International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2019.394.402

Research Article
Liver Protection Effect of Steamed *Codonopsis lanceolata* on Alcohol-induced Liver Injury in Mice and its Main Components by LC/MS Analysis

[1]Shu-quan Xin, [2]Zi Wang, [2]Wei-Nan Hao, [2]Xiao-Tong Yan, [2]Qi Xu, [2]Ying Liu, [2]Wei Liu, [2]Yan-Fei Li, [2]Xin-Dian Li and [2]Wei Li

[1]College of life Science, Changchun Normal University, Changchun, Jilin, China
[2]College of Chinese Medicinal Materials, Jilin Agricultural University, No. 2888 Xincheng street, 130118 Changchun, China

Abstract

Background and Objective: The roots of *Codonopsis lanceolata* have been used in traditional medicine for treatment of many diseases in China. Till now, there is no evidence on the liver protection effect of *C. lanceolata* on alcohol-induced liver injury. The purpose of this study was to investigate the hepatoprotective effect of steamed *C. lanceolata* (SCL) on ethanol induced liver injury in mice. **Materials and Methods:** Experimental mice were pretreated with different doses of SCL (100-400 mg kg^{-1}) for 2 weeks by gavage feeding. Biochemical markers and enzymatic antioxidants from serum, liver tissue were determined. **Results:** The results showed that the activities of ALT, AST and TG in serum, MDA level in liver tissue, decreased significantly ($p<0.05$) in the SCL-treated group compared with the alcohol group. On the contrary, the GSH level was increased markedly ($p<0.05$). Histopathological examination revealed that SCL (400 mg kg^{-1}) pre-treatment noticeably prevented alcohol-induced hepatocyte apoptosis and fatty degeneration. Moreover, LC-MS/MS analysis of SCL showed that it mainly contain Lobetyolin, Tangshenoside, Lancemasides F, Lancemasides B (Lancemasides E, Codonolaside), Lancemasides G, Lancemasides A (Codonoposide, Codonolaside) and Codonoposide. **Conclusion:** These results provide the evidence on possible application of SCL on acute alcohol-induced oxidative stress and liver damage in clinic.

Key words: Steamed *Codonopsis lanceolata*, acute alcoholic liver injury, LC-MS/MS

Received: June 23, 2018 Accepted: July 23, 2018 Published: March 15, 2019

Citation: Shu-quan Xin, Zi Wang, Wei-Nan Hao, Xiao-Tong Yan, Qi Xu, Ying Liu, Wei Liu, Yan-Fei Li, Xin-Dian Li and Wei Li, 2019. Liver protection effect of steamed *Codonopsis lanceolata* on alcohol-induced liver injury in mice and its main components by LC/MS analysis. Int. J. Pharmacol., 15: 394-402.

Corresponding Author: Wei Li and Xin-Dian Li, College of Chinese Medicinal Materials, Jilin Agricultural University, No. 2888 Xincheng street, 130118 Changchun, China Tel: +86-0431-84533304(8011)

Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Generally, the alcohol is deemed as a well-known liver carcinogen which results in oxidative damage. Alcoholic Liver Disease (ALD) is a leading cause of chronic liver disease worldwide and remains a major global health concern. The ALD includes a spectrum of liver diseases that starts with mild steatosis and progresses to steatohepatitis, fibrosis and cirrhosis. Early studies advanced in mechanisms related to 5 major areas of direct relevance to ALD: Oxidative stress; gut-liver axis and cytokine signaling; malnutrition; fibrin/clotting and stellate cell activation fibrosis[1]. Unfortunately, there are no effective and specific therapies for the treatment of ALD[2,3]. Therefore, investigation and discovery of liver protection therapeutic agents is of paramount importance in the protection of live damage. To date, natural remedies from traditional plants are seen as effective and safe alternative treatments for hepatotoxicity.

Codonopsis lanceolata (Campanulaceae), a plant with both medicinal and nutritive values is widely distributed in China, Korea and Japan. The roots of *C. lanceolata* (CL) have been used in traditional medicine for treatment of asthma, tonsillitis and pharyngitis[4,5], fatigue, bronchitis and cough[6,7]. The main pharmacological properties included anti-inflammatory properties, a protective effect against ischemic damage, alcoholic fatty liver[8,9] and affect apoptosis, cell cycle arrest in colon cancer and leukemia cells[10], antioxidant, anti-mutagenic[11], antimicrobial and anti-inflammatory effects[12,13]. The roots of CL contain various biologically active compounds including saponins, polyphenols, tannins, triterpene, alkaloids and steroids[14].

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 the most effective methods for Chinese medicines[15,16]. Taking steamed ginseng (red ginseng) as an example, a steaming process could cause extensive conversion of ginsenosides in unsteamed ginseng (white ginseng) to new less polar degradation compounds. Red ginseng is widely known to contain more pharmacologically activities than white ginseng[17,18]. In the past decades, the investigations on dried CL have received more and more attention because of its better bioactivities. However, most investigations mainly focused on fresh or dried CL but paid little attention to steamed ones[19-21].

In the present paper, tested the hypothesis that a steaming process affects the chemical profile and hepatoprotective effect of SCL. Moreover, a LC-MS/MS method was developed to qualitatively analyze its chemical constituents.

MATERIALS AND METHODS

Chemicals and reagents: The roots of CL, three-years old, were collected in Jilin province. The specimens were identified and authenticated by Prof. Wei Li, associate professor, Jilin Agricultural University. Silymarin (>85.0%, UV) was separated and supplied by the Institute of Special Wild Economic Animals and Plant. The alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), reduced glutathione (GSH) and Triglyceride (TG) assay kits were acquired from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). The HPLC-grade acetonitrile was purchased from Fisher Chemicals (USA). Other chemicals, such as alcohol were all of analytical grade from Beijing Chemical Factory.

Animals: Male ICR mice (Experimental Animal Holding of Jilin University with Certificate of Quality No. of SCXK (JI) 2011-0004), 22-25 g were housed individually in cages in a temperature-controlled room with a 12 h light/dark cycle. After 1 week of acclimation with free access to regular rodent chow and water, the mice were used for further experiment.

Sample preparation: The fresh roots of CL were washed clean. The SCL was manufactured by steaming fresh CL in an autoclave steam boiler at 105°C for 1 h. After cooling to room temperature, SCL was air dried at room temperature (25°C) until the weight was constant. The SCL powders were ultrasound-assisted extraction with 10 volumes of methanol at the temperature of 40°C for 90 min. After extracting for 3 times, the obtained filtrate was concentrated under reduced pressure in a rotary evaporator to give a crude extract and make up solution to the animal test.

Acute alcoholic liver injury experiment: The extract of SCL were dissolved in CMC-Na liquor to make up the low, medium and high dosage solution (100, 200 and 400 mg kg^{-1}) for the animal test.

After an acclimatization period of 1 week, the animals were randomly divided into 6 groups (7 mice per group) and treated for 15 days as follows: (i) The normal control group and the alcohol model group: Animals received saline intragastrically, (ii) The Silymarin-treated group (positive control group): Animals received Silymarin (50 mg kg^{-1}/day) intragastrically, (iii) Low, medium and high dosage SCL-treated groups, animals received SCL test solution (100, 200 or 400 mg kg^{-1}) intragastrically. After the last administration for 1 h, except the normal control group, other groups were intragastrically administered a one-time grant of 50% alcohol (4.8 g kg^{-1}) shock to induced acute

alcohol liver injury in mice. The animals were fasted for 12 h and sacrificed on the next day by cervical dislocation immediately after withdrawal of blood from the retrobulbar vessels. The serum was separated by centrifugation at 1500 rpm for 10 min. Liver samples were dissected out and washed immediately with saline. One fraction of the liver samples was immediately stored at -80°C for future analysis and another fraction was excised and fixed in a 10% formalin solution for histopathologic analysis.

Histological analysis of liver sections: Take the middle of the left lobe of the liver of mice do cross-section of subjects. Sudan III staining was used for the assessment of the fat deposition in the liver. Simply put, 5 μm sections were cut from frozen samples, affixed to microscope slide and allowed to air-dry at room temperature. The liver sections were firstly stained in Sudan III for 2 min and then counter stained with hematoxylin. The hepatic fat accumulation was observed under light microscope. Mainly observe the size of lipid droplets, the scope and area of distribution in the liver and each liver section was assigned a grade from 0-IV, where, 0 = Lipid droplets scattered and rare, I = Lipid droplets of no more than 1/4, II = Lipid droplets of no more than 1/2, III = Lipid droplets of no more than 3/4, IV = Liver tissue was almost instead of lipid drops. Statistical analysis was used Ridit analysis.

LC/MS analysis

Liquid chromatographic conditions: Samples were analyzed on Agilent HPLC system. Separation was achieved on hypersil ODS2 column (4.6×250 mm, 5 μm) from Dalian Elite Analytical Instruments Co., Ltd. The column temperature was set at 30°C 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: 0-20 min, 11-16% A; 20-35 min, 16-28% A; 35-60 min, 28-60% A.

MS conditions: The HPLC-UV system was interfaced to the MS detector. 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}.

Statistical analysis: Statistical analysis was performed using SPSS 16.0. All values were expressed as the means±SD. Statistical significance of the differences between groups was assessed by Student's t-test. A level of $p<0.05$ was considered statistically significant.

RESULTS

Observation of mice behaviors: After one-time impact heavy alcohol for half an hour, mice were appeared quadriplegia or adrenaline surge. After 1 h, the most of the mice were observed drunken sleepiness and loss of the righting reflex which was consistent with literature reports[22]. The results showed that the acute alcoholic liver injury was successfully established.

Effect of SCL on organ index and weight in mice: Organ indexes of the liver and spleen were evaluated in mice. Similar to previous studies[23,24], liver and spleen indexes were significantly increased ($p<0.05$) in mice that were exposed to alcohol. As shown in Table 1, the alcohol-induced increase in the liver coefficient was reduced by the Silymarin. Meanwhile, 400 mg kg^{-1} SCL-treated group prior to alcohol model group caused a significant increase ($p<0.05$) in liver index and spleen index. In addition, treatment with 200 and 400 mg kg^{-1} of SCL showed a significant decrease ($p<0.05$) on spleen index.

As showed in Table 1, there was no significant difference among the mean initial body weights of all groups. However, body weights of alcohol and SCL-treatment groups were lower than that of the normal control group by the end of the study.

Effects of SCL on ALT, AST and TG in serum: The results showed that AST and ALT levels were significantly increased after the administration of alcohol compared with the normal control group ($p<0.05$), which confirmed the successful establishment of liver injury. Treatment with 400 mg kg^{-1}

Table 1: Effects of SCL on weight, liver index and spleen index in mice

Groups	Dosage (mg kg^{-1})	Body weight (g) Before	Body weight (g) After	Liver index (×100, mg g^{-1})	Spleen index (×100, mg g^{-1})
Normal	-	26.02±1.10	34.46±1.82	4.82±0.15	0.32±0.02
Model	-	26.92±1.87	30.43±1.82	5.53±0.21*	0.46±0.14*
Silymarin	50	26.87±1.22	32.85±2.59	4.98±0.09#	0.34±0.04#
SCL	100	25.91±0.69	30.28±1.90	5.21±0.12	0.33±0.06
	200	26.91±1.42	31.05±2.79	5.02±0.11#	0.32±0.05#
	400	26.84±1.34	31.62±2.03	5.02±0.09#	0.29±0.04#

Values are expressed as the Mean±SD, n = 7, *$p<0.05$ vs. normal group, #$p<0.05$ vs. model group

of SCL significantly reduced the elevation of ALT ($p<0.05$) and 200 and 400 mg kg^{-1} of SCL significantly reduced the elevation of AST ($p<0.05$) compared with the alcohol group. Similarly, treatment with Silymarin significantly reduced the elevation of AST and ALT ($p<0.05$).

As shown in Table 2, TG activities were found to increase in serum of the ethanol treated group relative to the normal control. Treatment with SCL (100, 200, 400 mg kg^{-1}) and Silymarin during ethanol exposure resulted in significant protection of the liver as indicated by reductions in the elevated level of TG ($p<0.05$).

Effects of SCL on MDA and GSH in liver: As shown in Table 3, administration of alcohol caused a significant decrease ($p<0.05$) in the level of GSH and an increase in the MDA concentration compared with the normal control group. Treatment with SCL (100, 200 and 400 mg kg^{-1}) significantly raised ($p<0.05$) the level of GSH compared with the alcohol group. In addition, mice treated with 200 and 400 mg kg^{-1} of SCL showed a significant reduction ($p<0.05$) in MDA level in the liver homogenate compared with the alcohol-exposed group.

Histological analysis of liver sections

Liver pathological classification and grading: Systems for grading and staging incorporate the view that necro-inflammation is not only a measure of severity but also of ongoing disease activity and the parameter most potentially responsive to therapy[25]. As shown in Table 4, pathological changes in liver is mainly hepatic steatosis and mainly in the central veins around. Alcohol group prior to normal control group presented a significant liver injury; Silymarin group observably attenuated the accumulation of fat in the liver; no severe steatosis was observed in the 400 mg kg^{-1} of SCL group.

Histopathological examination: Histological changes were assessed using HE-stained liver tissue sections from each treatment group. The normal control group had normal lobular architecture with central veins and radiating hepatic cords (Fig. 1a). The alcohol group had enlargement of liver cells and blurred boundaries between cells and appeared more irregular vacuoles in cells (Fig. 1b). Mice treated with Silymarin showed the normal appearance of liver without any

Table 2: Effects on of SCL on serum ALT, AST and TG in mice

Groups	Dosage (mg kg^{-1})	n	TG (mmol L^{-1})	ALT (U L^{-1})	AST (U L^{-1})
Normal	-	7	0.74±0.15	32.09±2.31	18.70±6.00
Model	-	7	1.60±0.62*	49.00±5.05*	26.18±4.48*
Silymarin	50	7	1.11±0.19#	40.85±5.76#	19.06±2.11#
SCL	100	7	1.02±0.34#	48.25±3.86	25.22±4.32
	200	7	0.89±0.28#	40.16±3.56#	20.52±6.93#
	400	7	0.92±0.43#	38.00±6.34#	20.08±1.63#

Values are expressed as the Mean±SD, n = 7, *$p<0.05$ vs. normal group, #$p<0.05$ vs. model group

Table 3: Effects of SCL on liver GSH and MDA in mice

Groups	Dosage (mg kg^{-1})	n	GSH (mg g^{-1} prot)	MDA (nmol mg^{-1} prot)
Normal	-	7	104.40±11.72	15.93±9.66
Model	-	7	42.95±16.59*	53.12±29.70*
Silymarin	50	7	84.74±15.15#	11.51±4.60#
SCL	100	7	63.12±12.43#	9.58±0.50
	200	7	80.18±17.62#	7.30±2.26#
	400	7	93.42±12.11#	8.35±3.57#

Values are expressed as the Mean±SD, n = 7, *$p<0.05$ vs. normal group, #$p<0.05$ vs. model group

Table 4: Pathological changes in the liver of different doses mice that after given once ethanol by mouth

			Steatosis grade					
Groups	Dosage (mg kg^{-1})	Animal numbers	0	I	II	III	IV	Ridit analysis
Normal	-	7	0	7	0	0	0	0.32
Model	-	7	0	1	3	2	1	0.76*
Silymarin	50	7	0	5	2	0	0	0.44#
SCL	100	7	0	4	3	0	0	0.49
	200	7	1	2	2	2	0	0.57
	400	7	2	2	3	0	0	0.41#

Grading standard: Level 0: Lipid droplets scattered, rare and normal in the cell of liver; Level I: Liver cells containing lipid droplets of no more than ¼, Level II: Liver cells containing lipid droplets of no more than ½, Level III: Liver cells containing lipid droplets of no more than ¾, Level IV: Liver tissue was almost instead of lipid drops, statistical analysis was used Ridit analysis, *$p<0.05$ vs. normal group, #$p<0.05$ vs. model group

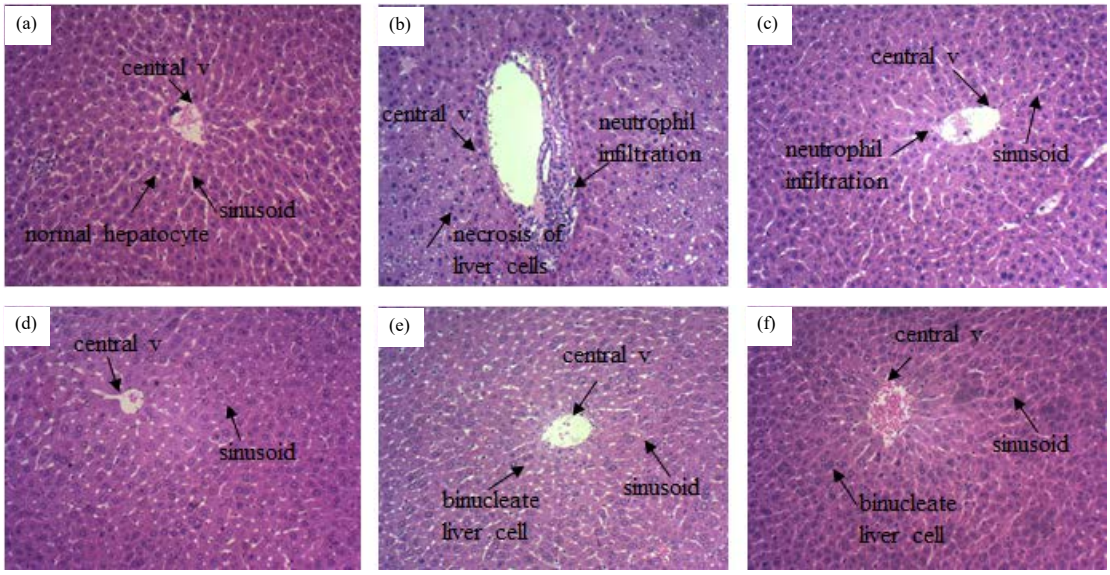

Fig. 1(a-f): Histologic results of tissues stained with HE under light microscopy in liver injury mice (100X), (a) Normal control group, (b) Model group, (c) Silymarin-treated group, (d) 100 mg kg^{-1} SCL-treated group, (e) 200 mg kg^{-1} SCL-treated group and (f) 400 mg kg^{-1} SCL-treated group

Table 5: Related substances of SCL identified by LC-MS/MS

Compounds	t_R/min	Parent ion (m/z)	Formula	Ion type	Mr	Product ion(m/z)
Tangshenoside I	23.4	696	$C_{29}H_{42}O_{18}$	$[M+NH_4]^+$	678	193, 511, 513, 515, 678
Lobetyolin	28.49	414	$C_{20}H_{28}O_8$	$[M+NH_4]^+$	396	295
Lancemasides F	43.61	1532	$C_{69}H_{109}O_{36}$	$[M+NH_4]^+$	1514	267, 1371
Lancemasides B						
Lancemasides E	44.14	1370	$C_{63}H_{99}O_{31}$	$[M+NH_4]^+$	1352	-
Codonolaside III						
Lancemasides G	44.21	1224	$C_{57}H_{89}O_{27}$	$[M+NH_4]^+$	1206	-
LancemasidesA						
Codonoposide III	45.14	1208	$C_{57}H_{89}O_{26}$	$[M+NH_4]^+$	1190	-
Codonolaside						
Codonoposide I	47.85	1222	$C_{58}H_{92}O_{26}$	$[M+NH_4]^+$	1204	-

histological alterations as like normal control mice (Fig. 1c). The 100 mg kg^{-1} of SCL group did not prevent the toxic effect of alcohol with large necrotic areas still present (Fig. 1d). The 200 mg kg^{-1} of SCL group produced a scanty normal lobular pattern with a very mild degree of hepatic injury (Fig. 1e). The high dose of SCL (400 mg kg^{-1}) prevented liver necrosis almost completely, showing lowermost hepatic damage and modified disruption of the liver architecture (Fig. 1f).

LC-MS/MS analysis of SCL: The spectrogram of positive ion of SCL extraction is obtained by LC-MS/MS as shown in Fig. 2. Information was obtained from the primary and secondary mass spectrum (Table 5).

In the MS/MS spectrum of compound 1, the ion at m/z 678 is due to the loss of NH_4 from the $[M+NH_4]^+$ ion at m/z 696 and fragment ions at m/z 193, 511, 513, 515 and 678 were also generated, according to the literature, compound 1 was identified as Tangshenoside I[26]; compound 2, the ion at m/z 396 is due to the loss of NH_4 from the $[M+NH_4]^+$ ion at m/z 414 and fragment ions at m/z 295 were also generated, according to the reported in the literature[27], compound 2 was identified as Lobetyolin; compound 3, the ion at m/z 1514 is due to the loss of NH_4 from the $[M+NH_4]^+$ ion at m/z 1532 and fragment ions at m/z 267 and 1371 were also generated. Comparing with the literature, compound 3 has the same MS/MS fragmentation patterns. Compound 3 was identified as Lancemasides[14] F; compound 4, the ion at m/z 1352 was produced by loss of the NH_4 from the $[M+NH_4]^+$ ion at m/z 1370, which is the same data as reported in the literature. Compound 4 was determined to Lancemasides B, Lancemasides E or Codonolaside[14,28]; compound 5, the ion at m/z 1206 was produced by loss of the NH_4 from the

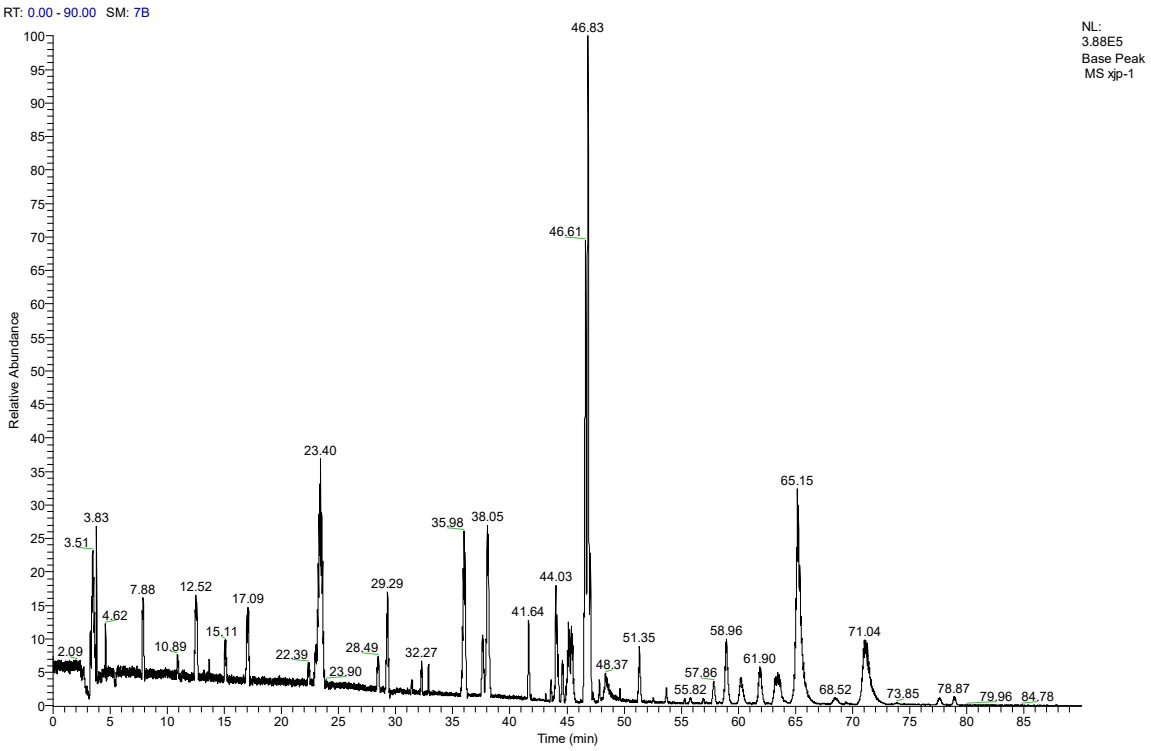

Fig. 2: Total ion chromatogram of SCL (ESI^+)

$[M+NH_4]^+$ ion at m/z 1224. Comparing with the reported in the literature, Compound 5 was identified as Lancemasides G[14]; Compound 6, the ion at m/z 1190 was produced by loss of the NH_4 from the $[M+NH_4]^+$ ion at m/z 1208, according to the literature, compound 6 was identified as Lancemasides A, Codonolaside or Codonoposide III[29-31]; compound 7, the ion at m/z 1204 is due to the loss of NH_4 from the $[M+NH_4]^+$ ion at m/z 1222, which is the same data as reported in the literature, compound 7 was evaluated as Codonoposide I[30].

DISCUSSION

Alcohol Liver Disease (ALD) is a major cause of chronic liver disease worldwide and can lead to fibrosis and cirrhosis[32]. Consumption of alcohol affects the liver and other organs and could contribute to the development of ALD. For the etiology of ALD, oxidative stress, increased expression of proinflammatory cytokines, liver apoptosis and apoptosis have been described[33,34]. The presence of syringin in the extract used in the present study, may explain the effectiveness of CL for liver injury[35].

According to a report in the literature, steam method can enhance the biological activities to increase the efficacy[36,37]. So this study was performed to investigate the protective effect of SCL on animal model after alcohol administration. Well known biomarkers of liver injury and histological changes were examined to evaluate the protective effect of SCL on alcoholic induced liver injury in mice in present study.

Accumulation of fat is the earliest and most common response to heavy alcohol ingestion[38]. The ALD is usually characterized by enlargement of the liver, increased serum TG level and the presence of a high number of fat droplets in liver sections[39]. It was noticed that ethanol-exposed led to a significant increase ($p<0.05$) in the level of TG compared to normal control group, which was obviously attenuated by all dose SCL-treatment groups showing the prevention effects of SCL on lipid accumulation caused by alcohol.

Hepatocytic injury is characterised by hepatic marker enzymes including ALT, AST. When liver cells are damaged, these enzymes leak into the bloodstream from liver tissue and produce markedly elevated serum levels[40]. Results of this study offer evidence that alcohol administration causes severe acute liver injury in mice. The reduction in activities of these biochemical parameters by 200 and 400 mg kg^{-1} of SCL treatment is an indication of prevention of hepatic tissue injury caused by alcohol.

The GSH is an important constituent of cellular protective mechanisms in effecting detoxification of reactive metabolites from cells. The observed decrease in GSH level might have been due to enhanced scavenging of reactive substances that

were produced as a result of ethanol metabolism[41]. In this study, the activity of antioxidant enzyme GSH was significantly decreased ($p<0.05$) in alcohol-treated group compared with normal control group. While intragastric administration of SCL reversed the alcoholic-induced effects, as this treatment resulted in significantly increased ($p<0.05$) concentration of GSH.

The level of MDA has been widely used as a biomarker of LPO for many years[42]. Current study revealed that mice treated with alcohol showed a significant increase ($p<0.05$) in MDA level compared to the normal control group. Treatment with SCL (200 and 400 mg kg^{-1}) significantly reduced ($p<0.05$) the alcoholic-induced hepatic MDA elevation, meaning that SCL could provide protective effects against alcoholic-induced liver injury.

Histological changes including cell necrosis, fatty metamorphosis in adjacent hepatocytes and infiltration of lymphocytes have been observed in the liver tissue section of the alcoholic model group and these histopathologic changes were significantly receded by SCL treatment, which further attested the beneficial effects of SCL on liver injury and retarding the progression to hepatic fibrosis induced by alcoholic.

CONCLUSION

On the whole, the SCL have protective effect on alcoholic hepatic in jury. The result of SCL analysis by LC-MS/MS showed the main components involved in the process of protecting the liver. The acute alcoholic hepatic injury was normalized by SCL administration. Thus SCL should be regarded as a new and promising agent with a high potential in the prevention and treatment of acute alcoholic liver injury.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (NO. 31360370, 31201331), the Youth Foundation of Jiangxi Educational Committee (NO. GJJ13023) and Jilin Province Science and Technology Development Plans (NO. 20130303096YY, 201201102).

SIGNIFICANCE STATEMENT

The present work clearly indicated that steamed method can produce more active constituents from the roots of *Codonopsis lanceolata*. Furthermore, the LC/MS analysis indicated that SCL had more saponins than un-steamed one. Importantly, these results showed the beneficial effect of SCL 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 *Codonopsis lanceolata* could prevent alcohol-induced liver injury.

REFERENCES

1. Beier, J.I., G.E. Arteel and C.J. McClain, 2011. Advances in alcoholic liver disease. Curr. Gastroenterol. Rep., 13: 56-64.
2. Schwartz, J.M. and J.F. Reinus, 2012. Prevalence and natural history of alcoholic liver disease. Clin. Liver Dis., 16: 659-666.
3. Fujii, H. and N. Kawada, 2014. Fibrogenesis in alcoholic liver disease. World J. Gastroenterol., 20: 8048-8054.
4. Weon, J.B., B.R. Yun, J. Lee, M.R. Eom and H.J. Ko *et al*., 2014. Neuroprotective effect of steamed and fermented *Codonopsis lanceolata*. Biomol. Therap., 22: 246-253.
5. Ichikawa, M., S. Ohta, N. Komoto, M. Ushijima and Y. Kodera *et al*., 2009. Simultaneous determination of seven saponins in the roots of *Codonopsis lanceolata* by liquid chromatography-mass spectrometry. J. Natural Med., 10.1007/s11418-008-0294-4
6. Hyam, S.R., S.E. Jang, J.J. Jeong, E.H. Joh, M.J. Han and D.H. Kim, 2013. Echinocystic acid, a metabolite of lancemaside A, inhibits TNBS-induced colitis in mice. Int. Immunopharmacol., 15: 433-441.
7. Yongxu, S. and L. Jicheng, 2008. Structural characterization of a water-soluble polysaccharide from the roots of *Codonopsis pilosula* and its immunity activity. Int. J. Biol. Macromol., 43: 279-282.
8. Cho, K., S.J. Kim, S.H. Park, S. Kim and T. Park, 2009. Protective effect of *Codonopsis lanceolata* root extract against alcoholic fatty liver in the rat. J. Med. Food, 12: 1293-1301.
9. Byeon, S.E., W.S. Choi, E.K. Hong, J. Lee, M.H. Rhee, H.J. Park and J.Y. Cho, 2009. Inhibitory effect of saponin fraction from *Codonopsis lanceolata* on immune cell-mediated inflammatory responses. Arch. Pharm. Res., 32: 813-822.
10. Wang, W.Y., S.C. Zhao and D.X. Liu, 2011. Study on the chemical constituents of *Codonopsis lanceolata*. Zhong Yao Cai [J. Chinese Med. Mater.], 34: 553-555.
11. Han, C., L. Han, M. Ye, L. Li and Y. Han, 2004. Experimental studies on the Anti-mutagenic effect of the total saponin of *Codonopsis lanceolata*. J. Environ. Occupat. Med., 21: 397-400.
12. Cha, A., Y. Choi, Y. Jin, M.K. Sung, Y.C. Koo, K.W. Lee and T. Park, 2011. Antilipogenic and anti-inflammatory activities of *Codonopsis lanceolata* in mice hepatic tissues after chronic ethanol feeding. BioMed Res. Int., Vol. 2012. 10.1155/2012/141395.

13. He, X., Y. Zou, W.B. Yoon, S.J. Park, D.S. Park and J. Ahn, 2011. Effects of probiotic fermentation on the enhancement of biological and pharmacological activities of *Codonopsis lanceolata* extracted by high pressure treatment. J. Biosci. Bioeng., 112: 188-193.
14. Ushijima, M., N. Komoto, Y. Sugizono, I. Mizuno and M. Sumihiro *et al*., 2008. Triterpene glycosides from the roots of *Codonopsis lanceolata*. Chem. Pharm. Bull., 56: 308-314.
15. Cheng, X.L., Q. Liu, Y.B. Peng, L.W. Qi and P. Li, 2007. Steamed ginger (*Zingiber officinale*): Changed chemical profile and increased anticancer potential. Food Chem., 129: 1785-1792.
16. Lin, A.S., K. Qian, Y. Usami, L. Lin and H. Itokawa *et al*., 2008. 5-Hydroxymethyl-2-furfural, a clinical trials agent for sickle cell anemia and its mono/di-glucosides from classically processed steamed rehmanniae radix. J. Nat. Med., 62: 164-167.
17. Jiao, L., X. Zhang, M. Wang, B. Li, Z. Liu and S. Liu, 2014. Chemical and antihyperglycemic activity changes of ginseng pectin induced by heat processing. Carbohydr. Polym., 114: 567-573.
18. Park, J.H., H.Y. Cha, J.J. Seo, J.T. Hong, K. Han and K.W. Oh, 2005. Anxiolytic-like effects of ginseng in the elevated plus-maze model: Comparison of red ginseng and sun ginseng. Prog. Neuropsychopharmacol. Biol. Psychiatry, 29: 895-900.
19. Du, Q.Q., S.Y. Liu, R.F. Xu, M. Li, F.R. Song and Z.Q. Liu, 2012. Studies on structures and activities of initial Maillard reaction products by electrospray ionisation mass spectrometry combined with liquid chromatography in processing of red ginseng. Food Chem., 135: 832-838.
20. Kim, G.N., J.S. Lee, J.H. Song, C.H. Oh, Y.I. Kwon and H.D. Jang, 2010. Heat processing decreases Amadori products and increases total phenolic content and antioxidant activity of Korean red ginseng. J. Med. Food., 13: 1478-1484.
21. Li, X., Y. Zheng, M. Liu and L. Zhang, 1999. [A study on maillard reaction and its products during processing of red ginseng]. Zhongguo Zhong yao za zhi, 24: 274-278, 318.
22. Crabbe, J.C., L.C. Kruse, A.M. Colville, A.J. Cameron and S.E. Spence *et al*., 2012. Ethanol sensitivity in high drinking in the dark selectively bred mice. Alcoholism: Clin. Exp. Res., 36: 1162-1170.
23. Tsai, C.F., Y.W. Hsu, W.K. Chen, W.H. Chang, C.C. Yen, Y.C. Ho and F.J. Lu, 2009. Hepatoprotective effect of electrolyzed reduced water against carbon tetrachloride-induced liver damage in mice. Food Chem. Toxicol., 47: 2031-2036.
24. Gao, H.Y., G.Y. Li, M.M. Lou, X.Y. Li, X.Y. Wei and J.H. Wang, 2012. Hepatoprotective effect of matrine salvianolic acid B salt on carbon tetrachloride-induced hepatic fibrosis. J. Inflammat., Vol. 9. 10.1186/1476-9255-9-16.
25. Brunt, E.M., 2000. Grading and staging the histopathological lesions of chronic hepatitis: The Knodell histology activity index and beyond. Hepatology, 31: 241-246.
26. Mizutani, K., M. Yuda, O. Tanaka, Y.I. Saruwatari, M.R. Jia, Y.K. Ling and X.F. Pu, 1988. Tanghenosides I and II from Chuan-Dangshen, the root of codonopsis tangshen oliv. Chem. Pharm. Bullet., 36: 2726-2729.
27. Ishida, S., M. Okasaka, F. Ramos, Y. Kashiwada, Y. Takaishi, O.K. Kodzhimatov and O. Ashurmetov, 2008. New alkaloid from the aerial parts of *Codonopsis clematidea*. J. Natural Med., 62: 236-238.
28. Xu, L.P., H. Wang and Z. Yuan, 2008. Triterpenoid saponins with anti-inflammatory activity from *Codonopsis lanceolata*. Planta Medica, 74: 1412-1415.
29. Lee, K.W., H.J. Jung, H.J. Park, D.G. Kim, J.Y. Lee and K.T. Lee, 2005. β-D-xylopyranosyl-(1→ 3)-β-D-glucuronopyranosyl echinocystic acid isolated from the roots of *Codonopsis lanceolata* induces caspase-dependent apoptosis in human acute promyelocytic leukemia HL-60 cells. Biol. Pharm. Bull., 28: 854-859.
30. Lee, K.T., J. Choi, W.T. Jung, J.H. Nam, H.J. Jung and H.J. Park, 2002. Structure of a new echinocystic acid bisdesmoside isolated from *Codonopsis lanceolata* roots and the cytotoxic activity of prosapogenins. J. Agric. Food Chem., 50: 4190-4193.
31. Yuan, Z. and Z. Liang, 2006. A new triterpenoid saponin from *Codonopsis lanceolata*. Chin. Chem. Lett., 17: 1460-1462.
32. Gao, B. and R. Bataller, 2011. Alcoholic liver disease: Pathogenesis and new therapeutic targets. Gastroenterology, 141: 1572-1585.
33. Darwish, H.A., N.R. Abd Raboh and A. Mahdy, 2012. Camel's milk alleviates alcohol-induced liver injury in rats. Food Chem. Toxicol., 50: 1377-1383.
34. Zhu, L., L. Wang, X. Wang, X. Luo and L. Yang *et al*., 2011. Hepatic deletion of Smad7 in mouse leads to spontaneous liver dysfunction and aggravates alcoholic liver injury. Plos One, Vol. 6. 10.1371/journal.pone.0017415.
35. Gong, X., L. Zhang, R. Jiang, C.D. Wang, X.R. Yin and J.Y. Wan, 2014. Hepatoprotective effects of syringin on fulminant hepatic failure induced by D galactosamine and lipopolysaccharide in mice. J. Applied Toxicol., 34: 265-271.
36. Weon, J.B., B.R. Yun, J. Lee, M.R. Eom and J.S. Kim *et al*., 2013. The ameliorating effect of steamed and fermented *Codonopsis lanceolata* on scopolamine-induced memory impairment in mice. Evidence-Based Complement. Alternat. Med., Vol. 2013. 10.1155/2013/464576
37. Weon, J.B., B.R. Yun, J. Lee, M.R. Eom and H.J. Ko *et al*., 2013. Effect of *Codonopsis lanceolata* with steamed and fermented process on scopolamine-induced memory impairment in mice. Biomol. Therapeut., 21: 405-410.

38. Adiels, M., S.O. Olofsson, M.R. Taskinen and J. Boren, 2008. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arteriosclerosis, Thrombosis Vascul. Biol., 28: 1225-1236.
39. Zeng, M.D., Y.M. Li, C.W. Chen, L.G. Lu, J.G. Fan, B.Y. Wang and Y.M. Mao, 2008. Guidelines for the diagnosis and treatment of alcoholic liver disease. J. Digest. Dis., 9: 113-116.
40. Kasdallah-Grissa, A., B. Mornagui, E. Aouani, M. Hammani and M. El-May *et al*., 2007. Resveratrol, a red wine polyphenol, attenuates ethanol-induced oxidative stress in rat liver. Life Sci., 80: 1033-1039.
41. Lieber, C.S., 1997. Role of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver diseases. Adv. Pharmacol., 38: 601-628.
42. Lykkesfeldt, J., 2007. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin. Chim. Acta, 380: 50-58.