Hindawi
Journal of Food Biochemistry
Volume 2023, Article ID 9952583, 13 pages
https://doi.org/10.1155/2023/9952583

Research Article

Morroniside Delays NAFLD Progression in Fructose-Fed Mice by Normalizing Lipid Metabolism and Inhibiting the Inflammatory Response

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Received 10 January 2023; Revised 17 April 2023; Accepted 12 May 2023; Published 18 August 2023

Academic Editor: Anandakumar Pandi

Chronic fructose consumption is becoming one leading risk factor for NAFLD due to its hazard on cellular lipid metabolism, which plays an essential role in the pathogenesis of NAFLD. Morroniside, an iridoid glycoside from *Cornus officinalis*, has been suggested to have potent in regulating cellular glucolipid metabolism. However, the effect and mechanism of morroniside on improving fructose-induced hepatic steatosis and retarding NAFLD progression remain ambiguous. In this study, we illustrate the efficacy of morroniside in alleviating fructose-triggered hepatic steatosis in mice. Mechanically, morroniside suppressed de novo lipogenesis and promoted β-oxidation by inhibiting the activation of PGC1β. Additionally, morroniside was found to work in attenuating hepatic inflammation in response to long-term fructose intake. Taken together, the current study reveals that morroniside is a promising food and medicinal therapy for NAFLD treatment and is effective in delaying the progression of NAFLD to NASH, especially in subtypes caused by excessive carbohydrate intake.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a chronic pathological syndrome characterized by excessive lipid accumulation in hepatocytes leading to hepatic steatosis in the absence of alcohol consumption [1]. With the changes in people's living and eating habits, the incidence of NAFLD is gradually increasing in recent years and showing a tendency toward younger age. Epidemiological studies showed that nearly 2 billion people worldwide suffer from NAFLD with a global prevalence of about 25% and continuously rising [2, 3]. Although NAFLD has become a world health-threatening epidemic, its complex and unclarified pathogenesis makes the development of specific and effective drugs for clinical NAFLD treatment extremely urgent in the field of liver metabolism.

Fructose, a common sweetener in food and beverage, is considered an independent risk factor for multiple metabolic diseases, such as obesity and diabetes [4, 5]. The high-fat diet was previously widely believed to be a major cause of NAFLD, but it is worth noting that long-term fructose consumption is considered a more important driver of NAFLD than a high-fat diet with the large increase in global consumption of soft drinks and high-fructose corn syrup [6, 7]. After ingestion, fructose is absorbed through the intestine and mostly into the liver, where it is metabolized

and converted into acetyl-CoA under the action of various functional enzymes to provide a substrate for fatty acid synthesis, and activates lipid transcription factors such as sterol regulatory elements binding protein 1 (SREBP-1) and carbohydrate response element binding protein (ChREBP), to promote de novo lipogenesis in hepatocytes [8, 9]. As the central regulatory organ of lipid metabolism, the liver plays a critical role in monitoring lipid metabolic homeostasis by coordinating biological processes such as fatty acid uptake, lipid synthesis, and β-oxidation in hepatocytes [10]. Studies have suggested that metabolites derived from long-term fructose intake can also impair hepatic fatty acid oxidation, leading to dysregulation of lipid metabolism homeostasis [11, 12]. Therefore, the maintenance of hepatic lipid metabolism homeostasis emerges as a novel insight into the prevention and treatment of chronic fructose intake-induced NAFLD.

Morroniside is a natural active iridoid glycoside mainly present in the edible and medicinal plant *Cornus officinalis Sieb*. et Zucc and has a variety of benefits for body health, including anti-inflammatory, improving diabetes, and regulating lipid metabolism [13–15]. Substantial research reveals that the traditional Chinese medicine *Cornus officinalis*, the main source of morroniside, has good biological activity and biosafety in improving obesity and reducing liver lipid accumulation in high-fat mice [16–18]. Additionally, morroniside has also been found to be effective in ameliorating glycolipid metabolism disorder in diabetic mice [15], indicating that morroniside has the potential to modulate lipid metabolism to prevent NAFLD development. However, the effect and mechanism of morroniside in improving dietary fructose intake-induced hepatic steatosis in mice remain unclear. In this study, we established a mouse model of hepatic steatosis using a chronic fructose diet to elucidate the role and molecular mechanism of morroniside in attenuating fructose-induced hepatic steatosis in mice from a new perspective of sustaining hepatic lipid metabolism homeostasis, whereby morroniside suppresses SREBP-1-driven de novo lipogenesis and promotes fatty acid oxidation. Notably, our results showed that the improving effect of morroniside was counteracted by PGC1β overexpression, indicating that morroniside ameliorates fructose-induced lipid metabolism disorders by inhibiting the activation of PGC1β. Our research reveals that supplementation with morroniside or morroniside-rich foods may be a promising therapeutic strategy for NAFLD.

2. Reagents and Methods

2.1. Reagents. Morroniside (purity ≥98%) was purchased from Shanghai Nature Standard Technology Co., Ltd. (Shanghai, China). Fructose (purity ≥99%) was obtained from Aladdin biological technology Co., Ltd. (Shanghai, China). Commercial kits for biochemical detection were bought from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), including alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (T-CHO), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), total superoxide dismutase (T-SOD), and malondialdehyde (MDA). ELISA kits of TNF-α, IL-6, and IL-1β were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. All other reagents were of analytical grade.

2.2. Animal Treatment. Male C57BL/6 mice were bought from the Center of Experimental Animals of Hubei Province (Wuhan, China). All mice were monitored under standard mouse nutrient conditions and randomly divided into four groups ($n = 8$ per group). The control group was free access to plain water, and the model and induced groups were fed 30% fructose water for 10 weeks. Meanwhile, the induced groups were intragastric gavage of morroniside (10 or 20 mg/kg) daily for consecutive 10 weeks [19, 20]. After administration, all mice were executed with 3% isoflurane to collect liver and serum samples for the following detections. All involved animal experiments were performed humanely according to protocols approved by the animal ethics committee of Hangzhou Xixi Hospital.

2.3. Cell Culture and Treatment. Human hepatoma cell lines HepG2 and normal mouse liver cell lines AML-12 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (Gibco, NY, USA) and Penicillin-Streptomycin (1 mM) at 37°C and 5% CO_2. HepG2 and AML-12 cells were incubated with 5 mM fructose solution and treated with different concentrations of morroniside (0, 10, 25, and 50 μM) for 24 h, then harvested for further investigations. For PGC1β transfection, transfection reagent Lipofectamine™ 2000 (Waltham, MA) and PGC1β plasmids were mixed and incubated for 20 min, then transfected into HepG2 cells. Moreover, the PGC1β-overexpressed cells were also incubated with fructose solution and morroniside (50 μM) for 24 h, then harvested for further analysis. All cell experiments were repeated at least three times.

2.4. Histological Analysis. Hematoxylin-eosin (*H* & *E*) staining was performed for histological assessment. In detail, fresh liver tissues were fixed in 4% paraformaldehyde for at least 24 h, embedded in paraffin, and cut into 5 μm sections. Liver sections were heated at 60°C for 30 min, then deparaffinized in xylene for 10 min, followed by rehydrating in ethanol at decreasing concentration grade for 5 min each and stained with *H* & *E* dye. Oil red O (ORO) staining was processed to evaluate the degree of lipid steatosis in the liver. The frozen liver slices were dyed within ORO solution, washed with 60% isopropanol, and counterstained the nucleus with hematoxylin. All these slices were mounted on cover glasses and observed using an Olympus DP50 (Tokyo, Japan). The hepatic steatosis of NAFLD mice was scored according to the criteria of Kleiner et al. [21]. The scoring system incorporated three histological features: steatosis (0–3), lobular inflammation (0–3), and hepatocellular ballooning (0–2), showing the progress of NAFLD.

2.5. Nile Red Staining. HepG2 cells were cultured in a 6-well plate and incubated with 5 mM fructose in the presence or absence of morroniside (50 μM) for 24 h. HepG2 cells were then fixed with paraformaldehyde and stained with Nile red solution for 15 min. Images were observed and captured using a fluorescence microscope.

2.6. Biochemical Detection and ELISA Analysis. Biochemical indices in serum were measured using commercial kits according to manufacturers' instructions, including AST, ALT, TG, T-CHO, HDL-c, LDL-c, MDA, and T-SOD. The TG contents in HepG2 cells were also detected and normalized according to the protein concentration. ELISA kits of TNF-α, IL-6, and IL-1β were used to determine the content of inflammatory factors in the liver following the protocols.

2.7. Reactive Oxygen Species (ROS) Assay. Hepatic ROS contents were detected in fresh liver tissues via a flow cytometer. In detail, primary hepatocytes were extracted from fresh liver tissue, then incubated with DCFH-DA at 37°C for 30 min and washed with PBS to remove probes that did not enter the cells, followed by analysis under a flow cytometer.

2.8. Western Blotting and Immunohistochemistry (IHC). Western blotting was performed to determine the protein expression in liver tissues and liver cells. Liver homogenates and cell suspensions were prepared to extract cytosolic protein using a Cytoplasmic Protein Extraction Kit (Beyotime, Shanghai, China). Extracted proteins were denatured at a constant 95°C in the metal bath for 5 min, then separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred into PVDF membranes. After blocking in blocking solution for 1 h, the membranes were incubated in specific primary antibodies at 4°C overnight, followed by incubating in the corresponding secondary antibodies. The protein blots would be visualized using an ECL western blot detection kit. The semi-quantification of the target protein and reference protein levels was performed using the Image*J* grayscale scanning software.

For immunohistochemical staining, rehydrated liver slides were boiled in the antigen retrieval solution for antigen retrieval. Immunohistochemistry analysis was then processed using SP Rabbit & Mouse HRP Kit (Cwbio, Beijing) according to protocol. The slices were then observed under a microscope (Olympus DP50, Tokyo, Japan). All involved antibodies were listed in Supplementary Table 1.

2.9. Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR). Total RNA was extracted from liver homogenate using a TRIzol™ Reagent and converted into complementary DNA (cDNA). The RT-qPCR assay was then performed on a CFX96 real-time PCR instrument (Bio-Rad) by using FastStart Universal SYBR Green Master Mix (Roche, Lewes, UK). Relative gene expression was based on GAPDH (glyceraldehyde 3-phosphate dehydrogenase) level as a reference control for quantitative analysis. The identified primers were listed in Supplementary Table 2.

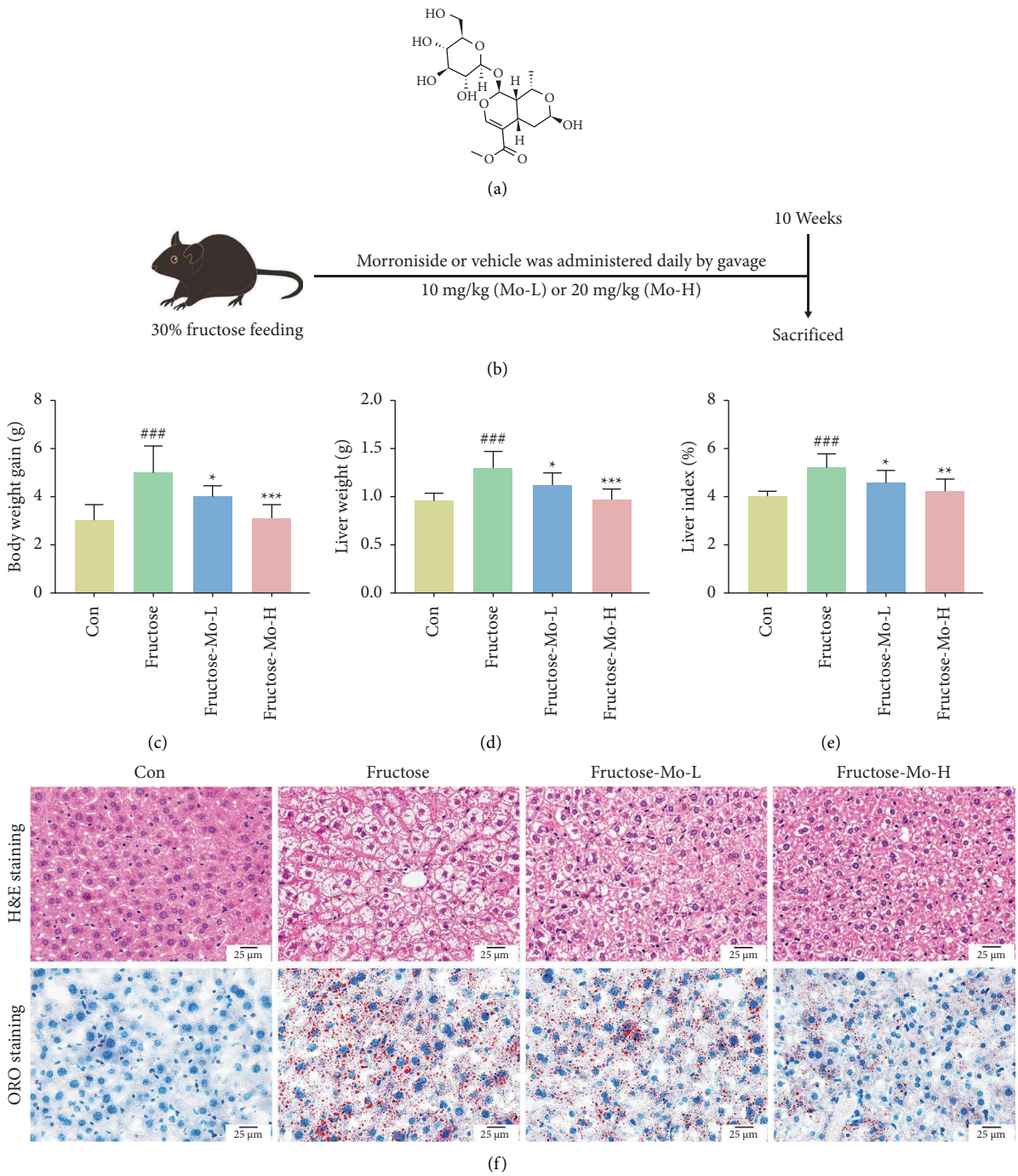
2.10. Statistical Analysis. Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analysis. Two-tailed unpaired *t*-test and ANOVA were performed between two groups and among three or more groups, respectively. All data were expressed as Mean ± S. D. $P < 0.05$ represents statistical significance.

3. Results

3.1. Morroniside Attenuates Fructose-Induced Hepatic Steatosis in Mice. Chronic fructose intake has become an independent risk factor for NAFLD. This study aims to clarify the effect of morroniside, an edible active iridoid glycoside (Figure 1(a)) on ameliorating fructose-induced NAFLD in a fructose diet-induced NAFLD mice model by feeding with 30% fructose solution and administrating morroniside (10 or 20 mg/kg) for 10 weeks (Figure 1(b)). Our results showed that after 10 weeks of fructose consumption, the mouse body weight, liver weight, and liver index were significantly increased compared with that in the control group, while markedly decreased after morroniside treatment (Figures 1(c)–1(e), Supplementary Figure 1). Moreover, compared with the control group, there were severe lipid accumulation and steatosis in the liver along with elevated liver and serum TG content, which were alleviated by morroniside administration (Figures 1(f)–1(i)). These results demonstrated that morroniside is effective in improving fructose-induced hepatic steatosis in mice.

3.2. Morroniside Improves Liver Dysfunction and Dyslipidemia in Fructose-Fed Mice. Next, we evaluated the changes in liver function and lipid index in the absence or presence of morroniside intervention in fructose-induced NAFLD mice. The results indicated that compared with the control group, serum AST and ALT activities along with the AST/ALT ratio were significantly elevated in the fructose-fed mice while those were reduced after morroniside treatment (Figures 2(a)–2(c)). Meanwhile, serum levels of T-CHO and LDL-c were decreased in morroniside-administrated mice than those in fructose-fed mice, accompanied by increased HDL-c (Figures 2(d)–2(f)). Our findings indicate that morroniside can normalize liver function and lipid metabolism.

3.3. Morroniside Enhances Hepatic Lipogenesis and Impairs β-Oxidation in Mice. The ingested fructose is mainly metabolized in the liver to enhance de novo lipogenesis and impair β-oxidation, leading to hepatic lipid metabolic disorder. To further investigate the mechanism underlying the ameliorative effect of morroniside in fructose-induced hepatic steatosis, we found that long-term fructose consumption increased both the mRNA and protein levels of PGC1β, SREBP-1, FASN, and ACC, whereas morroniside



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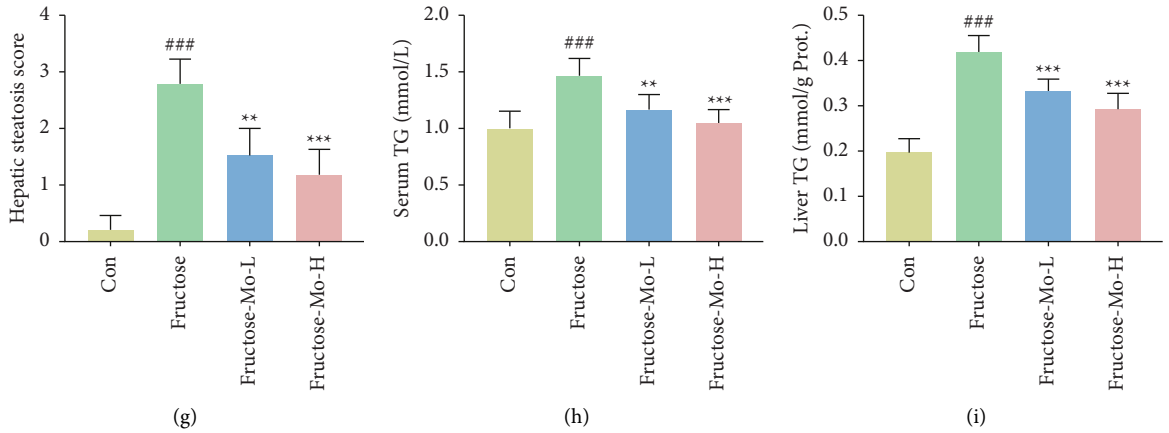


Figure 1: Morroniside attenuates fructose-induced hepatic steatosis in mice. (a) Molecular structure of morroniside. (b) Study design. Body weight (c), liver weight (d), and the liver index (e). (f) Hepatic hematoxylin and eosin (*H* & *E*) and oil red O (ORO) staining (400×, scale bars: 25 μm). (g) Hepatic steatosis score. Triglyceride (TG) content in serum (h) and liver tissue (i). Mean ± S.D., $n = 8$, $^{###}P < 0.001$ vs. the con group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the fructose-fed group. Con: control.

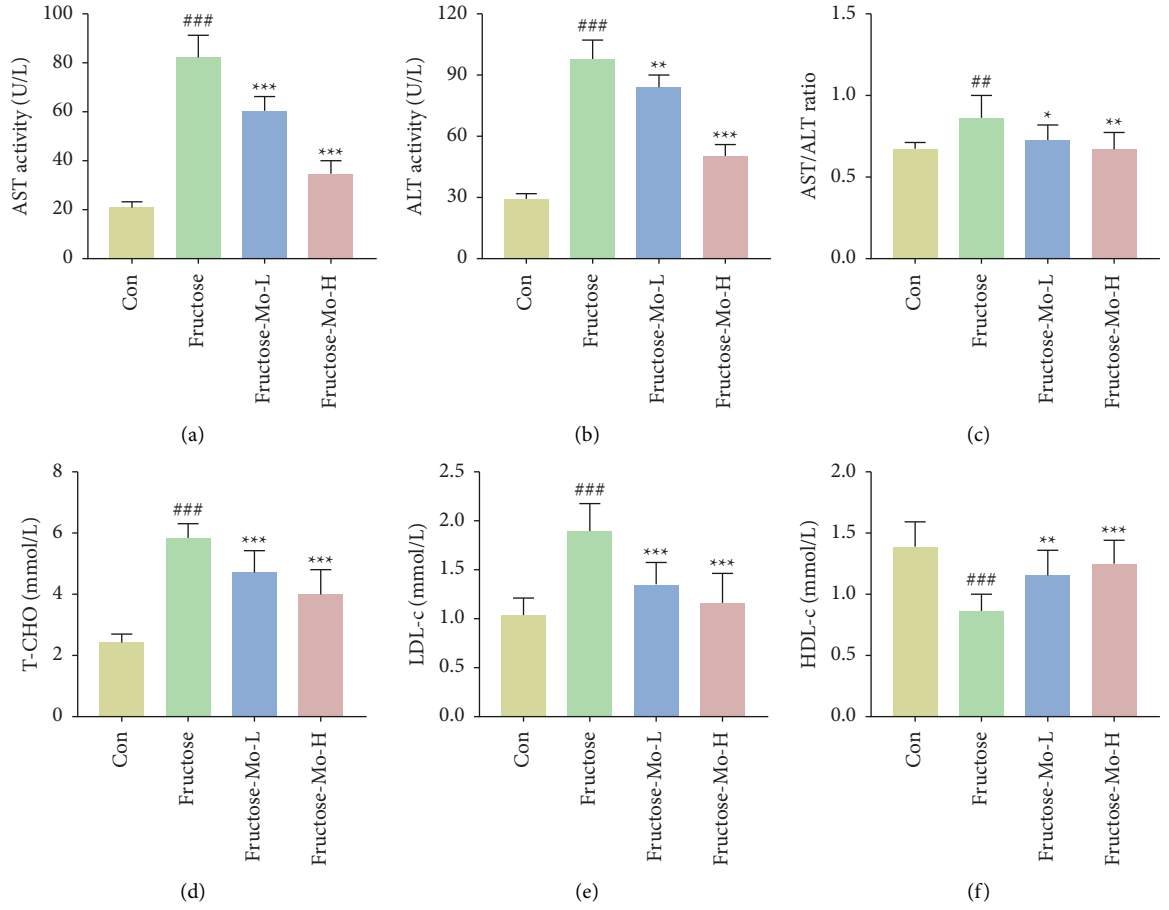


Figure 2: Morroniside improves liver dysfunction and dyslipidemia in fructose-fed mice. Activity of aspartate aminotransferase (AST). (a) and alanine aminotransferase (ALT) (b). (c) The ratio of AST to ALT. Serum levels of total cholesterol (T-CHO) (d), low-density lipoprotein cholesterol (LDL-C) (e), and high-density lipoprotein cholesterol (HDL-C) (f). Mean ± S.D., $n = 8$, $^{##}P < 0.01$, $^{###}P < 0.001$ vs. the con group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the fructose-fed group. Con: control.

made a great reduction in that (Figures 3(a)–3(f)). Immunohistochemical analysis of FASN and ACC expression showed similar results (Figure 3(g)). Moreover, compared with the control group, morroniside promoted the expression of PPARα and CPT1α in both transcriptional and translational levels, along with increased positive expression of PPARα and CPT1α in liver sections of morroniside-treated mice (Figures 3(h)–3(j)). All that showed that morroniside can maintain hepatic lipid homeostasis in fructose-triggered lipid anabolic and catabolic disorders.

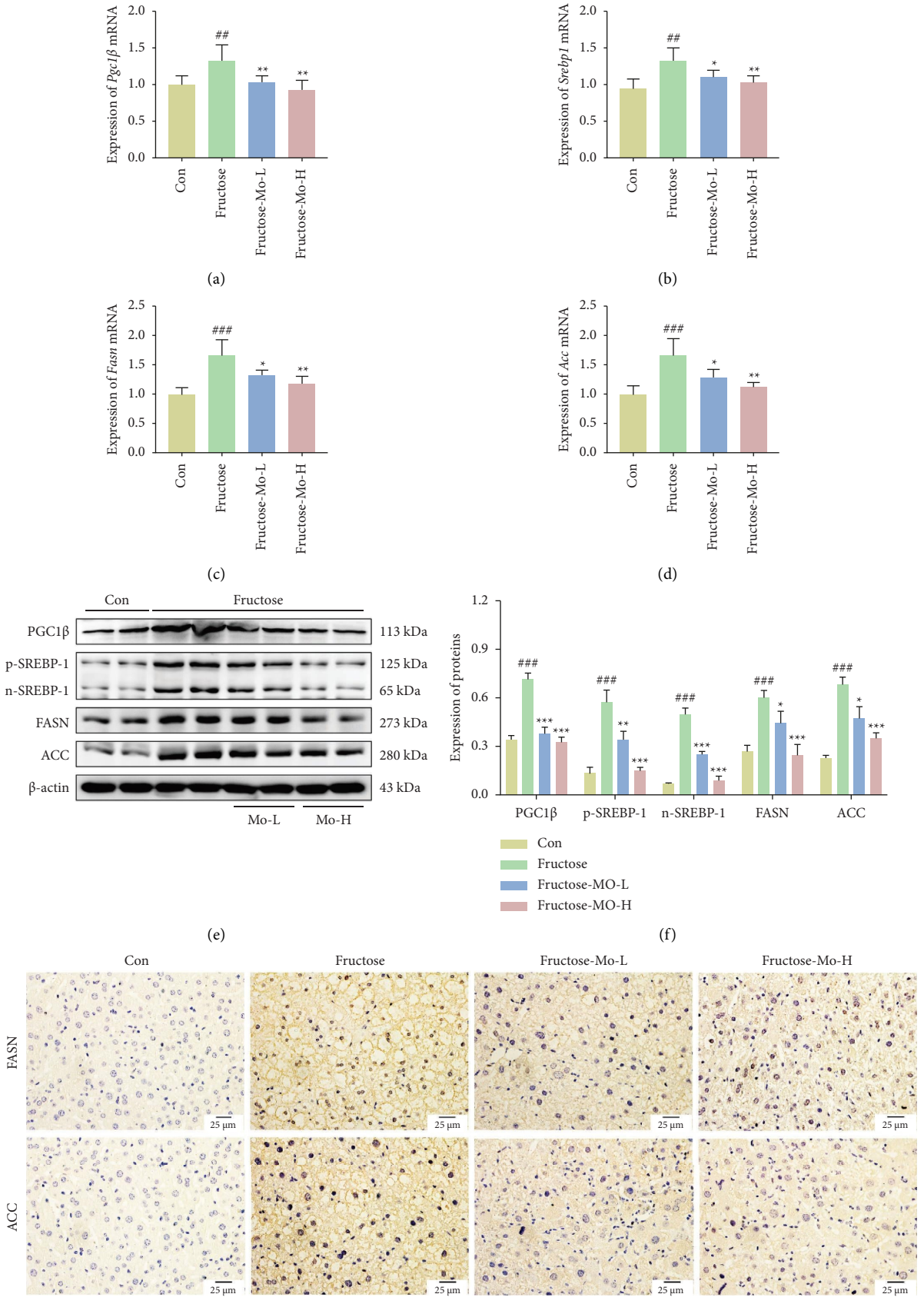
3.4. Morroniside Mitigates Oxidative Stress and Inflammatory Response in the Liver. Substantial evidence suggested that the enhancement of fructose intake was also linked with cellular oxidative stress and inflammatory response in the liver, which further aggravated hepatic lipid accumulation [8]. Similarly, our study demonstrated that compared with the control group, the ROS and MDA contents were elevated along with decreased T-SOD activity in the liver of fructose-fed mice, whereas these were reversed by morroniside treatment (Figures 4(a)–4(c)). Moreover, the hepatic levels of inflammatory factors TNF-α, IL-6, and IL-1β were significantly increased in the fructose-fed mice in comparison with those in the liver of the control group, but those were decreased by morroniside administration (Figures 4(d)–4(f)). Additionally, the hepatic protein expressions of NLRP3, Caspase-1, and IL-1β were reduced in morroniside-treated mice (Figures 4(g) and 4(h)). These findings suggest that morroniside can suppress the oxidative stress and inflammatory response in the liver of fructose-fed mice.

3.5. Morroniside Normalizes PGC1β-Mediated Lipid Metabolic Disorder in Vitro. CCK-8 assay was performed to determine suitable concentrations of morroniside in cell-based evaluations (Supplementary Figure 2). To further validate the mechanism underlying, Nile red staining of fructose-incubated HepG2 cells in the absence or presence of morroniside showed that compared with the control group, fructose-incubated cells had severe lipid accumulation, whereas morroniside treatment attenuated fructose-caused lipid accumulation in HepG2 cells (Figure 5(a)), along with reduced TG content (Figure 5(b)). Moreover, fructose incubation could significantly increase the protein expressions of PGC1β, SREBP-1, FASN, and ACC but increased PPARα and CPT1α expressions in fructose-incubated HepG2 and AML-12 cells in comparison with the control group, while the protein expressions of PGC1β, SREBP-1, FASN, and ACC were decreased, and PPARα and CPT1α expressions were increased after morroniside treatment (Figures 5(c)–5(f)). PGC1β has been reported to be a critical molecule that involves in cellular lipid metabolism. Next, we further used the PGC1β plasmid to construct a PGC1β-overexpressed HepG2 cell model by cell transfection to validate the important role of PGC1β in fructose-induced NAFLD (Supplementary Figure 3). Our results showed that the decreased protein expression of SREBP-1 and FASN, and the elevation in PPARα and CPT1α by morroniside treatment were partly counteracted after PGC1β overexpression (Figures 5(g) and 5(h)). It further indicated that morroniside alleviated lipid accumulation in hepatocytes by restoring disordered lipid metabolism and emphasized the important role of PGC1β in these processes.

4. Discussion

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of liver dysfunction worldwide and encompasses a spectrum of liver diseases with diverse histopathological liver phenotypes from simple lipid steatosis, fatty infiltration, nonalcoholic steatohepatitis (NASH), fibrosis, eventually progressive to hepatocellular carcinoma (HCC) [3, 22]. NAFLD is characterized by a complex liver metabolic dysfunction resulting from the interaction between multiple independent risk factors, such as chronic fructose consumption [7]. Growing evidence suggests the tight links between increased fructose consumption and the dramatic rise in the prevalence of NAFLD that makes high-fructose diet the closest recapitulation of the human NAFLD phenotype for translational hepatology research in animal models [6, 23]. *Cornus Officinalis*, a traditional Chinese herb and food plant, is widely used in the treatment of liver diseases [16, 24]. As the main component of *Cornus Officinalis*, morroniside has also been suggested to be effective in liver protection. Previous research has suggested that morroniside was effective in regulating metabolic disorders in the liver of diabetic mice [15], which mainly emphasized its effect on normalizing dyslipidemia and ameliorating the lipid accumulation phenotype, but the mechanism underlying was not well elucidated. Therefore, the present study focused on the effect and mechanism of morroniside on fructose-induced steatosis hepatis.

Dietary fructose is considered to have detrimental health consequences and is implicated in the development of metabolic diseases, including NAFLD [25]. The ingested fructose is firstly absorbed by the small intestine under the action of Glucose Transporter 5 (GLUT5), and via Glucose Transporter 2 (GLUT2), and the majority is transported into the liver where it is rapidly metabolized and cleared [4]. Fructose metabolism in the liver is initially involved in the phosphorylation of fructose by ketohexokinase into fructose 1-phosphatase (F1P), which undergoes a series of subsequent metabolic conversions to generate a large amount of acetyl-CoA, whose carbon precursor can be converted into fatty acids, thus entering the tricarboxylic acid (TCA) cycle undergoing oxidative metabolism. Studies have revealed that acetyl-CoA can not only be regenerated via ATP citrate lyase (ACLY) but also by the gut microbiota that dietary fructose intake can be transformed into acetate to provide lipogenic acetyl-CoA independently of ACLY [9], ultimately promoting de novo lipogenesis. Since this cycle has a relative maximum rate to deal with its substrates, the exceeding acetyl-CoA exits into the cytosol in the form of citrate and then dimerized and decarboxylated through an effect of acetyl-CoA carboxylase (ACC) to form malonyl-CoA as the substrate of lipogenesis, leading to increased intracellular



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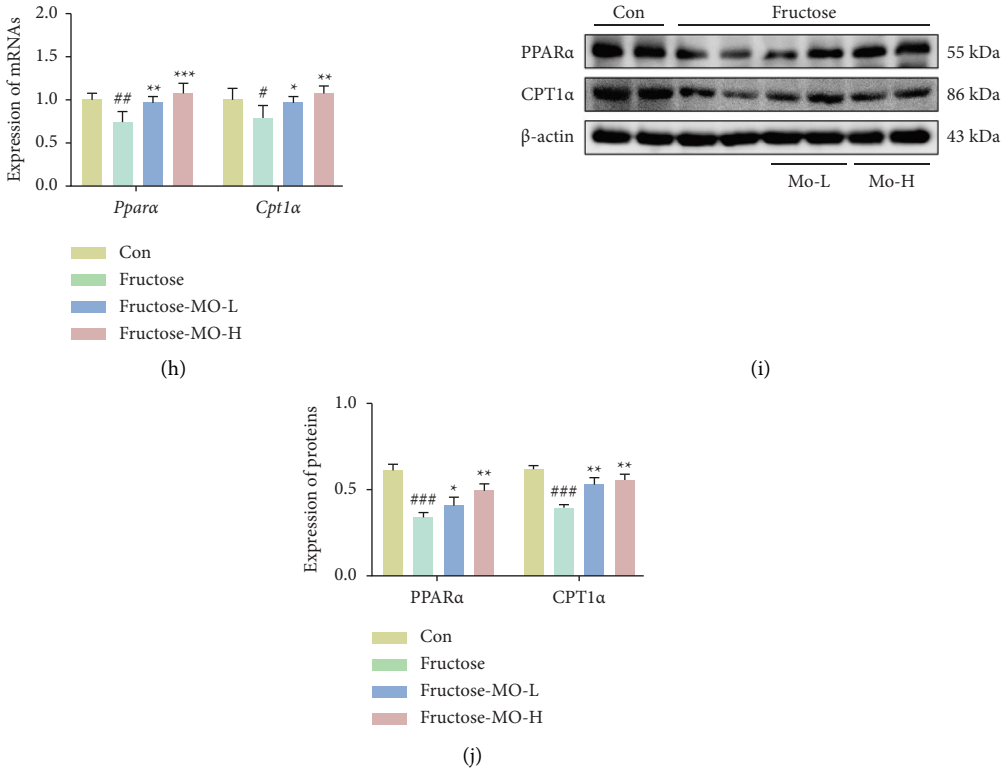
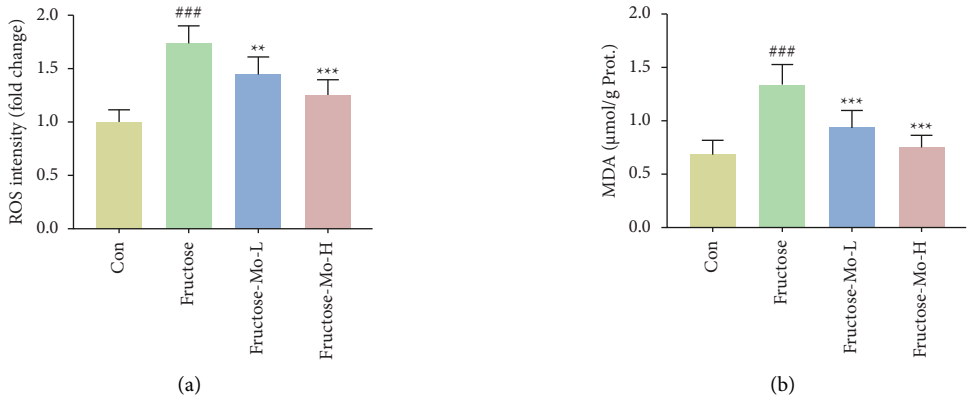


Figure 3: Morroniside enhances hepatic lipogenesis and impairs β-oxidation in mice. (a)–(d) The mRNA expression of Pgc1β, Srebp1, Fasn, and Acc from the con or fructose-fed mice with intragastric administration of either vehicle or morroniside, respectively. (e, f) Representative immunoblots and quantification of PGC1β, p-SREBP-1 (precursor), n-SREBP-1 (nuclear), FASN, and ACC in liver. (g) Representative images of immunohistochemistry assay of FASN and ACC in liver. (h) The mRNA expression of Pparα and Cpt1α from the con or fructose-fed mice with intragastric administration of either vehicle or morroniside, respectively. (i, j) Representative immunoblots and quantification of PPARα and CPT1α in liver. Mean ± S.D., $n = 8$ for RT-qPCR quantification analysis and $n = 3$ for liver protein expression analysis. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the con group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the fructose-fed group. Con: control.



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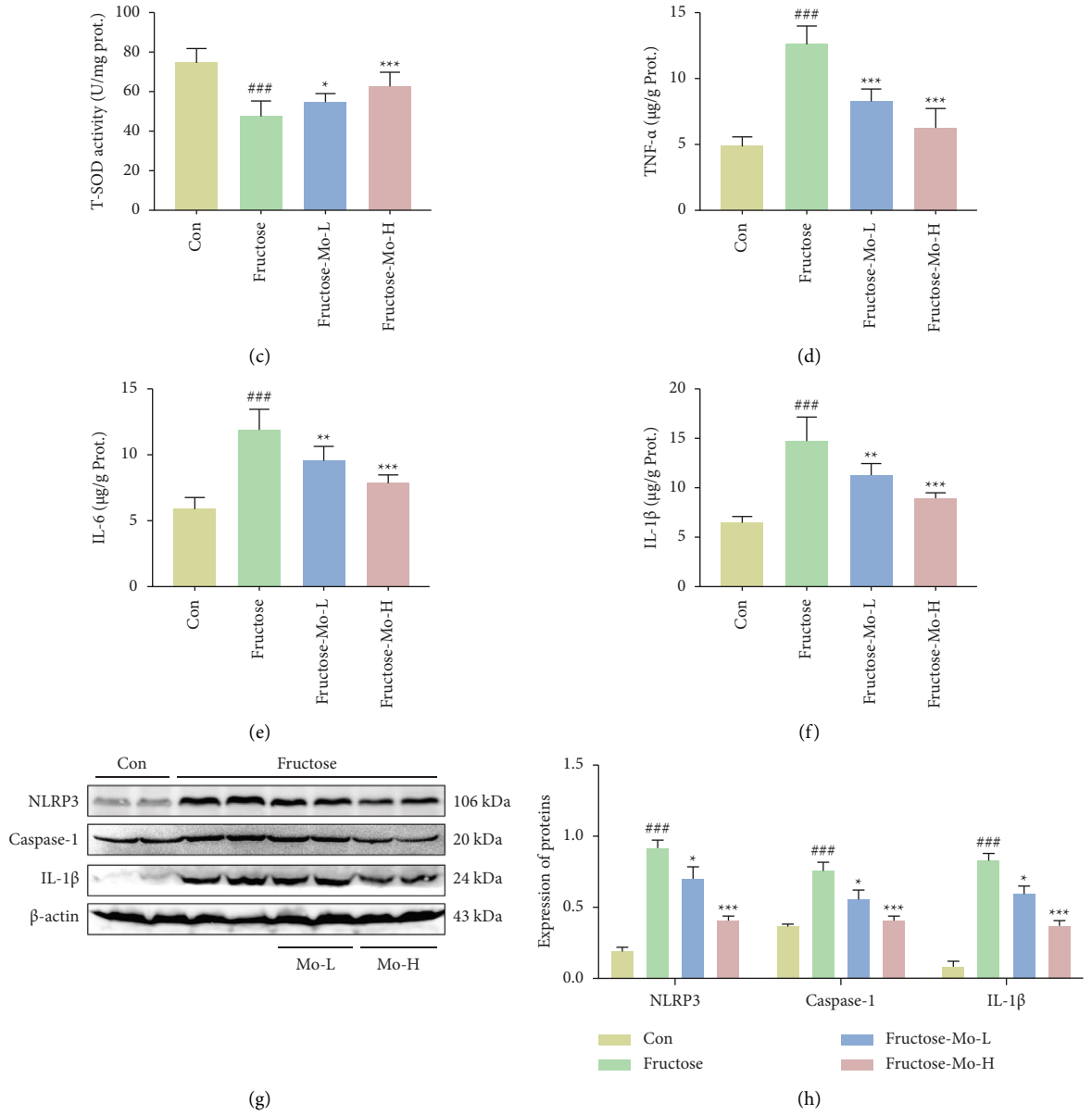
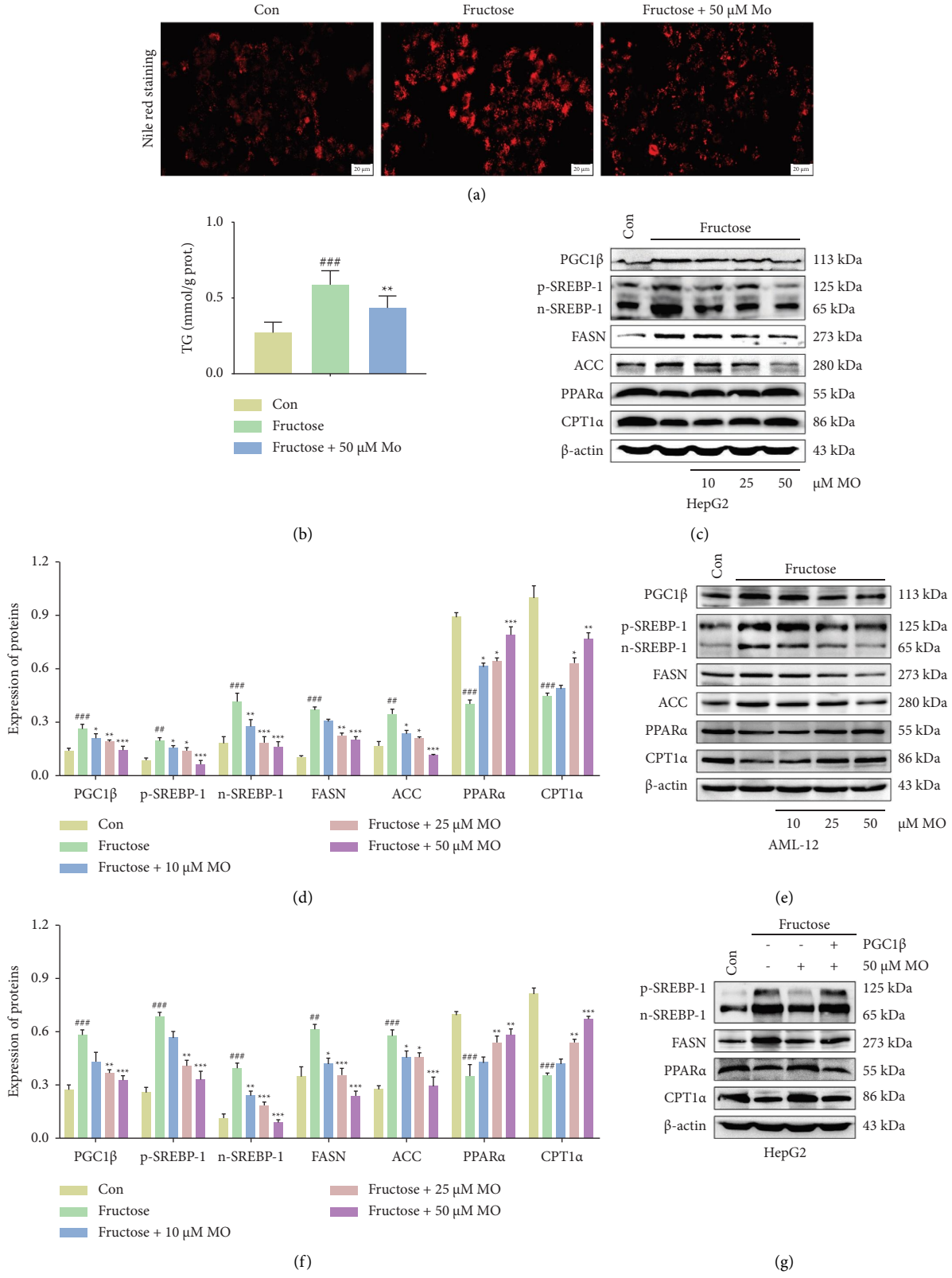


Figure 4: Morroniside mitigates oxidative stress and inflammatory response in the liver. The ROS intensity (a), content of MDA (b), and T-SOD activity (c) in the liver of the con or fructose-fed mice with intragastric administration of either vehicle or morroniside, respectively ($n = 8$). (d-f) The contents of inflammatory factors TNF-α, IL-6, and IL-1β in the liver of the con or fructose-fed mice with intragastric administration of either vehicle or morroniside, respectively ($n = 8$). (g, h) Representative immunoblots and quantification of NLRP3, caspase-1 and IL-1β in liver ($n = 3$). Mean ± S.D., $^{###}P < 0.001$ vs. the con group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the fructose-fed group. Con: control.

lipid formation [26]. Moreover, fructose robustly promotes lipogenesis not only by providing a substrate for fatty acid synthesis but also by stimulating the activation of lipogenic transcription factors, such as carbohydrate-responsive element-binding protein (ChREBP) and sterol regulatory element binding protein 1 (SREBP-1), which further activate the activity of enzymes (FASN and ACC) involved in de novo lipogenesis [9, 27, 28]. Furthermore, F1P stimulates the activation of peroxisome proliferator-activated receptor γ coactivator 1β (PGC1β), a transcriptional coactivator for SREBP-1, which can deteriorate lipogenesis by binding with ChREBP1 and SREBP-1 to enhance their transcriptional activities [29, 30]. Intriguingly, our results showed that the hepatic expressions of PGC1β, SREBP-1, FASN, and ACC were increased in the fructose group, while were decreased after morroniside treatment, indicating that morroniside is capable of reducing hepatic lipogenesis in fructose-fed mice.

Overloaded fructose intake has also been confirmed to impair mitochondrial oxidation [12]. Notably, malonyl-CoA inhibits the activity of carnitine palmitoyl transferase 1 α (CPT1α) and prevents β-oxidation that alters the balance between lipogenesis and fatty acid oxidation [31],



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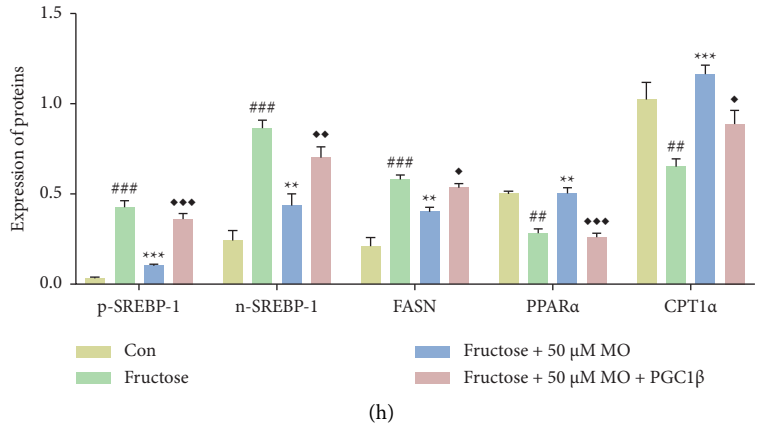

Figure 5: Morroniside normalizes lipid metabolic disorder in vitro. (a) Morroniside attenuated fructose-induced lipid accumulation in HepG2 cells by Nile red staining. (b) Morroniside reduced triglyceride (TG) content in fructose-induced HepG2 cells. (c, d) Representative immunoblots and quantification of key proteins in lipogenesis (PGC1β, p-SREBP-1 (precursor), n-SREBP-1 (nuclear), FASN, and ACC) and fatty acid oxidation (PPARα and CPT1α) in the fructose-induced HepG2 cells. (e, f) Representative immunoblots and quantification of key proteins in lipogenesis (PGC1β, p-SREBP-1 (precursor), n-SREBP-1 (nuclear), FASN, and ACC) and fatty acid oxidation (PPARα and CPT1α) in the fructose-induced AML-12 cells. (g, h) Representative immunoblots and quantification of p-SREBP-1 (precursor), n-SREBP-1 (nuclear), FASN, PPARα, and CPT1α in fructose-treated HepG2 cells by morroniside under PGC1β overexpression. Mean ± S.D., $n = 3$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the con group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the fructose-treated group; $^{\blacklozenge}P < 0.05$, $^{\blacklozenge\blacklozenge}P < 0.01$, $^{\blacklozenge\blacklozenge\blacklozenge}P < 0.001$ vs. the fructose and morroniside coprocessing group. Con: control.

contributing to lipid accumulation. In this study, the expression of CPT1α was decreased after long-term fructose intake but increased by morroniside. Considering that CPT1α is the target gene of proliferator-activated receptor alpha (PPARα), a master-regulated gene for fatty acid oxidation [32], the expression of PPARα was also reduced in response to fructose induction, whereas increased after morroniside administration. Moreover, studies had revealed that fructose (5 mM) could lead to lipid metabolism disorder and lipid deposition in liver cells [33, 34]. By that, we performed fructose (5 mM) induction in HepG2 and AML-12 cells and intervened with morroniside to further validate the effective mechanism, and ultimately, consistent results were found in vitro. Altogether demonstrated that fructose-induced enhanced lipogenesis in hepatocytes was accompanied by impaired fatty acid oxidation. In contrast, morroniside improved these dysregulations of lipid metabolism. Notably, to validate the role of PGC1β and the exactly targeted mechanism in the ameliorative effect of morroniside on fructose-induced lipid metabolic disorder in hepatocytes, we further overexpressed PGC1β in HepG2 cells and found that the positive effect of morroniside on regulating the imbalance between lipogenesis and β-oxidation was counteracted after PGC1β overexpression. It is suggested that morroniside might improve fructose-triggered lipid metabolism disorders by targeted inhibiting the activation of PGC1β, thus ameliorating the progression of NAFLD.

Enhanced lipogenesis along with impaired β-oxidation seems to exert only a minor impact on the whole process of NAFLD development but might be arguably enhanced by a variety of endogenous defense mechanisms and vicious cycles, expediting the progression towards NASH [35, 36]. In this study, we found that the serum levels of liver function indicators (AST and ALT) and triglyceride transfer protein (HDL-c and LDL-c) were influenced in fructose-fed mice, while tending to be normal after morroniside treatment, indicating that morroniside could improve fructose-induced liver dysfunction and accelerate triglyceride export, thus attenuating hepatic lipid accumulation. In lipid-engorged hepatocytes, the overreduction of upstream oxidation and excess de novo lipid synthesis exceeding the oxidative capacity increase mitochondrial oxidative stress, contributing to the cellular inflammatory response which further aggravates lipid accumulation and accelerates the progression of NAFLD [8]. Here, we found that fructose intake could enhance the liver ROS and MDA content but inhibit T-SOD activity, while these poor indicators of oxidative stress were improved by morroniside treatment. Moreover, fructose could also stimulate the activation of NOD-like receptor protein 3 (NLRP3) and promote the release of inflammatory factors that play a critical role in modulating the inflammatory response, such as caspase-1, TNF-α, IL-6, and IL-1β, whereas morroniside alleviated the activated hepatic oxidative stress and inflammation, suggesting that morroniside is effective in mitigating fructose-caused hepatic inflammatory response. Comprehensively, the regulatory effect of morroniside on lipid metabolism is integrated with its mitigation on lipid accumulation-triggered oxidative and inflammatory response, thus slowing down the progression of NAFLD.

5. Conclusion

In summary, our findings suggest the ameliorative effect of morroniside in fructose-induced hepatic steatosis and inflammatory response. In vivo and in vitro data demonstrated that morroniside can reduce hepatic de novo lipogenesis and promote β-oxidation, which may be related to the inhibitive

effect of morroniside on PGC1β activation. Additionally, morroniside is also effective in diminishing hepatic inflammation. Therefore, the current study reveals that morroniside is a promising therapeutic drug for NAFLD and delays the progression from NAFLD to NASH, especially for those resulting from overconsumption of carbohydrates.

Abbreviations

ACC: Acetyl-CoA carboxylase
ALT: Alanine transaminase
AST: Aspartate transaminase
ChREBP: Carbohydrate response element binding protein
CPT1α: Carnitine palmitoyl transferase 1α
FASN: Fatty acid synthase
FFA: Free fatty acid
HDL-C: High-density lipoprotein cholesterol
H & *E*: Hematoxylin-eosin
IL-1β: Interleukin-1β
IL-6: Interleukin-6
LDL-C: Low-density lipoprotein cholesterol
NAFLD: Nonalcoholic fatty liver disease
ORO: Oil red O
PGC1β: Peroxisome proliferator-activated receptor γ coactivator 1β
PPARα: Peroxisome proliferator-activated receptor α
ROS: Reactive oxygen species
SREBP-1: Sterol regulatory element-binding protein-1
T-CHO: Total cholesterol
TG: Triglyceride
TNF-α: Tumor necrosis factor α
Mo: Morroniside.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Q. T. conceptualized the study, investigated the study, and curated the data; J. X., Y. C., and R. H. developed methodology and provided formal analysis; Y. Z., J. S., and X. P. provided software and visualized the study; J. H. conceptualized the study and administrated the project; Y. C. and R. Z. provided funding acquisition, wrote the original draft, and reviewed and edited the article. All authors have read and agreed to the published version of the manuscript. Qiao Tong and Jianjun Xi contributed equally to this work.

Acknowledgments

This study was supported by the Research Project on the Application of Public Welfare Technology supported by the Science and Technology Department of Zhejiang Province (LGF21H300002), Zhejiang Provincial Key Laboratory for Accurate Diagnosis and Treatment of Chronic Liver Diseases (2020E10014), the Agricultural and Social Development Fund of the Hangzhou Science and Technology Committee (20201203B211), and the Medical Health Science and Technology Major and General Projects of Hangzhou Health Commission (Z20200022, Z20210004, A20220159).

Supplementary Materials

Effect of morroniside (Mo) on final body weight in fructose-fed mice (Supplementary Figure 1). Effects of morroniside (Mo) on cell viability of HepG2 cell lines (Supplementary Figure 2). Transfection efficiency of PGC1 overexpression (Supplementary Figure 3). List of the primary antibodies used for Western blot (WB) analysis and immunohistochemistry (IHC) (Supplementary Table 1). Sequences of primers used in RT-qPCR (Supplementary Table 2). (*Supplementary Materials*)

References

[1] R. Loomba, S. L. Friedman, and G. I. Shulman, "Mechanisms and disease consequences of nonalcoholic fatty liver disease," *Cell*, vol. 184, no. 10, pp. 2537–2564, 2021.
[2] Q. Ye, B. Zou, Y. H. Yeo et al., "Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis," *The Lancet Gastroenterology & Hepatology*, vol. 5, no. 8, pp. 739–752, 2020.
[3] Z. M. Younossi, "Non-alcoholic fatty liver disease- a global public health perspective," *Journal of Hepatology*, vol. 70, no. 3, pp. 531–544, 2019.
[4] M. A. Febbraio and M. Karin, ""Sweet death": fructose as a metabolic toxin that targets the gut-liver axis," *Cell Metabolism*, vol. 33, no. 12, pp. 2316–2328, 2021.
[5] L. Tappy and K. A. Le, "Metabolic effects of fructose and the worldwide increase in obesity," *Physiological Reviews*, vol. 90, no. 1, pp. 23–46, 2010.
[6] T. Jensen, M. F. Abdelmalek, S. Sullivan et al., "Fructose and sugar: a major mediator of non-alcoholic fatty liver disease," *Journal of Hepatology*, vol. 68, no. 5, pp. 1063–1075, 2018.
[7] K. W. Ter Horst and M. J. Serlie, "Fructose consumption, lipogenesis, and non-alcoholic fatty liver disease," *Nutrients*, vol. 9, no. 9, p. 981, 2017.
[8] J. Todoric, G. Di Caro, S. Reibe et al., "Fructose stimulated de novo lipogenesis is promoted by inflammation," *Nat Metab*, vol. 2, no. 10, pp. 1034–1045, 2020.
[9] S. Zhao, C. Jang, J. Liu et al., "Dietary fructose feeds hepatic lipogenesis via microbiota-derived acetate," *Nature*, vol. 579, no. 7800, pp. 586–591, 2020.
[10] V. T. Samuel and G. I. Shulman, "Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases," *Cell Metabolism*, vol. 27, no. 1, pp. 22–41, 2018.
[11] M. A. Herman and M. J. Birnbaum, "Molecular aspects of fructose metabolism and metabolic disease," *Cell Metabolism*, vol. 33, no. 12, pp. 2329–2354, 2021.
[12] S. Softic, J. G. Meyer, G. X. Wang et al., "Dietary sugars alter hepatic fatty acid oxidation via transcriptional and post-translational modifications of mitochondrial proteins," *Cell Metabolism*, vol. 30, no. 4, pp. 735–753.e4, 2019.
[13] J. Gao, P. Liu, Z. Shen et al., "Morroniside promotes PGC-1α-mediated cholesterol efflux in sodium palmitate or high

glucose-induced mouse renal tubular epithelial cells," *BioMed Research International*, vol. 2021, Article ID 9942152, 14 pages, 2021.

[14] C. H. Park, J. S. Noh, J. H. Kim et al., "Evaluation of morroniside, iridoid glycoside from Corni Fructus, on diabetes-induced alterations such as oxidative stress, inflammation, and apoptosis in the liver of type 2 diabetic db/db mice," *Biological and Pharmaceutical Bulletin*, vol. 34, no. 10, pp. 1559–1565, 2011.

[15] C. H. Park, N. Yamabe, J. S. Noh, K. S. Kang, T. Tanaka, and T. Yokozawa, "The Beneficial Effects of Morroniside on the Inflammatory Response and Lipid Metabolism in the Liver of db/db Mice," *Biological and Pharmaceutical Bulletin*, vol. 32, no. 10, pp. 1734–1740, 2009.

[16] L. Cao, Y. Wu, W. Li et al., "Cornus officinalis vinegar reduces body weight and attenuates hepatic steatosis in mouse model of nonalcoholic fatty liver disease," *Journal of Food Science*, vol. 87, no. 7, pp. 3248–3259, 2022.

[17] E. Park, C. G. Lee, H. Jeon et al., "Anti-obesity effects of combined Cornus officinalis and ribes fasciculatum extract in high-fat diet-induced obese male mice," *Animals*, vol. 11, no. 11, p. 3187, 2021.

[18] E. Park, C. G. Lee, J. Kim, J. H. Kang, Y. G. Cho, and S. Y. Jeong, "Efficacy and safety of combined extracts of Cornus officinalis and ribes fasciculatum for body fat reduction in overweight women," *Journal of Clinical Medicine*, vol. 9, no. 11, p. 3629, 2020.

[19] C. H. Park, J. S. Noh, T. Tanaka, and T. Yokozawa, "Effects of morroniside isolated from Corni Fructus on renal lipids and inflammation in type 2 diabetic mice," *Journal of Pharmacy and Pharmacology*, vol. 62, no. 3, pp. 374–380, 2010.

[20] N. Yamabe, J. S. Noh, C. H. Park et al., "Evaluation of loganin, iridoid glycoside from Corni Fructus, on hepatic and renal glucolipotoxicity and inflammation in type 2 diabetic db/db mice," *European Journal of Pharmacology*, vol. 648, no. 1-3, pp. 179–187, 2010.

[21] D. E. Kleiner, E. M. Brunt, M. Van Natta et al., "Design and validation of a histological scoring system for nonalcoholic fatty liver disease," *Hepatology*, vol. 41, no. 6, pp. 1313–1321, 2005.

[22] S. L. Friedman, B. A. Neuschwander-Tetri, M. Rinella, and A. J. Sanyal, "Mechanisms of NAFLD development and therapeutic strategies," *Nature Medicine*, vol. 24, no. 7, pp. 908–922, 2018.

[23] Y. R. Im, H. Hunter, D. de Gracia Hahn et al., "A systematic review of animal models of NAFLD finds high-fat, high-fructose diets most closely resemble human NAFLD," *Hepatology*, vol. 74, no. 4, pp. 1884–1901, 2021.

[24] N. H. Lee, C. S. Seo, H. Y. Lee et al., "Hepatoprotective and antioxidative activities of Cornus officinalis against acetaminophen-induced hepatotoxicity in mice," *Evidence-based Complementary and Alternative Medicine*, vol. 2012, Article ID 804924, 8 pages, 2012.

[25] J. S. Lim, M. Mietus-Snyder, A. Valente, J. M. Schwarz, and R. H. Lustig, "The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome," *Nature Reviews Gastroenterology & Hepatology*, vol. 7, no. 5, pp. 251–264, 2010.

[26] M. A. Herman and V. T. Samuel, "The sweet path to metabolic demise: fructose and lipid synthesis," *Trends in Endocrinology and Metabolism*, vol. 27, no. 10, pp. 719–730, 2016.

[27] D. W. Foster, "Malonyl-CoA: the regulator of fatty acid synthesis and oxidation," *Journal of Clinical Investigation*, vol. 122, no. 6, pp. 1958-1959, 2012.

[28] S. Softic, D. E. Cohen, and C. R. Kahn, "Role of dietary fructose and hepatic de novo lipogenesis in fatty liver disease," *Digestive Diseases and Sciences*, vol. 61, no. 5, pp. 1282–1293, 2016.

[29] J. Lin, R. Yang, P. T. Tarr et al., "Hyperlipidemic effects of dietary saturated fats mediated through PGC-1β coactivation of SREBP," *Cell*, vol. 120, no. 2, pp. 261–273, 2005.

[30] M. Xia, Y. Liu, H. Guo, D. Wang, Y. Wang, and W. Ling, "Retinol binding protein 4 stimulates hepatic sterol regulatory element-binding protein 1 and increases lipogenesis through the peroxisome proliferator-activated receptor-γ coactivator 1β-dependent pathway," *Hepatology*, vol. 58, no. 2, pp. 564–575, 2013.

[31] J. D. McGarry, G. P. Mannaerts, and D. W. Foster, "A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis," *Journal of Clinical Investigation*, vol. 60, no. 1, pp. 265–270, 1977.

[32] A. Montagner, A. Polizzi, E. Fouche et al., "Liver PPARα is crucial for whole-body fatty acid homeostasis and is protective against NAFLD," *Gut*, vol. 65, no. 7, pp. 1202–1214, 2016.

[33] X. Zhang, J. H. Zhang, X. Y. Chen et al., "Reactive oxygen species-induced TXNIP drives fructose-mediated hepatic inflammation and lipid accumulation through NLRP3 inflammasome activation," *Antioxidants and Redox Signaling*, vol. 22, no. 10, pp. 848–870, 2015.

[34] Y. Zhou, Y. L. Ding, J. L. Zhang, P. Zhang, J. Q. Wang, and Z. H. Li, "Alpinetin improved high fat diet-induced non-alcoholic fatty liver disease (NAFLD) through improving oxidative stress, inflammatory response and lipid metabolism," *Biomedicine & Pharmacotherapy*, vol. 97, pp. 1397–1408, 2018.

[35] S. Ducheix, M. C. Vegliante, G. Villani, N. Napoli, C. Sabba, and A. Moschetta, "Is hepatic lipogenesis fundamental for NAFLD/NASH? A focus on the nuclear receptor coactivator PGC-1beta," *Cellular and Molecular Life Sciences*, vol. 73, no. 20, pp. 3809–3822, 2016.

[36] I. Pierantonelli and G. Svegliati-Baroni, "Nonalcoholic fatty liver disease: basic pathogenetic mechanisms in the progression from NAFLD to NASH," *Transplantation*, vol. 103, no. 1, pp. e1–e13, 2019.