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Research Article

Liquid Chromatography/Mass Spectrometry Analysis and Hepatoprotective Effect of Steamed Platycodi Radix on Acute Alcohol-induced Liver Injury

[1]Li-Chun Zhao, [2]Ying Liu, [2]Zi Wang, [1]Nong Tang, [1]Jing Leng, [4]Bing Zheng, [3]Ying-Ying Liu and [2]Wei Li

[1]College of Pharmacy, Guangxi University of Chinese Medicine, 530011 Nanning, China
[2]College of Chinese Medicinal Materials, Jilin Agricultural University, 130118 Changchun, China
[3]Department of Nephrology, China-Japan Union Hospital of Jilin University, 130033 Changchun, China
[4]School of Business Administration, Zhejiang Gongshang University, 310018 Hangzhou, China

Abstract

Background and Objective: Platycodi Radix (PR), a famous traditional medicine and folk food, has been used in China. PR exerted numerous pharmacological activities including anti-tumor, anti-obesity and anti-inflammation. The present study aimed to investigate the active constituents by Liquid Chromatography/Mass Spectrometry (LC/MS) analysis and protective effects of saponins from the steamed Platycodi Radix (SSPR) on acute alcohol-induced liver injury in mice. **Materials and Methods:** Pretreatment with SSPR (100, 200 and 400 mg kg^{-1}) prior to alcohol administration significantly prevented the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and triglyceride (TG) in serum, malondialdehyde (MDA) level in liver tissue compared with the alcohol group ($p<0.05$). **Results:** The level of enzymatic antioxidants glutathione (GSH) was increased noticeably ($p<0.05$) in SSPR pretreatment mice liver tissue. Histopathological examination revealed that pretreatment with 400 mg kg^{-1} of SSPR markedly ameliorated acute alcohol-induced hepatocyte apoptosis and fatty degeneration. The findings from LC/MS analysis indicated that SSPR had more saponins than unheated-processed one and SSPR mainly contain a polyacetylene (Lobetyolin) and 22 saponins. **Conclusion:** These results showed the beneficial effect of SSPR on acute alcohol-induced oxidative stress and liver damage.

Key words: LC/MS analysis, saponins, lobetyolin, steamed platycodi radix, acute alcohol-induced liver injury

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Corresponding Authors: Wei Li, College of Chinese Medicinal Materials, Jilin Agricultural University, 130118 Changchun, China Tel/Fax: +86-431-84533304
Ying-Ying Liu, China-Japan Union Hospital of Jilin University, 130033 Changchun, China

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

It has been evidenced widely that long-term alcohol dependence and high alcohol consumption results in many disorders in the body, including malnutrition, sleep problems and even alcoholic liver diseases (ALD)[1-3]. ALD is becoming the most common alcohol-related disease and causes substantial morbidity and mortality all over the world[4]. Many pathways are thought to be involved in ALD, including alcohol-induced oxidative stress, lipid peroxidation generation of free radicals and inflammation[5,6]. Since oxidative stress is involved in the development of ALD, using the antioxidants would potentially blunt ethanol-induced oxidative stress and prevent pathogenesis[7,8]. Therefore, the predominant source of antioxidants and their role in preventing ethanol-induced liver injury is an important target.

Platycodon grandiflorum A.DC is a species of perennial flowering plant of the family Campanulaceae. Platycodi Radix (PR), the dried roots of *P. grandiflorum* has been used as a food and a traditional medicine for asthma and bronchitis in China[9,10]. Recently, it has shown that PR contains abundant of pharmacological active ingredients such as triterpenoid saponins which exhibiting the treatment of metabolic diseases including obesity, hyperlipidemia, tumor, inflammatory and diabetes[11-14]. In addition, it also showed a wide range of protective effect alcohol-induced and tetrachloride-induced hepatotoxicity in animal models[15,16].

Heat-processing method is thought to have the following functions: reduction of toxicity and side effects, to change properties or functions, to preserve the active constituents and to correct an unpleasant taste[17-19]. In addition, heat-processing method can affect the chemical profile of herbals and lead to the changes of bioactivities. Among of these heat-processing methods, steaming treatment is one of the most effective methods for Chinese medicines[20,21]. According to the reports on processing of red ginseng, steaming and drying process may lead to hydrolysis reaction of ginsenosides. During this process, the original ginsenosides (e.g. Rb1, Rc, Rd and Rb3) are converted into rare saponins (e.g. Rg3, Rk1 and Rg5) with less sugar link. In fact, the above rare saponins are more easily absorbed in the human body and show better pharmacological activities. Previous studies have clearly demonstrated that the pharmacological activity of steamed ginseng is far better than that of un-steamed one in terms of enhancing immunity and anti-tumor effects[22,23].

In the past decades, the investigations on dried PR have received more and more attention because of its better bioactivities. However, most investigations mainly focused on fresh or dried PR but paid little attention to steamed ones[24-26]. Based on the above facts, it was speculated that the steamed PR may exert more powerful hepatoprotective effects on alcohol-induced liver injury. In fact, several previous studies have revealed that Platycodi Radix is useful as a therapeutically potent natural ingredient for the prevention of alcohol-induced oxidative stress and liver damage[27]. In previous work, platycodin D (a marker saponin of PR) was isolated from the aerial parts of *P. grandiflorum* and reported to exert protective effects on alcohol-induced liver injury in mice[28]. The current study was performed to investigate whether steamed Platycodi Radix has protective effect on acute alcohol-induced liver injury in mice. Importantly, the change of constituents between the steamed and un-steamed one was observed for the first time. Moreover, LC/MS analysis was employed to determine the chemical constituents from SSPR and 22 saponins and lobetyolin were identified.

MATERIALS AND METHODS

Chemicals and reagents: The present work started at July, 2014 and ended at February 2015. In other words, the entire experiment lasted 6 months. Silymarin (purity>85.0%, UV) was separated and supplied by the Institute of Special Wild Economic Animals and Plant, Changchun, China. Assay kits for determining alanine aminotransferase (ALT), aspartate aminotransferase (AST) and malondialdehyde (MDA) and glutathione (GSH) content were obtained from the Nanjing Jiancheng Institute of Biotechnology, Nanjing, China. The triglyceride (TG) assay kit was purchased from Beijing BHKT Clinical Reagent Co. Ltd., Beijing China. Acetonitrile and methanol (HPLC-grade) were purchased from Fisher Chemicals (USA). All other chemicals used were of analytical grade and obtained from Beijing Chemical Factory, Beijing, China.

Plant material and preparation of extract: The fresh roots of *Platycodon grandiflorum* (Platycodi Radix), three-years old, were purchased from Niuyingzi planting base of Traditional Chinese Medicines, Inner Mongolia, China. The specimens were identified and authenticated by Prof. Wei Li, Jilin Agricultural University. The fresh Platycodi Radix was rinsed, removed of the peel and steamed in an autoclave sterilizer at the temperature of 105°C for 60 min. Then the steamed Platycodi Radix (SPR) was dried at a 45°C air dryer less than 24 h and ground to a 40 mesh powder and then preserved in the dryer and set aside.

Powdered SPR (500 g) was extracted by ultrasound-assisted extraction with 10 volumes of methanol at the

temperature of 40°C for 30 min. After extracting for 3 times, the obtained filtrate was concentrated under reduced pressure in a rotary evaporator to give a crude saponins fraction (SSPR).

HPLC and LC/MS analysis of SSPR: Samples were analyzed on Agilent 1100 HPLC system. Separation was achieved on Hypersil ODS2 column (250 mm×4.6 mm, 5 μm) from Dalian Elite Analytical Instruments Co. Ltd. The column temperature was set at 30 and detection wavelength was set 210 nm. The mobile phase was consisted of acetonitrile (A) and water (B) with flow rate of 1.0 mL min^{-1}. The gradient elution was programmed as follows: 0-30 min, 18-25% A; 30-60 min, 25-28% A; 60-70 min, 28-28% A; 70-75 min, 28-18% A.

Pneumatic assisted electrospray positive ionization (ESI^+) detection and cracking voltage is 160 V, atomizing air pressure is 276 kPa (40 psi) and drying temperature is 350°C, drying gas flow rate is 12 L min^{-1}.

Animals and treatments: Male ICR mice, 22-25 g, were obtained from Jilin University with Certificate of Quality No. of SCXK (JI) 2011-0004. The mice were kept in standard laboratory conditions with free access to diet and tap water and acclimated to a temperature-controlled room at 23±2°C with a 12 h light/dark cycle for one week prior to formal experiment. The experiments were conducted according to the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China). All experimental procedures were approved by the ethical committee for laboratory animals of Jilin Agricultural University.

A total of 48 male Institute of Cancer Research (ICR) mice were randomly assigned to six groups (8 mice per group). The experimental groups were as follows: Group I and II were administrated only 0.9% serving as control group and alcohol group, respectively. Group III was positive group being pretreated with silymarin (50 mg kg^{-1}). Groups IV-VI was gavaged with SSPR at doses of 100, 200 and 400 mg kg^{-1}, respectively. The dose selection was based on previous preliminary experiment. Each group was administered intragastrically once a day continuously for up to 14 days. After the last administration for 1 h, except the control group, other groups were intragastrically administered a one-time grant of 50% alcohol (4.8 g kg^{-1}) shock to induce acute alcohol liver injury in mice.

At the final stage of the experiment, the mice were fasted for 12 h and followed by cervical dislocation for sacrifice after obtained blood from the retrobulbar vessels. The serum was separated by centrifugation at 1500 rpm for 10 min at 4°C and stored at -80°C for analysis. Then, the mice were dissected immediately and sacrificed to obtain the liver. One fraction of the liver samples was immediately frozen in liquid nitrogen, before being stored at -80°C for future analysis; another fraction was excised and fixed in a 10% formalin solution for histopathologic analysis.

Measurement of serum biochemical index: Liver injury was assessed by measuring serum activities of ALT and AST using commercially available test kits (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China) according to the manufacturer's instructions. Besides, the content of TG was also calculated in accordance with the assay kit specification.

Measurement of GSH and MDA in liver homogenate: Liver tissues were homogenized on ice in Tris-HCl buffer (5 mM containing 2 mM EDTA, pH 7.4) to obtain a 10% (w/v) liver homogenate. Homogenates were then centrifuged at 1, 500 rpm for 15 min at 4°C and the supernatants were used immediately for the assays of MDA and GSH level. All these enzymes were determined using commercially available kits (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China) according to the manufacturer's instructions and previous study.

Histopathological examination and scoring for fat accumulation in the liver: The liver tissue was instantly fixed in 10% phosphate buffered formalin, processed by routine histology procedures and then embedded in paraffin. Tissue sections (5 μm) were stained with hematoxylin and eosin (H&E) and subsequently observed using a light microscope for histopathological examination.

The stained liver sections were evaluated for hepatic fat accumulation and ranked according to the distribution of the fat accumulation as follows: I =no fat droplets in the lobules (1-25% of the cells in the specimen have fat droplets), II =few fat droplets in the lobules (26-50%), III =moderate fat droplets in the lobules (51-75%), IV =numerous fat droplets (55-100%).

Statistical analysis: All experiments were performed in duplicate. All data were presented as the Means±standard deviations (SD). Statistically significant differences between the groups were determined by Student's t-test using SPSS 16.0. The p-value less than 0.05 were considered statistically significant.

RESULTS

HPLC analysis of SSPR: It is a challenging job for researchers to study the detection and structural characterization of chemical constituents contained in herbal and become the major obstacle for the further pharmacological investigation. Therefore, it is necessary to develop sensitive and reliable analytical methods for the identification of pharmacological active constituents. Here, the coupling of HPLC and ESI-MS has been used for online identification of the constituents in SSPR. According to the HPLC method, SSPR had more and higher chromatographic peaks than the un-steamed one (Fig. 1). In the present investigation, LC/MS method was employed to identify the main constituents in SSPR (Fig. 2-3). Lobetyolin and 22 saponins were identified according to the references[29,30] (Table 1). In summary, SSPR mainly contained the platycodin D (2.0640 mg g^{-1}), deapio-platycodin D (1.9924 mg g^{-1}) and lobetyolin (0.6098 mg g^{-1}).

Effect of SSPR on body weight and organ index: After one-time impact heavy alcohol, the mice did not die and most of the mice began to be observed quadriplegia or excitement. Furthermore, alcohol group was observed drunken sleepiness and the loss of righting reflex, followed by waking up after alcohol gavage 6 h in mice. The results indicated that the acute alcoholic liver injury was successfully established.

As shown in Table 2, body weights of the mice were not significantly affected by the pretreated administration with different doses of SSPR in comparison with control group. However, a significant elevation of liver and spleen indices were observed at the end of the experimental procedure, indicating that alcohol exposure induced hypertrophy of these tissues. By contrast, the mice in the positive group and SSPR groups noticeably reduced the elevated weight of liver ($p<0.05$) in a dose-dependent manner, suggesting their possible protective effects against alcohol-induced liver injury.

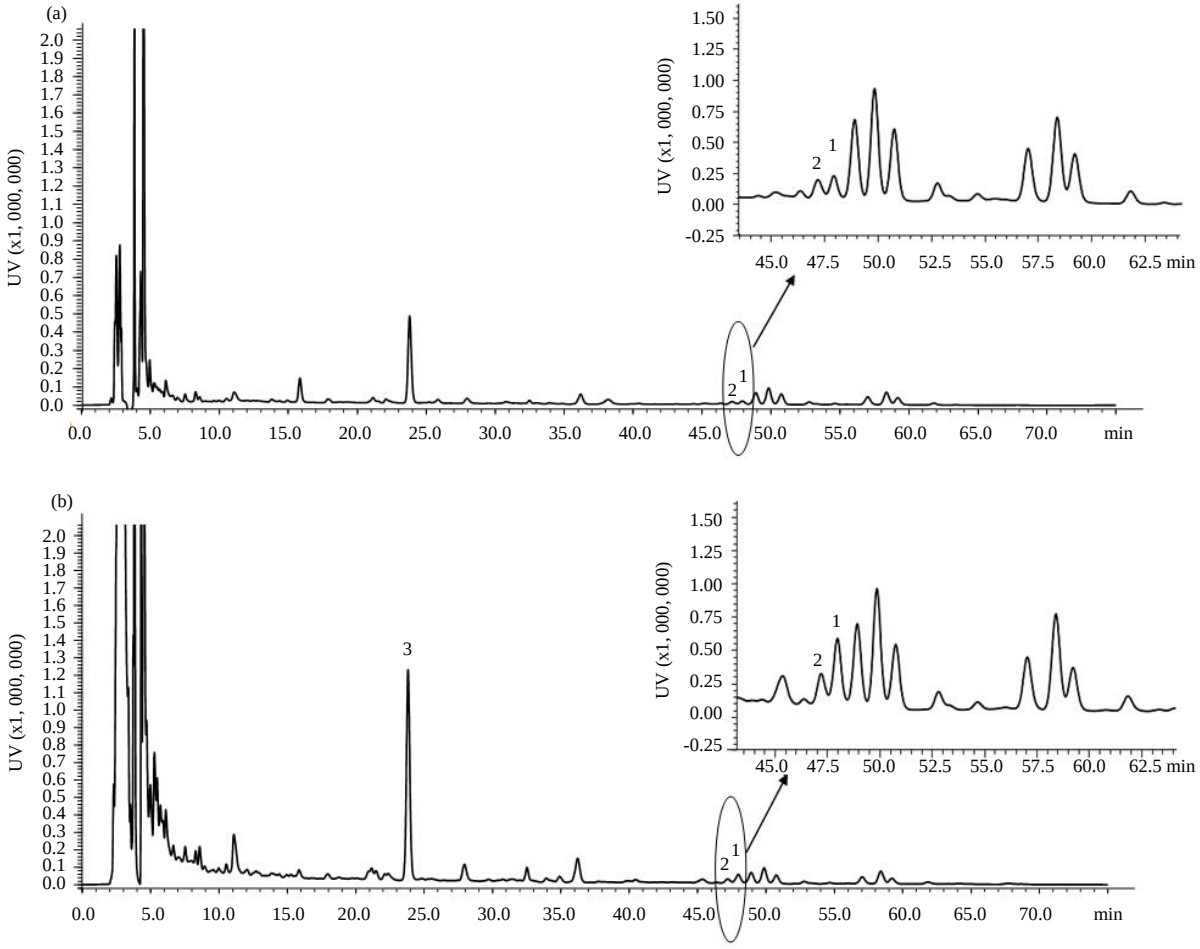

Fig. 1(a-b): HPLC chromatograms of saponins of (a) Un-steamed platycodi radix and (b) Steamed platycodi radix (SSPR). (1) Platycodin D with 2.0640 mg g^{-1}, (2) Deapio-platycodin D with 1.9924 mg g^{-1}, (3) lobetyolin with 0.6098 mg g^{-1}

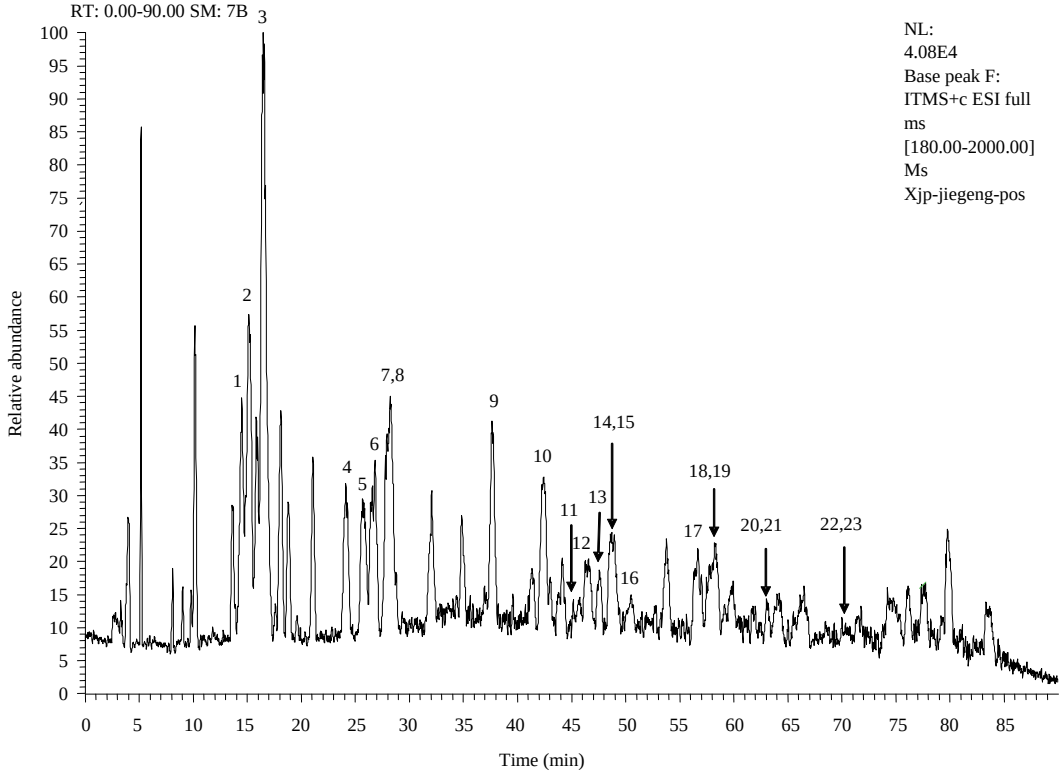

Fig. 2: Total ion chromatogram of saponins of steamed platycodi radix (SSPR) in positive mode

Table 1: Related substances of SSPR identified by LC-MS/MS

Compounds	t_R/min	Parent ion (m/z)	Formula	Ion type	Mr
Deapio-platycoside E	14.34	1417.7	$C_{64}H_{104}O_{34}$	$[M+H]^+$	1416.6
Lobetyolin	15.17	419.7	$C_{20}H_{28}O_8$	$[M+Na]^+$	396.5
Platycoside E	15.79	1549.7	$C_{69}H_{112}O_{38}$	$[M+H]^+$	1548.7
Deapio-platycodin D_3	24.16	1255.7	$C_{58}H_{94}O_{29}$	$[M+H]^+$	1254.6
β-Gentiobiosyl-platycodigenin	25.72	829.5	$C_{42}H_{68}O_{16}$	$[M+H]^+$	828.5
Platycodin D_3	26.53	1387.5	$C_{63}H_{102}O_{33}$	$[M+H]^+$	1386.6
β-Gentiotriosyl-platycodigenin	28.00	1007.7	$C_{48}H_{78}O_{22}$	$[M+H]^+$	1006.5
3″-*O*-Acetyl platycodin D_3	28.86	1429.4	$C_{65}H_{104}O_{34}$	$[M+H]^+$	1428.6
2″-*O*-Acetyl platycodin D_3	37.04	1429.6	$C_{65}H_{104}O_{34}$	$[M+H]^+$	1428.6
Deapio-platycodin D	43.00	1115.8	$C_{52}H_{84}O_{24}$	$[M+Na]^+$	1092.5
Deapio-3-*O*-acetyl-platycodin D	45.17	1135.5	$C_{54}H_{86}O_{25}$	$[M+H]^+$	1134.6
Platycodin D	46.27	1247.8	$C_{57}H_{92}O_{28}$	$[M+Na]^+$	1224.6
3″-*O*-Acetyl platycodin D_2	47.45	1429.1	$C_{65}H_{104}O_{34}$	$[M+H]^+$	1428.6
2″-*O*-Acetyl-platycodin D	48.48	1289.8	$C_{59}H_{94}O_{29}$	$[M+Na]^+$	1266.6
3″-*O*-Acetyl-platycodin D	48.48	1289.8	$C_{59}H_{94}O_{29}$	$[M+Na]^+$	1266.6
Deapio-2″-*O*-Acetyl-platycodin D	50.81	1135.4	$C_{54}H_{86}O_{25}$	$[M+H]^+$	1134.6
3″-*O*-Acetyl platycodin D_2	56.46	1429.2	$C_{65}H_{104}O_{34}$	$[M+H]^+$	1428.6
2″-*O*-Acetyl-platycodin D	57.83	1267.6	$C_{59}H_{94}O_{29}$	$[M+H]^+$	1266.6
3″-*O*-Acetyl-platycodin D	57.83	1267.6	$C_{59}H_{94}O_{29}$	$[M+H]^+$	1266.6
Dexyl-2″-*O*-Acetyl-polygalacin D_3	63.09	1149.4	$C_{55}H_{88}O_{25}$	$[M+H]^+$	1148.6
Dexyl-3″-*O*-Acetyl-polygalacin D_3	63.09	1149.4	$C_{55}H_{88}O_{25}$	$[M+H]^+$	1148.6
Deapio-2″-*O*-Acetyl-polygalacin D_2	70.33	1281.4	$C_{60}H_{92}O_{29}$	$[M+H]^+$	1280.6
Deapio-3″-*O*-Acetyl-polygalacin D_2	70.33	1281.4	$C_{60}H_{92}O_{29}$	$[M+H]^+$	1280.6

Effect of SSPR on the serum ALT, AST and TG levels: The results showed that the serum levels of ALT, AST and TG were significantly increased in mice of the alcohol group compared with that in control group ($p<0.05$). However, SSPR pretreatment for 14 days provided a significant hepatoprotective effect as evidenced by decreased ALT, AST and TG levels in dose dependent manner ($p<0.05$) (Table 3).

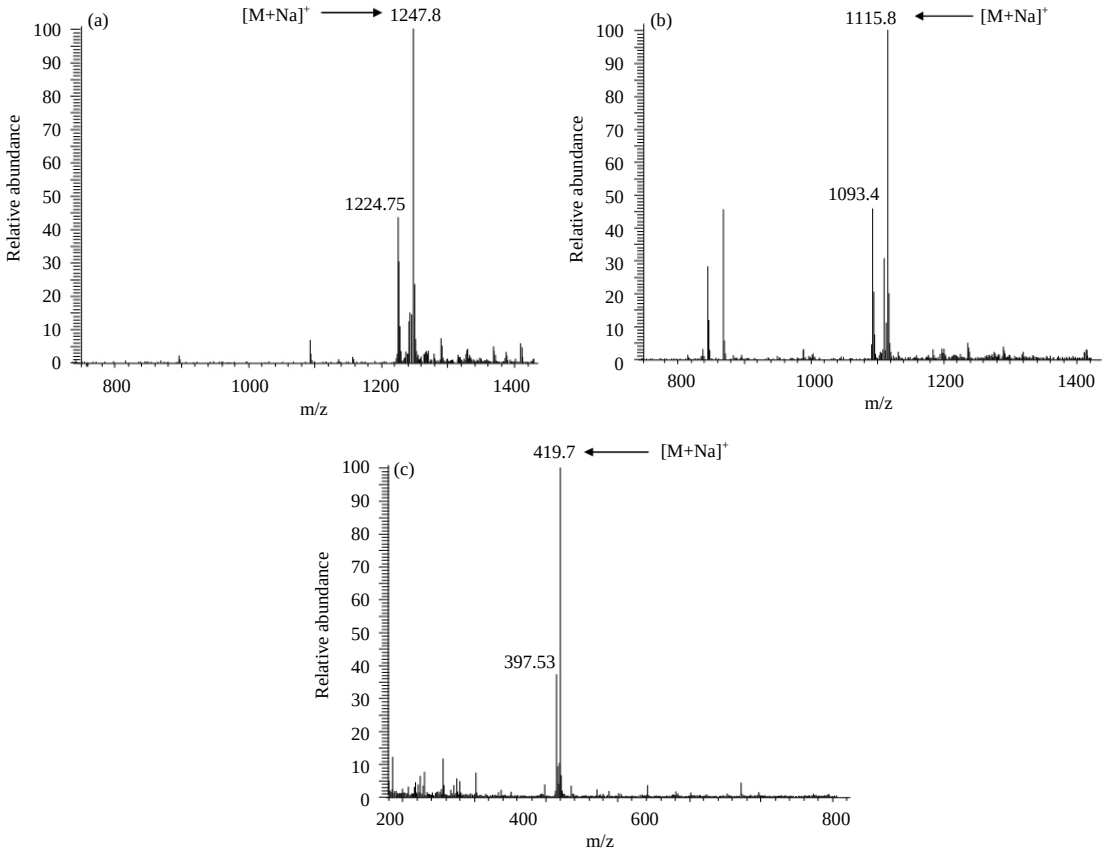

Fig. 3(a-c): Positive ESI-MS spectra of (a) Deapio-platycodin D, (b) Platycodin D and (c) Lobetyolin with $[M+Na]^+$ ion

Table 2: Effect of SSPR on body weight and index of immune organs in mice

Groups	Dosage (mg kg^{-1})	Body weight (g) Initial	Body weight (g) Finial	Liver index (mg g^{-1})	Spleen index (mg g^{-1})
Control	-	20.18±1.08	31.46±1.96	4.37±0.09	0.34±0.04
Alcohol	-	20.13±1.08	32.53±1.67	4.96±0.30#	0.44±0.08#
Silymarin	50	20.07±1.31	32.26±2.60	4.56±0.09*	0.34±0.04*
SSPR+alcohol	100	20.97±1.01	30.06±1.98	4.64±0.33*	0.38±0.03*
	200	20.32±1.25	31.26±2.59	4.59±0.29*	0.38±0.02*
	400	20.54±1.05	31.72±1.82	4.50±0.23*	0.37±0.02*

#Significance $p<0.05$, compared with control group, *Significance $p<0.05$, compared with alcohol group, n = 8

Table 3: Effect of SSPR on serum ALT, AST and TG level in mice

Groups	Dosage (mg kg^{-1})	ALT (U L^{-1})	AST (U L^{-1})	TG (mmol)
Control	-	24.84±9.52	28.20±6.80	0.74±0.15
Alcohol	-	59.79±15.40#	72.07±14.04#	1.87±0.62#
Silymarin	50	31.37±4.64*	32.80±9.33*	0.99±0.15*
SSPR+alcohol	100	34.11±1.83*	36.48±13.14*	1.29±0.58
	200	33.52±5.15*	34.09±11.41*	1.18±0.18*
	400	31.73±6.42*	33.51±10.54*	1.06±0.33*

#Significance $p<0.05$, compared with control group, *Significance $p<0.05$, compared with alcohol group, n = 8

Effect of SSPR on the Level of MDA and GSH in liver homogenate: The effects of SSPR on the levels of MDA and GSH in liver tissues were presented in Fig. 4. A significant increase in MDA content and decrease in GSH level were found in liver of the mice in alcohol group relative to that in control ($p<0.05$). While pretreated with different doses of SSPR reversed this biochemical activities significantly towards control level in a dose dependent manner ($p<0.05$).

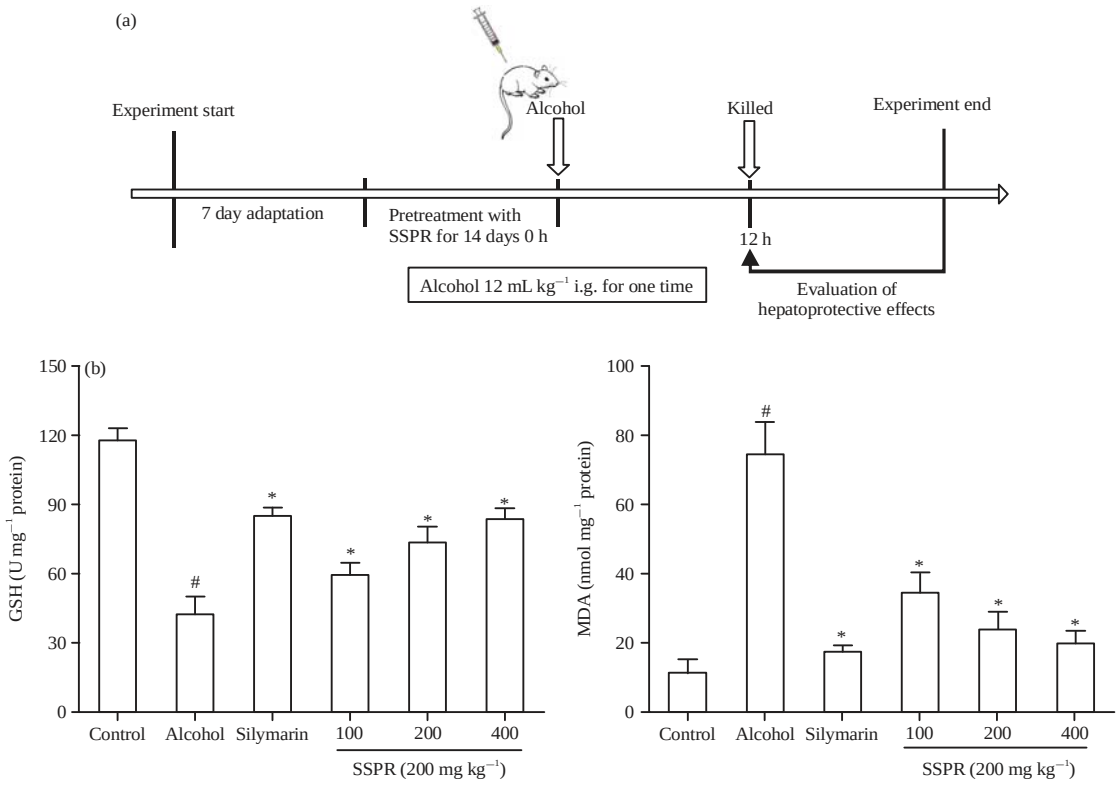

Fig. 4(a-b): Experimental design on (a) Alcohol-induced liver injury and (b) Effects of SSPR on liver MDA and GSH level in mice

#Significance p<0.05, compared with control group, *significance p<0.05, compared with alcohol group

Table 4: Pathological changes and grading results of liver pathological changes in mice

			Steatosis rank					Steatosis grade	
Group	n	Dosage (mg kg^{-1})	0	I	II	III	IV	Grade	Mean rank
Control	8	-	2	6	0	0	0	10	10.60
Alcohol	8	-	0	0	1	6	1	16	30.14#
Silymarin	8	50	1	5	2	0	0	10	18.38*
SSPR+alcohol	8	100	0	2	4	1	1	14	26.17*
	8	200	0	3	4	1	0	13	20.50*
	8	400	1	4	3	0	0	11	20.38*

Ranked standard: 0 = No fat droplets in the lobules (fat droplets in cell scattered, scarce and essential normal), I: Minor fat droplets in the lobules (1-25% of the cells in the specimen have fat droplets), II: Few fat droplets in the lobules (26-50%), III: Moderate fat droplets in the lobules (51-75%), IV: Numerous fat droplets (75-100%), Grading results: grade 0 = 0 score, grade I = 1 score, grade II =2 score, grade III =3 score, grade IV = 4 score. #Significance p<0.05, compared with control group, *Significance p<0.05, compared with alcohol group

Effect of SSPR on liver histopathology and fat deposition: Histopathologic examinations were assessed by H&E staining in liver tissue sections and observed in Fig. 5. The control mice had normal lobular architecture with central veins and orderly cell cord (Fig. 5a). However, in the alcohol group, hepatocytes exhibited extensive vacuolar degeneration, necrosis and some inflammatory cells or inflammatory foci. (Fig. 5b). Pretreatment of SSPR and silymarin clearly improved the histopathological alterations caused by alcohol, which may be due to the attenuation of alcohol-mediated oxidative stress (Fig. 5c-d). In particular, on the basis of liver tissues, a marked reduction in the degree of extensive vacuolar degeneration and necrosis was noted in the livers of mice from the high-dosage SSPR treated group compared with alcohol group mice. Liver fatty degeneration is observed as the major pathological changes in liver cells, which mainly distributed in the surrounding central veins of liver tissues. The alcohol group showed significant liver damage compared with the control group. As shown in Table 4, fatty degeneration

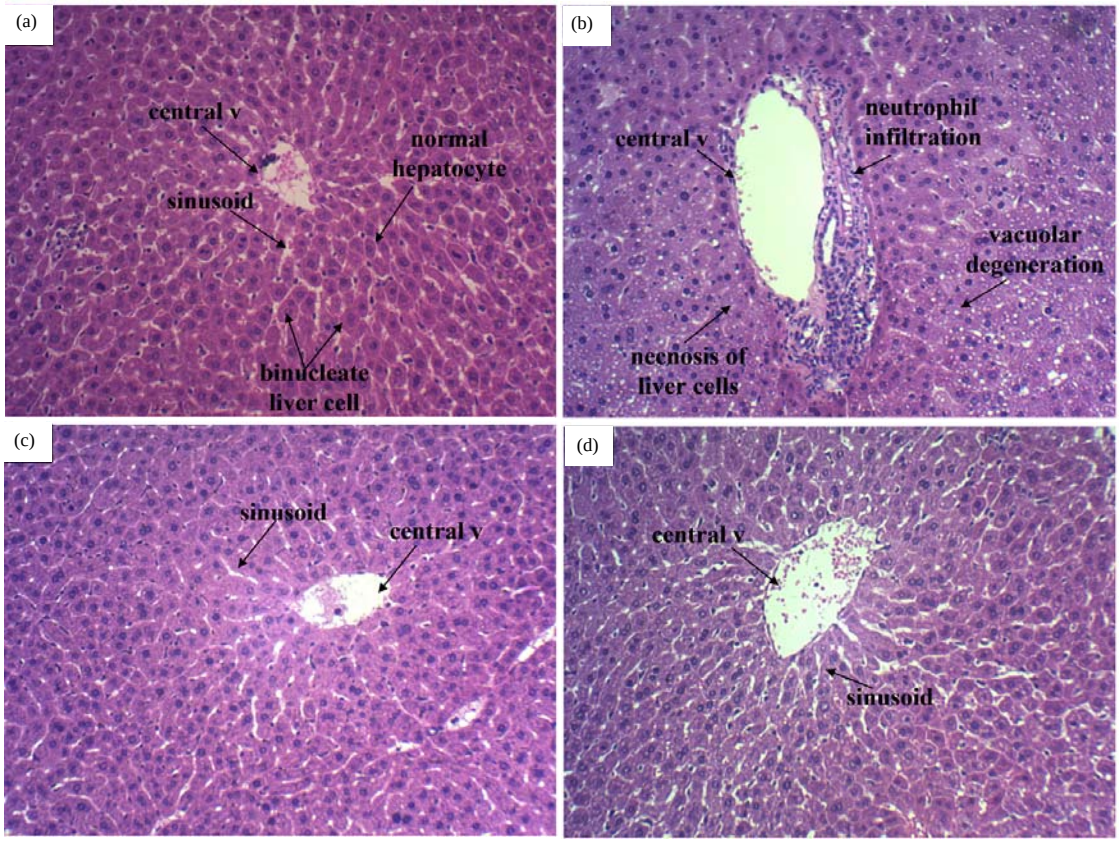

Fig. 5(a-d): Photomicrographs of liver sections obtained from (a) Control group, (b) Alcohol group, (c) Positive group (Silymarin, 50 mg kg^{-1}) and (d) SSPR+Alcohol (400 mg kg^{-1}) (magnification, all 200×)

grades were examined and exhibited a significant difference between the control group and alcohol group ($p<0.05$).

DISCUSSION

The HPLC analysis indicated that the major constituents of SSPR are saponins, including platycodin D (1), deapio-platycodin D (2) as well as lobetyolin (3), etc. The results also showed that some new components were generated and the amounts of several major components have changed in SPR during the heat-treatment compared to the dried one. Among these, deapio-platycodin D and platycodin D were increased to a certain extent compared with PR. That is to say that the steamed *P. grandiflorum* was found to have more PD than un-steamed one. Furthermore, the steamed one can give more edible effects for persons. Particularly, the concentration of lobetyolin in SPR was higher than that in PR. Therefore, the results showed that lobetyolin is one of the main active ingredients of PR. Moreover, the LC-MS analysis of the total ion chromatogram of SSPR has shown in Fig. 2 and Table 1. Comparison of MS fragmentation pattern along with molecular peaks was used for the identification of the chemical compounds with those of literatures[29,30].

ALI is a liver disease caused by long-term heavy drinking. The initial stage is usually fatty liver, which can develop into alcoholic hepatitis, hepatic fibrosis and cirrhosis. ALI is one of the common liver diseases in China and it seriously endangers people's health[1,4]. The pathogenic factors of ALI are relatively single, but the pathogenesis is more complex and has not yet been fully elucidated. At present, many natural components isolated from herbal medicines have a good preventive and therapeutic effect on ALI[8]. It is well known that Platycodi Radix has protective effects in the liver against oxidative damage caused by alcohol, possibly by inhibiting lipid accumulation and peroxidation and preserving the antioxidant defense system[31-33]. The results of present study demonstrated that acute alcohol exposure caused acute liver injury as evidenced by the elevation of serum ALT, AST and TG levels in serum and MDA level in liver and the reduction of GSH in liver and also showed that acute alcohol-induced hepatocyte swelling, fatty degeneration and apoptosis, reflecting early biochemical and pathological changes in alcoholic liver disease. Taken

together, SSPR pretreatment offered significant protection to acute alcohol-induced mice by attenuating ALT, AST, TG elevation and hepatic MDA accumulation and preventing GSH reduction in a dose-dependent manner. Histopathological changes after alcohol exposure were also remarkably improved by SSPR pretreatment for 14 days. The results clearly exhibited that SSPR has hepatoprotective effects in the prevention of alcohol-induced oxidative damage.

Hepatic damage is characterized by leakage of hepatic enzymes, such as ALT and AST, into plasma. Therefore, the serum activities of ALT and AST were used as biochemical markers for the early acute hepatic damage[34,35]. A single alcohol exposure significantly increased the levels of these markers, indicating that liver damage was induced by acute alcohol administration. However, pretreatment of SSPR especially with middle dose and high dose notably prevented the elevation of ALT and AST levels ($p<0.05$), suggesting that SSPR can provide a hepatoprotective effect against acute alcohol-induced liver injury.

Since overdose alcohol consumption is metabolized in the liver, excessive alcohol intake leads to alcoholic liver diseases[36]. In the initial stages of liver disease, TG is accumulated in hepatocytes, leading to the lipid accumulation and even development of a fatty liver. Therefore, the mice in alcohol treated groups had greater hepatic TG concentrations than that in control group. While TG concentrations in SSPR (200 and 400 mg kg^{-1}) groups were significantly lower than alcohol group, showing preventative effects of SSPR on lipid accumulation caused by alcohol.

MDA as a secondary product of lipid peroxidation is a low-molecular-weight substance possessing potential cytotoxicity[6,37]. Alcohol treatment caused an increase in the concentration of hepatic MDA, indicating peroxidation of hepatic lipids (Fig. 4). However, when mice were pretreated with SSPR for two week, MDA levels were significantly lower than in the alcohol-treated alcohol group mice. In particular, there was a marked inhibition of alcohol-induced lipid peroxidation, indicating that the protective effects of SSPR may be associated with its antioxidant potential in the liver tissues. Reduced GSH is one of the main antioxidants and plays a protective role in the metabolism of alcohol. Overdose alcohol intake has followed by the GSH decrease, which may reflect the consumption of GSH caused by the over production of ROS and subsequent oxidative stress caused by alcohol[5,38]. In this study, the activity of GSH in liver homogenates was significantly decreased ($p<0.05$) in alcohol-treated groups compared to control group. The present results showed that SSPR pretreatment inhibited the alcohol-induced depletion of hepatic GSH, indicating SSPR exerted a protective effect on antioxidant defense system.

Ingested excessive alcohol was metabolized in the liver and led to hepatic steatosis (fat accumulation in the liver), inflammation, hepatitis, fibrosis and cirrhosis. Therefore, to determine alcohol-induced histological changes and hepatic lipid accumulation, H&E and oil red O staining were performed in alcohol-treated mice liver. The results indicated that alcohol exposure in liver resulted in apoptosis, whereas treatment with SSPR before alcohol exposure noticeably attenuated the apoptotic cells, which may be representing a defense mechanism against alcohol-induced oxidative stress.

It is the first reports on the hepatoprotective effect of steamed Platycodi Radix and the data clearly clarified that SPR at the present dosage has a definite liver protective effect. In addition, compared with the previous study of Platycodi Radix, the present findings further elucidated that steaming method can significantly increase the content of active compounds.

CONCLUSION

In conclusion, these findings suggest that SSPR has protective effects on the liver against oxidative damage caused by alcohol exposure, possibly via inhibiting lipid accumulation and peroxidation and preserving the antioxidant defense system. Thus, SSPR might be a potent natural ingredient for the prevention of oxidative stress-induced liver diseases. The molecular mechanism of action underlying interactions involving oxidation stress, lipid accumulation, fatty degradation and hepatocyte apoptosis with SSPR will be further investigated in future.

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SIGNIFICANCE STATEMENT

The present work discovered that steaming method can produce more active constituents from the roots of *Platycodon grandiflorum*. Furthermore, the LC/MS analysis indicated that SSPR had more saponins than unheated-processed one. Importantly, these results showed the

beneficial effect of SSPR on acute alcohol-induced oxidative stress and liver damage. Comparing to the previous work, the present findings can help the people to recognize that diary Platycodi Radix could prevent alcohol-induced liver injury.

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