RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2024.112

ScienceAsia 50 (6): 2024: ID 2024112: **1–9**

Protective effect and mechanisms of *Chimonanthus salicifolius* polysaccharide on acute alcoholic liver injury in mice

Lin Chen[a,b,*], **Lu Huang**[a]

[a] Department of Food Research, Zhejiang Business College, Zhejiang 310018 China
[b] Research and Develop Department, Zhejiang Tact Artiste Biotechnology Group Co., Zhejiang 310053 China

*Corresponding author, e-mail: chenlinlucky@163.com

Received 9 Mar 2024, Accepted 16 Oct 2024
Available online 16 Dec 2024

ABSTRACT: *Chimonanthus salicifolius*, a traditionally unique medicinal herb in China and Asian countries, is capable of inhibiting pathogenic bacteria; and, hence, has been applied for healthy food. To explore its functional characteristics, the present study analyzed the main components of *C. salicifolius* polysaccharides (CSP) and examined the hepatoprotective effect of CSP through using an established mouse model of acute alcoholic liver injury. Additionally, the oxidative stress and inflammatory indicators were measured, and the hepatoprotective mechanism of CSP was revealed. The results indicated that the main components in CSP were galactose, glucose, arabinose, and mannose. After administration, CSP significantly decreased the levels of alanine aminotransferase, aspartate aminotransferase, triglyceride, and total cholesterol in alcoholic liver injury mice. CSP could remarkably alleviate liver damage from histopathological examination; increase the antioxidant activity of superoxide dismutase, glutathione, and glutathione peroxidase; decrease the levels of hepatic inflammation of TNF-α, IL-1β, and IL-6; and regulate the NF-κB signaling pathway. These findings demonstrated that CSP effectively improved acute alcoholic liver injury in mice and could be considered as a potential candidate for ALI treatment.

KEYWORDS: *Chimonanthus salicifolius*, polysaccharide, alcoholic liver injury; hepatoprotective effect

INTRODUCTION

Liver injury, a common liver disease affected by excessive alcohol, drug abuse, and environmental pollutants, gives rise to hepatocyte rupture injury, inflammation, necrosis, and even apoptosis [1–3]. Alcoholic liver injury (ALI) caused by acute or chronic alcohol abuse is mainly related to oxidative stress and lipid peroxidation [4]. According to the statistics of World Health Organization, more than 7.5 million of the world's drinkers have abuse alcohol problems, and the incidence of ALI shows an increasing trend [5]. The symptom can develop from fatty liver to alcoholic hepatitis, hepatic fibrosis, cirrhosis, and eventually causing liver cancer leading to liver failure and death [6]. Natural active products are found to have a protective effect against liver damage induced by ethanol, for example, polysaccharides from *Coprinus comatus* and *Dendrobium nobile*, which have shown strong antioxidant and immunomodulatory effects [7]. Therefore, there is of great significance to discover natural products as a potential treatment of ALI.

The pathogenesis of ALI is complex and variable. Ethanol is mainly metabolized by the liver, and short-term intake of large quantities can generate a large amount of reactive oxygen species (ROS), leading to a dynamic imbalance of oxidation-antioxidation in the liver system. Meanwhile, excessive accumulation of ROS in the liver may induce lipid peroxidation and damage the liver [8, 9]. Most of the available treatments for ALI including corticosteroids, glutathione and biphenyl diester expose limitations and side effects [10, 11]. Some herbs have certain curative effects on alcohol-induced liver injury without adverse reaction, and their specific mechanisms have been focused on oxidative stress response and expression of cellular inflammatory factors. Different doses of *C. comatus* extract changed the mitochondria resulting in the oxidation of fatty acids and accumulated fat in cells and causing recovery of alcoholic liver damage in rats [12]. *Gardenia jasminoides Ellis* polysaccharide could alleviate liver injury by inhibiting the TLR4/NF-κB signaling pathway [13]. Thus, it is necessary to reveal the hepatoprotective mechanisms of functional ingredients.

Chimonanthus salicifolius, belonging to Calycanthaceae family, is a traditional medicinal herb applied for drinking tea in China and Southeast Asian countries [14]. Through genome sequencing of 36,651 annotated protein-coding genes [15], it has been confirmed that this herb is rich in a variety of active ingredients with functional properties such as being antibacterial, anti-inflammatory, antiviral, immune-enhancing, etc [16]. Other herbal polysaccharides could have the ability to modulate cytokine release and the immunomodulatory. The polysaccharide produced by *Allium mongolicum* Regel as a xerophytic herb promoted T cell proliferation and IL-2 secretion and inhibited pathogenic bacteria [17].

Chimonanthus nitens Oliv. leaf extracts were found to inhibit α-glucosidase activity, regulate glycolipid metabolism, and elevate antioxidant capacity [18, 19]. With regard to the *C. salicifolius*, reported articles

mainly focused on flavonoids and anthraquinones extracted from its leaf and stem, which showed antioxidant and anti-inflammatory characteristics [20]. Few reports focused on the composition of polysaccharide and monosaccharides. For functionality tends to verify gastrointestinal discomfort symptom, it has been rarely any report on liver disease and other application of *C. salicifolius* extract. However, it was found that the extract of *C. salicifolius* could alleviate intestinal mucositis in mice induced by 5-fluorouracil, and this protective effect might be associated with flavonoids [21, 22]. In our previous study, the extract of *C. salicifolius* with identified rutin as a main ingredient effectively improved loperamide-induced constipation in mice [23]. In addition, *C. salicifolius* extract could alleviate dextran sodium sulfate (DSS)-induced colitis, contributing to remarkably higher abundance of *Lactobacillaceae* and *Bifidobacterium* in intestinal tract of mice [24]. Based on the reported antioxidant effects, we inferred that polysaccharides might be associated with liver protection. The aim of this study was to evaluate the effect of CSP on ALI by analyzing the main functional components of CSP and comparing the protective effect between CSP and bifendate. Furthermore, the histological changes of CSP on alcoholic liver injury and the protection mechanism employing on alcoholic liver injury in mice were revealed.

MATERIALS AND METHODS

Materials and animals

C. salicifolius leaves were obtained from Zhejiang TactArtiste Biotechnology Group Co., Ltd., China. Galactose, glucose, arabinose, and mannose (purity⩾98.0%) were acquired from Sigma-Aldrich, USA. Alanine aminotransferase (AST, C010-2-1), aspartate aminotransferase (ALT, C009-2-1), triglyceride (TG, F001-1), total cholesterol (TC, F002-1), superoxide dismutase (SOD, A001-3), glutathione (GSH, A006-2-1), glutathione peroxidase (GSH-Px, A005-1), and malondialdehyde (MDA, A003-1) assay kits were purchased from Nanjing Jiancheng Biotechnology Co., Ltd., China. Relevant antibodies used in the experiments were purchased from Sigma-Aldrich.

The animal experiments were conducted in strict accordance with the European Community guidelines. The protocol was reviewed and approved by Ethics Committee of Zhejiang Chinese Medical University, China (JN. No 202310606).

Preparation of *C. salicifolius* polysaccharide (CSP)

The fresh *C. salicifolius* leaves were crushed through a 60-mesh sieve, and the resulting powder was soaked in sterile water overnight to remove grease, pigments and other substances to collect dry powder. The CSP was then extracted at a solid-liquid ratio of 1:40, under ultrasonic temperature of 65 °C for 40 min. The extracted solution was concentrated under pressure to obtain crude polysaccharide concentrated solution. The solution then precipitated and redissolved by adding 95% ethanol for 3 times. Further, with papain (1%, w/v) addition, the solution was heated at 60 °C for 1.5 h, frozen and deactivated at −20 °C. The extract was centrifuged (8000×*g* for 15 min). After the protein was removed by savage method for 6 times, the centrifugation suspension was concentrated under pressure twice and dialyzed for 48 h. The CSP powder was obtained using the freeze dryer (Christ Delta LSC, German).

The crude polysaccharide powder was redissolved in distilled water to remove impurities, and the solution was added slowly from the top of DEAE fiber column until the sample was adsorbed and saturated. The gradient elution was performed with sodium chloride solution at a flow rate of 1 ml/min, and the absorbance of the eluate was determined by anthranone sulfuric acid method [25]. The eluate was collected and concentrated under reduced pressure at 55 °C, freeze-dried, and placed in a desiccator.

The CSP powder was dissolved with sterile water and made to 50 ml volume. According to the anthrone-sulfuric acid method, the absorbance was determined by chromogenic reaction (CSP purity was calculated by concentration and dilution ratio).

Identification of CSP

2 mg CSP powder was hydrolyzed in 5 ml trifluoroacetic acid (2 mol/l) at 100 °C for 6 h, and then centrifuged (10000×*g* for 10 min) to collect the polysaccharide. The CSP and monosaccharide standards of 5 μg/ml were prepared, using 75% acetonitrile as solvent. The main compounds of CSP were separated and analyzed using HPLC system (Agilent 1260) at 25 °C, with ZORBAX Extend-C18 liquid chromatography column (250 mm × 4.6 mm, 5 μm). The flow rate of carrier gas was 1.5 ml/min and the injection volume was 10 μl. Mobile phase consisted of different percentage of acetonitrile (A) in 0.2% glacial acetic acid (B). The linear gradient elution started at 15.5%–17.5% (A), then changed to 17.5%–40% (A) after 20 min, and 40%–15.5% (A) after 30 min. The standard substances of rhamnose, fucose, arabinose, xylose, mannose, glucose, and galactose were determined by HPLC. The CSP composition was analyzed by comparing the peak time of monosaccharides in the samples with the standard substances [26].

Animal treatment procedures

Fifty male KM mice (20 ± 1.0 g, 7 weeks old, inbred strain), provided by Shanghai Laboratory Animal Center (Shanghai, China), were housed in a standard rodent cage at a constant temperature (22 °C ± 2 °C) and humidity (55% ± 10%) under conventional standard laboratory conditions (basal maintenance fodder:

corn, bran, soybean, chicken, and fish meal).

All experimental procedures were conducted according to the guideline approved by Laboratory Animal Ethics of Zhejiang Chinese Medical University, China. After 7 days of adaptation to the new environment, the mice were randomly divided into 5 groups as follows: control group, model group, bifendate group (150 mg/kg bifendate), CSP-L group (150 mg/kg CSP), and CSP-H group (300 mg/kg CSP), with 10 mice in each group. Mice in CSP-L, CSP-H, and bifendate groups were gavaged with CSP 150 mg/kg bw, CSP 300 mg/kg bw, and bifendate (150 mg/kg bw), respectively. The control and the model groups were administrated with equal amounts of sterile water at regular intervals every day.

The animals were treated daily for 30 days. At 1 h after the last treatment and except for the control group, the other groups were gavaged with 50% ethanol (20 ml/kg bw). All mice were then strictly fasted but given free water as usual. After 24 h of acute alcohol modeling, the liver samples were collected and weighed using EP tube; and the blood samples were collected and centrifuged (2000×g for 15 min) at 4 °C to obtain the serum which was stored at −80 °C.

Histological analysis of liver injury

Histopathological evaluation was performed on liver tissue sections stained with hematoxylin-eosin (H&E). The liver tissues were embedded in paraffin (5 μm) and stored in formaldehyde solution. Each section was deparaffinized and rehydrated under different alcohol gradients followed by H&E staining [27]. The specimens were assessed under Olympus IX83 microscope (Tokyo, Japan).

Determination of biochemical parameters in serum

Serum ALT, AST, TC, and TG were measured using commercial kits according to the manufacturer's protocols.

Measurement of antioxidant capacity and inflammatory factors in liver

0.2 g liver tissue was added to pre-cooled sterile saline water at a ratio of 1:9 (g/ml) and homogenized with a tissue homogenizer in an ice bath. The homogenate was centrifuged (3500×g, 15 min), and the supernatant was collected. Oxidative stress indicators including MDA, SOD, GSH and GSH-Px were detected in the liver tissues according to the kit instructions.

The sample isolated from 30 mg liver tissue was adopted for extracting RNA using the TRIzol™ reagent. The cytokine levels (IL-1β, IL-6, TNF-α) in liver tissue were analyzed by real-time PCR by a previous method [24]. The expression level of the target gene was calculated using the $2^{-\Delta\Delta Ct}$ method [28, 29].

Western blot analysis

Liver tissue (100 mg) was placed in liquid nitrogen, fully ground into powder, and 0.25 ml RIPA lysate (containing 1% protease inhibitor) was then added and left in an ice bath for 30 min. After complete cracking, the supernatant was collected by centrifugation (10000×g, 5 min) at 4 °C.

The expression levels of inhibitors including kappa B-α (IKBα), phosphorylated IKB kinase-β (p-IKKβ), and phosphorylated p65 (p-p65) were analyzed by western blot. Autoradiograms were recorded by a ChemiDoc Touch imaging system (Bio-Rad Inc, USA) and quantified with the Compass for SW 3.1.8.0 software package. Equal lane loading was assessed using GAPDH [30, 31].

Table 1 Effects of CSP on liver weight.

Group	Body weight (g)	Liver weight (g)
Control	41.34 ± 1.32	1.51 ± 0.06
Model	38.08 ± 1.23**	2.07 ± 0.22**
Bifendate	40.08 ± 1.76	1.60 ± 0.06
CSP-L	38.45 ± 2.01**	1.78 ± 0.11**
CSP-H	38.68 ± 1.66**	1.62 ± 0.02

Data are shown as mean ± SD. ** in each row indicate significant difference ($p < 0.01$) among groups.

Statistical analyses

The data results were expressed as the mean value ± SD. $p < 0.05$ was regarded as statistically significant. GraphPad 10.1 software was employed for statistical analysis. The data was calculated using one-way ANOVA with significant differences among groups displayed.

RESULTS AND DISCUSSION

Main components in CSP

Results of main components in CSP were organized in Fig. 1. Four components of crude polysaccharide were observed after purification from elution curve. The water-washed layer was the most abundant, and the eluent of the water-washed layer was collected, dialyzed, concentrated, and freeze-dried to obtain CSP, a purity of 82.6%. The monosaccharide composition was analyzed by retention time of monosaccharide standards and CSP. The CSP were mainly arabinose (Ara), mannose (Man), glucose (Glu), and galactose (Gal); the contents of which were 24.6%, 6.7%, 18.8%, and 38.2%, respectively.

Histological analysis of liver tissues

The effects of CSP on liver weight were shown in Table 1. The body weight of the CSP-L, the CSP-H, and the model groups were significantly lower than the control and the bifendate groups. The liver of the model group appeared enlarged resulting in increases of liver weights. However, there was no significant difference between the liver weights of the CSP-H and the bifendate groups in comparing to the control.

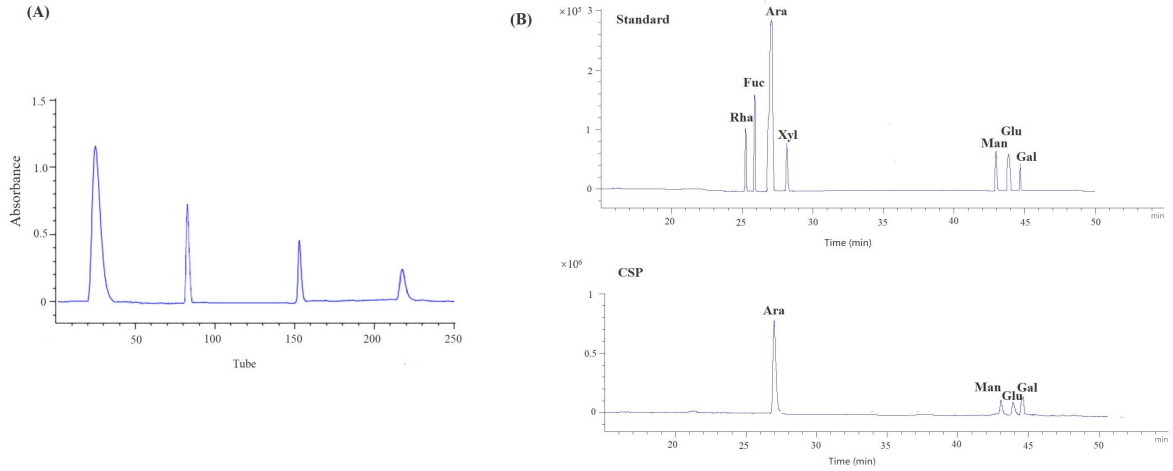

Fig. 1 Analysis of CSP's main components ($n = 3$). (A), Elution curve of CSP; (B), HPLC analysis of CSP.

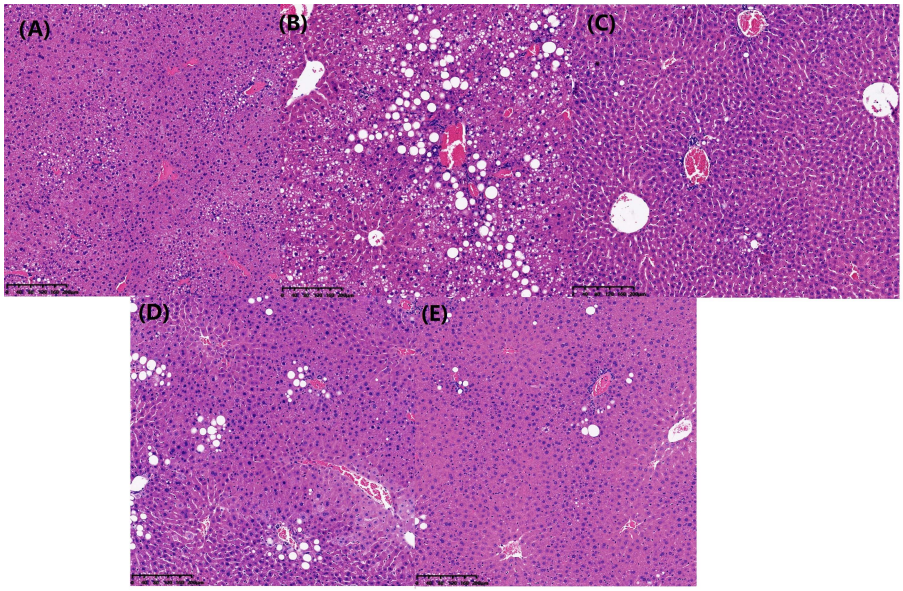

Fig. 2 Histomorphology images of liver tissue ($n = 3$). (A), Control group; (B), model group; (C), bifendate group; (D), CSP-L group; and (E), CSP-H group.

Histopathological observation is generally an effective method to evaluate alcoholic liver injury. The histopathological results of the liver tissues of mice in each group were shown in Fig. 2. The control group presented the full liver tissue and normal hepatic sinusoids, without inflammatory cell infiltration. However, the mice of ALI model group indicated diffuse watery degeneration of hepatocytes and disturbed arrangement of perivascular hepatocytes. Compared with the model group, the bifendate group and the two CSP groups all showed clear borders of hepatocytes and intact liver cells. The results proved that the CSP treatment could relieve liver tissue damage in ADI mice.

Effect of CSP on biochemical parameters in serum

ALT and AST are specific indicators of hepatocellular injury, and the concentration of ALT changes more significantly than that of AST. ALT is mainly distributed in hepatocyte cytoplasm, while AST exists in hepatocyte mitochondria. Once the liver is damaged, hepatocytes release ALT and AST into the blood, resulting in increased concentrations of blood ALT and AST [32]. The results of CSP effect on biochemical parameters in serum were shown in Fig. 3. Compared with the control group, the serum levels of ALT and AST were significantly elevated in the model group ($p < 0.01$). Compared with the model group, after administration,

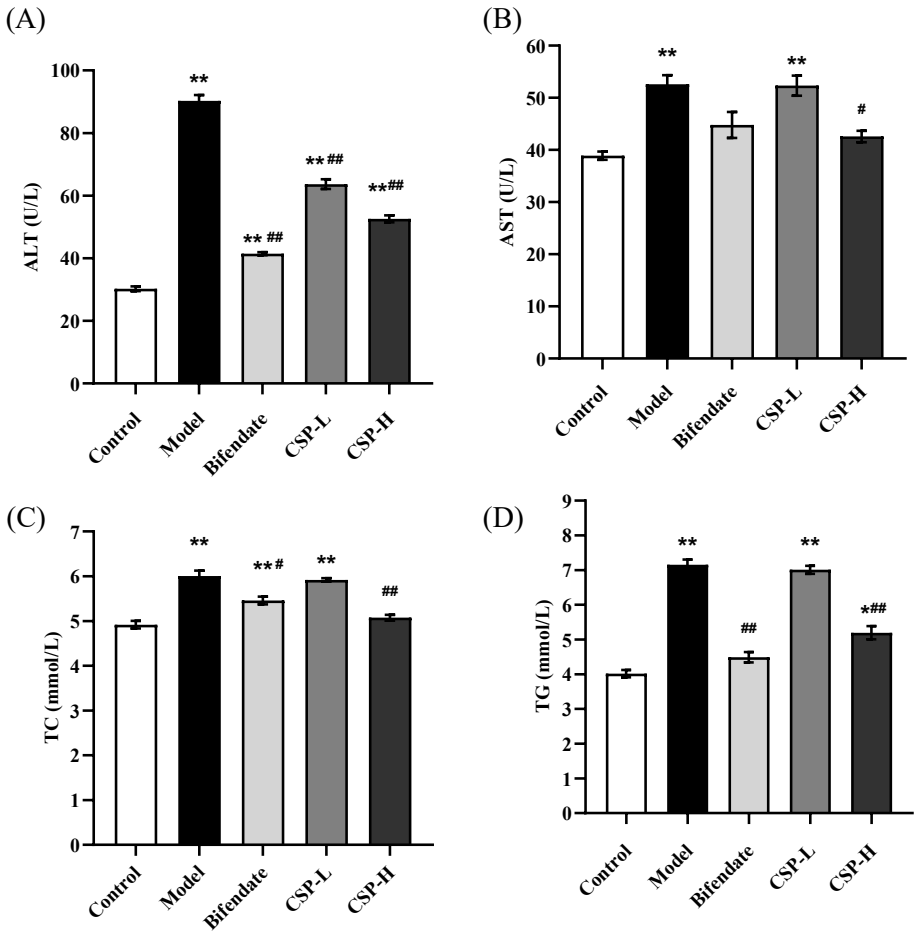

Fig. 3 Effect of CSP on biochemical parameters in serum ($n = 3$). (A), ALT; (B), AST; (C), TC; (D), TG; compared with control group, * $p < 0.05$, ** $p < 0.01$; and compared with model group, # $p < 0.05$, ## $p < 0.01$.

the ALT levels of bifendate and CSP groups showed significant decreases. The AST levels of bifendate and CSP-L groups were insignificantly reduced by 15.8% and 2.6%, respectively ($p > 0.05$); and significantly reduced by 20.2% ($p < 0.05$) in the CSP-H group. Alcoholic liver is closely related to blood lipids. TC and TG were evaluated for hepatic steatosis in this research. The TC and the TG levels of bifendate and CSP-H groups significantly decreased as compared with the model group ($p < 0.05$), with the CSP-H group showing the most significant difference ($p < 0.01$); and no notable changes were observed from the CSP-L and the model groups. The results indicated that CSP could reduce the content of blood lipid and exhibited a protective effect on ALI mice.

Effect of CSP on antioxidant capacity

Analyzing the contents of MDA, SOD, GSH, and GSH-Px in liver is a common method to evaluate antioxidant capacity. MDA is the main product of lipid peroxidation; and SOD and GSH-Px belong to antioxidant enzyme [33]. Compared with the control group (Fig. 4), MDA content in liver tissue of the model group was significantly increased, while the contents of GSH, GSH-Px and SOD were significantly decreased ($p < 0.01$). Compared with the model group, MDA contents of bifendate, CSP-L and CSP-H groups showed significant reduction ($p < 0.01$), while CSP-H and bifendate groups displayed extremely significant increases for SOD, GSH and GSH-Px ($p < 0.01$), with CSP-H reaching the maximum value. The results suggested that the CSP-H group played an antioxidant role and promoted the expression of antioxidant enzymes.

Effect of CSP on the expression of cytokines in liver tissue

TNF-α, as a polypeptide regulator, is induced by a variety of stimulants and distributed throughout the body, most of which is in the liver. IL-1β has strong pro-inflammatory activity for producing cytokines and chemokines. IL-6 can stimulate the proliferation of T and B cells and secrete antibodies to participate

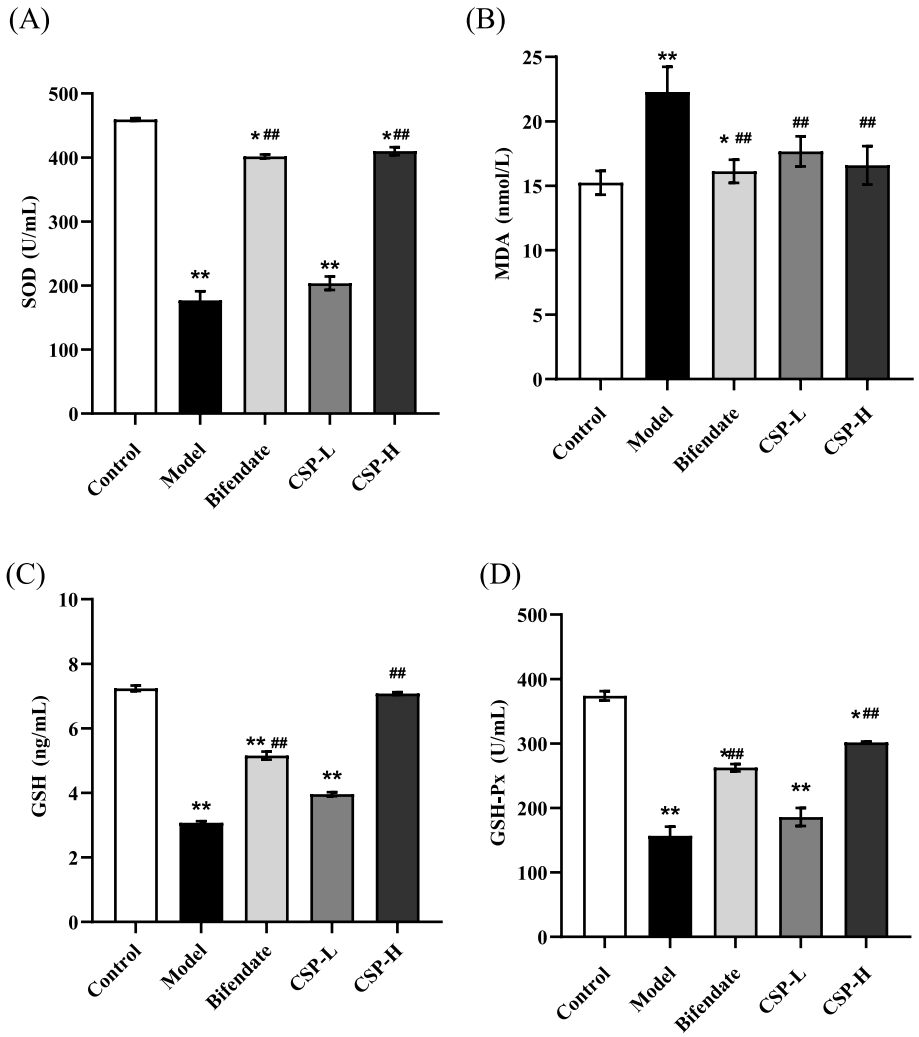

Fig. 4 Effect of CSP on antioxidant capacity ($n = 3$). (A), SOD; (B), MDA; (C), GSH; (D), GSH-Px; compared with control group, * $p < 0.05$, ** $p < 0.01$; and compared with model group, # $p < 0.05$, ## $p < 0.01$.

Table 2 Effects of CSP on cytokines in liver tissue.

Cytokine	Control	Model	Bifendate	CSP-L	CSP-H
IL-1β	1.34 ± 0.12^{a}	52.68 ± 8.67^{c}	30.62 ± 5.68^{c}	28.56 ± 1.98^{bc}	19.65 ± 3.04^{ab}
IL-6	1.36 ± 0.11^{a}	12.15 ± 1.14^{c}	5.36 ± 0.34^{b}	5.28 ± 1.22^{b}	3.36 ± 0.23^{ab}
TNF-α	4.65 ± 0.12^{a}	12.23 ± 2.89^{b}	4.62 ± 0.09^{a}	4.76 ± 0.22^{a}	3.22 ± 0.16^{c}

Data are shown as mean ± SD. Different letters in each row indicate significant difference ($p < 0.05$) among groups.

in the inflammatory response. According to previous studies, these cytokines commonly are used as pro-inflammatory factors to evaluate immunomodulatory function in liver [34, 35].

The levels of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) in liver tissues were described in Table 2. Compared with the model group, different treatments could drastically decrease the expression levels of IL-6, IL-1β and TNF-α cytokines ($p < 0.01$). There was no significant differences in TNF-α and IL-6 levels between bifendate and CSP-L groups ($p > 0.05$). Notably, the CSP-H group showed the lowest expression levels of IL-6, IL-1β and TNF-α. Therefore, it was inferred that CSP could protect against acute alcoholic liver injury by regulating NF-κB signaling pathway related to inflammation.

Western blot analysis

NF-κB is a class of transcription factors widely found in eukaryotic cells, and its pathway regulation is closely

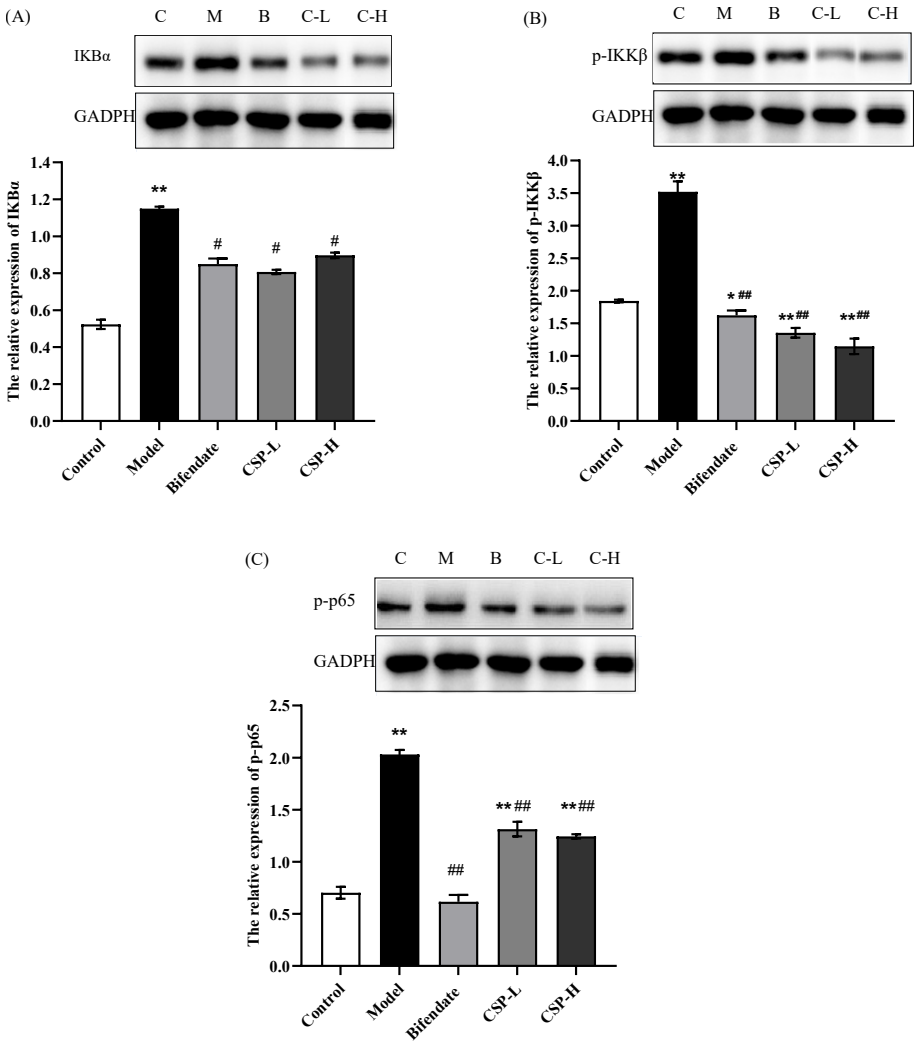

Fig. 5 Analysis of protein expressions (IKBα, p-IKKβ and p-p65) ($n = 3$). (A), IKBα; (B), p-IKKβ; (C), p-p65; compared with control group, * $p < 0.05$, ** $p < 0.01$; and compared with model group, # $p < 0.05$, ## $p < 0.01$.

related to immune response and other physiological processes of cells such as proliferation, transformation and apoptosis. As a key regulator of the classical NF-κB signalling pathway, IKKβ plays a role in recognizing and phosphorylating downstream substrate IκB family proteins. Phosphorylated IκB family proteins, i.e., IKBα and p65 proteins, release NF-κB molecules with transcriptional initiation activity into the nucleus through ubiquitination modification and proteasome-dependent degradation pathways, initiating the expression of relevant genes [13].

The levels of IKBα, p-IKKβ and p-p65 were evaluated by Western blot analysis (Fig. 5). The IkBα, p-IKKβ and p-p65 protein expression levels in the liver tissue of ADI model group were significantly increased compared with the control group ($p < 0.01$). The IkBα protein expression of bifendate and CSP treated groups were significantly lower than that of the model group ($p < 0.01$) and no significant difference was observed from that in the control group ($p > 0.05$). The p-IKKβ and p-p65 protein expression levels of CSP-L and CSP-H groups significantly changed compared with both of control and model groups ($p < 0.01$). Notably, the CSP-H group manifested the lowest p-IKKβ protein expression and the bifendate appeared the minimum value of p-p65 protein expression. The results indicated that CSP could effectively down-regulate the expression levels of IkBα, p-IKKβ and p-p65 in the liver cells of ADI mice.

NF-κB, a multifunctional transcription factor, regulates the inflammatory response and immunity through an important signaling pathway involved in the inflammatory response [35]. Therefore, the activation of the NF-κB signaling pathway is related to the activity of IKBα and p-IKKβ proteins upstream, as well as the regulation of p65 protein after entering the nucleus.

Negative feedback regulation of the NF-κB signaling pathway plays an important role in maintaining its proper activation. On the one hand, activation of NF-κB increases IKBα gene transcription, and newly synthesized IKBα protein enters the nucleus and binds to NF-κB, leading to its dissociation from DNA molecules. The nuclear export signal sequence in IKBα molecules can induce NF-κB to return to the cytoplasm, ensuring the activity of the NF-κB pathway and amplifying the inflammatory effect [36, 37].

In this study, the expression levels of IKBα, P-IKKβ and p-p65 proteins in mouse liver cells were significantly down-regulated by the intervention of CSP, suggesting that CSP could inhibit the activity of IKK, thereby reducing the phosphorylation of IκB and inhibiting the activation of NF-κB signalling pathway. Meanwhile, CSP weakened the activity of p-p65 protein and its negative regulation of NF-κB signaling pathway, which further reduced the inflammation-related effect. The results demonstrated that the pretreatment of CSP could effectively inhibit the activation of NF-κB signaling pathway, suppress inflammation in ADI mice, and prevent alcoholic liver disease.

Notably, the CSP-H (300 mg/kg CSP) exhibited remarkable treatment on relieving acute alcoholic liver injury compared with the other groups, as evidenced by biochemical and antioxidant properties. Different doses of *C. salicifolius* extract could protect against gastrointestinal discomfort. The 20 g/kg *C. salicifolius* extract significantly suppressed inflammation cellular proliferation, supporting the protect effect of gastric mucosa [15]. The 9 g/kg of *C. salicifolius* extract identified as flavonoids and coumarins could alleviate the intestinal mucositis in mice induced by 5-fluorouracil [16]. The result of this paper is consistent with our result in a previous study, i.e., for the relieving constipation and inflammatory bowel disease, an application of high-dose CSP has better prospects [23, 24].

CONCLUSION

In this paper, the main components of polysaccharides from *C. salicifolius* (CSP) were analyzed, and the hepatoprotective effect in acute alcoholic liver injury mice was evaluated. Further, this study investigated the capacities of antioxidants (MDA, SOD, GXH and GXH-Px), examined the serum indexes (ALT, AST, TC, and TG), and measured the inflammatory levels of TNF-α, IL-1β and IL-6. Additionally, the liver-protective mechanism of CSP regulated by NF-κB signaling pathway was verified. The results indicated that CSP presented the potential to be developed as a candidate for treating acute alcoholic liver injury.

Acknowledgements: This research was supported by Zhejiang Provincial Natural Science Foundation of China under Grant (No. LTGN24C200009); Zhejiang Provincial Philosophy and Social Sciences Planning Project (No. 24NDJC184YB). Zhejiang Business Collage Foundation of China (SZYZD202305, 3010305026, 3010305037, 3010306025).

REFERENCES

1. Aron-Wisnewsky J, Gaborit B, Dutour A, Clement K (2013) Gut microbiota and non-alcoholic fatty liver disease: New insights. *Clinl Microbiol Infec* **19**, 338–348.
2. Masarone M, Rosato V, Dallio M, Abenavoli L, Federico A, Loguercio A, Persocp M (2016) Epidemiology and natural history of alcoholic liver disease. *Rev Recent Clin Trials* **11**, 167–174.
3. Ellis JL, Yin C (2017) Histological analyses of acute alcoholic liver injury in zebrafish. *Jove-J Vis Exp* **25**, 55630.
4. Meritxell VC, Josepmaria A, Patricia D, Carolin L, Mohamed EH, Juan GA, Edilma A, Ana C (2022) Clinical, histological and molecular profiling of different stages of alcohol-related liver disease. *Gut* **71**, 1856–1866.
5. Yokoda RT, Rodriguez EA (2020) Review: pathogenesis of cholestatic liver diseases. *World J Hepatol* **12**, 423–435.
6. Appanna G, Kallis Y (2020) An update on the management of cholestatic liver diseases. *Med Clin* **5**, 513–516.
7. Hasegawa S, Yoneda M, Kurita Y, Nogami A, Honda Y, Hosono K, Nakajima A (2021) Cholestatic liver disease: current treatment strategies and new therapeutic agents. *Drugs* **81**, 1181–1192.
8. Zhou C, Lai Y, Huang P, Xie LP, Lin HY, Zhou Z, Mo C, Deng GH (2019) Naringin attenuates alcoholic liver injury by reducing lipid accumulation and oxidative stress. *Life Sci* **216**, 305–312.
9. Tu Y, Zhu S, Wang J, Burstein E, Jia D (2019) Natural compounds in the chemoprevention of alcoholic liver disease. *Phytother Res* **33**, 2192–2212.
10. Madrigal-Santillán E, Madrigal-Bujaidar E, Martínez MT (2014) Review of natural products with hepatoprotective effects. *World J Gastro* **20**, 14787–14804.
11. Yi Z, Xia Y, Liu XF, Wang QG, Xiong ZQ, Ai LZ (2020) Antrodin A from mycelium of *Antrodia camphorata* alleviates acute alcoholic liver injury and modulates intestinal flora dysbiosis in mice. *J Ethnopharmacol* **254**, 112681.
12. Ozalp FO, Canbek M, Yamac M, Kanbak G, Leo JLD, Griensven V (2014) Consumption of *Coprinus comatus* polysaccharide extract causes recovery of alcoholic liver damage in rats. *Pharm Biol* **2**, 994–1002.
13. Fang S, Wang T, Li Y, Xue H, Zou J, Cai J (2022) *Gardenia jasminoides* ellis polysaccharide ameliorates cholestatic liver injury by alleviating gut microbiota dysbiosis and inhibiting the tlr4/nf-κb signaling pathway. *Int J Biol Macromol* **205**, 23–36.
14. Liang XY, Zhao C, Su WK (2016) A fast and reliable UPLC-PAD fingerprint analysis of *Chimonanthus salicifolius* combined with chemometrics methods. *J Chromatogr Sci* **54**, 1213–1219.
15. Liu G, Fu J (2018) Squalene synthase cloning and functional identification in wintersweet plant (*Chimonanthus zhejiangensis*). *Bot Studs* **59**, 30.
16. Lv Q, Qiu J, Liu J, Zheng L, Chenget KJ (2020) The *Chimonanthus salicifolius* genome provides insight into magnoliid evolution and flavonoid biosynthesis. *Plant J* **l103**, 1910–1923.

17. Hu RP, Xu SY, Ao YH, Ma YH, Bao SH, Sun JT, Yuan H, Xue HT, et al (2022) Extraction and evaluation of biological activities of a polysaccharide from *Allium mongolicum* Regel. *ScienceAsia* **48**, 340–347.
18. Chen H, Jiang Y, Yang Z, Hu W, Xiong L, Wang N, Liu X, Zheng GD, et al (2017) Effects of *Chimonanthus nitens* Oliv. leaf extract on glycolipid metabolism and antioxidant capacity in diabetic model mice. *Oxid Med Cell Longev* **2017**, 7648505.
19. Chen H, Ouyang K, Jiang Y, Yang Z, Hu W, Xiong L, Wang N, Liu X, et al (2017) Constituent analysis of the ethanol extracts of *Chimonanthus nitens* Oliv. leaves and their inhibitory effect on α-glucosidase activity. *Int J Biol Macromol* **98**, 829–836.
20. Wang KW, Li D, Wu B, Cao XJ (2016) New cytotoxic dimeric and trimeric coumarins from *Chimonanthus salicifolius*. *Phytochem Lett* **16**, 115–120.
21. Wen H, Lei W, Hou J, Leqin KE (2021) Main components of ethyl acetate extract of *Chimonanthus salicifolius* and its effects on intestinal mucositis in mice induced by 5-fluorouracil. *Food Sci Technol Camp* **7**, 55720.
22. Liu Z, Xi J, Schröder S, Wang W, Xie T, Wang Z, Bao SS, Fei J (2013) *Chimonanthus nitens* var. *salicifolius* aqueous extract protects against 5-fluorouracil induced gastrointestinal mucositis in a mouse model. *Evid Based Complementary Altern Med* **2013**, 789263.
23. Chen L, Li X (2023) *Chimonanthus salicifolius* S. Y. Hu extract improve constipation symptoms and regulate intestinal microbiota in mice. *Food Sci Technol Res* **29**, 101–112.
24. Chen L, Li X, Gu Q (2023) *Chimonanthus salicifolius* extract alleviates DSS-induced colitis and regulates gut microbiota in mice. *Food Sci Nutri* **11**, 3019–3030.
25. Gao Z, Zhang JJ, Jin HJ, Li SS, Ren ZZ (2017) Hepatoprotection of enzymatic-extractable mycelia zinc polysaccharides by *Pleurotus eryngii* var. *tuoliensis*. *Carbohyd Polym* **157**, 196–206.
26. Yang Z, Wang J, Li J, Xiong L, Chen H, Liu X (2018) Antihyperlipidemic and hepatoprotective activities of polysaccharide fraction from *Cyclocarya paliurus* in high-fat emulsion-induced hyperlipidaemic mice. *Carbohyd Polym* **183**, 11–20.
27. Hatware KV, Sharma S, Patil K, Shete M, Karri S, Gupta G (2018) Evidence for gastroprotective, anti-inflammatory and antioxidant potential of methanolic extract of *Cordia dichotoma* leaves on indomethacin and stress induced gastric lesions in Wistar rats. *Biomed Pharm* **103**, 317–325.
28. Johnson AH, Frierson HF, Zaika A, Powell SM, Roche J, Crowe S (2005) Expression of tight-junction protein claudin-7 is an early event in gastric tumorigenesis. *Am J Pathol* **167**, 577–584.
29. Schmittgen TD, Livak K (2008) Analyzing real-time PCR data by the comparative ct method. *Nat Protoc* **3**, 1101–1108.
30. Singh LP, Kundu P, Ganguly K, Mishra A, Swarnakar S (2007) Novel role of famotidine in downregulation of matrix metalloproteinase-9 during protection of ethanol-induced acute gastric ulcer. *Free Radical Bio Med* **43**, 289–299.
31. Ganguly K, Kundu P, Banerjee A, Reiter RJ, Swarnakar S (2006) Hydrogen peroxide-mediated downregulation of matrix metalloprotease-2 in indomethacin-induced acute gastric ulceration is blocked by melatonin and other antioxidants. *Free Radical Biol Med* **41**, 911–925.
32. Yu J, Sun J, Sun M, Li W, Qi D, Zhang Y, Han C (2024) Protective mechanism of *Coprinus comatus* polysaccharide on acute alcoholic liver injury in mice, the metabolomics and gut microbiota investigation. *Food Sci Hum Well* **13**, 401–413.
33. Mao J, Zhan H, Meng F, Wang G, Huang D, Liao Z, Chen M (2022) Costunolide protects against alcohol-induced liver injury by regulating gut microbiota, oxidative stress and attenuating inflammation *in vivo* and *in vitro*. *Phytother Res* **3**, 1268–1283.
34. Kobayashi R, Kobayashi T, Sakai F, Hosoya T, Yamamoto M, Kurita-Ochiai T (2017) Oral administration of *Lactobacillus gasseri* SBT2055 is effective in preventing *Porphyromonas gingivalis*-accelerated periodontal disease. *Sci Rep* **7**, 545.
35. Liu M, Ding J, Zhang H, Shen J, Hao Y, Zhang X (2020) *Lactobacillus casei* lh23 modulates the immune response and ameliorates dss-induced colitis via suppressing jnk/p-38 signal pathways and enhancing histone h3k9 acetylation. *Food Funct* **11**, 5473–5485.
36. Dasgupta D, Roy N, Datta S, Chowdhury A, Bhattacharya N, Banerjee S (2015) Differential binding ability of nfκβ to the functional snp in the promoter of tnf-α (238g/a) increases the risk of alcoholic liver diseases. *J Clin Exp Hepatol* **5**, S16.
37. Lee HJ, Shin JS, Kyoung-Goo L, Sang C, Park Y (2017) Ethanol extract of *Potentilla supina* Linne suppresses lps-induced inflammatory responses through nf-κb and ap-1 inactivation in macrophages and in endotoxic mice. *Phytother Res* **31**, 475–487.