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Freshly pressed camellia seed oil rich in polyphenolic lipid concomitant ameliorates non-alcoholic steatohepatitis induced by western high-fat diet in mice

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Abstract

Objective: Study on Freshly Pressed Camellia seed oil (FP-CSO) which is rich in polyphenolic lipid concomitants in western high fat diet The protective effect of HFDs on the inflammatory response and liver damage of non-alcoholic fatty liver disease (NAFLD) induced in mice. Methods: C57BL/6 mice were fed a high fat diet (HFD-L) based on lard (high proportion of saturated fatty acids) for 12 weeks to induce the development of NAFLD. HFD-fed mice were then divided into three groups: a) continued to receive a high fat diet based on pig oil (HFD-L, n=10); B) HFD-FP-CSO, n=10, was replaced with fresh pressed camellia seed oil (rich in polyphenolic lipid concomitants) based high-fat diet with the same energy ratio; C) Refined camellia seed oil (R-CFO) refined camellia seed oil (HFD-R-CSO, n=10) refined camellia seed oil (R-CFO, lower polyphenolic lipid concomitants) high fat diet (HFD-R-CSO, n=10) continued feeding for 12 weeks. A normal control group (LOW fat diet, LFD, n=10) was fed a standard diet (low fat energy ratio diet). Body weight changes of mice in each group were recorded. Four items of blood lipid were detected by biochemical analyzer. Serum alanine transaminase (ALT) was detected to evaluate liver injury. Serum il-6, TNF-α, IFN-γ and other inflammatory cytokines were detected by ELISA. The mRNA expression of hepatic fatty acid translocase CD36(CD36) was detected by fluorescence quantitative PCR. The changes of fatty acid spectrum in liver were detected by GC-MS. Results: Fat metabolism disorder caused by HFD-L could be improved by replacing lard with CSO. CSO diet can improve hyperlipidemia, reduce body weight, and reduce serum INF-γ, IL-6 and other inflammatory cytokines. In addition, the expression of liver fatty acid translocase CD36 gene was up-regulated in HFD-FP-CSO group. Conclusion: Eating fresh camellia seed oil rich in polyphenolic lipid concomitant can repair liver damage caused by HFD. The mechanism may be related to the comprehensive effect of anti-inflammatory effect in adipose tissue and promoting the repair of lipid metabolism signaling pathway..

Keywords

High-fat diet, Inflammation, Freshly pressed camellia seed oil, Nonalcoholic fatty liver disease, Polyphenol lipid concomitants.

1. Introduction

Oil Camellia is a large evergreen shrub or small tree in the genus Camellia of Theaceae. Camellia oleica has a history of cultivation for more than 2300 years in China, which integrates economic, ecological and social benefits. It is a unique high-quality woody oil tree species in China. Camellia, oil palm, olive and coconut are known as the world's four major woody oil plants.

Camellia seed oil is rich in unsaturated fatty acids, with oleic acid content as high as 75%~80%. Because of its physical and chemical properties similar to Mediterranean olive oil, it enjoys the

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reputation of "Oriental olive oil". Camellia oil contains oleic acid, linoleic acid, linolenic acid, tocopherol, phytosterol, keratosallene and other nutrients. Long-term consumption can reduce cholesterol and prevent cardiovascular diseases[1]. Newly developed freshly pressed Camellia seed oil (FP-CSO) is rich in naturally active substances and its quality is comparable to that of extra virgin olive oil. A freshly squeezed camellia seed oil and extra virgin olive oil comparison study found [2], freshly squeezed camellia seed oil on the physical and chemical indicators like extra virgin olive oil, fatty acid composition and extra virgin olive oil are similar, but the oleic acid content is higher than that of extra virgin olive oil, palmitic acid and stearic acid (mainly two kinds of saturated fatty acid) content is lower than the extra virgin olive oil. The content of proanthocyanidins, vitamin E, polyphenols, total saponins, total triterpenes and coenzyme Q10 in fresh camellia seed oil was higher than that in extra virgin olive oil.

Nonalcoholic fatty liver disease (NAFLD) is a condition in which excess fat is deposited in the liver cells in the absence of high alcohol intake[3]. NAFLD is considered to be the liver manifestation of metabolic syndrome, which has become one of the major consequences of chronic overnutrition. NAFLD can progress to severe nonalcoholic steatohepatitis (NASH), which is characterized by inflammation with or without fibrosis and can lead to cirrhosis, liver cancer, and liver failure. Regarding the pathogenesis and progression of NAFLD, the molecular pathways behind it are not fully understood. In fact, the mechanisms involved in its pathophyphysiology have evolved under a more complex "multi-hit" hypothesis that includes nutritional factors, genetic and epigenetic mechanisms, changes in the gut microbiome, insulin resistance, oxidative stress, and cytokines secreted from adipose tissue.

Dietary fat intake may be an important modifiable factor in the development of NAFLD [4]. High fat diets (HFDs), especially those rich in saturated fatty acids (SFAs), can lead to increased liver fat accumulation and aggravate liver cell damage. The regulation of hepatic lipid metabolism by fatty acids is complex. Dietary fatty acid composition affects hepatic lipid metabolism. For example, omega-3 fatty acids inhibit the accumulation of lipids in the liver. On the other hand, Monounsaturated fatty acids (MUFAs) inhibit liver lipid synthesis by reducing the activation of sterol regulatory element-binding proteins (SREBP). In addition, oleic acid reduced the expression of genes related to lipid synthesis in the liver of Zucker obese rats. There is strong evidence that extra virgin olive oil reduces HFD-induced cirrhosis. When used in combination with other compounds, such as hydroxytyrosol at low doses, additional benefits were found in liver fat accumulation. Since dietary fat changes can prevent and/or reverse fat accumulation in the liver, we speculated that the addition of CSO may be beneficial to NAFLD patients.

The purpose of this study was to explore the role of CSO in the occurrence and development of NAFLD and its related molecular mechanisms. In addition, given the similarity of physicochemical properties between FP-CSO and extra virgin olive oil, we investigated the effect of the composition of polyphenolic lipid concomitants of FP-CSO on these mechanisms..

2. Materials and methods

2.1. Animal feeding and experimental design

Forty SPF male C57BL/6 mice, weighing 20 g ~ 25 g and 6 w years old, were purchased from Beijing Vitong Lihua Experimental Animal Technology Co., LTD., Animal Production License Number: SCXK (Beijing) 2016-0011. Raised in the SPF animal Experiment Center of Jianghan University. The ambient temperature was 24 ℃ ~ 26 ℃, and the humidity was 50% ~ 65%. All the mice could get water and food freely. The animal care and experimental program is formulated in accordance with the care and use guidelines of experimental animals of Jianghan University. Mice were given either a standard diet (13% of energy from fat, LFD) or a high fat diet based on lard (HFD-L; HFD with 60% fatty energy) to 12 weeks to promote the development of NAFLD. The HFD-receiving animals were then divided into three groups

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according to their diets: a) HFD-L diet; B) HFD based on FP-CSO (HFD-FP-CSO); C) HFD (HFD-R-CSO) based on refined camellia seed oil and continued feeding for 12 weeks. The concentrations of total phenolic compounds in FP-CSO and R-CSO were 538 ppm and 125 ppm, respectively.

2.2. Serum biochemical analysis and cytokine determination

On the weekend of 24th, the mice were fasting without water for 8h. After CO2 anesthesia, orbital blood was collected. Blood samples were kept at room temperature for 30 min, centrifuged at 3 000 r/min for 15 min, and serum samples were collected. Triglyceride (TG), total cholesterol (TCh), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) concentrations were detected using a biochemical analyzer. Serum inflammatory cytokines IL-6, IL-1β, TNF-α, INF-γ were detected by ELISA kit (Shenzhen Xinbosheng Biotechnology Co., LTD.)

2.3. The gene expression of liver tissue was analyzed by real-time fluorescence quantitative PCR

The mRNA expression of peroxisome proliferative factor activated receptor A(Ppara), triglyceride lipase Pnpla3 and hepatic fatty acid translocase Cd36 were detected by fluorescence quantitative PCR. Total LIVER RNA was extracted using the Animal Tissue/cell RNA Extraction Kit (Kangwei Century). The concentration and purity of the extracted RNA were determined by Nanodrop (Thermo Fisher). CDNA was synthesized by reverse transcription using the SuperRT cDNA First strand Synthesis Kit (Kang Wei Century). Fluorescence quantitative qPCR was performed by SYBR method in CFX96 RealTime PCR tester (Biorad), and each sample was tested in triplications. Gene expression was calibrated by endogenous glyceraldehyde 3-phosphorus dehydrogenase (Gapdh).

2.4. Statistical analysis

All data were expressed as X ± S, and GraphPad Prism 8.0 software was used for statistical analysis. Multivariate analysis of variance was used to determine the differences between groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of CSO on body weight and organ index in mice

HFD-L mice had a significantly higher body weight (BW) at the end of the study compared to LFD mice (Figure 1A) (Figure 1A, P<0.001). In addition, we did not observe any BW differences in HFD-FP-CSO mice compared to HFD-R-CSO mice. Epididymal fat weight in HFD-L mice was significantly higher than in LFD mice at the end of the study (Figure 1B; P< 0.001). However, no significant differences in epididymis weight were found in all HFD mouse populations. Liver weight (FIG. 1C) did not differ significantly between the HFD-fed groups. However, liver weight index was significantly reduced in the HFD-FP-CSO group compared to the HFD-L group (Figure 1D, P<0.05).

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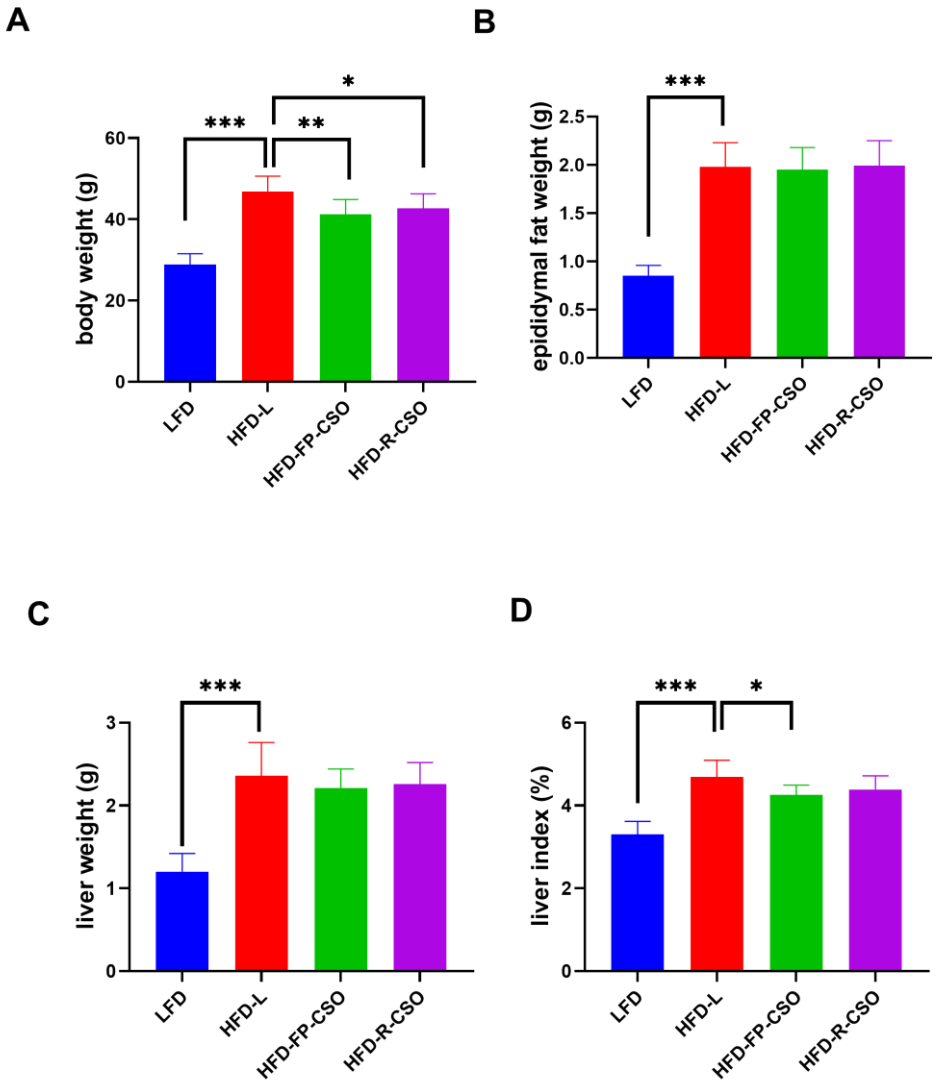

Figure 1: Body and organ weights in HFD-fed mice.

(A) Changes in body weight at the end of the study. (B) epididymal fat weight at 24 weeks. (C) liver weight after 12 weeks of experiment. (D) Liver mass index at the end of the study. *P<0.05, **P<0.01, ***P<0.001.

3.2. Effect of CSO on blood lipid in mice

Compared with LFD mice, HFD-L mice had significantly higher serum total cholesterol, low density lipoprotein cholesterol, and triglyceride levels (P<0.001). Compared with HFD-L mice, HFD-FP-CSO mice had significantly lower total cholesterol and low density lipoprotein cholesterol (P<0.001), HFD-R-CSO showed a significant decrease in triglyceride levels (P<0.05). HFD-fp-cso mice had significantly higher HDL cholesterol levels than HFD-L mice (P<0.05).

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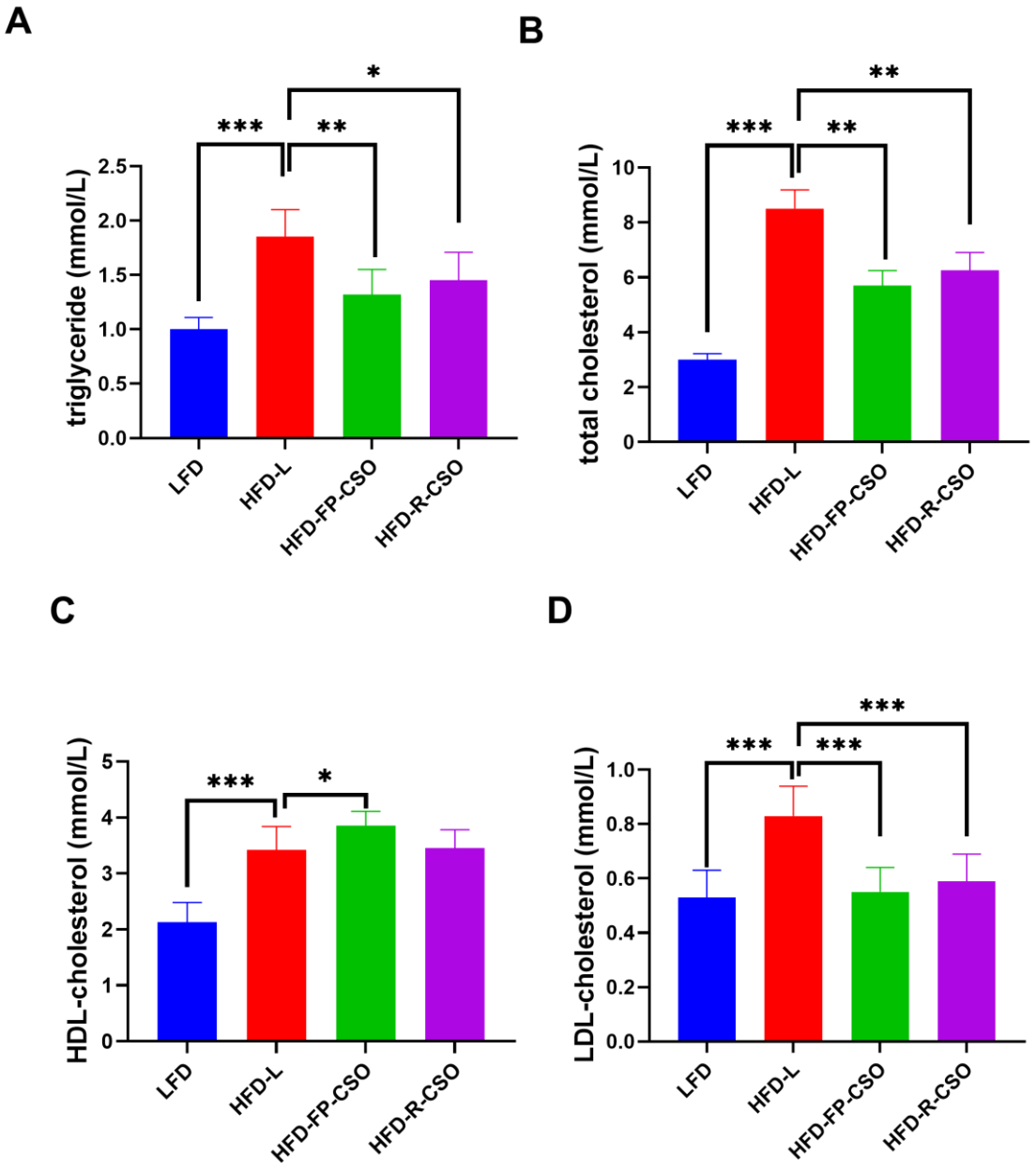

Figure 2: Effects of different diets on lipid status in mice after 24 weeks of experiment. Serum triglycerides (A), total cholesterol (B), high density lipoprotein cholesterol (C), low density lipoprotein cholesterol (D). *P<0.05, **P<0.01, ***P<0.001.

3.3. Inflammatory cytokines: Adipose tissue response to HFD

Figure 3 shows serum cytokine levels in mice at the end of the study. IFN-γ levels in HFD-L mice were significantly higher than those in other groups (P<0.001), Il-6 levels were significantly higher in LFD, HFD-FP-CSO and HFD-R-CSO groups (P<0.05). The levels of IL-1β and TNF-α did not differ significantly between groups.

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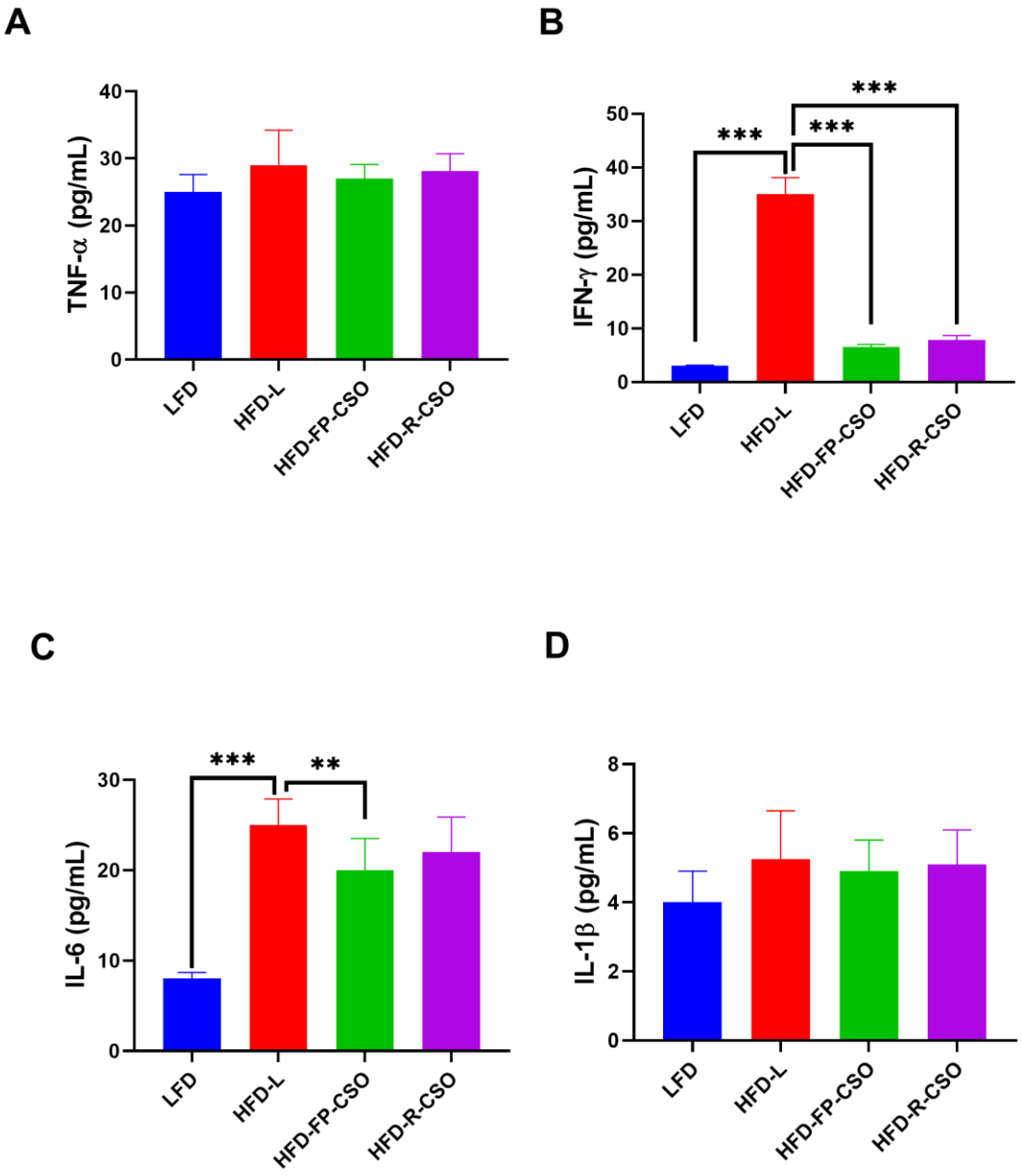

Figure 3: Serum INF-γ, IL-6, IL-1β and TNF-α levels
*P<0.05, **P<0.01, ***P<0.001.

3.4. Regulation of genes involved in hepatic lipid metabolism

Analysis of mRNA expression levels of Cd36, Pnpla3 and Ppara, known to regulate lipid balance in the liver, showed that after 24 weeks of nutritional intervention (Figure 4A), the expression of fatty acid transporter Cd36 was significantly increased in the HFD-FP-CSO group (P<0.05). In addition, HFD-FP-CSO and HFD-R-CSO mice increased the expression of fatty acid metabolism genes Ppara and Pnpla3 (P<0.05, Figure 4B). The HFD-R-CSO group showed an increased expression trend of Ppara (Figure 4C).

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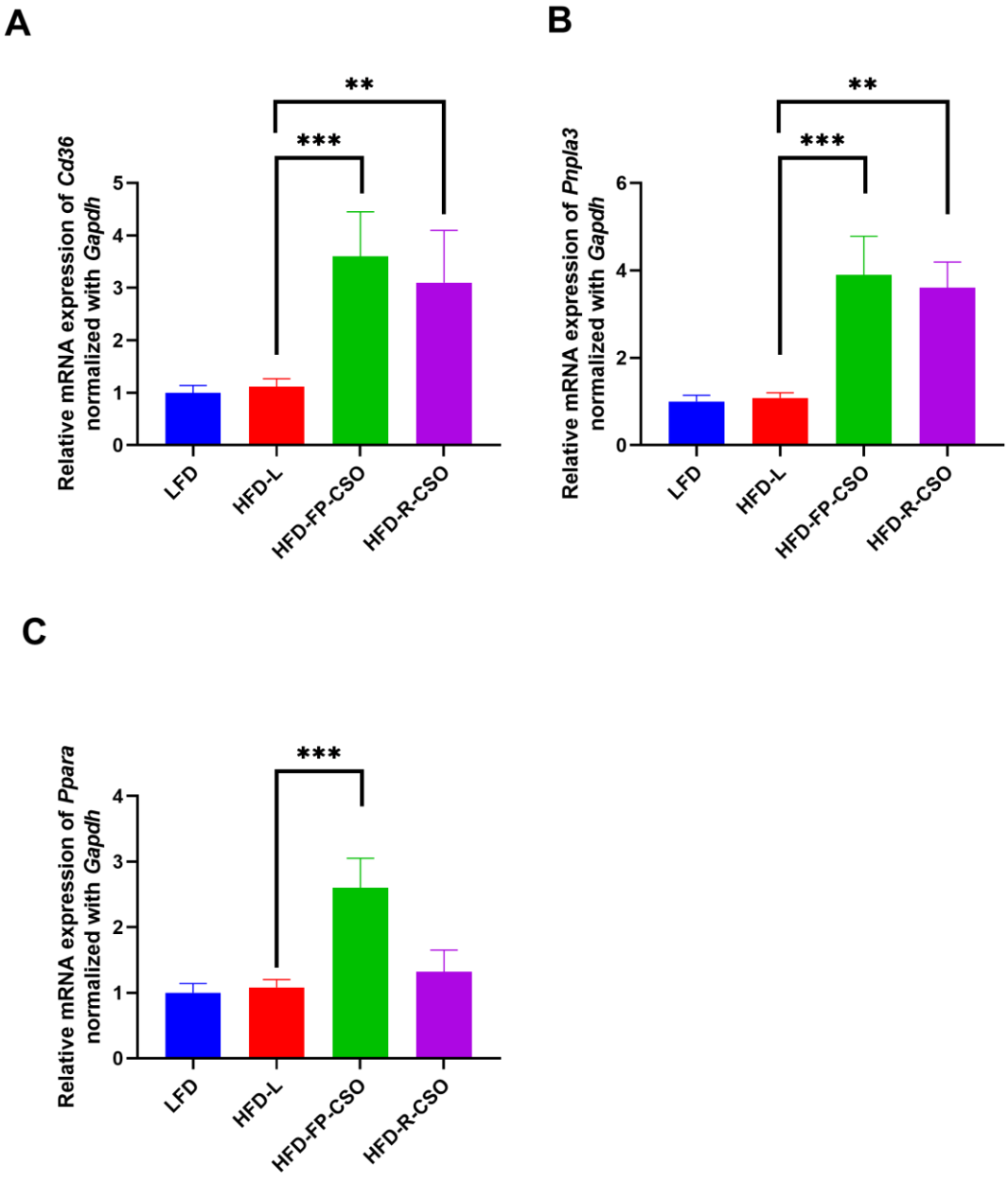

Figure 4: After 24 weeks of experiment, mRNA expression of Cd36 (A), Pnpla3 (B) and Ppara (C), genes that regulate lipid metabolism in mice, was detected in the liver.

4. Conclusion

Substitution of dietary fat with phenolic rich CSO reversed HFD-induced cirrhosis in mice. In addition, use of phenol-rich FP-CSO instead of R-CSO improved plasma lipid profile and adipose tissue cytokine expression in mice with NAFLD. It must be pointed out that one of the effective measures to prevent and treat NAFLD is to change inappropriate eating habits. In this regard, our data suggest that muFA-rich diets, especially those rich in phenolic CSO, should be encouraged in patients with NFLD. In order to further clarify the new mechanism of the effect of CSO on the progression of NAFLD, subsequent studies will comprehensively analyze the transcriptome level and the metabolome level of phenol derived metabolites.

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