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Original Article

Characterization and hepatoprotective activity of anthocyanins from purple sweet potato (*Ipomoea batatas* L. cultivar *Eshu* No. 8)

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ABSTRACT

The hepatoprotective activity of anthocyanin-rich purple sweet potato extract (APSPE) was demonstrated. Sixty mice were randomly divided into six groups: control group [without carbon tetrachloride (CCl_4) or APSPE]; model group (with CCl_4 only); positive control group (50 mg/kg body weight silymarin); low-dose group (100 mg/kg body weight APSPE); medium-dose group (200 mg/kg body weight APSPE); and high-dose group (400 mg/kg body weight APSPE). After 10 days intragastric administration of the respective supplements, the mice in all groups except control were injected intraperitoneally with CCl_4 (0.15% in arachis oil, 10 mL/kg body weight, intravenous). Twelve hours after CCl_4 injection, the mice were measured in terms of liver index, levels of aspartate aminotransferase and alanine aminotransferase in serum, as well as glutathione, superoxide dismutase, and malondialdehyde in liver homogenate. Additionally, the livers of mice were stained with hematoxylin and eosin and sectioned for observation. Nineteen purple sweet potato anthocyanins were identified from the purple sweet potato cultivar *Eshu* No. 8 and analyzed by liquid chromatography– electrospray ionization–tandem mass spectrometry. Peonidin 3-coumaryl-p-hydroxybenzoyl sophoroside-5-glucoside was first identified in purple sweet potato. The results showed that anthocyanins in *Eshu* No. 8 had good hepatoprotective activity.

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1. Introduction

Organisms need the energy from oxidation reactions to sustain metabolism [1]. However, excessive oxidative products can be generated sometimes in our body such as superoxide anion and hydroxyl radicals, which are known as reactive oxygen species (ROS). These oxidative products can directly attack the phospholipid molecules of cell membrane, leading to lipid peroxidation and changes in the structure and function of cell membrane. These changes can result in cellular damage [2,3], and a variety of chronic diseases such as inflammation, liver diseases, diabetes, renal failure, aging and cancer [4]. With more studies conducted on the relationship between oxidative stress injury and chronic disease, antioxidants from nature have received increasing attention [5,6].

Liver disease is a quite common threat to human health [7]. However, due to lack of reliable liver protective drugs, compounds extracted from plants such as silymarin are extremely attractive to the prevention and treatment of liver disease [8]. Carbon tetrachloride (CCl_4) is a classic liver poison, which can be easily absorbed by the organism through the digestive tract and respiratory tract, and transformed into trichloromethyl radical ($CCl_3\cdot$) through cytochrome oxidase P450 in liver microsomes [9]. A previous study reported that ROS are closely linked with lesions of liver pathology, especially with alcohol and drug-induced liver injury [10]. CCl_4-induced acute or chronic liver injuries were widely used as a model to establish experimental animal for filtering liver diseases drugs and ingredients with hepatoprotective activity in food [11,12].

Purple sweet potato has a high nutritional value due to its abundance of phytochemicals beneficial to human health and it is thus regarded as a functional food [13]. Since its introduction from Japan to China, purple sweet potato has received a lot of attention because of its high content of anthocyanins [14]. Anthocyanins are flavonoids with a positive charge with C6-C3-C6 structure, and one of the most common water-soluble pigments in nature. Anthocyanins are also one of the most common daily intakes of flavonoids [15]. The basic structures of purple sweet potato anthocyanins are cyanidin, peonidin, and pelargonidin. Among them, the content of pelargonidin is relatively low [16]. The C3 and C5 were combined separately with sophorose and glucose to form glycosidic bond. The sophorose in C6 were combined with *p*-hydroxybenzoic acid, ferulic acid, coumaric acid, or caffeic acid to form one or two monoacylated bonds [17]. In recent years, many reports have been published on the structure of purple sweet potato anthocyanins. In 2012, the library of purple sweet potato anthocyanins was concluded by Kim et al [18]. Another six novel purple sweet potato anthocyanins were separated and characterized by Lee et al [16]. Purple sweet potato anthocyanins were found to be quite stable and have good biological activities, such as antioxidation, antimutation, and antidiabetes [19–22].

The planting distribution of purple sweet potato is increasingly wide in China, and *Eshu* No. 8 is a new species cultivated in Hubei, China, with a quite good yield [23]. So far, there is no report on anthocyanins from *Eshu* No. 8 except that our laboratory did research into its effects on serum uric acid level and xanthine oxidase activity in hyperuricemic mice [24]. A study reported that purple sweet potato anthocyanins can effectively remove hydroxyl radicals and reduce the occurrence of lipid peroxidation [19], suggesting that purple sweet potato anthocyanins may have protective effects in liver damage induced by CCl_4. However, to our knowledge, no information is available about the content and hepatoprotective activity of purple sweet potato anthocyanins in *Eshu* No. 8. Therefore, it is necessary to extract the anthocyanins from *Eshu* No. 8, identify the structures of anthocyanins and their hepatoprotective activity. In this article, anthocyanins were separated and purified from *Eshu* No. 8. The structures of individual anthocyanins were analyzed by high-performance liquid chromatography (HPLC)–diode array detection (DAD)–electrospray ionization (ESI)–tandem mass spectrometry (MS/MS). The hepatoprotective activity of anthocyanins was also studied in CCl_4-induced Kunming mice.

2. Materials and methods

2.1. Materials and chemicals

Dried purple sweet potato powder (*Ipomoea batatas* L. cultivar *Eshu* No. 8) was donated by Puzetian Food Co. Limited (Wuhan, China). Assay kits for determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) were purchased from Nanjing Jiancheng Biotechnology Institute (Nanjing, China). Silymarin was bought from MADAUS GmbH (Germany). All other reagents were of analytical grade.

2.2. Extraction and enrichment of anthocyanins

The extraction and enrichment of anthocyanins used the method as described previously with some modifications [16]. Solvent extraction was carried out using a solvent of 40% 0.1M HCl in anhydrous ethanol (v/v). The dried purple sweet potato powder was weighed and 5 g placed in a capacity tube, followed by the addition of 50 mL extracting solvent into the sample tube. After being capped and mixed briefly, the sample tubes were placed on magnetic stirrers at 60°C for 30 minutes, followed by centrifugation at 2057 *g* for 10 minutes. The supernatant extract was transferred into another tube. The extraction process was repeated several times until no red color was observed in the solvent. Finally, all the extracts were evaporated and pooled into one tube.

The 50 mL aqueous solution of the crude anthocyanins was loaded onto an AB-8 resin (weak polarity macroporous resin, 0.3–1.25 mm particle size, Nankai Hecheng Science & Technology Co., Tianjin, China) column (25 mm × 100 mm). After that, the column was water washing. Finally, the fraction eluted with 70% ethanol was collected. After the eluent was evaporated and freeze-dried, extract of purple sweet potato anthocyanins (APSPE) was obtained and stored at −20°C in sealed aluminum bags for future use.