Dendrobium huoshanense polysaccharide prevents ethanol-induced liver injury in mice by metabolomic analysis

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ABSTRACT

The prevalence of alcohol consumption has increased in modern dietary life and alcoholic liver injury can follow. *Dendrobium huoshanense* polysaccharide (DHP) is a homogeneous polysaccharide isolated from *Dendrobium huoshanense*, which possesses hepatoprotection function. In this study, we investigated the metabolic profiles of serum and liver tissues extracts from control, ethanol-treated and DHP\ethanol-treated mice using a UHPLC/LTQ Orbitrap XL MS-based metabolomics approach. Our results indicated that DHP alleviated early steatosis and inflammation in liver histology and the metabolomic analysis of serum and hepatic tissue revealed that first, ethanol treatment mainly altered phosphatidylcholines (PCs) including PC (13:0) and phosphocholine, arachidonic acid metabolites including 20-ethyl PGF2α and amino acids including L-Proline; Second, DHP supplementation ameliorated the altered metabolic levels particularly involved in phosphocholine and L-Proline. These data suggested that DHP might restore the perturbed metabolism pathways by ethanol exposure to prevent the progression of alcoholic liver injury.

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1. Introduction

Alcohol is widely consumed almost everywhere and approximately 2.5 million people die from too much or frequent alcohol consumption each year in the world [1–3]. Epidemiological evidence indicates that alcoholic liver injury from harmful alcohol consumption is one of leading risk factors for the occurrence of liver diseases and many other diseases [4,5]. Therefore, a meaningful management strategy that identifies effective natural compounds for alcohol consumers to prevent or slow down the progression of alcoholic liver injury in the early stage would be beneficial.

Dendrobium huoshanense C.Z. Tang and S.J. Cheng (Orchidaceae) is a valuable perennial herb distributed mainly in southeast China. Its stems have been traditionally used as herbal medicine, tea drinks or soup ingredients in China for centuries because of its tonic function for assisting in curing diseases, strengthening the body and prolonging life, which were recorded in the books such as "*Shennong's Classic of Materia Medica*" from the Eastern Han Dynasty and the "*Compendium of Materia Medica*" from the Ming Dynasty.

Polysaccharides as the principal bioactive compounds existing in *D. huoshanense* have been shown to have hepatoprotective activities in selenium-induced, carbon tetrachloride-induced and alcohol-induced liver injury [6–8].

Metabolomics is an effective systems biology approach to characterize metabolic profiles of organs, tissues or cells when subjected to pathophysiological stimuli or genetic modifications by multiparametric statistical analysis [9,10]. This technique focuses on the changes of endogenous small molecular metabolites (MW < 1000) during metabolic processes. Metabolomics provides a new view, which is distinct from other omics technologies (genomics, transcriptomics and proteomics). The liver plays the most important roles in substance metabolism and energy metabolism and is the source of massive endogenous metabolites [11]. When alcohol intake occurs, the liver as the most important organ responsible for the metabolism of alcohol unavoidably suffers "multi-hits" induced by alcohol exposure [12–14]. Therefore, metabolic processes in the liver are certainly disturbed in the pathogenesis of alcoholic liver injury [15].

In the previous study [8], we investigated the effects of orally administrated *D. huoshanense* polysaccharide on ethanol-induced subacute liver injury in mice using a proteomic approach. In the present study, we further employed the UHPLC-LTQ Orbitrap XL MS-based metabolomics approach to analyze the serum and

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hepatic metabolic profiles of the mice with ethanol-induced subacute liver injury after oral administration of *D. huoshanense* polysaccharide. This study would help to get a better insight into the protection mechanisms of *D. huoshanense* polysaccharide against alcohol-induced liver injury based on the changes in the endogenous small molecular metabolites in the liver.

2. Materials and methods

2.1. Chemicals and reagents

HPLC grade methanol, acetonitrile and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Analytical grade chloroform was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultra-pure water was prepared by filtering double distilled water through a Milli-Q water purification system (Millipore Corp., Billerica, MA, USA). Sampling vials and cylinder filters were purchased from Thermo (San Jose, USA) and Jinteng Experiment Equipment Co. Ltd. (Tianjin, China), respectively. The homogeneous *Dendrobium huoshanense* polysaccharide (DHP), which is composed of a backbone consisting of →1)-α-D-glucose-(6→ with O-acetyl groups, →1)-α-D-glucose-(4→ and →1,3)-α-D-mannose-(6→ in the molar ratio of 2.4: 1: 1 and branches of →1)-α-D galactose with a relative molecular weight of 2.2×10^4 Da based on HPGPC, GC-MS and NMR analysis, was obtained by hot water extraction, ethanol precipitation and fractionation on DEAE-Cellulose anion-exchange column and Sephacryl S-200 gel column according to our previous report [16]

2.2. Animal experiments and sample collection

Animal experiments were carried out according to the design including DHP administration and energy balance of different groups as our previous study [8]. In short, SPF Kunming mice (male, 6 to 8 weeks old) from Laboratory Animal Center (Anhui Medical University, China) were housed in the surroundings with a 12 h light/12 h dark cycle, room temperature of $23 \pm 2\,°C$ and relative humidity of $55 \pm 5\%$. Mice were housed for acclimatization for 5 days prior to all experiments. Then, mice were randomly divided into control group, ethanol group and DHP/ethanol group ($n = 12$ per group). DHP/ethanol group mice received oral administration of DHP (400 mg/kg b. wt) once daily for 45 days. Control group and ethanol group mice received an equal volume of vehicle. From the 31st day, the mice in ethanol group and DHP/ethanol group suffered intragastric administration with ethanol (2400 mg/kg b. wt) once daily for consecutive 15 days to induce subacute alcoholic liver injury. On the 45th day, all mice were sacrificed and blood was obtained by eyeball removal to harvest the serum. The left lobes of livers were fixed in 10% neutral formalin for histological analysis. The right lobes of the livers were frozen immediately in liquid nitrogen and then stored at $-80\,°C$ until LC-MS analysis.

2.3. Histological analysis

The fixed liver samples were embedded in paraffin, sectioned with a thickness of 5 μm and stained with haematoxylin and eosin (H&E) according to the conventional procedure. The degree of liver injury was assessed based on histopathology examinations using a BX43 light microscope (Olympus, Japan).

2.4. Sample preparation for metabolomic analysis

Extraction methods were carried out with minor modification according to protocols as described previously [17]. Serum samples (100 μl of each) were thawed at room temperature, added with 300 μl pre-chilled methanol, vortexed for 2 min, incubated

for 30 min at 4 °C and centrifuged at 12,000 g for 10 min at 4 °C. The supernatants were collected and filtrated through 0.22 μm cylinder filters to sampling vials for UHPLC-LTQ Orbitrap XL MS analysis. Frozen liver tissues (50 mg per sample) were incubated with 1.5 ml of pre-chilled extraction solution containing trichloromethane-methanol-water (2/5/2, v/v/v), homogenized with tissue grinders (Kimble, California, USA) on ice, vortexed for 2 min, incubated for 30 min at 4 °C and centrifuged at 12,000 g for 10 min at 4 °C. The supernatants were also obtained for UHPLC-LTQ Orbitrap XL MS analysis. QC sample was a mixture of equal volume of all serum samples and prepared as described above. The QC sample was analyzed at every sixth injection of samples throughout the whole analytical run.

2.5. UHPLC conditions

Metabolite separation was performed using an Accela UHPLC system equipped with an Accela 1250 pump and autosampler (Thermo Fisher Scientific, San Jose, CA, USA). The LC conditions were
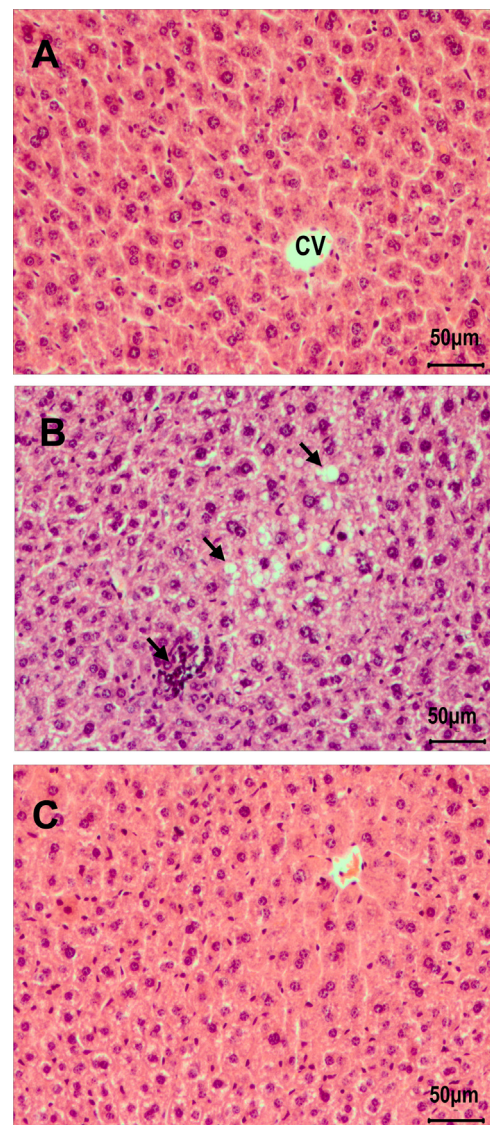

Fig. 1. Effect of DHP on hepatic pathological changes of mice induced by ethanol exposure. (A) Control mice showed normal hepatic cell structure and hepatic lobular architecture (200 ×). (B) Ethanol-treated mice showed steatosis and inflammation (arrow) (200 ×). (C) DHP/ethanol-treated mice showed lesser steatosis and lesser inflammation (200 ×) (CV = central vein).