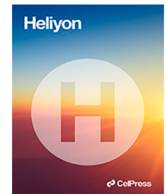
Heliyon 10 (2024) e31195

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Penthorum chinense Pursh extract ameliorates alcohol-related fatty liver disease in mice via the SIRT1/AMPK signaling axis

Hui Zhuge [a,d,1], Yan Pan [a,d,1], Shanglei Lai [a,d], Kaixin Chang [a,d], Qinchao Ding [b,c,d], Wenjing Cao [b], Qing Song [b], Songtao Li [b], Xiaobing Dou [a,d,**], Bin Ding [a,d,*]

[a] *College of Life Science, Zhejiang Chinese Medical University, Hangzhou, 310053, China*
[b] *College of Public Health, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China*
[c] *College of Animal Science, Zhejiang University, Hangzhou, 310058, China*
[d] *Molecular Medicine Institute, Zhejiang Chinese Medical University, Hangzhou, 310053, China*

A R T I C L E I N F O

Keywords:
Penthorum chinense Pursh
Alcohol-related fatty liver disease
SIRT1
AMPK
Lipid metabolism
Graphical abstract

A B S T R A C T

Penthorum chinense Pursh (*P. chinense*), a functional food, has been applied to protect the liver against alcohol-related fatty liver disease (ALD) for a long history in China. This study was designed to evaluate the ameliorative activity of the polyphenolic fraction in *P. chinense* (PGF) depending on the relief of ALD. The ALD mouse model was established by exposing the mice to a Lieber-DeCarli alcohol liquid diet. We found that PGF administration significantly ameliorated alcohol-induced liver injury, steatosis, oxidative stress, and inflammation in mice. Furthermore, alcohol-increased levels of the critical hepatic lipid synthesis proteins sterol regulatory element binding transcription factor (SREBP-1) and diacylglycerol *o*-acyltransferase 2 (DGAT2) were attenuated by PGF. Similarly, PGF inhibited the expression of the lipid transport protein very low-density lipoprotein receptor (VLDLR). Interestingly, PGF restored alcohol-inhibited expression of carnitine palmitoyltransferase 1 (CPT1) and peroxisome proliferator-activated receptor alpha (PPARα), essential fatty acid β-oxidation proteins. Mechanistic studies revealed that PGF protects against alcohol-induced hepatocyte injury and lipid deposition via the SIRT1/AMPK signaling pathway. In sum, this research clearly demonstrated the protective effects of PGF against ALD, which was mediated by activating SIRT1/AMPK pathways in hepatocytes. We provide a new theoretical basis for using *P. chinense* as a functional food in ALD.

1. Introduction

Excessive alcohol consumption is recognized as a major social and health problem worldwide [1], causing damage to various tissues and organs, especially the liver. Research studies have shown that alcohol-related liver disease (ALD) has become the leading cause of death from alcohol-related diseases [2]. This disease encompasses a wide range of clinical features, including fatty liver, advanced hepatitis, and fibrosis, which may progress to cirrhosis and even hepatocellular carcinoma [3–5]. In the clinic, abstinence of alcohol combined with some liver health beneficial therapy is an effective treatment to improve ALD. However, some of the drugs have

* Corresponding author.
** Corresponding author.
E-mail addresses: xbdou77@163.com (X. Dou), db@zcmu.edu.cn (B. Ding).
[1] These authors contribute equally to this paper.

https://doi.org/10.1016/j.heliyon.2024.e31195
Received 8 December 2023; Received in revised form 10 May 2024; Accepted 13 May 2024
Available online 16 May 2024
2405-8440/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

adverse effects. To our knowledge, corticosteroids, applied for severe alcoholic hepatitis, lead to immune suppression and increase the risk of unwished infections. N-acetylcysteine and ursodeoxycholic acid may induce gastrointestinal discomfort and allergic reactions [6]. Therefore, it is critical to discover novel medications for the prevention and treatment of ALD.

Penthorum chinense Pursh (*P. chinense*), widely distributed in East Asia, is the typical Chinese medicinal food homology species [7]. The Miao people commonly use it as a preventive and therapeutic material for liver disease [8]. Previous studies have found that the aqueous extract of *P. chinense* has a protective effect against alcohol-induced liver injury in mice [9,10]. Instead, another study showed that the ethanol extract of *P. chinense* had significantly superior protective activity than that of the aqueous extract [7]. Interestingly, we found that the ethanol extract of *P. chinense* possessed antimicrobial activity that was not present in the aqueous extract [11]. Furthermore, we prepared the ethanolic extract of *P. chinense* with ethyl acetate extract, followed by HDP 500 column chromatography purification, to obtain 80 % ethanol elution fraction, in which the pinocembrin-7-O residues (PGF) were enriched with remarkable antibacterial, antioxidant, and anti-lipid accumulation activities [12]. However, the beneficial efficiency of PGF on ALD is still unclear. Therefore, the current study was undertaken to evaluate the protective activity of PGF on alcohol-induced hepatocyte steatosis and explore the potential mechanisms.

In the present study, we investigated and provided strong evidence of the protective ability of PGF against hepatic steatosis in chronically alcohol-fed mice. We also found that the SIRT1/AMPK signaling pathway is the crucially regulated pathway of PGF. This study provided a novel insight of the mechanisms, which contributed to the application of *P. chinense*.

2. Experimental section

2.1. Preparation of ethanol fraction from P. chinense

The preparation of the PGF fraction of *P. chinense* stem and identification the major components were carried according to the protocol of the previous publication [12]. In brief, the 70 % ethanolic extract of *P. chinense* stem, which was reflux extracted for 1.5 h with 1:10 solid liquid ratio, was successively extracted with petroleum ether and ethyl acetate. This ethyl acetate extract was further loaded into HDP 500 chromatographic column. The polar ingredients flowed through the column with 30 % ethanol. The PGF fraction was eluted with 80 % ethanol. The phytochemical ingredients in this fraction were comparatively identified by HPLC analysis with previously published methods, and with the previously collected and identified three major ingredients as standards [11].

2.2. Animal treatments

The animal study was reviewed and approved by the Animal Ethical and Welfare Committee of Zhejiang Chinese Medical University (ZSLL-2017-150). Twenty-four C57BL/6 male mice (8-week-old) were randomly divided into four groups: pair-fed control group (PF), alcohol-fed group (AF), alcohol-fed/low dose PGF group (LDF), and alcohol-fed/high dose PGF group (HDF), and housed in a temperature-controlled room (24 °C) with a 12 h light-dark cycle under specific-pathogen-free condition. Lieber-DeCarli ALD model was established as described previously [13]. In brief, the mice in the AF, LDF, and HDF groups were fed the Lieber-DeCarli alcohol diet (Trophic Animal Feed High-Tech Co., NanTong, China). In the first week, the alcohol content gradually increased from 0 % to 11 % of total calories. In the subsequent 2–4 weeks, the alcohol increased to accounting for 22 %, 27 %, and 32 % of the total calories. In comparison, the mice in the PF group were given the same calories as the Lieber-DeCarli control diet. From the second week, mice in the LDF and HDF groups were intragastricly administrated 80 mg/kg and 160 mg/kg PGF in every other days, and mice in the PF and AF groups simultaneously received the same volume of saline. At the end of the treatment, the mice were weighed and sacrificed with pentobarbital solution (40 mg/kg body weight) after 4 h of fasting. The blood samples were collected from the inferior vena cava and centrifuged at 3000 rpm for 10 min. The supernatant plasma was stored at −20 °C. The livers were collected and fixed in 4 % formaldehyde or frozen in −80 °C refrigerator.

2.3. Biochemical analyses

The concentrations of alanine transaminase (ALT) and aspartate transaminase (AST) in plasma were determined by the respective determination kits (Nanjing Jiancheng Bio Co., Nanjing, China). The levels of TG in the liver tissues were determined with the specific kit (Applygen, Beijing, China). Additionally, we used the commercial kits to detect the enzyme activities of MDA, CAT, and T-SOD, respecitvely. (Nanjing Jiancheng Bio Co., Nanjing, China).

2.4. Histological analysis

The formaldehyde-fixed liver was embedded in paraffin wax and cut into 4 μm serial sections, and stained with hematoxylin and eosin (H&E) solutions for microscopic observation. The collected hepatic tissues were Tissue-Tek OCT embedded and cryosectioned into 8 μm, which were subjected to Oil Red O staining.

2.5. RNA extraction and real-time quantitative PCR

Total RNA in the liver was isolated with a Trizol reagent (Invitrogen, NY, USA). Nanodrop (Thermo Fisher Scientific, MA, USA) was applied to determine the quality and quantity of the isolated RNA. The real-time PCR was performed with the iQ-SYBR Green PCR

Supermix (Bio-rad Laboratories Ltd., CA, USA) on a StepOnePlus real-time PCR system (Applied Biosystems, CA, USA). The primer sequences were listed in Table 1. The transcriptional level of the gene was semi-quantified with the $2^{-(\triangle\triangle CT)}$ value, compared with that of the internal reference.

2.6. Western blot

Western blot is based on SDS polyacrylamide gel electrophoresis, which can separate proteins according to their molecular weights. The proteins were separated with 10 % SDS-PAGE and transferred onto a PVDF membrane, which would be hybridized with the specific antibody and detected with the secondary antibody, respectively. The following antibodies were used: anti-*p*-ACC (dilution of 1:1000; Cell Signaling Technology,#11818), anti-ACC (dilution of 1:1000; Cell Signaling Technology, #3662), anti-*p*-AMPK$^{Thr\ 172}$ (dilution of 1:1000; Cell Signaling Technology, #2535), anti-AMPK (dilution of 1:1000; Cell Signaling Technology, #2532), anti-SIRT1 (dilution of 1:500; Santa Cruz Biotechnology, sc-74465), anti-PPAR-α (dilution of 1:500; Santa Cruz Biotechnology, sc-398394), anti-DGAT2 (dilution of 1:500; Santa Cruz Biotechnology, sc-293211), anti-SREBP-1 (dilution of 1:500; Santa Cruz Biotechnology, sc-365513), CD36 (dilution of 1:500; Santa Cruz Biotechnology, sc-7309), anti-CPT1(dilution of 1:500; Proteintech, 15184-1-AP), anti-VLDLR (dilution of 1:500; Proteintech, 19493-1-AP) and anti-actin (dilution of 1:500; ABclonal, AC026). Anti-actin was used as the internal control. The engaged secondary antibodies were anti-rabbit (dilution of 1:5000; Boster Biological Tcchnology, BA1054) and anti-mouse (dilution of 1:5000; Boster Biological Tcchnology, BA1050).

2.7. AML-12 cell culture

AML-12, a nontransformed mouse hepatic cell line, was obtained from the American Type Culture Collection (ATCC, Manassas, VA). AML-12 cells were cultured in Dulbecco's Modified Eagle Medium/Ham's Nutrient Mixture F-12 (DMEM/F12, 1: 1, Hyclone, Logan, UT, USA) containing 10 % (v/v) fetal bovine serum, 5 mg/mL insulin (Solarbio, Beijing, China), 5 μg/mL transferrin (Solarbio, Beijing, China), 5 ng/mL selenium (Solarbio, Beijing, China), 40 ng/ml dexamethasone (Sigma-Aldrich, MO, USA), 100 U/mL penicillin (Solarbio, Beijing, China) and 100 μg/mL streptomycin (Solarbio, Beijing, China), 2 mmol/L glutamine (Sigma-Aldrich, MO, USA), in a humidified incubator at 37 °C with 5 % CO_2.

2.8. Cell viability assays

Cell viability was determined by measuring lactate dehydrogenase (LDH) release in the cultured and MTT test. For the LDH assay, the culture medium was collected and detected with an LDH assay kit (Thermo Scientific Inc, VA) according to the manufacturer's instructions. For the MTT test, the cultured cells with indicated treatment were incubated with fresh MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) at 37 °C for 4 h to allow incorporation and conversion of MTT to formazan derivative. After incubation at room temperature on the rocker, the absorbance values were measured using FLUOstar Omega (BMG Labtech, Offenburg, Germany).

2.9. Lipid deposition assay

For intracellular TG detection, cells were collected and lysed. The contents of TG and protein in the lysate were determined by the TG assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and BCA kit (Beyotime, Shanghai, China), respectively, according to the manufacturer's instructions. The TG level was calculated and expressed as the relative TG content in per gram protein.

2.10. Statistical analyses

In this study, more than three repeats were conducted. All data were presented by mean value ± standard deviation. The data were statistically analyzed using one-way analysis of variance (ANOVA) with IBM-SPSS statistics software version 22.0 (IBM-SPSS Inc, Armonk, NY). When $p < 0.05$, the differences were considered statistically significant.

Table 1
Primer sequence for qRT-PCR.

Target genes	Forward primer	Reverse primer
18s	F:GAATGGGGTTCAACGGGTTA	R:AGGTCTGTGATGCCCTTAGA
Adh1	F:CCATCGAGGACATAGAAGTCGC	R:TGGTTTCACACAAGTCACCCC
Aldh2	F:TTCCCACCGTCAACCCTTC	R:CCAATCGGTACAACAGCCG
Tnf-α	F:GTCCCCAAAGGGATGAGAAG	R:TTTTGCTACGACGTGGGCTAC
Il-1β	F:GAAATGCCACCTTTTGACAGTG	R:TGGATGCTCTCATCAGGACAG
Il-6	F:AGTTGCCTTCTTGGGAVTGA	R:TCCACGATTTCCCAGAGAAC

3. Results

3.1. PGF attenuated chronic alcohol consumption-induced liver injury

In this study, chronic alcoholic liver disease murine model was prepared by liber liquid feed, and the model pattern diagram and intervention were shown in Fig. 1A. To assess the role of PGF in chronic alcohol consumption-induced liver injury in mice, the plasma ALT and AST, which were considered biochemical markers of liver injury, were assayed. And LDF and HDF significantly reversed the alcohol exposure induced high content of ALT and AST in plasma (Fig. 1B&C). To further evaluate the protective effect of PGF against alcohol-induced liver injury, we comparatively observed the pathophysiological changes of the H&E stained murine livers in different groups (Fig. 1D). The AF group exhibited significant hepatocyte vacuolization compared to the PF group, which was significantly ameliorated by PGF intervention (LDF and HDF group).

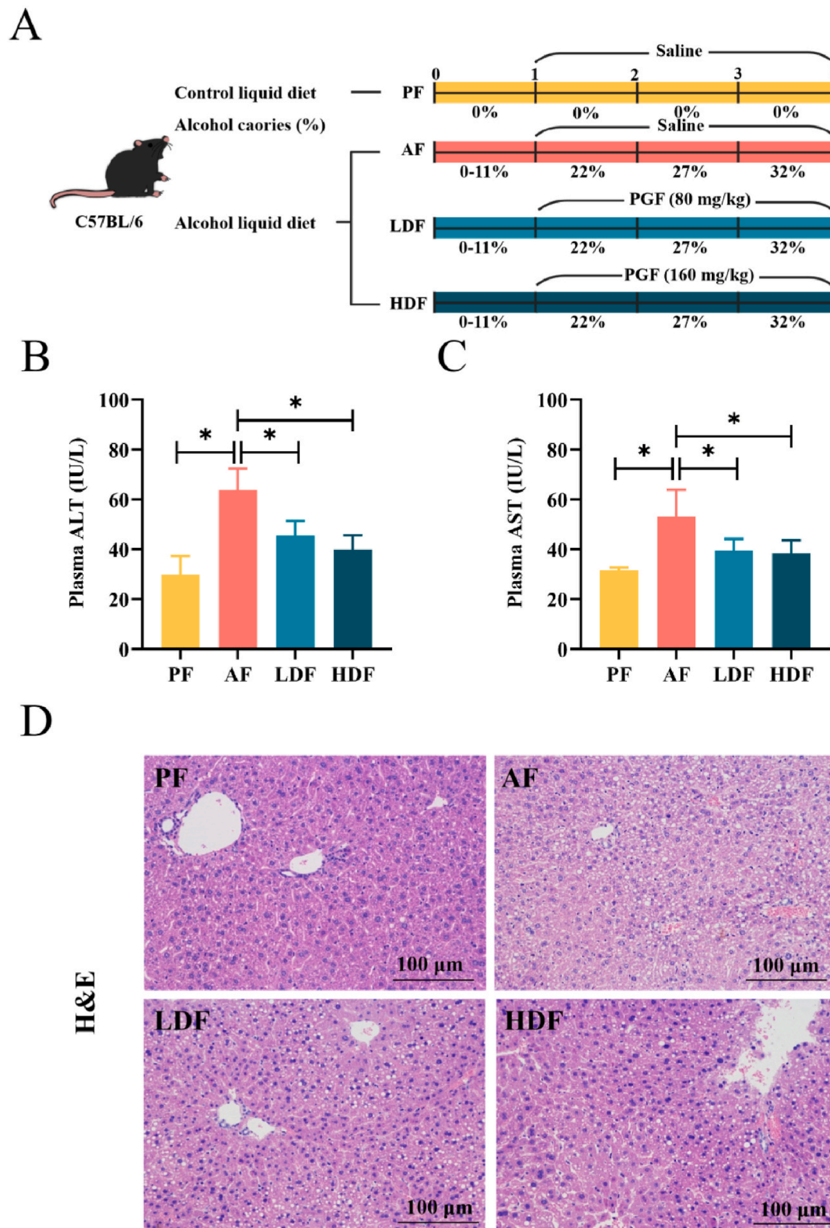


Fig. 1. PGF attenuated chronic alcohol consumption-induced liver injury. (A) Liber liquid diet model and intervention pattern diagram; (B) plasma ALT; (C) plasma AST; (D) Representative H&E staining of the liver. The scale bar length represents 100 µm *$P < 0.05$ vs. corresponding control.

3.2. PGF improved chronic alcohol consumption-induced hepatic oxidative stress and inflammation

It is well known that oxidative stress is an essential pathological mechanism of alcohol-induced liver dysfunction [13]. Therefore, we evaluated the level of oxidative stress in hepatocytes by detecting the lipid peroxidation product malondialdehyde (MDA), the antioxidant-related enzyme systems superoxide dismutase (SOD) and catalase (CAT). Alcohol exposure significantly increased the MDA level and decreased the enzyme activities of SOD and CAT in hepatocytes. Conversely, PGF ameliorated the aforementioned liver oxidative stress parameters (Fig. 2A–C). Meanwhile, the increased expression of inflammatory factors (*Tnf-α, Il-1β,* and *Il-6*) in the liver due to alcohol consumption was also restored by PGF intervention (Fig. 2D–F). These results suggested that PGF has excellent anti-oxidant and anti-inflammatory capacity. Further, we also comparatively verified the transcription of ethanol dehydrogenase (*Adh1*) and acetaldehyde dehydrogenase (*Aldh2*), the major enzyme response for ethanol catabolism in mice. The negative result was showed in (Supplementary figure 1).

3.3. PGF ameliorated chronic alcohol consumption-induced hepatic steatosis

The intervention of PGF significantly attenuated alcohol consumption resulting in the increase of murine liver weight, and the liver-body ratio (Fig. 3A&B). Considering alcohol aggravated lipid accumulation in the liver, we further observed the lipid deposition in the liver with oil-red O staining. The results showed that the density of red-stained lipid droplets in the AF group was significantly increased, and some even fused into clumps. On the contrary, the lipid droplets in the LDF and HDF groups were significantly reduced compared to the AF group (Fig. 3C). Importantly, PGF attenuated alcohol-induced accumulation of TG and FFA in the liver (Fig. 3D&E). These data implied that PGF significantly improved hepatic steatosis.

3.4. PGF regulated lipid metabolism associated proteins in the liver

To further explore the underlying mechanism(s) by which PGF promoted alcohol-induced lipid accumulation in the murine liver, we examined the expression of the proteins, involved in lipid synthesis, transport, and catabolism by Western blot. The results showed that *m*-SREBP-1, regulating fatty acid synthesis, has been significantly elevated under alcohol-mediated conditions, whereas it could be statistically reduced by the intervention of PGF. While at the same time, DGAT2, an essential protein in lipid synthesis [14], was

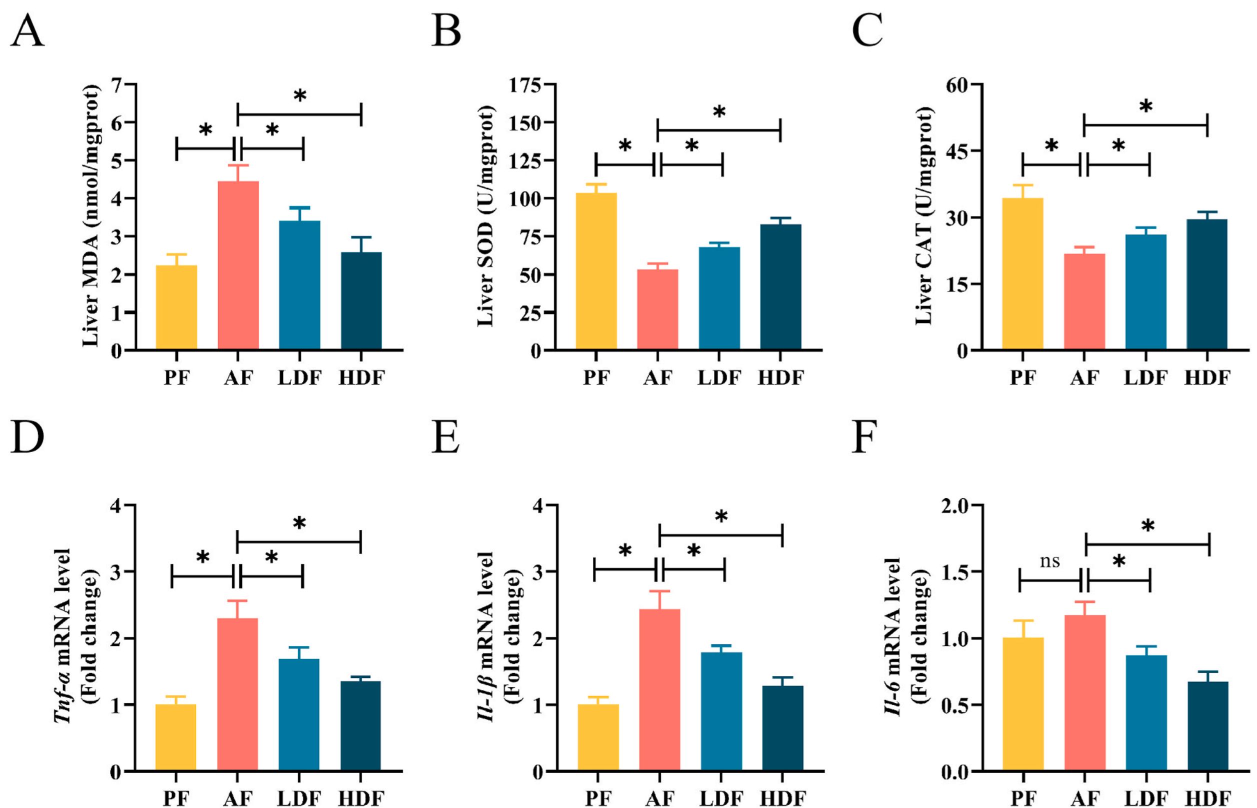


Fig. 2. PGF improved chronic alcohol consumption-induced hepatic oxidative stress and inflammation. (A) MDA content in the liver; (B) T-SOD activity in the liver; (C) CAT activity in the liver; (D–F) qRT-PCR results of the expressions of *Tnf-α*, *Il-1β*, and *Il-6* in the murine liver of each group. *$P < 0.05$ vs. corresponding control.

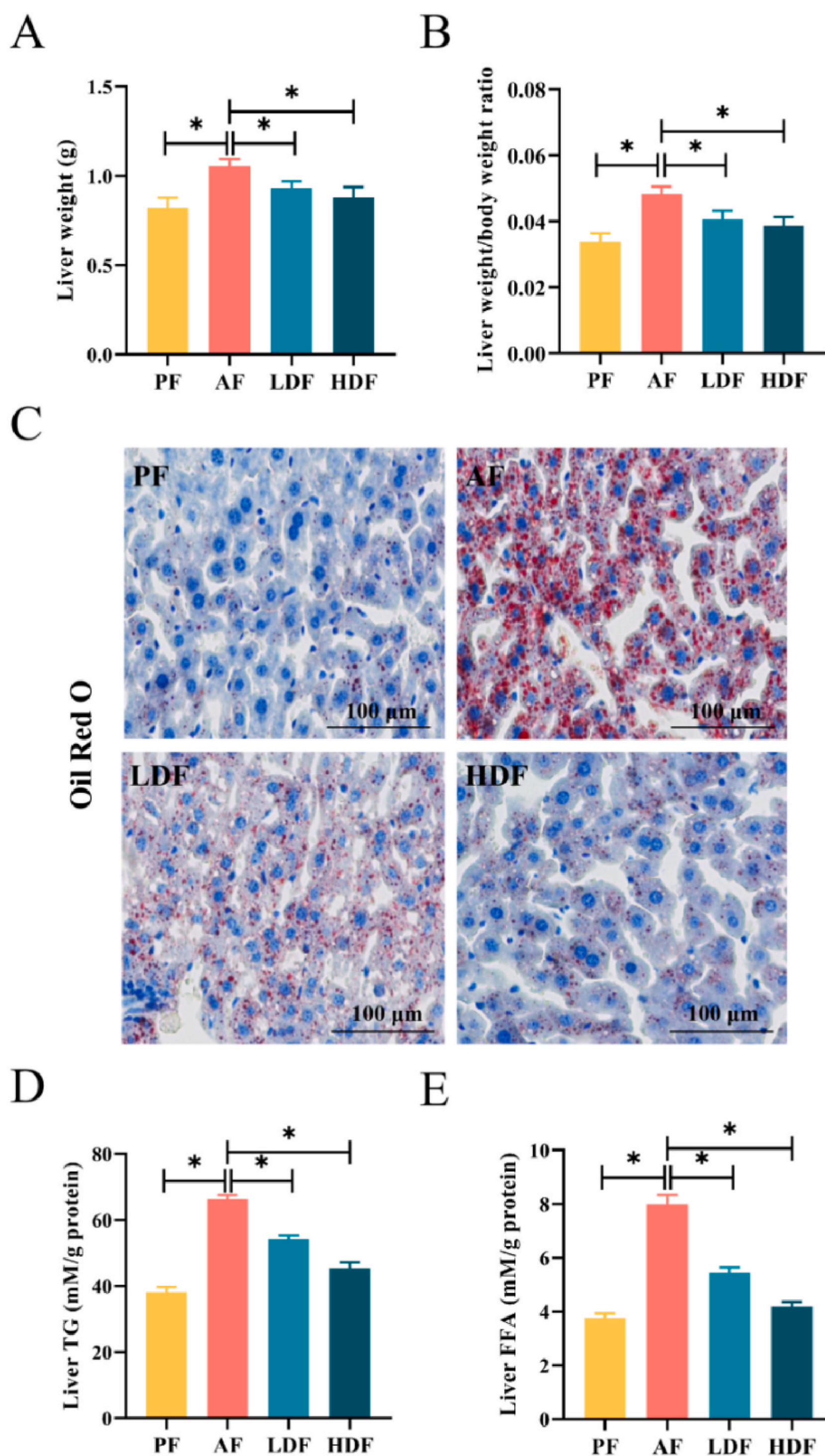


Fig. 3. PGF ameliorated chronic alcohol consumption-induced hepatic steatosis. (A) Liver weight; (B) The liver-to-body-weight ratio of mice; (C) Representative Oil Red O staining of the liver. The scale bar length represents 100 μm; (D) Liver TG; (E) Liver FFA. *$P < 0.05$ vs. corresponding control.

similarly down-regulated by PGF (Fig. 4A). VLDLR and CD36 are two critical receptors that play important roles in hepatocyte lipid uptake [15,16]. VLDLR, a key protein for hepatic triglyceride transport [17], was elevated after alcohol ingestion, which was significantly inhibited by PGF application. Interestingly, CD36, a fatty acid transporter [18], was not influenced by PGF (Fig. 4B). In

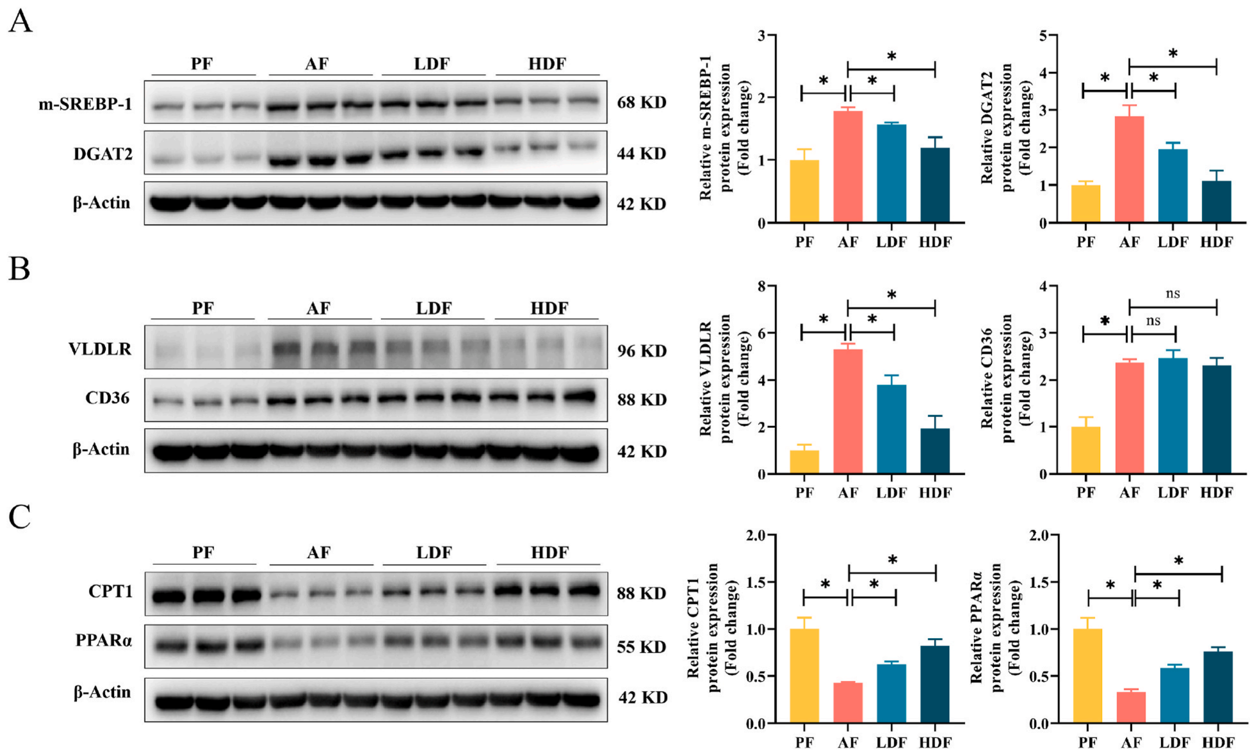


Fig. 4. PGF regulated the lipid metabolism associated proteins in the liver. Western blotting evaluated the expression of (A) *m*-SREBP-1 and DGAT2; (B) VLDLR and CD36; (C) CPT1 and PPARα in liver. The intensity of the protein bands was quantified using ImageJ. *$P < 0.05$ vs. corresponding control.

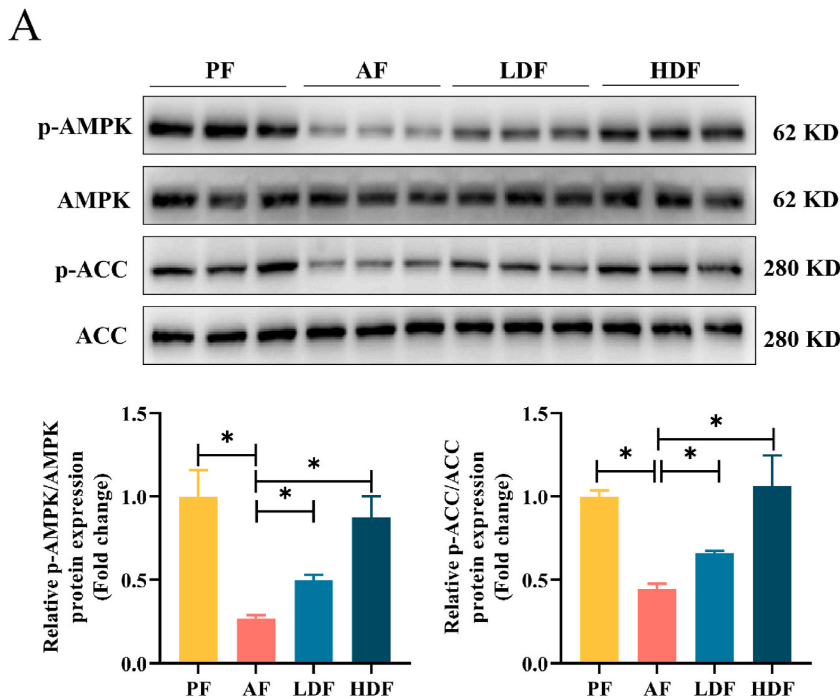


Fig. 5. PGF activated the AMPK-ACC signaling pathway in the liver. Western blot analysis of (A) *p*-AMPK, AMPK, *p*-ACC, and ACC in murine liver. *$P < 0.05$ vs. corresponding control.

addition, CPT1 is the rate-limiting enzyme for fatty acid oxidation and catalyzes the transport of fatty acids to the mitochondrial matrix for β-oxidation [19]. As shown in Fig. 4C, PGF restored the CPT1 expression, which was obviously low in AF. On the contrary, PPARa, an essential protein for β-oxidation, was elevated by PGF, suggesting that the stimulating energy metabolism might be the key way of PGF on amelioration of alcohol-induced lipid deposition.

3.5. PGF activated the AMPK-ACC signaling pathway in the liver

It is well known that AMPK is one of the core molecules regulating biological energy metabolism and plays a crucial role in lipid metabolism in the liver [20,21]. Therefore, we next evaluated the regulative capability of PGF on the AMPK signaling pathway in the liver of different groups of mice. The alcohol consumption decreased the phosphorylation level of AMPK, which was reversed by both low and high doses of PGF (Fig. 5 A). ACC (the rate-limiting enzyme of fatty acid metabolism) is one of the downstream targets of AMPK. Consistent with the results of AMPK phosphorylation, PGF treatment resulted in a significantly increased ACC phosphorylation level (Fig. 5 A).

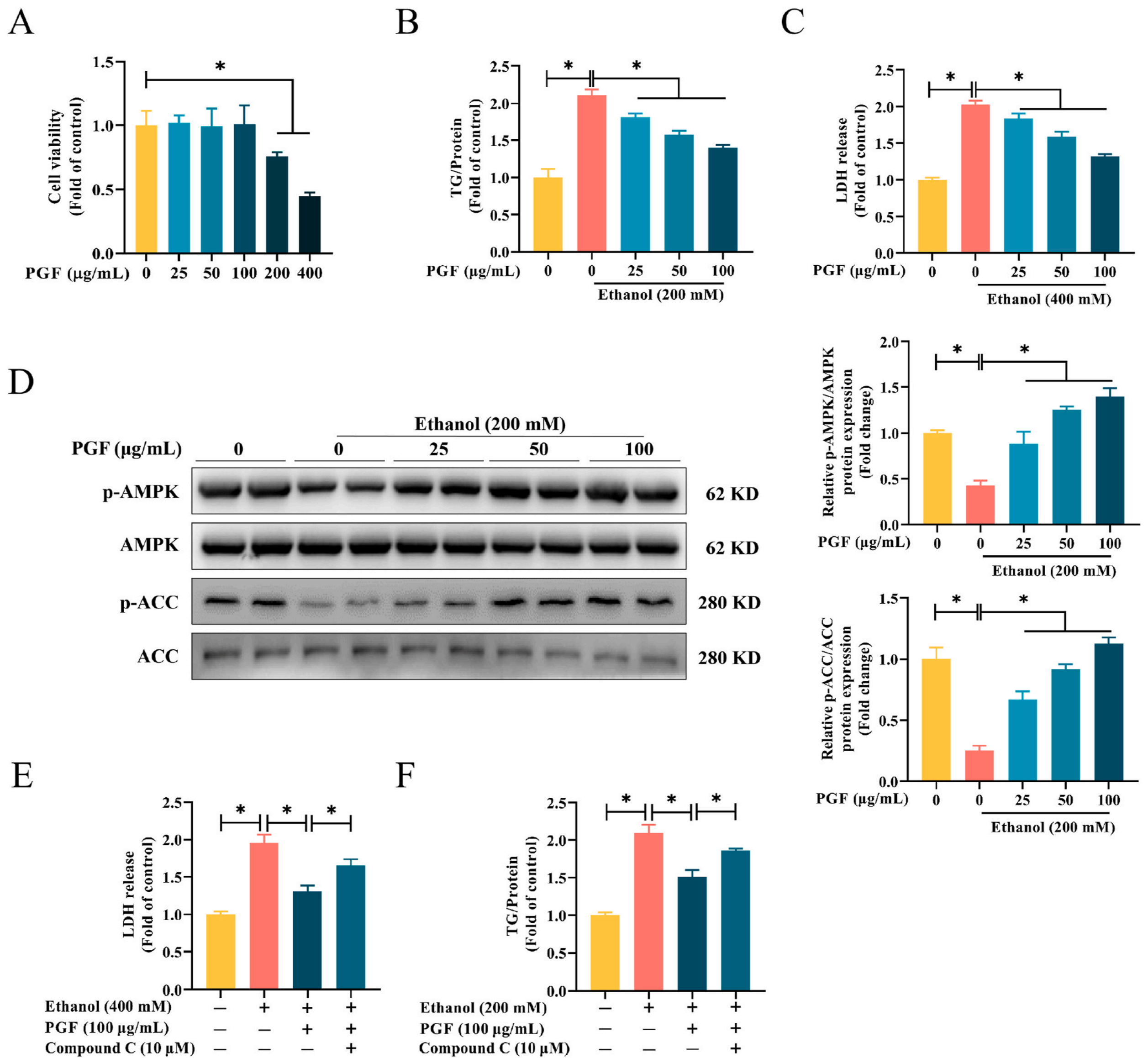


Fig. 6. PGF-activated AMPK phosphorylation contributed to the protection against lipid deposition in hepatocytes. (A) Cell viability in AML-12 cell assays with different concentrations of PGF (0, 25, 50, 100, 200, and 400 μg/mL) was determined using MTT assay; the intracellular concentration of TG (B) and LDH (C) in AML-12 cell assays, which were pretreated with PGF (0, 25, 50, and 100 μg/mL) 2 h following by ethanol exposure for 48 h; (D) Western blot analysis of *p*-AMPK, AMPK, *p*-ACC, and ACC in AML-12 cells; The LDH (E) and TG (F) levels in different AML-12 cells assays. *$P < 0.05$ vs. corresponding control.

3.6. PGF-activated AMPK phosphorylation contributed to the protection against lipid deposition in hepatocytes

To confirm the regulative capability of PGF on AMPK, we prepared the ethanol treated AML-12 cell assays *in vitro*. We first evaluated a safe dose of PGF on hepatocytes with MTT assay and found that PGF has no cytotoxic on AML-12 at doses even to 100 μg/mL (Fig. 6A). The protective effect of PGF against ethanol-induced hepatocyte injury and lipid deposition was demonstrated by evaluating the intracellular TG and LDH release (Fig. 6B&C). Our data suggest that PGF treatment significantly reversed ethanol-induced TG accumulation and LDH release dose-dependently. Consistent with the *in vivo* results, phosphorylation levels of AMPK and ACC were also increased by PGF (Fig. 6D). Interestingly, PGF treatment could dose dependently stimulate the AMPK phosphorylation even in normal cell assays, without ethanol incubation (Supplementary Figure 2). In addition, an AMPK inhibitor (compound C) significantly blocked beneficial effect of PGF on the ethanol-induced high level of TG and LDH in AML-12 cells. (Fig. 6 E&F). These results demonstrated that AMPK was crucial for PGF to show the protective capability on ethanol-induced hepatic lipid deposition.

3.7. PGF activated the AMPK signaling pathway via stimulating SIRT1 expression

AMPK activity is regulated by many factors, among which SIRT1 is one of the important regulators. We then evaluated the

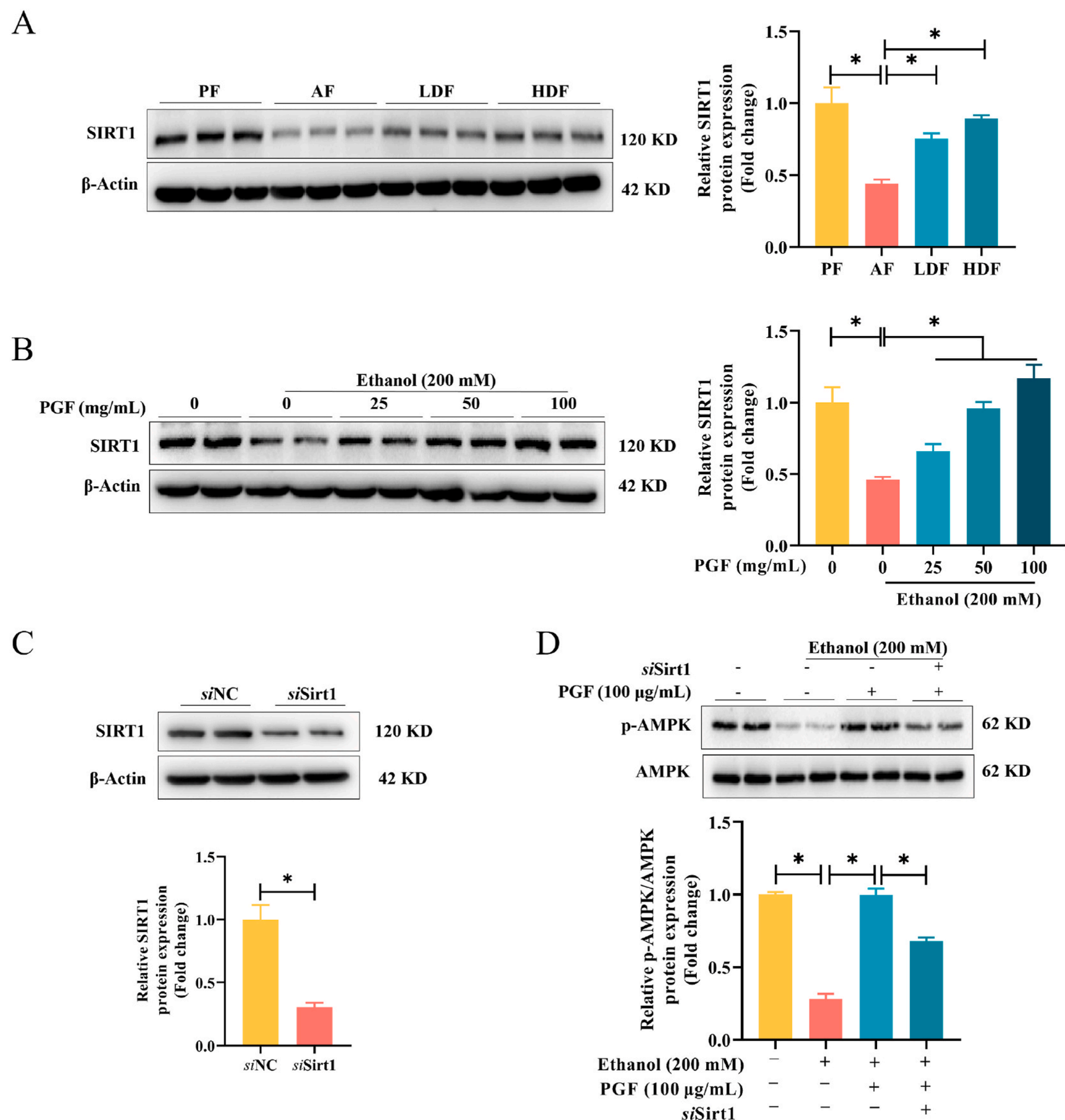


Fig. 7. PGF activated the AMPK signaling pathway *via* stimulating SIRT1 expression. Western blot analysis of SIRT1 in murine liver (A) and AML-12 cells (B); (C) The expression of SIRT1 were knocked down with SIRT1 siRNA (*si*Sirt1) in AML-12, in compared with unspecific siRNA (*si*NC) treatment for 48 h; (D) The phosphorylation level of AMPK in different cell assays was determined with Western blot. *$P < 0.05$ vs. corresponding control.

regulative capability of PGF on the expression of SIRT1 *in vivo* and *in vitro*. Our results showed that PGF significantly restored the alcohol and inhibited the SIRT1 expression (Fig. 7A&B). To confirm our finding, we engaged the SIRT1 knockdown AML-12 cell assays. The expression of SIRT1 was knocked down with the specific si-RNA in AML-12 cells. The result represented that PGF lost the regulative capability on AMPK phosphorylation (Fig. 7C&D), which implied that the PGF stimulated the phosphorylation of AMPK through increasing the SIRT1 expression.

4. Discussion

In this study, we demonstrated for the first time that PGF fraction in *P. chinense* extracts protected liver injury in chronic alcohol-fed mice through amelioration of the hepatic lipid deposition. In addition, PGF significantly possessed antioxidant, and anti-inflammatory activities and regulatory effects on fatty acid synthesis and catabolic processes in hepatocytes. And the SIRT1/AMPK signaling pathway might be the key regulative target of PGF.

P. chinense, riching in natural antioxidants, has recently been focused on by the nutraceutical and food industries [22]. *P. chinense* was widely used as an alcohol detoxification herb by the Miao people in China. Due to its high safety profile, *P. chinense* has been approved as a new functional food in recent years and is gradually being developed into a functional food for alcohol detoxification and liver protection [23]. Although *P. chinense* is widely known for its good detoxification ability, the underlying mechanisms are not fully understood. Previous studies have shown that the aqueous extract of *P. chinense* protects against ethanol-induced liver injury by reducing CYP2E1-mediated oxidative stress and enhancing oxidative defense mechanisms by activating the Nrf2/HO-1 pathway [10]. Yunbin Jiang et al. performed network pharmacological analysis and animal experiments to validate the liver protective capability of *P. chinense*, and that quercetin might be the main ingredient [24]. However, Tian-Tian Zhang et al. found that the alcohol extracts showed more effective than the aqueous extracts in protecting against CCl_4-mediated hepatotoxicity. Additionally, the 70 % ethanol extract presented better effect than that of the 95 % ethanol extract [7]. Consistently, our previous study found that the PGF fraction had the variety bioactivities [12]. In this study, we found that (1) PGF significantly improved alcohol-induced hepatic steatosis and liver injury; (2) reversed alcohol-induced oxidative stress; (3) inhibited alcohol-induced lipid accumulation and the expression of the inflammatory factors in the liver; (4) reduced the expression of crucial proteins for fatty acid synthesis and stimulated the lipid catabolism through regulating SIRT1/AMPK pathway.

Accumulating evidence suggested that hepatic oxidative stress and inflammatory responses play a central and causal role in the pathological process of ALD [25,26]. Malondialdehyde (MDA), a lipid peroxidation product, can unspecifically bind with proteins to form adducts, resulting in dysfunction of protein and cellular homeostasis disorders [27,28]. Our data showed that PGF significantly inhibited MDA levels in the alcoholic liver. Furthermore, PGF restored the vitality of the antioxidant enzymes, e.g. SOD and CAT, the critical defense systems against oxidative stress. Subsequently, one of the pathological characteristics of the alcoholic liver damage is high inflammatory responses. In fact, PGF reduced the expression of inflammatory factors (*Tnf-α, Il-1β,* and *Il-6*) in the liver of the alcohol feed mice.

Hepatocyte steatosis is considered a symptom of long-term excessive alcohol consumption [13]. Hence, the avoid lipid deposition in hepatocytes is critical in controlling the development of ALD. However, alcoholic liver steatosis is a multifactorial disease, and the involved process including such as lipogenesis [29–31], lipid transport [32,33], and lipid degradation [34,35]. SREBP-1 is a key transcriptional factor regulating the fatty acid synthesis. Moreover, the activated SREBP-1 can translocate into the nucleus to promote some enzyme expression, which responds for fatty acid synthesis, including FAS and SCD1 [14,36]. Hence, we proved that the regulative capability of PGF on expression of SREBP-1 and DGAT2. These data are consistent with a therapeutical role of PGF on ALD treatment. In addition, it was known that CD36 and VLDLR could mediate the necessary fatty acid and lipoprotein/lipid transport into hepatocytes and increase intracellular triglyceride accumulation [37]. The evidence suggests that the feeding of alcohol leads to up-regulation of the expression of CD36 and VLDLR in hepatocytes [15,38]. In the present study, we indicated that PGF attenuated alcohol-induced high levels of hepatic VLDLR expression. However, PGF did not affect the protein level of CD36 in the mouse liver, suggesting that PGF could alleviate alcohol-induced TG accumulation by improving the alcohol-induced imbalance of lipid transport. Furthermore, PPARα is a key transcription factor that regulates the expression of proteins involved in fatty acid uptake and β-oxidation [39], whereas CPT1 is a key regulator and rate-limiting enzyme for fatty acid β-oxidation in hepatocytes, which also regulates PPARα expression [40]. Therefore, CPT1 and PPARα are commonly used to evaluate the capacity of fatty acid oxidative catabolism. Previous reports have shown that excessive alcohol consumption decreased the expression of CPT1 and PPARα [41,42]. Whereas in the present study, we observed that PGF rescued the alcohol-induced low level of CPT1 and PPARα, which suggested a regulatory effect of PGF on fatty acid oxidation. The aforementioned data suggested that PGF protected alcohol-induced intracellular TG accumulation in hepatocytes by inhibiting fatty acid synthesis, lipid transport, and improving fatty acid β-oxidation.

To further clarify the mechanism of PGF in regulating lipid metabolism, we found that the expression of both SREBP-1 and CPT1 associated with the activation of AMPK. AMPK has emerged as an essential regulator of various functions in hepatocytes, especially the lipid metabolism [43,44]. The phosphorylated AMPK suppresses the cleavage and translocation of SREBP-1 [45], and contributes to the phosphorylation and inactivation of ACC, resulting in the decrease of malonyl-CoA. Malonyl-CoA is an inhibitor of CPT1 [3,46], which decreases CPT1 activity, thus interfering with the β-oxidation pathway. Based on these evidences, we tested the phosphorylation of AMPK and ACC in PGF treated ALD mice. Compared with the PF group, PGF intervention could effectively ameliorate ethanol-induced AMPK and ACC phosphorylation in the murine liver. This is consistent with the results of our *in vitro* assay experiment. Notably, AMPK inhibitor (Compound C) blocked the protective activity of PGF on both ethanol-induced hepatocyte damage and lipid accumulation, which implied that AMPK was mechanistically involved in the beneficial role of PGF.

AMPK activity is regulated by a variety of factors, of which SIRT1 is one of the key regulators [46–48]. SIRT1 is a NAD^+ dependent

deacetylase, and alcohol consumes NAD^+ during oxidative metabolism, resulting in reduced NAD^+ levels and inhibiting SIRT1 activity [49]. Our results showed that PGF restored alcohol decreasing SIRT1 expression in a dose-dependent manner; moreover, knockdown of SIRT1 suppressed the regulation of PGF on AMPK phosphorylation, suggesting a mechanistic involvement in the protective effects of SIRT1 in PGF. In this study, we found that PGF had the efficacy of significantly improving alcoholic liver injury and lipid deposition, and the data at this study showed that SIRT1/AMPK was the key signaling pathway of PGF. However, how PGF regulates SIRT1/AMPK, still needs to be explored. In addition, the ingredients in PGF, and their specific function still need to be verified.

5. Conclusions

In summary, our study provided the evidence for the first time that the PGF application ameliorated chronic alcohol consumption-induced liver injury and steatosis by activating the SIRT1/AMPK signaling pathway. Our new findings provided a valuable support for the application of PGF as a promising functional food in prevention of ALD.

Animal ethics review

The animal study was reviewed and approved by the Animal Ethical and Welfare Committee of Zhejiang Chinese Medical University (ZSLL-2017-150).

Data availability statement

The data associated with the study not been deposited yet into a publicly available repository. If you have any needs, please contact the corresponding author by email to obtain more information about the data.

CRediT authorship contribution statement

Hui Zhuge: Methodology, Formal analysis, Data curation. **Yan Pan:** Writing – original draft, Formal analysis. **Shanglei Lai:** Formal analysis, Data curation. **Kaixin Chang:** Formal analysis, Data curation. **Qinchao Ding:** Methodology, Data curation. **Wenjing Cao:** Methodology. **Qing Song:** Writing – review & editing, Methodology, Funding acquisition. **Songtao Li:** Funding acquisition, Formal analysis. **Xiaobing Dou:** Funding acquisition, Conceptualization. **Bin Ding:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by grants from the Natural Science Foundation of China (82273625 82103838), Zhejiang Natural Science Foundation (LZ21H030001 and LR20H260001). Traditional Chinese Medicine Science and Technology Program of Zhejiang Province (2023ZF011). We also thank the great help from the Medical Research Center, Academy of Chinese Medical Sciences, Zhejiang Chinese Medical University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at. https://doi.org/10.1016/j.heliyon.2024.e31195.

References

[1] L.Z. Kong, et al., Pathogenesis, Early Diagnosis, and therapeutic Management of alcoholic liver disease, Int. J. Mol. Sci. 20 (11) (2019).
[2] B. Gao, R. Bataller, Alcoholic liver disease: pathogenesis and new therapeutic targets, Gastroenterology 141 (5) (2011) 1572–1585.
[3] J. Altamirano, R. Bataller, Alcoholic liver disease: pathogenesis and new targets for therapy, Nat. Rev. Gastroenterol. Hepatol. 8 (9) (2011) 491–501.
[4] X. Chen, et al., Dihydroartemisinin attenuates alcoholic fatty liver through regulation of lipin-1 signaling, IUBMB Life 71 (11) (2019) 1740–1750.
[5] Q. Song, et al., ER stress-induced upregulation of NNMT contributes to alcohol-related fatty liver development, J. Hepatol. 73 (4) (2020) 783–793.
[6] A.K. Singal, V.H. Shah, Therapeutic Strategies for the treatment of alcoholic hepatitis, Semin. Liver Dis. 36 (1) (2016) 56–68.
[7] T.T. Zhang, et al., Hepatoprotective function of Penthorum chinense Pursh, Food Funct. 4 (11) (2013) 1581–1585.
[8] A. Wang, L. Lin, Y. Wang, Traditional Chinese herbal medicine Penthorum chinense Pursh: a phytochemical and pharmacological review, Am. J. Chin. Med. 43 (4) (2015) 601–620.
[9] Y.W. Cao, et al., The hepatoprotective effect of aqueous extracts of Penthorum chinense Pursh against acute alcohol-induced liver injury is associated with ameliorating hepatic steatosis and reducing oxidative stress, Food Funct. 6 (5) (2015) 1510–1517.
[10] Y.W. Cao, et al., Protective effects of Penthorum chinense Pursh against chronic ethanol-induced liver injury in mice, J. Ethnopharmacol. 161 (2015) 92–98.

[11] B. Ding, et al., Characterization of the anti-Staphylococcus aureus fraction from Penthorum chinense Pursh stems, BMC Complement Altern Med 19 (1) (2019) 219.
[12] Q. Ding, et al., Bioactivity evaluation of pinocembrin Derivatives from Penthorum chinense Pursh stems 14 (9) (2019) 1934578X1987589.
[13] Q. Ding, et al., Lactobacillus plantarum ZY08 relieves chronic alcohol-induced hepatic steatosis and liver injury in mice via restoring intestinal flora homeostasis, Food Res. Int. 157 (2022) 111259.
[14] S. Jeon, R. Carr, Alcohol effects on hepatic lipid metabolism, J. Lipid Res. 61 (4) (2020) 470–479.
[15] Z. Wang, et al., Nuclear factor (erythroid-derived 2)-like 2 activation-induced hepatic very-low-density lipoprotein receptor overexpression in response to oxidative stress contributes to alcoholic liver disease in mice, Hepatology 59 (4) (2014) 1381–1392.
[16] E. Barroso, et al., SIRT3 deficiency exacerbates fatty liver by attenuating the HIF1α-LIPIN 1 pathway and increasing CD36 through Nrf2, Cell Commun. Signal. 18 (1) (2020) 147.
[17] J. Heeren, L. Scheja, Metabolic-associated fatty liver disease and lipoprotein metabolism, Mol Metab 50 (2021) 101238.
[18] M.Y. Pepino, et al., Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism, Annu. Rev. Nutr. 34 (2014) 281–303.
[19] M. Schreurs, F. Kuipers, F.R. van der Leij, Regulatory enzymes of mitochondrial beta-oxidation as targets for treatment of the metabolic syndrome, Obes. Rev. 11 (5) (2010) 380–388.
[20] G.R. Steinberg, D.G. Hardie, New insights into activation and function of the AMPK, Nat. Rev. Mol. Cell Biol. 24 (4) (2023) 255–272.
[21] C. Fang, et al., The AMPK pathway in fatty liver disease, Front. Physiol. 13 (2022) 970292.
[22] L. Liu, et al., Macroporous Resin-Assisted Enrichment and isolation of antioxidant and cytotoxic Phenolics from Penthorum chinense 31 (6) (2021) 854–858.
[23] A. Wang, et al., A review of Penthorum chinense Pursh for hepatoprotection: Traditional use, phytochemistry, pharmacology, toxicology and clinical trials, J. Ethnopharmacol. 251 (2020) 112569.
[24] Y. Jiang, et al., Integrated strategy of network pharmacology, molecular docking, HPLC-DAD and mice model for exploring active ingredients and pharmacological mechanisms of Penthorum chinense Pursh against alcoholic liver injury, J. Ethnopharmacol. 298 (2022) 115589.
[25] Y.M. Yang, Y.E. Cho, S. Hwang, Crosstalk between oxidative stress and inflammatory liver injury in the pathogenesis of alcoholic liver disease, Int. J. Mol. Sci. 23 (2) (2022).
[26] S. Li, et al., The role of oxidative stress and antioxidants in liver diseases, Int. J. Mol. Sci. 16 (11) (2015) 26087–26124.
[27] A. Dey, A.I. Cederbaum, Alcohol and oxidative liver injury, Hepatology 43 (2 Suppl 1) (2006) S63–S74.
[28] M. Chen, W. Zhong, W. Xu, Alcohol and the mechanisms of liver disease, J. Gastroenterol. Hepatol. (2023).
[29] L. Kang, et al., Chronic ethanol and triglyceride turnover in white adipose tissue in rats: inhibition of the anti-lipolytic action of insulin after chronic ethanol contributes to increased triglyceride degradation, J. Biol. Chem. 282 (39) (2007) 28465–28473.
[30] Z.G. Wang, et al., Adipose tissue-liver axis in alcoholic liver disease, World J. Gastrointest. Pathophysiol. 7 (1) (2016) 17–26.
[31] X. Wei, et al., Chronic alcohol exposure disturbs lipid homeostasis at the adipose tissue-liver axis in mice: analysis of triacylglycerols using high-resolution mass spectrometry in combination with in vivo metabolite deuterium labeling, PLoS One 8 (2) (2013) e55382.
[32] K.K. Kharbanda, et al., Betaine attenuates alcoholic steatosis by restoring phosphatidylcholine generation via the phosphatidylethanolamine methyltransferase pathway, J. Hepatol. 46 (2) (2007) 314–321.
[33] K.K. Kharbanda, et al., Betaine administration corrects ethanol-induced defective VLDL secretion, Mol. Cell. Biochem. 327 (1–2) (2009) 75–78.
[34] A. Xu, et al., The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice, J. Clin. Invest. 112 (1) (2003) 91–100.
[35] Z. Zhong, et al., Acute ethanol causes hepatic mitochondrial depolarization in mice: role of ethanol metabolism, PLoS One 9 (3) (2014) e91308.
[36] Siqingaowa, et al., Sterol regulatory element-binding protein 1 inhibitors decrease pancreatic cancer cell viability and proliferation, Biochem. Biophys. Res. Commun. 488 (1) (2017) 136–140.
[37] M.K. Jang, et al., Protective effects of Alisma orientale extract against hepatic steatosis via inhibition of Endoplasmic Reticulum stress, Int. J. Mol. Sci. 16 (11) (2015) 26151–26165.
[38] R.D. Clugston, et al., CD36-deficient mice are resistant to alcohol- and high-carbohydrate-induced hepatic steatosis, J. Lipid Res. 55 (2) (2014) 239–246.
[39] G.Z. Liu, et al., Fenofibrate inhibits atrial metabolic remodelling in atrial fibrillation through PPAR-α/sirtuin 1/PGC-1α pathway, Br. J. Pharmacol. 173 (6) (2016) 1095–1109.
[40] H. Yang, et al., Overexpression CPT1A reduces lipid accumulation via PPARα/CD36 axis to suppress the cell proliferation in ccRCC, Acta Biochim. Biophys. Sin. 54 (2) (2022) 220–231.
[41] G.V. Ronnett, et al., Fatty acid metabolism, the central nervous system, and feeding, Obesity 14 (Suppl 5) (2006) 201s–207s.
[42] T. Nakajima, et al., Peroxisome proliferator-activated receptor alpha protects against alcohol-induced liver damage, Hepatology 40 (4) (2004) 972–980.
[43] K.M. Lee, et al., Disruption of the cereblon gene enhances hepatic AMPK activity and prevents high-fat diet-induced obesity and insulin resistance in mice, Diabetes 62 (6) (2013) 1855–1864.
[44] M. Varela-Rey, et al., Role of AMP-activated protein kinase in the control of hepatocyte priming and proliferation during liver regeneration, Exp Biol Med (Maywood) 236 (4) (2011) 402–408.
[45] Y. Li, et al., AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice, Cell Metab 13 (4) (2011) 376–388.
[46] S. Herzig, R.J. Shaw, AMPK: guardian of metabolism and mitochondrial homeostasis, Nat. Rev. Mol. Cell Biol. 19 (2) (2018) 121–135.
[47] Q. Ding, et al., Hepatic TRPC3 loss contributes to chronic alcohol consumption-induced hepatic steatosis and liver injury in mice, Life Metabolism (2023).
[48] R.B. Ding, J. Bao, C.X. Deng, Emerging roles of SIRT1 in fatty liver diseases, Int. J. Biol. Sci. 13 (7) (2017) 852–867.
[49] R. Ren, et al., Emerging roles of SIRT1 in alcoholic liver disease, Int. J. Biol. Sci. 16 (16) (2020) 3174–3183.