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Dragon's blood dropping pills have protective effects on focal cerebral ischemia rats model☆

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ABSTRACT

Dragon's blood is a bright red resin obtained from *Dracaena cochinchinensis* (Lour.) S.C.Chen (Yunnan, China). As a traditional Chinese medicinal herb, it has great traditional medicinal value and is used for wound healing and to stop bleeding. Its main biological activity comes from phenolic compounds. In this study, phenolic compounds were made into dropping pills and their protective effects were examined by establishing focal cerebral ischemia rats model used method of Middle Cerebral Artery Occlusion (MCAO), and by investigating indexes of neurological scores, infarct volume, cerebral index, cerebral water content and oxidation stress. Compared to model group, high, middle and low groups of Dragon's blood dropping pills could improve the neurological function significantly ($p < 0.01$) and reduce cerebral infarct volume of focal cerebral ischemia rats remarkably ($p < 0.05$–0.01). Meanwhile, each group could alleviate cerebral water content and cerebral index ($p < 0.05$–0.01) and regulate oxidative stress of focal cerebral ischemia rats obviously ($p < 0.05$–0.01). Activities of middle group corresponded with that treated with positive control drug. The results obtained here showed that Dragon's blood dropping pills had protective effects on focal cerebral ischemia rats.

Introduction

Ischemic cerebrovascular disease (ICVD) is harm to human health seriously. It is one of the three major diseases which have the characteristics of high incidence, disability, fatality and recurrence, next only to heart disease and cancer (Diener et al. 2008). In clinic, most cerebral infarction is due to cerebral arterial thrombosis. It can make cell apoptosis of cerebral ischemic and hypoxic regions with "ischemic waterfall-like reaction" immediately. Ischemic stroke accounts for 70–80% of the stroke incidence and its prevention and treatment are necessary without delay. Therefore, it is a very important theoretical research and practical applications on complicated mechanisms and effective drugs study of ICVD.

Chinese herbal medicine has a long history in China, and is still in wide use up to now because of its definite therapeutic effect, wide indications, safety and convenient application. Dragon's blood is a kind of red resin obtained from *Dracaena cochinchinensis* (Lour.) S.C.Chen (Yunnan, China). And it is a recently discovered native dracaena species that has become the main source of dragon's blood in China from then on (Cai and Xu 1979; Fan et al. 2008; Wang et al. 2010, 2011; Zheng et al. 2012). As a "panacea of blood activating" resin, it has great medicinal value and its main biological activity comes from phenolic compounds (Zhou et al., 2001; Zhu et al. 2002). Pharmacological studies have showed that it has positive effects on treatment of blood stasis syndrome, trauma, tumors, inflammation, gynecopathy, allergic dermatitis and so on. It can promote blood circulation and serve as an antithrombotic, antioxidant, antiseptic, and antiinflammatory compound (Choy et al. 2008; Gurgel et al. 2001; Jones 2003; Peres et al. 1997; Rao et al. 2007; Zheng et al. 2005). It can improve blood circulation, metabolism, immune function and so on. Our previous study showed that Dragon's blood also had radioprotective effects in radiation-induced neuro-injury

Abbreviations: ATP, adenosine triphosphate; CAT, catalase; CCA, common carotid artery; ECA, external carotid artery; GSH-PX, glutathione peroxidase; ICA, internal carotid artery; ICVD, ischemic cerebrovascular disease; MCAO, Middle Cerebral Artery Occlusion; MDA, malondialdehyde; NO, nitric oxide; NOS, nitric oxide synthase; SOD, superoxide dismutase; TTC, 2,3,5-triphenyltetrazolium chloride; V, cerebral infarction volume.

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in rat (Xin et al. 2012). All these indicate that Dragon's blood has enormous potential for further study.

In this study, phenolic compounds from Dragon's blood were made into dropping pills and their effects on focal cerebral ischemia rats model were examined by establishing focal cerebral ischemia rats model used method of Middle Cerebral Artery Occlusion (MCAO). The protective effects were further determined to validate the new dosage forms of dropping pills and to obtain information for further drug development.

Materials and methods

Materials

Reagents

Sodium carboxymethyl cellulose (CMC-Na, No. 050803), sodium chloride (No. 20081205), 2,3,5-triphenytetrazolium chloride (TTC, No. 20070601) and chloral hydrate (No. 30037517) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Xuesaitong tablets (No. 20071201), as a positive control drug (Wei 2004), were purchased from Weihe Pharmaceutical Co. Ltd. (Yuxi, China). Superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-PX), nitric oxide synthase (NOS) and catalase (CAT) kits (No. 20070329) were supplied from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Else: MCAO monofilaments (No. 2634-100, AAAA grade, diameter of headend: 0.34 ± 0.02 mm; diameter of body: 0.26 mm) were purchased from Beijing Sunbio Biotech Co., Ltd. (Beijing, China).

Sample preparation

The multi-phenolic fraction of crude Dragon's blood was prepared for this study. Crude Dragon's blood extract was dissolved in 40% ethanol and kept for 24 h at room temperature to separate a clear supernatant. The clear supernatant was eluted with 95% ethanol on macroporous adsorption resin and prepared by concentration and desiccation. Some of phenolic compounds were identified by Beijing BIT&GY Pharmaceutical R&D Co., Ltd to be the active components of Dragon's blood (Xin et al. 2011) (Fig. 1).

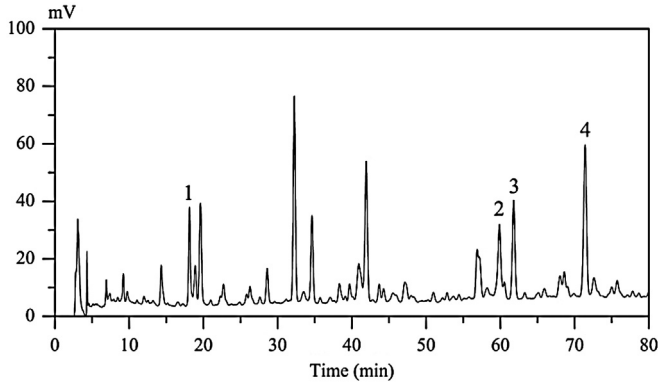

Fig. 2. High-performance liquid chromatogram of Dragon's blood extracts at 280 nm. 1: Resveratrol; 2: Loureirin A; 3: Loureirin B; 4: Pterostilbene.

Chromatographic conditions were as follows (Teng et al. 2011). Column: Ultimate XB-C_{18} (250 mm × 4.6 mm, 5 μm) column (Welch Materials, MD, USA); eluent: A: 1% glacial acetic acid water solution, B: acetonitrile; linear gradient elution was adopted starting from 25% to 55% B in 80 min; flow rate was 1.0 mL min^{-1} and the column temperature was 25 °C. The monitoring wavelength was set at 280 nm. The main phenolic compositions of Dragon's blood extracts were Resveratrol (No. 1, 3.6 mg g^{-1}), Loureirin A (No. 2, 6.9 mg g^{-1}), Loureirin B (No. 3, 6.2 mg g^{-1}) and Pterostilbene (No. 4, 5.0 mg g^{-1}) of the dry extracts. The retention time of 1–4 compounds were 18.11, 59.93, 61.56 and 71.51 min, respectively (Fig. 2).

Dragon's blood dropping pills (No. 20071201) contained 30% multi-phenolic fraction of crude Dragon's blood and was provided by Beijing BIT&GY Pharmaceutical R&D Co., Ltd.

Animals

The study complied with the "Guide for the Care and use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1985) and all animals was approved by the institutional animal experiments committee. SD rats (230–250 g body weight, 6–8 weeks old) and their special

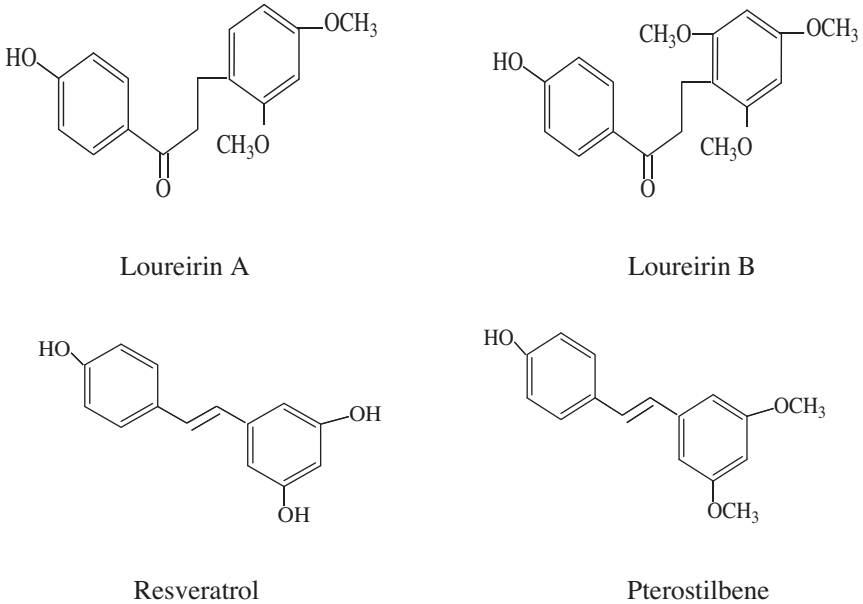

Fig. 1. Chemical structures of the main components in Dragon's blood extracts (patent of BIT&GY: A Dragon's blood extract and its preparation method and uses, Patent No. ZL 2008 1 0182971.7).