Protective effect of total flavonoid C-glycosides from Abrus mollis extract on lipopolysaccharide-induced lipotoxicity in mice

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[ABSTRACT] Abrus mollis is a widely used traditional Chinese medicine for treating acute and chronic hepatitis, steatosis, and fibrosis. It was found that the total flavonoid C-glycosides from Abrus mollis extract (AME) showed potent antioxidant, anti-inflammatory, and hepatoprotective activities. To further investigate the hepatoprotective effect of AME and its possible mechanisms, lipopolysaccharide (LPS)-induced liver injury models were applied in the current study. The results indicated that AME significantly attenuated LPS-induced lipid accumulation in mouse primary hepatocytes as measured by triglyceride (TG) and total cholesterol (TC) assays and Oil Red O staining. Meanwhile, AME exerted a protective effect on LPS-induced liver injury as shown by decreased liver index, serum aminotransferase levels, and hepatic lipid accumulation. Real-time PCR and immunoblot data suggested that AME reversed the LPS-mediated lipid metabolism gene expression, such as sterol regulatory element-binding protein-1 (SREBP-1), fatty acid synthase (FAS), and acetyl-CoA carboxylase 1 (ACC1). In addition, LPS-induced overexpression of activating transcription factor 4 (ATF4), X-box-binding protein-1 (XBP-1), and C/EBP homologous protein (CHOP) were dramatically reversed by AME. Furthermore, AME also decreased the expression of LPS-enhanced interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2). Here, it is demonstrated for the first time that AME ameliorated LPS-induced hepatic lipid accumulation and that this effect of AME can be attributed to its modulation of hepatic de novo fatty acid synthesis. This study also suggested that the hepatoprotective effect of AME may be related to its down-regulation of unfolded protein response (UPR) activation.

[KEY WORDS] Flavonoid C-glycosides; Endotoxin; Unfolded protein response; Lipid metabolism; Inflammation


Introduction

Abras mollis Hance is a member of the Fabaceae family that has been commonly used in traditional Chinese medicine in southwestern China for centuries [1]. Because of its bioactivities in reducing fever, removing dampness, and detoxification, Abrus mollis is a main plant-based source of some well-known patented Chinese medicines, including “Jigucao capsules” for treating acute and chronic hepatitis, steatosis and fibrosis in China [2-5]. A. mollis is rich in flavonoid C-glycosides [6]. Pharmacological studies indicate that the flavonoid C-glycosides possess various biological activities, including antioxidant, antimicrobial, cytotoxic, and hepatoprotective activities [7-10]. Therefore, it was speculated that the flavonoid C-glycosides might be the bioactive compounds of A. mollis. The total flavonoid C-glycoside fraction from A. mollis extract (AME) was enriched and purified in this laboratory. The main components of AME are vicenin-2 (apigenin-6,8-di-C-β-D-glucopyranoside, I), isoschaftoside (apigenin-6-C-α-L-arabinopyranosyl-8-C-β-D-glucopyranoside, II), and...
regulator of lipotoxicity and oxidative damage [20-22]. Previous studies from this laboratory indicated that AME possesses anti-inflammatory, antioxidant and hepatoprotective activities [12-13]. However, the mechanisms underlying the AME-induced amelioration of liver dysfunction remain unclear.

Cyclooxygenase is an enzyme, officially known as prostaglandin-endoperoxide synthase. Pharmacological inhibition of COX can cause inflammation and pain relief. In a previous study, AME also showed an effect on inhibiting COX-1 and COX-2, which indicated that AME might be a non-selective COX inhibitor. Numerous human and animal studies indicated that inflammation is a critical factor involved in the pathogenesis of liver disease [14-16]. Therefore, it was considered that AME might be developed as a new natural drug for the treatment of liver dysfunction by ameliorating hepatic inflammation.

Lipopolysaccharide (LPS) is a primary cell-wall component of Gram-negative bacteria and activates key mediators of inflammatory responses, such as tumor necrosis factor-α (TNF-α) and IL-6. LPS administration is considered as an experimental model of inflammation and infection. In addition, accumulating reports have indicated that LPS-induced inflammation plays an important role in the pathogenesis of metabolic disease, such as obesity, diabetes, and liver dysfunction [15-16]. Recent studies demonstrated that single dose treatment of LPS induced hepatic lipid accumulation in mice, and further found that SREBP-1c, FAS and ACC were significantly up-regulated in the livers of mice injected with LPS [17]. Liu et al. reported that fortifying the cellular antioxidant defense system can prevent LPS-induced liver injury in murine animals [18-19]. A previous study here also showed that LPS can affect expressions of the key genes involved in lipid metabolism (data not shown). Here, LPS-induced liver injury models were applied to investigate the hepatoprotective effect of AME. The key genes involved in lipid metabolism were further detected to study the underlying mechanism.

UPR activation plays an important role in regulating lipogenesis. It is reported that C/EBP homologous protein (CHOP), a downstream UPR pathway target, is a key regulator of lipotoxicity and oxidative damage [20-22]. Emerging evidence also indicates that major UPR members, including activating transcription factor 4 (ATF4) and X-box-binding protein-1 (XBP-1), may regulate glucose and cholesterol metabolism-related genes, as well as fatty acid synthesis during endoplasmic reticulum (ER) stress [23-25]. In the present study, the key UPR-related genes were detected to test whether UPR was involved in the AME-mediated alleviation of liver dysfunction.

The objective of the current study was to investigate whether AME attenuated LPS-induced hepatic lipid metabolism dysregulation, and to further study a potential mechanism. The in vitro and in vivo results indicated that AME significantly decreased the LPS-induced hepatic lipid accumulation and inflammatory responses. Further studies on the key genes involved in lipid metabolism demonstrated that AME-ameliorated LPS-induced hepatic lipotoxicity was attributed to its regulation of hepatic de novo fatty acid synthesis. UPR-related gene expressions were altered by LPS and AME, indicating that the UPR pathway might be involved in the modulation of lipid metabolism and inflammation.

Materials and Methods

Materials

Medicinal raw material samples of Abrus mollis were gifts from Senior Engineer YE Wen-Zhi, Guangxi Yulin Pharmaceutical Co., Ltd. The aerial parts of A. mollis were collected from Yulin city of Guangxi province in Sep. 2010, and identified by Senior Engineer YE Wen-Zhi, Guangxi Yulin Pharmaceutical Co., Ltd.. The dry aerial parts of Abrus mollis without seeds were ground to a powder and extracted in 70% ethanol under reflux for 2 h, repeated twice. The filtrates were combined, evaporated at 60 °C in vacuum and then suspended in water. The solution was filtered and was chromatographed by HPD-100 macroporous resin with gradient elution of aqueous ethanol and AME was obtained in the 50% aqueous ethanol elution, as described previously [11]. According to the HPLC-UV analysis results, the total flavonoid C-glycosides content of purified AME was over 50% (51.3%), including 14.3% vicenin-2, 21.9% isoschaftoside, and 15.1% schaftoside [26].

Animal studies

CD-1 (ICR) mice (male, 8 weeks old) were obtained from SIPPR-BK Experimental Animal Co., Ltd. (Shanghai, China). All of the experiments and procedures involving mice were approved by the China Pharmaceutical University and Laboratory Animal Management Committee of Jiangsu Province Ethical Committee (Approval number: 2110693), and were conducted in accordance with the standard ethical guidelines under the control of the Ethical Committee mentioned above and all applicable regulations.

To examine the effect of AME treatment on LPS-induced hepatic lipid accumulation, CD-1 mice were randomly assigned to four groups (n = 5): 1) control, 2) LPS (Escherichia coli LPS, serotype 0127: B8, Sigma-Aldrich, St. Louis, MO, USA), 3) AME, and 4) AME + LPS. Mice were fed a standard diet and gavaged daily with vehicle control (distilled water) or AME (200 mg·kg⁻¹) for 7 days. At day 6, the LPS and AME + LPS groups were injected intraperitoneally with LPS (2 mg·kg⁻¹), while the other 2 groups were injected intraperitoneally with vehicle control (distilled water) 22 h before sacrifice. All of the mice were housed under identical conditions in an aseptic facility, and were given free access to water and food. Mice were weighted daily to adjust drug intake. At the end of the period, the mice were fasted for 16 h. Blood and liver tissues were collected when sacrificed.

Isolation and culture of mouse primary hepatocytes

Mouse primary hepatocytes (MPHs) were isolated from CD-1 mice (male, 8-week old) and cultured as described previously with slight modifications [27]. Cells were cultured...
in serum-free Williams E media containing dexamethasone (0.1 μmol·L⁻¹), penicillin (100 U·mL⁻¹), and thyroxine (1 μmol·L⁻¹), and were treated with control (distilled water), AME (100 μg·mL⁻¹), LPS (500 ng·mL⁻¹), or AME (100 μg·mL⁻¹) + LPS (500 ng·mL⁻¹) for 24 h in 5% CO₂ at 37 °C.

**Immunoblot analysis**

Total protein lysates were prepared from liver tissue using RIPA buffer (20 mmol·L⁻¹ Tris-HCl, 150 mmol·L⁻¹ NaCl, 2 mmol·L⁻¹ EDTA, 20 mmol·L⁻¹ NaF, 1 mmol·L⁻¹ NaVO₄, 1% NP40, and 0.1% SDS, pH 8.0). Protein concentration was determined using Bio-Rad protein assay reagent. Total protein lysates were resolved on 10% SDS-PAGE gels and transferred to nitrocellulose membranes. The membranes were incubated with CHOP, ATF4, p-JNK, COX-2, SREBP-1, and FAS antibodies (Santa Cruz, CA, USA) overnight at 4 °C. All the primary antibodies were used at a ratio of 1 : 400. After washing twice with phosphate-buffered saline (PBS), membranes were incubated with goat anti-rabbit or goat anti-mouse antibodies at a ratio of 1 : 10 000 (KeyGEN Biotech, Nanjing, China) for 2 h. The membranes were washed twice with PBS followed by development using a Bio-Rad ECL detection kit (Bio-Rad, Hercules, CA, USA). After exposure, the membranes were stripped with stripping buffer (62.5 mmol·L⁻¹ Tris-HCl, pH 6.8 containing 100 mmol·L⁻¹ β-mercaptoethanol and 2% SDS) and reprobed with antibodies against the internal control β-actin (1 : 500). Immunoblot band density was analyzed using Image Lab computer software (Bio-Rad, Hercules, CA).

**Measurement of triglyceride and cholesterol**

After 24 h of treatment, the MPHs were treated with PBS twice and harvested with 300 μL RIPA buffer. Liver tissues were homogenized in RIPA buffer (100 mg·mL⁻¹). The amount of TG or TC was measured using the corresponding assay kit (Whitman Biotech, Nanjing, China), and the results were normalized to the total protein amounts of sample pellets.

**Real-time quantitative PCR**

Total cellular RNA was isolated from MPHs or liver tissues using Trizol reagent (Invitrogen, Madison, WI, USA). Total RNA (2 μg) was used for first-strand cDNA synthesis using the Prime Script RT-PCR kit (TaKaRa, Otsu, Japan). The mRNA levels of key hepatic lipid metabolism genes, such as FAS, SREBP-1, peroxisome proliferator-activated receptor α (PPARα), carnitine palmitoyl transferase 1α (CPT1α), acetyl-CoA carboxylase 1 (ACC1), TNF-α, and IL-6 were quantified using specific primers for each gene [22, 28-29]. SsoFast™ EvaGreen® Supermix (Bio-Rad, Hercules, CA, USA) was used as a fluorescent dye to detect double-stranded DNA. The mRNA values for each gene were calculated by the delta delta Ct method and normalized to GAPDH mRNA as an internal control.

**Histopathology analysis**

Hepatic tissue sections were collected and fixed in 4% paraformaldehyde in PBS at room temperature overnight. Specimen regions were standardized for all of the mice. Paraffin-embedded tissue sections (≤5 μm) were stained with hematoxylin and eosin (HE) according to standard techniques. The images were taken using a microscope (Olympus, Tokyo, Japan).

**Oil Red O staining**

Liver tissue sections were stained with Oil Red O, as described previously [22]. The MPHs were fixed in 4% paraformaldehyde for 10 min and rinsed with PBS. Intracellular lipids were stained with 0.5% Oil Red O in 60% 2-propanol for 1 h. The images were taken using a microscope (Olympus, Tokyo, Japan).

**Blood Chemistry**

Whole blood samples were centrifuged after collection at 3 000 r·min⁻¹ for 10 minutes at 4 °C. Mice sera were transferred into clean tubes and stored at –80 °C. An Olympus AU1000 automated biochemical analyzer (Olympus, Tokyo, Japan) was used to measure serum biochemical parameters including TG, TC, alanine aminotransferase (ALT) and aspartate transaminase (AST) according to the manufacturer’s instructions.

**Statistical analysis**

All of the results were expressed as the x ± SEM. One-way ANOVA analysis of variance was used to determine the statistical significance among treatments, while t-test analysis of variance was used to analyze the differences between the LPS and AME + LPS groups. Statistics were performed using GraphPad Prism5 (GraphPad Software Inc., San Diego, CA, USA). P < 0.05 was considered to be statistically significant.

**Results**

**Effect of AME treatment on LPS-induced lipid accumulation in vitro**

To determine whether AME treatment alleviated LPS-induced lipid accumulation, MPHs were treated with each agent for 24 h, and cellular TG and TC levels were quantified by corresponding kit. As shown in Fig. 1B, the LPS-induced TG and TC level increases were markedly ameliorated by AME (P < 0.05 and ***P < 0.001, respectively). Intracellular lipids were further stained using Oil Red O, as described previously. LPS treatment significantly increased intracellular lipids (Fig. 1A); however, this effect of LPS was mostly diminished by AME.

**Effect of AME treatment on LPS-induced hepatic lipid metabolism gene dysregulation in vitro**

To further identify the underlying cellular mechanisms, key cholesterol and fatty acid metabolism gene expressions were detected by real-time PCR. As shown in Fig. 1C, the LPS-induced increase in SREBP-1, FAS, and ACC1 expression was blunted by AME (**P < 0.01 and ***P < 0.005). LPS-induced CPT1α inhibition, which is a key enzyme that is involved in mitochondrial fatty acid oxidation, was partially reversed in the AME + LPS group. Similarly, AME treatment also increased PPARα expression (**P < 0.05), which was suppressed by LPS (###P < 0.005). These results suggest that AME affects fatty acid synthesis and metabolism in hepatocytes.
Effect of AME treatment on LPS-induced UPR activation in vitro

Accumulating evidence indicates that steatosis-related liver diseases produce UPR activation [30-31]. To determine whether AME affected LPS-induced UPR activation, MPHs were treated with drugs for 24 h. As demonstrated in Fig. 1D, although CHOP expression still showed a significant difference between the control and AME + LPS groups ($^\# P < 0.01$), AME markedly decreased the LPS-induced overexpression in ATF4, XBP-1 and CHOP ($^\# P < 0.05$, $^# P < 0.05$ and $^{###} P < 0.001$). IL-6 expression in the AME + LPS group was much lower than in the LPS group ($^# P < 0.01$). These results suggest that UPR activation is related to AME-mediated hepatoprotection in MPHs, indicating it may be a potential mechanism.

Effect of AME on LPS-Induced hepatic steatosis in vivo

Mouse body and liver weights were assessed at the end of the experimental period. As demonstrated in Table 1, LPS significantly increased the liver index ($^{***}P < 0.001$), serum ALT ($^\# P < 0.01$), and AST ($^{***}P < 0.001$) levels, which were diminished in AME-treated mice ($^{**}P < 0.01$). The TG and TC results indicated that AME dramatically decreased LPS-induced hepatic lipid accumulation by 50% and 40%, respectively (Fig. 2C). The HE and Oil Red O staining results further confirmed that LPS-induced hepatic lipid accumulation was significantly reduced after AME treatment (Fig. 2A, 2B). Hepatic lipid metabolism gene expression levels were further analyzed by real-time PCR. Consistent with the in vitro results, AME blunted the LPS-induced increase in SREBP-1, FAS, and ACC1 mRNA expression ($^\# P < 0.05$, $^# P < 0.05$ and $^{###} P < 0.001$). Similarly, AME also reversed LPS-induced PPAR-α and CPT-1α down-regulation (Fig. 2D). In addition, LPS-induced CHOP, ATF4 and XBP-1 overexpression was alleviated by AME ($^\# P < 0.05$, $^# P < 0.05$ and $^{###} P < 0.001$), which again indicated that AME-mediated hepatoprotection was related to the UPR activation (Fig. 2E). As demonstrated in Fig. 2F, AME blocked the LPS-induced increase in SREBP-1 and FAS protein expression levels, as well as the high expression of key UPR genes ($^\# P < 0.05$). These results further confirmed that AME ameliorated LPS-induced lipid metabolism dysregulation, which was related to UPR pathway modulation.
Fig. 2  Effect of AME treatment on LPS-induced hepatic injury in vivo. Mouse liver sections were stained using HE or Oil Red O, as described in the Methods. The images were taken with an Olympus microscope equipped with an image recorder using a 20× lens. Representative photomicrographs for each treatment are shown. A) HE staining. B) Oil Red O staining. C) Hepatic TG and TC levels. D) Relative mRNA levels of key genes that are related to hepatic lipid metabolism. E) Relative mRNA levels of key hepatic genes related to UPR and inflammation. F) Protein was isolated from livers for immunoblot analysis. β-Actin was used as a loading control, except for p-JNK (t-JNK was used as loading control for p-JNK). Representative immunoblots for each protein and statistical bar graph are shown.

Effect of AME treatment on LPS-induced hepatic inflammation in vivo

Accumulating evidence has indicated that ER stress or UPR activation is an important inflammation inducer, and is associated with numerous pathologies, including obesity, atherosclerosis, diabetes, and liver disease [19, 32]. To determine whether AME reduces the LPS-induced inflammatory response, TNF-α and IL-6 levels were measured by real-time PCR. COX-2 and JNK expression were detected by immunoblot. As shown in Fig. 2E, AME significantly inhibited LPS-induced hepatic IL-6 expression by 67%, while there was no significant change in TNF-α (data not shown). AME also alleviated hepatic p-JNK and COX-2 expression (†P < 0.05, ‡P < 0.01) (Fig. 2F). These results
revealed that AME treatment diminished the LPS-induced inflammatory response.

**Discussion**

*Abrus mollis* is a traditional Chinese medicine that has been widely used for treating acute and chronic hepatitis, steatosis, and fibrosis. The total flavonoid C-glycosides from *Abrus mollis* extract (AME) was enriched and purified in this laboratory. In a previous study, AME showed potent antioxidant, anti-inflammatory, and hepatoprotective activities. However, the underlying mechanism remained unknown. In the present study, the hepatoprotective activity of AME in LPS-induced liver injury models was confirmed. Moreover, this study demonstrated that AME significantly decreased LPS-induced hepatic lipid accumulation and inflammatory responses in vivo, and further indicated that AME-ameliorated LPS-induced hepatic lipotoxicity could be attributed to modulation of hepatic de novo fatty acid synthesis. It was also suggested that the hepatoprotective effect of AME might be related to the down-regulation of UPR activation.

Numerous clinical and basic studies indicated that inflammation is a critical factor involved in the pathogenesis of liver diseases. Accumulating evidence has proposed LPS as an important factor in alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) [33-36]. A recent clinical study reported that serum LPS concentration was increased in patients with NAFLD [37]. In this study, LPS significantly increased TG and TC levels in MPH, which was confirmed by Oil Red O staining. AME treatment dramatically diminished LPS-induced intracellular lipid accumulation, which indicated that AME might protect against LPS-induced hepatic lipotoxicity (Fig.1A and Fig.1B). To further confirm the hepatoprotective effect of AME in vivo, CD-1 mice were applied and treated with the drugs mentioned previously. The results indicated that single dose administration of LPS accelerated lipid accumulation, detected by TG and TC analysis and Oil Red O staining, which further confirmed the work of Endo et al. [38]. AME alleviated increased liver index and serum aminotransferase levels induced by LPS, indicating its protective activity (Table 1). Similarly, LPS-induced hepatic TG and TC level increases were blunted by AME treatment (Fig. 2C). HE and Oil Red O staining also confirmed the suppressive effect of AME on LPS-induced hepatic lipid accumulation (Fig. 2A and Fig. 2B). Taken together, it was demonstrated that AME attenuated the LPS-induced hepatic lipotoxicity both in vitro and in vivo.

Hepatic steatosis is the result of dysregulation of lipid metabolism caused by excessive lipid uptake, increased de novo lipid synthesis, and/or reduced lipid oxidation and metabolism. Studies have demonstrated that hepatic de novo fatty acid syntheses play an important role in fatty liver disease. A study by Xu et al. demonstrated that ACC1 depletion alleviated hepatic TG accumulation. Miao et al. reported that hepatic FAS levels were up-regulated in high-fat diet-induced NAFLD [39-40]. Further analysis of key lipid metabolism genes demonstrated that AME significantly decreased the LPS-mediated SREBP-1, FAS, and ACC1 overexpression. The LPS-induced CPT1a and PPARγ down-regulation were reversed by AME (Fig. 1C, Fig. 2D). All of these results indicated that AME played a critical role in regulating fatty acid synthesis and metabolism. In 2007, Gregor et al reported that SREBP protein activation occurred during ER stress, and was critical for the regulation of cholesterol metabolism (SREBP1α, SREBP2) or lipid synthesis (SREBP1c) target genes [41]. However, whether AME affects cholesterol metabolism remains to be further studied. In summary, these studies suggested that the protective effect of AME on LPS-induced hepatic lipid accumulation were attributed to its inhibition of hepatic de novo fatty acid synthesis.

The ER is a principal cellular organelle for protein synthesis and maturation. Disruption of any ER function causes ER stress and activates the UPR. Recently, several studies have been focused on the contributions of both inflammation and ER stress/UPR activation to metabolic diseases [42]. In hepatocytes, XBP1 is downstream of the UPR sensor IRE1α and regulates the transcription of many genes that are involved in fatty acid synthesis, such as ACC. Lee et al. demonstrated that when fed a high carbohydrate diet to stimulate hepatic lipogenesis, liver-specific XBP1-deficient mice showed lower levels of serum triglycerides and cholesterol, and did not develop hepatic steatosis. The previous work also indicated that CHOP, downstream of ATF4, plays a critical role in HIV protease inhibitor-induced dyslipidemia [22, 25]. To further investigate whether AME-mediated hepatoprotection was related to UPR activation, expressions of major UPR-involved transcription factors were detected by real-time PCR and immunoblot analysis. In the present study, AME blunted the LPS-enhanced ATF4, XBP-1, and CHOP overexpression both at the mRNA level and protein level (Fig. 1D, Fig. 2E, and Fig. 2F). Combined with the previous findings, it was suggested that the hepatoprotective activity of AME might be related to the AME inhibition of UPR activation. However, whether AME down-regulation of the major UPR pathway genes represents a molecular mechanism underlying AME-mediated protection against hepatic lipotoxicity remains obscure, and is an ongoing project. In addition, the specific AME target genes also need to be further studied.

UPR activation interacts with inflammatory pathways, which is another important contributor to hepatic lipotoxicity [41-44]. This laboratory has reported that expression of inflammatory cytokines TNF-α and IL-6 is linked to hepatic UPR activation [14, 22, 45]. AST and ALT are considered as specific indicators of liver inflammation and are commonly measured clinically to diagnose liver function. In this study,
we further demonstrated that the LPS-induced inflammatory response was consistent with its effect on UPR activation (Table 1, Fig. 1D, Fig. 2E, and Fig. 2F). These studies suggest that the UPR is emerging as a potential site for the intersection of inflammation and metabolic disease.

Taken together, these studies demonstrated for the first time that AME could alleviate LPS-induced hepatic lipid accumulation and further indicated that this effect of AME was attributed to the inhibition of hepatic de novo fatty acid synthesis. In addition, it was suggested that the hepatoprotective activity of AME may be related to its down-regulation of the major UPR pathway genes, providing important information for future mechanistic studies of Chinese traditional medicine. However, whether down-regulating UPR activation represents an important molecular mechanism underlying AME-mediated protection against hepatic lipotoxicity still needs further study.

Nonstandard Abbreviations

AME: Total flavonoids C-glycosides from *Abras mollis* Extract; LPS: lipopolysaccharide; TG: triglyceride; TC: total cholesterol; SREBP-1: sterol regulatory element-binding protein-1; FAS: fatty acid synthase; ACC1: acetyl-CoA carboxylase 1; ATF4: activating transcription factor 4; XBP-1: X-box-binding protein-1; CHOP: C/EBP homologous protein; IL-6: interleukin-6; COX-2: cyclooxygenase-2; UPR: unfolded protein response; TNF-α: tumor necrosis factor-α; ER: endoplasmic reticulum; JNK: J- Jun N-terminal kinase; MPH: mouse primary hepatocyte; PBS: phosphate-buffered saline; PPARα: peroxisome proliferator-activated receptor α; CPT-1α: carnitine palmitoyl transferase 1α; HE: hematoxylin and eosin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALD: alcoholic liver disease; NAFLD: non-alcoholic fatty liver disease.

References


