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# *n*-Butanol soluble fraction of the water extract of Chinese toon fruit ameliorated focal brain ischemic insult in rats via inhibition of oxidative stress and inflammation

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ABSTRACT

*Aim of the study:* *Toona sinensis* Roem. (*Meliaceae*; *Toona sinensis*; Chinese toon) is a type of arbor that is widely distributed in Asia. The fruits of *Toona sinensis* Roem has been traditionally recognized for treatment of cerebrovascular diseases. To evaluate the potential clinical use of the fruits of *Toona sinensis* Roem, we determined the dose dependence of the neuroprotective efficacy in a focal cerebral ischemic reperfusion model of rats and explored the underlying mechanisms.

*Materials and methods:* Rats were subjected to occlusion of the middle cerebral artery (MCAO) by a nylon filament and treated with different doses (20 mg/kg and 30 mg/kg) of *n*-butanol soluble fraction of the water extract of Chinese toon fruit or the vehicle for 1 week before induction of ischemia, s.i.d..

*Results:* *n*-Butanol soluble fraction of the water extract of Chinese toon fruit reduced in a dose-dependent manner the ischemia-induced cerebral infarct and edema volume and attenuated neurological deficits observed at 6 h point after ischemia. *n*-Butanol soluble fraction of the water extract of Chinese toon fruit reduced the levels of nitrate, nitrite, lipid peroxidation, cyclooxygenase-1, thromboxane in post-ischemic brain. *n*-Butanol soluble fraction of the water extract of Chinese toon fruit adjusted the elevation of the activity of glutathione peroxidase and superoxide dismutase in ischemic brain.

*Conclusions:* The present study was the first evidence of effectiveness of *n*-butanol soluble fraction of the water extract of Chinese toon fruit in the rat stroke models, as it reduced infarct volume, inhibited the oxidative stress and inflammation.



## 1. Introduction

Stroke is the third leading cause of death in developed countries. Until now there is less effective neuroprotective therapy for treatment of stroke. Involvement of oxidative stress in neuronal loss after stroke is well established. Several components of reactive oxygen species-ROS (superoxide, hydroxyl radical, hydrogen peroxide and peroxynitrite radical) have been found to be generated after ischemic reperfusion (IR) injury and play an important role in the neuronal loss after cerebral ischemia (Meenakshisundaram and Shyam, 2004). Superoxide and hydroxyl radical are potent in producing destruction of cell membrane by inducing lipid peroxidation. Accumulation of hydrogen peroxide was reported to impair the mitochondrial function. Superoxide along with hydroxyl radical produces modification in the primary, secondary and tertiary structure and aggregation and/or fragmentation of cellular proteins. Reactive nitrogen species (RNS) such as nitric oxide (NO) are another key factor in the pathophysiological response of the brain after IR injury. Inducible nitric oxide synthase (iNOS) is upregulated after IR injury. This results in the excessive nitric oxide (NO) production. This excess NO reacts with superoxide to form peroxynitrite, one of the potent radical which produces neuronal death after cerebral ischemia. Peroxynitrite can directly hydroxylate and nitrate the aromatic residues of amino acids and nucleotides in cytosol and nucleus. These reactions result in dysfunction of cellular machinery followed by neuronal loss (Isabelle et al., 2005; Samuel et al., 2011; Chen et al., 2012). So, antioxidative strategy plays an important role for the treatment of stroke.

A number of drugs with potential neuroprotective activity including many antioxidants have been used in the treatment of stroke. Recently, intense interest has been focused on the neuroprotective properties of a series of natural products. In particular, some natural products act as neuroprotecting agents by enhancing

*Abbreviations:* CTFBE, (*n*-Butanol soluble fraction of the water extract of Chinese toon fruit)

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the survival of neurons while preventing their death and apoptosis (Zhu et al., 2004). Chinese toon (*Toona sinensis* Roem.; *Meliaceae*; *Toona sinensis*) is a type of arbor that is widely distributed in Asia. It has long been used as a traditional Chinese medicine for a wide variety of conditions in Chinese society and is very popular in vegetarian cuisine in Taiwan, Hubei, Sichun, Chongqing region. The edible leaves have been used as an oriental medicine for treating rheumatoid arthritis, cervicitis, urethritis, tympanitis, gastric ulcers, enteritis, dysentery, itchiness, and cancer (Xien, 1996; Yang et al., 2011). Moreover, the safety levels and nontoxic characteristics of aqueous extracts of *Toona sinensis* were evaluated using a bacterial reverse mutation assay (Ames test), and both acute and subacute toxicity studies were conducted in mice (Liao et al., 2006). Notably, our previous study have shown that people in Sanxia region (China three gorges region, Yichang, Hubei province, China) in China, water extract of Chinese toon fruit (seeds of Chinese toon), contains volatile oils, which consist mainly of flavone, polyphenol and sesquiterpene, were used for traditional medicine preparation for curing cerebrovascular and cardiovascular diseases (Chen et al., 2008; Hou et al., 2011; Liu et al., 2002; Li and Chen, 2009). While the underlying pharmacological mechanisms of this drug are still a matter of debate. Our previous studies have shown that Chinese toon fruit extracts exerts antioxidative, antithrombotic, anti-diabetic effects, as well as the leaf and/or shoots, and the research results of antithrombotic effect has been patented by the Chinese Patent Office (Chen and Sun, 2007).

It is well-established that oxidative stress and inflammation are part of the major pathobiological mechanisms of ischemia/reperfusion (I/R) injury (Ozbal et al., 2008; Yousuf et al., 2009). Present study was undertaken to evaluate the neuroprotective potential of Chinese toon fruit in middle cerebral artery occlusion induced focal cerebral IR model in rats and the underlying pharmacological mechanisms by assessing the effects of Chinese toon fruit extracts on ROS/RNS and pro-inflammatory mediators (COX-1/COX-2, PGI2/TXA2).

## 2. Materials and methods

### 2.1. Drugs and reagents

Ripen and dried Chinese toon fruits were collected in autumn from sanxia region (Hubei Province, China). Aspirin was purchased from BAYER (Leverkusen, Germany). 2,3,5-Triphenyltetrazolium chloride (TTC) was purchased from Sigma (Saint Louis, U.S.A.). Assay kits for malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase, nitrogen monoxidum (NO) and total protein quantitation kit were purchased from Naijing Jiancheng Bio-Tek Co (Nanjing, China). Enzyme-Linked Immunosorbent Assays (ELISA) kits for COX-1, COX-2, 6-keto-PGF1α and TXB2 were purchased from Shanghai Blue Gene Biotech CO., LTD (Shanghai, China). All other reagents were of analytical grade and purchased from Shanghai chemical engineering (Shanghai, China).

### 2.2. Animals

Adult male Sprague-Dawley rats weighing 250–280 g were obtained from Experimental Animal Center, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). All animals were housed in groups of five or six with continuous access to food and water ad libitum and were maintained on a 12 h light/dark cycle regulated at 23 °C room temperature. The experiments began at least 7 days after their arrival. Handling and experimental procedures on all animals were in accordance with the institutional and National Research Council's guideline for animal experiments, which comply with international rules and policies.

### 2.3. Plant material and preparation

Ripen and dried Chinese toon fruit were collected in autumn from sanxia region, Hubei Province, China. Botanical identification of the plant was carried out by Hubei Key Laboratory of Natural Products Research and Development, China three gorges University, China, and a voucher specimen of the plant material has been deposited there. Air-dried seeds of Chinese toon (2 kg) were simmered in hot filtered water for a period of 3 h. The clear solution was collected and heated at 50 °C to reduce the volume to 30%. The concentrated solution was partition extracted with chloroform, followed by ethyl acetate, and finally by *n*-butanol. The *n*-butanol soluble part was evaporated under reduced pressure, and the residue was lyophilized to obtain 92.7 g of a crude brown powder. It was dissolved in 2% Tween 80 before use.

### 2.4. Middle cerebral artery occlusion model (MCAO)

Focal cerebral ischemia was induced using the intraluminal filament technique. Anesthesia was administered with chloral hydrate (350 mg/kg, i.p.); it was maintained throughout the operation. The right common carotid artery was exposed through a midline cervical incision. A heparinized intraluminal filament (φ0.265 mm, rounded tip) coated with paraffin was introduced via the external carotid artery. The rectal temperature was monitored and maintained at 37 °C using a heating pad. After 120 min of occlusion, the filament was gently pulled out and the external carotid artery was permanently closed by cauterization (Zuo et al., 2012). In sham-operated rats, the right common carotid artery was exposed and the external carotid artery was opened without introducing the filament into the internal carotid artery. After the operation, the animals were allowed to wake up in the incubator (37 °C) and were then moved to their home cages.

### 2.5. Experimental design

Rats were divided into six groups. The experimental group was treated with CTFBE (20 mg/kg and 30 mg/kg, ig, CTFBE group ($n=6$)) for 1 week before induction of ischemia, s.i.d.. The vehicle group was treated with Tween 80 (2% Tween 80 5 ml/kg, ig, 2% Tween 80 5 ml/kg group ($n=6$)) for 1 week before induction of ischemia. The positive control group was treated with Aspirin (Aspirin 10 mg/kg, ig, Aspirin 10 mg/kg group ($n=6$)) for 1 week before induction of ischemia. CTFBE, Tween 80 and Aspirin were administered intragastrically everyday in the morning. The sham operated control group (SHAM, $n=4$) was not treated with any drug for 1 week before induction of sham-operated ischemia.

### 2.6. Evaluation of the neurological deficit scores

Neurological deficits evaluations were carried out at 6 h after MAC occlusion by an observer masked to the identity of experimental groups using the following criteria as described by Longa et al. (1989):0, no neurologic deficit or normal function; 1, failure to extend right forepaw fully; 2, circling to right; 3, vert to right; 4, coma or absence of spontaneous motor activity; 5, death. Hence, the higher the score the poor the neurological function is.

### 2.7. Determination of cerebral infarct and edema

Animals were reanesthetized with chloral hydrate (350 mg/kg) and decapitated after 6 h of modeling. The brains were carefully removed and then sectioned coronally in 2-mm thick sections