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Research report

Icariin, a major constituent from *Epimedium brevicornum*, attenuates ibotenic acid-induced excitotoxicity in rat hippocampus

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HIGHLIGHTS

- Icariin attenuated learning and memory impairments induced by ibotenic acid in rats.
- Icariin protected against neuronal loss and apoptosis in the hippocampus of ibotenic acid-induced rats.
- Icariin promoted the calbindin expression in the hippocampus of ibotenic acid-induced rats.
- Icariin repressed the activation of MAPK pathway induced by ibotenic acid.
- Icariin inhibited the ibotenic acid induced NF-κB phosphorylation.

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ABSTRACT

Excitotoxicity is one of the most extensively studied causes of neuronal death and plays an important role in Alzheimer's disease (AD). Icariin is a flavonoid component of a traditional Chinese medicine reported to possess a broad spectrum of pharmacological effects. The present study was designed to investigate the effects of icariin against learning and memory impairment induced by excitotoxicity. Here, we demonstrated that rats receiving intracerebroventricular injection of excitatory neurotoxin ibotenic acid exhibited impaired learning and memory. Oral administration of icariin at doses of 20 and 40 mg/kg rescued behavioral performance and protected against neurotoxicity in rat hippocampus by suppressing ibotenic acid induced pro-apoptosis. Furthermore, Western blott of hippocampal specimens revealed that icariin up-regulated the expression of calbindin-D28k protein following ibotenic acid administration. Additionally, icariin inhibited mitogen-activated protein kinase (MAPK) family phosphorylation and nuclear factor kappa B (NF-κB) signaling, implicating the MAPK signaling and NF-κB signaling pathways were involved in the mechanism underlying icariin-mediated neuroprotection against ibotenic acid-induced excitotoxicity. These data suggested that icariin could be a potential agent for treatment of excitotoxicity-related diseases, including AD.

1. Introduction

Alzheimer's disease (AD) is a degenerative disease of the central nervous system, characterized by early dementia and cognitive dysfunction, including impairment of learning and memory loss and other associated cognitive deterioration. The incidence of AD is rapidly increasing as the global population ages, putting pressure on social care systems and families [1], and representing an immense economic burden.

A distinctive pathological feature of AD patients is neuronal degeneration and death in certain areas of the hippocampus [2]. The role of the hippocampus in learning and memory has been extensively studied [3], and its susceptibility to excitotoxicity has been well documented [4]. Excitotoxic cell death in the hippocampus is dependent upon excitatory glutamatergic neurons, in particular *N*-methyl-D-aspartate (NMDA)-type glutamate receptors [5]. Neurotoxins such as kainic acid and ibotenic acid (IBO) are used experimentally to evoke seizures, which are followed by extensive neuronal damage [6,7]. These agents induce neurodegeneration primarily by activating NMDA receptors, mediating massive intracellular accumulation of calcium (Ca^{2+}). Ca^{2+} overload can reproduce many of the key features of in *vivo* excitotoxicity, particularly mitochondrial dysfunction, and activate pro-apoptotic Bcl-2

Abbreviations: AD, Alzheimer's disease; IBO, ibotenic acid; NMDA, *N*-methyl-D-aspartate; Aβ, beta-amyloid; MAPK, mitogen-activated protein kinase; Erk, extracellular-regulated kinase; JNK, c-Jun N-terminal kinase; ICA, icariin; ICV, intracerebroventricular; ANOVA, one-way analysis of variance; CNS, central nervous system; NF-κB, nuclear factor κB.

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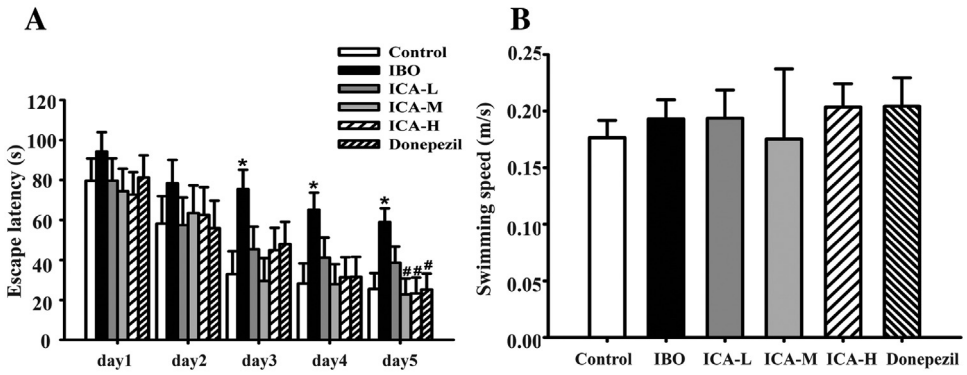

Fig. 1. Effect of icariin on Morris water maze escape latency in IBO memory impaired rats. Rats were administered ibotenic acid and/or 10, 20 or 40 mg/kg icariin (ICA-L, ICA-M and ICA-H, respectively) or 0.6 mg/kg donepezil. After 15 days administration, Morris water maze escape latency was recorded during five consecutive days of training. (A) The time taken to reach a submerged platform (escape latency); (B) The swimming speed. Data were expressed as mean ± SEM. $n = 10$ in each group (* $P < 0.05$ *vs* control group; $^{\#}$ $P < 0.05$ *vs* IBO group).

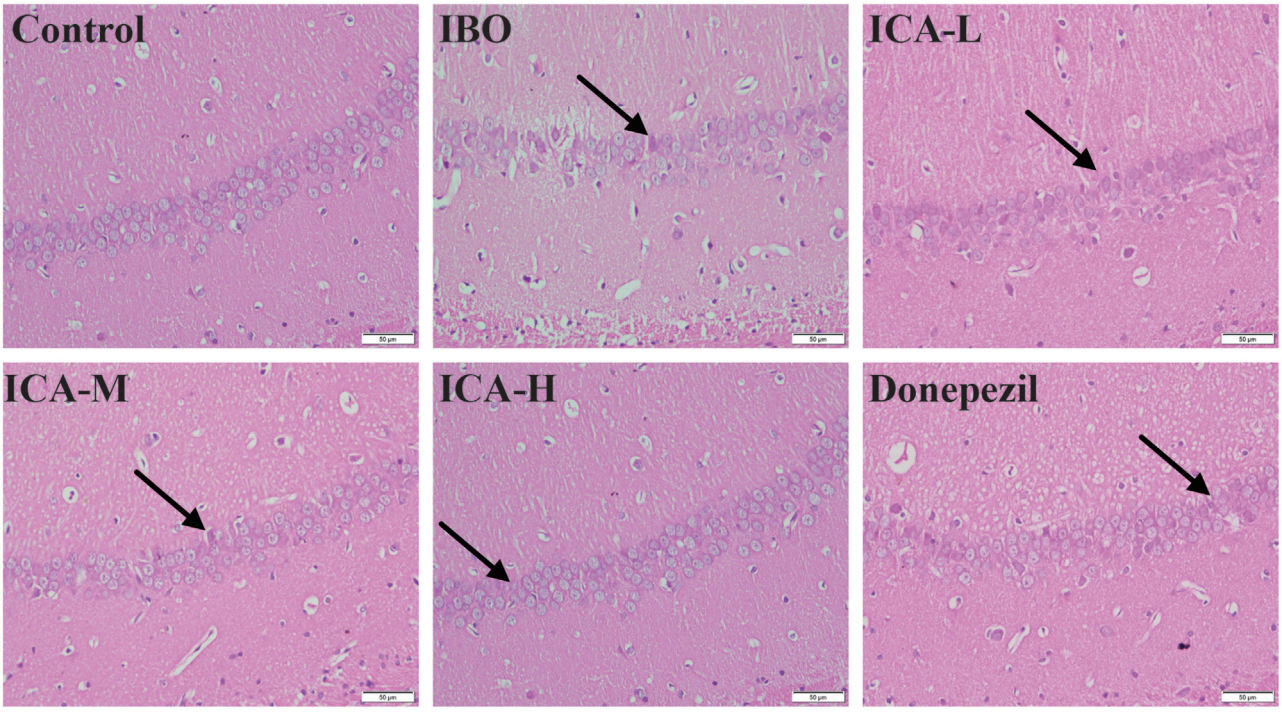

Fig. 2. Effect of icariin on hippocampus CA1 region morphology in IBO memory impaired rats. Representative photomicrographs of HE staining of brain tissue sections across hippocampus CA1 region (magnification 400 ×) 21 days after administration of ibotenic acid and/or 10, 20 or 40 mg/kg icariin (ICA-L, ICA-M and ICA-H, respectively) or 0.6 mg/kg donepezil. Scale bar = 50 μm.

family proteins and cysteine-requiring aspartate proteases (caspases) [8]. These findings suggest that processes reducing calcium influx to cells, or enhancing calcium efflux may be critical for neuronal survival.

Icariin (ICA), a flavonoid extracted from *Berberidaceae Epimedium* plants (*Herba epimedii*) [9], has been reported to exert antioxidative and immunoregulatory effects and regulate neuroendocrine functions *in vitro* and *vivo* [10]. Recently, the effects of icariin on learning and memory have been widely explored in animal models. Administration of icariin improved spatial learning and memory in a rat model of AD established by infusion of β-amyloid protein (Aβ), and appeared to inhibit the production of insoluble fragments of Aβ by suppressing beta-secretase expression [11]. In addition, $A\beta_{1\sim42}$-induced atrophies of axons and dendrites were restored in 5 × FAD mice by post-treatment with icariin [12]. Furthermore, icariin upregulated phosphorylated cyclic adenosine monophosphate response element binding protein level and improved learning and memory function in hippocampus of the senescence-accelerated mouse [13]. However, whether icariin exerts its effect on ameliorating excitatory amino acid toxicity in the hippocampus has not been determined.

Therefore, this study was aimed to investigate the neuroprotective properties of icariin on IBO-induced excitotoxicity in rats, and determine the underlying mechanisms.

2. Materials and methods

2.1. Animals

Ninety adult male Sprague-Dawley (SD) rats (3 months old; Animal Center of the Third Military Medical University, Chongqing,