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.

2.3. Quantitation of total anthocyanin

The quantitation of total anthocyanins was determined using a modified pH differential method as described previously [25]. Briefly, 10 mg/mL APSPE was aliquoted in duplicate. One was diluted with pH 1.0 buffer, and the other with pH 4.5 buffer. The absorbance of each solution was measured after equilibration for 15 minutes using a spectrophotometer (UV-2000; UNICO, Shanghai, China) at a wavelength of 525 nm and 700 nm versus a blank cell filled with distilled water. The absorbance was calculated as follows:

$$A = (A_{525} - A_{700})_{pH\ 1.0} - (A_{525} - A_{700})_{pH\ 4.5.}$$

Total anthocyanins were calculated using the following equation and expressed as mg of cyanidin 3-glucoside per 100 g:

$$C = (A/eL) \times M \times D \times (V/W) \times 100$$

where C is the total anthocyanin content (mg/100 g), A is the absorbance measured, e is the molar absorbance of cyanidin 3-glucoside (26,900 L/cm/mol), L is cell path length (1 cm), M is the molecular weight of cyanidin 3-glucoside (449.2 g/mol), D is dilution multiple, V is the final volume (mL), and W is the sample weight (mg).

2.4. Analysis and characterization of anthocyanins by LC-ESI-MS/MS

The individual anthocyanins were separated by an Agilent 1100 HPLC equipped with a diode array detector connected to an Agilent 6300 mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA), and a 250-cm × 4.6-mm inner diameter 5-μm TSKgel C18 column (Tosoh Corporation, Tokyo, Japan). The temperature of column was set at 30°C during the HPLC analysis. The injection volume of the 2 mg/mL solution of APSPE was 10 μL. The individual anthocyanins were obtained by HPLC-DAD at 525 nm. Solvent A was 1% formic acid in water (v/v), and solvent B was purified acetonitrile. The solvent gradient was 0–10 minutes, 90 to 85% A; 10–20 minutes, 85% to 80% A; 20–30 minutes, 80% to 75% A; 30–40 minutes, 75% to 70% A; 40–45 minutes, 75% to 70% A. Mass spectra in the m/z range of 200–1600 were obtained by electrospray ionization in positive-ion mode. The mass spectrometric conditions were optimized as follows: the capillary voltage of 4.5 kV, pressure of nebulizing nitrogen of 275.8 kPa, capillary temperature of 350°C, and dry gas flow at 10 L/min.

2.5. Tested animals

A total of 60 Kunming mice (males, specific-pathogen-free, 6-week-old, 18–22 g body weight) were purchased from Hubei Center for Disease Control and Prevention Animal Center (Certificate No. SCXK 2008-0005, Wuhan, China). All animals were handled in accordance with the standards for laboratory animals established by the People's Republic of China (GB14925-2001) and the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals. Animals were kept under standard laboratory conditions (12 hours light/dark cycles, temperature 25 ± 2°C, relative humidity 50%) with food and water provided ad libitum.

2.6. CCl₄-induced hepatotoxicity in mice

All the mice were randomized into six groups ($n = 10$): normal control group; CCl₄-injured model group; low-dose, medium-dose, and high-dose groups with APSPE (100 mg/kg body weight, 200 mg/kg body weight, and 400 mg/kg body weight); and positive control group with silymarin (50 mg/kg body weight). The mice in every group were fed with standard mice feed with distilled water freely available. After adaptation for 1 week, the mice in the normal control group and the CCl₄-injured model group were administered with 10 mL/kg body weight of normal saline by gavage once a day. The mice in low-dose, medium-dose and high-dose groups were administrated separately with 100 mg/kg body weight, 200 mg/kg body weight, and 400 mg/kg body weight of APSPE every day. The mice in the positive control group were administrated with 50 mg/kg body weight of silymarin once a day. After 10 days of such successive treatment and 4 hours after the last intragastric administration, the mice in each group were intraperitoneally injected with 10 mL/kg body weight 0.15% CCl₄ solution (dissolved in the oil), except that the mice in the normal control group were treated with normal saline at the same dosage. After diet fasting (with distilled water still available) for 12 h, the blood samples of the mice were collected, and then the mice were sacrificed by cervical dislocation. After that, the livers were taken out, rinsed with normal saline solution, and weighed one by one. One liver lobe from each mouse was used for determination of biochemical indicators, and the remaining liver lobes were put into formalin for histopathological analysis.

2.7. Detection of liver index

The liver index on a fresh weight basis was calculated according to the following formula:

$$\text{Liver index (\%)} = [\text{weight of liver (g)/final body weight (g)}] \times 100$$

2.8. Preparation of serum and liver homogenate samples

After blood samples were centrifuged at 1575 g at below 4°C for 15 minutes, serum samples were separated for ALT and AST determination.

Liver samples (0.5 g wet weight) were taken from one lobe of each liver, and homogenized in 4.5 mL precooling normal saline. The liver homogenates were centrifuged at 1157 g at 4°C for 15 minutes before supernatants were separated for MDA, GSH, and SOD determination in the liver homogenate.

2.9. Biochemical assays

All the assessments of biochemical assays (ALT and AST in serum, MDA, GSH, and SOD in liver tissues) were performed

using the assay kits according to the manufacturer's instructions.

2.10. Histopathological analysis

The piece of liver, once taken out, must be fixed in formalin immediately. After 48 hours' fixation, liver tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin for microscopic observation at 200× magnification.

2.11. Statistical analysis

Data are expressed as the mean ± standard deviation values. Analysis of variance (ANOVA) followed by a t test was used to evaluate the significant differences between groups using SPSS version 16.0 for Windows. A p-value < 0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Separation of individual anthocyanins by HPLC and characterization of anthocyanins by HPLC-ESI-MS/MS

As reported in previous studies, individual APSPE were separated by HPLC with a C18 column [18,26,27]. Thus, the C18 column was used in our experiment. As shown in Figure 1, 19 individual anthocyanins were separated. Among them, Peaks 13, 14, and 15 were the largest in area, indicating that these three anthocyanins were the most abundant in *Eshu* No. 8, and the other kinds of APSPE were low in content. The anthocyanins from *Eshu* No. 8 were a little different from anthocyanins in other purple sweet potato varieties. For instance, as reported previously, the most abundant anthocyanins in purple sweet potato cultivar *Zami* were cyanidin 3-caffeoyl-p-hydroxybenzoyl sophorside-5-glucoside, cyanidin 3-caffeoyl-feruloyl sophorside-5-glucoside, and cyanidin 3-caffeoyl sophorside-5-glucoside [16]. In *Eshu* No. 8, they were peonidin 3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside, peonidin 3-

caffeoyl-feruloyl sophoroside-5-glucoside and peonidin 3-dicaffeoyl sophoroside-5-glucoside. Besides, there were some anthocyanins with the basic structure of pelargonidin in *Borami*, *Molpo 62*, and *Zami* [16], while *Eshu* No. 8 was without pelargonidin. Peonidin 3-coumaryl-p-hydroxybenzoyl sophoroside-5-glucoside was first reported in purple sweet potato.

The structures of the 19 anthocyanins were identified by HPLC-ESI-MS/MS. ESI in positive-ion mode was adopted to catch the molecular fragments. Mass scan spectra were acquired with a mass scan range of 200–1600 m/z. Previous studies reported that there is p-hydroxybenzoylsoph (m/z 120), caffeoyl (m/z 162), feruloyl (m/z 176), coumaryl (m/z 146), glucoside (m/z 162) or sophoroside (m/z 324) in the fragments of purple sweet potato anthocyanins [16,18]. These reported data greatly facilitated our analyses, and all of the structures of anthocyanins are shown in Table 1. It was found Peaks 2 and 7, Peaks 4, 5 and 11, and Peaks 12 and 13. Figures 2–4 illustrated the analysis processes separately.

The molecular weights of Peaks 2 and 7 were 935, respectively, indicating that they were isomers. As seen in Figure 2A, the MS/MS of Peak 2 was 773 ([M − $C_6H_{10}O_5$]$^+$), 449 ([M − $C_{12}H_{20}O_{11}$ − $C_9H_6O_3$]$^+$), and 287 ([M − $C_{12}H_{20}O_{11}$ − $C_9H_6O_3$ − $C_6H_{10}O_5$]$^+$), indicating that Peak 2 consisted of cyanidin, sophoroside, glucoside, and caffeoyl. The MS/MS of Peak 7 was 773 ([M − $C_6H_{10}O_5$]$^+$), 461 ([M − $C_{12}H_{20}O_{10}$ − $C_9H_6O_3$]$^+$) and 287 ([M − $C_{12}H_{20}O_{10}$ − $C_9H_6O_3$ − $C_6H_{10}O_5$]$^+$), showing that Peak 7 was composed of cyanidin, sophoroside, glucoside, and caffeoyl. According to the report by Lee et al [16], the structure of Peak 2 was cyanidin 3-(6‴-caffeoyl sophoroside)-5-glucoside, and the Peak 7 was cyanidin 3-(6″-caffeoyl sophoroside)-5-glucoside (Figure 3B).

As shown in Table 1, the molecular weights of Peaks 4, 5, and 11 were all 949, so they were isomers. As shown in Figure 3A, the fragments of 787 ([M − $C_6H_{10}O_5$]$^+$), 463 ([M − $C_{12}H_{20}O_{10}$ − $C_9H_6O_3$]$^+$) and 300 ([M − $C_6H_{10}O_5$ − $C_9H_6O_3$ − $C_{12}H_{20}O_{10}$]$^+$) were caught in the fragment of Peak 4, indicating that this peak was composed of peonidin, sophoroside, glucoside, and caffeoyl. The MS/MS of Peak 5 was 787 ([M − $C_6H_{10}O_5$]$^+$), 449 ([M − $C_{10}H_8O_3$ − $C_{12}H_{20}O_{10}$]$^+$), and 287 ([M − $C_{10}H_8O_3$ − $C_{12}H_{20}O_{10}$ − $C_6H_{10}O_5$]$^+$), showing that Peak 5
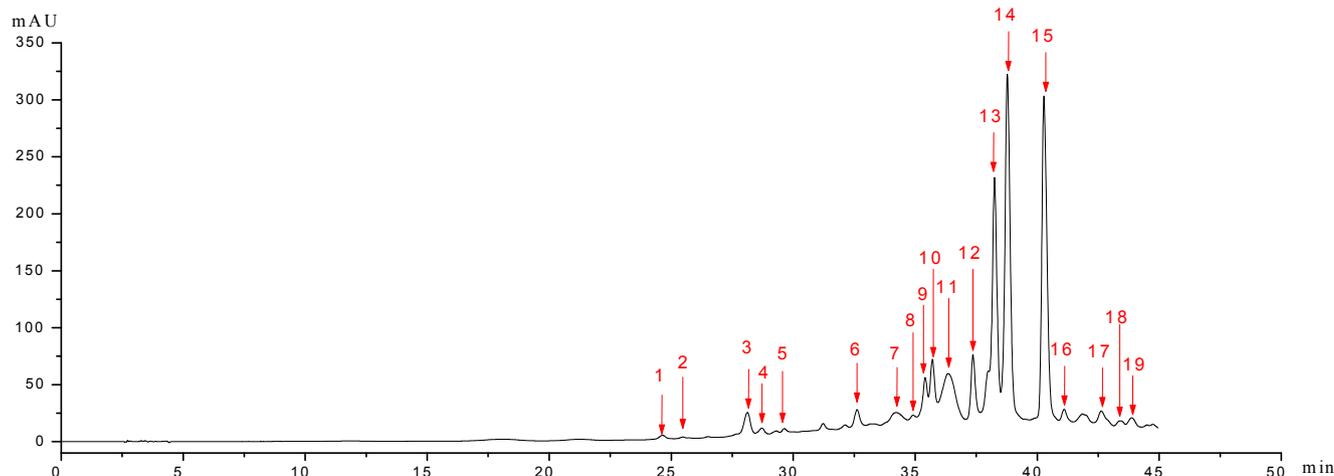
Figure 1 − **High-performance liquid chromatography−diode array detection chromatograms of anthocyanins from purple sweet potato powder (*Ipomoea batatas* L. cultivar *Eshu* No. 8) detected at 525 nm.**

Table 1 – Identification of anthocyanins from purple sweet potato powder (*Ipomoea batatas* L. cultivar *Eshu* No. 8) by high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry.

Peak	t_R (min)	Formula	[M$^+$] (m/z)	Major fragment ion (m/z)	Identification	Quantitation (mg/g)	Reference
1	24.6	$C_{40}H_{45}O_{23}$	893	731, 449, 287	Cyanidin 3-p-hydroxybenzoyl sophoroside-5-glucoside	0.858	16
2	25.5	$C_{42}H_{47}O_{24}$	935	773, 449, 287	Cyanidin 3-(6'''-caffeoyl sophoroside)-5-glucoside	0.339	16
3	28.1	$C_{41}H_{47}O_{23}$	907	745, 463, 301	Peonidin 3-p-hydroxybenzoyl sophoroside-5-glucoside	7.10	16
4	28.7	$C_{43}H_{49}O_{24}$	949	787, 463, 301	Peonidin 3-(6'''-caffeoyl sophoroside)-5-glucoside	2.29	16
5	29.6	$C_{43}H_{49}O_{24}$	949	787, 449, 287	Cyanidin 3-feruloyl sophoroside-5-glucoside	2.04	16
6	32.6	$C_{44}H_{51}O_{24}$	963	801, 463, 301	Peonidin 3-feruloyl sophoroside-5-glucoside	7.12	16
7	34.2	$C_{42}H_{47}O_{24}$	935	773, 449 287	Cyanidin 3-caffeoyl sophoroside-5-glucoside	13.1	16
8	34.9	$C_{33}H_{41}O_{21}$	773	611, 449, 287	Cyanidin 3-sophoroside-5-glucoside	4.44	16
9	35.4	$C_{51}H_{53}O_{27}$	1097	935, 449, 287	Cyanidin 3-dicaffeoyl sophoroside-5-glucoside	12.2	16
10	35.7	$C_{49}H_{51}O_{26}$	1055	893, 449, 287	Cyanidin 3-caffeoyl-p-gydroxybenzoyl sophoroside-5-glucoside	14.8	16
11	36.4	$C_{43}H_{49}O_{24}$	949	787, 463, 301	Peonidin-3-caffeoyl sophoroside-5-glucoside	32.5	16
12	37.4	$C_{52}H_{55}O_{27}$	1111	949, 449	Cyanidin 3-caffeoyl-feruloyl sophoroside-5-glucoside	16.2	16
13	38.2	$C_{52}H_{55}O_{27}$	1111	949, 463	Peonidin 3-dicaffeoyl sophoroside-5-glucoside	57.9	16
14	38.8	$C_{50}H_{53}O_{26}$	1069	907, 463, 301	Peonidin 3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside	75.7	16
15	40.8	$C_{53}H_{57}O_{27}$	1125	963, 463	Peonidin 3-caffeoyl-feruloyl sophoroside-5-glucoside	69.2	16
16	41.1	$C_{52}H_{55}O_{26}$	1095	933, 463, 301	Peonidin 3-caffeoyl-p-coumaryl sophoroside-5-glucoside	5.59	16
17	42.6	$C_{51}H_{55}O_{26}$	1083	921, 463, 301	Peonidin 3-feruloyl-p-hydroxybenzoyl sophoroside-5-glucoside	5.81	18
18	43.4	$C_{50}H_{53}O_{25}$	1053	891, 463, 301	Peonidin 3-coumaryl-p-hydroxybenzoyl sophoroside-5-glucoside	1.81	*
19	43.9	$C_{54}H_{59}O_{27}$	1139	977, 463	Peonidin 3-(6'', 6'''-diferuloyl sophoroside)-5-glucoside	2.43	18

*Anthocyanin identified for the first time in purple sweet potato in this paper.
t_R = retention time.
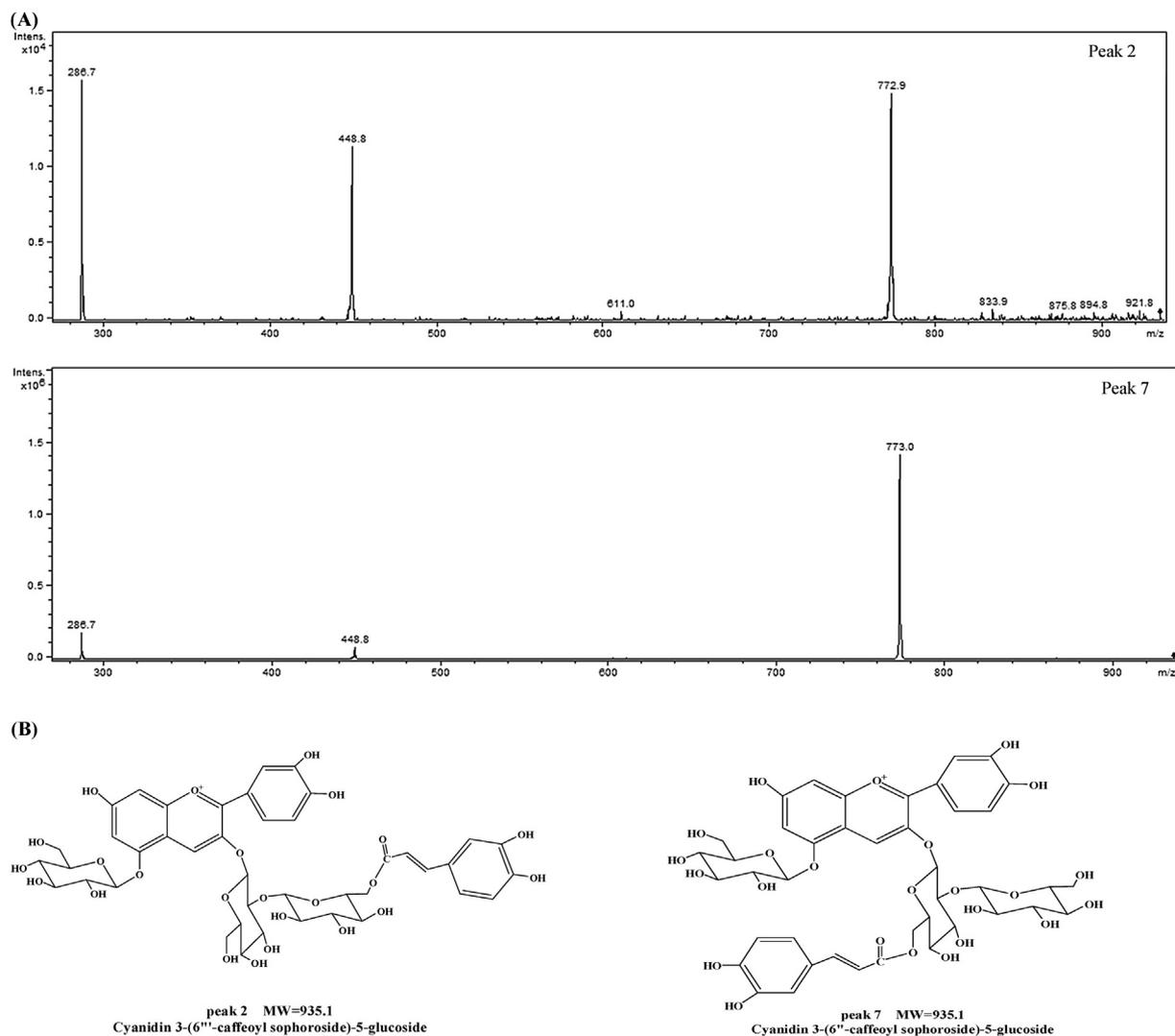
Figure 2 – (A) High-performance liquid chromatography–tandem mass spectrometry; (B) Chemical structures of Peaks 2 and 7.

consisted of cyanidin, sophoroside, glucoside, and feruloyl. The MS/MS of Peak 11 was 787 ($[M - C_6H_{10}O_5]^+$), 463 ($[M - C_9H_6O_3 - C_{12}H_{20}O_{10}]^+$), and 301 ($[M - C_9H_6O_3 - C_{12}H_{20}O_{10} - C_6H_{10}O_5]^+$), indicating that Peak 11 consisted of peonidin, sophoroside, glucoside, and caffeoyl. Also, as shown in Figure 3B, with 1% formic acid solution (v/v) as phase A and acetonitrile as phase B in mobile phases, it can be deduced from the sequences of the peaks of individual anthocyanins that the structure of Peak 4 was peonidin 3-(6″-caffeoylsophoroside)-5-glucoside, the structure of Peak 5 was cyanidin 3-feruloyl sophoroside-5-glucoside, and the structure of Peak 11 was peonidin 3-caffeoyl sophoroside-5-glucoside.

As shown in Table 1, the molecular weights of Peaks 12 and 13 were 1111. As seen in Figure 4A, the fragment of Peak 12 was 949 ($[M - C_6H_{10}O_5]^+$), and 449 ($[M - C_{10}H_8O_3 - C_9H_6O_3 - C_{12}H_{20}O_{10}]^+$), indicating that Peak 12 consisted of cyanidin, sophoroside, glucoside, caffeoyl and feruloyl. The fragment of Peak 13 was 949 ($[M - C_6H_{10}O_5]^+$), and 463 ($[M - C_6H_{10}O_5 - C_9H_6O_3 - C_9H_6O_3 - C_{12}H_{20}O_{10}]^+$), suggesting that Peak 14 consisted of peonidin, sophoroside, glucoside, and difcaffeoyl. Their structures are shown in Figure 4B. According to previous

reports [16], they were cyanidin 3-caffeoyl-feruloyl sophoroside-5-glucoside and peonidin 3-dicaffeoyl sophoroside-5-glucoside.

As we can see in Table 1, the molecular weight of Peak 18 was 1053. As shown in Figure 5A, the fragments of Peak 18 was 891 ($[M - C_6H_{10}O_5]^+$), 463 ($[M - C_9H_6O_2 - C_7H_4O_2 - C_{12}H_{20}O_{10}]^+$), and 301 ($[M - C_9H_6O_3 - C_7H_4O_2 - C_{12}H_{20}O_{10} - C_6H_{10}O_5]^+$), showing that Peak 18 was composed of peonidin, sophoroside, glucoside coumaryl and hydroxybenzoyl. There were no reports about the structure of Peak 18 in purple sweet potato. This indicates that the structure of Peak 18 was peonidin 3-coumaryl-p-hydroxybenzoyl sophoroside-5-glucoside (Figure 5B).

3.2. Liver index

The health status of viscera can be intuitively shown by organ index. Twelve hours after CCl$_4$ injection, the livers of mice would be damaged by acute toxicity, and the liver index of mice would obviously increase [28]. After dissection, livers were taken out for liver index determination. The damage
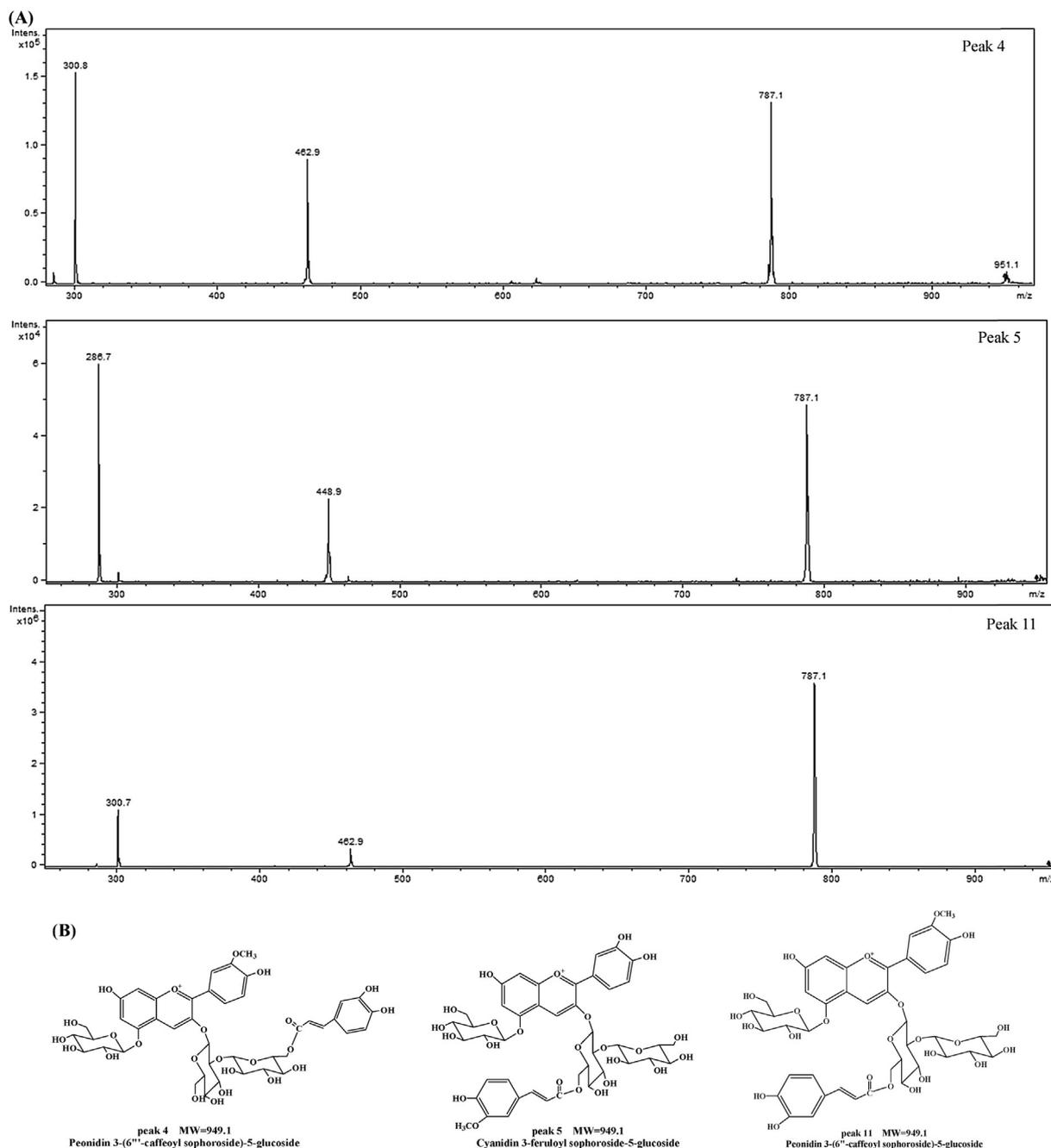
Figure 3 – (A) High-performance liquid chromatography–tandem mass spectrometry; **(B)** Chemical structures of Peaks 4, 5, and 11.

degree of livers was intuitively reflected. Figure 6 shows that the liver index of the control group mice without CCl_4 injection was apparently lower than that of the model group mice with CCl_4 injection ($p < 0.05$), suggesting that our model was successful. The liver indices of mice in middle-dose and high-dose groups were markedly lower than that of the model group ($p < 0.05$), and they were no significant with that of the control group. The data in Figure 6 reveal that APSPE has good hepatoprotective activity against CCl_4-induced liver damage by inhibiting the increase of liver weight effectively.

3.3. Effects of APSPE on AST and ALT in mice serum

AST and ALT are important enzymes in human cells and are involved in the synthesis of important amino acids in the human body. AST and ALT exist in mitochondria and liver cytoplasm, respectively. Usually, the contents of AST and ALT are stable in serum. However, when the structures of parenchymal hepatic cells were damaged, especially when the membranes were damaged, AST from mitochondria and ALT from liver cytoplasm would release into serum, resulting in
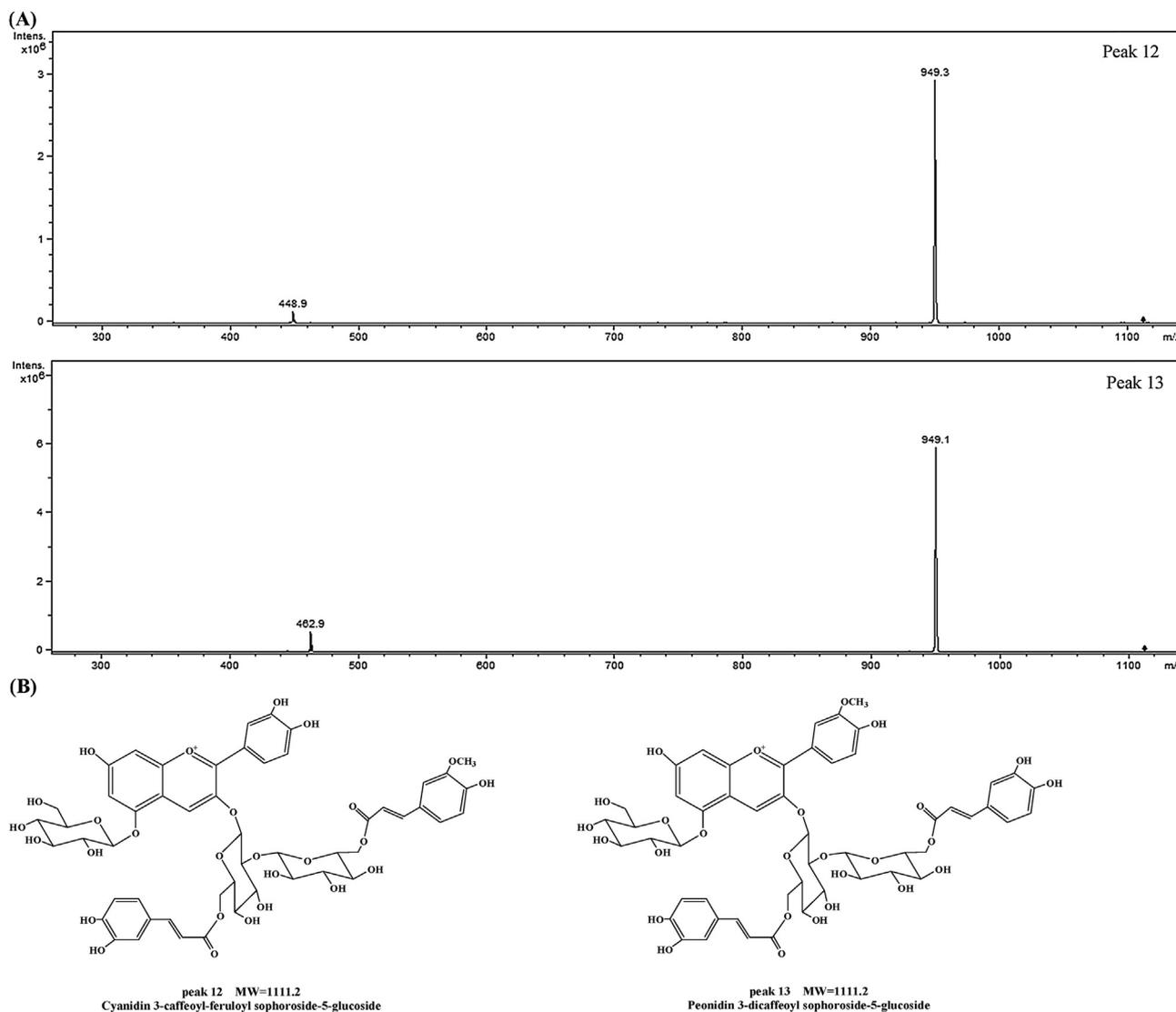
Figure 4 – **(A) High-performance liquid chromatography–tandem mass spectrometry; (B) Chemical structures of Peaks 12 and 13.**

the increase of AST and ALT in serum, which were quite sensitive indexes to reflect the liver damage [29].

As shown in Table 2, when compared with the normal group, the activity of AST and ALT in serum from the model group increased significantly ($p < 0.05$), indicating that the CCl₄-induced liver toxicity was obvious. The levels of AST and ALT in groups treated with APSPE were all apparently lower than those of the model group ($p < 0.001$–0.05) in a dose-dependent manner. The levels of AST and ALT in the medium-dose group were close to those of the positive control group, and the effect of the high-dose group was even better than that of the positive control group. Therefore, APSPE could effectively protect liver and decrease the release of AST and ALT.

3.4. Effects of APSPE on SOD, GSH, and MDA in mouse liver

There are important active substances such as SOD, GSH, and MDA in the human body. SOD plays an important role in maintaining the oxidation balance of organism, which can clear away O_2^- to protect cells from damage. GSH is an important oligopeptide, which can clear away a variety of free radicals, stabilize mercapto enzymes, and protect hemoglobin and other auxiliary factors from oxidative damage. GSH can decompose peroxide from the process of lipid peroxidation, which has great hepatoprotective activity against chemical substance-induced cell toxicity [30]. Organisms can generate ROS through enzyme systems and nonenzyme systems, attacking polyunsaturated fatty acids in the biofilms and causing lipid peroxidation. MDA is the typical product of lipid peroxidation [31]. The antioxidant ability of organism is directly reflected by contents of SOD and GSH, while the degree of lipid peroxidation is reflected by the content of MDA, and the degree of cell damage is reflected indirectly.

As shown in Table 3, when compared with the control group, the content of MDA in mice livers from the model group increased markedly, and the contents of SOD and GSH decreased significantly ($p < 0.05$). When compared with the

(A)

(B)

peak 18   MW=1053.1
Peonidin 3-coumaryl-p-hydroxybenzoyl sophoroside-5-glucoside
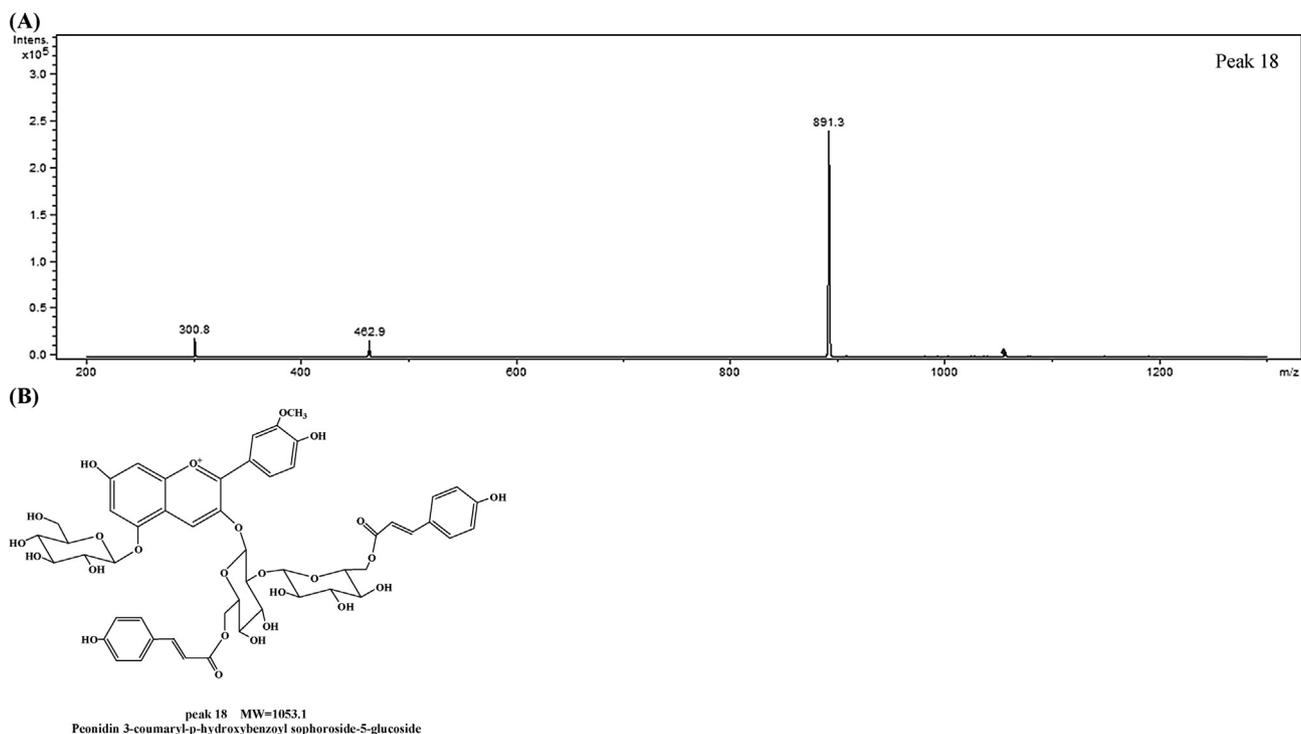
Figure 5 – (A) High-performance liquid chromatography–tandem mass spectrometry; (B) Chemical structures of Peak 18.
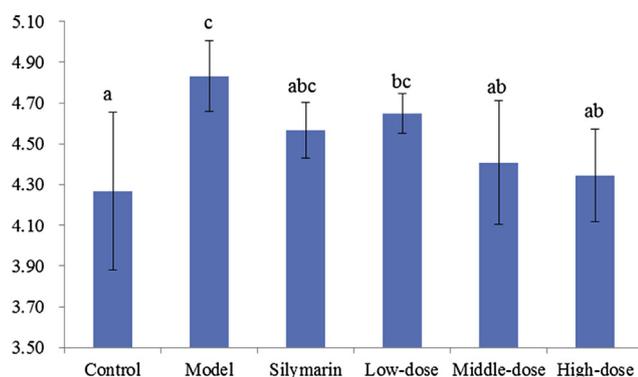
Figure 6 – Effect of the liver index on carbon tetrachloride (CCl_4)-induced damage in mice. The liver index on a fresh weight basis was calculated according to the following formula: Liver index (%) = [weight of liver (g)/final body weight (g)] × 100. model group: CCl_4 (0.15% in arachis oil, 10 mL/kg body weight, intravenous); silymarin (50 mg/kg body weight) + CCl_4 injection; low-dose group: anthocyanin-rich purple sweet potato extract (APSPE; 100 mg/kg body weight) + CCl_4 injection; medium-dose group: APSPE (200 mg/kg body weight) + CCl_4 injection; high-dose group: APSPE (400 mg/kg body weight) + CCl_4 injection.

model group, the contents of MDA in the groups treated with APSPE apparently decreased, and the contents of SOD and GSH increased obviously in a dose-dependent manner. The effect of the medium-dose group was close to that of the positive control group, while the effect of the high-dose group was a little higher than that of the positive control group. Therefore, the activity of SOD and the content of GSH were increased, and the content of MDA in mice livers in APSPE-treated-groups was decreased. This result illustrated that APSPE can effectively increase the vitality and content of reducing substances against CCl_4-induced liver damage.

3.5. Histopathological examination

As shown in Figure 7, the liver and liver nuclei of the control group were clear, abundant in cytoplasm, and showed no changes in cell alignment. Hepatic cord and sinusoid were clearly visible. However, it can be seen in Figure 7B that the hepatocytes of the model group swelled severely, like a hydropic degeneration or vacuolation. Cytoplasm was suffused with fat droplets and showed obvious steatosis. The alignments of hepatocytes were disordered; cytoplasm was uneven; cell nuclei were fused; and necrotic foci appeared. All these observations suggest that model was successful in the form of histomorphology. APSPE treatment groups decreased, to varying degrees, the damage induced by CCl_4. With the dose of APSPE increasing, the liver damage degree gradually decreased. For instance, the microscopic examination of the mice in the low-dose group showed that hepatocytes were moderately damaged, including steatosis, acute coagulative necrosis, neutrophils infiltration and other cells. However, few hepatocytes from the high-dose group were damaged. The result of histopathology was consistent with the biochemical criterion of mice and demonstrates that APSPE can effectively protect liver against CCl_4-induced liver damage.

Table 2 – Effects of anthocyanin-rich purple sweet potato extract (APSPE) administration on the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), in serum in the liver damaged mice induced by carbon tetrachloride (CCl₄).

Group	Treatment	AST (U/L)	ALT (U/L)
I	Control	28.4 ± 7.93 [a]	39.1 ± 3.74 [a]
II	CCl$_4$ (10 mL/kg bw, i.p.)	107 ± 14.3 [d]	298 ± 16.9 [e]
III	CCl$_4$ + silymarin (50 mg/kg bw, p.o.)	79.2 ± 11.9 [b]	230 ± 14.9 [c]
IV	CCl$_4$ + APSPE (100 mg/kg bw, p.o.)	91.2 ± 9.07 [c]	269 ± 16.3 [d]
V	CCl$_4$ + APSPE (200 mg/kg bw, p.o.)	82.2 ± 9.90 [b]	231 ± 21.5 [c]
VI	CCl$_4$ + APSPE (400 mg/kg bw, p.o.)	71.3 ± 8.55 [b]	176 ± 9.68 [b]

Values are expressed as mean $\pm$ standard deviation ($n = 10$).
Different letters indicate significant differences ($P < 0.05$).
bw = body weight; i.p. = intraperitoneally; p.o. = *per os*.

Table 3 – Effects of anthocyanin-rich purple sweet potato extract (APSPE) administration on malondialdehyde (MDA) and glutathione (GSH) levels as well as superoxide dismutase (SOD) activities in liver homogenate in the liver damaged mice induced by carbon tetrachloride (CCl₄).

Group	Treatment	MDA (nmol/mg protein)	SOD (U/mg protein)	GSH (nmol/mg protein)
I	Control	4.52 ± 0.38 [a]	320 ± 6.17 [ab]	2.02 ± 0.16 [a]
II	CCl$_4$ (10 mL/kg bw, i.p.)	7.04 ± 0.66 [d]	280 ± 4.88 [c]	1.58 ± 0.12 [c]
III	CCl$_4$ + silymarin (50 mg/kg bw, p.o.)	5.18 ± 0.49 [b]	319 ± 11.58 [a]	1.73 ± 0.15 [ab]
IV	CCl$_4$ + APSPE (100 mg/kg bw, p.o.)	5.98 ± 0.47 [c]	288 ± 8.68 [c]	1.67 ± 0.11 [bc]
V	CCl$_4$ + APSPE (200 mg/kg bw, p.o.)	5.32 ± 0.37 [b]	317 ± 9.32 [a]	1.81 ± 0.15 [b]
VI	CCl$_4$ + APSPE (400 mg/kg bw, p.o.)	5.15 ± 0.48 [ab]	330 ± 7.53 [b]	2.06 ± 0.12 [a]

Values are expressed as mean $\pm$ standard deviation ($n = 10$).
Different letters indicate significant differences ($P < 0.05$).
bw = body weight; i.p. = intraperitoneally; p.o. = *per os*.
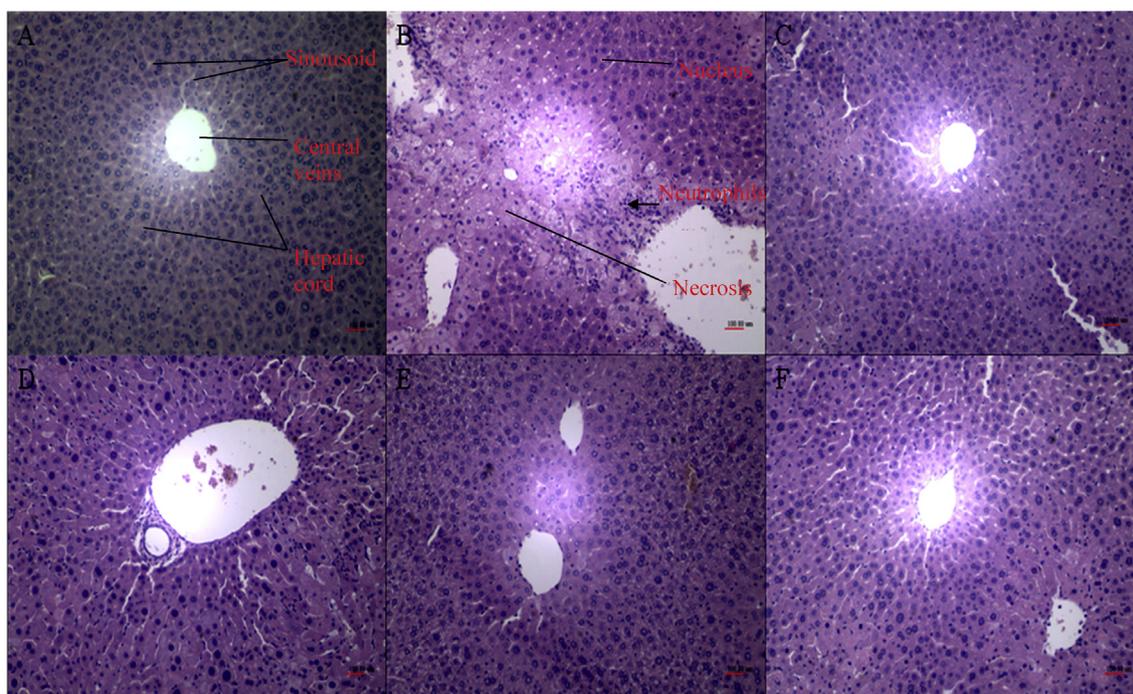
Figure 7 – Effect of anthocyanin-rich purple sweet potato extract (APSPE; 100 mg/kg body weight, 200 mg/kg body weight, and 400 mg/kg body weight) and silymarin on liver histopathology of carbon tetrachloride (CCl₄)-treated mice. (A) liver section of the normal control group; (B) liver section of the CCl₄-injured model group; (C) liver section of the positive control group with silymarin (50 mg/kg body weight) + CCl₄ injection; (D) liver section of the low-dose group with APSPE (100 mg/kg body weight) + CCl₄ injection; (E) liver section of the medium-dose group with APSPE (200 mg/kg body weight) + CCl₄ injection; (F) liver section of the high-dose group with APSPE (400 mg/kg body weight) + CCl₄ injection. Magnification 200×. Scale bar: 100 μm.

4. Conclusion

In summary, 19 purple sweet potato anthocyanins were identified from the purple sweet potato cultivar *Eshu* No. 8 and analyzed by the HPLC-DAD-ESI-MS/MS method. Peonidin 3-coumaryl-p-hydroxybenzoyl sophoroside-5-glucoside was identified for the first time in purple sweet potato. The hepatoprotective activity of APSPE was also studied in CCl_4-induced Kunming mice. In the *in vivo* assay, pretreatment with APSPE significantly decreased the levels of AST, ALT, and MDA against CCl_4 injuries, and restored the activities of defense antioxidant substances SOD and GSH towards normalization. These results support the effect of APSPE of *Eshu* No. 8 in hepatoprotective use with scientific evidence. As for diet, the effective dose of APSPE was 1–2 g/d. Every day, taking 20–40 g raw purple sweet potato powder is a good choice to protect the liver against CCl_4 damage.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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