Arctiin attenuates lipid accumulation, inflammation and oxidative stress in nonalcoholic fatty liver disease through inhibiting MAPK pathway

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a common chronic liver disease that predominantly affects the adult population. It was reported that arctiin improves functioning of the liver through multiple pathways, but the exact molecular mechanism is not clear yet. First, a mouse obesity model was successfully constructed, and hematoxylin and eosin staining and enzyme-linked-immunosorbent serologic assay (ELISA) were used to assess the levels of alanine aminotransferase and aspartate aminotransferase, respectively, in liver tissue damage. Then lipid accumulation in liver tissues was detected by immunohistochemistry (IHC) staining, and detection kits were used to determine the levels of triglycerides and total cholesterol in serum. Western blotting was used to detect the expression of adipose synthesis proteins, sterol regulatory element-binding protein-1, stearoyl-CoA desaturase-1 and fatty acid synthase, in liver tissues. Further, the levels of glutathione peroxidase, malondialdehyde, superoxide dismutase, catalase and reactive oxygen species as well as that of interleukin 2 (IL-2), IL-1β, IL-6 and tumor necrosis factor-α, and the expression of p-P65 and P65, in liver tissues were measured by ELISA and IHC, respectively. Finally, the protein expression levels of extracellular signal-regulated kinase (ERK), phospho-ERK, Jun N-terminal kinase, phospho-JNK, p38 and phospho-p38 in liver tissues were examined by WB. The results showed that relative to normal diet, mice on high-fat diet had increased body weight as well as fat content in liver tissues, increased liver tissue damage, decreased oxidative stress capacity and enhanced inflammatory response, while arctiin changed these adverse effects and inhibited mitogen-activated protein kinase (MAPK) pathway. Arctiin exerts hepatoprotective effects by inhibiting MAPK pathway and improving lipid accumulation, inflammatory response and oxidative stress.

Keywords: arctiin; nonalcoholic fatty liver disease; lipid accumulation; inflammation; oxidative stress

Introduction

Nonalcoholic fatty liver disease (NAFLD) is currently one of the most prevalent chronic liver diseases worldwide, affecting approximately one-quarter of the adult population (Younossi et al., 2016). NAFLD is a state of simple steatosis to nonalcoholic steatohepatitis (commonly known as NASH), characterized by hepatic cellular damage and inflammation, which may eventually progress to cirrhosis and hepatocellular carcinoma.
In addition, NAFLD is an important risk factor for type 2 diabetes (Song et al., 2021), atherosclerosis, cardiovascular disease, and chronic kidney disease (Chalasani et al., 2012; Musso et al., 2014). However, its pathogenesis is still unclear and current treatment options are relatively limited. Therefore, there is an urgent need to develop effective pharmacological treatments for NAFLD.

Arctiin, a lignan isolated from burdock (Arctium lappa), is known to have antirotal and anti-inflammatory effects (Lee et al., 2011; Wu et al., 2009). It has been shown that burdock sapogenins reduce neuronal damage in the prefrontal cortex (PFC) of the brain and inhibit inflammatory response because of microglia activation in mice under chronic unpredictable mild stress by modulating the signaling of high mobility group box 1–toll-like receptor 4–nuclear factor kappa B (HMGB1/TLR4/NF–κB) pathway, thereby alleviating depressive symptoms (Xu et al., 2020). Burdock glucoside can also improve lung injury by inhibiting phosphatidylinositol 3-kinase–protein kinase B (PI3K/AKT) pathway and suppressing lipopolysaccharide (LPS)-induced inflammation and oxidative stress in lung tissues (Zhou et al., 2018). In terms of myocardial protection, burdock glucoside improves cardiac function by inhibiting mitogen-activated protein kinase (MAPK) pathway and ameliorating cardiac hypertrophy (Golec et al., 2021) and cardiac fibrosis (Li et al., 2017). Burdock glucoside significantly reduces adipogenesis in fibroblast 3T3-L1 preadipocytes, and it has a potential role in preventing obesity (Min et al., 2014). In addition, burdock sapogenins also have hepatoprotective effects. It was shown that burdock sapogenins antagonize the hepatotoxicity induced by raglanolactone by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway (Zhou et al., 2020).

Current therapeutic strategies for treatment include the use of natural chemical components, such as the extraction of active ingredients from plants for treating NAFLD. In addition to natural ingredients, aerobic exercise (Rahmati et al., 2021), resistance exercise (Rahmati et al., 2022) and herbal medicine (Keshvari et al., 2020) are alternative choices that can successfully inhibit inflammatory responses in chronic diseases. Miah et al. (2021) found that supplementation with cumin seed powder prevented oxidative stress, hyperlipidemia and NAFLD in mice fed with a high-fat diet. Wu et al. (2021b) found anti-obesity effects of six-bubble tea extract by modulating lipid metabolism and oxidative stress in high-fat diet-induced obese mice. Therefore, natural ingredients have good prospects for treating NAFLD. The aim of this study was to investigate the molecular mechanism of hepatoprotective effect of arctiin for treating NAFLD.

Methods

Animals

In all, 30 C57BL/6 mice, each weighing about 20 g, were obtained from Speifu (Beijing, China). The acclimation period was 2 weeks. The animals were kept in 22–25°C environment, with a 12-h light–12-h dark cycle. Food and water were provided freely. When the mice were 8-week old, they were divided equally into five groups of six mice each, and maintained on high-fat diet for 16 weeks to establish an obesity model; three of these groups were treated with different doses of arctiin (Solarbio, Beijing, China) by gavage while being fed with a high-fat diet (Li et al., 2019). The grouping scheme was as follows: (1) standard chow diet (SCD), (2) high-fat diet (HFD), (3) HFD + 25-mg/kg arctiin, (4) HFD + 50-mg/kg arctiin, and (5) HFD + 100-mg/kg arctiin. After 16 weeks, the mice were executed and liver tissues were taken for experimental purposes. Serum and tissue samples were collected and stored for further study. All experiments were approved by the Ethics Committee of Wuhan No. 1 Hospital and conformed to ethical standards as required by law and guidelines regarding laboratory animals.

Liver histology

Paraffin sections (3–4 μm) were stained with hematoxylin and eosin (H&E) and examined under a Leica Aperio Versa microscope (Leica; Wetzlar, Germany). All observations were performed by experienced laboratory staff.

Biochemical assays

To assess fasting blood glucose levels, fasting blood sugar (FBS) levels were measured in mice after restraint using an automated glucometer (MK1236; Everlab, Beijing, China). Mice were fasted for 4 h prior to evaluation. Approximately 0.1 g of liver tissue was weighed for biochemical assays. First, 2 mL of chloroform–methanol (2:1, v/v) solution was used to dissolve liver tissue homogenate and centrifuged at 3,000 rpm for 10 min at room temperature. The material from the lower clear layer was carefully transferred to a new tube using a pipette and dried by nitrogen. Subsequently, methanol was used to dissolve and analyze subsequently. Analytical protocols were performed according to the manufacturer’s instructions. Triglyceride (TG) and total cholesterol (TC) levels were analyzed using the Solarbio kit (Beijing, China). To detect aspartate aminotransferase (AST) levels, an AST assay kit (Solarbio) was used, and alanine aminotransferase (ALT) levels were analyzed using an ALT assay kit (Lablead, Beijing, China). Approximately 0.1 g of liver
tissue was homogenized in 1 mL of 1.15% potassium chloride (Solarbio). Then, 0.2 mL of 8.1% sodium dodecyl sulfate solution (SDS; Lablead, Beijing, China), 1.5 mL of 20% acetic acid (Lablead), 1.5 mL of 0.8% aqueous thio-barbituric acid reactive (TBA) solution (Solarbio) and 0.7 mL of distilled water were added to liver homogenate. The mixture was heated in a water bath at 95°C for 30 min. Thereafter, the heated mixture was immediately cooled in ice. Then, 1 mL of distilled water and 5 mL of n-butanol (Lablead) were added and centrifuged at 4,000 rpm for 20 min. The absorbance of the clarified supernatant was measured at 532 nm using a microplate reader (SpectraMax i3X; Molecular Devices, CA). Liver malondialdehyde (MDA) content was expressed as nmol MDA per gram of liver tissue. A standard curve was calculated using 1,1,3,3-tetraethoxypropane (Lablead). To quantify antioxidant activity, liver tissues were homogenized with phosphate-buffered saline (PBS) and centrifuged at 1,500 g for 15 min. Subsequently, the clarified supernatant was collected and used as a sample. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) of the liver were measured using a colorimetric assay kit (Lablead). Interleukin 2 (IL-2), IL-1β, IL-6 and tumor necrosis factor-α (TNF-α) levels in liver tissues were determined by enzyme-linked-immunosorbent serologic assay (ELISA) kits (Solarbio).

**Immunofluorescence analysis**

Next, levels of lipid droplet formation in liver tissues as well as reactive oxygen species (ROS) were analyzed by immunofluorescence. Bovine serum albumin (BSA)-conjugated palmitate (250 μM) was incubated for 24 h in the presence or absence of arctiin. For lipid droplet staining, liver tissues were stained with 0.01-mg/mL BODIPY™ 493/503, a lipophilic bright green fluorescent dye (Lablead). Subsequently, dichlorofluorescein (DCF)-specific antibodies (ab113851; Abcam, Cambridge, UK) were used to stain for 24 h, followed by incubation with Alexa Fluor 488 anti-mouse secondary antibody for 1 h. Liver tissues were stained with 4',6-diamidino-2-phenylindole (Sigma, St Louis, MO, USA). Fluoroshield sealer containing 4',6-diamidino-2-phenylindole (Sigma) was used to seal all liver tissues. Fluorescence images were obtained using a laser scanning confocal microscope (FV3000, Olympus, Japan). The absolute number of cells was counted manually, And TOTAL number of stained cells were included in the analysis.

**Western blotting analysis**

Protein obtained from liver tissues using radioimmunoprecipitation assay (RIPA) buffer (Cat No. 89901; Thermo Fisher, MA, USA) was followed by centrifuging at 16,000×g at 4°C for 15 min. To quantify protein concentration, bicinchoninic acid (BCA) protein assay (Thermo Fisher) was performed. Denaturation of total proteins was processed in a metal bath at 95°C for 5 min; then, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (7.5%) was operated, and 30 μg of protein was separated per lane. Afterwards, proteins were wet transferred to polyvinylidine fluoride (PVDF) membranes (Billerica, MA, USA), which were blocked by 5% skimmed milk dissolved in tris buffered saline with tween (TBST), with continuous shaking in a shaker at room temperature for 2 h. Related primary antibodies were applied to incubate with membranes.

The following primary antibodies were included: β-actin (1:2,000; Cat No. CL594-66009; Proteintech, Shanghai, China), sterol regulatory element-binding protein-1 (SREBP1; 1:2,000; Cat No. K106528P; Solarbio), stearoyl-CoA desaturase-1 (SCD1; 1:2,000; Cat No. K007822P; Solarbio), fatty acid synthase (FAS; 1:2,000; Cat No. K000322P; Solarbio), p-p65 (1:2,000; Cat No. K006209P; Solarbio), p-p53 (1:2,000; Cat No. K003407P; Solarbio), phospho-extracellular signal-regulated kinase (ERK; 1:2,000; Cat No. K006234P; Solarbio), ERK (1:2,000; Cat No. K106589P; Solarbio), Jun N-terminal kinase (JNK; 1:2,000; Cat No. K109227P; Solarbio), phospho-JNK (1:2,000; Cat No. K009325P; Solarbio), p-ERK (1:2,000; Cat No. K006186P; Solarbio), and p-38 (1:2,000; Cat No. K106589P; Solarbio). Then, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:1,000; Cat No. K106528P; Solarbio) and P38 (1:2,000; Cat No. K106589P; Solarbio). The Easysee Western Blot Kit (Lablead) was used to measure chemiluminescence signals. ImageJ version 1.53 was used to analyze the expression of proteins in each group.

**Statistical analysis**

All data were presented as mean ± standard deviation (SD) for experiments performed in triplicate. Unpaired or paired Student’s t-test or one-way analysis of variance (ANOVA) was utilized to analyze results followed by Tukey’s test to compare each group. The results were tested for normality, and t-test was performed for the data that conformed to normality and homogeneity of variance. Otherwise, the Mann–Whitney U test was performed. If not, the interquartile range was used. Chi-square (χ²) test was used to compare three groups of data. Data were analyzed, and graphs were plotted, by using GraphPad Prism version 6.0 software (GraphPad)
Results

Arctiin ameliorates liver tissue damage in high fat-fed mice

Mice were fed with normal diet and high-fat diet, and the effects of arctiin on body weight and liver tissues of mice were investigated. The results showed that the body weight of mice increased significantly relative to SCD, and fasting blood sugar (FBS) and liver weight–total body weight also increased significantly due to high-fat diet. In other HFD groups, the FBS of mice fed with 50 mg/kg arctiin was significantly lower relative to the HFD group. When the amount of arctiin feeding reached 100 mg/kg, the body weight, FBS and liver weight–total body weight of mice were significantly reduced (relative to the HFD group; Figures 1A–C). To further investigate the effect of high-fat diet on liver tissues of mice, H&E staining observed no significant abnormalities in the liver tissues of SCD group, while a large number of white areas with more severe damage were present in the liver tissues of the HFD group, and a gradual decrease in the damaged areas of liver tissue could be observed with increasing amounts of arctiin feeding (Figure 1D). When liver tissues showed functional abnormalities, enzyme activities of ALT and AST, were elevated. The results showed that HFD significantly increased the activities of ALT and AST relative to SCD, but the enzyme activities of ALT

![Figure 1](image_url)
and AST gradually decreased with increasing amount of arctiin feeding (Figure 1E).

**Arctiin improves liver tissue lipid metabolism in high fat-fed mice**

The effect of both feeding methods on fat accumulation in mice liver tissues was studied. The results of immunohistochemistry (IHC) staining showed that no green areas stained with BODIPY™ 493/503 were seen in the SCD group, indicating no significant fat accumulation. In contrast, a large number of green areas were present in HFD, with significant fat accumulation. Moreover, with increase in the amount of arctiin feeding, a gradual decrease in fat accumulation in liver tissues could be observed (Figure 2A). Further quantification of TG and TC in liver tissues showed that HFD significantly elevated the content of TG and TC relative to SCD, while the content of TG and TC in liver tissues gradually decreased with increasing amounts of arctiin feeding (Figure 2B). Western blotting (WB) results showed that

![Image](image-url)

**Figure 2.** Changes in lipid metabolism of liver tissues of mice under different diets and drug administration. (A) Lipid accumulation in liver tissues. (B) Changes in the levels of triglycerides and total cholesterol. (C) Changes in the protein expression levels of SREBP1, SCD1 and FAS using β-actin as an internal reference for upper samples. N = 3, ***P < 0.001 vs. the standard chow diet group, *P < 0.05 vs. the high-fat diet (HFD) group, ###P < 0.001 vs. the HFD group.
the relative protein expression levels of SREBP1, SCD1 and FAS were significantly increased in the HFD group, indicating that HFD diet exacerbated pressure on liver tissues to metabolize fat, thus causing an increase in the expression level of protein. In contrast, the relative protein expression levels of SREBP1, SCD1 and FAS in liver tissues decreased significantly with increasing amount of arctiin feeding (Figure 2C).

Arctiin improves oxidative stress in liver tissues of high fat-fed mice

The effect of high fat on the level of oxidative stress in mice liver tissues continue to be investigated. Under normal conditions, MDA levels are not elevated and the levels of GPx, SOD and CAT are in a relatively stable state, representing the ability of liver tissues to handle peroxides. HFD caused a significant increase in MDA levels and a significant decrease in GPx, SOD and CAT levels. It is noteworthy that the level of MDA decreased gradually with increasing amount of arctiin feeding. The levels of GPx and SOD increased significantly when arctiin feeding was 50 mg/kg, while CAT levels increased significantly when arctiin feeding was 100 mg/kg. These results suggested that arctiin could promote the oxidative stress capacity of liver tissues (Figure 3A). We further found by IHC that the ROS levels in SCD were inactive, while HFD greatly activated ROS levels in liver tissues. As the amount of arctiin feeding increased, ROS levels decreased gradually (Figure 3B).

Arctiin ameliorates inflammatory factors in liver tissues of high fat-fed mice

In order to investigate whether arctiin had an effect on high fat-induced inflammatory response, the levels of inflammatory factors in liver tissues (Figure 4A) and \( \text{p-P65/P65} \) expression levels in inflammatory response

![Figure 3. Changes in oxidative stress response in mice liver tissues under different diets and drug administration methods.](image)

(A) Changes in the levels of MDA, GPx, SOD and CAT. (B) DCF fluorescence response to reactive oxygen species (ROS) level. \( N = 3, ***P < 0.001 \) vs. the standard chow diet group, \(^*P < 0.05 \) vs. the HFD group, \(^{#}P < 0.01 \) vs. the high-fat diet (HFD) group, \(^{###}P < 0.001 \) vs. the HFD group.
(Figure 4B) were examined using ELISA. The results showed that high-fat diet significantly increased inflammatory response in mice liver tissues relative to normal diet, that is, elevated levels of IL-2, IL-1β, IL-6 and TNF-α and p-P65/P65 expression levels. Notably, with arctiin feeding of 25 mg/kg, relatively insignificant improvement was observed in the inflammatory response of mice liver tissues, and only decreased the IL-6 content. When arctiin feeding was 50 mg/kg or even higher, these inflammatory factors, as well as p-P65/P65 expression levels, were significantly reduced, suggesting that burdock sapogenins could improve inflammatory factors in liver tissues of high fat-fed mice.

**Burdock sapogenins can inhibit MAPK pathway**

Previous experiments demonstrated the protective effect of arctiin on the liver, and the signaling pathway it regulates triggered our interest. The protein expression levels of p-ERK, ERK, JNK, p-JNK, p-P38 and P38 in liver tissues were examined, and the phosphorylation levels of the corresponding proteins were transformed. It was found that high-fat diet compared to normal diet significantly elevated the levels of p-ERK/ERK, JNK/p-JNK, and p-P38/P38. When arctiin feeding was 25 mg/kg, it significantly reduced the levels of p-ERK/ERK and JNK/p-JNK in mice liver tissues but did not significantly alter the levels of p-P38/P38. When arctiin feeding was 50 mg/kg or even higher, the levels of p-ERK/ERK, JNK/p-JNK, and p-P38/P38 were significantly reduced (Figure 5). These results suggested that burdock sapogenins could inhibit MAPK pathway and thus exert their hepatoprotective effects.

**Discussion**

In this study, an obese animal model was constructed by feeding mice with a high-fat diet for 16 weeks to study the effects of different doses of arctiin on the liver tissues of mice. Body weight, FBS and liver weight–total body weight of mice on a high-fat diet were higher than those of mice on normal diet, and the fat content of liver tissues was higher than those on normal diet. The levels of ALT and AST were also significantly high, indicating that mice obesity model was successfully constructed. In this study, arctiin showed its protective effect on liver tissues (Yu et al., 2021). We then investigated the effects of a high-fat diet on fat metabolism, oxidative stress and inflammatory response in liver tissues in three ways (Najafi et al., 2020).

BODIPY™ 493/503 staining showed an accumulation of lipid droplets in liver tissues, a significant increase in serum TG and TC levels, and an increase in the expression levels of lipid-synthesizing proteins SREBP1, SCD1 and FAS in mice on a high-fat diet. This suggested a reduced ability of the liver to metabolize fat. A review
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has shown that high fat affects the normal physiological processes of liver tissues and produces lesions, which decrease the fat metabolizing capacity of liver tissues (Landeche et al., 2019). Arctiin feeding to mice resulted in an increased utilization of fat metabolized by the liver, indicating an enhanced fat metabolizing capacity of the liver. High-fat diet, on the other hand, affected the oxidative stress capacity of liver tissues (Piché et al., 2020). Our experimental results showed that MDA levels increased in mice fed with a high-fat diet, while the levels of GPx, SOD and CAT decreased significantly, indicating a decrease in the oxidative stress capacity of liver tissues. It was observed that ROS levels were lowest in mice fed with a normal diet but increased in mice fed with a high-fat diet. It was found that the capacity for oxidative stress in mice that with a high-fat diet was impaired but got improved by feeding with natural compounds. This validated the role of arctiin in our study.

High-fat diet also increased the levels of inflammatory factors IL-2, IL-1β, IL-6 and TNF-α in mice liver tissues, with increased levels of p-P65/P65. This suggested that inflammatory response in obese mice was in an activated state. It was shown that high-fat diets caused inflammatory response in various metabolic diseases, such as hypothyroidal inflammation in obesity and metabolic diseases (Jais and Brüning, 2017). High-fat diets exacerbated early psoriatic skin inflammation unrelated to obesity (Herbert et al., 2018). Our study also showed that inflammatory response in mice liver tissues was activated by a high-fat diet, but arctiin altered this inflammatory response. This further illustrated the hepatoprotective effect of arctiin.

Finally, we found that the protein expression levels of p-ERK/ERK, JNK/p-JNK, and p-P38/P38 were significantly increased in mice fed with a high-fat diet, and arctiin treatment reduced this elevation, suggesting that the hepatoprotective effect of arctiin was mainly due to the inhibition of the MAPK signaling pathway. It has been shown that a high-fat diet targets key proteins in the MAPK signaling pathway to cause brain insulin resistance in mice (Kothari et al., 2017). Wu et al. (2021a) found that diet-induced obesity could be changed by feeding induced hepatic factor Manf targeting the p38 MAPK pathway to promote fat browning. This suggested that MAPK was activated in obesity, and liver tissue damage might be altered by inhibiting the MAPK signaling pathway.

However, there are flaws in this study. First, little research has been done on arctiin, a natural product with many beneficial effects, and whether arctiin can treat NAFLD is not studied in detail. Although this study found the hepatoprotective effect of arctiin, it did not compare difference in action between arctiin and other natural compounds with hepatoprotective effects. Recently, Tarantino et al. (2021) reviewed the pathogenesis of NAFLD and existing therapeutic drugs, such as metabolism-targeted therapy, oxidative stress-targeted therapy, inflammation-targeted therapy, etc., and revealed emerging therapies and development strategies. Negi et al. (2022) reviewed the
progress of various natural products in treating NAFLD. These current drugs are worthy of being compared. Second, although this study found that arctiin could have a protective effect on the liver, the safety evaluation of natural compounds also requires consideration. Finally, although this study found that arctiin could inhibit the MAPK signaling pathway, the specific target may be more complex, and could be determined in the future studies. In the prospective studies, we would compare the efficacy of arctiin with these existing natural medicines to complete our research.

Conclusion

In conclusion, arctiin can inhibit MAPK pathway and improve lipid accumulation, inflammatory response and oxidative stress, which can have hepatoprotective effects.

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Competing Interests

The authors state that there were no conflicts of interest to disclose.

Data Availability

The authors declared that all data supporting the findings of this study are available in the paper, and any raw data could be obtained on request from the corresponding author.

Author Contributions

Lin Li and Ying Zhang designed and carried out the experiments. Fangxi Xiao and Zhigang Wang analyzed and interpreted the data, and Ju Liu prepared the manuscript.

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