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# Naringin attenuates alcoholic liver injury by reducing lipid accumulation and oxidative stress

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ABSTRACT

*Aims:* Alcoholic liver disease (ALD) is a leading health risk worldwide, which can induce hepatic steatosis, progressive fibrosis, cirrhosis and even carcinoma. As a potential therapeutic drug for ALD, naringin, an abundant flavanone in grapefruit, could improve resistance to oxidative stress and inflammation and protects against multiple organ injury. However, the specific mechanisms responsible for protection against alcoholic injury remain not fully understood. In this study, we aim to investigate the effect and the regulatory mechanisms of naringin in the liver and whole body after alcohol exposure under zebrafish larvae system.

*Main methods:* At 96 h post fertilization (hpf), larvae from wild-type (WT) and transgenic zebrafish, with liver-specific eGFP expression (Tg(lfabp10α:eGFP)), were exposed to 2% ethanol for 32 h to establish an ALD model. Different endpoints, such as morphological changes in liver shape and size, histological changes, oxidative stress-related free radical levels, apoptosis and the expression of certain genes, were chosen to verify the essential impact of naringin in alcohol-induced liver lesions.

*Key findings:* Subsequent experiments, including Oil red O, Nile red, pathological hematoxylin and eosin (H&E), and TUNEL staining and qPCR, revealed that naringin treatment reduced alcoholic hepatic steatosis, and this inhibitory effect was dose dependent. Specifically, a 25 mg/L dose resulted in an almost normal response.

*Significance:* This finding suggested that naringin may inhibit alcoholic-induced liver steatosis and injury by attenuating lipid accumulation and reducing oxidative stress and apoptosis.

*Abbreviations:* ALD, Alcoholic liver disease; GK, glucokinase; PPAR γ, peroxisome proliferator-activated receptor γ; GLUT4, glucose transporter-4; GLUT2, glucose transporter-2; G6Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase; FAS, fatty acid synthase; G6PD, glucose-6-phosphate dehydrogenase; ACAT, acyl-coenzyme A:cholesterol acyltransferase; VCAM-1, vascular cell adhesion molecule-1; MCP-1, monocyte chemotactic protein-1; ICAM-1, intercellular adhesion molecule-1; IL-8, interleukin-8; MIP-1α, macrophage inflammatory protein-1 alpha; ERK1/2, extracellular signal-regulated kinase 1/2; JNK, c-Jun N-terminal kinase; p38 MAPK, p38 mitogen-activated protein kinase; LPS, lipopolysaccharide; hpf, hours post fertilization; EGFP, enhanced green fluorescent protein; WT, wild-type; PFA, paraformaldehyde; H&E, hematoxylin and eosin; PBS, phosphate-buffered saline; OCT, optimal cutting tissue; DHE, dihydroethidium; ANOVA, analysis of variance; dpf, days post fertilization; cyp2e1, cytochrome P450 family 2 subfamily E member 1; cyp2y3, cytochrome P450 family 2 subfamily Y polypeptide 3; Fabps, Fatty acid-binding proteins; ROS, reactive oxygen species

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## 1. Introduction

Alcoholic liver disease (ALD), the most common cause of hepatic steatosis and a predisposition for severe liver disease, is a leading health risk worldwide [1]. ALD, a condition caused by excessive alcohol consumption, involves a spectrum of disease states, ranging from simple hepatic steatosis, progressive fibrosis, cirrhosis. Often, as a person continues to drink heavily, cirrhosis will ultimately progress to hepatocellular carcinoma [2]. Considering the growing prevalence of ALD, more efficacious therapy is needed.

A considerable amount of experimental and epidemiological research has found that reduced risk of coronary heart disease, diabetes and non-alcoholic fatty liver diseases is positively correlated with dietary flavonoid intake [3]. Naringin (4′,5,7-trihydroxyflavone-7-rhamnoglucoside), which is abundant in grapefruit and related citrus species [3], has been cogently reported to normalize blood glucose and cholesterol levels and hepatic lipid levels and to improve insulin signaling [4–6]. Through naringin's colonic metabolite, naringenin, this flavonoid also effectively attenuates inflammation and protects against oxidation and lipoperoxidation [7–9]. In addition, naringin has been experimentally documented to have potential therapeutic advantages for relieving hepatotoxicity and preventing liver injuries in liver cancer through antioxidative and antilipoperoxidative activities [10]. Increased glucokinase (GK), peroxisome proliferator-activated receptor gamma (PPAR γ), and glucose transporter-4 (GLUT4) expression and decreased glucose transporter-2 (GLUT2), glucose-6-phosphatase (G6Pase), and phosphoenolpyruvate carboxykinase (PEPCK) expression, which are thought to correlate with glucose modulation, were detected in type 2 diabetic rabbit models after naringin administration. A concurrent decrease in fatty acid and cholesterol metabolism-related factors, such as fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PD), and hepatic fatty acid β-oxidation, was also observed after naringin administration in the same experiment [11]. In response to high-cholesterol consumption, the cholesterol-lowering effects of naringin are mediated by decreased hepatic acyl-coenzyme A:cholesterol acyltransferase (ACAT) activity and increased fecal sterol excretion [4]. Interestingly, a downregulation of ACAT activity induced by naringin administration was also found in Lee CH's research, along with downregulated vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemotactic protein-1 (MCP-1) gene expression [12]. Naringin, not lovastatin, was proven to exert significant liver protective properties by preventing fatty liver and increasing liver enzyme levels. This effect was mediated via the downregulation of fatty streak formation, neointimal macrophage infiltration and intercellular adhesion molecule-1 (ICAM-1) expression, which appears to play a crucial part in anti-atherosclerosis [13]. Regarding anti-inflammation, decreased interleukin-8 (IL-8), MCP-1, and macrophage inflammatory protein-1 alpha (MIP-1α) secretion and gene translation and suppressed extracellular signal-regulated kinase 1/2(ERK1/2), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation were identified in vitro in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages treated with 50-200 μM naringin [14]. Consistent with these findings, naringin works in an analogous manner in vivo, as evidenced by the variety of biological targets and complicated mechanisms found in previous studies [15–19]. In a variety of disorders, such as toxic chemical-induced nephrotoxicity [20] and hepatotoxicity [10], radiation-induced damage [21, 22] and renal/testicular ischemia-reperfusion injury [23, 24], naringin exerts significant protective action by ameliorating injury markers and lipid peroxidation, as well as scavenging free radicals and enhancing oxidation resistance. Together, these findings clearly demonstrate that naringin is a potent protector against oxidation.

Recently, zebrafish have been used as model organisms to investigate human diseases and toxicology because of their suggested high genetic similarity to humans and their superior laboratory model characteristics, such as improved modeling speed and model quantity and the opportunity for continuous in vivo observation [25–28]. Primary zebrafish liver morphogenesis is complete at 48 h post fertilization (hpf) and full liver function is detected at 72 hpf [28]. In our study, a transgenic line of zebrafish that expresses enhanced green fluorescent protein (EGFP) in the liver was selected to label liver size, shape and location in zebrafish larvae. Thus, zebrafish models show promise for liver disease and drug screening studies.

Since naringin may be a superior therapeutic for alcohol-induced hepatic steatosis, exploring this incompletely understood molecular mechanism is greatly important. In our present study, we carried out a series in vivo experiments with zebrafish larvae to investigate the hepatoprotective role of naringin in an ALD model. Different endpoints, such as morphological changes in liver shape and size, histological changes, oxidative stress-related free radical levels, apoptosis and the expression of certain genes, were chosen to verify the essential impact of naringin on alcohol-induced liver lesions. Considering the ability of naringin in this ALD model, we suggest that citrus flavonoids may be effective tools for identifying regulators of alcohol metabolism, lipid homeostasis and liver lesions.

## 2. Materials and methods

### 2.1. Zebrafish husbandry and exposure protocols

All our studies were carried out using adult wild-type (WT) zebrafish (AB strain) and liver-specific EGFP transgenic zebrafish (*Tg (lfabp10α:eGFP)*), obtained from the Key Laboratory of Zebrafish Modeling and Drug Screening for Human Diseases of Guangzhou Higher Education Institute, Southern Medical University. This transgenic line contains the *lfabp10α* promoter that drives hepatocyte-specific expression and an enhanced-GFP tag for visualizing the cells that incorporate the transgene. Zebrafish were maintained on a 14-h light:10-h dark cycle at 28 °C. All zebrafish experimental protocols were approved by the Institutional Animal Care and Use Committee of Southern Medical University.

At 96–98 hpf, zebrafish larvae were randomly divided into two groups: one control group and one model group. Zebrafish larvae in the model group were incubated in a 2% ethanol solution for up to 32 h in an incubator at 28 °C, while the control group was treated with fish water only [29]. Then, the model group was separated into five groups and respectively exposed to fish water, 0.1% DMSO or 6.25, 12.5, or 25 mg/L naringin for 48 h. Larvae were collected and observed, and the general status of each group was recorded.

### 2.2. H&E staining

Zebrafish larvae were fixed in 4% paraformaldehyde (PFA) at 4 °C overnight and later embedded in paraffin, and 4-μm sections were mounted on slides. Then, sections were routinely processed for hematoxylin and eosin (H&E) staining and were observed and photographed using a light microscope (Nikon Eclipse Ni-U; Nikon, Tokyo, Japan).

### 2.3. Whole fish oil red O staining

After collecting and fixing in 4% PFA, zebrafish larvae from each group were washed three times with phosphate-buffered saline (PBS) and respectively infiltrated with 20, 40, 80 and 100% 1,2-propylene glycol (Sigma, USA) at room temperature for 15 min. Then, larvae were incubated in 0.5% Oil red O (Sigma, USA) diluted in 100% 1,2-propylene glycol at 65 °C in the dark for 1 h. To fade the background colour, we washed the stained larvae with 100, 80, 40 and 20% 1,2-propylene glycol respectively for approximately 25 min. Finally, the stained larvae were washed three times with PBS and stored in 70% glycerol (Sigma, USA). Images of liver lipid droplets were taken and observed using a bright-field dissecting microscope (Olympus szx10, Tokyo, Japan).

### 2.4. Oil red O staining of cryosections

Fresh larvae were fixed in 4% PFA overnight at 4 °C and incubated with 30% sucrose at 4 °C for 3 days. Then, the zebrafish were embedded in optimal cutting tissue (OCT) compound (Leica, Germany), sectioned at 15 μm and stored at −20 °C. Before staining, slides were warmed to room temperature and washed with water for 15 s to remove OCT. Slides were permeated twice in 100% 1,2-propylene glycol for 5 min and stained with 0.7% Oil red O (Sigma, USA) in the dark at 60 °C for 10 min. Slides were immediately destained in 85% 1,2-propylene glycol for 3 min and washed with water to clean the background. The slides were photographed using a light microscope (Nikon Eclipse Ni-U; Nikon, Tokyo, Japan).

### 2.5. Nile red staining of cryosections

We conducted Nile red staining using the abovementioned cryosections. The slides were first stained with DAPI (Solarbio Life Science, China), a nuclear staining dye, in the dark for 4 min at room temperature and washed with PBS once. Thereafter, Nile red dye (Sigma USA), at a final concentration at 0.5 μg/mL in acetone, was used to stain the lipid droplets on cryosections for 10 min. Imaging was performed with a light microscope (Nikon Eclipse Ni-U; Nikon, Tokyo, Japan) after washing the slides with PBS once.

### 2.6. Quantitative real-time PCR

Total RNA was extracted from 10 larvae, purified according to the standard procedure [30] and subsequently reverse-transcribed with qScript cDNA using a PrimeScript™ RT-PCR kit (Takara). qPCR was carried out on a LightCycler 96 instrument (Roche, Switzerland) using a SYBR Green kit (Takara Biotechnology, Inc.). Target gene expression was calculated by the comparative CT method. Gene *rppo* was used as a reference, and the primers for each gene are listed in Table 1.

### 2.7. Superoxide detection

After naringin treatment, live larvae were immediately transferred to 24-well plates and incubated with 10 μm dihydroethidium (DHE, Beyotime) solution in the dark at 28 °C for 10 min. After incubation, larvae were washed with fish water twice and anesthetized using 0.2% tricaine (Sigma, USA). DHE fluorescence distribution was recorded by a light microscope (Nikon Eclipse Ni-U; Nikon, Tokyo, Japan) at 100× magnification.

### 2.8. TUNEL staining cryosections

TUNEL assays were used to determine whether naringin was able to reverse apoptosis in zebrafish larvae. TUNEL staining was performed on cryosections using an In Situ Cell Death Detection kit (Roche). Slides were permeabilized in 0.01% Triton X-100, washed with PBS and stained with 1:10 TUNEL working solution in the dark at 37 °C for 1 h. Nuclei were stained by DAPI in the dark at room temperature for 4 min and were counted. The slides were immediately photographed with a light microscope (Nikon Eclipse Ni-U; Nikon, Tokyo, Japan).

**Table 1**
Primers used to quantify mRNA levels.

| Gene | FP sequence (5′-3′) | RP sequence (5′-3′) |
|---|---|---|
| rppo | ctgaacatctcgcccttctc | tagccgatctgcagacacac |
| Cyp2y3 | tattcccatgctgcactctg | aggagcgtttacctgcagaa |
| Fabp 10α | ttacgctcaggagaactacga | ggatgtgggagaatcggtcag |

### 2.9. Statistical analysis

Statistical tests were executed using SPSS statistical software (version 16.0). The results are presented as the means ± SD. Differences between Oil red O staining in the control and ethanol group were quantified at the gray level and evaluated with Student's *t*-test. One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, was used to assess the qPCR, Oil red O staining and superoxide detection results for differences among the control group, ethanol group and naringin group. Values were considered statistically significant when $P < 0.05$.

## 3. Results

### 3.1. Establishment of the ALD model

To avoid the metabolic influence of fasting, larvae was subjected to ethanol at 96–98 hpf, a window of time after the liver is formed but before all the yolk is utilized (5.5–6 days post fertilization (dpf)) [31]. To differentiate between acute and chronic alcohol exposure, we exposed zebrafish larvae to ethanol for 32 h.

Based on a considerable body of research, the characteristic morphological phenotype, hepatomegaly and behavioral abnormalities were observed in the majority of zebrafish larvae after a 32-h exposure to 2% ethanol [32, 33]. At 96–98 hpf, we chose to expose *Tg(lfabp10α:eGFP)* larvae to 2% ethanol for 32 h to further verify the hepatic morphological phenotype. Consistent with our previous results, hepatomegaly lordosis occurred in the majority of larvae after the 32-h ethanol treatment. After the 32-h exposure to 2% ethanol, severe lipid sedimentation was obtained using frozen whole fish Oil red O and H&E (Fig. 1A) staining. Positive Oil red O staining in the liver was quantified by ImageJ software (Fig. 1B). Consistently, Nile red staining on cryosections revealed that the model group exhibited more severe lipid accumulation (Fig. 1C). Considering our findings, an ALD model was successfully established following 2% ethanol exposure for 32 h.

### 3.2. Effect of naringin on downregulating lipid accumulation

As demonstrated above, major lipid accumulation was observed in the liver of zebrafish larvae after ethanol stimulation. Notably, a prominent dose-dependent decrease in lipid deposition was visualized via Oil red O staining on whole fish and cryosections (Fig. 2A, B). Hepatic steatosis severity was quantified according to the gray level, and ultimately, the results revealed that naringin substantially downregulated hepatic steatosis(Fig. 2C). Our findings indicate that 25 mg/L naringin can reverse alcoholic lipid accumulation in zebrafish larvae. Furthermore, histopathological studies on liver lesions, as assessed by H&E staining of paraffin-embedded larvae sections, were consistent with the above results (Fig. 2D).

### 3.3. Effect of naringin on regulating alcoholic injury- and lipid metabolism-related genes

To investigate whether naringin attenuates lipid metabolism, improving lipid homeostasis and reducing alcohol-induced hepatic steatosis, we quantified related gene (*cyp2y3, fabp10α*) levels via qPCR. Cytochrome P450 family 2 subfamily E member 1 (*cyp2e1*) is believed to be the dominant enzyme that regulates the oxidative stress response by mediating alcohol metabolism in mammals. Prior studies have proven that cytochrome P450 family 2 subfamily Y polypeptide 3 (*cyp2y3*) shares homology with *cyp2e1* in zebrafish [31]. Increased *cyp2y3* expression indicates accelerated alcohol metabolism and aggravated liver injury [31]. Fatty acid-binding proteins (Fabps), which are abundant in tissues, have been verified to be involved in fatty acid metabolism, lipid uptake and transport and overall lipid homeostasis [34]. As shown in the qPCR results (Fig. 3), a significant increase in the

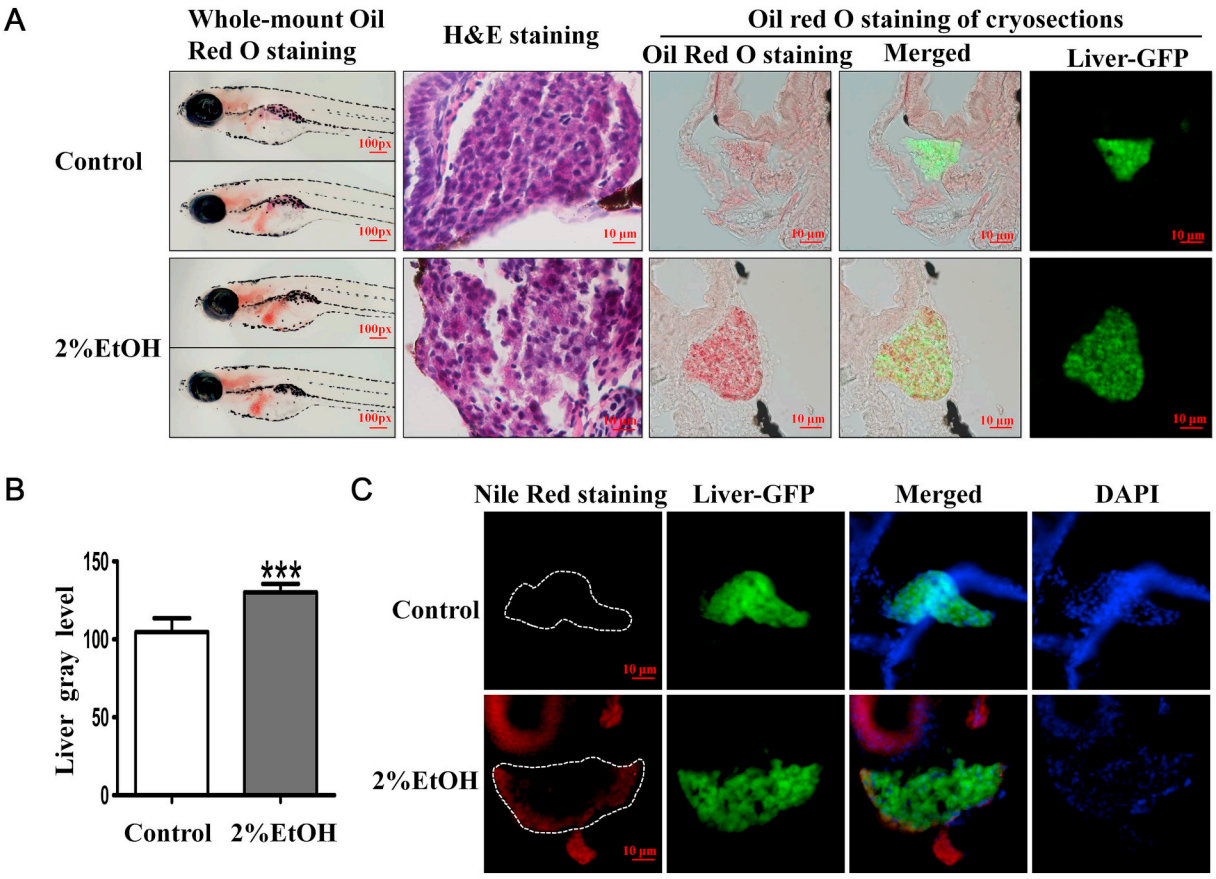


**Fig. 1.** An alcoholic fatty liver disease model was established in zebrafish larvae.
(A)Whole-mount Oil red O staining, Oil red O staining of liver cryosections and H&E staining of paraffin liver sections of zebrafish larvae exposed to 2% ethanol and control larvae. (B) Quantitative gray levels in livers subjected to Oil red O staining; data are expressed as the mean ± SD, n = 20 per group from three experiments. * < 0.05 vs the control group, Student's *t*-test. (C) Nile red staining for intracellular lipid droplets in *Tg(lfabp10α:eGFP)* larvae.

*cyp2y3*, *fabp10α* level was noticed in zebrafish larvae treated with ethanol compared with control larvae. Furthermore, naringin treatment decreased these genes to near normal levels. Our findings indicate that naringin may exert potential therapeutic benefits by improving alcohol and lipid metabolism and reducing toxic substances.

### 3.4. Protective effect of naringin against oxidative stress

Lipid metabolism and oxidative stress are closely related [35, 36]. The superoxide anion is clearly the primary reactive oxygen species (ROS) that exists in mitochondria [37], and superoxide release leads to oxidative stress and cell death [37]. To visualize the degree of liver damage, a liver-specific EGFP transgenic zebrafish (*Tg(lfabp10α:eGFP)*) was used to identify the liver location, and DHE dye was used to detect superoxide radical distribution and amounts. The results revealed that superoxide radical levels in zebrafish livers were significantly increased after ethanol exposure and reversed after treatment with 25 mg/L naringin (Fig. 4A). The distribution and amounts of superoxide anions was quantified according to the fluorescence intensity (Fig. 4B) and suggested a protective effect of naringin on antioxidation.

### 3.5. Protective effect of naringin against apoptosis

Oxidation is usually accompanied by DNA damage. Several studies have now clearly demonstrated that endoplasmic reticulum stress and DNA damage play an important etiologic role in alcoholic liver injury development [31, 38]. TUNEL assays were conducted to elucidate the anti-apoptotic role of naringin on alcoholic liver steatosis. As shown in the results, after ethanol exposure, zebrafish livers presented with increased histological injury severity and apoptotic cell death (Fig. 5). In conclusion, we support the importance of naringin for inhibiting apoptosis.

## 4. Discussion

Hepatic steatosis is the earliest sign of alcohol abuse and may develop into more severe hepatopathy [39]. Chronic hepatic steatosis is the condition that occurs prior to steatosis hepatitis and cirrhosis and makes hepatocytes susceptible to lesions [40]. Therefore, further hepatic lesions triggered by alcohol can be hindered by the blockade of lipid accumulation. Naringin is reportedly able to ameliorate hepatic steatosis in vivo by regulating oxidative stress and inflammation [41]. In addition, recent studies have identified a diverse range of complicated mechanisms of action that suggest naringin is an optimal molecule for protecting against lipid homeostasis and inflammation [4, 7, 9, 20, 42]. However, the effectiveness of naringin on alcoholic and metabolic abnormalities has not been depicted clearly.

On the basis of previous studies [32,33], the method of 2% ethanol exposure for 32 h was selected to establish an acute alcoholic fatty liver zebrafish model. Subsequent experiments, including Oil red O, Nile red, H&E, and TUNEL staining and qPCR, revealed that naringin treatment reduced alcoholic hepatic steatosis, and the inhibitory effect of naringin was dose dependent. Specifically, a 25 mg/L dose resulted in an almost normal response. To further understand the mechanism by which naringin reduces steatosis, qPCR, superoxide detection and TUNEL staining were conducted to identify the effect of naringin on lipid metabolism and oxidative stress and damage, and all the changes support our hypothesis regarding lipid metabolism improvement and antioxidative

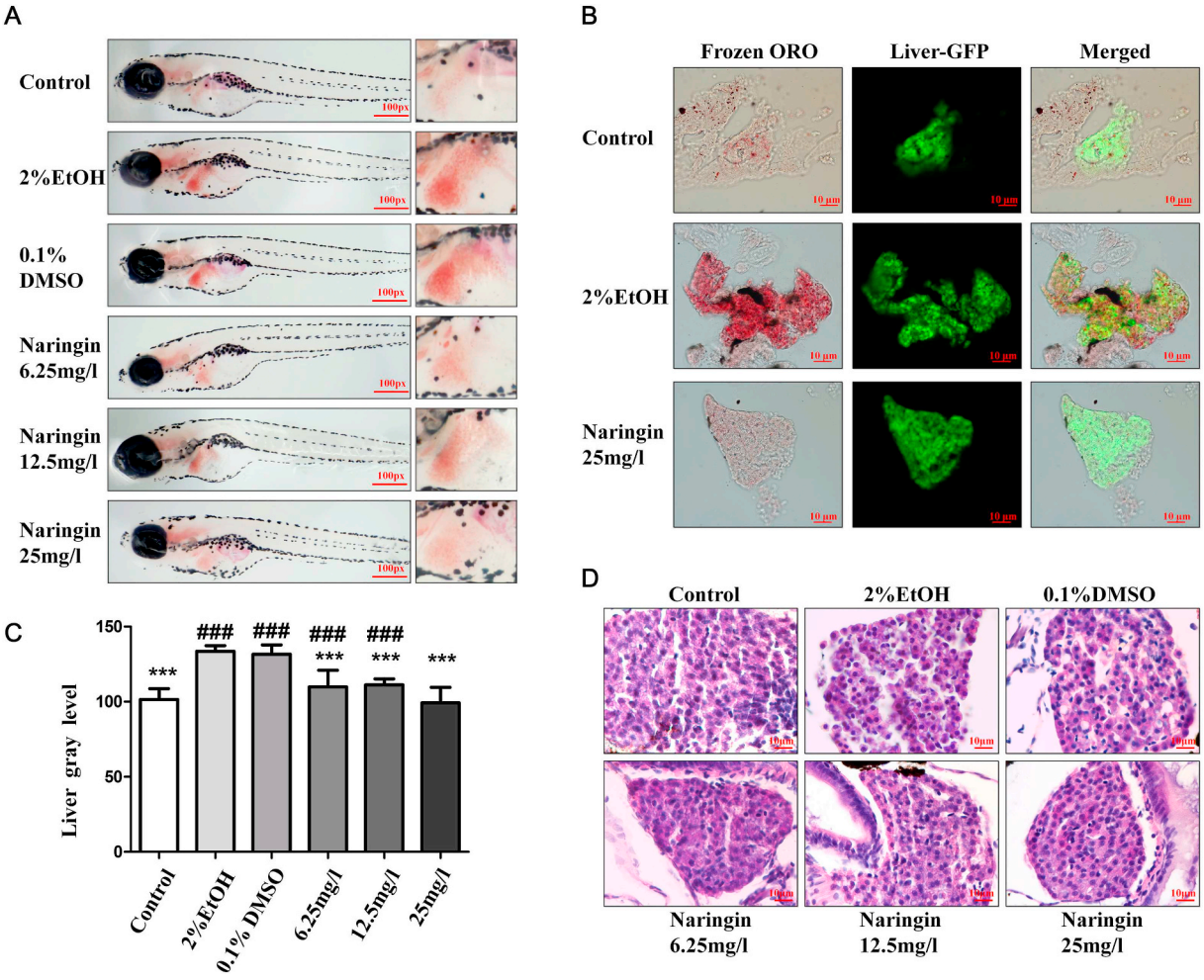


**Fig. 2.** Naringin down-regulated lipid accumulation in zebrafish larvae induced by 2% ethanol treatment in zebrafish larvae.
Whole-mount Oil red O staining for detection of lipid accumulation in zebrafish livers after naringin treatment. (B) Oil red O staining of liver cryosections treated with 2% ethanol and 25 mg/L naringin and control sections. (C) Quantitative analysis of Oil red O staining in larvae treated with 2% ethanol, 0.1% DMSO, and 6.25 mg/L, 12.5 mg/L and 25 mg/L naringin and control larvae. Data are expressed as the mean ± SD, n = 10 per group from two experiments. * < 0.05 vs the 2% ethanol group, # < 0.05 vs the control group using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. (D) H&E staining of paraffin liver sections on zebrafish larvae to detect changes of liver histopathology.

and antiapoptotic effects.

Excessive free radical production leads to oxidative stress, a key factor that leads to many diseases, such as liver disease and cancer. Superoxide is a major oxidation product of lipid accumulation and is an important indicator of oxidative damage in lipid metabolism [35, 36, 43]. Inspired by Chang YC et al.'s study [44], we used DHE (Beyotime) to detect the distribution and amounts of superoxide. Our results suggested that superoxide generation was prominently increased and that

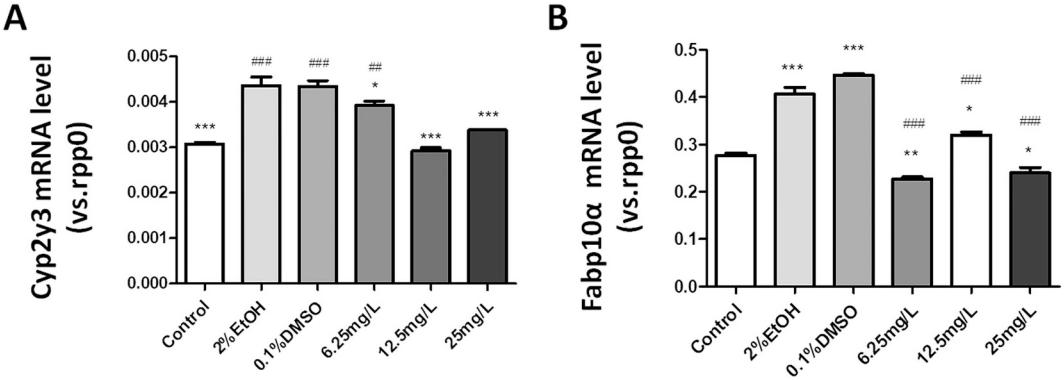


**Fig. 3.** Naringin contributes to downregulation alcoholic injury- and lipid metabolism-related genes.
(A) and (B) Real-time PCR analysis of the mRNA levels of alcohol metabolism(*cyp2y3*) and lipid metabolism (*fabp 10α*) inelated genes in zebrafish larvae treated with 2% ethanol, 0.1% DMSO, and 6.25 mg/L, 12.5 mg/L or 25 mg/L naringin and control larvae. Data are expressed as the mean ± SD, n = 10 from two experiments. * < 0.05 vs 2% ethanol group, # < 0.05 vs the control group according to one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

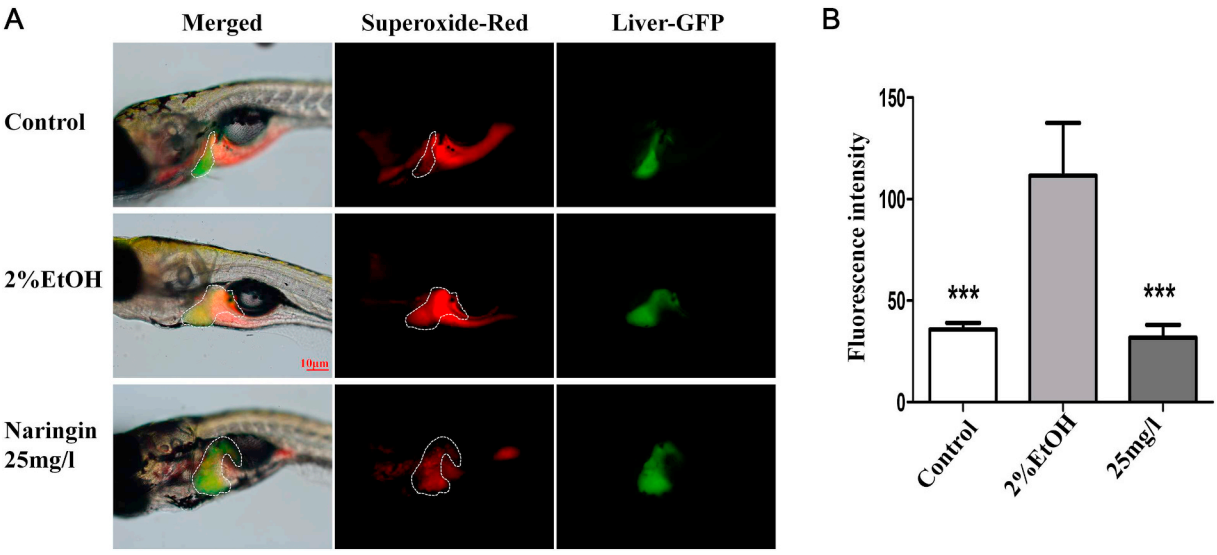


**Fig. 4.** Naringin protected zebrafish larvae against oxidative stress after alcohol administration.
(A)Fluorescence micrographs of superoxide radical generation in zebrafish larvae treated with 2% ethanol or 25 mg/L naringin and control larvae. (B) The distribution and amounts of superoxide anions were quantified according to the fluorescence intensity. Data are express as the mean ± SD. * < 0.05 vs the 2% ethanol group.

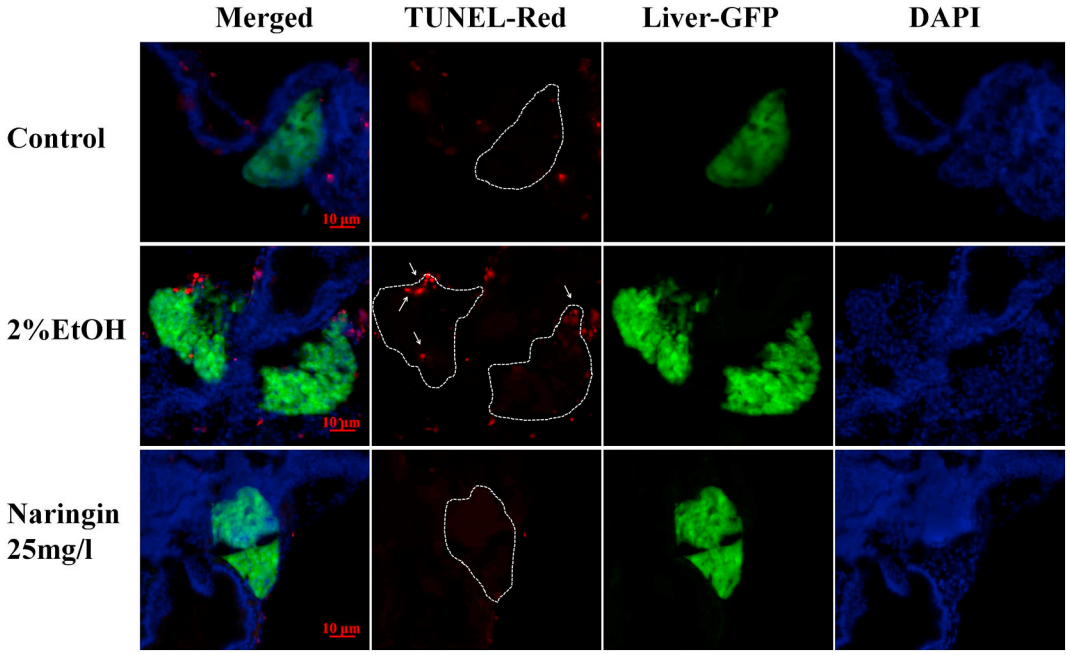


**Fig. 5.** Naringin protected zebrafish larvae against apoptosis during acute alcoholic injury.
In situ detection of cell apoptosis by TUNEL staining of paraffin liver sections in zebrafish larvae treated with 2% ethanol or 25 mg/L naringin and control sections. Apoptotic cells are indicated by white arrowheads.

oxidative stress was present in zebrafish exposed to 2% ethanol. Meanwhile, the *cyp2y3* level was significantly increased. *Cyp2e1*, a homologous *cyp2y3* gene, is involved in generating intramitochondrial ROS [45]. Previous studies revealed plentiful evidence that plants contain antioxidants, such as flavonoids, that are able to scavenge free radicals [46]. In our results, 25 mg/L naringin almost returned these changes to normal levels, which indicated that naringin exerts potential therapeutic benefits in reducing oxidative stress. The fluorescent detection of free radicals in our research was fast, simple and convenient and shows promise for drug screening, including for dosage and chronic toxicity evaluations.

*Cyp2y3* shares homology with *cyp2e1* and is critical for alcohol metabolism in zebrafish liver. *Cyp2y3* shares 43% similarity with *cyp2e1*, which makes it the closest *cyp2e1* homologue [31]. Thus, alcohol metabolism and oxidative stress can be suppressed via *cyp2y3* inhibition. Notably, our present study revealed that naringin protects zebrafish liver from ethanol-mediated damage by downregulating *cyp2y3* mRNA expression. *Fabp10α*, an intracellular fatty acid-binding protein, is associated with fatty acid and intracellular lipid metabolism. Considering all we found, alcoholic liver injury in zebrafish larvae is a collection of aggravated lipid accumulation, alcoholic toxicity, oxidative stress and apoptosis, and naringin contributes to downregulation of mRNA expression of alcoholic injury- and lipid metabolism-related genes.

As far as we know, this is the first experiment to investigate the beneficial role of naringin in the mechanistic link among alcohol and

lipid metabolism, oxidative stress and apoptosis in an ALD zebrafish model. This ALD model is generated easily and quickly. Compared with rodent models, the ALD zebrafish larvae model is hindered by certain limitations. For example, it was not possible to obtain individual liver tissues on such a little larvae and blood from zebrafish larvae, which made it insufficient to detect mRNA and protein expression in the liver and valuate the serum level of biochemical markers of liver damages. However, the zebrafish larvae model has emerged as a powerful model for many diseases, due to its high genetic similarity, short generation time, high fertility and low husbandry cost. We can obtain many larvae in a short time, and screening for abnormal phenotypes is easy since surgery is not needed, as zebrafish larvae are transparent. In summary, zebrafish models show promise for disease research and drug screening.

## 5. Conclusion

In conclusion, our study demonstrated that naringin administration attenuates hepatic steatosis and lesions in an ALD model in zebrafish larvae. Naringin lowered lipids levels, inhibited alcoholic injury, and attenuated oxidative stress and apoptosis, which are the probable mechanisms by which alcohol-induced hepatic steatosis is reversed. However, further studies are warranted to identify the pathways by which naringin balances alcohol and lipid metabolism and reduces oxidative stress and apoptosis. Therefore, we suggest the possibility that citrus flavonoids, such as naringin, may be potential potent therapeutic drugs against alcohol-induced liver injury. Further pre-clinical and clinical trials that establish the safety and efficiency of these compounds are required.

## Author contributions statement

LG, and ZL:conception and design of the study; CZ and YL performed major experiments; CZ, YL, PH, LX, HL and YC organized generation, collection, assembly, and interpretation of data; LG, GD, CM and ZZ participated in the drafting and revision of the manuscript; LG, ZL and SH obtained funding; ZG, YC and XS provided scientific advices; LG and ZL participated in study supervision.

## Conflict of interest

Authors declare no conflict of interest.

## Acknowledgments

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