RESEARCH ARTICLE

The co-extract from *Hovenia dulcis* Thunb. and *Phyllanthus emblica* L. alleviated ethanol-induced liver injury *via* accelerated ethanol metabolism and synergistic hepatoprotection in zebrafish

Mei Zhang[1, *], Hongyuan Zhang[1, 2], Rongjian Sa[1], Yanjie Zhang[1], Ling Liang[1], Zhiwei Huang[2], Benyong Lou[1]

[1]Department of Pharmaceutical Engineering, College of Materials and Chemical Engineering, Research and Testing Center of Pharmaceuticals, Minjiang University, Fuzhou, Fujian, China. [2]Department of Food Engineering, College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China.

Received: November 26, 2025; **accepted:** March 11, 2026.

Excessive alcohol consumption causes alcoholic liver disease (ALD), a severe disorder characterized by toxic hepatocellular injury. The pathogenesis of ALD is complex, and currently, there remains no safe and specific drug for its clinical treatment. Thus, the development of drug-food homologous products for alleviating ALD-induced liver damage holds broad market prospects. This study investigated the hepatoprotective effects of co-extracts from *Hovenia dulcis* Thunb. and *Phyllanthus emblica* L. (HPE) against ethanol-induced liver injury using a zebrafish ALD model. The results showed that zebrafish larvae exposed to 2% ethanol exhibited significant liver damage as manifested by hepatic lipid accumulation and degeneration. High-performance liquid chromatography (HPLC) analysis revealed that gallic acid and dihydromyricetin were the major components of HPE. HPE treatment significantly reduced hepatocellular vacuolar degeneration and steatosis, downregulated the expression of lipid metabolism-related genes of *HMGCRa*, *HMGCRb*, and *FASN*, and lowered triglyceride (TG) and total cholesterol (TC) levels. Moreover, HPE enhanced ethanol metabolism by increasing the activities of alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH), upregulating *ADH8a* and *ADH8b* expression, and downregulating *CYP2y3* and *CYP3a65* expression. Additionally, HPE mitigated hepatocellular injury by reducing alanine aminotransferase (ALT) and aspartate aminotransferase (AST) leakage and alleviated oxidative stress and inflammation by modulating superoxide dismutase (SOD) activity, malondialdehyde (MDA) levels, and the expression of *Nrf2*, *IL-1β*, and *IL-6*. These results demonstrated that HPE had potential as a functional food for managing ALD by improving lipid and ethanol metabolism and attenuating oxidative stress and inflammatory responses.

Keywords: alcoholic liver disease; *Hovenia dulcis* Thunb.; *Phyllanthus emblica* L.; zebrafish; ethanol metabolism; hepatoprotection.

[*]**Corresponding author:** Mei Zhang, Department of Pharmaceutical Engineering, College of Materials and Chemical Engineering, Research and Testing Center of Pharmaceuticals, Minjiang University, Fuzhou, Fujian 350108, China. Email: TimWard757@hotmail.com.

Introduction

Excessive alcohol intake induces the development of alcoholic liver disease (ALD), a toxic hepatic injury that represents a prominent global health issue. ALD encompasses a spectrum of pathological changes ranging from simple alcoholic fatty liver to alcoholic hepatitis, liver fibrosis, and ultimately hepatocellular carcinoma [1, 2]. The pathogenesis of ALD is complex and

involves multiple mechanisms including direct hepatocellular damage caused by ethanol and its metabolites [3], oxidative stress induced by the overproduction of reactive oxygen species (ROS) and free radicals during ethanol metabolism [4], lipid metabolism disorders that lead to lipid accumulation and peroxidation [5], and inflammatory liver damage triggered by endotoxins that activate Kupffer cells to release cytokines and inflammatory factors [6]. Currently, there are no safe and specific drugs for the clinical treatment of ALD. Therefore, developing natural and low-toxic hepatoprotective active substances has become a research hotspot in this field.

Extensive studies have been conducted on the individual hepatoprotective activities of *Hovenia dulcis* seeds (HD) and *Phyllanthus emblica* fruits (PE). HD contains flavonoids such as dihydromyricetin and myricetin, which exhibit alcohol-detoxifying, liver-protective, antioxidant, and anti-inflammatory properties [7]. These components facilitate ethanol metabolism, scavenge excessive free radicals, resist lipid peroxidation, and reduce inflammation, thereby playing a role in protecting the liver [8]. Similarly, PE is rich in polyphenols, which are known for their hepatoprotective, antioxidant, anti-inflammatory, and antitumor effects [9]. Studies have suggested that PE achieves its hepatoprotective effects through antioxidant activity, anti-apoptosis, lipid metabolism regulation, detoxification, and inflammation reduction [10, 11]. Based on the principle of synergistic effect, plant compounding has become a research trend. Studies have confirmed that the combined extract of *Pueraria lobata* and HD has notable antioxidative and anti-inflammatory properties, effectively alleviating ethanol-induced liver damage *in vitro* [12]. However, no research has been reported on the combination of HD and PE, and their synergistic hepatoprotective effects and specific mechanisms remain unclear. The zebrafish ALD model offers advantages over traditional rodent models including simplicity in establishing liver injury, high reproductive capacity, low cost, and optical transparency of embryos and larvae for real-time imaging [13, 14].

This study explored the protective effect and synergistic mechanism of HD-PE co-extract (HPE) using an acute zebrafish ALD model. Liver histopathological analysis, biochemical assays, and real-time quantitative PCR (RT-qPCR) were employed to assess indicators associated with liver injury, lipid metabolism, ethanol metabolism, and inflammatory responses. The results of this study provided a scientific foundation for the development of highly effective and low-toxic food-medicinal homologous hepatoprotective products and a new approach for the adjunctive prevention and treatment of ALD.

Materials and methods

HPE preparation

HD and PE were purchased from Haozhou Xiantai Biopharmaceutical Co., Ltd. (Bozhou, Anhui, China). The materials were crushed separately, passed through a 40-mesh sieve, and mixed in a 1:1 mass ratio. The mixture was extracted with 60% ethanol (v/v) under continuous stirring at room temperature for 24 h. Subsequently, the mixture was centrifuged at 4,000 rpm for 20 min using TGL-20bR centrifuge (Shanghai Anting, Shanghai, China). The supernatant was evaporated to a paste at 60℃ using SENCO RE-201 rotary evaporator (Shanghai, China). HPE was finally obtained using Yamato DC801 freeze dryer (Minami Alps, Tokyo, Japan).

High-performance liquid chromatography (HPLC) analysis

Shimadzu LC-20AD HPLC (Shimadzu, Nakagyo, Kyoto, Japan) with Thermo BDS HYPERSIL C18 column (4.6 × 250 mm, 5 μm) was used to determine gallic acid and dihydromyricetin in HPE under the mobile phases of methanol (A) and 2% formic acid (B) with the elution gradient of 1 - 10% A at 0 - 30 min, 10 - 20% A at 30 - 40 min, 20 - 25% A at 40 - 50 min, 25 - 30% A at 50 - 80 min, 30 - 64% A at 80 - 90 min, 64 - 80% A at

90 - 100 min. The column temperature was 30℃, and the injection volume was 10 µL with the flow rate of 1.0 mL/min and the detection wavelength of 285 nm. Standard curves were established with peak area versus concentration as gallic acid $Y = 2.49108 \times 10^7X - 45,755$ ($R^2 = 0.9999$, linear range of 0 to 2.50 µg/µL) and dihydromyricetin $Y = 3.16873 \times 10^7X - 882,060$ ($R^2 = 0.9998$, linear range of 0 to 0.85 µg/µL). All used reagents were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China).

Experimental animals

The wild AB line zebrafish were purchased from the China National Zebrafish Resource Center (Wuhan, Hubei, China). The zebrafish embryos and juveniles were cultivated in Danieau's buffer solution containing 1,017 mg/L NaCl, 15.6 mg/L KCl, 42.5 mg/L $Ca(NO_3)_2{\cdot}4H_2O$, 29.6 mg/L $MgSO_4{\cdot}7H_2O$, and 356 mg/L HEPES with pH adjusted to 7.2 at 28.5℃. The experimental protocol was approved by the Experimental Animal Welfare Ethics Committee of Fuzhou Cold-Spring Biology Co., LTD (Fuzhou, Fujian, China) with the approval number of 2024073002.

Determination of safe concentration of HPE for zebrafish embryos

The embryos were disinfected with 0.003% NaClO. The embryos that developed normally 24 hours after fertilization were seeded into a 6-well plate with 20 embryos per well and incubated in HPE solutions for 48 hours. The animals were divided into control (Danieau's buffer solution) and HPE-treated groups at the gradient concentrations of 20, 40, 60, 80, 100, 120, 140 µg/mL with three parallel wells for each group. The 6-well plates were then placed in a constant-temperature incubator at 28.5℃ for 48 hours. The embryonic mortality rate was recorded. A probit analysis was used to fit the dose-response curve between embryonic mortality and HPE concentration, and the median lethal concentration (LC_{50}) was calculated.

Ethanol-induced zebrafish liver injury model

An ethanol-exposed liver injury model was established in zebrafish larvae. Four-day post-fertilization (dpf) zebrafish larvae were randomly divided into a control group and a model group. The control group was cultured in Danieau's buffer, while the model group was exposed to 2% ethanol (EtOH) in the same buffer at 28.5°C for 32 hours. After ethanol exposure, the model group was further subdivided into five groups including model group treated with Danieau's buffer, positive control group treated with 0.5 mM N-acetylcysteine (NAC), and three HPE treatment groups at the concentrations of 8.4, 16.8, and 42.0 µg/mL, respectively. Zebrafish larvae were transferred to 6-well plates with 30 larvae per well and incubated at 28.5°C for 40 hours before collecting larvae for histological and biochemical analyses.

Hematoxylin and eosin (H&E) staining

Collected zebrafish larvae were fixed in 4% paraformaldehyde (PFA) at 4°C overnight and then embedded in paraffin. Four-micrometer-thick tissue sections were prepared for H&E staining, and liver sections were observed and imaged by using a Nikon SMZ800N optical microscope (Nikon Corporation, Shinagawa-ku, Tokyo, Japan).

Whole mount oil red O (ORO) staining

Collected zebrafish larvae were fixed in 4% PFA at 4°C overnight and then washed three times with 1× phosphate-buffered saline (PBS). After fixation, the larvae were permeabilized with 60% propylene glycol for 30 min and then stained with 0.5% ORO solution for 3 h at room temperature. The samples were rinsed in 60% propylene glycol, washed with water to remove excess stain, and finally photographed using Nikon SMZ800N optical microscope.

Biochemical analyses

Zebrafish larvae were rinsed three times with PBS and then homogenized in 500 µL PBS. The homogenate was centrifuged at 4°C and 3,000 rpm for 10 min to collect the supernatant. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alcohol dehydrogenase (ADH), and acetaldehyde dehydrogenase (ALDH), as well as the activity of

Table 1. The sequences of primers (5' to 3') used for RT-qPCR.

Gene	Forward primer	Reverse primer
RPP0	CTGAACATCTCGCCCTTCTC	TAGCCGATCTGCAGACACAC
CHOP	AGGAAAGTGCAGGAGCTGAC	CTCCACAAGAAGAATTTCCTCC
GADD45αa	TGGCTTTGTTTGTGGGACTT	TGGAAAACAGTCCACTGAGA
HMGCRa	CTGAGGCTCTGGTGGACGTG	GATAGCAGCTACGATGTGGCG
HMGCRb	CCTGTTAGCCGTCAGTGGA	TCTTTGACCACTCGTGCCG
FASN	GAGAAAGCTTGCCAAACAGG	GAGGGTCTTGCAGGAGACAG
ADH8a	TGGTTGATGGGGACAAGCGAGG	CCACACTGAGAGAGGAAGAGGG
ADH8b	CTTGGAGTCGACAGACAATAAAGG	CTGCACTGACAGCCAGAATTTGG
CYP2y3	TATTCCCATGCTGCACTCTG	AGGAGCGTTTACCTGCAGAA
CYP3a65	AAACCCTGATGAGCATGGAC	CAAGTCTTTGGSGATGAGGA
Nrf2	CTCCAAACCTCCGTTCACCA	GTCGTCTACGGGCAGATTGA
IL-1β	TGGACTTCGCAGCACAAAATG	CACTTCACGCTCTTGGATGA
IL-6	TCAACTTCTCCAGCGTGATG	TCTTTCCCTCTTTTCCTCCTG

superoxide dismutase (SOD) and the levels of total cholesterol (TC), triglycerides (TG), and malondialdehyde (MDA) in the supernatant were determined in accordance with the instructions of the corresponding kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

RT-qPCR analysis

Total RNA was extracted from zebrafish larvae using RNAiso Plus reagent (Takara Bio Inc., Kusatsu, Shiga, Japan) following the manufacturer's instructions. After confirming the purity and integrity of the extracted RNA with electrophoresis, RNA was reverse transcribed into complementary DNA (cDNA) using a NovoScript® 1st Strand cDNA Synthesis SuperMix (Novoprotein Technology Co., Ltd., Suzhou, Jiangsu, China). *RPP0* gene was employed as a reference gene. The primer sequences of all tested genes were listed in Table 1. RT-qPCR was performed in a 20 µL total volume by using Archimed™ X4 real-time fluorescent qPCR instrument (Kunpeng Gene Technology Co., Ltd., Beijing, China). The RT-qPCR reaction conditions were set as 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min with a final stage of 95°C for 15 s and 60°C for 10 s.

Statistical analysis

SPSS Statistics 27 (IBM, Armonk, NY, USA) was employed for the statistical analysis of this study, while GraphPad Prism 10.1 (https://www.graphpad.com) and Origin 9.8.5 (https://www.originlab.com) were used for graphing. All data were presented as mean ± standard deviation (SD). Statistically significant differences were determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test with *P* value less than 0.05 being considered as statistically significant.

Results

HPLC analysis of HPE

HPLC chromatograms demonstrated two major peaks at retention times of 10.677 and 45.260 min for standard gallic acid and dihydromyricetin, respectively (Figure 1A). In contrast, HPLC analysis of HPE revealed multiple distinct peaks at various retention times with gallic acid and dihydromyricetin eluting at approximately 10.754 and 45.228 min, respectively (Figure 1B). Moreover, the contents of gallic acid and dihydromyricetin in HPE were 75.701 ± 0.186 and 41.905 ± 0.132 mg/g, respectively. The results showed that gallic acid and dihydromyricetin were the two major compounds in HPE.

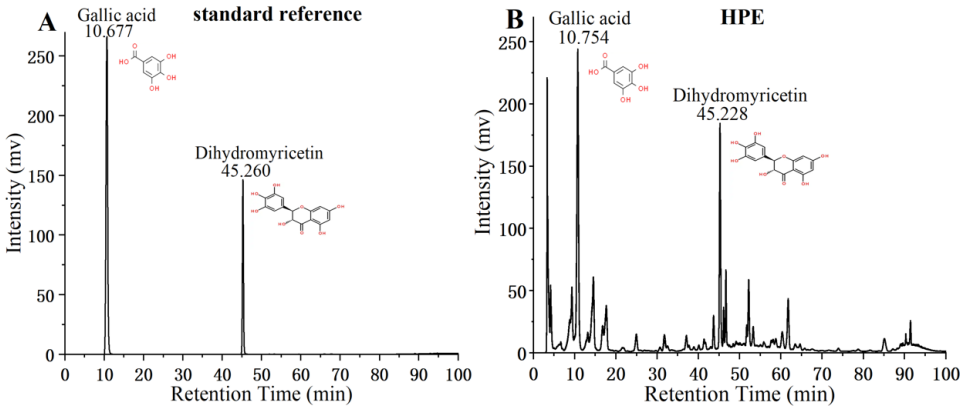

Figure 1. HPLC chromatograms of standard reference (A) and HPE (B).

Determination of safe concentration of HPE in zebrafish embryos

After zebrafish embryos were exposed to HPE solutions for 48 hours, those in the control group and 20 μg/mL HPE group developed normally with no developmental abnormalities or deaths observed. When the HPE concentration exceeded 40 μg/mL, the embryos began to exhibit decreased vitality, abnormal yolk extension, somite deformities, and even death. The embryonic mortality rate increased in an HPE concentration-dependent manner (Figure 2). At an HPE concentration of 100 μg/mL, the mortality rate was 48.3 ± 7.6%. The median lethal concentration (LC_{50}) of HPE for zebrafish embryos was calculated as 83.7 μg/mL using the probit method. Therefore, HPE concentrations of 8.4, 16.8, and 42.0 μg/mL were selected as the low, medium, and high-dose treatments for subsequent zebrafish experiments.

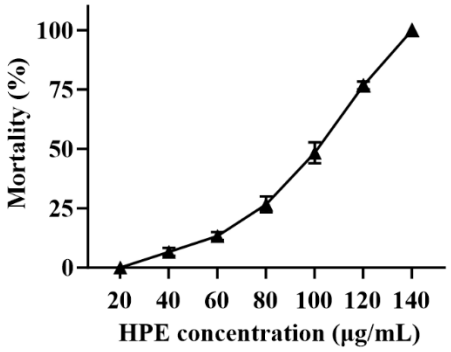

Figure 2. Mortality of zebrafish embryos with HPE treatments.

HPE ameliorated ethanol-induced hepatic steatosis in zebrafish

Liver tissue sections of zebrafish in each treatment group were analyzed by H&E staining (Figure 3A). The results showed that 2% EtOH treatment induced hepatocellular damage, lipid accumulation, and aggravated inflammation in zebrafish larvae, confirming the successful establishment of an acute alcoholic liver injury model. In the positive control group with 0.5 mM NAC and HPE treatment groups, the hepatocellular structures were significantly improved with reduced vacuolar degeneration and steatosis, orderly cell arrangement, and improved hepatocyte morphology. Whole zebrafish ORO staining was performed to visually evaluate changes in liver morphology and hepatic steatosis (Figure 3B). The results showed that the livers of zebrafish larvae were enlarged, while the yolk sacs exhibited severe edema, and the liver staining intensified in the 2% EtOH group. Quantitative analysis of gray values revealed a significant increase in the 2% EtOH group, accompanied by obvious punctate lipid droplet deposition, indicating severe liver injury (Figure 3C). The liver and yolk sac morphology of larvae in the NAC and HPE treatment groups was significantly improved, approaching normal conditions. Gray values were significantly reduced in all treatment groups with the high-dose HPE group showing the most pronounced reduction in lipid accumulation, even exceeding that of the positive control group.

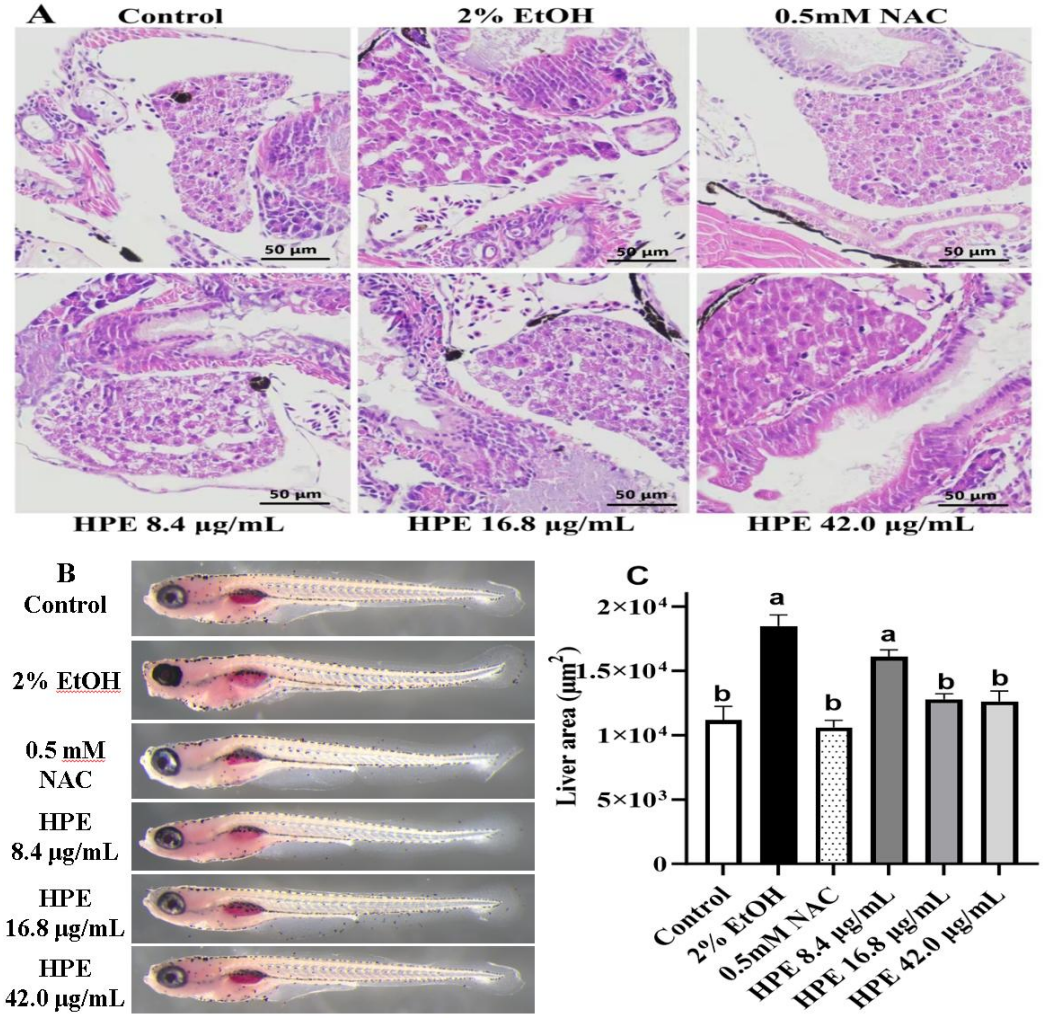

Figure 3. HPE ameliorated ethanol-induced hepatic steatosis in zebrafish. **A.** H&E staining showed pathological changes. **B.** Whole-mount oil red O staining. **C.** Quantitative analysis of liver gray value. Data were presented as mean ± SD (n = 3). Values with different lowercase letters indicated significantly different ($P < 0.05$).

HPE alleviated ethanol metabolism in alcoholic liver injury zebrafish

In the model group, 2% EtOH exposure markedly downregulated the expression levels of *ADH8a* and *ADH8b*, along with a significant decrease in ADH and ALDH activities (Figures 4A-4D), which was accompanied by a marked upregulation of *CYP2y3* and *CYP3a65* expression (Figures 4E and 4F). These results indicated that acute ethanol treatment inhibited the ADH/ALDH ethanol metabolism pathway in zebrafish, triggered the overexpression of *CYP2y3* and *CYP3a65*, and led to the generation of hydroxyl radicals, superoxide anions, and other toxic metabolites. Collectively, these changes exacerbated oxidative stress and hepatocellular damage. In contrast, HPE treatment significantly upregulated the expression levels of *ADH8a* and *ADH8b* while concurrently downregulating the expression of *CYP2y3* and *CYP3a65*.

HPE diminished ethanol-induced lipid accumulation in zebrafish

The results of hepatic TG and TC levels and the expression of lipid metabolism-related genes (*HMGCRa*, *HMGCRb*, *FASN*) showed that the expression levels of *HMGCRa*, *HMGCRb*, and *FASN* were considerably upregulated in zebrafish

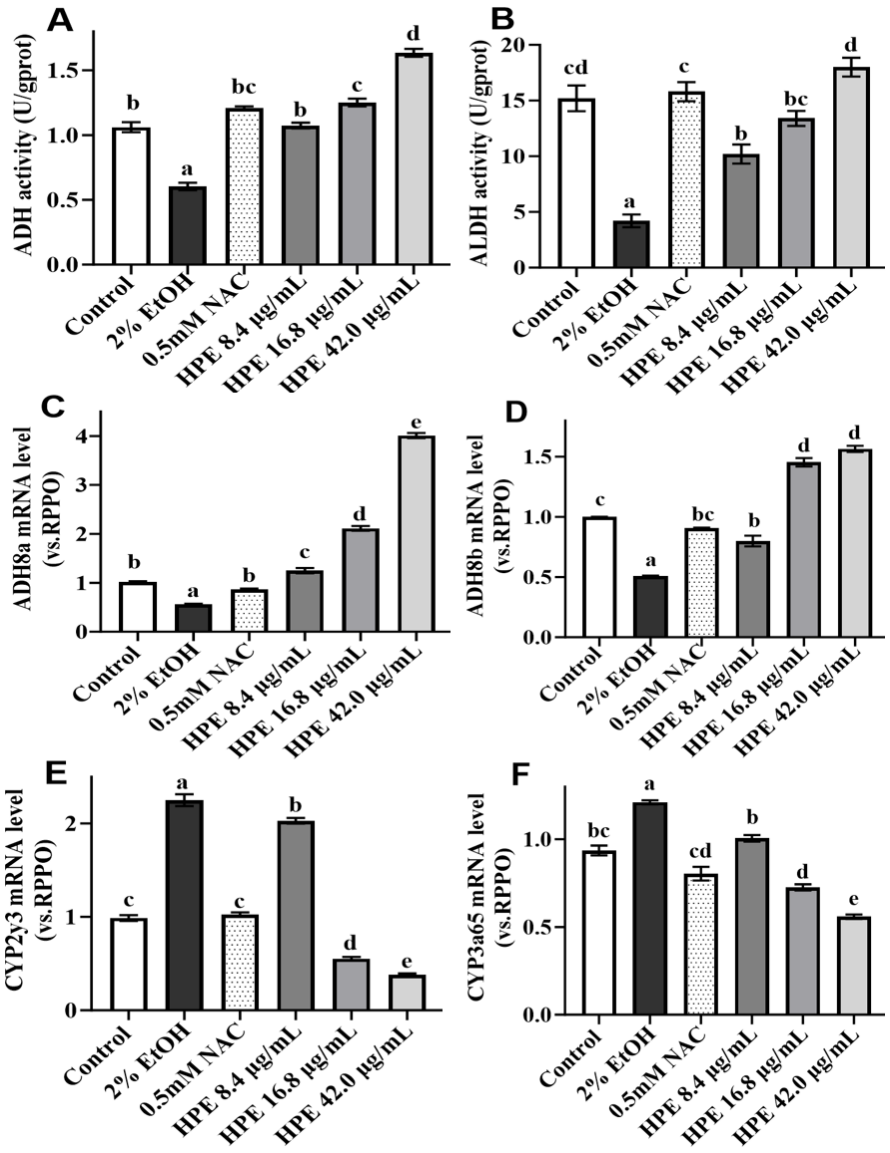

Figure 4. HPE alleviated ethanol metabolism in alcoholic liver injury zebrafish. **A.** ADH activity. **B.** ALDH activity. **C.** The expression level of *ADH8a*. **D.** The expression level of *ADH8b*. **E.** The expression levels of *CYP2y3*. **F.** The expression level of *CYP3a65*. Data were presented as mean ± SD (n = 3). The different lowercase letters indicated significantly difference ($P < 0.05$).

larvae exposed to 2% EtOH with a concurrent increase in TG and TC levels (Figure 5). In contrast, the expression levels of *HMGCRa*, *HMGCRb*, and *FASN* were significantly downregulated in the NAC and medium-dose HPE treatment groups, while TG and TC levels were notably reduced.

HPE ameliorated ethanol-induced liver damage in zebrafish

In the 2% EtOH group, the expression levels of *CHOP* and *GADD45αa* were markedly upregulated with a significant elevation in ALT and AST activities. Conversely, the NAC and HPE treatment groups significantly downregulated the ethanol-induced overexpression of *CHOP* and *GADD45αa* along with a decrease in ALT and AST activities. Furthermore, the HPE treatment groups significantly reduced ALT and AST activities, exhibiting a dose-dependent protective effect. Among these groups, the high-

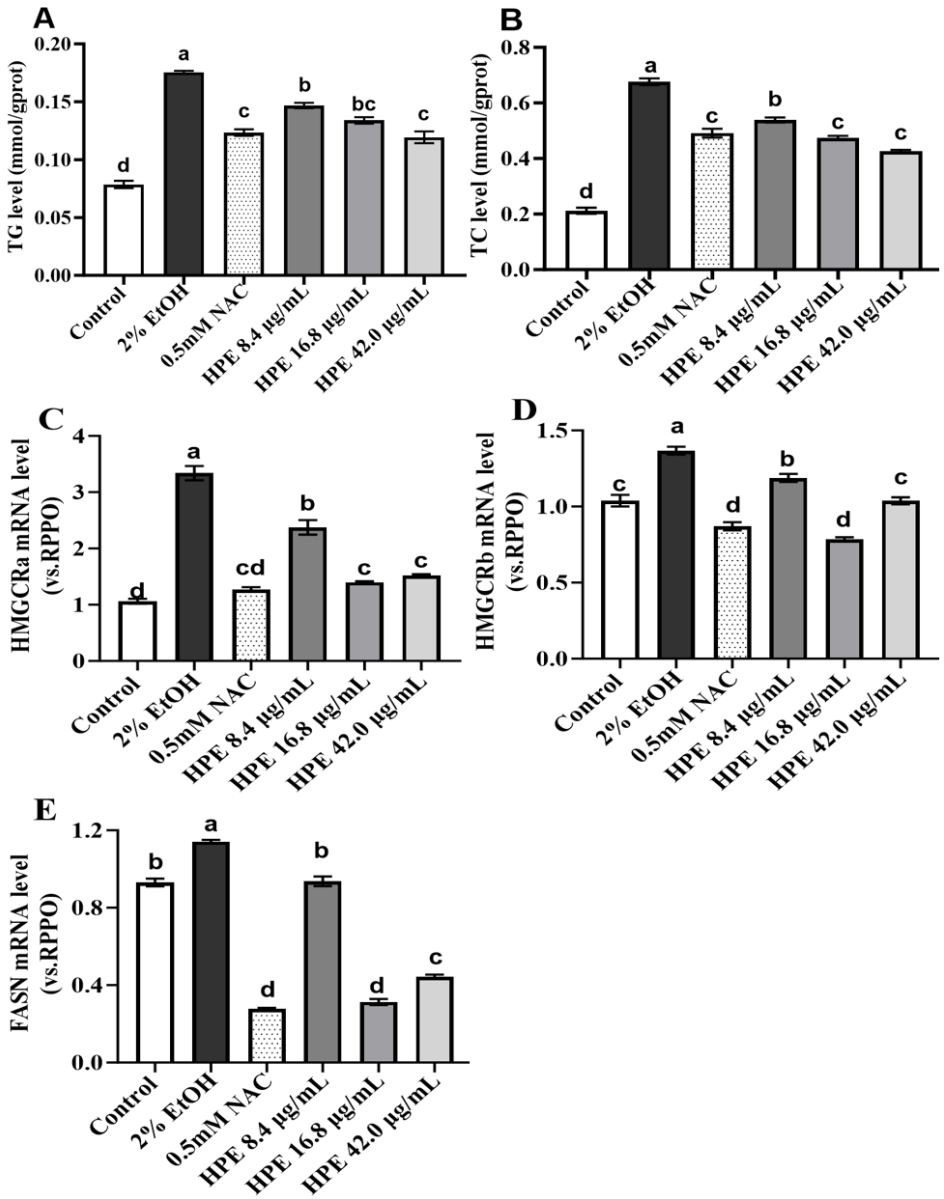

Figure 5. HPE diminished ethanol-induced lipid accumulation in zebrafish. **A.** TG level. **B.** TC Level. **C.** The expression level of *HMGCRa*. **D.** The expression level of *HMGCRb*. **E.** The expression level of *FASN*. Data were presented as mean ± SD (n = 3). The different lowercase letters indicated significantly difference ($P < 0.05$).

dose HPE treatment group demonstrated the most pronounced reduction in ALT and AST activities, nearly reaching those observed in the NAC group (Figure 6).

HPE inhibited ethanol-induced oxidative stress responses in zebrafish

The results showed that SOD activity was markedly diminished, while MDA content was notably elevated, and *Nrf2* expression levels were upregulated in zebrafish larvae in the model group. The results indicated that acute ethanol treatment reduced antioxidant enzyme activity in larvae, causing excessive accumulation of free radicals, exacerbating lipid peroxidation, and leading to a marked increase in MDA content and hepatocellular oxidative stress damage. The HPE treatment groups showed a significant dose-

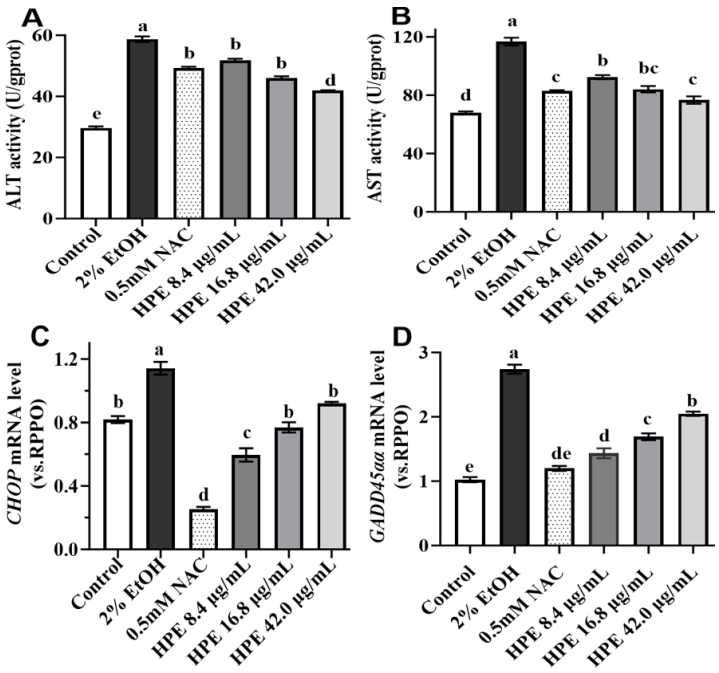

Figure 6. HPE ameliorated ethanol-induced liver damage in zebrafish. **A.** Effect of HPE on the activity of ALT. **B.** Effect of HPE on the activity of AST. **C.** Effect of HPE on the expression of *CHOP*. **D.** Effect of HPE on the expression of *GADD45αα*. Data were presented as mean ± SD (n = 3). The different lowercase letters indicated significantly difference ($P < 0.05$).

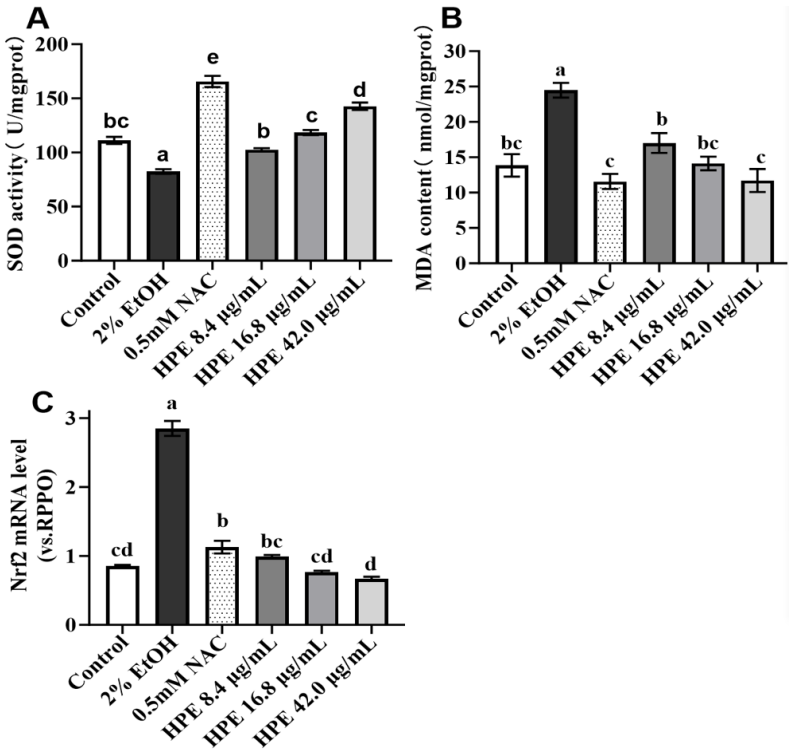

Figure 7. HPE inhibited ethanol-induced oxidative stress responses in zebrafish. **A.** The activity of SOD. **B.** MDA content. **C.** The expression of *Nrf2*. Data were presented as mean ± SD (n = 3). The different lowercase letters indicated significantly difference ($P < 0.05$).

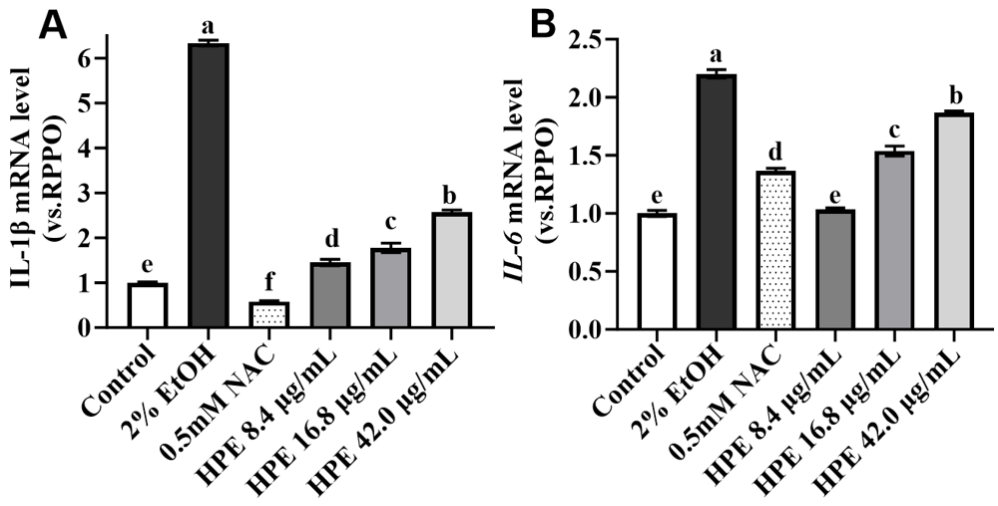

Figure 8. HPE reduced ethanol-induced inflammatory responses in zebrafish. **A.** The expression level of *IL-1β*. **B.** The expression level of *IL-6*. Data were presented as mean ± SD (n = 3). The different lowercase letters indicated significantly difference ($P < 0.05$).

dependent increase in SOD activity, a reduction in MDA content, and a decrease in ethanol-induced *Nrf2* overexpression (Figure 7).

HPE reduced ethanol-induced inflammatory responses in zebrafish

The expression levels of *IL-1β* and *IL-6* were markedly upregulated in the model group, indicating enhanced inflammatory activity in zebrafish larvae. However, the positive control group and all HPE treatment groups significantly downregulated the ethanol-induced overexpression of *IL-1β* and *IL-6* with the most prominent efficacy observed in the high-dose HPE group (Figure 8).

Discussion

ALD is one of the liver diseases that currently affect human health. It is a series of liver damaging lesions caused by excessive or frequent alcohol consumption [16]. Both *Hovenia dulcis* and *Phyllanthus emblica* are medicinal and edible plants, which possess health-beneficial effects such as promoting alcohol metabolism, hepatoprotection, antioxidation, and inflammation [9]. Our previous research confirmed the presence of gallic acid, dihydromyricetin, corilagin, paclitaxel, and ellagic acid in HPE [14]. Gallic acid and dihydromyricetin are the main components of HPE, forming stable O-H••O hydrogen bonds. These interactions may promote the formation of supramolecular structure, potentially enhancing the synergistic effects of the active ingredients in HPE [17]. Acute ethanol treatment aggravates hepatic lipid accumulation and triggered hepatic steatosis in zebrafish larvae [18]. In contrast, HPE intervention attenuates ethanol-induced hepatotoxicity and steatosis induced in this model. Based on the compatibility theory of traditional Chinese medicine and supramolecular fractionation technology, this study prepared HPE and established an acute alcohol-induced hepatic injury zebrafish model to examine the protective effect of HPE against hepatic damage in the zebrafish ALD model to search potential drugs for alleviating liver injury caused by ALD.

Approximately 90% of the alcohol consumed by the human body is metabolized in the liver. Ethanol metabolism in human hepatocytes primarily occurs through two enzymatic pathways including ADH and the microsomal ethanol oxidizing system. These pathways convert ethanol into acetaldehyde, which is then metabolized by ALDH into non-toxic acetic acid for excretion. In zebrafish, *ADH8a* and *ADH8b* are class I alcohol dehydrogenases that share high

similarity with *ADH1*, the primary ethanol-metabolizing enzyme in humans. Additionally, *CYP2y3* facilitates the oxidative metabolism of ethanol, leading to the progressive accumulation of acetaldehyde and reactive oxygen species (ROS), thereby inducing oxidative stress [19]. *CYP3a65* is crucial for the biotransformation of both endogenous and exogenous substrates [20]. The overexpression of *CYP2y3* and *CYP3a65* can cause severe hepatocellular damage. Based on the pathogenesis of ALD, this research found that HPE upregulated the expression of *ADH8a* and *ADH8b* and enhanced ADH and ALDH activities in zebrafish liver, which accelerated ethanol decomposition, shortened intoxication recovery time, and increased the ethanol metabolism rate in the zebrafish. Meanwhile, HPE alleviated ethanol-induced metabolic toxicity by downregulating the expression of *CYP2y3* and *CYP3a65*. These effects might constitute the initial protective mechanism by which HPE mitigated ethanol-induced liver damage in ALD. In zebrafish, *HMGCRa* and *HMGCRb* serve as key regulatory genes for lipid metabolism and cholesterol biosynthesis [21]. Similarly, *FASN* mediates *de novo* long-chain fatty acid synthesis, and its overexpression is associated with TG accumulation [22]. Past research found that ethanol intervention disrupted the tricarboxylic acid cycle and fatty acid oxidation and, therefore, upregulated the expressions of *HMGCRa*, *HMGCRb*, and *FASN*, promoting the synthesis of TG and TC, and exacerbating hepatic fat accumulation and steatosis [23]. HPE reduced the synthesis rate of TG and TC by inhibiting the ethanol-induced upregulation of *HMGCRa*, *HMGCRb*, and *FASN*, thereby reducing hepatic TG and TC production and relieving excessive lipid deposition in hepatocytes. These results indicated that HPE alleviated ethanol-induced hepatic steatosis in zebrafish larvae by suppressing the overexpression of these key lipogenic genes, which represented a potential mechanism for its anti-steatotic effect. Endoplasmic reticulum (ER) stress and DNA damage caused by ethanol metabolism are closely related to liver dysfunction. *CHOP* is a major signaling molecule involved in ER stress-mediated apoptosis, which can activate pro-apoptotic factors [24]. *GADD45αa* functions as a DNA damage-inducible gene that mediates cell cycle arrest and induces apoptosis. The upregulated expression of *CHOP* and *GADD45αa* leads to cell growth arrest, apoptosis, and necrosis in zebrafish hepatocytes, ultimately resulting in liver injury [25]. ALT and AST activities are common biochemical indicators used to evaluate the extent of hepatocellular damage. This study found that ethanol treatment induced the overexpression of *CHOP* and *GADD45αa* and increased ALT and AST activities, indicating that zebrafish experienced severe ER stress and DNA damage. However, HPE protected cells from ethanol-induced cell death and damage by regulating ER stress-related genes. Specifically, it achieved this anti-apoptotic effect by downregulating the expression of *CHOP* and *GADD45αa* and reducing the activity of ALT and AST. Collectively, these results indicated that HPE protected zebrafish from acute ethanol-induced hepatocellular injury, probably by alleviating ER stress and DNA damage and maintaining cellular integrity. Excessive ethanol intake generates large amounts of ROS during liver metabolism, upsetting the oxidative-antioxidant balance, which may induce hepatic oxidative stress and lipid peroxidation. SOD activity and MDA content are critical biomarkers that reflect the body's antioxidant capacity, lipid peroxidation level, and oxidative stress status. *Nrf2* is a key transcription factor that regulates cellular responses to oxidative damage by controlling the expression of antioxidant and detoxification-related proteins [26]. Oxidative stress injury is one of the main causes of ALD. This study showed that HPE reduced the oxidative stress level in zebrafish liver with acute alcoholic liver injury by suppressing *Nrf2* overexpression, increasing SOD activity, and reducing MDA content in hepatocytes. These effects promoted the breakdown of excessive free radicals in the liver, reduced lipid peroxidation, and thereby enhanced the body’s antioxidant capacity. The results of this study indicated that the antioxidant effect might be the primary mechanism underlying HPE’s protective role

against ethanol-induced hepatocellular injury. IL-1β plays a pivotal role in driving pro-inflammatory responses and acts as a key regulator of secondary inflammatory mediators. IL-6 is involved in immune response and inflammatory processes, contributing to hepatocellular damage by promoting immune cell differentiation and lysosomal release reactions [27]. Excessive ethanol intake can induce the synthesis and release of the downstream pro-inflammatory cytokines IL-1β and IL-6, leading to significant hepatic inflammatory damage. Notably, HPE alleviated ethanol-induced inflammatory injury in zebrafish larvae by suppressing the overexpression of *IL-1β* and *IL-6*. This downregulation of inflammatory gene expression reduced the overall inflammatory response, thereby protecting hepatocytes from ethanol-induced damage, which might be an important mechanism underlying HPE's hepatoprotective effect.

This study elucidated that gallic acid and dihydromyricetin were major compounds in HPE and identified the mechanisms by which HPE alleviated acute alcoholic liver injury in zebrafish, focusing on hepatic steatosis, lipometabolism and alcohol metabolism, oxidative stress, inflammatory responses, and hepatocyte injury. The study demonstrated that HPE could significantly reduce ethanol-induced lipid accumulation, enhance antioxidant enzyme activities, mitigate inflammatory responses, and alleviate hepatocellular injury. These findings suggested that HPE represented a promising functional candidate for ameliorating acute alcohol-induced liver injury by accelerating ethanol metabolism and exerting synergistic hepatoprotective effects through multiple pathways. Future research in mammalian models and clinical trials is essential.

Acknowledgements

This research was supported by the Science and Technology Planning Project of Fujian Province (Grant No. 2023Y0076, 2024S0009, 2024S0006, 2026N0029).

References

1. Singal A. 2021. Similarities and differences between non-alcoholic fatty liver disease (NAFLD) & alcohol-associated liver disease (ALD). Transl Gastroenterol Hepatol. 6:1-1.
2. Rigopoulou EI, Gatselis N, Arvaniti P, Koukoulis GK, Dalekos GN. 2021. Alcoholic liver disease and autoimmune hepatitis: Sometimes a closer look under the surface is needed. Eur J Intern Med. 85(4):86-91.
3. Axley PD, Richardson CT, Singal AK. 2019. Epidemiology of alcohol consumption and societal burden of alcoholism and alcoholic liver disease. Clin Liver Dis. 23(1):39-50.
4. Novitskiy G, Traore K, Wang L, Trush MA, Mezey E. 2010. Effects of ethanol and acetaldehyde on reactive oxygen species production in rat hepatic stellate cells. Alcohol Clin Exp Res. 30(8):1429-1435.
5. Wu J, Danielsson A, Zern MA. 2005. Toxicity of hepatotoxins: New insights into mechanisms and therapy. Expert Opin Investig Drugs. 8(5):585-607.
6. Ryu T, Kim K, Choi SE, Katherine PS, Won-Il. 2023. New insights in the pathogenesis of alcohol-related liver disease: The metabolic, immunologic, and neurologic pathways. Liver Res. 7(1):1-8.
7. Li Z, Shao Z, Li S, Hua M, Sun Y. 2019. Protective effect of extract from *Panax* ginsen and *Hovenia dulcis* Thunb against alcohol-induced liver injury in mice. Sci Technol Food Ind. 4:302-306.
8. Zhang N, Yang H, Yan X, Zhao J, Zhao Y, Yu Z. 2020. Liver metabolomics study on protection of *Hoveniae semen* on alcoholic liver disease in rats. J Shenyang Pharm Univ. 37(11):1003-1008.
9. Hsu CL, Yen GC. 2007. Effect of gallic acid on high fat diet-induced dyslipidaemia, hepatosteatosis and oxidative stress in rats. Br J Nutr. 98(4):727-735.
10. Lu CC, Yang SH, Hsia SM, Wu CH, Yen GC. 2016. Inhibitory effects of *Phyllanthus emblica* L. on hepatic steatosis and liver fibrosis *in vitro*. J Funct Foods. 20:20-30.
11. Thilakchand KR, Mathai RT, Simon P, Ravi RT, Baliga-Rao MP, Baliga MS. 2013. Hepatoprotective properties of the Indian gooseberry (*Emblica officinalis gaertn*): A review. Food Funct. 4(10):1431-1441.
12. Chen Z, Yue Y, An H, Yan H, Park HJ, Lin P. 2025. Therapeutic effects of *Pueraria lobata* (Willd.) Ohwi root and *Hovenia dulcis* Thunb. extracts on alcoholic liver disease: Network pharmacology and experimental validation. J Tradit Chin Med Sci. 12(1):100-111.
13. Morrison JK, DeRossi C, Alter IL, Nayar S, Giri M, Zhang C. 2022. Single-cell transcriptomics reveals conserved cell identities and fibrogenic phenotypes in zebrafish and human Liver. Hepatol Commun. 6(7):1711-1724.
14. Wang X, Zhang JB, He KJ, Wang F, Liu CF. 2021. Advances of zebrafish in neurodegenerative disease: From models to drug Discovery. Front Pharmacol. 12:1802-18010.

15. Zhang M, Zhang H, Huang Z, Zhang Y, Liang L, Lou B. 2024. Analysis of targeted components in the combination extract of *Hovenia dulcis* and *Phyllanthus emblica* by UPLC-MS/MS. J Biobased Mater Bioenergy. 18(1):151-156.
16. Chen C, Zuo Y, Hu H, Li X, Zhang L, Yang D. 2023. Hepatic lipid metabolism disorders and immunotoxicity induced by cysteamine in early developmental stages of Zebrafish. Toxicology. 493:153555.
17. Yin H, Wu N, Zhou B, Hong M, Zhu B, Qi M, *et al*. 2021. Slow-release drug-drug cocrystals of oxaliplatin with flavonoids: Delaying hydrolysis and reducing toxicity. Cryst Growth Des. 21:75-85.
18. Wang ZL, Tao L, Yang M, Wang YL, Wang Y, Tian Y. 2024. Research progress on the intervention of food-borne polyphenols in alcoholic liver disease. J Chin Inst Food Sci Technol. 24:479-491.
19. Tsedensodnom O, Vacaru AM, Howarth DL, Yin C, Sadler KC. 2013. Ethanol metabolism and oxidative stress are required for unfolded protein response activation and steatosis in alcoholic liver disease. Dis Model Mech. 6(5):1213-1226.
20. Lee M, Nam S, Yoon HG, Kim S, You Y, Choi KC. 2022. Fermented *Curcuma longa* L. prevents alcoholic fatty liver disease in mice by regulating *cyp2e1*, *srebp-1c*, and *ppar-α*. J Med Food. 254:456-463.
21. Jung YS, Radhakrishnan K, Hammad S, Müller S, Müller J, Noh JR, *et al*. 2024. Errγ-inducible *fgf23* promotes alcoholic liver injury through enhancing *cyp2e1* mediated hepatic oxidative stress. Redox Biol. 71:103107.
22. Wu N, Sarna LK, Hwang SY, Zhu Q, Karmin O. 2013. Activation of 3-hydroxy-3-methylglutaryl coenzyme a (HMG-CoA) reductase during high fat diet Feeding. BBA-Mol Basis Dis. 1832(10):1560-1568.
23. Christoph D, Marc-Oliver R, Georgi K, Michael S, Kathrin S, Weiss TS, *et al*. 2010. Expression of fatty acid synthase in nonalcoholic fatty liver disease. Int J Clin Exp Patho. 3(5):5-14.
24. Hyun J, Han J, Lee C, Yoon M, Jung Y. 2021. Pathophysiological aspects of alcohol metabolism in the liver. Int J Mol Sci. 22(11):5717.
25. Liu YS, Yuan MH, Zhang CY, Liu HM, Liu JR, Wei AL. 2021. *Puerariae lobatae* radix flavonoids and puerarin alleviate alcoholic liver injury in zebrafish by regulating alcohol and lipid metabolism. Biomed Pharmacother. 134:111121.
26. Gao T, Lin L, Yang Q, Zhu Z, Wang S, Xie T. 2024. The raw and Vinegar-processed *Curcuma phaeocaulis* val. ameliorate TAA-induced zebrafish liver injury by inhibiting TLR4/MyD88/NF-κB signaling pathway. J Ethnopharmacol. 319 (Part2):14.
27. Leeyeon K, Weon LJ, Hyeyoung K. 2018. Docosahexaenoic acid inhibits IL-6 and IL-1β expression by suppressing production of mitochondrial reactive oxygen species in alcoholic pancreatitis. Free Radic Bio Med. 120:S94-S95.