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Purple Sweet Potato (*Ipomoea batatas* L.) Anthocyanins: Preventive Effect on Acute and Subacute Alcoholic Liver Damage and Dealcoholic Effect

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S Supporting Information

ABSTRACT: This study aimed to investigate the dealcoholic effect and preventive effect of anthocyanins from purple sweet potato (PSPAs) on acute and subacute alcoholic liver damage (ALD). Seven-week-old male inbred mice were grouped into five groups: control group (without PSPAs and ethanol treatments), model group (with ethanol treatment only), low-dose group (50 mg PSPAs/kg body weight), middle-dose group (125 mg PSPAs/kg body weight), and high-dose group (375 mg PSPAs/kg body weight), and the mice in all groups were administered intragastrically. Biochemical parameters of serum and liver were determined, and the histopathological changes of liver tissue were also analyzed. Results showed that all tested parameters were ameliorated after consumption of PSPAs. Therefore, PSPAs have preventive effect on acute and subacute ALD. It is suggested that PSPAs could be used as a supplementary reagent during prophylactic and curative managements of ALD.

KEYWORDS: purple sweet potato anthocyanins, biochemical parameters, histopathological analysis, dealcoholic effect, alcoholic liver damage

INTRODUCTION

Normal cell metabolism generates reactive oxygen species (ROS). As a secondary messenger, ROS regulates several different normal physiological functions in organisms and participates in a variety of cellular redox-regulatory mechanisms to defend against oxidative stress and maintain redox balance. However, only at low or medium levels with the beneficial effects of ROS happen, and a high level of ROS and/or inadequate antioxidant defense will cause oxidative stress, inducing damages to cellular structure including phospholipid bilayer, protein, and deoxyribonucleic acid (DNA).¹ The cellular antioxidant system consists of antioxidant [e.g., superoxide dismutase (SOD) and glutathione S-transferase (GST)] and some small molecular substances (e.g., glutathione, uric acid, and coenzyme). Besides, natural bioactive compounds including polyphenols, carotenoids, and ascorbic acid in vegetables, fruits, tea, and other plant origin products also play important roles in the prevention of oxidation-related diseases.²

Alcohol is a common liver unwanted substance, and long-term drinking will cause different degrees of damage to the digestive system, circulatory system, urinary system, and blood system. After absorption, 90% of alcohol will be oxidized and decomposed in the liver. The metabolism of alcohol leads to the generation of free radicals and consumption of cellular antioxidant activity, further resulting in the generation of alcohol-related diseases.³ Alcoholic liver damage (ALD) caused by excessive alcohol consumption has long been a serious threat to human health. Alcoholic liver disease includes alcoholic hepatitis (AH), alcohol hepatic fibrosis (AHF), alcoholic fatty liver (AFL), alcoholic liver cirrhosis (ALC), and slight alcoholic liver damage (SALD).⁴ In the United States, alcoholic cirrhosis is one of the

top seven higher mortality rate diseases, accounting for >80% of liver cirrhosis.⁵ In China, the morbidity of alcoholic liver disease also shows an increasing trend year by year and has become the second largest cause of liver damage after hepatitis virus.⁶ A large number of studies have shown that alcohol consumption will result in an imbalance of the antioxidant system in the body, which will cause the formation of oxidative stress. Continuous oxidative stress will cause liver steatosis, resulting in hepatitis, hepatic fibrosis, liver cirrhosis, liver cancer, etc.^{7,8}

Anthocyanins, as a category of natural polyphenols, are widespread throughout vegetables and fruits. Recent studies have shown that anthocyanins have many biological activities, for example, antioxidant activity, memory-improving activity, and immunity-enhancing activity.^{9,10} In recent years, purple sweet potato (*Ipomoea batatas* L.) (PSP) has received more attention because of the special color, high nutritional value, and vital function for human health. As anthocyanins in PSP are more stable than those in strawberry, perilla, red cabbage, etc.,¹¹ PSP is considered an outstanding source of anthocyanins as a food dye. Anthocyanins from purple sweet potatoes (PSPAs) have many biological activities, for example, antioxidant activity, antihypertension, memory enhancing, antiatherosclerosis.^{12–15} Because of their naturally nontoxic character, PSPAs are allowed to be used as natural edible pigments by the World Health Organization (WHO) and the U.S. Food and Drug Administration (FDA).

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PSPAs can scavenge oxygen free radicals, reduce lipid peroxides in blood and liver, and prevent liver damage induced by dimethylnitrosamine (DMA), *tert*-butyl hydroperoxide (T-BHP), and acetaminophen (APAP).^{16–18} However, the pathological process of liver damage induced by chemicals is not the same as that induced by alcohol consumption and thus could not simulate the process of ALD. Besides, there is still no study of the preventive effect of PSPAs on acute and subacute ALD. Therefore, we carried out righting reflex loss and recovery experiments of mice under alcoholic intoxication to investigate the potential dealcoholic effect of PSPAs. In addition, to investigate the preventive effect on acute and subacute ALD of PSPAs, we tested the liver function index, lipid peroxidation degree, antioxidant activity, and liver histopathology in the body of mice treated by PSPAs by establishing acute and subacute ALD mice models.

MATERIALS AND METHODS

Plant Materials. PSP (cultivar 'YAN No. 176') was obtained from the Academy of Agricultural and Forestry Sciences in Hebei Province. They were harvested at the beginning of October 2011 and immediately kept at 10–14 °C. Within 2 weeks, the harvested PSPs were washed thoroughly, sliced, and freeze-dried. All samples were ground using a domestic grinder and stored in sealed aluminum foil bags at –20 °C prior to extraction. The proximate composition of the obtained PSP powder, which was already published by us, was as follows: moisture, 10.82 ± 0.12% (w/w); starch, 65.35 ± 1.25% (w/w); protein, 8.21 ± 0.14% (w/w); fat, 0.7 ± 0.02% (w/w); ash, 3.89 ± 0.02% (w/w); and crude fiber, 4.87 ± 0.09% (w/w).¹⁹

Preparation of PSPAs. Anthocyanins were extracted from PSP according to published methods.¹⁹ Briefly, the dried PSP powder, ammonium sulfate, deionized water, and ethanol were mixed according to the quality proportion of 2:4:11:5, and the mix mentioned above was blended well and then kept still to form two phases completely. The formed aqueous two-phase system was kept in a dark place for 30 min at room temperature and then centrifuged at 4 °C and 5000g for 10 min. A separating funnel was used to separate the above-mentioned two phases. The upper phase was concentrated in a rotary evaporator, adsorbed by macroporous resin (AB-8), and eluted by 70% of ethanol solution at an elution flow rate of 1 mL/min. The effluent was collected, concentrated, freeze-dried, and stored at –20 °C in sealed aluminum bags.

Total Anthocyanin Quantification. A pH-differential method was used to determine the total anthocyanin content.²⁰ Briefly, 0.0125 g of the extract was dissolved in 1 mL of distilled water. Two water solutions were carried out on the sample. Potassium chloride (0.025 M) and sodium acetate (0.4 M) were used to set the pH values of the two solutions at 1.0 and 4.5, respectively. The two solutions were diluted to make the absorbance at 520 nm <1.2. The diluted solutions were equilibrated for 15 min, and then absorbances at 520 and 700 nm were recorded using an UV-vis spectrophotometer model UI-trospec 2000 (Amersham Pharmacia Biotech, Dubendorf, Switzerland). Absorbance was calculated as $A = (A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH}1.0} - (A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH}4.5}$, and total anthocyanin content was calculated using the following formula and expressed as milligrams of cyanidin 3-glucoside equivalent (CGE)/100 g PSPAs:

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

C is total anthocyanin content (mg CGE/100 g PSPAs), A is the absorbance mentioned above, e is the molar absorbance of cyanidin 3-glucoside (26900 L/cm/mol), L is cell path length (1 cm), M is the molecular weight of cyanidin 3-glucoside (432 g/mol), D is dilution multiple, V is the final volume (mL), and W is the sample weight (mg).

HPLC-ESI-MS/MS Analysis of Anthocyanins. The anthocyanins in purple sweet potato extract were analyzed using an Agilent 1200 series high-performance liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) connected to an Agilent 6410 triple-quadrupole mass spectrometer. The column used was an Agilent Zorbax SB-C18 column (250 mm × 4.6 mm i.d., 5 μm). The operating conditions were as

follows: flow rate, 0.5 mL/min; injection volume, 20 μL; and column temperature, 25 °C. The elution solvents were (A) 0.1% v/v trifluoroacetic acid in water and (B) acetonitrile. The gradient was as follows: from 5 to 30% B from 0 to 5 min; from 30 to 45% B from 5 to 45 min; from 45 to 90% B from 45 to 50 min. Spectra from 200 to 600 nm were scanned, and anthocyanins were detected at 254 nm. To identify PSP anthocyanins, electrospray ionization (ESI) was run in the positive ion mode in the mass range from 400 to 2000 (*m/z*) and under the following conditions: flow rate, 8.0 L/min; drying gas temperature, 350 °C; nebulizer pressure, 40 psi; and capillary voltage, 4500 V.¹⁹

Experimental Animals and Growth Conditions. Seven-week-old male inbred (C57BL/6) mice were obtained from Vital River Laboratories (VRL) (Beijing, China). The animals were kept under controlled conditions at 18–26 °C and 40–70% relative humidity with a 12 h dark-light cycle and acclimated for 2 weeks prior to use. Feed pellets and disinfected tap water were provided for cafeteria feeding. Experiments were carried out on the basis of the regulations of the Experimental Animal Welfare and Ethics Committee, Chinese Association for Laboratory Animal Sciences (CALAS).

Alcoholic Intoxication Experiments. For the righting reflex loss experiment of alcoholic intoxication, C57BL/6 mice were separated into four groups, 10 animals in each group: control group (same volume of saline), low-dose group (50 mg PSPAs/kg body weight), middle-dose group (125 mg PSPAs/kg body weight), and high-dose group (375 mg PSPAs/kg body weight). All groups were fasted for 12 h, and then PSPAs in saline were administered intragastrically. Thirty minutes later, mice were treated with 50% ethanol solution (18 mL/kg body weight). Duration of the ethanol-induced loss of righting reflex was recorded. Judgment standard of lost righting reflex was as follows: the ethanol-treated mice were placed back down, and the righting reflex was judged as lost if the above pose was maintained for >30 s.

For the righting reflex recovery experiment of alcoholic intoxication, the grouping method was the same as that used in the righting reflex loss experiment of alcoholic intoxication. All groups were fasted for 12 h and then treated with 50% ethanol solution (18 mL/kg body weight). Thirty minutes later, all groups were administered intragastrically PSPAs in saline once. The duration of recovery of righting reflex was recorded.

The righting reflex for each control or treatment mouse was performed in triplicate. The duration of ethanol-induced loss of righting reflex and the duration of recovery of righting reflex for each mouse were recorded as means of three experiments.

Acute and Subacute ALD Mice Models. For the prevention experiment of PSPAs on acute ALD, C57BL/6 mice were divided into five groups of 12 animals: negative control (without anthocyanins and ethanol treatments), acute ALD mice model group (with ethanol treatment only), low-dose group (50 mg PSPAs/kg body weight), middle-dose group (125 mg PSPAs/kg body weight), and high-dose group (375 mg PSPAs/kg body weight). The same volumes of saline were administered intragastrically for the negative control and acute ALD mice model group, whereas PSPAs were administered intragastrically for low-, middle-, and high-dose groups once daily for 30 consecutive days; food and water intakes of mice were recorded once a day. Four hours after the last administration, the negative control group was fed by gavage using an equal amount of purified water, whereas the other groups were treated with a 50% ethanol solution (12 mL/kg body weight). All groups were fasted for 16 h, and then anesthesia was carried out by injecting 60 mg/kg body weight pentobarbital sodium into the abdominal cavity of the mice. Blood was sampled from the abdominal aorta to determine activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and lactate dehydrogenase (LDH). Livers were weighed and cut into slices, some of which were kept in buffered formalin for histological observation, and 10% of liver tissue homogenates was obtained from the remaining liver sections and stored at –75 °C.

For the prevention experiment of PSPAs on subacute ALD, the grouping method was the same as that used in the prevention experiment of PSPAs on acute ALD. The same volumes of saline were administered intragastrically for the negative control and acute ALD model group, whereas PSPAs were administered intragastrically for low-,

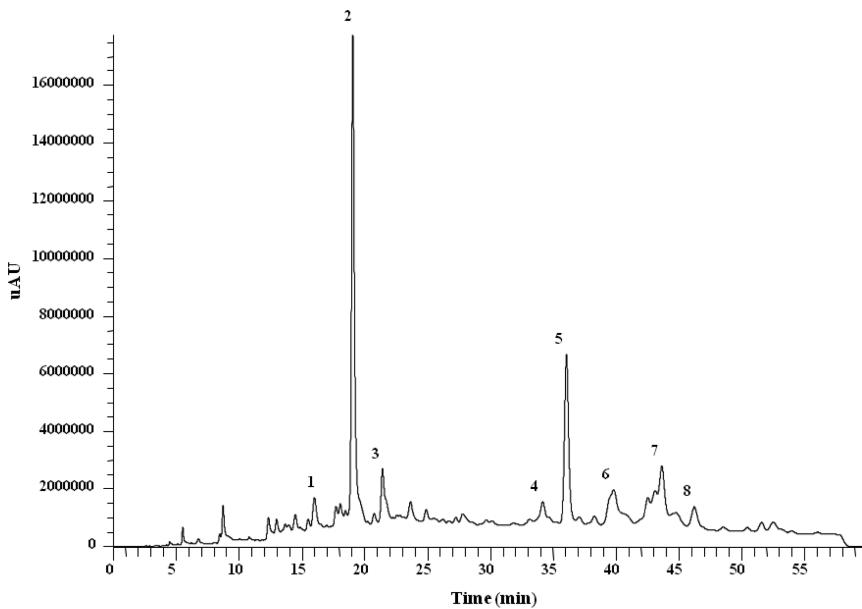


Figure 1. HPLC chromatogram of purple sweet potato anthocyanin compounds detected at 254 nm. Peak numbers refer to Table 1.

Table 1. Mass Spectrometric Data and Identification of Purple Sweet Potato Anthocyanins

peak	retention time (min)	[M ⁺] (m/z)	major fragment ion (m/z)	identification	ref
1	16.02	471	448, 297	cyanidin-3-glucoside	22
2	19.07	730	713, 685, 377	unknown	
3	21.43	907	745	peonidin-3-p-hydroxybenzoylsophoroside-5-glucoside	23
4	34.19	516	499, 342, 301	peonidin-3-methoxy-glucoside	22
5	36.07	1111	949	peonidin 3-(6'',6''-dicaffeoyl sophoroside)-5-glucoside	23
6	39.83	949	787, 463, 301	peonidin 3-caffeoyle sophoroside-5-glucoside	23
7	43.66	1069	907, 447, 301	peonidin 3-caffeoyl-p-hydroxybenzoylsophoroside-5-glucoside	23
8	46.26	1125	963, 463	peonidin 3-(6''-caffeoyl-6''-feruloylsophoroside)-5-glucoside	23

middle-, and high-dose groups once daily for 30 consecutive days. A 30% ethanol solution was fed by gavage at 10 mL/kg body weight once a day to the model group and anthocyanins groups for 14 days before the finish of this experiment, and the duration between the administration of saline or anthocyanins and the administration of ethanol was >4 h. All groups were fasted for 4 h, and then anesthesia was carried out by injecting 60 mg/kg body weight pentobarbital sodium into the abdominal cavity of mice. Blood was sampled from the abdominal aorta to determine the activities of serum ALT, AST, LDH, TCH, TG, and LDL-C. Livers were weighed and cut into slices, some of which were kept in buffered formalin for histological observation, and 10% of liver tissue homogenates were obtained from the remaining liver sections and stored at -75 °C.

Determination of Liver Index. The liver index of mice was calculated according to the following equation: liver index = liver weight/body weight × 100%.

Determination of Serum ALT, AST, LDH, TCH, TG, and LDL-C Activities. Activities of serum ALT, AST, LDH, TCH, TG, and LDL-C were determined using the corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Determination of Hepatic MDA, SOD, GST, and ADH Levels. Mice liver samples were blended with 30 mM cold phosphate buffer and 1% TritonX-100 and ground in a tissue grinder. The supernatant was obtained for assay of malondialdehyde (MDA), SOD, GST, and alcohol dehydrogenase (ADH) according to the instructions of corresponding kits (Nanjing Jiancheng Bioengineering Institute).

Histopathological Observation. Mice liver sections were fixed for 24 h in 10% formalin buffer, embedded in paraffin for hematoxylin and eosin staining, and then observed using a light microscope. The magnification was 100 times, and at least 10 areas of each tissue slice were observed.

Statistical Analysis. Experiments were performed in at least triplicate. The results were expressed as means ± SD (standard deviation). Statistical analysis was carried out with SAS software (version 8.1, SAS Institute Inc., Cary, NC, USA). Significant differences were decided at *p* < 0.05.

RESULTS AND DISCUSSION

Total Anthocyanins and Identification of PSPAs. On the basis of the result of the pH-differential method, the total anthocyanin content was 3.02×10^4 mg cyanidin 3-glucoside equiv/100 g PSPAs, and the yield of PSPAs corresponded to 0.93% of the PSP powder. Figure 1 shows the HPLC chromatogram of PSPAs. Eight constituents were separated. From the UV-visible spectra, LC-ESI/MS spectra, and some literature sources (Table 1 and Supporting Information Figure 1), seven constituents were identified. Peaks 1 and 4 were tentatively identified as cyanidin-3-glucoside and peonidin-3-methoxyglucoside, respectively.²¹ Peaks 3, 5, 6, 7, and 8 were tentatively identified as peonidin 3-p-hydroxybenzoyl sophoroside-5-glucoside, peonidin 3-(6'',6''-dicaffeoyl sophoroside)-5-glucoside, peonidin 3-caffeoyle sophoroside-5-glucoside, peonidin 3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside, and peonidin 3-(6''-caffeoyl-6''-feruloyl sophoroside)-5-glucoside, respectively.²² Peak 2 was a new discovery, which will be further investigated.

Alcoholic Intoxication Experiments. The loss of righting reflex indicates that mice have become drunk, whereas the recovery of righting reflex means that mice have sobered up.²³

Table 2 shows the effect of PSPAs on durations of ethanol-induced loss and recovery of righting reflex. Compared with

Table 2. Effect of PSPAs on the Durations of Ethanol-Induced Loss and Recovery of Righting Reflex^a

treatment group	duration of loss of righting reflex (min)	duration of recovery of righting reflex (min)
control	5.97 ± 2.83	445.55 ± 24.87
low-dose PSPAs	10.22 ± 3.16#	423.51 ± 15.12
middle-dose PSPAs	11.77 ± 2.10#	383.76 ± 13.22#
high-dose PSPAs	13.69 ± 2.75##	382.73 ± 18.08#

^aValues are the mean ± standard deviation of 10 determinations. Compared with control #: $p < 0.05$; ##, $p < 0.01$.

control, intake of PSPAs extended the duration of ethanol-induced loss of righting reflex significantly ($p < 0.05$), especially for the high-dose PSPAs group ($p < 0.01$), where the duration of ethanol-induced loss of righting reflex was extended to 13.69 ± 2.75 min [2.29 times control (5.97 ± 2.83 min)], indicating that PSPAs may have potential prevention effect on alcoholic intoxication. Besides, in middle- and high-dose PSPA groups, the durations of the recovery of the righting reflex were both shortened significantly ($p < 0.05$), indicating that PSPAs may have a potential curative effect on alcoholic intoxication.

Growth and Liver Index of C57BL/6 Mice. The liver index can partly reflect the level of ALD.²⁴ Table 3a shows the effects of PSPAs on original body weight, final body weight, liver weight, and liver index of mice in the acute ALD experiment. No significant difference was found for the changes of body weight between different treatment groups, and no significant difference was found for the final body weight between the different treatment groups, either. Compared with control, acute alcohol intake resulted in a significant increase of liver weight (1.27 ± 0.12 g) and liver index ($5.92 \pm 0.17\%$) ($p < 0.01$), which were 117.59 and 123.08% of control (liver weight and liver index were 1.08 ± 0.10 g and $4.81 \pm 0.22\%$, respectively). Compared with the model, liver weights in the low-, middle-, and high-dose groups were reduced by 3.15, 3.15, and 11.81%, respectively, and the liver indices in the low-, middle-, and high-dose groups were reduced by 6.25, 7.09, and 7.60%, respectively. However, even in

the high-dose PSPA group, the liver weight (1.12 ± 0.07 g) and liver index ($5.47 \pm 0.12\%$) were still 3.70 and 13.72% higher than those of the control. Our result was in agreement with that reported by McKim et al.,²⁵ who found that antioxidants such as cocoa flavonoids could reduce acute ALD.

Table 3b shows the effects of PSPAs on original body weight, final body weight, liver weight, and liver index of mice in the subacute ALD experiment. Compared with control, the body weight of mice in the model group (20.82 ± 0.78 g) was reduced significantly by 6.68% ($p < 0.05$). The possible reason was that the metabolism of alcohol changed the digestive function of the stomach and intestine, resulting in changes of food consumption. The liver index can partly reflect the severity of ALD. Compared with control, subacute alcohol intake resulted in a significant increase of liver weight (1.16 ± 0.09 g) and liver index ($5.57 \pm 0.33\%$) ($p < 0.01$). Compared with model, liver weights in the middle- and high-dose PSPA groups were reduced significantly ($p < 0.01$) by 2.59 and 4.31%, respectively, and the liver indices in the middle- and high-dose PSPA groups were reduced significantly ($p < 0.01$) by 9.87 and 11.13%, respectively. Particularly, no significant difference was found between the liver index of the high-dose PSPA group and that of control.

Serum ALT, AST, and LDH Activities. The change of serum biochemical indices is an important symbol of liver damage. Serum transaminases (i.e., ALT and AST) are essential catalysts in human metabolic process. Increasing ALT is a mark of liver cell membrane damage, and increasing AST is a mark of liver cell mitochondria damage. When damages such as inflammation and necrosis happen to liver cells, ALT and AST are released into the blood, causing an increase of serum transaminase.²⁶ The effect of PSPAs on the activity of serum ALT and AST of the acute ALD mice is shown in Figure 2a. Compared with control, the activities of ALT and AST in the model group increased significantly ($p < 0.01$), indicating that acute alcoholism caused damages of liver cell membrane and mitochondria. Compared with the model group, the activity of ALT in low-, middle-, and high-dose groups of PSPAs decreased by 47.28, 35.15, and 45.04%, respectively, and the activity of AST decreased by 17.67, 28.39, and 30.21%, respectively, indicating that PSPAs had a preventive effect on acute ALD. Our result was similar to the effect of genistein on ALD.²⁷ Figure 2c shows the effect of PSPAs on the activity of serum ALT and AST of the subacute ALD mice. Compared with control, the activity of AST in the model group increased, but not

Table 3. Effect of PSPAs on Body Weight, Liver Weight, and Liver Index of Acute (a) and Subacute (b) ALD Mice^a

treatment group	body wt		liver wt (g)	liver index (%)
	initial wt (g)	final wt (g)		
(a) Acute ALD Mice				
control	19.15 ± 0.32	22.41 ± 0.91	1.08 ± 0.10	4.81 ± 0.22
model	18.72 ± 0.37	21.39 ± 0.78	1.27 ± 0.12**	5.92 ± 0.17**
low-dose PSPAs	19.00 ± 0.56	22.11 ± 1.12	1.23 ± 0.17**	5.55 ± 0.14**,#
middle-dose PSPAs	18.70 ± 0.43	22.35 ± 1.04	1.23 ± 0.08**	5.50 ± 0.19*,#
high-dose PSPAs	18.88 ± 0.46	22.17 ± 1.06	1.12 ± 0.07*	5.47 ± 0.12*,#
(b) Subacute ALD Mice				
control	19.05 ± 0.32	22.31 ± 0.91	1.08 ± 0.10	4.81 ± 0.22
model	18.82 ± 0.37	20.82 ± 0.78*	1.16 ± 0.09**	5.57 ± 0.33**
low-dose PSPAs	19.00 ± 0.56	22.01 ± 1.12	1.15 ± 0.06**	5.23 ± 0.12**
middle-dose PSPAs	18.95 ± 0.43	22.46 ± 1.04	1.13 ± 0.17*,##	5.02 ± 0.68##
high-dose PSPAs	18.88 ± 0.46	22.43 ± 1.06	1.11 ± 0.14*,##	4.95 ± 0.42##

^aValues are the mean ± standard deviation of 10 determinations. Compared with control: *, $p < 0.05$; **, $p < 0.01$. Compared with model: #, $p < 0.05$; ##, $p < 0.01$.

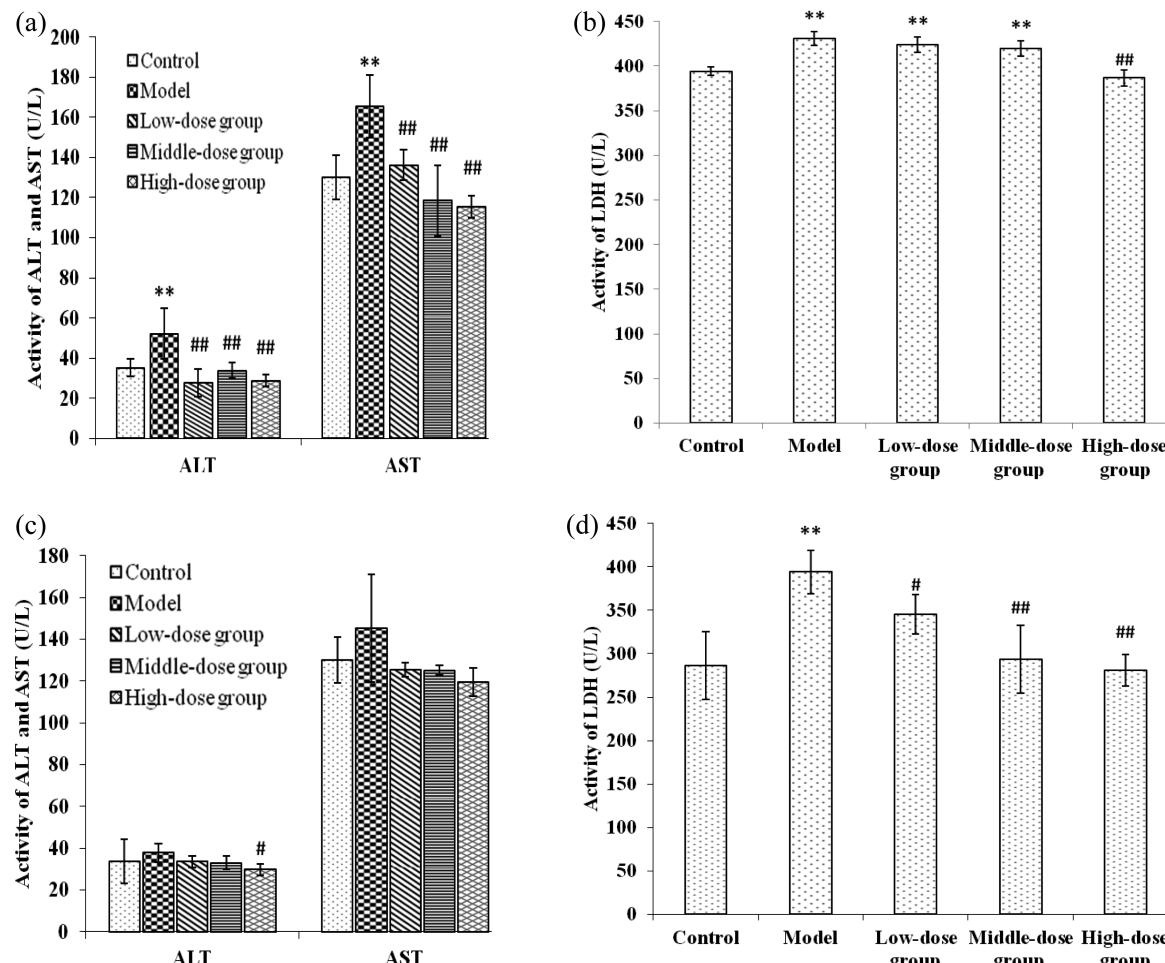


Figure 2. Effect of PSPAs on the activity of serum ALT, AST, and LDH of ALD mice: (a) effect of PSPAs on the activity of serum ALT and AST of acute ALD mice; (b) effect of PSPAs on the activity of serum LDH of acute ALD mice; (c) effect of PSPAs on the activity of serum ALT and AST of subacute ALD mice; (d) effect of PSPAs on the activity of serum LDH of subacute ALD mice. Compared with control: *, $p < 0.05$; **, $p < 0.01$. Compared with model: #, $p < 0.05$; ##, $p < 0.01$.

significantly, indicating that the intake of alcohol had caused slight damage of liver cell membrane and mitochondria. Compared with the model group, the activity of ALT in low-, middle-, and high-dose groups of PSPAs decreased by 11.11, 12.70, and 21.30%, respectively, and the activity of AST decreased by 13.60, 13.94, and 17.73%, respectively, indicating that PSPAs had a preventive effect on subacute ALD.

Besides, under normal circumstances, LDH contained in living cells could not penetrate the cell membrane; when cells were affected by outside poisonous substances, the cell membrane would be damaged, and the permeability of the cell membrane would be changed; thus, the intracellular LDH would be released into extracellular fluid, resulting in the increase of LDH activity.²⁸ The effect of PSPAs on the activity of serum LDH of the acute ALD mice is shown in Figure 2b. Compared with control, the LDH level of the model group showed a significant increase ($p < 0.01$). Intake of PSPAs in advance could inhibit the increase of LDH, especially in the high-dose group, for which the activity of LDH (386.80 ± 14.94 U/L) was 10.21% lower than that of model (430.80 ± 37.75 U/L), and no significant difference was found between the LDH level of the high-dose PSPAs group and that of control (394.20 ± 24.94 U/L) ($p > 0.05$). The effect of PSPAs on the activity of serum LDH of the subacute ALD mice was shown in Figure 2d. Compared with control, the LDH level of the model group showed a significant increase ($p < 0.01$),

indicating that intake of alcohol caused damage to the cell membrane. Intake of PSPAs in advance could inhibit the activity of LDH, especially in middle- and high-dose groups, as there was no significant difference between the LDH activities of the middle-dose (293.60 ± 39.29 U/L) and high-dose (281.00 ± 18.51 U/L) groups and that of control (286.40 ± 38.88 U/L) ($p > 0.05$). Our result was in agreement with that reported by Tang et al.,²⁹ who studied the effect of quercetin on ALD and found that quercetin could alleviate the release of liver-specific aminotransferases induced by ethanol. The possible reason is that anthocyanins and quercetin are both flavonoids, which possess strong antioxidant activity (Supporting Information Figure 2), and one of the mechanisms of alleviating ALD is inhibiting the increase of oxidative stress.

Serum TG, TCH, and LDL-C Levels. At the primary phase of alcoholic liver disease, TG and TCH will accumulate massively, which is a sensitive indicator of reflecting lipometabolism. LDL-C is endogenous TG, and excessive intake of alcohol will cause the increase of synthesis of LDL-C. The effect of PSPAs on serum TCH, TG, and LDL-C levels of acute ALD mice is shown in Figure 3a,b. Compared with control, the levels of TCH and TG in the model group increased by 4.39 and 12.96%, respectively. Compared with the model group, the levels of serum TCH in low-, middle-, and high-dose anthocyanin groups decreased by 11.34, 13.03, and 20.59%, respectively, and the levels of serum

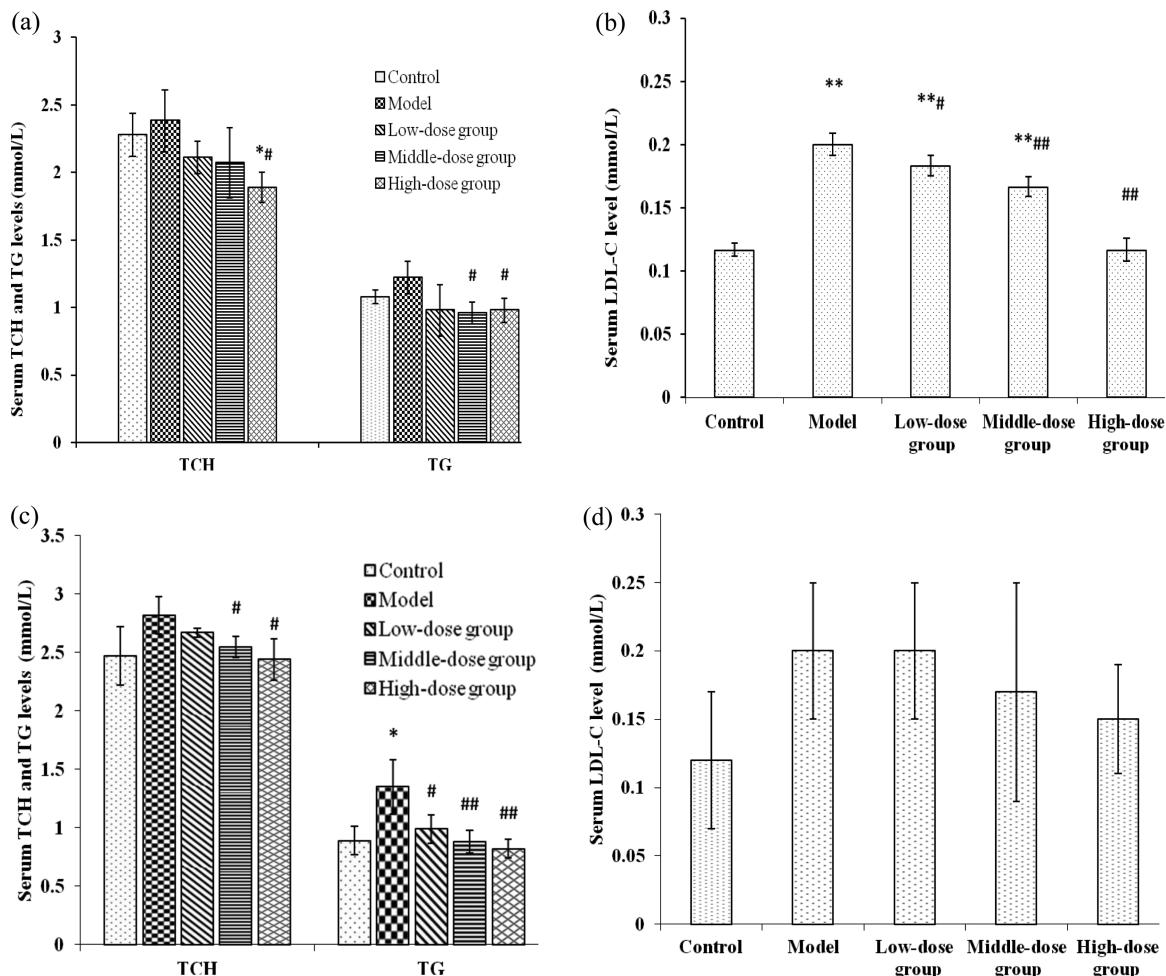


Figure 3. Effect of PSPAs on the serum TCH, TG, and LDL-C levels of ALD mice: (a) effect of PSPAs on serum TCH and TG levels of acute ALD mice; (b) effect of PSPAs on serum LDL-C level of acute ALD mice; (c) effect of PSPAs on serum TG and TCH levels of subacute ALD mice; (d) effect of PSPAs on serum LDL-C level of subacute ALD mice. Compared with control: *, $p < 0.05$; **, $p < 0.01$. Compared with model: #, $p < 0.05$; ##, $p < 0.01$.

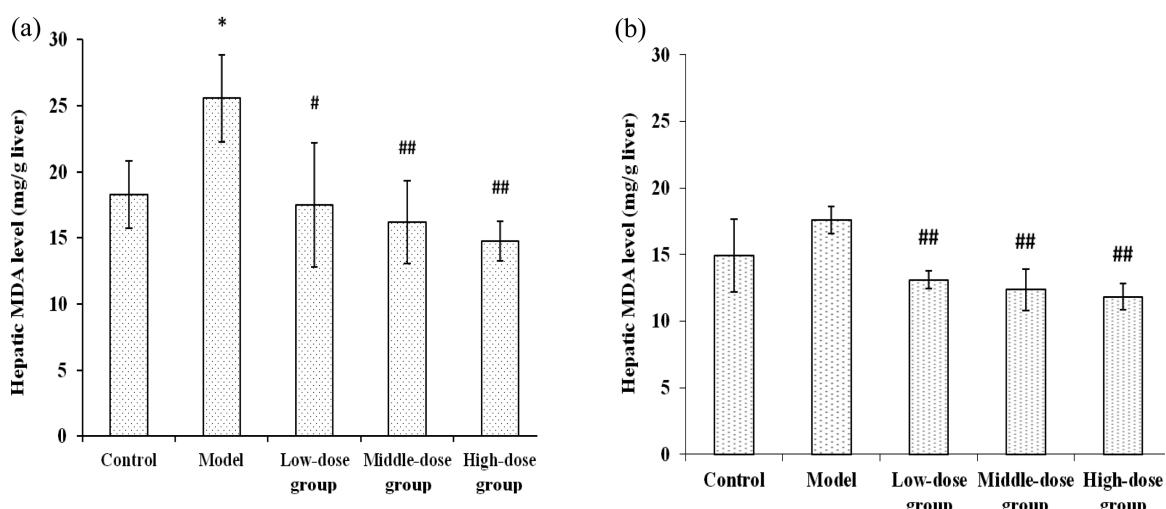


Figure 4. Effect of PSPAs on the hepatic MDA level of the ALD mice: (a) effect of PSPAs on hepatic MDA level of acute ALD mice; (b) effect of PSPAs on hepatic MDA level of subacute ALD mice. Compared with control: *, $p < 0.05$; **, $p < 0.01$. Compared with model: #, $p < 0.05$; ##, $p < 0.01$.

TG in low-, middle-, and high-dose PSPA groups decreased by 19.67, 21.31, and 19.67%, respectively. Moreover, the level of TCH in the high-dose PSPA group was 17.11% lower than control ($p < 0.05$), and no significant difference was found

between the levels of TG in all PSPA groups and control. These results indicate that intake of PSPAs can alleviate alcohol-induced accumulation of TCH and TG. Our results were in accordance with those reported by Zhang et al.,⁶ who found

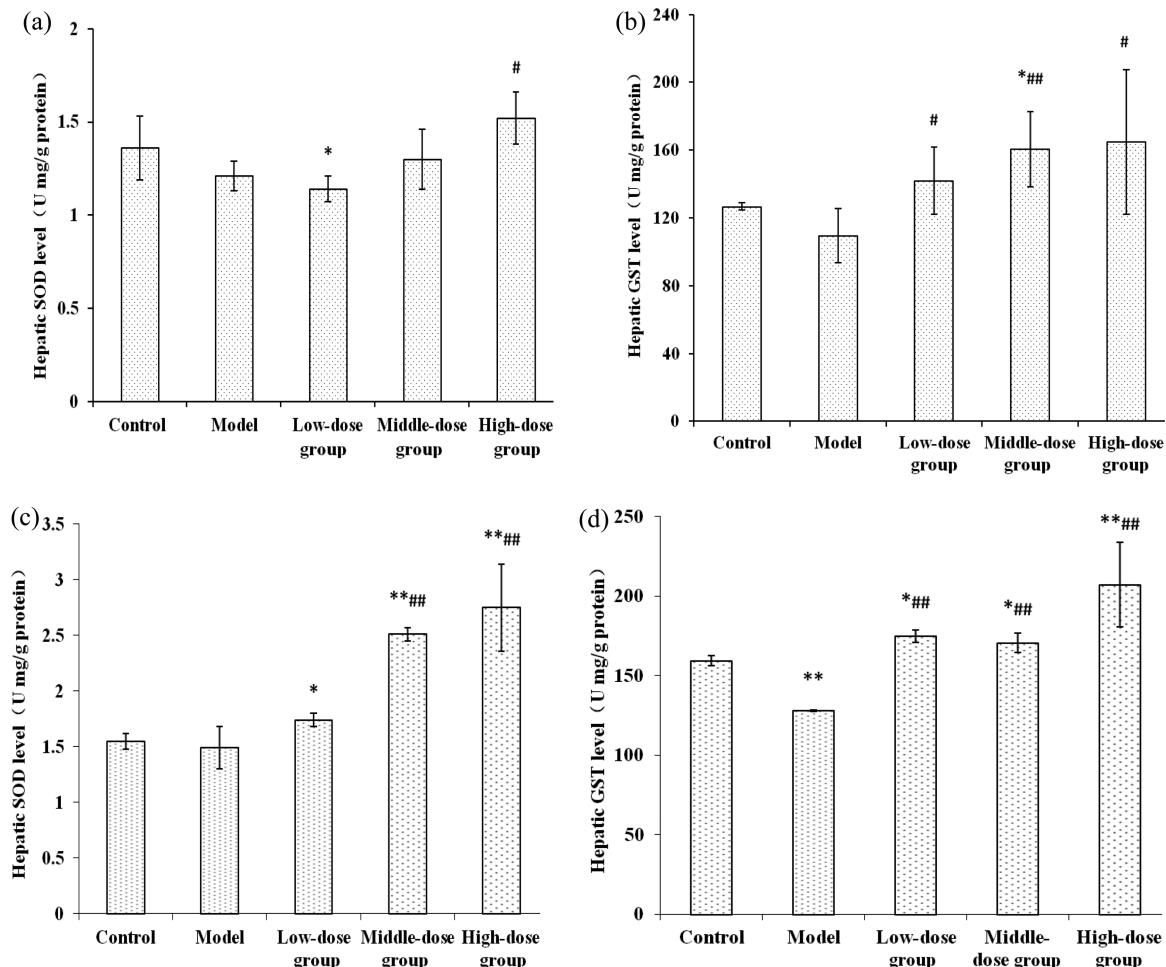


Figure 5. Effect of PSPAs on the hepatic SOD and GST levels of the ALD mice: (a) effect of PSPAs on hepatic SOD level of acute ALD mice; (b) effect of PSPAs on hepatic GST level of acute ALD mice; (c) effect of PSPAs on hepatic SOD level of the subacute ALD mice; (d) effect of PSPAs on hepatic GST level of subacute ALD mice. Compared with control: *, $p < 0.05$; **, $p < 0.01$. Compared with model: #, $p < 0.05$; ##, $p < 0.01$.

ostruthin could inhibit the increase of TCH, TG, and LDL-C induced by alcohol and alleviate acute alcohol intoxication and hepatic lipid metabolism. The possible reason was that anthocyanins and ostruthin both belong to flavonoids and possess strong antioxidant activity (Supporting Information Figure 2).

Studies showed that long-term intake of alcohol would increase the NADH/NAD⁺ ratio, up-regulate lipogenesis, and down-regulate transport capacity of fatty acid, causing hepatitis.³⁰ Therefore, chronic intake of alcohol is often accompanied by the increase of blood lipids, but antioxidants can reduce this trend. Taurine³¹ and fermented barley³² could reduce the increase of blood lipids induced by ALD significantly because of their antioxidant activity. Our result was similar to reported results. Subacute intake of alcohol increased the levels of serum TCH and TG by 14.17 and 51.69%, respectively. Compared with the model, the levels of serum TCH in low-, middle-, and high-dose PSPA groups were decreased by 5.32, 9.57, and 13.48%, respectively, and the levels of serum TG in low-, middle-, and high-dose PSPA groups were decreased by 26.67, 34.81, and 39.26%, respectively. Furthermore, there was no significant difference between the serum TCH and TG levels of low-, middle-, high-dose PSPA groups and control (Figure 3c,d).

Hepatic MDA Level. After a lot of alcohol intake in a brief period, alcohol is metabolized in the liver, producing some aldehyde compounds, which can combine with protein, release a large amount of reactive oxide species (ROS), and increase internal oxidative stress level and lipid peroxidation. MDA is the main product of lipid peroxidation induced by ROS, and the content of MDA can reflect the oxidative stress level of liver.³³

Figure 4a shows the effect of PSPAs on the hepatic MDA level of the acute ALD mice. Compared with control, MDA in the model group increased significantly ($p < 0.05$) by 39.69%, indicating that alcohol intake increased the oxidative stress level of liver. Compared with the model group, MDA levels in low-, middle-, and high-dose PSPA groups decreased significantly ($p < 0.05$) by 31.55, 36.56, and 42.31%, respectively, and no significant difference was found between all PSPA groups and control. The possible reason is that PSPAs possess strong antioxidant activity. Our result was similar to the result of Bharrhan et al.,³⁴ who found that catechin could decrease MDA in alcohol-induced damaged liver of rats.

Figure 4b shows the effect of PSPAs on the hepatic MDA of the subacute ALD mice. Compared with control, MDA in the model group increased by 17.97%. Pretreated by PSPAs, lipid peroxidation of mice was inhibited significantly, and thus MDA was decreased significantly ($p < 0.01$) by 25.47, 29.73, and 32.75% for low-, middle-, and high-dose PSPA groups,

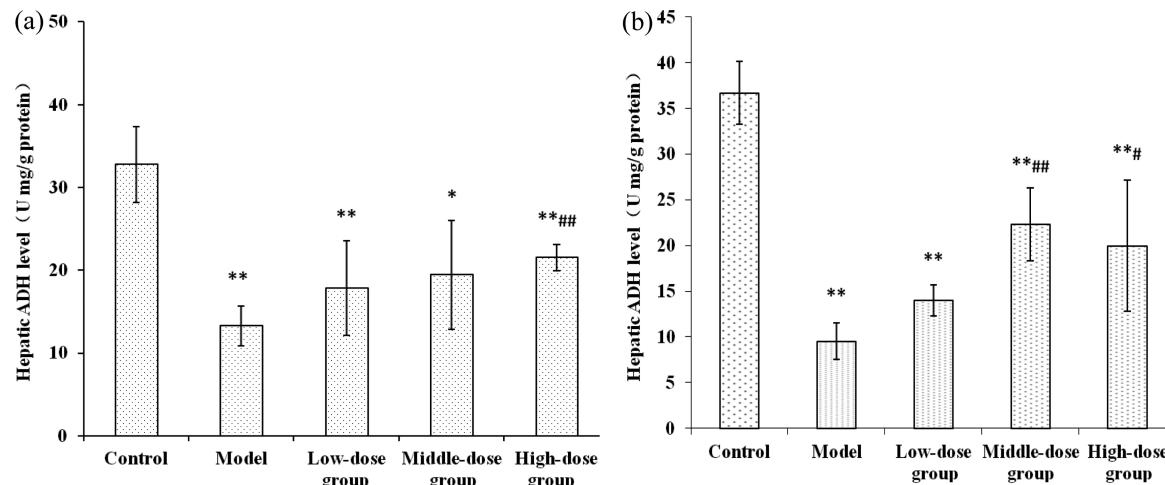


Figure 6. Effect of PSPAs on the hepatic ADH level of the ALD mice: (a) effect of PSPAs on hepatic ADH level of acute ALD mice; (b) effect of PSPAs on hepatic ADH level of the subacute ALD mice. Compared with control: *, $p < 0.05$; **, $p < 0.01$. Compared with model: #, $p < 0.05$; ##, $p < 0.01$.

Table 4. Effect of PSPAs on the Histopathology of Acute (a) and Subacute (b) ALD Mice^a

treatment group	parallel 1	parallel 2	parallel 3
(a) Acute ALD Mice			
control	—	—	—
model	hepatocyte edema +++	hepatocyte edema ++	hepatocyte edema +++
low-dose PSPAs	hepatocyte edema ++	hepatocyte edema +	hepatocyte edema ++
middle-dose PSPAs	hepatocyte edema +	—	—
high-dose PSPAs	—	—	—
(b) Subacute ALD Mice			
control	—	—	—
model	hepatocyte edema +++	hepatocyte edema +++++	hepatocyte edema +++
low-dose PSPAs	hepatocyte edema ++	hepatocyte edema +	hepatocyte edema ++
middle-dose PSPAs	hepatocyte edema +	—	—
high-dose PSPAs	hepatocyte edema +	—	—

^a— indicates no lesion; +, minor lesion; ++, mild lesion; +++, moderate lesion; +++++, severe lesion.

respectively. Particularly, the MDA level in the high-dose PSPA group was 20.66% lower than that in control. This can be attributed at least partly to the capacity of eliminating active oxygen radicals of anthocyanins. Our result is in agreement with the findings of You et al.³⁵ and López et al.,³⁶ who found that the antioxidants in taraxacum and amaranth had some preventive effects on ALD.

Hepatic SOD and GST Levels. In recent years, people are paying more attention to the effect of oxidative stress and lipid peroxidation on ALD. Alcohol can produce a large amount of radicals during its metabolism in the liver, for example, O_2^- , H_2O_2 , and OH^- . When the radicals go beyond the elimination ability of human body, liver cells will be damaged.³⁷ GST and SOD are important internal antioxidants, which can alleviate oxidative stress caused by alcohol. Some studies have already showed that intake of antioxidants such as vitamin E and vitamin C in advance could alleviate alcohol-induced liver damage.³⁸

The effect of PSPAs on the hepatic SOD level of the acute ALD mice is shown in Figure 5a. SOD activity in the model group (1.21 ± 0.08 U mg/g protein) was 11.03% lower than that in control, indicating that the elimination ability of mice on O_2^- was weakened by alcohol intake. Compared with the model, SOD activity in the high-dose PSPA group was increased significantly ($p < 0.05$) by 25.62% and was even 11.76% higher than normal level (control, 1.36 ± 0.17 U mg/g protein); thus,

the ability to eliminate O_2^- was also improved, and the liver damage was alleviated.

Intake of alcohol will increase the internal oxidation products, and GST can eliminate oxygen free radicals effectively, prevent oxidative stress, and inhibit lipid peroxidation. Acetaldehyde is an alcohol metabolite, which can combine with GST, resulting in changes of membrane permeability and fluidity; thus, the structure and function of cells are damaged. Moreover, when the structure and function of cells are damaged, free radicals will not be eliminated; thus, liver damage will be aggravated. At present, the decrease of GST content is considered to be one of the mechanisms of ALD. Figure 5b shows the effect of PSPAs on the hepatic GST level of the acute ALD mice. Compared with control, the GST level in the model group decreased by 13.67%, indicating that the liver cell membrane was damaged. The GST content in anthocyanin groups was increased significantly in a dose-dependent relationship, and the GST contents in low-, middle-, and high-dose PSPAs groups were even 11.89, 26.62, and 30.08% higher than that in control, indicating that PSPAs could alleviate alcohol-induced oxidative stress, thus alleviating alcohol-induced liver damage. Our result is in agreement with that reported by Tang et al.,²⁹ who found that taurine could decrease alcohol-induced dyslipidemia and oxidative damage.

The effects of PSPAs on hepatic SOD and GST levels of the subacute ALD mice are shown in Figure 5c,d. Compared with control, the SOD and GST levels in the model group decreased.

Intake of PSPAs in advance increased the SOD and GST activities significantly ($p < 0.01$) to normal levels, and the SOD and GST levels in the high-dose group were 77.42 and 29.94% higher than those in the control group.

Hepatic ADH Level. ADH is a metalloenzyme existing in liver cell cytoplasm and mitochondria, which can transform ethanol into acetaldehyde and then oxidize acetaldehyde into acetic acid. In mitochondria, some acetic acid can enter into the tricarboxylic acid cycle and be oxidized into CO_2 and H_2O , which are removed from the body.³⁹ The effect of PSPAs on the hepatic ADH level of the acute ALD mice is shown in Figure 6a. Compared with control, the ADH level of the model group decreased significantly ($p < 0.01$) by 59.56%, indicating that a large amount of alcohol could not be metabolized into CO_2 and H_2O , causing hepatotoxicity easily. Compared with the model group, ADH activities in low-, middle-, and high-dose PSPA groups were increased by 34.31, 46.76, and 62.14%, respectively. However, the ADH activity in the high-dose PSPA group was still 34.43% lower than in the control group.

The effect of PSPAs on the hepatic ADH level of the subacute ALD mice is shown in Figure 6b. Compared with control, ADH activity in the model group decreased significantly ($p < 0.01$). Compared with the model group, ADH activity of anthocyanin groups increased significantly ($p < 0.05$). However, ADH activity of high-dose PSPA group was still 45.59% lower than that of the control group.

Histopathological Analysis. Table 4a and supplement Figure 3 showed the effect of PSPAs on the histopathology of the acute ALD mice. The liver cells of C57BL/6J mice in control group had complete structure, similar size, tight arrangement, uniform staining, and no liver pathological changes. In model group, the mice liver cells showed pathological changes, e.g. loose arrangement and cellular edema. In anthocyanins low-dose group, the pathological changes of mice liver cells were similar to that in model group, e.g. disordered arrangement of cells and cellular edema, but the degree thereof was reduced. In anthocyanins middle and high-dose groups, no pathological changes was observed, and the cellular morphology thereof was close to that of control group.

The effect of PSPAs on the histopathology of subacute ALD mice is shown in Table 4b and Supporting Information Figure 3f–i. Compared with control, pathological changes such as liver cell swelling and fatty degeneration happened to mouse liver in the model group, which included mice that were treated with alcohol continuously. After the intake of PSPAs, liver cell swelling was alleviated significantly, and the improving pathological effect showed a dose-dependent relationship. Liver tissue morphology after high-dose anthocyanin treatment was similar to that after normal treatment.

ASSOCIATED CONTENT

Supporting Information

Additional experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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ABBREVIATIONS USED

ADH, alcohol dehydrogenase; ALD, alcoholic liver damage; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GST, glutathione S-transferase; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; PSPAs, purple sweet potato anthocyanins; TCH, total cholesterol; TG, triglyceride

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