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# Escin attenuates cognitive deficits and hippocampal injury after transient global cerebral ischemia in mice via regulating certain inflammatory genes

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ABSTRACT

Considerable evidence has been accumulated demonstrating an important role for inflammation in ischemic brain injury and its contribution to greater cerebral damage after ischemia. Blocking the inflammatory reaction promotes neuroprotection and shows therapeutic potential for clinical treatment of ischemic brain injury. Escin, a natural mixture of triterpenoid saponin isolated from the seed of the horse chestnut, demonstrates antiedematous and anti-inflammatory effects. Here we assessed neuroprotective effects of escin with a transient global cerebral ischemia model. Global cerebral ischemia was induced by occluding both common carotid arteries and withdrawing 0.3 ml of blood from the tail vein in mice. Treatment with escin was initiated 0.5 h after ischemia induction and given once a day for three consecutive days. Then animals were assessed using the Morris water–maze test and step-down passive avoidance test. Acetylcholinesterase (AChE) activity, histological pathology, and expression of inflammatory genes in the hippocampus were determined. The results showed escin significantly improved learning and memory recovery and reduced hippocampal damage in the cerebral ischemic mice. However, donepezil merely improved learning and memory recovery but did not ameliorate hippocampal damage in the cerebral ischemic mice. Furthermore, we found escin significantly downregulated certain inflammatory gene expression and upregulated expression of granulocyte-macrophage colony-stimulating factor (GM-CSF), which was recently reported as a neuroprotective protein in the brain. Our results indicate that inhibition of inflammation and protection of hippocampal neurons by escin may be a potentially useful therapy for ischemic brain injury.


## 1. Introduction

Ischemic brain injury, caused by stroke or cardiac arrest, is considered one of the most serious threats to human health; however, modern medicine has not found an effective cure for this condition and research to develop neuroprotective drug therapy for acute cerebral ischemia has not been satisfactory (Gorelick, 2002; Fisher, 2008; Püttgen et al., 2009). Evidence suggests that neuronal cell death occurs following ischemia in susceptible brain regions, such as the hippocampus and the striatum. Morphological manifestations of neuronal cell death have been observed in the ischemic brain and genetic and biochemical evidence further supports the role of neuronal cell death in neurological diseases including ischemia (Honkaniemi et al., 1996; Ruan et al., 2003). The deficits in learning and memory induced by ischemia show a close correlation with neuronal death in the hippocampal CA1 region (Block, 1999). Additionally, the cholinergic system projecting to the hippocampus plays a crucial role in cognitive function, and presynaptic cholinergic terminals are sensitive to cerebral ischemia (Haba et al., 1991; Ishimaru et al., 1995).

Evidence suggests that post-ischemic death of neuronal cell is mediated by multiple mechanisms. One of the processes that may play an important role in the delayed progression of the cell death is post-ischemic inflammation which is initiated by expression of cytokines, adhesion molecules, and other inflammatory mediators (Brea et al., 2009; Dos-Anjos et al., 2009; Yrjänheikki et al., 1999; Liao et al., 2001).

The hallmark of the inflammatory reaction in the brain is the activation of resident microglia and the infiltration and recruitment of peripheral inflammatory cells. Activation of inflammatory cells stimulates large production of pro-inflammatory and cytotoxic factors, including pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), monocyte chemoattractant protein 1 (MCP-1). These substances, together with other inflammatory mediators, such as complement, contribute to the late stages of ischemic injury. Therefore, interventions in post-ischemic inflammation provide insight into potential therapeutic targets for ischemic brain injury, and preclinical studies have

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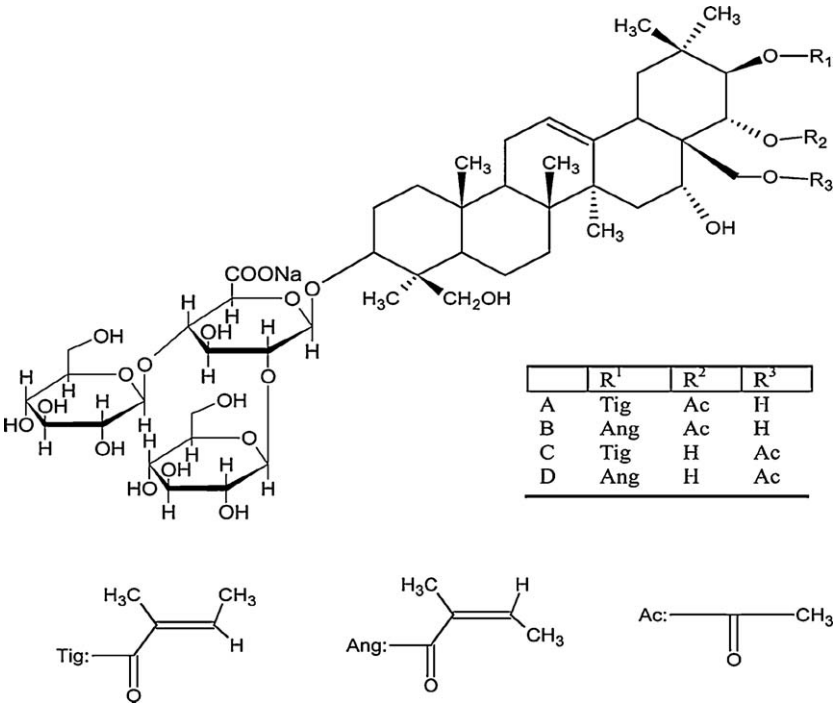


**Fig. 1.** Chemical structure of escin.

suggested that interventions that attenuate inflammation may reduce the progression of ischemia-induced brain damage (Ooboshi et al., 2005; Webster et al., 2009; Son et al., 2009; Capone et al., 2007).

Escin is a natural mixture of triterpene saponins, which mainly consist of A, B, C and D escin (Fig. 1). Accumulating experimental evidence suggests that escin exerts anti-inflammatory and anti-edematous effects. Escin has been shown to prevent edema in animal models of inflammation that reproduce the initial exudative phase, such as paw edema induced by irritative agents (Guillaume and Padioleau, 1994). Escin also inhibits acetic acid-induced increase in capillary permeability and adhesion formation in animal model (Fu et al., 2005). In another research escin can downregulate ICAM-1 and E-selectin protein expression, and reduce the adhesiveness and migration of neutrophils (Hu et al., 2004; Guillaume and Padioleau, 1994). According to Matsuda et al. (1997), the anti-inflammatory effects of escin are mainly dependent on their antihistaminic and antiserotoninergic activities. Arnould et al. (1996) found that escin dose-dependently prevented hypoxia-induced activation of human endothelial cells, as evidenced by the inhibition of hypoxia-increased phospholipase $A_2$, an enzyme responsible for the release of precursors of inflammatory mediators.

Taken as a whole, these results suggest a potential use for escin in ischemic brain injury therapy. In this study, we evaluated the effect of escin on the recovery of learning and memory, AChE activity, and on hippocampal damage in a mouse model of transient global cerebral ischemia. We also estimated the effect of escin on the mRNA expression of some inflammatory genes including cytokines, chemokines, adhesion molecules and complement components known to be induced after cerebral ischemia.

## 2. Methods

### 2.1. Animals and drugs

Male Swiss mice weighing 18–22 g were provided by the Experimental Animal Center of Shandong Engineering Research Center for Natural Drugs (certificate number, 200106003). They were kept in air conditioned rooms (temperature, 23 ± 2 °C) on a 12 h light–dark cycle, with free access to food and water. Animal experimental procedures were carried out in strict accordance with the National Institutes of Health regulations on the use and care of animals for scientific purposes. Surgical procedures, stroke induction, and animal sacrifice (at the end of the observation period) were performed under general anesthesia with intraperitoneal (i.p.) injection of chloral hydrate (400 mg/kg).

Sodium salt of escin (i.e., sodium escinate, consisting of A, B, C, and D and containing at least 65% A and B) was obtained as a lyophilized powder in a 5 mg vial from Luye Pharmaceutical Company Limited (China) and donepezil hydrochloride tablets were provided by Eisai (China) Pharmaceutical Company Limited.

### 2.2. Transient global cerebral ischemia model

To induce ischemia, animals were anesthetized with chloral hydrate (400 mg/kg) under spontaneous respiration. A midline neck incision was made, then the bilateral common carotid arteries (CCA) were carefully isolated and occluded by artery clips (Xinhua Surgical Instrument, China) for 20 min. While the arteries were clamped, 0.3 ml of blood was withdrawn from the tail vein. Then the artery clips were removed and cerebral blood flow was restored. The incision was closed and body temperature was maintained at approximately 37 °C with heating lamps. The control animals received the same surgical procedure except that the carotid arteries were not occluded (Watanabe et al., 2003).

### 2.3. Assessment of spatial learning and memory (Morris water–maze test)

The effect of escin on spatial learning and memory in mice was determined using the Morris water–maze test. Sixty male mice were divided randomly into six groups ($n = 10$): the sham operation group (sham), the ischemia placebo-treated group (ischemia), the ischemia 0.625 mg/kg donepezil group (donepezil), the ischemia 0.45 mg/kg escin group (0.45), the ischemia 0.9 mg/kg escin group (0.9), and the ischemia 1.8 mg/kg escin group (1.8). Escin was administered intravenously 30 min after ischemia and donepezil was administered orally. Treatments were administered once daily for three consecutive days.

After a 3-day course of drug therapy, the spatial memory of mice was measured in the Morris water–maze (Institute of Materia Medica, Academy of Medical Science, China). Before initiating the maze test, mice were allowed to swim freely in a pool of water (diameter, 90 cm; depth, 19 cm; temperature, 26 ± 1 °C) for 60 s without an escape platform. The pool was placed in a large dimly lit test room and surrounded by visual cues. A platform (diameter, 5 cm) was placed 1 cm below the surface of the water. The pool was divided into four quadrants with the platform in a fixed position in one quadrant. Learning consisted of four trials each day on five consecutive days; mice were placed in the water from four different starting points and the latency to escape onto the platform was recorded. Mice that were unable to find the platform within 60 s were placed on the platform for 20 s, and their escape latent period was recorded 60 s.

### 2.4. Step-down passive avoidance test

The drug treatments were the same as those in Morris water–maze test. On day 8 and day 14 after escin administration, latency and error number was determined