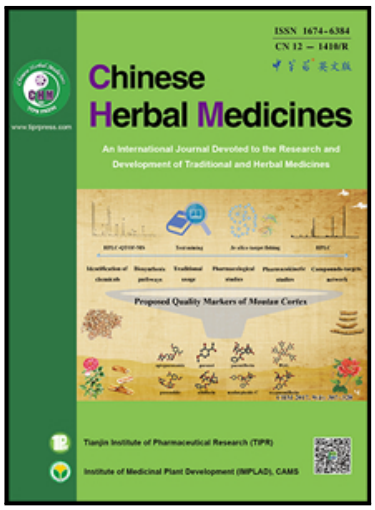


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# Protective effect of icaritin on focal cerebral ischemic-reperfusion mice

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**Abstract**: **Objective** The purpose of this study was to investigate the protective effects of icaritin (ICT), one of the active ingredients in *Epimedii Folium*, on mouse model of cerebral ischemia-reperfusion (I/R) in *vivo*. **Methods** ICR mice were subjected to an 1 h transient middle cerebral artery occlusion (MCAO) and followed by 24 h of reperfusion. Neurological deficits, infarct volume, brain edema and survive rate were measured, respectively. The levels of brain IL-1β, TNF-α, ROS and DNA-binding activity of NF-κB p65 were measured by ELISA kits. The levels of malondialdehyde (MDA) and activities of superoxide dismutase (SOD) were detected by spectrophotometry, and the release of nitric oxide (NO) were detected by Griess kit. **Results** The results showed that ICT markedly reduced the neurological deficit scores, brain edema, infarct volume and increased the survival rate of the cerebral I/R mice. The expression of IL-1β, TNF-α, NO, MDA and DNA-binding activity of NF-κB p65 were significantly inhibited by ICT, while the activity of SOD were up-regulated at the same time. **Conclusion** These results suggested that ICT possessed significant neuroprotective effects in cerebral I/R mice, which might be related to prevent neuroinflammatory and oxidative damage.

**Key words**: icaritin, focal cerebral ischemic-reperfusion, neuroinflammation, oxidative damage, brain edema, brain infarct

## Introduction

Stroke is the third leading cause of death and a leading cause of disability worldwide (Petrea and Beiser, 2009). Between 1990 and 2010, the number of stroke-related deaths increased by 26 % and disability-adjusted life-years by 19 % (Lai and Ye, 2013). Ischemia and reperfusion is a pathological condition, which is characterized by an initial restriction of blood supply to an organ followed by the subsequent restoration of perfusion and concomitant reoxygenation.

Cessation of blood flow to the brain leads to energy loss and necrotic cell death (Alexandrova and Bochev, 2005). This initiates the proinflammatory immune response, which activates inflammatory cells including microglia/macrophages and generates ROS. These activated inflammatory cells further release proinflammatory cytokines, MMPs, nitric oxide (NO) and more ROS in a feed-back fashion (Alexandrova and Bochev, 2005). This proinflammatory cytokines including IL-1β, TNF-α and IL-6 induce sever ischemia-reperfusion (I/R) injury (Baeuerle and Henkel, 1994). NF-κB, which has been shown to be induced in glial cells in human cerebral infarctions, also upregulates gene expression of many kinds of proinflammation cytokines in cerebral ischemic stroke (Terai and Matsuo, 1996).

Icaritin (Figure 1), hydrolyzed by icariin, is recognized as a major active ingredient of *Herba Epimedii* (Liu and Ye, 2005). As a highly interesting natural flavonoid compound for drug development, icaritin has a broad spectrum of established pharmacological functions, including inhibition of many kinds of human cancers, such as breast cancer cells (Guo and Zhang, 2011), human endometrial cancer cells (Tong and Zhang, 2011) and chronic myeloid leukemia (Zhu and Li, 2011). Recent studies have reported that icaritin reduces incidence of steroid-associated osteonecrosis with inhibition of both thrombosis and lipid-deposition in a dose-dependent manner (Zhang and Qin, 2009). In addition, icaritin also shows an immunosuppressive effect on T cells by interfering T cells activation (Li and Hu, 2012) and on macrophages by inhibiting phospho-p38 and phospho-JNK2 (Lai and Ye, 2013). But there is no report on whether icaritin has effects on brain cerebral I/Rinjury.

In current study, middle cerebral artery occlusion