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Quercetin alleviates ethanol-induced liver steatosis associated with improvement of lipophagy

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ABSTRACT

Although emerging evidence demonstrated that quercetin could be explored as a potential candidate for the early intervention of alcoholic liver disease (ALD), the exact mechanisms against ethanol-induced hepatic steatosis haven't been fully elucidated. Herein, we investigated the effect of quercetin on liver steatosis caused by chronic-plus-single-binge ethanol feeding, focusing on lipophagy. Adult male mice were pair-fed with liquid diets containing ethanol (28% of total calories) and treated with quercetin for 12 weeks. Chronic-plus-binge ethanol consumption led to lipid droplets accumulation and liver damage as evidenced by histopathological changes, the increased content of triglyceride in serum and liver, and the elevated of serum ALT and AST level, which were greatly attenuated by quercetin. Moreover, quercetin blocked autophagy suppression by chronic-binge ethanol intake as manifested by the morphological improvement of mitochondrial characteristics, the increased number of autolysosome and restoration of autophagy-related protein expression. Furthermore, quercetin promoted lipophagy confirmed by the decreased perilipin 2 (PLIN2) level, activated AMPK activity and increased co-localization of liver LC3II and PLIN2 proteins. Collectively, these findings suggest that regular consumption of dietary quercetin has a role in preventing hepatic steatosis induced by chronic-plus-binge ethanol feeding, which mechanism may associate with the evident regulatory effect of quercetin on lipophagy.

1. Introduction

Most of the medical treatments achieved great success in ancient times, which seemed to be the use of phytochemicals. Nowadays, a great deal of scientific research has been emerged to explore the bioactivity of phytochemicals, including various flavonoids, in the diet of medicinal herbs against acute and chronic disease (D'Andrea, 2015). Quercetin, a dietary flavonol, is abundant in onions, broccoli, tomatoes, apples, berries wine, and black or green tea, and accounts for about 75% of human total flavonol consumption (Sampson et al., 2002). Most studies showed that quercetin exerted a substantially beneficial role in diabetic, nonalcoholic fatty liver disease (NAFLD) or alcoholic liver disease (ALD) mechanically by regulating the expression of lipid metabolism related genes and the secretion of inflammatory mediators, which manifested as diminishing the accumulation of hepatic fat droplets, ameliorating inflammatory stimuli, and inhibiting the deposition of hepatic collagen and the progression of hepatic fibrosis (Pisonero-Vaquero et al., 2015; Tang et al., 2012; Wang et al., 2013b; Ying et al., 2013; Zhang et al., 2015).

Global burden of disease (2018) reported that alcohol use was a leading risk factor for disease burden worldwide, accounting for nearly 10% of global deaths among populations aged 15–49 years in 2016. ALD caused by chronic alcohol abuse has been one of global public health problems because of its worldwide consumption and addition. Ethanol-provoked dyslipidemia and lipid accumulation as lipid droplets (LDs) in liver are the prominent pathophysiological manifestations of

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Abbreviations

ALD	alcoholic liver disease
LDs	lipid droplets
NAFLD	nonalcoholic fatty liver disease
PLIN2	Perilipin 2
ROS	reactive oxygen species
TC	total cholesterol
TG	total triglyceride

ALD. And excessive LDs, especially ectopically accumulated lipids, are susceptible to the attack of reactive oxygen species (ROS) resulted from ethanol metabolism, which synergistically aggravates oxidative damage of liver and other tissues, contributes to the cellular signaling impaired, organelle dysfunction, and cell injury and death (Fisher-Wellman and Neufer, 2012; Furukawa et al., 2004; Jaishy and Abel, 2016; Mota et al., 2016; Musso et al., 2018). Consequently, removal of excess lipid droplets is beneficial to the balance of lipid (Wang et al., 2017). Studies showed that quercetin supplement reduced the size/number of lipid droplet and alleviated hepatic lipid accumulation by inhibiting the ROS and thioredoxin-interacting protein pathway (Zhang et al., 2015), regulating the expression of lipid metabolism gene and lipoperoxidation (Jung et al., 2013; Porras et al., 2017; Zhang et al., 2016), or regulating ATP-binding cassette transporter A1, liver X receptor-α and proprotein convertase subtilisin/kexin type 9 (Li et al., 2018). However, the exact mechanisms of quercetin against ethanol-induced hepatic steatosis have not been fully elucidated. Therefore, it's vital to explore the potentially mechanisms of quercetin to prevent ALD.

Autophagosomes selectively target LDs and transport them to the lysosome, in which LDs are degraded by the lysosome acid lipase. This process is termed as lipophagy (Singh et al., 2009). Obviously, lipophagy plays an indispensable role in lipid metabolism. Rasineni et al. (2017) reported that chronic alcohol intake inhibited lipophagy by depleting the lysosomes, aggravating liver damage and steatosis. And other studies showed that enhacement of lipophagy reduced ethanol-induced hepatotoxicity and alleviated hepatic lipid accumulation (Lu and Cederbaum, 2015; Wang et al., 2013a). Our previous data demonstrated that quercetin was conducive to maintain homeostatic balance of redox status by regulating autophagy and mitophagy (Yu et al., 2016). Recent researches suggested that flavonoid, including quercetin, prevented ectopic lipid accumulation through promoting lipophagy in palmitic acid-challenged β-cells and isolated pancreatic islets or high-fat diet induced NAFLD (Varshney et al., 2018; Zhu et al., 2018). To the best of our knowledge, however, little attention has been focused on the effect of quercetin on lipophagy in the chronic-binge ethanol feeding mouse model. Herein, we investigated the protective role of quercetin on the chronic plus a single binge ethanol-induced liver fat accumulation and liver damage by focusing on the lipophagy regulation.

2. Materials and methods

2.1. Chemicals and reagents

Quercetin (purity ≥ 98%, HPLC) was from Sigma-Aldrich (St Louis, Missour, USA). Ethanol (purity ≥ 99.7%, AR) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Anti-GAPDH mouse monoclonal antibody, anti-p62 rabbit monoclonal antibody, anti-*p*-AMPKα rabbit monoclonal antibody, anti-rabbit IgG (secondary antibody) and anti-mouse IgG (secondary antibody) were obtained from Cell Signaling Technology (USA). Anti-LC3II rabbit and anti-LAMP1 mouse monoclonal antibody were provided by Sigma-Aldrich (USA) and Santa Cruz Biotechnology (USA), respectively. The kits of aspartate (AST) and alanine aminotransferases (ALT) were purchased from the Nanjing Jiancheng Corporation (Nanjing, China). Total triglyceride (TG) and cholesterol (TC) were obtained from Biosino Biotechnology Co., Ltd. (Beijing, China). Other chemicals and organic solvents were of analytical grade and purchased from local reagent retailer.

2.2. Animal treatment

Forty adult male C57BL/6J mice (6–8 weeks old), weighing 18–20 g, were purchased from Beijing Vital Rival Laboratory Animal Technology Co., Ltd. Using chronic-plus-single-binge ethanol feeding, the mice were pair-fed with either regular or ethanol-containing Lieber De Carli liquid diets (Lieber and DeCarli, 1986) for 12 weeks. The compositions of Lieber De Carli liquid diets were shown in Table 2, and we adjusted the ethanol concentration. After a week of acclimatization with ethanol-free Lieber De Carli liquid diets (Beijing HFK Bioscience Co., Ltd., Beijing, China), weight-matched animals were randomly divided into four groups as the following: 1) normal control group (C), 2) ethanol group (E, 28% of total calories as ethanol), 3) ethanol plus quercetin group (EQ, quercetin: 100 mg/kg body weight), 4) quercetin control group (Q). After 12 weeks, the animals were orally administered with ethanol or maltose solutions at 7:00 a.m. in the morning. At 9 h post-gavage, mice were sacrificed, and serum was separated from whole blood and stored in −20 °C. Livers were weighted and stored in −80 °C for further analysis.

All animals were treated in accordance with the Guiding Principles in the Care and Use of Laboratory Animals published by the US National Institutes of Health, and all animal procedures were approved by the Tongji Medical College Council on Animals Care Committee. All animals were housed in a temperature-controlled room (25 ± 2 °C) and a relative humidity (55%–65%) with a 12-h light/dark cycle. Body weight was measured weekly, and food consumption was calculated daily through taking the volume of the bottle before and after a 24-h period.

2.3. Hematoxylin & eosin and oil-red O staining of the liver

Liver tissue samples fixed in 4% paraformaldehyde were embedded with paraffin and sectioned into consecutive 5-μm thick sections at −20 °C. The sections were stained with Hematoxylin and eosin staining (H & E) and observed by light microscope (Olympus, Japan).

Cryostat sections of liver tissue were stained with Oil-red O solution and followed by washing with 60% isopropyl alcohol. The sections were counterstained with hematoxylin and observed by light microscope (Olympus, Japan).

2.4. Determination of TC and TG levels of serum and hepatic

Plasma samples from six mice per experimental condition were assayed. Liver homogenates (10%) were prepared with precooled isopropanol on ice and stewed for 48 h at 4 °C. Following centrifuge at 1150 g for 15 min at 4 °C, the supernatants were collected to measure hepatic total cholesterol (TC) and triglycerides (TG) in the light of the instruction of reagent kit.

2.5. Determination of the biomarkers for liver injury

Levels of ALT and AST were measured by the methods of Reitman and Frankel (1957).

2.6. Transmission electron microscope

After animals were sacrificed, the trimmed liver tissues were fixed in glutaraldehyde (2.5%) and stored in 4 °C. The post-fixation were immobilized in 0.1M osmium tetroxide prepared in 0.1M phosphate buffer (pH 7.4) for 2 h at room temperature followed by dehydration, permeation and embedding in araldite. The tissues were polymerized at

60 °C for 48 h and cut into ultrathin sections (80–100 nm) using an ultramicrotome (Leica EM UC7, Germany). Subsequently, the thin slices on copper mesh grid were stained with uranyl acetate (2%) and lead citrate for 15 min at room temperature, and observed under a transmission electron microscope (Tecnai G^2 20 TWIN, USA).

2.7. Double immunofluorescence and co-localization studies

The embedded paraffin blocks of liver tissues were sliced into 5 μm sections and de-paraffinized and rehydrated with xylene and ethanol. For a microwave antigen retrieval step, the slices were immersed in a 0.01M Tris-EDTA solution (pH 9.0), heated until boiling, held 2 min on a low heat and then heated 5 min with low fire in 5 min later. Finally, the sections were blocked with 10% normal goat serum blocking solution for 20 min at room temperature, followed by overnight incubation with primary rabbit anti-PLIN2 (1/100, Abcam, USA) and mouse anti-LC3B (1/50, Santa Cruz, USA). The following day, the slides were washed three times in PBS and incubated with Alexa fluro 488-conjugated goat anti-rabbit IgG (1/200, Proteintech, China) and Alexa fluor 594-conjugated goat anti-mouse IgG (1/200, Proteintech, China) for 30 min at 37 °C. After rinsed with PBS, the slices were incubated with DAPI for 5 min and mounted with an anti-fluorescent quenching sealing agents, and subsequently photographed under a fluorescent microscope (Olympus, Japan).

2.8. Western blot analysis

Liver tissues were lysed in the radio immunoprecipitation assay lysis buffer supplemented with 1 mM phenylmethanesulfonyl fluoride. The lysates were centrifuged and protein concentrations were measured by BCA protein assay kit (Beyotime Biotechnology, shanghai). Equal amount of total protein was electrophoresed on a 12% acrylamide SDS gel and then electrotransferred to polyvinylidene fluoride membrane (Millipore, MA, USA). After being blocked with skimmed milk powder for 2 h at room temperature, the membranes were incubated overnight at 4 °C with the specific antibody for the target protein and 1 h with the corresponding secondary antibody. The membranes were washed and detected with EGL plus kit in Western Blotting Detection System (Amersham Bioscience, Little Chalford, UK). The density of each target band was quantified by Image Pro-Plus 6.0 software and normalized to GAPDH as optical density.

2.9. Statistical analysis

All data was showed as mean ± SD. Statistical difference comparison was made using one-way analysis of variance. To evaluate the significance of differences between the two groups, we used the Student-Newman-Keuls test and $P < 0.05$ was considered as significant.

3. Results

3.1. Effects of quercetin on body weight and liver index of mice fed with ethanol

All mice survived during the experimental period until sacrifice. There was no statistical difference in initial weight or food intake among four groups. As shown in Table 1, the weight gain of all ethanol-fed mice was lower than that of non-ethanol-challenged mice. Compared with normal control group, the liver weight and liver index of ethanol-fed mice were increased by 34.8% ($P < 0.01$) and 44.2% ($P < 0.01$), respectively; whereas quercetin significantly improved the negative effects caused by ethanol. There were no differences on mice weight, liver weight and liver index of quercetin alone in comparison with normal control.

3.2. The alleviated effects of quercetin on ethanol-mediated liver lipid accumulation and injury

As shown in Fig. 1A, chronic-plus-binge ethanol intake lead to liver structure disorder and large amount of macrovesicular lipid droplets infiltration. And Oil red O staining showed that chronic and a single binge ethanol intake caused hepatic lipid accumulation (Fig. 1B). For further quantitative analysis the level of mice hepatic lipid accumulation, we measured the TC and TG levels of serum and liver tissues. There were no differences in TC level of serum and liver tissues among four groups (Fig. 1S). Consistent with histopathological examination, serum and hepatic TG contents of ethanol feeding mice were increased by 80.9% ($P < 0.01$) and 24.7% ($P < 0.01$), respectively. On the contrary, quercetin treatment alleviated ethanol-induced morphological changes, and only a small amount of fat vacuoles could be observed. And daily quercetin intervention antagonized the ethanol-induced adverse effects on TG content of serum and hepatic tissues. No difference was found between normal mice and quercetin alone.

As depicted in Fig. 1D, long-term alcohol consumption plus a single binge ethanol feeding provoked mice liver damage manifested by a remarkable serum ALT and AST elevation. As expected, quercetin normalized ALT and AST level derived from ethanol. However, quercetin per se had no influence on serum ALT and AST levels compared to normal mice.

3.3. The ameliorated effects of quercetin treatment on the inhibition of autophagy induced by ethanol

To evaluate the effects of ethanol on autophagy, we observed liver ultrastructure changes through TEM images and detected the expression of autophagy-related proteins. As showed in Fig. 2A, there were lots of organelles rich in well-developed mitochondria with integral membrane and autolysosome in hepatocytes of normal control or quercetin alone group. However, chronic-plus-single-binge ethanol feeding resulted in evident mitochondria swell and marked loss of autolysosomes (disappeared in the most field of vision). Such ultrastructural abnormities were reversed by quercetin, especially on the morphological restoring of mitochondrial characteristics. As illustrated

Table 1
Effects of quercetin on body weight and liver ratio to weight in the mice chronically fed ethanol for 12 weeks.

	Initial weight (g)	Final weight (g)	Weight gain (g)	Average daily diet (ml)	Liver weight (g)	Liver ratio to weight (%)
C	23.2 ± 1.50	33.5 ± 1.95	10.8 ± 1.70	13.3 ± 2.14	0.92 ± 0.13	3.19 ± 0.16
E	23.9 ± 1.39	29.0 ± 1.26	6.9 ± 0.99**	12.6 ± 2.64	1.24 ± 0.23**	4.60 ± 0.39**
EQ	23.6 ± 1.44	30.3 ± 1.45	8.0 ± 1.31**	12.3 ± 3.06	1.17 ± 0.12$^{**\#\#}$	4.09 ± 0.15$^{**\#\#}$
Q	23.5 ± 1.65	31.8 ± 2.11	10.4 ± 2.14	13.3 ± 1.96	0.88 ± 0.15	3.08 ± 0.19

The male C57BL/6J mice were pair-fed with regular or ethanol-containing Lieber De-Carli liquid diets for 12 weeks. Quercetin was added to the liquids diets every day. Data were shown as mean ± SD, n = 10. C, normal control group; E, ethanol group; EQ, ethanol plus quercetin group; Q, quercetin group. $^{*}P < 0.05$, $^{**}P < 0.01$vs. C; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. E.

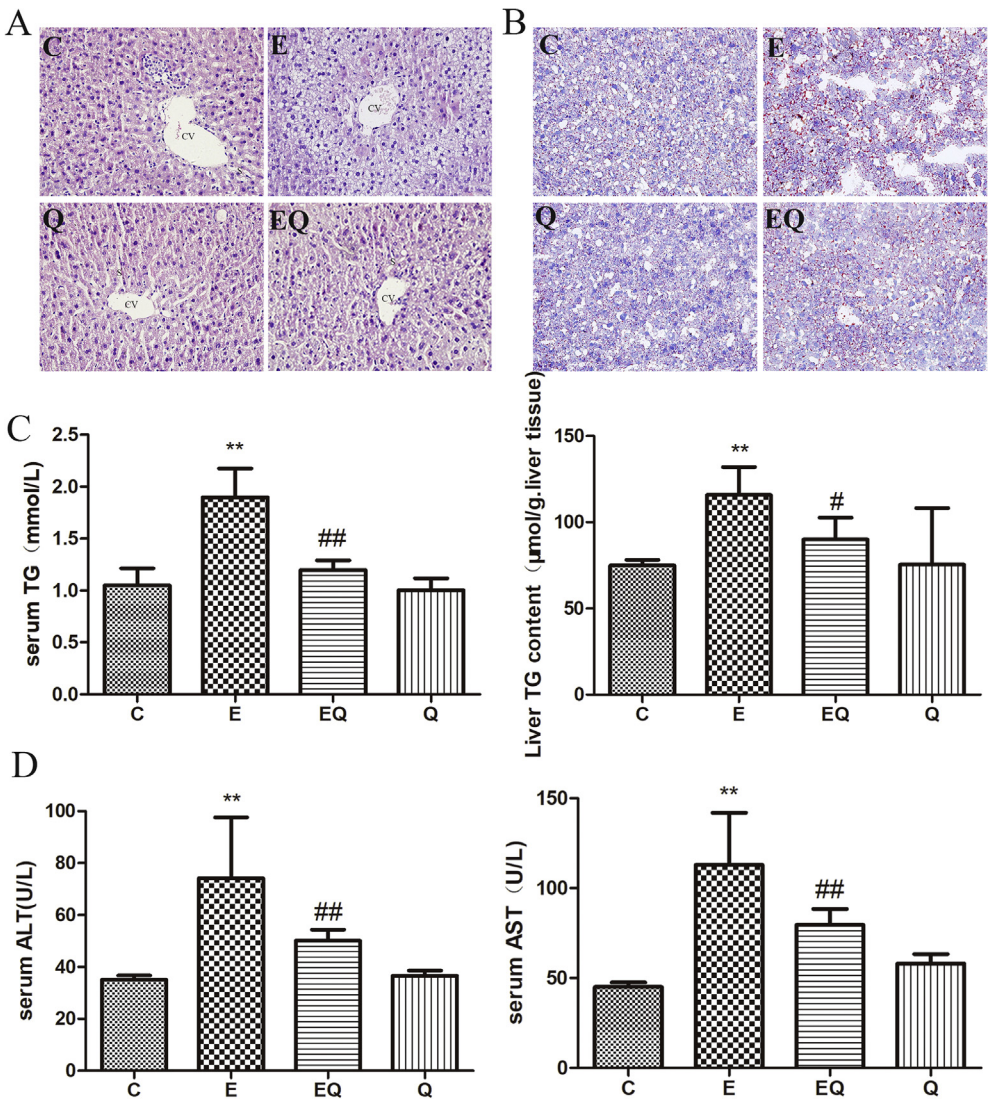

Fig. 1. Effects of quercetin treatment on histological findings disturbed by chronic and binge ethanol feeding. (A) Fixed liver tissue sections of mice stained with hematoxylin and eosin were observed by light microscope (magnification ×400). (B) Snap frozen liver tissue sections of mice were stained with Oil red O and observed by light microscope (magnification ×400). (C, D) Serum TG, liver TG, Serum ALT and AST were determined by standard commercial assays kits and the value were presented as mean ± SD (n = 6). CV: central vein; S: hepatic sinusoid; C, normal control group; E, ethanol group; EQ, ethanol plus quercetin group; Q, quercetin group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. C; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. E.

in Fig. 2B, mice fed with ethanol-contained liquid diets displayed down-regulation of the protein expression of liver LC3II and LAMP1, while the expression of p62 increased by 6.1 fold in comparison with the control group. Quercetin restored hepatic LC3II and LAMP1 protein expression, accompanied with the expression of p62 reduced by 66.6% ($P < 0.05$) when co-treated to ethanol-fed mice.

3.4. Quercetin promoted lipophagy to improve hepatic lipid accumulation caused by chronic-plus-single-binge ethanol feeding

To further investigate the mechanism of quercetin-mediated alleviation of ethanol-induced liver lipid accumulation and injury, lipophagy level was measured by western blot and immunofluorescent staining. TEM image analysis showed that marked increases on both numbers and size of LDs were observed in hepatocytes of ethanol-fed mice compared to smaller LDs in those of normal mice (Fig. 2A). Enhanced protein of the perilipin 2 (PLIN2), a LD-associated protein facilitating lipid storage, further confirmed this finding with a 1.98-fold increase in ethanol group compared to normal control (Fig. 3A). Chronic ethanol consumption plus a single binge alcohol feeding decreased the co-localization of hepatic LC3II and PLIN2 protein (Fig. 3C). In addition, the expression of *p*-AMPK protein was reduced by 52.7% in alcohol-fed group compared to normal control group (Fig. 3B). Conversely, quercetin treatment evidently decreased the numbers and size of LDs, normalized the PLIN2 protein and increased the co-localization of liver LC3II and PLIN2 protein. It's well known that AMPK activation regulates lipid metabolism and its inhibition is linked with obesity-related diseases in lipid overload conditions (Herzig and Shaw, 2018; Woods et al., 2017). In line with others, quercetin significantly increased the expression of *p*-AMPK when co-treated with ethanol (Fig. 3B).

4. Discussion

Quercetin, a naturally occurring bioflavonoid, has a wide range of well-characterized biological effects including the health benefits, the enhancement of physical and mental activity, and several different pharmacological effects (D'Andrea,2015). Many researches showed that quercetin could improve the lipid accumulation (Wang et al., 2013b; Zhang et al., 2015; Zhang et al., 2016). The present study indicated that quercetin alleviated mice hepatic steatosis and liver injury induced by chronic-plus-single-binge ethanol feeding, and the mechanism could be associated with AMPK-mediated promoting lipophagy.

The effect of drinking on health has been a controversial topic. The latest research found that the risk of all-cause mortality and cancers specifically rose with increasing levels of alcohol consumption, and the level of consumption that minimized health loss is zero (GBD, 2016 Alcohol Collaborators, 2018). The liver, as the main organ of ethanol metabolism, is also receiving the attention of researchers. Chronic-plus-binge alcohol feeding in mice is a newly developed modeling method

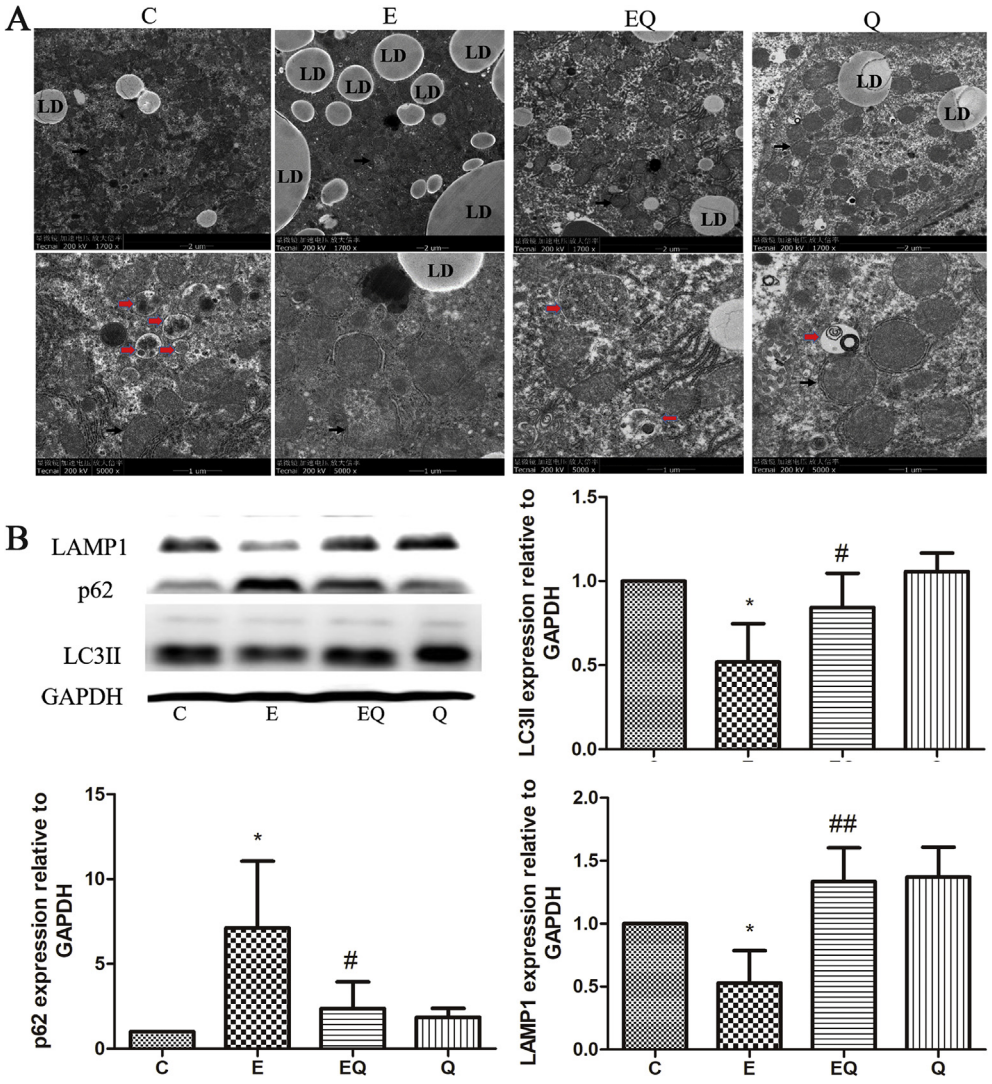

Fig. 2. Effects of quercetin on mice liver lipid droplets and autolysosome induced by chronic-plus-single-binge ethanol feeding. (A) Lipid droplets (LD indicated), mitochondria (black arrows indicated) and autolysosome (red arrows indicated) were observed from transmission electron microscope (TEM) images. (B) Western blot analysis was performed to measure LC3II, p62 and LAMP1 and quantified by Image-Pro Plus 6.0 software. Blotting with GAPDH was used as a protein loading control. Values were showed as mean ± SD. C, normal control group; E, ethanol group; EQ, ethanol plus quercetin group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. C; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. E.

for studying alcoholic liver injury in recent years. And this feeding synergistically induced liver injury, inflammation and fatty liver, which mimicked the drinking pattern in many alcoholic hepatitis patients who have a background of chronic intake for many years (chronic) and a history of recent heavy alcohol use (binge) (Bertola et al., 2013). Therefore, we adopted this modeling method to explore ALD and the intervention mechanism of quercetin.

Autophagy, a lysosomal degradation process to eliminate dysfunctional macromolecules and organelles, preserves cells from damage generated by stress conditions and takes on an adaptive pro-survival response (Klionsky et al., 2016). Impairment of autophagy has been found to relate with lipid metabolic disorders such as fatty liver and obesity (Singh and Cuervo, 2012). At present, however, the effects of ethanol on autophagy are still controversial. Several studies showed that binge drinking enhanced autophagy to remove ethanol-induced mitochondria damage and excess lipid droplets (Ge and Schekman, 2014; Li and Ding, 2016; Ni et al., 2013; Thomes et al., 2015). Attractively, two other independent studies reported that although long-term ethanol exposure increased autophagic flux and autophagosome numbers in mice liver, the increased p62 level and impaired autophagosome-lysosome fusion suggested that chronic ethanol consumption caused inhibition of autophagic vacuole degradation (Chao et al., 2018; Thomes et al., 2015). A little difference from these researches was that our present study showed that chronic ethanol consumption plus binge drinking not only reduced the numbers of lysosome, but also inhibited the formation of autophagosomes, as manifested by a decrease of LAMP1 and LC3II level. Indeed, autophagy can either be increased or declined by ethanol according to the model used, the dose and duration of ethanol treatment, the way of ethanol feeding, the tissue evaluated and the experimental condition.

Although the effects of alcohol on autophagy are affected by various factors, it is becoming clear that suppressing autophagy aggravates liver lipid accumulation and damage, while promoting autophagy is beneficial for the improvement. Quercetin, naturally occurring in various vegetables and fruits, makes a significant difference in the onset and development of autophagy (Calgarotto et al., 2018; He et al., 2017). Recently, our team demonstrated that quercetin mitigated mitochondrial damage induced by ethanol or high-fat diet through counteracting mitophagy suppression (Liu et al., 2018; Yu et al., 2016). In accordance with these studies, the current results showed that quercetin increased the numbers of lysosome and promoted the fusion of autophagosome-lysosome to restore ethanol-caused the inhibition of autophagy based on the analysis of TEM evaluation and molecular markers related to autophagy flux.

LDs, as a main form of lipid, are made of a neutral lipid core composed of TG and cholesterol esters surrounded by phospholipid monolayer and specific proteins. And the perilipin family of proteins plays the most important role in the biogenesis, stabilization, and degradation of LDs (Pawella et al., 2014). Perilipin 2 (PLIN2), one of the perilipin families, almost ubiquitously expresses in all tissues (Kaushik

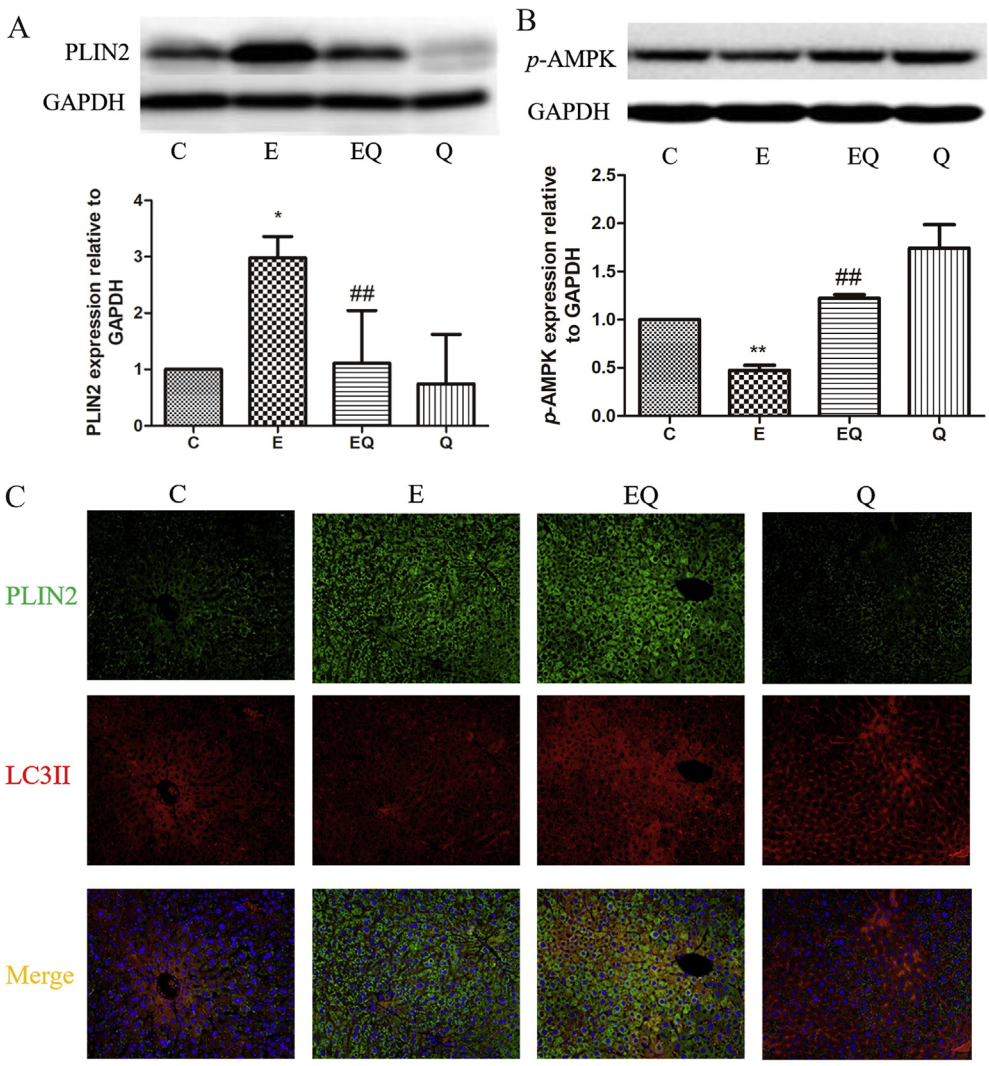

Fig. 3. Effects of quercetin treatment on mice liver lipophagy by chronic-plus-binge ethanol feeding. (A, B) Western blot analysis was performed to measure liver PLIN2 or *p*-AMPK expression, and quantified by Image-Pro Plus 6.0 software. Blotting with GAPDH was used as a protein loading control. Data was showed as mean ± SD. (B) Double immunofluorescent staining was used to observe co-localization of PLIN2 and LC3II in the embedded paraffin block of liver tissue under a fluorescent microscope (magnification ×400).C, normal control group; E, ethanol group; EQ, ethanol plus quercetin group; Q, quercetin group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. C; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. E.

and Cuervo, 2015) and has been well-recognized as a general marker for liver steatosis in adults and children (Carr et al., 2017; Pawella et al., 2014; Straub et al., 2013). In line with other studies (Carr and Ahima, 2016; Rasineni et al., 2014; Williams et al., 2018), our results showed that PLIN2 was increased by chronic and binge ethanol feeding. Varshney et al. (2018) reported that kaempferol, a natural flavonoid, significantly declined the level of PLIN2 mRNA and protein to exert the effect of hypolipid in palmitic acid-treated β-cell. Our present study demonstrated that when we used quercetin co-treatment for ethanol-challenged mice, the expression of PLIN2 was normalized by quercetin, along with a decreased TG content and LD size/numbers, collectively suggesting that quercetin did ameliorate alcohol-induced hepatic steatosis by lowering the PLIN2 expression.

Lipophagy, as a subtype of macroautophagy, refers to an intracellular lipid degradative process in which the LDs are engulfed by autophagosomes and subsequently fused with lysosomes to form autolysosomes (Schulze et al., 2017; Wang, 2016). As a major organ of lipid metabolism, the liver is particularly susceptible to lipotoxicity. By mobilizing LD catabolism and promoting secretion and assembly of density lipoprotein, lipophagy plays a significant role in the lipid metabolism and lipid homeostasis, and clinically development and progression of liver disease responsible for the lipotoxicity. Besides, Lu and Cederbaum (Lu and Cederbaum, 2015) speculated that autophagy-dependent processes such as mitophagy and lipophagy facilitated to minimize ethanol-induced CYP2E1-dependent oxidative stress and therefore the subsequent liver damage and steatosis. Rasineni et al. (2017) found that chronic ethanol administration retarded the rate of hepatocyte lipophagy owing in part to the lowered levels of phosphorylated Src kinase available to activating Dyn2 substrate, and thereby depleting lysosomes for LD breakdown. On the other side, pharmacological enhancement of lipophagy has been observed to alleviate ethanol-caused hepatotoxicity and steatosis in mice (Wang et al., 2013a). Zhu et al. (2018) demonstrated that quercetin ameliorated HFD-induced NAFLD through regulating hepatic VLDL assembly and lipophagy. Our findings showed that quercetin had a positive effect on lipophagy as manifested by the increased co-localization of hepatic LC3II and PLIN2 protein. It's well-known that the progression of hepatic steatosis is accelerated when the rate of fatty acids esterification into TG exceeds the rate of fatty acids output through lipolysis and mitochondrial fat acids oxidation (Kawano and Cohen, 2013). De Boer et al. (2006) showed that long-term quercetin intervention accelerated the breakdown of TGs and upregulated lung fatty acid catabolism pathways, like beta-oxidation and ketogenesis. And plasma free fatty acid levels in quercetin-treated rat were declined. Other similar studies also reported that quercetin could improve mitochondria function (de Oliveira et al., 2016; Yu et al., 2016), trigger mitochondria biogenesis (de Oliveira et al., 2016; Henagan et al., 2015), and decrease incomplete beta oxidation and fat mass accumulation. Therefore, we could speculated that quercetin might regulate cellular energy homeostasis by promoting the breakdown of TGs, on the one hand, and improving mitochondrial function, increasing the transfer of hydrolyzed fatty acids to mitochondria and further strengthening the rate of

mitochondrial β-oxidation on the other.

AMPK is a key regulator of cellular lipid metabolism and has a positive effect on autophagy (Chen et al., 2014; Herzig and Shaw, 2018; Woods et al., 2017; Yao et al., 2016). Zhang et al. (2018) found that dihydroquercetin ameliorated alcohol-induced liver steatosis by the activation of AMPK. Some studies have similarly reported that quercetin increased the expression of *p*-AMPK (Chen et al., 2014; Lee and Kim, 2018; Qiu et al., 2018). In line with others, our study found that quercein significantly abolished the inhibition of AMPK activity caused by chronic-plus-binge ethanol consumption, suggesting that activated AMPK might mediate quercetin-induced lipophagy.

Above all, our findings support a protective role for regular consumption of dietary quercetin in preventing hepatic lipid accumulation and liver injury caused by chronic-plus-binge ethanol feeding, and lipophagy may represent the potential therapeutic target in the pathogenesis of ALD.

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Appendix A. Supplementary data

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Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2018.12.028

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