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Atractylodes lancea rhizome water extract reduces triptolide-induced toxicity and enhances anti-inflammatory effects

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[ABSTRACT] The present study was designed to explore the influence of water extracts of *Atractylodes lancea* rhizomes on the toxicity and anti-inflammatory effects of triptolide (TP). A water extract was prepared from *A. lancea* rhizomes and co-administered with TP in C57BL/6 mice. The toxicity was assayed by determining serum biochemical parameters and visceral indexes and by liver histopathological analysis. The hepatic CYP3A expression levels were detected using Western blotting and RT-PCR methods. The data showed that the water extract of *A. lancea* rhizomes reduced triptolide-induced toxicity, probably by inducing the hepatic expression of CYP3A. The anti-inflammatory effects of TP were evaluated in mice using a xylene-induced ear edema test. By comparing ear edema inhibition rates, we found that the water extract could also increase the anti-inflammatory effects of TP. In conclusion, our results suggested that the water extract of *A. lancea* rhizomes, used in combination with TP, has a potential in reducing TP-induced toxicity and enhancing its anti-inflammatory effects.

[KEY WORDS] Triptolide; *Atractylodes lancea*; Licorice; CYP3A; Anti-inflammation effects

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Introduction

Triptolide (TP) is one of the major active components in *Tripterygium wilfordii* Hook F (TWHF). TWHF has been used for centuries in traditional Chinese medicine to treat inflammatory and autoimmune diseases, including rheumatoid arthritis (RA), immune complex nephritis, and systemic lupus erythematosus (SLE), and to prevent rejection following organ and tissue transplantations [1]. Clinical and experimental studies have demonstrated that TP has multiple pharmacological activities, such as anti-inflammation, anti-tumor, anti-fertility, and anti-rejection [2]. However, many cases of TP-induced toxicity affecting the digestive, urogenital, and blood circulatory systems have been reported [3-4]. The toxicity of TP can be affected by the expression level of hepatic cytochrome P450 (CYP450) enzymes, which are involved in metabolizing toxic compounds [5-6]. A recent study has shown that CYP3A2, one of the genes encoding for CYP450, has an important role in the sex-related metabolism of TP [7]. *In vitro* studies have also shown that CYP3A plays a major role in the hydroxylation of TP in the liver [8-9]. These observations suggest that induction of hepatic CYP3A may be able to reduce TP-induced toxicity.

In the clinical application of traditional Chinese medicine, TWHF is often used in combination with other herbal medicines (*e.g.,* licorice and paeony extracts) to reduce its toxicity [2, 10]. These herbal medicines usually reduce TP-induced toxicity by inducing hepatic CYP3A, and accelerating the clearance of TP. However, they may also reduce the anti-inflammatory effects of TP. Therefore, alternative combination therapies that do not reduce the efficacy of TP are still needed.

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Atractylodes lancea (Thunb.) DC, belonging to the family Compositae, is well documented in *Shen Nong Ben Cao Jing*, the first Chinese pharmacopoeia written in the Han dynasty around 200–100 BC [11]. The traditional medicine based on this species is called "Cangzhu" in China, "Khod-Kha-Mao" in Thailand, and "So-jutsu" in Japan [12]. The rhizomes from *A. lancea*, commonly called Rhizoma atractylodis, are used as an important drug in traditional Chinese medicine to revitalize the spleen and treat dyspepsia, gastroparesis, visceral hypersensitivity, rheumatic diseases, and night blindness [13-14]. These traditional uses are explained by the compound's ability to eliminate dampness, strengthen the spleen, expel wind-cold from the superficial parts of the body, and clear away the common cold [12]. *A. lancea* rhizomes also show pharmacological activities such as anti-inflammatory, antimicrobial, and anti-ulcer effects, and hepatoprotection [15-16]. It has been reported that the water extract of *A. lancea* rhizomes promotes liver protein synthesis, and induces CYP3A expression, resulting in both increased mRNA levels and increased enzyme activity, contributing to its hepatoprotective effect [17]. These findings suggest that when co-administered with TP, *A. lancea* rhizome extracts may reduce TP-induced toxicity by accelerating TP metabolism in the liver. Furthermore, the combined treatment with TP and *A. lancea* rhizome extracts may achieve synergistic or additive anti-inflammatory effects, as they both alleviate inflammation. The results from the present study demonstrated that the water extract of *A. lancea* rhizomes reduceed TP-induced toxicity, and enhances the anti-inflammatory effects of TP.

Materials and Methods

Chemicals and reagents

TP (> 99% purity) was purchased from ChromaDex (Irvine, CA, USA). Tween 80 and xylene were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Atractyloside A, vanillic acid, syringic acid, protocatechuic acid, protocatechuic aldehyde, and limonoids were purchased as reference substances from Jingzhu Bio-tech Co. (Nanjing, China). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and L-glutathione (GSH) analysis kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Plant materials and extraction

A. lancea rhizomes were collected from Maoshan region, Jiangsu Province, and authenticated by Prof. Zhen Ouyang (Department of Pharmacognosy, Jiangsu University, Zhengjiang China). Dried rhizomes of *A. lancea* (100 g) were ground into powder and refluxed with 800 mL of water for 2 h. After filtration, the water extraction procedure was repeated twice. The pooled water extracts were then combined and concentrated under reduced pressure, followed by freeze-drying. The volatile oil extract was prepared according to the Chinese Pharmacopoeia (2015). The yields of the water and volatile oil extracts were approximately 42.9% and 5.21% (*W/W*), respectively. Licorice was purchased from Haixin Chinese Herbal Pieces Co. (Bozhou, China). The licorice water extract was prepared according to the reported method [2].

Chemical analysis of the water extract of A. lancea *rhizomes*

Chromatographic experiments were performed on a Waters ACQUITY^{TM} UPLC^{TM} system (Waters Corp., Milford, MA, USA) equipped with a 2998 photodiode array detector (PDA) together with a quaternary pump, an auto-sample injector, an on-line degasser, and an automatic thermostatic column oven. The mass spectrometry instrument was consisted of a Waters Synapt^{TM} QTOF/MS (Waters Corp.). Ionization was performed in the negative electrospray (ESI) mode. The mass range was set at *m/z* 100–1 000 Da with a 0.5 s scan time. UPLC separation was achieved on a Waters ACQUITY^{TM} UPLC^{TM} BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm) with the column temperature being set at 25 °C. The mobile phase consisted of (A) water containing 0.1% formic acid and (B) acetonitrile. The following gradient elution was used: 95% A (0 min); 80% A (0–1 min); 75% A (1–5 min); 70% A (5–7 min); 40% A (7–10 min); 5% A (10–13 min); and 5% A (13–14 min). The flow rate was set at 0.4 mL·min^{-1}, and the injection volume was 2 μL. Representative chromatographs are shown in Fig. 1.

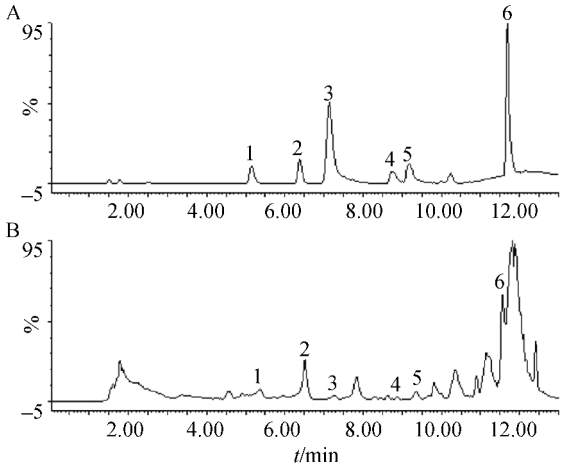

Fig. 1 The total ion current chromatograms of six constituents as mixed standards (A) and the *A. lancea* rhizome water extract (B). Peak 1: protocatechuic acid; 2: atractyloside A; 3: protocatechuic aldehyde; 4: syringic acid; 5: vanillic acid; and 6: limonoids

Animals and drug administration

Male C57BL/6 mice (weighing 20 ± 2 g, 8 weeks old) were obtained from the Model Animal Research Center of Nanjing University (Nanjing, China, SCXK2010-0001). They were housed for 1 week in an air-conditioned room (25 ± 1 °C, 60% relative humidity, and a 12 h light/12 h dark cycle). The mice had free access to tap water and a regular diet before the experiments. For the CYP3A induction analysis, the mice were treated with the water extract of *A. lancea* rhizomes or vehicle by oral gavage every day for 2 weeks. For the toxicity