Journal of Ethnopharmacology ■ (■■■■) ■■■–■■■

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

In vitro and *in vivo* hepatoprotective and antioxidant activity of ethanolic extract from *Meconopsis integrifolia* (Maxim.) Franch

Gao Zhou, Yuxin Chen, Song Liu, Xingcheng Yao, Youwei Wang *

Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (Wuhan University), Ministry of Education, and Institute of TCM & Natural Products, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, PR China

ARTICLE INFO

Article history:
Received 14 October 2012
Received in revised form
25 February 2013
Accepted 15 May 2013

Keywords:
Antioxidant activity
Carbon tetrachloride
Hepatoprotective activity
In vitro
In vivo
Meconopsis integrifolia

ABSTRACT

Ethnopharmacological relevance: *Meconopsis integrifolia* (Maxim.) Franch is a high mountain endemic species used as a traditional Tibetan and Mongolian herb to treat hepatitis, pneumonia, and edema. This study aims to investigate the hepatoprotective and antioxidant effects of *Meconopsis integrifolia* ethanolic extract (MIE) *in vitro* and *in vivo*.

Materials and methods: The *in vitro* antioxidant property of MIE was investigated by employing various established systems. Rats with carbon tetrachloride (CCl_4)-induced liver injury were used to assess the hepatoprotective and antioxidant effect of MIE *in vivo*. The level or activity of alkaline phosphatase (ALP), glutamate pyruvate transaminase (ALT), aspartate aminotransferase (AST), and total bilirubin (TB) in the blood serum and thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) in the liver and kidney of the rats were assayed using standard procedures.

Results: MIE exhibited strong antioxidant ability *in vitro*. In the rats with CCl_4-induced liver injury, the groups treated with MIE and silymarin showed significantly lower levels of ALT, AST, ALP, and TB. MIE demonstrated good antioxidant activities in both the liver and kidney of the rats *in vivo*.

Conclusions: MIE exhibits excellent hepatoprotective effects and antioxidant activities *in vitro* and *in vivo*, supporting the traditional use of *Meconopsis integrifolia* in the treatment of hepatitis.

1. Introduction

Liver diseases have become a health burden worldwide because of unhealthy dietary habits, environment pollution, and virus infections. The geographical environment in the Tibetan plateaus limits the agricultural development in the area. Therefore, traditional Tibetans have maintained a diet with high in saturated fat and low in fruits and vegetables (Moreno, et al., 2000; Owen and Johns, 2002), thereby resulting in a high incidence of liver and gallbladder diseases among the aboriginal adult people (Fang, 2009). The development and exchange of culture since the ancient times have greatly contributed to the preservation and dissemination of knowledge on traditional Tibetan medicine. The traditional Tibetan herbal medicines, which include numerous prescriptions used to treat liver diseases, are still widely used by the Tibetans. Several herbs, such as *Halenia elliptica* and *Swertia mussotii*, have been proven to possess good hepatoprotective activities based on modern pharmacology experiment method (Huang et al., 2010b; Lv et al., 2010).

The earliest record of the application of *Meconopsis integrifolia* (Maxim.) Franch can be traced back to the eighth century (Yue Wang Yao Zhen, eighth century A.D.). *Meconopsis integrifolia* has been utilized as *oubei* to treat hepatitis, pneumonia, and edema. *Meconopsis integrifolia*, as one of the most widely distributed species of the genus, is a flagship species of the alpine scree in the Qinghai–Tibetan Plateau. The bright yellow flowers and leaf blade margins of the species make it distinguishable from other species (Yang et al., 2012). *Meconopsis punicea*, *Meconopsis quintuplinervia*, *Meconopsis torquata*, and *Meconopsis lancifolia* in the same genus were also used as *oubei*. Together with *Meconopsis integrifolia*, these herbs were believed to contain therapeutic effects in the treatment of hepatitis (Luo et al., 1984; Du et al., 2011; Wu et al., 2011). Modern pharmacological research on *oubei* mainly focuses on *Meconopsis quintuplinervia*. The ethanolic extract of *Meconopsis quintuplinervia* has significant hepatoprotective effects on rats with carbon tetrachloride (CCl_4)-induced liver damage (Ding and Li, 2007). He et al. (2012) showed that the ethanolic extract of *Meconopsis quintuplinervia* exhibited strong *in vitro* and *in vivo* antioxidant activity. Zhou et al. (2009) compared the main alkaloids in six *Meconopsis* species. Their results showed that *Meconopsis integrifolia* contained less alkaloid compared with other species. Antioxidant effects have an important function in liver protection (Fraschini et al., 2002). This study aims to evaluate the *in vitro* and *in vivo* hepatoprotective and antioxidant activities of the *Meconopsis integrifolia* (Maxim.) Franch ethanolic extract (MIE).

* Corresponding author. Tel.: +86 276 875 9323; fax: +86 27 68759010.
E-mail addresses: wyw@wbgcas.cn, wyw@whu.edu.cn (Y. Wang).

0378-8741/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2013.05.027

2. Materials and methods

2.1. Chemicals

2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), α,α-Diphenyl-β-picrylhy-drazyl (DPPH), and Folin–Ciocalteau's reagent were purchased from Sigma-Aldrich (St. Louis, MO). β-carotene was purchased from Fluka (Menlo Park, CA). Linoleic acid was purchased from Aladdin^{tm}. Ascorbic acid (Vc), gallic acid, rutin, and butylated hydroxytoluene (BHT) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other chemicals used in the analysis were of analytical grade and were obtained from the China Medicine (Group) Shanghai Chemical Reagent Corporation (Shanghai, China).

2.2. Plant materials

Meconopsis integrifolia (whole plant) was purchased from the Tibetan Traditional Medicine Pharmaceutical Factory (Lhasa City, Tibet, China) and was authenticated under references and authoritative books (Flora of China, Pharmacopoeia Committee of Ministry of Health of China, 1995) by the corresponding author (Wuhan University, Wuhan, China). A specimen (No. 592) was deposited at the Institute of Traditional Chinese Medicine and Natural Products, School of Pharmaceutical Sciences, Wuhan University.

2.3. Preparation of plant extracts

The powder (100 g, gradient size of less than 0.25 mm) of *Meconopsis integrifolia* was extracted under supersonic washer using 70% ethanol (1:10) at room temperature for 45 min. The solvent was evaporated via rotary evaporation at 35 °C. The residue was lyophilized, and the resulting dry powder was stored at 4 °C. The ethanol extract yield from *Meconopsis integrifolia* was 16.04% relative to the dry starting material.

2.4. Determination of total phenolic and flavonoid content

The total phenolic content of the extract was determined using the Folin–Ciocalteau assay (Sabir and Rocha, 2008). The results were expressed in mg of gallic acid equivalents per g of dry extract. The total flavonoid content was determined using a colorimetric assay (Zheng et al., 2011), with rutin as standard. The results were expressed in mg of rutin equivalents per g of dry extract.

2.5. In vitro antioxidant activity

2.5.1. DPPH radical scavenging assay

The free-radical-scavenging activity of MIE was measured using an improved DPPH assay (Huang et al., 2010a). The extract solution with concentration of 0.3 mL was mixed with a solution of 0.2 mmol/L DPPH in methanol (2.7 mL). The mixture was mixed vigorously, and then left to stand for 1 h at room temperature before measuring the absorbance value at 517 nm. Radical scavenging activity was calculated using the following equation:

$$\text{Percent inhibition rate} = [(As - Ai/As)] \times 100,$$

where *As* is the absorbance of DPPH alone, and *Ai* is the absorbance of DPPH in the presence of various extracts. The concentrations of BHT and Vc identical to the experimental samples were used as reference.

2.5.2. ABTS radical scavenging assay

The ability of the extract to scavenge ABTS radical was determined according to a previously published method (Huang et al., 2011). ABTS was dissolved in deionized water at 7 mmol/L concentration, and potassium persulfate with a concentration of 2.45 mmol/L was added afterward. The reaction mixture was kept in the dark at room temperature for 16 h. The mixture was then diluted with 80% ethanol to obtain an absorbance value of 0.700 ± 0.005 at 734 nm. Test substances (0.3 mL) at various concentrations were incubated with ABTS+ solution (2.7 mL) in a 30 °C water bath for 30 min in the dark. The absorbance at 734 nm was immediately recorded. Samples of BHT and Vc at the same concentrations were used as references. The level of radical scavenging was calculated using the aforementioned equation for DPPH.

2.5.3. Super oxide radical scavenging assay

The capacity of MIE to scavenge superoxide radicals was examined using a pyrogallol auto-oxidation system (Xiang and Ning, 2008), with slight modifications. Reaction mixtures that contain test extracts (0.2 mg/mL) in Tris–HCl buffer (4.50 mL, 50 mmol/L, pH 8.2) were incubated for 10 min at 25 °C. Subsequently, 0.15 mL of pyrogallol (3 mmol/L, prepared in 10 mmol/L HCl) was added. The absorbance of the reaction mixture at 325 nm was measured immediately and at 30 s intervals thereafter. The auto-oxidation rate constant (*Kb*) of pyrogallic acid was calculated from the curve of 325 nm vs. time. The control did not contain test extracts, and concentrations of BHT and Vc identical to the samples were used as reference. The inhibitory actions of the test extracts on the auto-oxidation rate of pyrogallic acid correlated with their ability to scavenge superoxide radicals.

2.5.4. Reducing power assay

The reducing power of the extracts was estimated using the method by Oyaizu (1986). Various concentrations of 0.2 mL extracts were mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). After incubation at 50 °C for 20 min, 2.5 mL of trichloroacetic acid (10%) was added; each mixture was centrifuged at 1000 rpm for 10 min. Subsequently, 2.5 mL of the supernate was collected and mixed with 2.5 mL of deionized water and 0.5 mL of ferric chloride (0.1%). The absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicates an increase in reducing power. BHT and Vc were used as standards for comparison.

2.5.5. β-Carotene bleaching assay

The antioxidant activity of MIE was evaluated according to the β-carotene bleaching method (Shon et al., 2003), with slight modifications. A solution of β-carotene was prepared by dissolving 6 mg of β-carotene in 20 mL of chloroform. Approximately 4 mL of the β-carotene solution, 80 mg of purified linoleic acid, and 800 mg of Tween80 emulsifier were then pipetted into a 500 mL round-bottom flask. After the mixture was thoroughly incorporated, chloroform was removed via vacuum, and 200 mL of aerated distilled water was then added to the flask with vigorous shaking. Aliquots (3.0 mL) of the resulting emulsion were transferred into different test tubes containing 0.2 mL solution of MIE (0.2 mg/mL), and were incubated in a water bath at 50 °C. Absorbance readings were recorded at 30-min intervals for 2 h. BHT was used as standards for comparison. Lipid peroxidation (LPO) inhibition was calculated as follows: $\text{LPO inhibition} = [(As - Ai)/As] \times 100$, where *As* is the initial absorbance of the assay and *Ai* is the absorbance of the assay after 2 h.

Please cite this article as: Zhou, G., et. al., *In vitro* and *in vivo* hepatoprotective and antioxidant activity of ethanolic extract from *Meconopsis integrifolia* (Maxim.) Franch Journal Of Ethnopharmacology (2013), http://dx.doi.org/10.1016/j.jep.2013.05.027