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ORIGINAL ARTICLE

Ethanolic extract of root from *Arctium lappa* L ameliorates obesity and hepatic steatosis in rats by regulating the AMPK/ACC/CPT-1 pathway

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Abstract

Burdock (*Arctium lappa* L) root is eaten as a vegetable in many countries and used as an ethnomedicine because of its various pharmacological effects. The objective of this study was to investigate the underlying mechanisms of ethanolic extract of root from *Arctium lappa* L root (ALE) to lose weight and regulate lipid metabolism. The results showed that ALE can regulate lipid metabolism level and inhibit the weight gain of rats induced by the high-sugar and high-fat diet. The contents of triglyceride and cholesterol in the liver of obese rats significantly reduced, and hepatic steatosis was ameliorated. In addition, this study identified that ALE enhanced hepatic fatty acid β-oxidation and ameliorated hepatic steatosis by activating AMPK/ACC/CPT-1 pathway. These results indicated that ALE has a potential preventive and therapeutic effect on metabolic-associated fatty liver disease and obesity.

Practical Applications

Obesity is already a global health problem. Obesity causes accumulation of triglycerides, which leads to hepatic steatosis. Long-term steatosis causes liver damage and metabolic fatty liver disease. Plant-derived functional foods or herbal medicines have better effects on weight loss and liver protection, which are more conducive to long-term use with less toxic side effects. As a medicinal and edible plant material, *Arctium lappa* L root has the effect in losing weight. Our study showed that ethanolic extract of *Arctium lappa* L root effectively regulates lipid metabolism and inhibits hepatic steatosis. *Arctium lappa* L root may be used as a therapeutic drug and functional food raw material for obesity and fatty liver disease.

KEYWORDS
AMPK, *Arctium lappa* L, fatty acid β-oxidation, metabolic-associated fatty liver disease, obesity, steatosis

1 | INTRODUCTION

With the complication of daily diet, plenty of high-calorie foods are also intaken resulting in excessive fat accumulation and obesity. Obesity often associated with insulin resistance, metabolic-associated fatty liver disease (MAFLD), and hyperlipidemia and other disorders of glucose and lipid metabolism. Therefore, obesity has gradually become one of the important causes of chronic metabolic diseases,

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and the amelioration of obesity has gradually become the focus of research (Guerra et al., 2021). Obesity is mainly a state of obvious overweight and thick fat layer in the body (Gutiérrez-Cuevas et al., 2021). The accumulation of fat is mainly caused by the disorder of lipid metabolism in the body, such as the accumulation of triglycerides. The pathogenesis of diseases related to obesity is often accompanied by excessive accumulation of triglycerides in liver cells (Sakurai, Kubota, Yamauchi, & Kadowaki, 2021). The accumulation of a large amount of lipids in liver will cause steatosis and further accelerate the formation of liver disease. Metabolic-associated fatty liver disease (MAFLD) is a liver disease related to metabolism, especially related to obesity and lipid metabolism disorders. Therefore, reducing the lipotoxicity of triglycerides to liver cells and reducing hepatic steatosis is the key to the treatment of MAFLD and obesity (Lim, Kim, & Targher, 2021).

The metabolism of triglycerides is mainly carried out in the liver (Heeren & Scheja, 2021). Therefore, hepatic steatosis occurs most frequently in obesity and liver disease, but it is not irreversible (Maciejewska-Markiewicz, Stachowska, Hawryłkowicz, Stachowska, & Prowans, 2021). Lipid metabolism enzymes play an important role in this process, such as hormone-sensitive lipase (HSL), Acetyl CoA carboxylase (ACC), and carnitine palmitoyltransferase-1 (CPT-1) are related to the breakdown of fatty acids, Sterol regulatory element-binding protein 1 (SREBP-1) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR) regulate cholesterol synthesis, glycerol-3-phosphateacyl transferases (GPAT), and FAS (Fatty acid synthase) are involved to fatty acid synthesis, etc. (Mazzolini et al., 2020). These key enzymes and rate-limiting enzymes in the lipid metabolism process are importantly related to the regulation of 5′-adenosine monophosphate-activated protein kinase (AMPK) (von Loeffelholz, Coldewey, & Birkenfeld, 2021). AMPK is a key protein in the body to regulate glucose and lipid metabolism, especially the activity of AMPK in the liver is related to energy storage, fatty acid synthesis and decomposition, and cholesterol output (Hsiao et al., 2021). This indicates that the cascade of AMPK participation and activation is the key to regulating lipid metabolism disorders. From the mechanism point of view, the key enzymes, such as HMG-COAR, GPAT, and ACC, are all inhibited by AMPK activation, which in turn enhances the activity of HSL and CPT-1 that is a rate-limiting enzyme for fatty acid β-oxidation on mitochondria (Da Eira, Jani, Sung, Sweeney, & Ceddia, 2020). Fatty acid β-oxidation is the main way to dispose of fatty acids in the liver (Weinberg, 2006). Therefore, elucidating the molecular mechanism of reducing liver cell lipotoxicity and improving liver cell steatosis will help to find and develop specific therapeutic interventions for obesity and MAFLD.

Functional food and herbal medicine have good effects in ameliorating obesity and obesity-induced metabolic diseases (Chen et al., 2011; Jiang et al., 2019; Sham et al., 2014). Therefore, the search for natural ingredients to improve lipid metabolism disorders can reduce the use of synthetic drugs and potential damage to the liver and kidneys, contributing also to a healthy and effective way to ameliorate obesity. *Arctium lappa* L is a perennial herb of the Compositae family, which is popular as a functional food due to its potential pharmacological activities. In particular, *Arctium lappa* L root has the effect of regulating lipid metabolism, but its specific mechanism is still unclear (Miele & Beguinot, 2012). Ethanol extract of root from *Arctium lappa* L contains caffeic acid, chlorogenic acid, polyphenols, and other compounds that may have prebiotic function. (Moro & Clerici, 2021; Romualdo et al., 2020). In particular, plant polyphenols have obvious pharmacological effects on lowering of blood glucose, blood lipids, and blood pressure (Zhang, Xu, Zhang, Liu, & Chen, 2021). Caffeic acid and chlorogenic acid also can activate AMPK and cause AMPK-related cascade reactions (Ferracane, Graziani, Gallo, Fogliano, & Ritieni, 2010; Vasileva et al., 2020). However, the underlying mechanism of *Arctium lappa* L on obesity and lipid metabolism-related diseases such as MAFLD is still poorly understood. We guessed that *Arctium lappa* L improves obesity and obesity-induced related diseases may be achieved by activating AMPK signaling pathway to regulate glucose and lipid metabolism. We tried to study whether *Arctium lappa* L root has pharmacological effects to ameliorate obesity and obesity-related lesions by using a rat model of obesity induced by a high-sugar and high-fat diet, and explored underlying molecular mechanism.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

RIPA Lysis Buffer, Protease and phosphatase inhibitor and Bradford Protein Assay Kit were purchased from Beyotime. Ethanol (95% v/v, food grade), the total protein assay kit with standard BCA method, the total cholesterol assay kit, and triglyceride assay kit, and Tissue Mitochondria Isolation Kit were purchased from Nanjing Jiancheng Bioengineering Institute. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (Tris-Glycine SDS-PAGE Running Buffer), EZ-Buffers C Western Transfer Buffer, and enhanced chemiluminescence western blot detection reagents were purchased from Sangon Biotech. High purity total RNA extraction kit, ChamQ Universal SYBR qPCR Master Mix and RT-PCR and aHiScript III RT SuperMix for qPCR (+gDNA wiper) were purchased from Vazyme Biotech. Fatty acid β-oxidation rate colorimetric test kit was purchased from GenMed.

Primary antibodies against adenine monophosphate activated protein kinase (AMPK), Phospho-AMPKα (Thr172), Acetyl-CoA Carboxylase (ACC), and Phosphor-ACC (Ser79) were purchased from Cell Signaling Technology. Primary antibodies against carnitine palmitoyltransferase-1 (CPT-1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Proteintech. Horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Cell Signaling Technology. Primary antibodies were used at 1:1,000 dilutions whereas 1:5,000 dilutions were used for secondary antibodies.

2.2 | Preparation of *Arctium lappa L* root extracts

Arctium lappa L was harvested from Feng-county, Xuzhou city, P. R. China (30°55′00.00″N 121°27′00.00″E), in November 2020 and

Arctium lappa L root powder was obtained by superfine grinding. Approximately 10 g of the ground materials were extracted with 100 mL ethanol (95% v/v), the ratio of solid-to-liquid was 1:10 (Maeta, Ishikawa, & Takahashi, 2021). After the extraction was performed for 24 hr, all the extracts were evaporated to dryness using a rotary evaporator at 60°C and stored at −4°C until used (Gurunanselage Don & Yap, 2019). Ethanolic extract of root from *Arctium lappa* L (ALE) were dissolved in normal saline, at a stock concentration of 10 mg/mL.

2.3 | Animals and experimental design

Sprague–Dawley rats are able to adapt quickly to high-fat diets and gain weight rapidly. So SD rats were chosen to establish the obesity model in this study (Devan, Arumugam, Shankar, & Poosala, 2018). Sprague–Dawley (SD) rats (5 weeks old) were all obtained from the Shanghai SLAC Laboratory Animal Co., Ltd. Rats were bred and maintained in the Animal Resource Centre of Nanjing Medical University. Rats had free access to food and water in a room with an ambient temperature of 22 ± 2°C and a 12:12 hr light/dark cycle. All animal procedures were performed in strict accordance with the guideline of the Institutional Animal Care and Use Committee of Nanjing Medical University. The ethics approval number for the use of animals in this study is DWRL2020056. The experiment lasted for 6 weeks with the intervention of ethanolic extract of root from *Arctium lappa* L. Rat diets are divided into normal diets and high-calorie diets. The calorie of a high-calorie diets is 3.41 kcal/g and the calorie of a normal diets is 1.83 kcal/g. The specific composition of rat diets in this study are listed at the Table 1. Rat diets were provided by XIETONG.ORGANISM Co., Ltd.

Some studies found that the ethanol extract of plenty of plants has the function of treating hepatic steatosis (Abbas, Boby, Lee, Hong, & Park, 2022; Quan et al., 2022), but there is a lack of research on the treatment of obesity and liver steatosis with ethanol extract of *Arctium lappa* L root. Samples of 5 and 100 mg/kg•bw/day of ethanol extract of *Arctium lappa* L were used as the low monitoring dose and high monitoring dose respectively for the experiment.

A total of 60 healthy SD rats were chosen for experiment, divided 50 rats into high-sugar and high-fat feeding for 14 days, the obesity model of rats was evaluated by detecting body weight on the 14 days and eliminate 1/3 of obese-resistant rats with lower body weight gain. When α was at 0.05 and power ($1-\beta$) was 80%, the sample size was approximately 10 per group via power analysis. Finally, the total of 40 rats were divided into four groups, CON group, HSHSD group, low monitoring group (ALE-L), and the high monitoring group (ALE-H) for 14 days. HSHSD group and monitoring group kept a high-sugar and high-fat diet. The experiment continued for 42 days and the monitoring group were orally treated with ALE (50 and 100 mg/kg•bw/day) before feeding by gavage. The blank control group continued to be fed with a normal diet. The experiment lasted for a total of 56 days. During this period, food intake and body weight were tested. After blood sampling, all the rats were anesthetized and sacrificed. The liver, spleen, kidney, and thymus tissues of rats were removed, weighed, and stored at −80°C for further experiments.

TABLE 1 The composition of rat diets

Composition	Normal diets	High-calorie diets
Fat (calorie ratio)	17%	45%
Carbohydrates (calorie ratio)	64%	36%
Protein (calorie ratio)	19%	19%
Moisture content	6%	6%
Calcium	5,000 ppm	5,000 ppm
Phosphorus	3,000 ppm	3,000 ppm
Potassium	3,600 ppm	3,600 ppm
Sodium	1,039 ppm	1,039 ppm
Magnesium	513 ppm	513 ppm
Iron	45 ppm	45 ppm
Zinc	38 ppm	38 ppm
Manganese	10 ppm	10 ppm
Copper	6 ppm	6 ppm
Iodine	0.2 ppm	0.2 ppm
Vitamin A	4 IU/g	4 IU/g
Vitamin D	1 IU/g	1 IU/g
Vitamin E	0.075 IU/g	0.075 IU/g
Vitamin K	0.9 ppm	0.9 ppm
Thiamine, B1	5 ppm	5 ppm
Vitamin B6	6 ppm	6 ppm
Folic acid	2 ppm	2 ppm
Riboflavin	6 ppm	6 ppm
Niacin	30 ppm	30 ppm
Pantothenic acid	15 ppm	15 ppm

2.4 | Western blotting analysis

Liver tissues of rats were lysed in the RIPA Lysis buffer with protease and phosphatase inhibitor cocktail for general use according to the manufacturer's instructions. Protein concentrations were determined with the total protein assay kit with standard BCA method (Bradford, 1976). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis using polyacrylamide TGX gels and then transferred to polyvinylidene difluoride (PVDF) membranes by EZ-Buffers C Western Transfer Buffer. After blocking, PVDF membranes were incubated with various specific primary antibodies as described above in Tris Buffered Saline with Tween 20 at 4°C overnight. Membranes were washed and incubated in corresponding horseradish peroxidase-conjugated secondary antibodies for 1 hr at room temperature. Proteins were visualized and detected by enhanced chemiluminescence western blot detection reagents and analyzed with ImageQuant™ LAS 4000 imaging system (G.E. Healthcare).

2.5 | Histology analysis

Frozen sections of liver tissue were stained with hematoxylin and eosin (H&E). The sections were stained with hematoxylin at room temperature until the nuclei turned blue, and then transferred to eosin solution for staining at room temperature, dehydrated with an alcohol gradient and made transparent with xylene, and then sealed with neutral balsam (Kujiraoka et al., 2022). The stained sections will be stored at room temperature. Then oil red O staining was performed (Ma et al., 2022). The stained sections will be stored at 4°C. All photos were taken by an optical microscope (ECLIPSE 80i; Nikon), and the magnification was ×20.

2.6 | RNA reverse-transcription and quantitative RT-PCR analysis

Total RNA was extracted with High purity total RNA extraction kit. Total RNA (1 μg) of each liver samples was reverse-transcribed into cDNA and amplified using a ChamQ Universal SYBR qPCR Master Mix according to the manufacturer's directions. RT-PCR was measured using HiScript III RT SuperMix for qPCR (+gDNA wiper) with an ABI 7300 StepOneTM Fast Real-Time PCR System (Applied Biosystems). After the addition of primers and template DNA to the master, PCR thermal cycle parameters were as follows: 95°C for 3 min, 40 cycles of 55°C for 30 s, and 95°C for 15 s, and a melting curve from 60 to 95°C to ensure amplification of a single product. In each sample, the GAPDH gene was used as an endogenous control to normalize for differences in the amount of total RNA (Barber, Harmer, Coleman, & Clark, 2005). The primer sequences used in this study are listed at the Table 2.

TABLE 2 Sequences of quantitative PCR primers

Target gene	Primer	Sequence(5′-3′)
β-Actin	Forward Reverse	CGGTCAGGTCATCACTATCGGCA GCCACAGGATTCCATACCCAGGA
ACC	Forward Reverse	TGAGGAGGACCGCATTTATC AAGCTTCCTTCGTGACCAGA
CPT-1	Forward Reverse	CATTTCCGGGACAAAGGCAAGT CGTGGACTCGCTAGTACAGGAA
FAS	Forward Reverse	CTATTGTGGACGGAGGTATC TGCTGTAGCCCAGAAGAG
GPAT	Forward Reverse	CACCACATCAAGGATACAGCTC GCGTTGCGGATCTGAAGAAG
HMG-CoAR	Forward Reverse	TGGCAGGACGCCAACCTCTAT TGACGGCTTCACAAACCACA
HSL	Forward Reverse	CGCCT TACGG AGTCT ATGC GAGGA CACCT TGGCT TGA

2.7 | Detection of total cholesterol and triglyceride

The liver homogenate of rats was operated with the total cholesterol assay kit and Triglyceride assay kit according to the manufacturer protocol. Data were obtained through enzyme-labeled instrument and analyzed.

2.8 | Detection of fatty acid β-oxidation rate

Take the rat liver homogenate and extract the mitochondria through the Tissue Mitochondria Isolation Kit, and determine the protein content by Bradford Protein Assay Kit. After the mitochondrial separation solution is diluted to 10 mg/mL, it is repeatedly frozen and thawed three times to make it into mitochondrial fragments to achieve maximum enzyme activity. Detect fatty acid β-oxidation level in liver tissue by fatty acid β-oxidation rate colorimetric test kit and analysis by Automatic microplate reader (Wang et al., 2022). The ferricyanide in the colorimetric test kit is reduced by carnitine during the β-oxidation of fatty acids, so the rate of micromolar ferricyanide reduction can be used to express the ability of β-oxidation.

2.9 | Statistical analysis

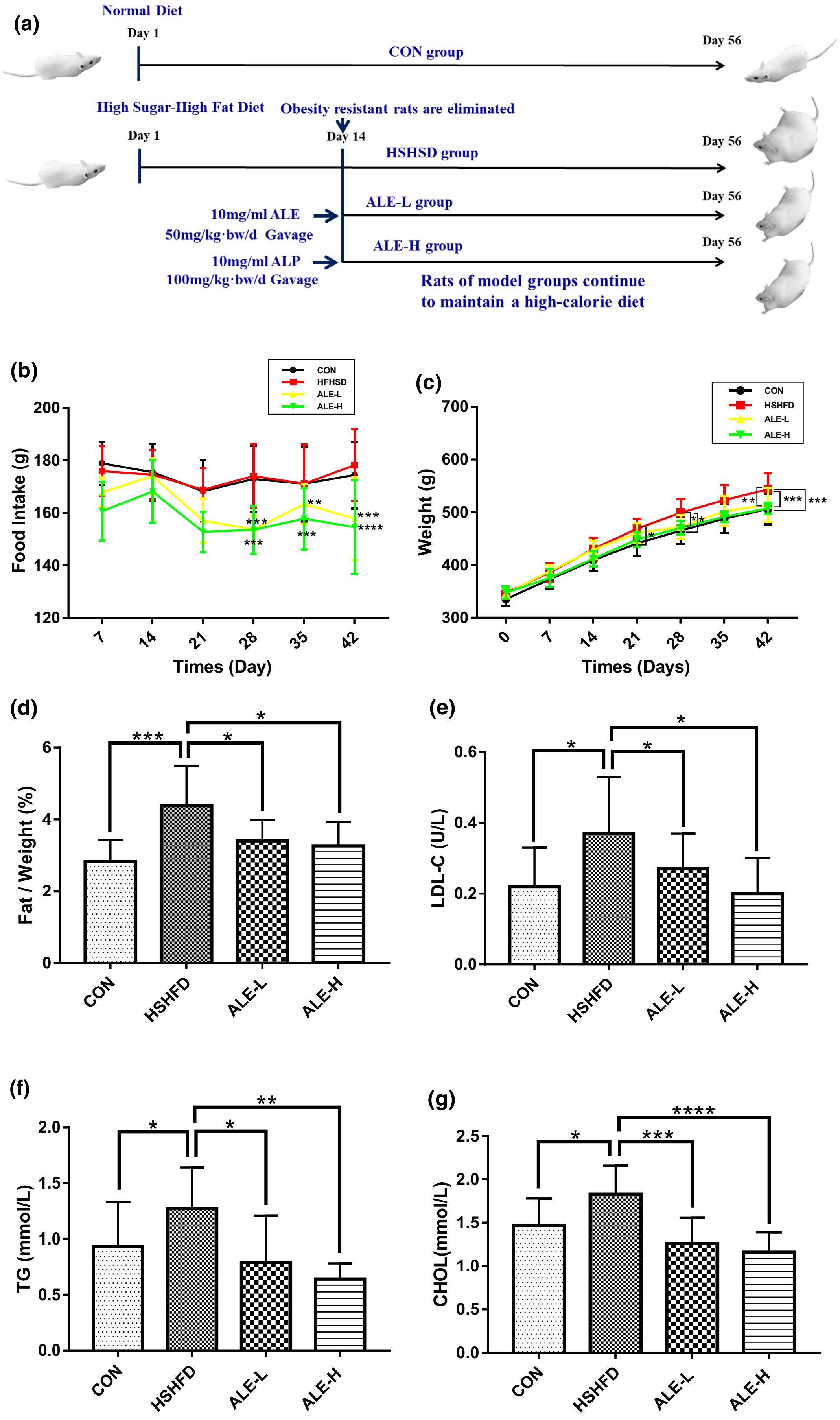
All data are presented as the means ± SD and were collected and analyzed in a blinded manner. Statistical analysis was performed using Student's *t* test and ANOVA. The tests used are indicated in the figure legends. In all studies, *n* indicates the number of samples per group, and cases in which $p < .05$ were considered statistically significant.

3 | RESULTS

3.1 | *Arctium lappa* L reduces bodyweight gain and plasma lipids in HSHFD-induced obese rats

Experiment procedure was as shown in Figure 1a, the data of total 42 days in Figure 1 were recorded after the rats were divided into four groups after 14 days. Compared with the CON

FIGURE 1 ALE reduces bodyweight gain and plasma lipids in HSHFD-induced obese rats. Forty rats were divided into three groups, CON group, HSHSD group, ALE-L group and ALE-H group on the 14th day. HSHSD group and ALE group kept a high-sugar and high-fat diet. The experiment continues for 42 days and ALP group were orally treated with ALE before feeding by gavage (a). Average food intake (b) and body weight (c) were observed in each week. Ratio of the fat/weight (d) was determined after the rats were sacrificed. LDL-c (e), TG (f), CHOL (g) levels were analyzed by automatic biochemical detector. Data in (d–g) were shown as mean ± SD. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$ vs HSHFD group, $n = 10$


(a)
Normal Diet
Day 1
CON group
Day 56
High Sugar-High Fat Diet
Obesity resistant rats are eliminated
Day 1
Day 14
HSHSD group
Day 56
ALE-L group
Day 56
10mg/ml ALE
50mg/kg·bw/d Gavage
ALE-H group
Day 56
10mg/ml ALP
100mg/kg·bw/d Gavage
Rats of model groups continue
to maintain a high-calorie diet
(b)
CON
HFHSD
ALE-L
ALE-H
Food Intake (g)
200
180
160
140
120
7
14
21
28
35
42
Times (Day)
**
***
***
***
***
****
(c)
CON
HSHFD
ALE-L
ALE-H
Weight (g)
700
600
500
400
300
0
7
14
21
28
35
42
Times (Days)
*
**
**
***
***
(d)
Fat / Weight (%)
6
4
2
0
***
*
*
CON
HSHFD
ALE-L
ALE-H
(e)
LDL-C (U/L)
0.6
0.4
0.2
0.0
*
*
*
CON
HSHFD
ALE-L
ALE-H
(f)
TG (mmol/L)
2.0
1.5
1.0
0.5
0.0
*
*
**
CON
HSHFD
ALE-L
ALE-H
(g)
CHOL(mmol/L)
2.5
2.0
1.5
1.0
0.5
0.0
*
***
****
CON
HSHFD
ALE-L
ALE-H

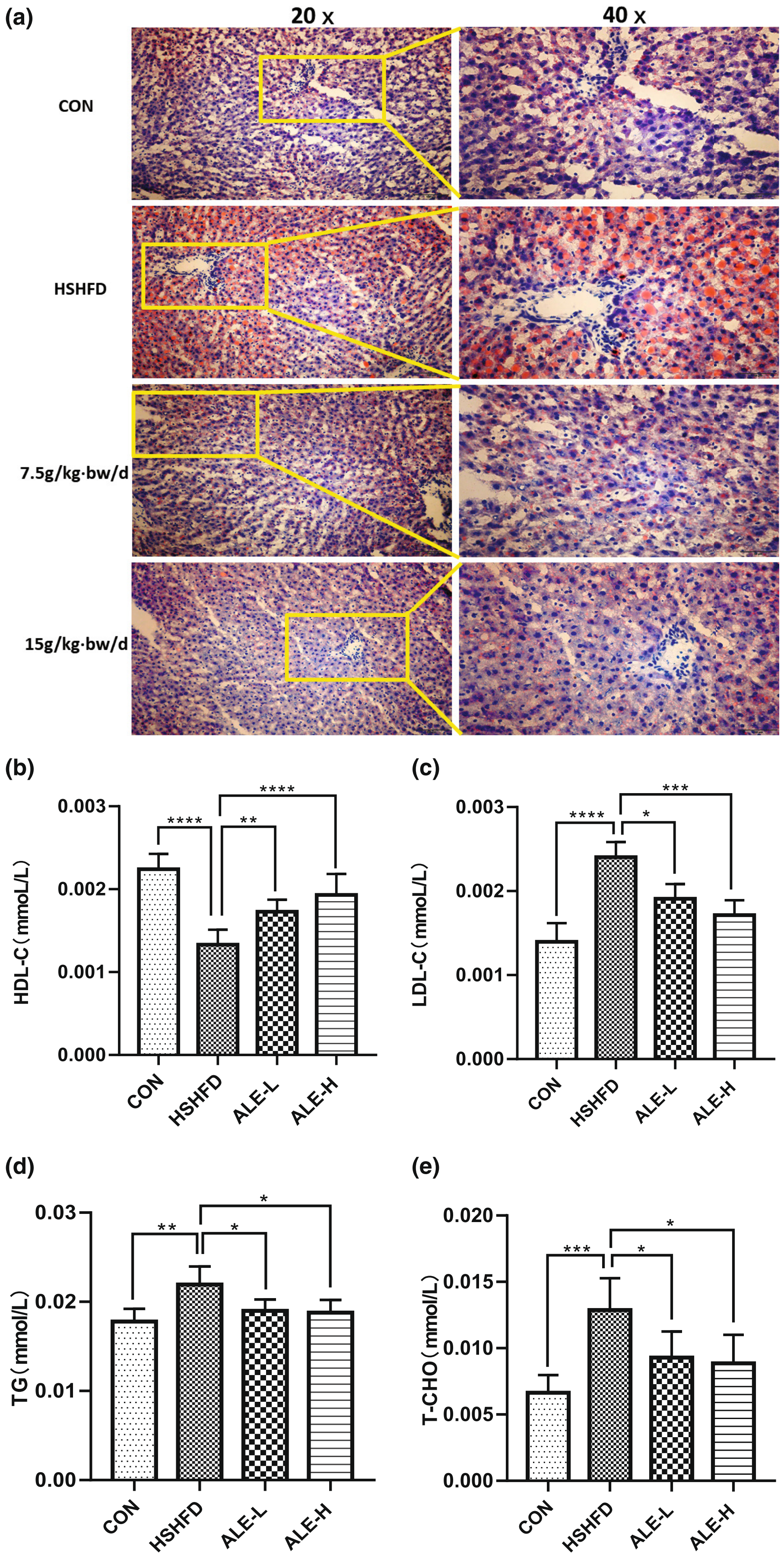

(a)
20 x
40 x
CON
HSHFD
7.5g/kg·bw/d
15g/kg·bw/d
(b)
HDL-C（mmoL/L）
0.003
0.002
0.001
0.000
****
****
**
CON
HSHFD
ALE-L
ALE-H
(c)
LDL-C（mmoL/L）
0.003
0.002
0.001
0.000
****
***
*
CON
HSHFD
ALE-L
ALE-H
(d)
TG（mmol/L）
0.03
0.02
0.01
0.00
**
*
*
CON
HSHFD
ALE-L
ALE-H
(e)
T-CHO（mmol/L）
0.020
0.015
0.010
0.005
0.000
***
*
*
CON
HSHFD
ALE-L
ALE-H

FIGURE 2 ALE reduces liver steatosis in HSHFD-induced obese rats. Histological examinations for liver were performed. Liver tissues of rats were stained with H&E and red O (a), images presented are in ×20 magnification and ×40 magnification. Scale bar: 40× microscope was 50 μm, 20× microscope was 100 μm. HDL-C, LDL-C, T-CHO, and TG contents of liver tissue were detected by using reagent test kit (b–e). Data were shown as mean ± SD, ($n = 6$ for panel b–e). $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$ vs HSHFD group, $n = 6$

group, the body weight of the HSHFD group ($p < .5$) significantly increased from the 21st day, but the food intake of the HSHFD group did not change significantly. However, it was found that the food intake (Figure 1b) and body weight (Figure 1c) of the ALE-intervention group ($p < .5$) both showed a significant downward trend from the 28th day compared with the HSHFD group, while the ALE-H group and the ALE-L group had no significant difference. And with the increase of experimental time, the body weight of the ALE-intervention group gradually recovered to the level of the control group. This result showed that ALE has the effect of reducing body weight and food intake to a certain extent, but there is no obvious dose relationship. After all group rats were sacrificed, serum biochemical results showed that LDL-C ($p < .05$), TG ($p < .05$), and TC ($p < .05$) in the HSHF group had a significant upward trend compared with the CON group, while the results of the ALP-intervention group showed that the concentrations of LDL-C ($p < .05$), TG (ALE-L, $p < .05$ & ALE-H, $p < .01$), and TC (ALE-L, $p < .001$ & ALE-H, $p < .0001$) in the rats had a significant downward trend relative to the HSHFD group (Figure 1e–g). In particular, the TC contents of the high-monitoring group were reduced approximately two times and CHOL contents of the ALE-H group were reduced approximately 1.5 times compared to the HSHFD group. In addition, comparing the body weight and weight of the testis and kidney fat of the rats, the fat-to-weight ratio of the HSHFD group ($p < .001$) was significantly higher than the control group, while the fat-to-weight ratio of the ALE-intervention group ($p < .05$) was significantly lower than that of the HSHFD group (Figure 1d).

3.2 | *Arctium lappa* L reduces hepatic steatosis in HSHFD-induced obese rats

If excessive fat is deposited in the liver, it will inevitably cause steatosis. It was found that the HSHFD group had a significant increase in lipid droplets compared to the control group by performing HE-oil red O staining on the rat liver. It showed that obvious steatosis has occurred, and the area around the liver sinusoids was obviously expanded to form fatty liver. However, the lipid droplets in the ALE-intervention group were significantly reduced compared to the HSHFD group, which indicated that the steatosis in the ALE group was significantly improved (Figure 2a). In order to further verify the changes in lipid content in the liver, TG and T-CHO of liver was detected. The results showed that the TG ($p < .01$) and T-CHO ($p < .05$) level of the HFHSD group were significantly higher than the control group, and the TG ($p < .05$) and T-CHO ($p < .05$) level of the ALE-intervention group were both significantly lower than the HSHFD group (Figure 2d,e). In particular, TG level of ALE-L and ALE-H were nearly restored to the level of CON group. In addition, LDL-C and HDL-C of liver were also detected. The results showed that HDL-C ($p < .001$) in HSHFD group was significantly lower than that in the CON group by nearly two times, and LDL-C ($p < .001$) was also significantly higher by two times. However, compared with HSHFD group, HDL-C were significantly higher (ALE-L, $p < .01$ and ALE-H, $p < .0001$) and LDL-C (ALE-L, $p < .05$ and ALE-H, $p < .001$) were significantly lower in ALE-intervention group, which was consistent with the results in animal serum (Figure 2b,c).

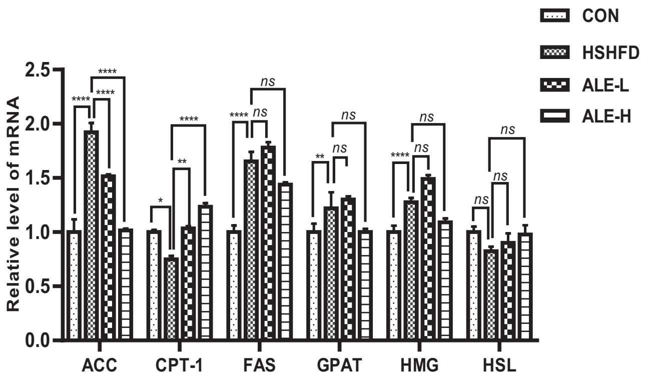


FIGURE 3 ALE modulated the mRNA level of lipid metabolism related genes in liver of the rats. mRNA levels of ACC, CPT-1, FAS, GPAT, HMG-CoAR, and HSL were detected by quantitative RT-PCR. There were significant changes in the mRNA levels of ACC and CPT-1. Values are means ± SD. $^{*}p < .05$, $^{**}p < .01$, $^{****}p < .0001$, $^{ns}p < .05$ vs HSHFD group, $n = 6$

3.3 | *Arctium lappa* L modulated the mRNA level of lipid metabolism-related genes in liver of the rats

Steatosis is usually related to disorder of lipid metabolism factors. The mRNA level of several key lipid metabolism-related factors in the rat liver was detected by RT-QPCR experiment on the rat liver, such as ACC, CPT-1, FAS, GPAT, HMG-CoAR, and HSL. It was found that the ACC ($p < .0001$) mRNA level of the HSHFD group was significantly higher than that of the control group and the mRNA level of CPT-1 ($p < .05$) was significantly lower compared with the control group. However, the ACC mRNA level of the ALE-intervention group ($p < .0001$) was significantly lower than that of HSHFD group, the mRNA level of CPT-1 was significantly higher than that of HSHFD group (ALE-L, $p < .01$ and ALE-H, $p < .0001$) (Figure 3). CPT-1 is the rate-limiting enzyme of fatty acid β-oxidation, which indicates that ALE can regulate the mRNA level of ACC and CPT-1 in rat liver to ameliorate the steatosis of rat liver.

3.4 | *Arctium lappa* L upregulates the protein expression of CPT-1 by enhancing the protein phosphorylation level of AMPK and ACC in the liver

The protein expression of ACC and CPT-1 was verified by Western Blotting experiment. The protein expression of CPT-1 in the HSHFD group was significantly decreased than that the control group ($p < .0001$), while the CPT-1 expression in the ALE-L ($p < .0001$) group and ALE-H ($p < .0001$) group both increased nearly two times than the HSHFD group (Figure 4a,c). The expression of CPT-1 is inhibited by ACC, and when the phosphorylation level of ACC increases, the expression of CPT-1 is also up-regulated. It was found that the phosphorylation level of ACC protein of the HSHF group was significantly decreased than that of the control group ($p < .0001$), while the phosphorylation level of ACC protein of the ALE-L ($p < .0001$) and ALE-H ($p < .0001$) groups were increased at least two times than that of the HSHF group (Figure 4a,b). The phosphorylation of ACC can be regulated by AMPK, which is related to glucose and lipid metabolism. It was found that the phosphorylation of AMPK was inhibited in the

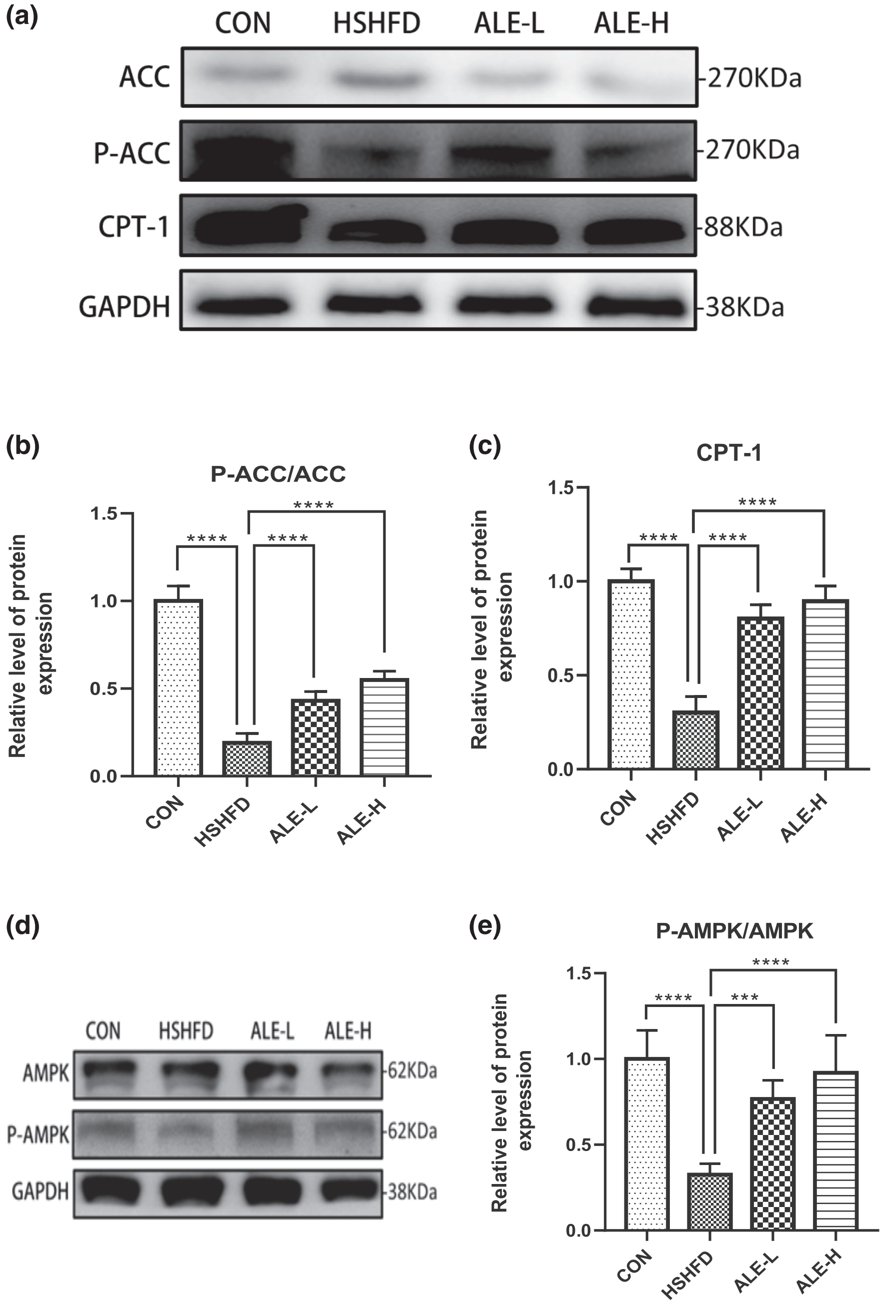


FIGURE 4 ALE upregulates the protein expression of CPT-1 by enhancing the protein phosphorylation level of AMPK and ACC in the liver. Western blot analysis was performed to access CPT-1protein expression (a,c), ACC protein phosphorylation level (a,b), AMPK protein phosphorylation level (d,e) in liver. Representative Western blot bands of (a) ACC, (b) CPT-1 and (d) p-ACC/ACC are shown. Data were shown as mean ± SD. $^{*}p < .05$, $^{**}p < .01$, $^{****}p < .0001$, $^{ns}p > .05$ vs HSHFD group, $n = 6$

HSHFD group compared to the CON group ($p < .0001$), while the phosphorylation level of AMPK in the liver of rats increase at least two times in the ALE-intervention group (ALE-L, $p < .001$ & ALE-H, $p < .0001$) than the HSHFD group (Figure 4d,e). The increasing trend of phosphorylated AMPK was almost consistent with that of ACC phosphorylation.

3.5 | *Arctium lappa* L promotes fatty acid β-oxidation in liver through AMPK/ACC/CPT-1 pathway

The mitochondria were extracted for detection of fatty acid β-oxidation rate detection experiment from the liver. The β-oxidation ability is expressed by the rate of micromolar ferricyanide reduction. It was found that the β-oxidation ability of the HSHFD group ($p < .01$) was significantly lower than that of the control group, while the β-oxidation rate of the ALE-L ($p < .1$) and ALE-H ($p < .01$) groups was significantly higher than that of the HSHFD group (Figure 5). Additionally, β-oxidation rate of the high-monitoring group was nearly three times higher than that of HSHFD group. This indicates that ALE can inhibit the reduction of fatty acid β-oxidation rate caused by high-sugar and high-fat diet.

4 | DISSCUSION

Various external and internal factors, such as dietary environment and genetics, have gradually significantly affected the pathological progress of obesity. In normal circumstances, triglycerides are absorbed and utilized by muscle cells and fat cells along with the blood circulation, and the remaining triglycerides gather in the liver for metabolism. However, the accumulation of excessive triglycerides causes hepatocyte lipotoxicity, hepatocyte steatosis, and reduces efficiency of fatty acids β-oxidation, which will increase concentration of free fatty acids in peripheral blood (Heeren & Scheja, 2021). Excess cholesterol and triglycerides cause the accumulation of triglycerides in fat cells and resulting in obesity (Rosengren, 2021). In particular, the accumulation of fat in the liver gradually leads to fatty liver (Geng, Faber, de Meijer, Blokzijl, & Moshage, 2021). This phenomenon is bound to have necessary risks to the glucose and lipid metabolism, so as to form a vicious circle (Kim et al., 2021). A number of basic experiments and clinical studies have confirmed that natural foods or natural products can improve obesity and MAFLD (Liu, Chen, Zhang, & Ni, 2021; Shin et al., 2021). It would be of great significance to find a botanical remedy with positive therapeutic effects and minimum side effects.

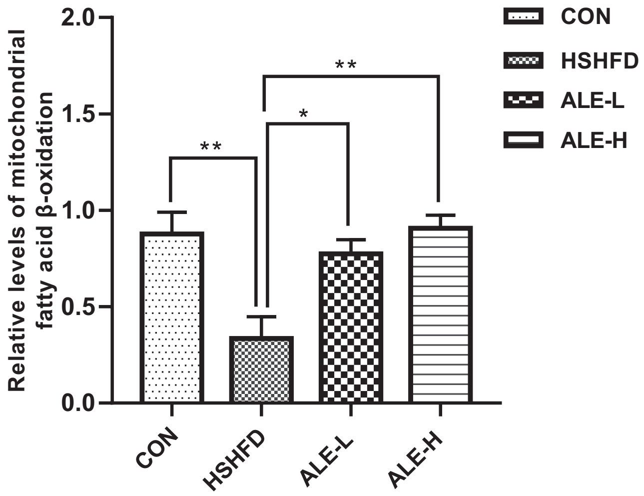


FIGURE 5 ALE promotes fatty acid β-oxidation in liver. Mitochondria were extracted from the three groups of liver tissues, and the rate of fatty acids β-oxidation in the liver was detected by using reagent test kit. The reduction rate of micromole ferricyanide was used to characterize the β-oxidation rate of fatty acids. Data were shown as mean ± SD, $^*p < .05$, $^{**}p < .01$, vs HSHFD group, $n = 6$

Arctium lappa L root benefits from its high content of polyphenols, polysaccharides, and dietary fiber, which leads to its potentially therapeutic effect in obesity, diabetes, and hyperlipidemia, etc (Chan et al., 2011). Therefore, *Arctium lappa* L root has become a new resource food with medicine and food homology. As a functional food, the effect of *Arctium lappa* L on the amelioration of obesity has been studied, and the protective effect on the liver has been reported, but the mechanism is not yet clear (Hong et al., 2015). Therefore, it is of great significance to study the mechanism of *Arctium lappa* L on obesity and its protective effect on the liver. This experiment has indeed proved this idea as shown in Figures 1 and 2, *Arctium lappa* L root can inhibit weight gain in rats and reduce the serum lipid level and the hepatic steatosis of the liver was ameliorated. Interestingly, *Arctium lappa* L root powder can inhibit the food intake of rats as shown in Figure 1b, which indicates that *Arctium lappa* L root powder may have the effect of suppressing appetite. Suppressing appetite will also be an important research direction in the future, because appetite is also an inevitable factor affecting obesity and lipid metabolism. This also indicated that *Arctium lappa* L may have a certain regulatory effect on the central nervous system.

According to the analysis of *Arctium lappa* L root components, it can be known that *Arctium lappa* L contains bioactive polysaccharides, polyphenols, and other substances. In fact, studies have found that plant polysaccharides and plant polyphenols have a certain effect on ameliorating glucose and lipid metabolism disorders, especially having anti-diabetic, anti-cancer, and ameliorating effects on obesity (Luo, Mu, & Sun, 2021; Vamanu, Dinu, Pelinescu, & Gatea, 2021). In particular, plant polysaccharides and plant polyphenols can regulate cell growth and proliferation, and regulate glucose and lipid metabolism by activating AMPK (Skrobuk, von Kraemer, Semenova, Zitting, & Koistinen, 2012; Wang et al., 2021), especially polyphenols such as chlorogenic acid and caffeic acid (Kong et al., 2021; Zheng, Choi, Lee, Lee, & Kang, 2021). Research on the activation of AMPK has been widely reported. Among them, the content of chlorogenic acid in the root is abundant in the whole plant of *Arctium lappa* L. This indicates that the effects of *Arctium lappa* L root in ameliorating obesity (Kuo et al., 2012) and MAFLD are most

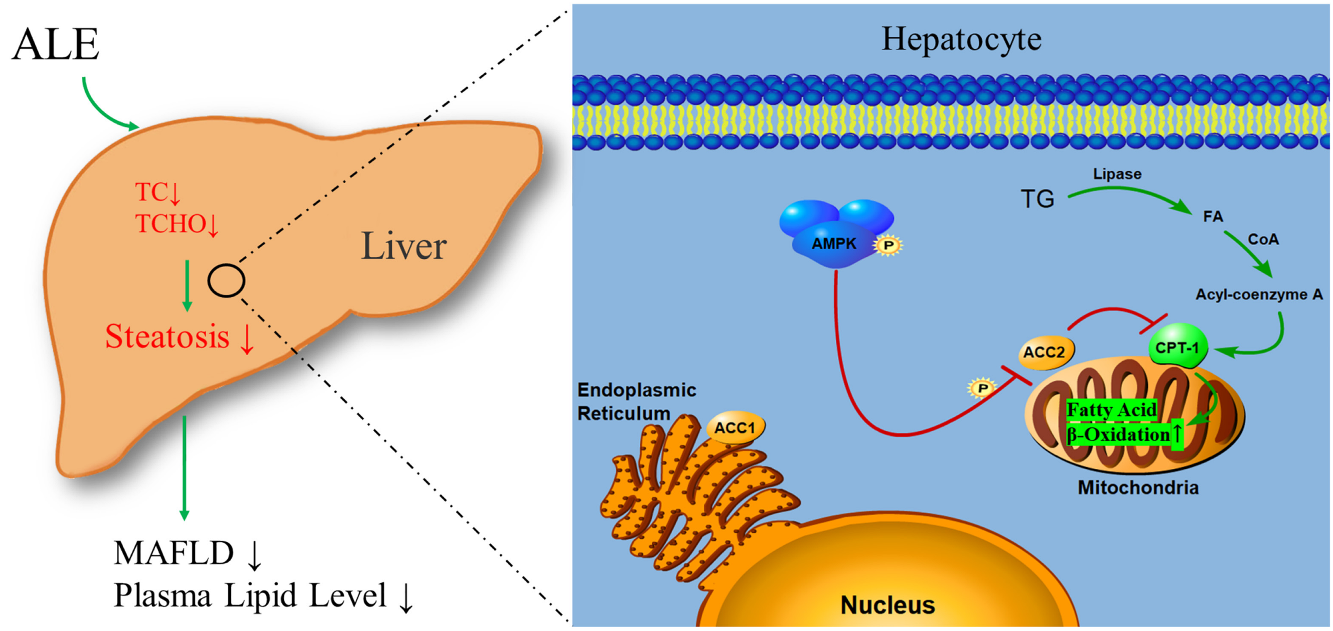


FIGURE 6 ALE ameliorates liver steatosis through AMPK/ACC/CPT-1 pathway. ALE enhances the phosphorylation of ACC by regulating the phosphorylation of AMPK in liver and up-regulates the expression of CPT-1. Triglycerides (TG) are broken down into fatty acids (FA) and glycerol under the action of lipase in hepatocellular. Acyl-coenzyme A formed by fatty acids and acetyl-CoA can be transported to mitochondria through increased CPT-1 for fatty acid β-oxidation. The ability of fatty acid β-oxidation is enhanced in the liver, which will reduce the accumulation of triglycerides and cholesterol

likely related to the activation of AMPK (Kuo et al., 2012). AMPK is a serine/threonine kinase, which is the central part of the protein kinase cascade that regulates the energy state of cells. Its activation or inhibition can produce complex cellular biological effects, so it is crucial in obesity and lipid metabolism in the body (Wu et al., 2021; Zhou et al., 2021). As we all know, the rate-limiting enzyme of fatty acids β-oxidation is CPT-1. The triglycerides in the liver are decomposed into fatty acids, which form acyl-CoA with CoA and enter the mitochondria for fatty acid β-oxidation through CPT-1. This process will reduce liver triglyceride accumulation, ameliorate steatosis, and lower blood lipids. The expression of CPT-1 is closely related to the activation of AMPK and ACC. Under normal circumstances, ACC can inhibit the expression of CPT-1, thereby inhibiting the transfer of acyl-coenzyme A to mitochondria (Nicholas et al., 2019). It reduces the ability of fatty acids β-oxidation. Correspondingly, when ACC is phosphorylated, the protein of ACC is down and the β-oxidation of fatty acids is enhanced. In fact, we indeed found that the phosphorylation level of ACC was significantly increased, and the expression of CPT-1 was significantly increased as shown in Figure 4. It was also found that fatty acid β-oxidation in liver tissue had been enhanced, which is shown in Figure 5. This indicates that ACC/CPT-1 may be the important way for *Arctium lappa L* root to enhance the fatty acids β-oxidation.

Our research showed that ALE can activate AMPK in the liver, enhance the phosphorylation level of ACC and the protein expression of CPT-1. In short, ALE enhances fatty acid β-oxidation in liver through AMPK-ACC-CPT-1 pathway as shown in the mechanism diagram of our research (Figure 6). This mechanism can enhance hepatic metabolism of fatty acids, accelerate lipolysis, reduce fat accumulation, reduce lipotoxicity, and ameliorate liver steatosis. It is important that the hepatic lipid metabolism will eventually affect the process of body fat accumulation. In the entire molecular mechanism, the activation of AMPK is very likely to be a key regulatory protein for ALE to lower lipids and ameliorate obesity. It also provides possibility that ALE can ameliorate MAFLD. The result of this research also shows that *Arctium lappa L* root has great potential as a functional food for obesity suppression and lipid-regulation. In addition, *Arctium lappa L* root contains many active factors, so looking for a single natural element in ALE are meaningful for the treatment of obesity and MAFLD. It also provides directional guidance for the research of natural plants and the assessment of nutritional factors, and provides a molecular basis for research of *Arctium lappa* L root on the targeting of obesity and lipid metabolism. In addition, *Arctium lappa* L root is not widely known and used in many countries, this research may provide a broader direction for the use.

AUTHOR CONTRIBUTIONS

Kaiyang Ma: Investigation, Writing original draft, Data curation, Conceptualization, Methodology, Validation, Visualization, Formal analysis. Weixi Sheng: Validation, Visualization, Software, Data curation, Formal analysis. Rong Gao: Methodology, Validation. Jin Feng: Methodology, Investigation. Wuyang Huang: Conceptualization, Methodology. Li Cui: Investigation. Jun Liu: Conceptualization. Ying Li: Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Resources.

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CONFLICT OF INTEREST

The all authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

ETHICS APPROVAL STATEMENT

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed, and all animal procedures were performed in strict accordance with the guideline of the Institutional Animal Care and Use Committee of Nanjing Medical University.

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