, VEGF, Bcl-2, and p-ERK1/2 were also measured in brain tissue.	The combined treatment of ICA and MSCs reduced brain infarction volume and improved neurological and behavioral outcomes in the animal model of ischemic stroke. The combined treatment increased the expression of the pro-angiogenic and pro-neurogenic factors VEGF and BDNF, respectively, by activating the PI3K and ERK1/2 signaling pathways in the hippocampus and frontal cortex. The combined treatment of ICA and MSCs also promoted both angiogenesis and neurogenesis in the animal model.

ISSN (online) 3047-7433
ISSN (print) 3047-7441

3	Deqing Xiong (Xiong et al., 2016)	2015	In vivo study (China)	Male adult Sprague-Dawley rats (4 months old, 250 to 280 g)	Male adult Sprague-Dawley rats pretreated with ICA at doses of 10, 30 mg/kg twice per day for 3 consecutive days followed by cerebral I/R injury induced by middle cerebral artery occlusion (MCAO). 24 h after reperfusion, the protein expression levels of interleukin-1β (IL-1β), transforming growth factor-β 1 (TGF-β 1), PPARα and PPARγ, inhibitory κB -α (IκB-α) degradation and nuclear factor κB (NF-κB) p65 phosphorylation were detected by Western blot, respectively	Pretreatment with icariin (ICA) at doses of 10 and 30 mg/kg twice per day for 3 consecutive days significantly improved neurological function and reduced infarct volume in a rat model of cerebral ischemia-reperfusion (I/R) injury. ICA pretreatment reduced the protein levels of IL-1β and TGF-β in a dose-dependent manner. It also suppressed the cerebral I/R-induced degradation of inhibitory κB-α (IκB-α) and the phosphorylation of the p65 subunit of NF-κB, indicating that ICA can inhibit the activation of the NF-κB pathway, and upregulated the protein expression of PPARα and PPARγ, but not PPARβ, in the brain tissue after cerebral I/R injury.
4	Shan Chen (Chen et al., 2024)	2024	In vitro study (China)	Mouse neuroblastoma N2a cell lines	N2a neuronal cells were used to create an in vitro oxygen-glucose deprivation (OGD) model. The effects of icariin on OGD cells were assessed using the CCK-8 kit and M2 pyruvate kinase isoenzyme (PKM2) expression were detected using western blotting, RT-qPCR, and flow cytometry. To investigate the underlying molecular mechanisms, the study used the PKM2 agonist TEPP-46 and	Icariin inhibited apoptosis and inflammation in OGD-induced neurons by downregulating the expression of PKM2 and pro-apoptotic proteins (caspase-3, Bax) while upregulating the anti-apoptotic protein Bcl-2. Activating PKM2 with the agonist TEPP-46 enhanced the apoptotic effects, indicating that icariin's neuroprotective effects are mediated through regulation of PKM2.
5	Zhiyong Zhou (Zhou et al., 2024)	2024	In vitro and in vivo studies (China)	PC-12 cells and male Sprague-Dawley rats induced MCAO (middle cerebral artery occlusion)	In vitro experiments using PC-12 cells, including an oxidative damage model with H2O2, treatment with ICT, and various assays to measure cell viability, ROS, apoptosis, mPTP status, and mitochondrial membrane potential. In vivo experiments using the MCAO rat model, including assessment of neurological function, infarct volume, histological analysis, and Western blotting to measure mPTP and apoptosis-related proteins.	In the MCAO rat model, ICA treatment reduces infarct volume and improves neuronal morphology in a dose-dependent manner. ICA and the mPTP inhibitor CsA both improve cerebral I/R injury by inhibiting excessive mPTP opening in MCAO rats.
6	Cheng-Tien Wu (Wu et al., 2021)	2021	In vivo study (China)	Male ICR mice	The animal model used was 4-5 week old male ICR mice were randomly divided into 5 groups: sham, I/R, I/R + edaravone, I/R + ICA, and I/R + ICT - Drugs were administered by intraperitoneal injection 1 hour before the MCAO surgery, with doses of 60 mg/kg for both ICA and ICT - The MCAO surgery involved anesthesia, neck incision, and insertion of a nylon thread into the left common carotid artery for 50 minutes of occlusion, with cerebral blood flow monitored using a laser Doppler	Pretreatment with ICA and ICT significantly improved the pathological changes and neurological deficits in the MCAO mouse model of ischemic stroke, including reduced body weight loss, infarct volume, and neurological severity score. ICA and ICT pretreatment effectively reduced apoptotic cell death in the hippocampus and cortex of MCAO mice, as shown by increased cleaved caspase-3, cleaved PARP, Bax; decreased Bcl-2. ICA and ICT pretreatment attenuated oxidative stress in the MCAO mice, as evidenced by increased SOD-1, catalase expression and reduced lipid peroxidation (MDA levels). ICA and ICT pretreatment inhibited endothelial-to-mesenchymal transition in the brains of MCAO mice, as demonstrated by reversal of the decreased endothelial marker CD31 and increased mesenchymal markers fibronectin and vimentin.
7	Ming-ming Dai (Dai et al., 2021)	2021	In vivo study (China)	Adult male Sprague-Dawley rats	An ischemia-reperfusion rat model was established and treated with mild hypothermia, ICA or JSH-23 (inhibitor of NF-κB). Thermistor probe, 2′3′5′-triphenyl tetrazolium chloride (TTC), 5/12-score system, and ELISA were used to detect temperature (rectum, cortex, striatum), infarct volume, neurological deficit, and cerebral cell death of these rats. The expressions of TNF-α, IL-6, NF-κB, Nrf2, PPARα, PPARγ, JAK2, p-JAK2, STAT3, and p-STAT3 were detected by Western blot or q-PCR.	ICA reduced the time for low physical temperature to reach target temperature during the mild hypothermia treatment in MCAO rats. The combination of ICA and mild hypothermia had a greater neuroprotective effect in reducing infarct volume and neurological deficits compared to either treatment alone.The combination of ICA and mild hypothermia also had a greater inhibitory effect on inflammatory and apoptotic factors compared to either treatment alone.

Source: obtained from primary data, 2025

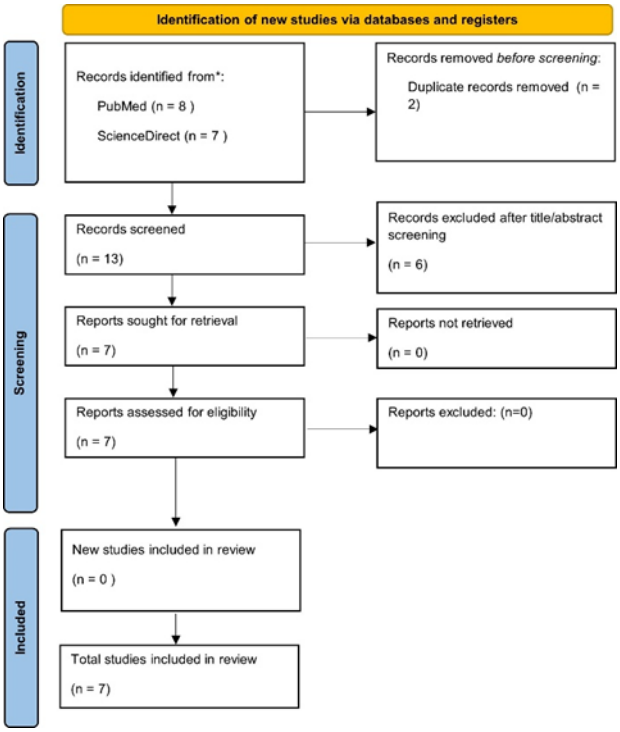

Figure 1. PRISMA flowchart search and selection process
Source: obtained from The PRISMA 2020 statement, 2020(Page et al., 2021)

ISSN (online) 3047-7433
ISSN (print) 3047-7441