WITHDRAWN:
LP-cs Ameliorated CCl 4 -Induced Acute Liver Injury by Suppression of Cellular Stress

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Abstract

Background: Acute liver injury (ALI) involves excessive oxidative stress(OS) and inflammatory responses, leading to a high mortality rate due to lack of effective therapy. Carbon tetrachloride (CCl_4) is widely used to induce ALI by induction of reactive oxygen species. Probiotics, including *Lactobacillus plantarum* ST-III, have been shown to produce antibacterial and antioxidant substances such as organic acids or bacteriocins that reduce liver damage. Nevertheless, the effect of *Lactobacillus plantarum* ST-III culture supernatant (L-P-cs) on CCl_4-induced liver injury remains unclear.

Methods: Mice were pretreated with L-P-cs or medium for 14days before one dose of 0.2% CCl_4 at 10ml/kg body weight delivered by intraperitoneal injection. CCl_4-induced liver injury was examined by measuring serum levels of liver transaminases and high mobility-group box 1 protein (HMGB1) and liver histological staining. Inflammation and apoptosis in liver were evaluated by real-time PCR, enzyme-linked immunosorbent assay (ELISA), and TUNEL staining. Apoptosis in NCTC 1469 cells was detected using CCK8 and western blotting (WB). In liver, OS and endoplasmic reticulum stress(ERS)-related proteins were measured using kits and WB.

Results: L-P-cs significantly ameliorated CCl_4-induced liver injury and reduced CCl_4- induced inflammatory response and apoptosis, consistent with NCTC 1469 cells' results. L-P-cs also restored CCl_4-induced increases in cell OS and ERS to normalize liver function. Specifically, L-P-cs pretreatment decreased CCl_4-induced increases in nuclear factor (erythroid-2 related) factor 2, HO-1, superoxide dismutase, glucose regulatory protein, and activating transcription factor 6.

Conclusion: L-P-cs synergistically improves liver lobule necrosis, hepatocyte inflammation, and apoptosis by reducing liver OS and ERS.

Introduction

Hepatic injury endangers human health and is increasing yearly. Acute liver injury (ALI) is characterized by abrupt hepatic dysfunction and inflammatory responses[1–3]. ALI may be caused by many factors, including viral infections[4], improper use of medications[5, 6], excessive intake of ethanol[7], and others. Within 72 hours of the initial insult, abnormal liver function indicators begin to appear, accompanied by jaundice and other manifestations. Several studies showed that oxidative stress and inflammatory responses are elevated in liver injury[8, 9].

Endoplasmic reticulum stress (ERS) is an essential mechanism of drug-induced ALI[10, 11]. Carbon tetrachloride (CCl_4), also known as tetrachloromethane, is a toxic compound that causes liver cell necrosis. When ingested accidentally, CCl_4 injures livers and kidneys within a short time. Also, CCl_4 is often used as a model agent in ALI models often used to study liver toxicity mechanisms[12].

Probiotics carry several benefits, including antioxidative[13] and anti-tumor properties[14], the ability to regulate intestinal flora, and improve immunity[15]. For these reasons, probiotics are a research focus. For alcoholic liver disease, probiotics reduce liver injury through oxidative stress and lactic acid bacteria such as *Lactobacillus plantarum*. Probiotics retard the progression of non-alcoholic steatosis by reducing cholesterol levels[16]. *Lactobacillus plantarum* produces biologically active metabolites such as short-chain fatty acids, conjugated fatty acids, extracellular polysaccharides, and neuroactive metabolites such as gamma-aminobutyric acid; it also produces a variety of bacteriocins whose antimicrobial peptides may be used as food preservatives or antibiotic supplements[17]. Therefore, probiotic metabolites' direct use is expected to constitute a better strategy for liver injury treatment.

Lactobacillus plantarum ST-III was first discovered in kimchi. Studies have shown that this probiotic lowers serum cholesterol and triglyceride levels[18, 19]. *Lactobacillus plantarum* ST-III promotes intracellular Ca^{2+} assimilation at the cellular level to reduce ERS caused by liver injury[20–22]. Therefore, in the present study, we investigated the effects of *Lactobacillus plantarum* ST-III culture supernatants (L-P-cs) in a mice model of CCl_4-induced liver injury.

Materials And Methods

Culture of L-P-ST-III

Lactobacillus plantarum ST-III was provided by the Biological Experimental Center of Wenzhou Medical University and was cultured in tomato medium at 37 ℃ according to the National Center for Medical Culture Collections' culture guidelines. The culture suspension was then centrifuged at 25°C at 5000 *g* for 10 minutes; the supernatant was removed and filtered through 0.22-μm membrane filters. L-P-cs from the culture was at 10^9 colony-forming units/mL bacterial cells.

Animal experiments

Eight-week-old male ICR mice were purchased from the Experimental Animal Center of Wenzhou Medical University, weighing 20–25 g. The mice were maintained at 25 ± 0.5 ˚C in 12-h light/dark cycle conditions. All animals were randomly divided into four groups (n = 10 in each group): control group, CCl_4 group, L-P-cs + CCl_4 group, and Medium + CCl_4 group. The first two groups drank normal drinking water, while the latter two groups were fed with a ratio of L-P-cs/medium and drinking water mixture for 14 days to ensure that one mouse consumed 1 ml supernatant per day. After 14 days, the control group was given corn oil (10 ml/kg, intraperitoneal injection). The other three groups were given an intraperitoneal injection of CCl_4 (0.2% CCl_4, 10 ml/kg, dissolved in corn oil). After 24 hours of the CCl_4 challenge, all mice were weighed and anesthetized with 5% chloral hydrate to collect blood samples through the orbital vein. Tissue samples were collected and weighed. Half were fixed in 4% paraformaldehyde, and the other half was frozen in liquid nitrogen and stored at -80°C (Fig. 6A).

Chemicals and reagents

Carbon tetrachloride (CCl_4, AR) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Corn oil (Sigma-C8267) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC) biochemical kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Tumor necrosis factor α (TNF-α, SEKM-0034), interleukin 6 (IL-6, SEKM-0007), and high-mobility-group protein B1 (HMGB1, SEKM-0145) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). Cell Counting Kit-8 (CCK8) was purchased from Dojindo (Japan).

Biochemical analysis

The blood samples were centrifuged (12000 r/min, 10 min, 4 ℃), and the serum was isolated. Serum levels of ALT, AST, and HMGB1 were measured using assay kits. Liver tissue was prepared to make 10% homogenates with cold 0.9% saline solution, and the homogenates were centrifuged at 3500 r/min for 10 min at 4 ℃. The supernatants were subjected to further measurement.

Histopathological examination

Liver tissue was fixed in 4% polyoxymethylene in phosphate-buffered saline for 24 h. Fixed liver tissues were dehydrated in alcohol and embedded in paraffin and cut into 5μm thickness sections using a microtome (RM2235, Leica). The sections were dewaxed and hydrated, then stained with hematoxylin and eosin (H&E) staining. The histopathological morphology of liver sections was observed using a Nikon ECLIPSE 80i light microscope (Nikon, Tokyo, Japan).

TUNEL

The liver tissue paraffin-embedded was sectioned at 5 μm. According to the operating instructions of the TUNEL Kit (Beyotime Biotechnology, C1088), the sections were prepared and observed using the Nikon confocal microscope. The positive cells were counted in at least five fields using Image J software.

Real-time PCR

Total liver RNA was extracted using the UNlQ-10 Column TRIzol Total RNA Isolation Kit (B511321, Sangon Biotech Shanghai Co., Ltd.). The extracted RNA was reverse transcribed into cDNA, according to the instructions of the PrimeScript™ RT reagent Kit (RR037A, Takara Biomedical Technology Beijing Co., Ltd). The concentration of DNA was measured and diluted to the same standard. Real-time PCR was performed using TB Green Fast qPCR Mix (Takara) on a Light Cycler 480II system (Roche, Mannheim, Germany), according to the manufacturer's instructions by 40 cycles of 95°C for 5 s and 60°C for 10 s. Sangon synthesizes the primers, and the specific sequences were as follows:

IL-6 forward 5'-ACAAGTCGGAGGCTTAATTACACAT-3';

IL-6 reverse 5'-AATCAGAATTGCCATTGCACAA-3';

TNF-α forward 5'-AGGGTCTGGGCCATAGAACT-3';

TNF-α reverse 5'-CCACCACGCTCTTCTGTCTAC-3';

GAPDH forward 5'-CCAGAACATCATCCCTGCAT-3';

GAPDH reverse 5'-GTTCAGCTCTGGGATGACCTT-3';

Cell culture

NCTC 1469 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's Modified Eagle's Medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), streptomycin (100 μg/ml) and penicillin sodium (100 U/ml). All cells were cultured under 37°C with 95 % air and 5% CO_2 in an incubator. The medium was replaced every three days, and the cells were harvested and diluted at a ratio of 1:2 every three days.

The cells in the logarithmic growth phase were cultured in 96-well plates (1 x 10^4 cells/well) for 24 h before treatment. The concentration of CCl_4 was determined to test the cytotoxicity in a pre-experiment. The gradient CCl_4 (5, 10, 10, 10, 20, and 40 mmol/L) was added to the cells. After 6 hours, cell viability was tested using CCK8 reagent at 450 nm. In the follow-up cell experiment, the cells were divided into four groups: control, CCl_4, L-P-cs + CCl_4, and culture medium + CCl_4. The cells were pre-protected with L-P-cs or medium for 24 hours before CCl_4 was given. After 6 hours, cells were collected for western blot analysis (Fig. 6B).

Western blot

The liver tissue was mechanically crushed in lysis buffer (RIPA:PMSF: NaF = 100:1:1) and centrifuged at 12000 r/min for 15 min at 4 ℃ to obtain protein supernatants. Total protein concentrations were measured using the BCA method. The prepared protein was resolved on SDS-PAGE and transferred to PVDF membranes. The primary antibodies were incubated overnight at 4 ℃. These included Cleaved-caspase3, Bax, Bcl-2, Nrf2, GRP78, ATF6 (1:1000, Abcam, USA), HO-1, SOD2, NQO1, PDI, GAPDH (1:800, Proteintech), and CHOP (1:1000, Cell Signaling Technology, USA). The secondary antibodies (Multi Sciences Biotech, Co., Ltd.) were incubated at room temperature for 2 hours. The protein signal was detected using ECL kits (Solarbio, PE0010), and the strips were processed and analyzed using Image Lab software.

Statistical analysis

The experimental data were analyzed using one-way ANOVA with GraphPad Prism 8. The results were expressed as the mean ± SEM. $P < 0.05$ was considered statistically significant.

Results

L-P-cs ameliorated CCl_4-induced organ injury in mice.

The thymus and spleen indexes decreased, and the liver index increased in the CCl_4 group. The ratio of LW/BW in the CCl_4 mice group was significantly higher than those of control mice, an effect that was markedly reduced by L-P-cs treatment (Fig. 1 A). The ratios of SW/BW and TW/BW in the CCl_4 mice group and Medium + CCl_4 group were significantly lower than those of the control group (Fig. 1 B & 1 C). L-P-cs pretreatment significantly increased the ratios. CCl_4 induced organ damage. The situation was reversed in the L-P-cs group, suggesting that L-P-cs protects against CCl_4-induced organ injury.

Transaminases are widely distributed in cells of the liver, heart, and other tissues. Changes in serum transaminase levels are often used to reflect the degree of liver injury[23] [24]. CCl_4 administration produced significant elevations of transaminases compared to the control group (Fig. 1 D & 1 E). L-P-cs administration attenuated transaminase elevations in mice treated with CCl_4, further suggesting that L-P-cs protects against CCl_4-induced liver injury. Changes in HMGB1 expression showed the same result (Fig. 1 F). H&E staining was used to determine the effect of L-P-cs on hepatocellular necrosis. Liver sections in the control group stained with H&E showed normal liver architecture. By contrast, liver tissue from the CCl_4 group showed centrilobular congestion, inflammatory cell infiltration, significant fatty changes (steatosis), increased mitotic activity, severe centrilobular necrosis, and blurred outlines of cells around the central vein (Fig. 1 G). This effect was significantly lower in the L-P-cs pretreatment group. Taken together, our results suggest that L-P-cs protected against CCl_4-induced liver and spleen injury.

L-P-cs ameliorated CCl_4-induced hepatic inflammation and apoptosis in mice

The early stage of ALI was accompanied by acute inflammation[25]. To determine whether L-P-cs ameliorated CCl_4-induced hepatocyte inflammation, two pro-inflammatory cytokines IL-6 and TNF-a, in the liver were analyzed using ELISA mRNA detection. Levels of IL-6 and TNF-α in the CCl_4 liver group were markedly higher than those of the control group; however, this change was smaller in the L-P-cs group. The same results were seen concerning mRNA detection (Fig. 2 A–2 D). Inflammatory indexes were upregulated in the CCl_4 group and significantly improved in the L-P-cs group. These findings suggest that L-P-cs prevents CCl_4-induced inflammation.

There are interactions between inflammatory signaling pathways and apoptosis. The occurrence of inflammation can lead to apoptosis[26]. Therefore, we studied the apoptotic pathway using our model. The number of TUNEL-positive cells in the CCl_4 and medium + CCl_4 groups was significantly greater than in the control group. The numbers of TUNEL-positive cells were significantly lower in the L-P-cs group (Fig. 2 E & 2 F). We also measured changes in apoptosis. In the CCl_4 group, Cleaved caspase-3 and pro-apoptotic protein Bax expression were greater, and anti-apoptotic protein Bcl-2 was lower (Fig. 2 I–2 L).

This change was reversed in the L-P-cs group. These findings suggest that L-P-cs reduces the apoptosis induced by CCl_4-induced ALI.

L-P-cs ameliorated CCl_4-induced NCTC 1469 cells apoptosis.

As shown in Figures 3 A and 3 B, L-P-cs and tomato culture medium groups at different concentrations retained the same cell activity as the control group in NCTC 1469 cells. The cell activity was significantly lower when the concentration of CCl_4 was 40 mmol/L (Fig. 3 C). We chose 40 mmol/L for the subsequent experiments. Cell activity was significantly restored at the L-P-cs concentrations of 5% and 10%, after L-P-cs pre-protection for 24 h and CCl_4 injury for 6 h (Fig.3 D). These findings suggest that L-P-cs had no toxicity cytotoxicity on NCTC 1469 cells and reversed cell injury caused by CCl_4. Cleaved-caspase 3, Bax, and Bcl-2 were observed using a western blot assay. In vitro levels of Cleaved-caspase 3 and Bax in the CCl_4 group were significantly greater than those of the control group. Protein expression levels of Bcl-2 were lower in the CCl_4 group. In contrast, treatment with L-P-cs significantly reversed these proteins' expression levels (Fig. 3 E –3 H).

Together, both in vivo and in vitro results suggested that L-P-cs reversed protein expression levels, and this tendency ameliorated CCl_4-induced liver apoptosis and contributed to its hepatoprotective activity.

L-P-cs ameliorated CCl_4-induced oxidative stress in mice

CCl_4-induced reactive oxygen species (ROS) participate in hepatic injury[27, 28]. Therefore, we measured oxidative stress-related proteins in mouse livers. CCl_4 administration resulted in a significant reduction in liver GSH-PX concentration and T-AOC and SOD activity compared with the control group. L-P-cs pretreatment group ameliorated the CCl_4-induced decrease by restoring the activity of SOD and normal concentrations of GSH-PX and T-AOC (Fig. 4 A–4 C). MDA is the end-product of lipid peroxidation in liver injury[29]. Liver MDA levels were significantly greater in the CCl_4 group than in the control group, whereas L-P-cs pretreatment abolished the CCl_4-induced increase in liver tissues' MDA contents (Fig. 4 D).

To elucidate the molecular mechanism of L-P-cs against CCl_4-induced oxidative stress, WB was used to measure the Nrf2/HO-1 signaling pathway expression. In comparison with the control group, CCl_4 exposure reduced Nrf2 and HO-1 in the liver. L-P-cs pretreatment resulted in a significant rise in the expression of Nrf2 and HO-1 in CCl_4-treated mice (Fig. 4 E–4 G). The levels of SOD2 and NQO1, when in the CCl_4 groups, also reduced significantly compared with the control group. L-P-cs pretreatment caused a significant increase in SOD2 and NQO1 expression in liver tissues induced by CCl_4 (Fig. 4 E & 4 G & 4 H). Strikingly, L-P-cs pretreatment significantly ameliorated CCl_4-induced oxidative stress in mice.

L-P-cs ameliorated CCl_4-induced ER stress in mice

CCl_4 is a chemical toxicant that causes ERS[30]. To measure L-P-cs pretreatment-mediated inhibition of CCl_4-induced ER stress in the liver, we measured expression levels of ER stress protein markers using a

western blot assay. Hepatic protein levels of glucose-regulated protein 78 (GRP78), ATF6, PDI, XBP1, and C/EBP-homologous protein (CHOP) were significantly greater in the CCl_4 group than in the control mice. These changes were reversed by L-P-cs pretreatment. The extent of these changes was significantly attenuated in mice receiving L-P-cs pretreatment (Fig. 5 A– 5 G). Taken together, these findings suggest that L-P-cs protects against CCl_4-induced ER stress.

Discussion

In the present study, we preliminarily explored the effect of L-P-cs on CCl_4-induced ALI in mice. We found that L-P-cs synergistically inhibited liver lobule necrosis, hepatocyte inflammation, and apoptosis by reducing liver oxidative stress and ERS.

Recent studies found that probiotics have a therapeutic effect on alcoholic liver disease, but their unstable nature and toxic side-effects have greatly limited their clinical application[31]. Studies confirmed that biologically-active metabolites produced by probiotics such as short-chain fatty acids, extracellular polysaccharides, and neuroactive metabolites have antioxidant, anti-inflammatory anti-tumor properties that benefit the host[32]. Probiotics also produce a variety of effective bacteriocins that may be used as food preservatives or antibiotic supplements[17]. These lines of evidence suggest that probiotic metabolites have the potential to serve as hepatoprotective microorganisms and may be less affected by environmental factors and more stable.

Lactobacillus plantarum ST-III is a probiotic with cholesterol-lowering activity[18]. Therefore, we hypothesized that *Lactobacillus plantarum* ST-III culture supernatant (L-P-cs) might have unexpected liver-protective effects. Our findings supported this hypothesis. Pretreatment with L-P-cs prevented alterations in serum levels of transaminases and HMGB1 in mice with acute CCl_4-induced liver injury. Histology showed that CCl_4 induced ALI in mice characterized by liver lobule congestion, inflammatory cell infiltration, and increased mitotic activity. These effects were significantly prevented by L-P-cs pretreatment. These are only a subset of some pathological results; therefore, we further explored the liver-protective mechanisms of L-P-cs.

After CCl_4 enters the body, it is metabolized to produce chloroform free radical (CCl3·), which causes lipid peroxidation in liver cells and damage to cell membranes and organelles under the influence of liver cytochrome P450. Recent research in free radical biology suggested an essential pathophysiological role of free radicals and oxidative stress in developing and progressing liver diseases[33]. Classical CCl_4-induced ALI in mice can be divided into two stages: the first stage is liver oxidative and lipid peroxidation damage. This damage leads to deficiency or depletion of endogenous antioxidant enzymes; therefore, liver oxidative injury can be assessed by measuring levels of the lipid peroxidation product MDA, superoxide dismutase (SOD), and GSH in liver tissue. MDA is an essential product of oxidative stress[34]. We found that L-P-cs significantly increased GSH-Px, T-AOC, and SOD in liver tissue, while MDA content decreased. These changes suggest that L-P-cs prevents liver injury by reducing oxidative stress.

Several studies confirmed that Nrf2 is highly expressed in the liver. When ROS or electrophiles attack cells, Nrf2 dissociates from Keap1 and translocates into the nucleus, first forming a heterogeneous relationship with the small Maf protein[35, 36]. The dimer, combined with the antioxidant response element, activates transcriptional activation of the antioxidant enzyme gene expression regulated by Nrf2. We found that CCl_4 stimulation reduced liver Nrf2 expression, and L-P-cs pretreatment significantly normalized these changes. We performed western blot analysis of the expression of HO-1, NQO1, and SOD2 downstream of Nrf2 and found that L-P-cs' antioxidant effect was achieved via the Nrf2 signaling pathway.

The second stage is CCl_4-induced inflammatory liver injury. Free radical metabolism not only directly damages liver tissue but also promotes inflammation. Therefore, CCl_4-induced liver inflammatory injury can be assessed by measuring the expression of the inflammatory factors TNF-α and IL-6 in serum and liver tissue. We found that the expression of TNF-α and IL-6 increased significantly in the injury group but decreased in the L-P-cs treatment group, suggesting that L-P-cs prevents CCl_4-induced liver injury by reducing inflammation.

ERS inhibits fatty acid oxidation in the liver[37]. We found that CCl_4-induced ALI also caused ERS and hepatocyte calcium overload. Intracellular calcium overload has been shown to induce cell death through ERS[38]. The mitochondria absorb the $Ca2^+$ released by the ER, and then the permeability transition pore is opened to release cytochrome c and activate the apoptotic caspase cascade[39]. At the cellular level, *Lactobacillus plantarum* ST-III promotes intracellular Ca^{2+} assimilation[40]. Therefore, we hypothesized that L-P-cs' protective effect on the liver would also be mediated by promoting intracellular Ca^{2+} assimilation to reduce ERS. This result was verified by our experiment involving the ATF6 signaling pathway.

Activated ATF6 enters the nucleus and binds to the promoter ER stress response element as a homologous or heterodimer with the universal transcription factor nuclear factor-Y. This binding induces the transcriptional expression of ERS genes such as GRP78, PDI, and CHOP[41] to protect cells from damage caused by ER and restore cell function[42].

GRP78 is an endoplasmic reticulum chaperone protein. GRP78 is an essential protein to main cellular homeostasis via ca-ATPase. Since CCl_4 can directly act on mitochondria, the GRP78 increase might result from stress response that occurred in the early stage[43]. Abnormal expression of GRP78 in the endoplasmic reticulum leads to misfolding. We found that CCl_4 treatment elevated levels of ERS markers ATF6, GRP78, PDI, and CHOP; after pretreatment with L-P-cs, these protein levels tended to normalize. These findings suggest L-P-cs resists CCl_4-induced ALI by regulating ERS in liver cells.

Ca^{2+} released by ER activates the caspase cascade of apoptosis that induces hepatocyte death. Early studies showed that oxidative stress is involved in apoptosis and can be triggered by promoting ROS production and reducing antioxidant function[44]. In this context, we explored the effect of CCl_4-induced

ALI on apoptosis. The pro-apoptotic protein Bax is overexpressed in CCl_4-induced ALI, and expression of its related anti-apoptotic protein Bcl-2 is suppressed, suggesting that CCl_4 exposure causes severe cell death. Importantly, through L-P-cs preprocessing, this situation is significantly improved. The ability of L-P-cs to reduce apoptosis was verified again in terms of Cleaved-caspase3. This finding also suggests that L-P-cs inhibits ERS through intracellular Ca^{2+} assimilation, thereby reducing cell apoptosis.

Caspases comprise a large family. We only explored Cleaved-caspase 3 under L-P-cs pretreatment, and this is insufficient. Further studies will determine whether L-P-cs pretreatment reduces apoptosis by affecting other factors levels in liver cells. Apoptosis is programmed cell death characterized by specific morphological features, including cell shrinkage, nuclear fragmentation, and chromatin condensation[43]. Indeed, this study confirmed that Cleaved-caspase 3 and Bax levels were increased in CCl_4-induced mice in vivo, as well as in NCTC1469 cells in vitro. Levels of Bcl-2 expression were lower in CCl_4-treated mice in vivo and NCTC1469 cells in vitro. Together, in vivo and in vitro results suggested that L-P-cs reversed these apoptotic indicators; this tendency ameliorated CCl_4-induced liver apoptosis and contributed to its hepatoprotective activity.

In summary, our findings suggest that L-P-cs protect against CCl_4-induced ALI. This finding was demonstrated by suppressing oxidative stress and ERS to reduce hepatocyte inflammation and apoptosis. It is generally believed that the intake of large amounts of probiotics entails many unstable factors that are reflected in the instability of its biological characteristics and their possible unpredictable side effects[31, 45, 46]. Although our research is currently limited to an overview of L-P-cs through the cell signal transduction system to treat CCl_4-induced ALI, it provides novel insights regarding L-P-derived products that reduce the development risk of CCl_4-induced ALI. Future studies focusing on probiotic supernatants may help broaden the scope of clinical use of probiotics (Fig. 7)

Declarations

Conflicts of interest

The authors declare no conflict of interest.

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Data availability statement

All data, models, and code generated or used during the study appear in this article.

Ethics approval and consent to participate- The animal study was reviewed and approved by the Laboratory Animal Ethics Committee of Wenzhou Medical University & the Laboratory Animal Centre of Wenzhou Medical University.

Consent for publication- Not applicable

Availability of data and material-All data, models, and code generated or used during the study appear in this article.

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Figures

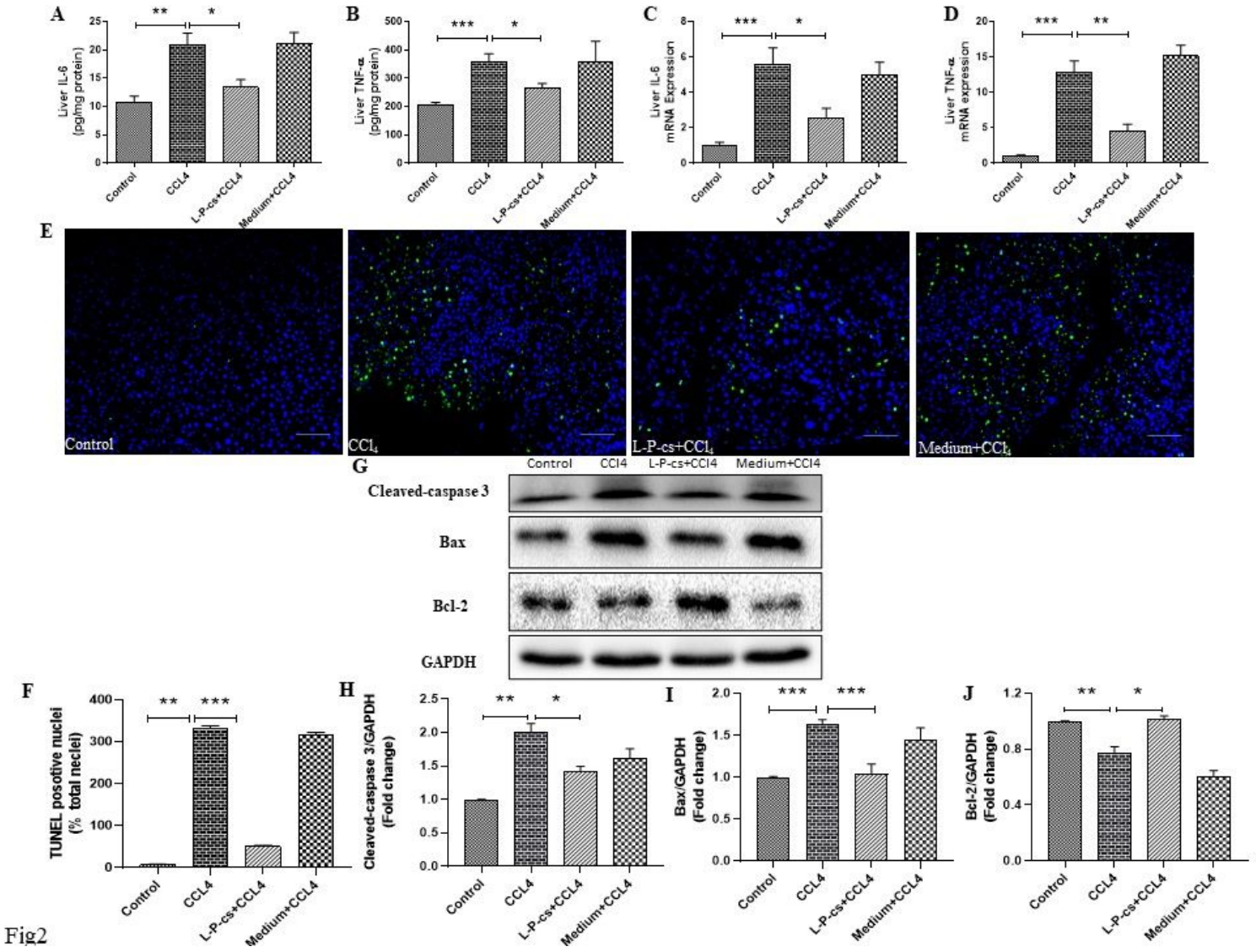

Fig2

Figure 1

L-P-cs ameliorated CCl4-induced organs injury in mice. (A) Liver index (liver weight/body weight ratios). (B) Spleen index (spleen weight/body weight ratios). (C) Thymus index (thymus weight/body weight ratios). (D) Plasma alanine aminotransaminase (ALT) levels. (E) Plasma aspartate aminotransferase (AST) levels. (F) Plasma high-mobility-group protein B1 (HMGB1) levels. (G) Hematoxylin and eosin (H&E) staining of liver tissue from control, CCl4, L-P-cs + CCl4, Medium + CCl4 mice (100 x, scale bars = 100μm). All data are expressed as means ± SEM, n = 8. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the control group or CCl4 model group.

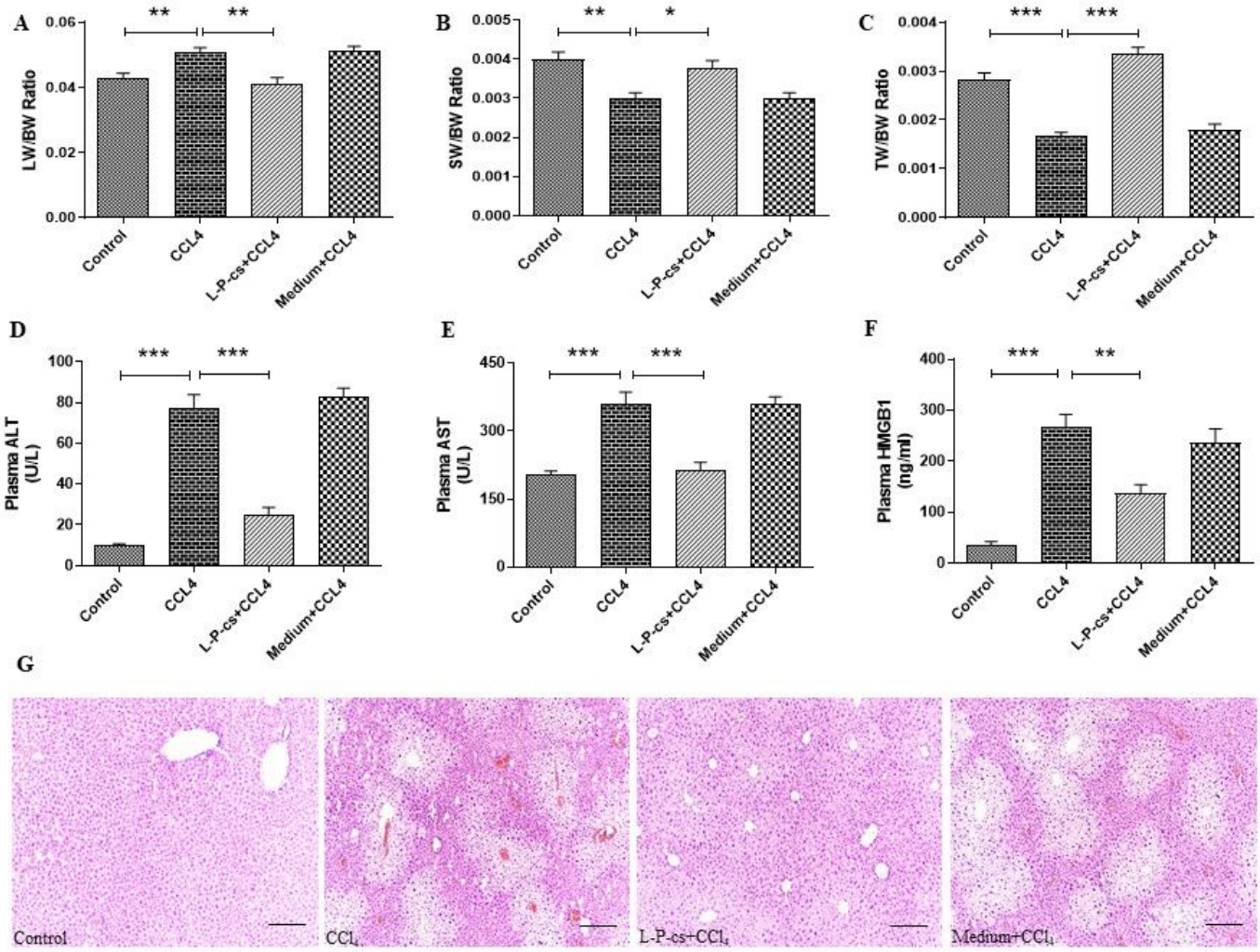

Fig1

Figure 2

L-P-cs ameliorated CCl4-induced hepatic inflammation and apoptosis in mice. (A, B) The expression of interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) in liver tissue by enzyme-linked immunosorbent assay (ELISA). (C, D) The mRNA expression levels of IL-6 and TNF-α in liver tissues. (E) TUNEL fluorescent images (100 x, scale bars = 100 μm). (F) Statistical analysis of TUNEL fluorescent images. (G) Western blot analysis of levels of Cleaved-caspase 3, Bax, and Bcl-2 in liver tissue. (H–J) Intensities of Cleaved-caspase 3, Bax, and Bcl-2 normalized to GAPDH. All data are expressed as means ± SEM, n = 8. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the control group or CCl4 model group.

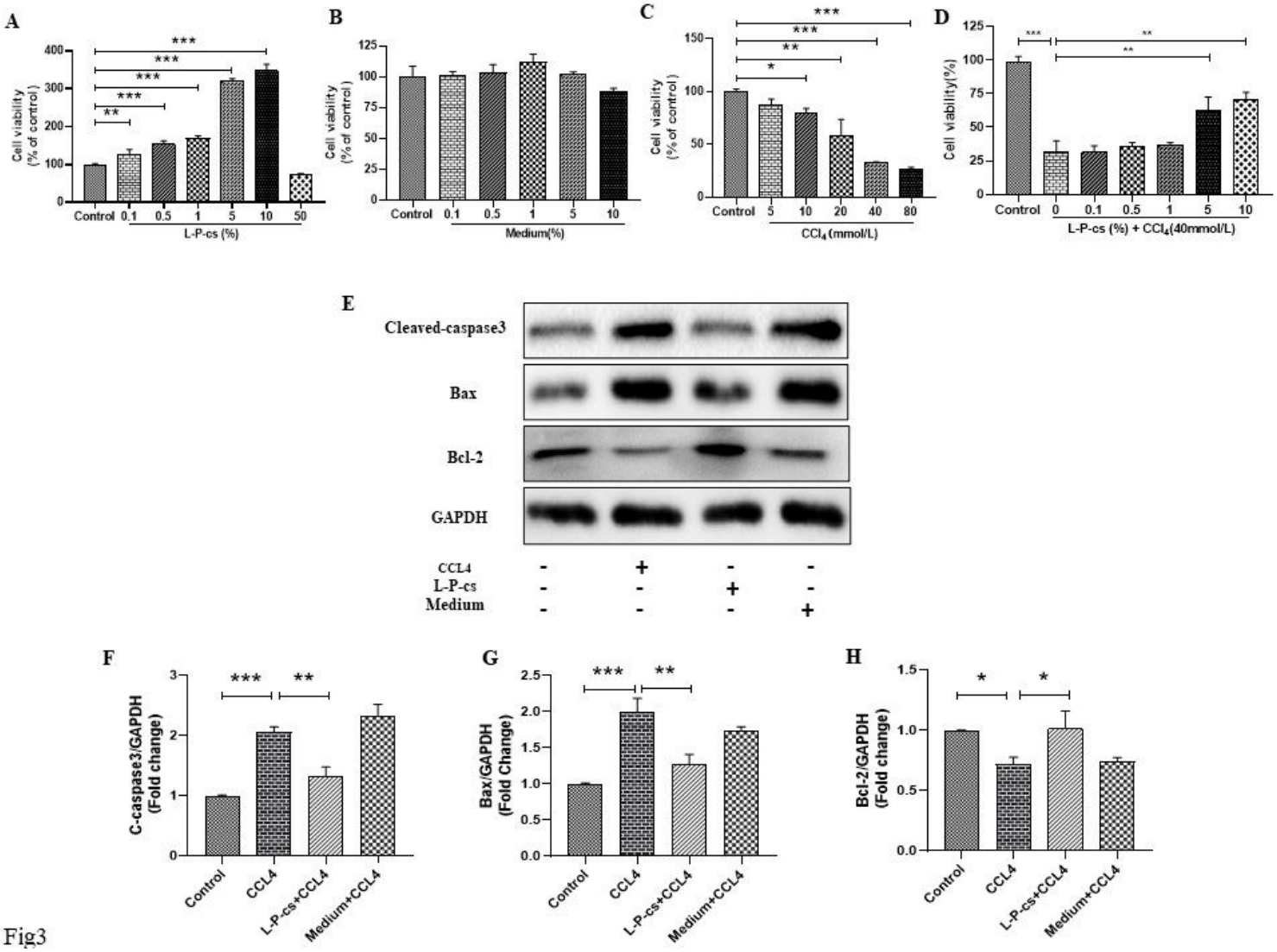

Fig3

Figure 3

L-P-cs ameliorated CCl4-induced apoptosis in NCTC 1469 cells. (A) Cell viability of NCTC 1469 cells under different concentrations of L-P-cs. (B) Cell viability of NCTC 1469 cells under different concentrations of the medium. (C) Cell viability of NCTC 1469 cells under different concentrations of CCl4. (D) Cell viability of different groups after pre-protection. (E) Western blot analysis of levels of Cleaved-caspase 3, Bax, and Bcl-2 in NCTC 1469 cells. (F–H) Intensities of Cleaved-caspase 3, Bax, and Bcl-2 normalized to GAPDH. All data are expressed as means ± SEM, n = 5. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the control group or CCl4 model group.

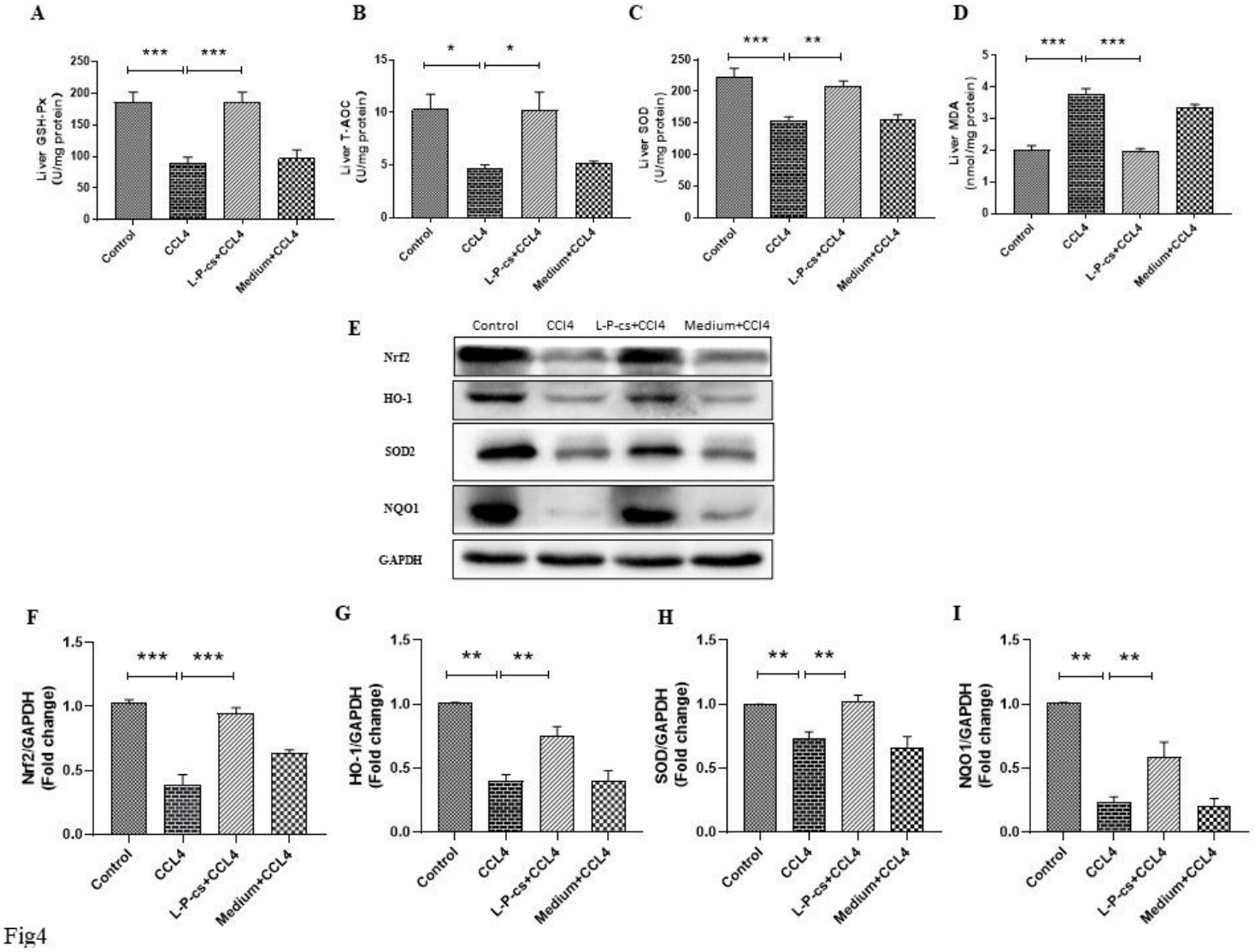

Fig4

Figure 4

L-P-cs ameliorated CCl4-induced oxidative stress in mice. (A) Liver glutathione peroxidase (GSH-PX) levels. (B) Liver total antioxidant capacity (T-AOC) levels. (C) Liver superoxide dismutase (SOD) levels. (D) Liver malondialdehyde (MDA) levels. (E) Western blot analysis of levels of Nrf2, HO-1, SOD2, and NQO1 in liver tissue. (F–I) Intensities of Nrf2, HO-1, SOD2, and NQO1 normalized to GAPDH. All data are expressed as means ± SEM, n = 8. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the control group or CCl4 model group.

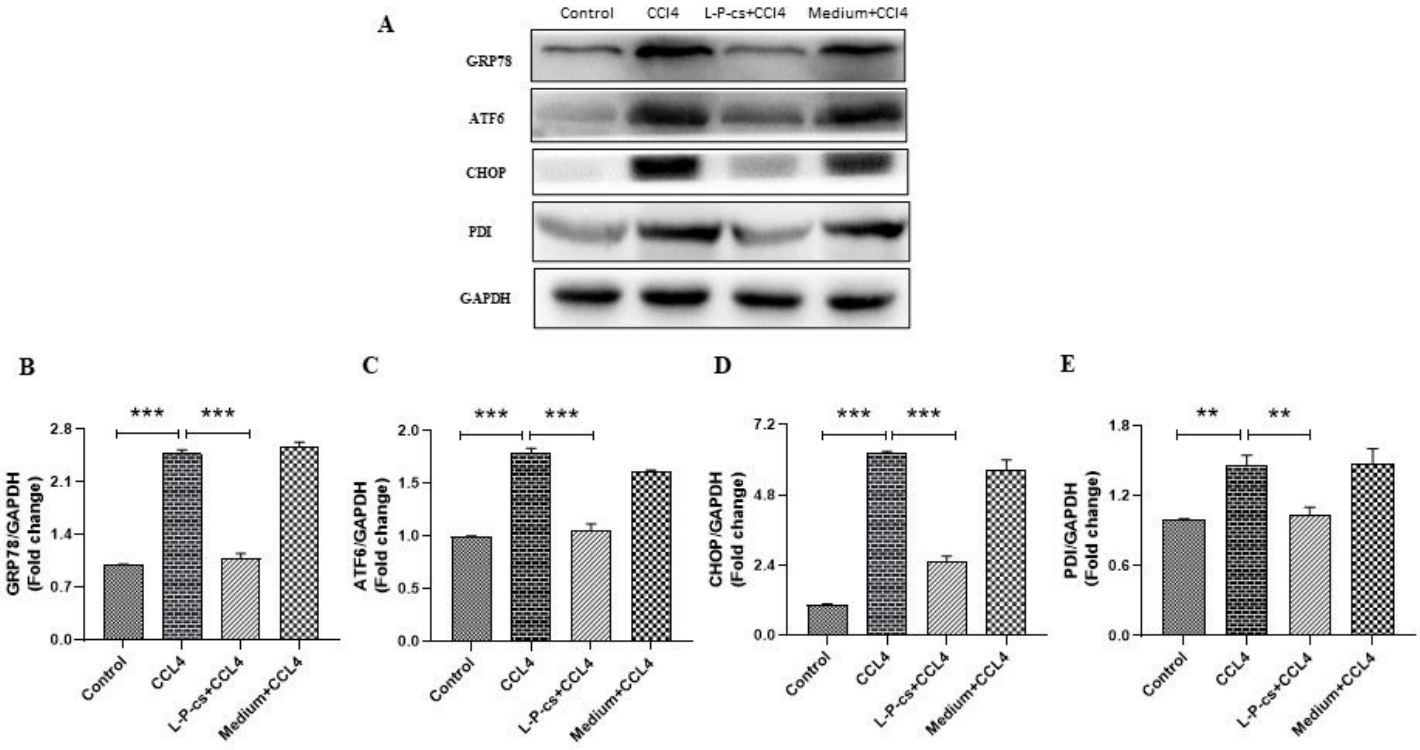

Fig5

Figure 5

L-P-cs ameliorated CCl4-induced ER stress in mice. (A) Western blot analysis of levels of GRP78, ATF6, CHOP, and PDI in liver tissue. (B–E) Intensities of GRP78, ATF6, CHOP, and PDI normalized to GAPDH. All data are expressed as means ± SEM, n = 8. **P < 0.01, and ***P < 0.001 vs. the control group or CCl4 model group.

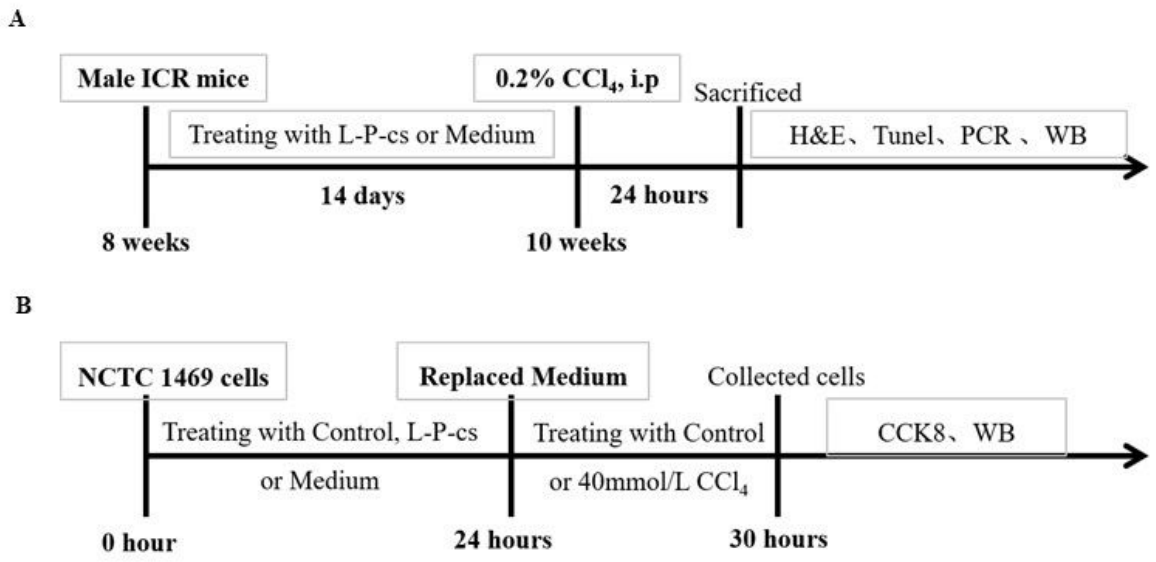

Fig6

Figure 6

Experimental process of the study.

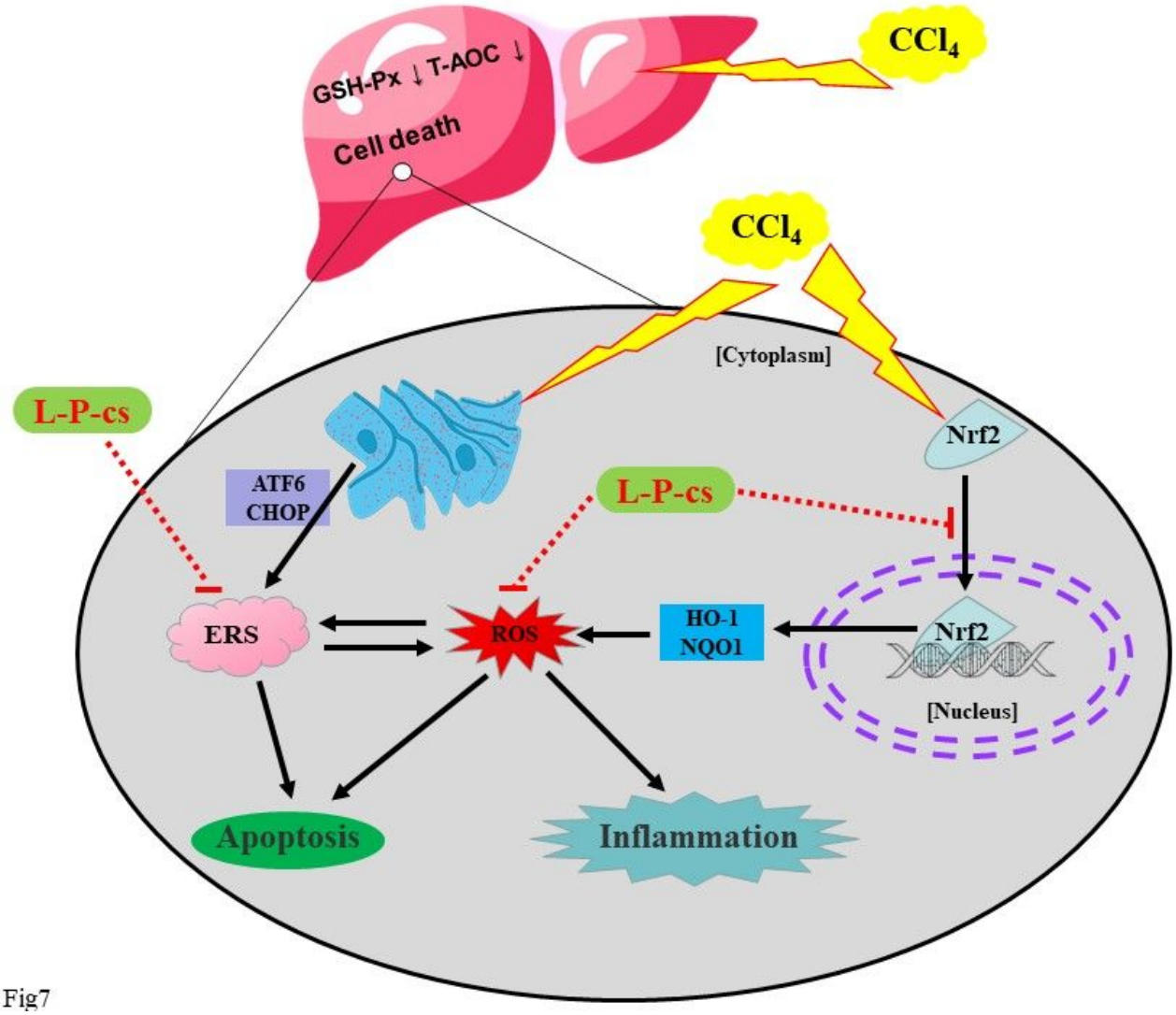

Fig7

Figure 7

A schematic showing L-P-cs ameliorates CCl4-induced acute liver injury (ALI).