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Polyphenols extracted from *Coreopsis tinctoria* buds exhibited a protective effect against acute liver damage

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ABSTRACT

The present study aims to investigate the constituents, antioxidant activities and hepatoprotective effect of polyphenols extracted from *Coreopsis tinctoria* buds (CTB-PE) against acute hepatic injury. HPLC analysis showed that six compounds were identified from polyphenolic extracts. The determination of antioxidant activities revealed that the CTB-PE had DPPH radical scavenging activity, ferric-reducing antioxidant power and oxygen radical absorbance capacity. The animal experiments verified that CTB-PE pretreatment ameliorated the D-galactosamine/lipopolysaccharide-induced alterations in histopathological changes, serum biochemical indicators, antioxidant capacity and lipid peroxidation. The potential mechanisms of the hepatoprotective effect might be that CTB-PE treatment significantly enhanced the protein and mRNA expression levels of NF-E2-related factor 2 (Nrf2), peroxisome proliferator-activated receptors α (PPARα) and γ (PPARγ). It suggested that the protective effect of CTB-PE against liver damage was likely attributed to eliminate free radicals, inhibit oxidative stress response and mediate inflammatory response.

1. Introduction

Acute liver disease, a distinct clinical syndrome, is a major cause of high morbidity and mortality (Alasmari et al., 2014). Diverse pathogenesis, such as virus infection, excessive drinking, fatty liver and toxic drugs/chemicals, are related to hepatotoxicity (Hung et al., 2017). Lipopolysaccharide (LPS), one of endogenous substances can cause liver injury (Hung et al., 2017). D-galactosamine (D-GalN) is a commonly chemical hepatotoxic drug. D-GalN/LPS-induced the hepatotoxicity was related with the imbalance of inflammatory cytokines, such as interleukin (IL-6), tumor necrosis factor-α (TNF-α), and monocyte chemotactic protein 1 (MCP-1) (Gong et al., 2017). Studies have shown that the abnormal activation of inflammatory response and oxidative stress are the potential pathogenesis of acute liver injury (Duan et al., 2011; Li et al., 2015). However, no effective treatment method has been available for acute liver disease without liver transplantation (Gong et al., 2017). Therefore, prevention and treatment of acute liver disease through dietary intervention might be a crucial therapeutic target.

Epidemiological and animal studies have shown that polyphenols possess protective effects on liver injury (Pereira, Barros, & Ferreira, 2016). Total polyphenols extracted from *Schisandra chinensis* pollen protected against CCl_4-induced acute hepatotoxicity in mice by inhibiting lipid peroxidation and increasing antioxidant activity (Cheng et al., 2013). Curcumin protected dimethylnitrosamine-induced hepatotoxicity *via* antioxidant response element- directed induction of heme oxygenase-1 (HO-1) protein expression (Farombi, Shrotriya, Na, Kim, & Surh, 2008). The polyphenolic extract of apple peel could be a chemopreventive or chemotherapeutic agent against liver injury induced by high oxidative stress (Nie, Ren, Lu, Sun, & Yang, 2015). Several reports have found that medicinal plants could be applied in the effective therapy of liver ailments (Alipour, Dashti, & Hosseinzadeh, 2014; Domitrović, & Potočnjak, 2016; Picking, Delgoda, Boulogne, & Mitchell, 2013). The polyphenolic compounds extracted from *Syzygium jambos* leaf played similar roles to silymarin in protecting rats from the acute CCl_4 intoxication (Sobeh et al., 2018). Therefore, plant rich in polyphenol components are emerged as the effective resources for the

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CAT, catalase; CTB-PE, polyphenols extracted from *C. tinctoria* buds; D-GalN, D-galactosamine; GSH, glutathione peroxidase; HO-1, heme oxygenase-1; LPS, lipopolysaccharide; MDA, malondialdehyde; Nrf2, nuclear factor-erythroid-2-related factor; PPARs, peroxisome proliferator-activated receptors; ROS, reactive oxygen species; SOD, superoxide dismutase

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prevention of acute liver injury.

Coreopsis tinctoria Nutt. (Compositae) is widely distributed in the alpine region of Kunlun Mountains in Xinjiang, China, with its dry buds being used for the treatment of hypertension and hyperlipidemia in Uyghur traditional medicine (Cao et al., 2011). Flavonoids and phenolic acids extracted from *Coreopsis tinctoria* Nutt. (*C. tinctoria*) were the major functional compounds, which exhibited antioxidant, anti-inflammatory, anti-cancer, antihypertensive, and hypoglycemic activities (Dias, Bronze, Houghton, Mota-Filipe, & Paulo, 2010; Guo, Zhang, Li, & Ho, 2015; Ma et al., 2016; Zălaru et al., 2014). The alcoholic extract of *C. tinctoria* buds showed promising antioxidant and antihypertensive effects (Sun et al., 2013). However, to the best of our knowledge, the hepatoprotective effect of polyphenols extracted from *C. tinctoria* buds (CTB-PE) is still unclear.

Therefore, in this study, the hepatoprotective effect of CTB-PE on D-GalN/LPS-induced mice was evaluated. The underlying mechanisms of the hepatoprotective activity were explored from the point of antioxidant and anti-inflammatory capacities *in vitro* and *in vivo*. The signaling pathways of NF-E2-related factor 2 (Nrf2), peroxisome proliferator-activated receptors α (PPARα) and γ (PPARγ) were further explored.

2. Materials and methods

2.1. Chemicals and reagents

DPPH, ABTS, Trolox, ABAP, D-GalN and LPS were obtained from Sigma-Aldrich, Inc. (St. Louis, USA). Commercial assay kits for ALT, AST, GSH, SOD, CAT, MDA, and protein extraction kits, BCA protein assay kits, Nrf2 antibody, PPARα antibody, PPARγ antibody were bought from Nanjing Jiancheng Bioengineering Co., Ltd (Nanjing, China). Silybin capsules were obtained from Tasly sants Pharmaceutical Co., Ltd (Tianjin, China). Horseradish peroxidase-conjugated goat anti-rabbit antibody was purchased from Signalway Antibody (Pearland, USA). All the other reagents were purchased from Kelong Chemical Reagent Factory (Chengdu, China) and were analytical grade.

2.2. Sample preparation

C. tinctoria buds were obtained and identified by Doctor Shengjun Zhao, Chinese Medicine Hospital of Xinjiang Uygur Autonomous Region. The sample was dried at 50 °C for 4 h and smashed by Chinese herbal medicine crusher sieving for 60 meshes until required.

2.2.1. Extraction of C. Tinctoria buds polyphenols

The polyphenols of *C. tinctoria* buds were extracted using the method reported by Okarter, Liu, Sorrells, & Liu, (2010) with a slight modification. The dried CTB-PE was blended with 80% frozen acetone, and mixed for 2 min by high-speed disperser (5810, Eppendorf Inc., Germany). Then the mixture was centrifuged at 3500*g* for 10 min, and the supernatant was collected. The residue was extracted twice under the same conditions. All the supernatants were combined, evaporated at 45 °C under vacuum, and freeze-dried to obtain the remaining residue. The residue was then reconstituted to 10 mL with deionized water and stored at −80 °C until analysis within three weeks.

2.2.2. Polyphenolic identification using HPLC

The constituents of the extracts were identified by the HPLC method reported by Liang, Cui, Li, Liu, & Zhao (2013) with a slight modification. The analysis was performed on the equipment (LC-20A, Shimadzu Inc., Japan). A Thermo BDS C_{18} column (250 × 4.6 mm i. d. 5 μm) was used for separation. Two mobile phases were 0.1% formic acid (phase A) and 100% acetonitrile (phase B) at a flow rate of 0.7 mL/min. All the injection volume was 10 μL, and the column temperature was 30 °C. The conditions of elution gradients were performed as follows: 0–5 min, 10% B; 5–50 min, 10–40% B; 50–55 min, 40–90% B; 55–62 min, 90% B; 62–65 min, 90–10% B; 65–75 min, 10% B. The polyphenols were monitored at 280 nm.

2.3. Antioxidant activities in vitro

2.3.1. DPPH radical scavenging activity (DPPH) assay

The DPPH assay was measured using the method reported by Cheung, Cheung, & Ooi (2003) with a slight modification. The polyphenolic extracts were diluted, and diluent polyphenolic extracts (1 mL) at different concentrations were added into a 10 mL test tube with 5 mL of 0.1 mmol/L DPPH solution. The mixture was incubated for 30 min in darkness and then immediately determined at the absorbance of 520 nm. Distilled water was used as the blank, and ascorbic acid was used as the control. The scavenging activity on DPPH radical was calculated by the formula (1):

$$SA(\%) = (1-A_1/A_0) \times 100 \quad (1)$$

where SA is the DPPH radical scavenging activity, A_1 is the absorbance of CTB-PE or the ascorbic acid, and A_0 is the absorbance of the blank. Results were expressed as the EC_{50} values (the concentration required to scavenge half of the free radicals).

2.3.2. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was measured using the method reported by Ardestani, & Yazdanparast, (2007) with a slight modification. Diluent polyphenolic extracts (1 mL) at different concentrations, 0.2 mol/L phosphate buffer (2.5 mL, pH 6.6) and 1% potassium ferricyanide (2.5 mL) were added into a 10 mL test tube. The mixture was warmed at 50 °C for 20 min, and then added into 2.5 mL of 10% acetocaustin solution. After blending, the reaction mixture was centrifuged at 3000*g* for 10 min. The supernatant was collected, and added to 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride solution. Distilled water was used as the blank, and ascorbic acid was used as the control. The absorbance was determined at 700 nm. The greater reducing power was correlated with the increased absorbance. Results were expressed as the EC_{50} values (the concentration required when the absorbance reached to 0.5).

2.3.3. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was measured using the method reported by Wolfe et al. (2008) with a slight modification. Trolox solutions (6.25, 12.5, 25, 50 μmol/L) or CTB-PE (1 μg/mL) were prepared in 75 mmol/L phosphate buffer (pH 7.4), respectively. The blank, sample solutions, or trolox solutions (20 μL) and fluorescein solution (200 μL, 0.96 μmol/L) were added to each well in 96-well microplate at the determined position in triplicate. The outside wells of plate were not used. The microplate was incubated for 20 min at 37 °C, and then ABAP solutions (20 μL, 119 mmol/L) were added into each well. The fluorescence intensity was immediately determined by Fluoroskan Ascent plate reader (SynegyH1MG, BioTek Instruments Co. Ltd, USA). The decay of fluorescence was recorded every 4.5 min for 2.5 h at 37 °C (excitation wavelength/incident wavelength: 485/538 nm). Results of ORAC values were expressed as μmol trolox equivalent (TE) per gram of CTB-PE (μmol TE/g PE).

2.4. Animal assay

Male Kunming mice (weight range at 20 ± 2 g) were obtained from the Chongqing company of Teng Xin Biotechnology. All mice were fed under the standard conditions (25 °C, 50% relative humidity) for a week, and allowed to free access to get water and food. Mice were randomly separated into five groups with nine mice in each group: (1) control group; (2) D-GalN/LPS group; (3) D-GalN/LPS + silybin capsules group (400 mg/kg bw, SC); (4) D-GalN/LPS + CTB-PE low dose group (150 mg/kg bw, CTB-L); (5) D-GalN/LPS + CTB-PE high dose group (600 mg/kg bw, CTB-H). The mice were administered