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Centella asiatica lowers body fat accumulation via regulating cholesterol homeostasis- and lipid metabolism-related genes in mice with high-fat, high-sugar diet-induced obesity

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Abstract

To understand the mechanisms involved in the anti-obesity effects *Centella asiatica* (CA), we examined body weight, serum levels, white adipose tissue (WAT) weight, histological analysis, and the expression of cholesterol homeostasis- and lipid metabolism-related genes in mice with high-fat, high-sugar diet (HFHSD)-induced obesity that were orally treated with CA for 12 weeks. Eight-week-old, male C57BL/6J mice were assigned to the following four groups (8 mice/group): NOR, normal diet; HFHSD (Control), HFHSD; CA-L, HFHSD + CA 300 mg/kg; CA-H, HFHSD+CA 600 mg/kg. The suspension of powdered CA leaf was fed using oral gavage. CA treatment significantly attenuated HFHSD-induced increase in body weight gain, serum glucose, triacylglycerol, and WAT weight ($p < 0.05$). Compared to that in HFHSD, adipocyte diameter and macrovesicular area of epididymal WAT significantly decreased with CA treatment ($p < 0.05$). The mRNA expression levels of peroxisome proliferator-activated receptor gamma (PPARγ), fatty acid synthase (FAS), cluster of differentiation 36 (CD36), 3- hydroxyl-3-methylglutaryl CoA reductase (HMGCR), and stearoyl CoA desaturase 1 (SCD 1) were significantly downregulated in the CA-H compared to the HFHSD ($p < 0.05$). CA exerts anti-obesity effects by lowering body fat accumulation via regulating gene expression and thus, is a potential lipid-lowering agent.

Keywords *Centella asiatica*, Body weight, Body fat, Obesity, Adipocyte

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Introduction

Obesity, defined as accumulation of excessive body fat, is driven by an imbalance in energy input and expenditure [1, 2]. It is a medical condition that raises the risk of several diseases such as diabetes, stroke, heart ailments, arthritis, inflammation, and even cancer [3]. Since the introduction of medicines to treat obesity in the 1930s, various substances have been tested, although most were only marginally effective in lowering body weight, and some are no longer marketed owing to their side effects [4, 5]. Alternative and complementary medicine has gained increasing attention because of the unsatisfactory

results and adverse reactions of medical intervention in obesity management [6, 7]. Epidemiological studies have recommended the use of plants to reduce the risk of obesity [8].

Plants are natural resources for medicine that exert no toxic or adverse effects. They synthesize hundreds of chemical compounds for various functions, including protection against fungi, insects, and disease. Medicinal plants, also known as medicinal herbs, have been discovered and used in traditional medicine since prehistoric times [9]. Numerous animal- and cell-based studies have been performed on medicinal plants and their active components to confirm their efficacy and mechanisms of action [10, 11].

Centella asiatica (CA) has been receiving widespread attention from researchers interested in both its phytochemical and biological properties [12, 13]. A perennial herbaceous plant belonging to the family Apiaceae, CA is a valuable medicinal herb mainly grown in swampy areas of India, China, Malaysia, Sri Lanka, Indonesia, Madagascar, and Korea. According to the Indian pharmacopeia, CA exhibits various therapeutic effects on psoriasis, diarrhea, fever, varicose ulcers, eczema, leprosy, lupus, and amenorrhea. Recent studies have reported various biological properties of CA, since it comprises several anti-oxidant, anti-inflammatory, anti-microbial, neuroprotective, memory improvement, and anti-depressant compounds [13–16]. Recent studies have shown that CA is effective for treating endocrine diseases, such as obesity, because it improves certain metabolic pathways and has fat-suppressing effects [17, 18]. There have been several claims regarding the underlying mechanisms involved in the anti-obesity effects of CA over the past few decades [19, 20]; however, more scientific data are needed to justify its ever-increasing use.

Some principal mechanisms of lipid metabolism are modulated by insulin signaling, adipogenesis, adipocyte differentiation, lipolysis, and β-oxidation of free fatty acid (FFA) in the obese state. Lipolysis refers to the degradation of triglyceride to yield FFA and glycerol. It can be induced by lipase, which are the direct transcriptional target of peroxisome proliferator activated receptor gamma (PPARγ) [21]. Excess FFA produced by lipolysis can migrate in the circulation and accumulate in the blood or the liver, resulting in upregulation of proteins involved in de novo lipogenesis, such as sterol regulatory element-binding protein (SREBP) and fatty acid synthase (FAS) [22]. Conversely, lipid accumulation can be reduced by upregulation of genes involved in β-oxidation of FFA in the adipose tissue and liver [23]. For this reason, controlling these factors has been an ideal therapeutic strategy to prevent or ameliorate obesity associated complications. Furthermore, the use of natural products as therapeutic agents in treating and preventing obesity has become popular [24]. Activated 5'adenosine monophosphate activated protein kinase (AMPK) triggers beneficial physiological effects, including reductions in fat deposition. AMPK knockdown upregulated FAS, and SREBP which is another key enzyme of fat synthesis [25]. The mechanistic study of CA on adipogenesis and lipolysis in white adipose tissue (WAT) in obese mice remains unresearched. Thus, in this study, we investigated its ameliorative effects in vivo to understand the underlying mechanisms involved in the anti-obesity effects of CA and provide scientific evidence to support CA as a supplement to prevent obesity. We examined body weight, serum levels, WAT weight, histological analysis, and the expression of cholesterol homeostasis- and lipid metabolism-related genes in obese mice treated orally with CA.

Materials and methods

Preparation of Centella asiatica (CA)

CA leaf was collected during early summer from Jeju (Republic of Korea) and provided by ASKBASE Co. (Seoul, Republic of Korea). Briefly, CA was cultivated in a pesticide-free greenhouse facility for 45 days. The harvested CA was immediately dried at 60 °C for 24 h in a forced-convection oven (VS-1202D4N; Vision Bionex, Bucheon, Korea). The dried CA leaf was pulverized using a food processor and passed through a 25-mesh sieve to collect CA particles than 0.70 mm. The dried and powdered CA leaf was weighed and suspended in distilled water. The CA leaf suspension was sonicated for 30 min in a water bath set at 37 °C just before feeding it.

Animals

Eight-week-old, C57BL/6J male mice were procured from Orient Bio (Seongnam, Korea). All animal use and euthanasia protocols were reviewed and approved by the Animal Care and Use Committee of Korea University (KUIACUC-2021-0098). In accordance with the institution's guidelines, the mice were individually housed in stainless steel cages and were maintained in standard environmental conditions (temperature: 24 ± 1℃, humidity: 50–60%, and light-dark cycle: 12/12 hour). After one week acclimatization, 32 mice were randomly assigned to four groups (8 mice/group) and fed with either a normal diet or high-fat high-sugar diet (HFHSD, 45 kcal% fat and 32 kcal% sucrose) for 12 weeks as follows: Group 1: mice fed with normal diet (NOR), Group 2: mice fed with HFHSD (HFHSD), Group 3: mice fed with HFHSD along with 300 mg/kg CA leaf suspension treatment (CA-L), Group 4: mice fed with HFHSD along with 600 mg/kg CA leaf suspension treatment (CA-H). The composition of the experimental diets (normal diet and HFHSD) is shown in Table 1. The dosage of CA (300 mg/kg or 600

Table 1 Composition of normal diet (ND) and high-fat, high-sugar diet (HFHSD, 45 kcal% fat and 32 kcal% sucrose)

Ingredient	Diet (g)	
	ND	HFHSD
Casein	22	22
L-Cysteine	0.18	0.18
Cornstarch	50	–
Maltodextrin	7.5	7.5
Soybean oil	4	2.5
Mineral mix	4	4
Sodium bicarbonate	1	1
Potassium citrate	0.4	0.4
Vitamin mix	1	1
Cholin bitatrate	0.2	0.2
Sucrose	10	45.1
Coconut oil	–	25.3
Total	100.28	109.18

mg/kg) was determined based on previous studies in consideration of its effectiveness and safety [17, 26, 27]. The CA suspension (200 µl/mice) was fed using oral gavage with a ball-tip needle of the same volume every day.

Body weight and food consumption were monitored weekly. At the end of the experiment, the mice were starved for 12 h and sacrificed by CO_2 asphyxiation. Blood samples were collected from inferior vena cava. Serum was separated by centrifugation at 3000*g* for 15 min at 4 °C. Serum glucose, triacylglycerol, and cholesterol levels were measured using FUJI DRI-CHEM 3500 (Fuji Photo Film, Osaka, Japan). After collecting blood, WAT (inguinal WAT, iWAT; mesenteric WAT, mWAT; epididymal WAT, eWAT) were removed and weighed immediately. They were then rinsed with phosphate-buffered saline, and stored at − 80 °C until analysis.

Histological analysis

Liver and eWAT were fixed in 10% neutral formalin for 42 h. The tissues were placed in cassettes, washed in phosphate-buffered saline with three changes, cleared in xylene for 30 min with two changes, and embedded in paraffin for 1 h with three changes. The tissues were blocked in paraffin and cut to 5 µm thickness. The sections were stained with hematoxylin and eosin (H&E) and viewed under a light microscope (Leica, Wetzlar, Germany). The tissues were photographed at 200× magnification. The diameter and macrovesicular area were evaluated using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Quantitative real-time polymerase chain reaction (PCR)

Total RNA was extracted from the liver using Trizol reagent (Gibco-BRL, Grand Island, NY, USA), according to the manufacturer's instructions. The extracted RNA was reverse-transcribed using Moloney murine leukemia virus transcriptase. The expression levels of genes of interest were determined from the synthesized cDNA using AccuPower GreenStar qPCR PreMix (Bioneer, Daejeon, Korea) on an Excycler 96 Real-Time Quantitative Thermal Block machine (Bioneer). The primer sequences used in the experiments is shown in Table 2. The PCR included the following steps: denaturing at 95 °C for 5 min followed by 50 cycles of 95 °C for 10 s, 60 °C for 40 s, and 72 °C for 10 s. Transcript concentrations were calculated as copies per µl using a standard curve. The mRNA expression was normalized

Table 2 List of primer sequences used in real-time PCR

Gene	Primer sequence (5′-3′)	
	Forward	Reverse
GAPDH	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG
PPARγ	GTACTGTCGGTTTCAGAAGTGCC	ATCTCCGCCAACAGCTTCTCCT
FAS	CTGCGATTCTCCTGGCTGTGAA	CAACAACCATAGGCGATTTCTGG
CD36	GGACATTGAGATTCTTTTCCTCTG	GCAAAGGCATTGGCTGGAAGAAC
AMPK	GGTGTACGGAAGGCAAAATGGC	CAGGATTCTTCCTTCGTACACGC
SREBP-2	AGAAAGAGCGGTGGAGTCCTTG	GAACTGCTGGAGAATGGTGAGG
HMGCR	GCTCGTCTACAGAAACTCCACG	GCTTCAGCAGTGCTTTCTCCGT
SREBP-1 C	CGACTACATCCGCTTCTTGCAG	CCTCCATAGACACATCTGTGCC
LDLR	GAATCTACTGGTCCGACCTGTC	CTGTCCAGTAGATGTTGCGGTG
SCD1	GCAAGCTCTACACCTGCCTCTT	CGTGCCTTGTAAGTTCTGTGGC

GAPDH glyceraldehyde-3-phosphate dehydrogenase, *PPARγ* peroxisome proliferator activated receptor gamma, *FAS* fatty acid synthase, *CD36* cluster of differentiation 36, *AMPK* 5′adenosine monophosphate activated protein kinase, *SREBP-2* sterol regulatory element binding protein 2, *HMGCR* 3-hydroxyl-3-methylglutaryl CoA reductase, *SREBP-1C* sterol regulatory element binding protein 1C, *LDLR* low-density lipoprotein receptor, *SCD1* stearoyl CoA desaturase 1

to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the results are presented as fold-changes relative to NOR.

Statistical analyses

Each result is expressed as mean ± standard error of mean (SEM). Statistical analyses were performed using the SPSS statistical analysis software (version 19.0; International Business Machines, Armonk, NY, USA). Differences between groups were evaluated statistically using one-way analysis of variance and Tukey's multiple tests. Differences between the HFHSD and others were evaluated using Student's *t*-test. Results with $p < 0.05$ were considered statistically significant.

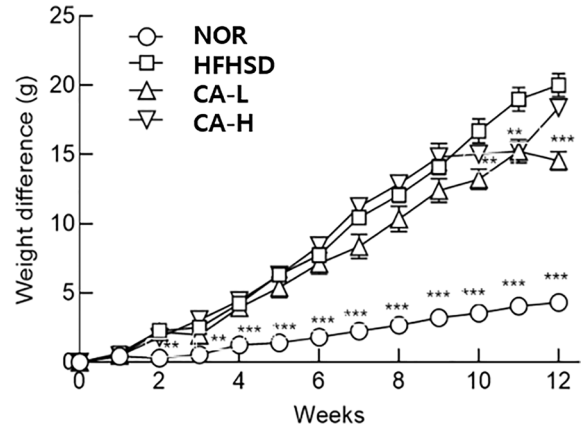

Fig. 1 Body weight gains in mice with high-fat, high-sugar diet (HFHSD)-induced obesity treated orally with *Centella asiatica* (CA) for 12 weeks. Values are the means ± standard error of mean (SEM) for eight mice. The asterisk indicates a significant difference compared to HFHSD (**p < 0.01, ***p < 0.001). NOR, normal diet; HFHSD **(**Control**)**, HFHSD; CA-L, HFHSD + CA 300 mg/kg; CA-H, HFHSD + CA 600 mg/kg

Results

Body weight gain and food consumption

No signs of toxicity, such as piloerection, alterations in locomotor activity, diarrhea, or deaths, were recorded during the 12 weeks of oral CA treatment. Figure 1 illustrates the effect of CA on body weight gain in mice with HFHSD-induced obesity. The initial weights of the mice were 22.70 g (NOR), 22.71 g (HFHSD), 22.80 g (CA-L), and 22.76 g (CA-H). HFHSD triggered significant changes in body weight gain pattern, with the weight gain in mice fed with HFHSD being considerably higher than in mice fed with normal diet throughout the study (12th week, NOR 4.32 g vs. HFHSD 20.00 g, $p < 0.001$). The average daily food consumption was 2.48 g/day (NOR), 2.72 g/day (HFHSD), 2.58 g/day (CA-L), and 2.55 g/day (CA-H) (data not shown). Food consumption did not differ between groups during the experimental period. CA treatment showed a tendency to attenuate HFHSD-induced body weight gain. Despite comparable food consumption, body weight gain in the CA-L was suppressed significantly from week 10 compared to the HFHSD (10th week, CA-L 13.22 g vs. HFHSD 16.70 g, $p < 0.01$; 11th week, CA-L 15.24 g vs. HFHSD 18.98 g, $p < 0.01$, 12th week, CA-L 14.56 g vs. HFHSD 20.00 g, $p < 0.001$).

Serum glucose, triacylglycerol, and cholesterol

The effects of CA treatment on serum glucose, triacylglycerol, and cholesterol levels in HFHSD-induced obese mice are shown in Fig. 2. HFHSD induced significant increases in serum glucose (NOR 183.38 mg/dl vs. HFHSD 266.83 mg/dl, $p < 0.001$), triacylglycerol (NOR 92.00 mg/dl vs. HFHSD 139.20 mg/dl, $p < 0.001$), and cholesterol levels (NOR 45.38 mg/dl vs. HFHSD 86.80 mg/dl, $p < 0.001$). CA treatment attenuated HFHSD-induced increase in serum glucose and triacylglycerol levels. In particular, CA-H had significantly lower both of serum glucose (CA-H 216.86 mg/dl vs. HFHSD 266.83 mg/dl, $p < 0.05$) and triacylglycerol (CA-H 115.20 mg/dl vs. HFHSD 139.20 mg/dl, $p < 0.05$) than the

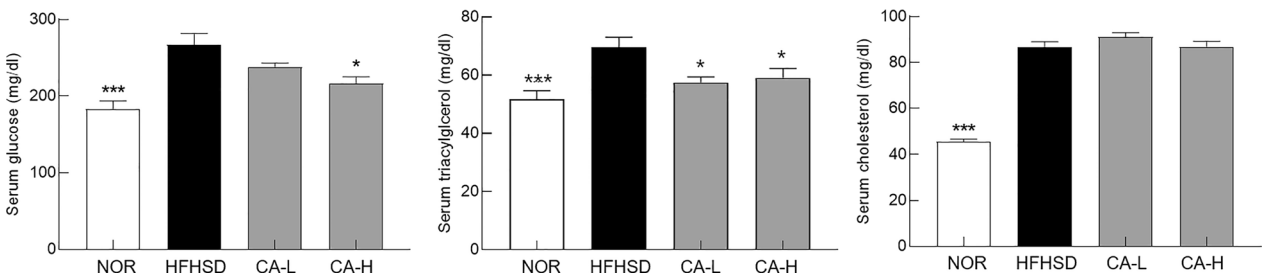

Fig. 2 Serum glucose, triacylglycerol, and cholesterol in mice with high-fat, high-sugar diet (HFHSD)-induced obesity orally treated with *Centella asiatica* (CA) for 12 weeks. Values are the means ± standard error of mean (SEM) for 8 mice. The asterisk indicates a significant difference compared to HFHSD (* p < 0.05, *** p < 0.001). NOR, normal diet; HFHSD **(**Control**)**, HFHSD; CA-L, HFHSD + CA 300 mg/kg; CA-H, HFHSD + CA 600 mg/kg

HFHSD. However, there were no significant differences in serum cholesterol levels between the HFHSD and groups treated with CA.

Relative weights of WAT

The relative weights of the iWAT, mWAT, and eWAT were measured to examine the effect of CA treatment on body fat distribution. Figure 3 shows the relative weights of iWAT, mWAT, and eWAT in mice with HFHSD-induced obesity that were orally treated with CA for 12 weeks. All relative weights of WAT were significantly lower in mice fed on normal diet than in mice fed HFHSD (iWAT, NOR 5.74 mg/g vs. HFHSD 44.14 mg/g, $p < 0.001$; mWAT, NOR 11.89 mg/g vs. HFHSD 25.33 mg/g, $p < 0.001$; eWAT, NOR 12.93 mg/g vs. HFHSD 57.76 mg/g, $p < 0.001$). Although CA treatment did not affect mWAT, it tended to lower the volume of iWAT and eWAT; CA treatment significantly reduced the relative weight of iWAT in mice with HFHSD-induced obesity (CA-L 37.30 mg/g vs. HFHSD 44.14 mg/g, $p < 0.05$; CA-H 36.36 mg/g vs. HFHSD 44.14 mg/g, $p < 0.01$) and relative weight of eWAT in CA-L group was significantly lower than that in the HFHSD (CA-L 48.65 mg/g vs. HFHSD 57.76 mg/g, $p < 0.01$).

Microphotographic observations

As illustrated in Fig. 4A, large macrovascular adipocytes were observed in the liver tissue of the HFHSD compared with those in the NOR. However, CA treatment markedly reduced hepatic steatosis in mice with HFHSD-induced obesity. The adipocyte size of eWAT in mice with HFHSD-induced obesity also increased compared to that in mice fed a normal diet (Fig. 4B). In the groups treated orally with CA, we observed a marked reduction in adipocyte hypertrophy compared to the HFHSD. The histological analysis of eWAT in HFHSD-induced obese mice treated orally with CA is shown in Fig. 4C. There was a significant increase in the adipocyte diameter of eWAT in mice fed on HFHSD compared to those fed on normal diet (NOR 39.50 μm vs. HFHSD 104.83 μm, $p < 0.001$). HFHSD also caused a significant increase in the macrovesicular area of eWAT compared to the normal diet (NOR 100% vs. HFHSD 841.51%, $p < 0.001$). CA treatment attenuated HFHSD-induced increase in adipocyte size in eWAT. In particular, CA-H had significantly smaller adipocyte diameter in eWAT than the HFHSD (CA-H 80.50 μm vs. HFHSD 104.83 μm, $p < 0.001$). Compared to that in the HFHSD, macrovesicular area of eWAT was decreased following CA treatment (CA-L 428.54% vs. HFHSD 841.51%, $p < 0.05$; CA-H 458.84% vs. HFHSD 841.51%, $p < 0.05$).

mRNA expression profile

We evaluated the effects of CA on cholesterol homeostasis- and lipid metabolism-related genes by analyzing mRNA expression in liver tissue using quantitative real-time PCR (Fig. 5). The mRNA expression levels of PPARγ, FAS, CD36, SREBP-2, HMGCR, SREBP-1 C, LDLR, and SCD1 were significantly higher in mice with HFHSD-induced obesity than in mice fed a normal diet ($p < 0.001$). In mice with HFHSD-induced obesity that were orally treated with CA, the mRNA expression levels of PPARγ, FAS, CD36, HMGCR, and SCD1 were downregulated in a dose-dependent manner; in the CA-H, the mRNA expression levels of PPARγ, FAS, CD36, HMGCR, and SCD 1 were significantly decreased compared to those in the HFHSD (PPARγ, CA-H 2.36 vs. HFHSD 3.66, $p < 0.01$; FAS, CA-H 2.36 vs. HFHSD 3.78, $p < 0.01$; CD36, CA-H 3.90 vs. HFHSD 5.41, $p < 0.05$; HMGCR, CA-H 2.64 vs. HFHSD 4.36, $p < 0.01$; SCD1, CA-H 2.90 vs. HFHSD 5.87, $p < 0.001$). During CA treatment, SREBP-2 expression was slightly reduced in the groups treated orally with CA compared to that in the HFHSD, without significant differences. In this study, we did not observe any effect of CA treatment on SREBP-1 C or LDLR in mice with HFHSD-induced obesity.

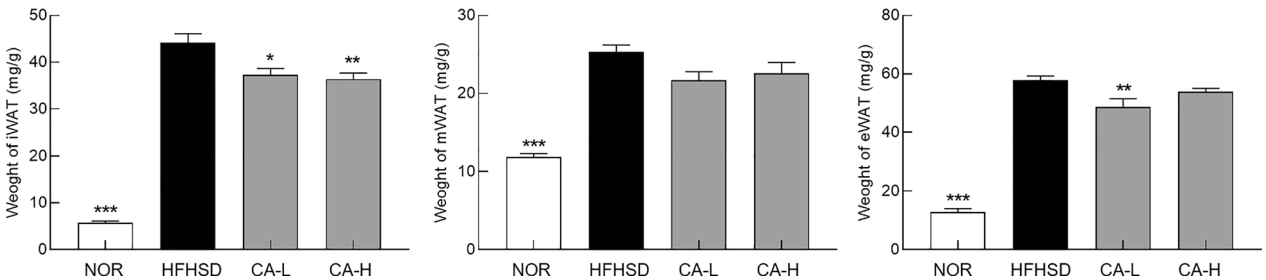

Fig. 3 Relative weight of white adipose tissue (WAT) in mice with high-fat, high-sugar diet (HFHSD)-induced obesity that were orally treated with *Centella asiatica* (CA) for 12 weeks. Values are the means ± standard error of mean (SEM) for eight mice. The asterisk indicates a significant difference compared to HFHSD (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). NOR, normal diet; HFHSD **(**Control**)**, HFHSD; CA-L, HFHSD + CA 300 mg/kg; CA-H, HFHSD + CA 600 mg/kg. iWAT, inguinal WAT; mWAT, mesenteric WAT; eWAT, epididymal WAT.

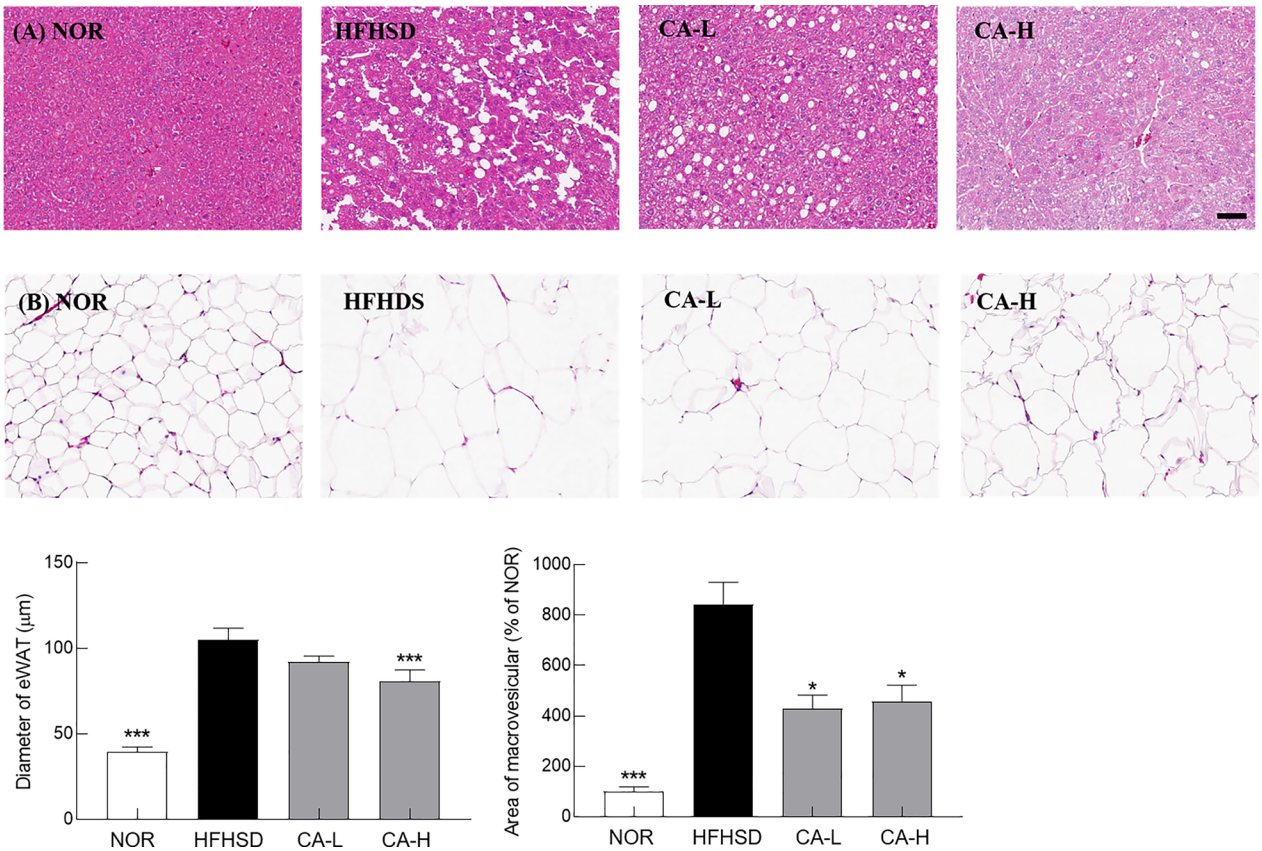

Fig. 4 Microphotographic observation in mice with high-fat, high-sugar diet (HFHSD)-induced obesity that were orally treated with *Centella asiatica* (CA) for 12 weeks. The liver tissue (**A**) and epididymal white adipose tissue (eWAT) (**B**) were stained with hematoxylin and eosin (H&E), and viewed under a microscope (×200) (scale bar = 100 μm). The diameter and macrovesicular area of eWAT (**C**) were evaluated using ImageJ. Values are the means ± standard error of mean (SEM) for eight mice. The asterisk indicates a significant difference compared to HFHSD (* $p < 0.05$, *** $p < 0.001$). NOR, normal diet; HFHSD **(**Control**)**, HFHSD; CA-L, HFHSD + CA 300 mg/kg; CA-H, HFHSD+CA 600 mg/kg

We also investigated the status of AMPK, which plays a role in cellular energy homeostasis by activating fatty acid uptake and oxidation when cellular energy is low. In mice with HFHSD-induced obesity, AMPK level was markedly reduced compared to mice fed with a normal diet (NOR 1.00 vs. HFHSD 0.22, $p < 0.001$). AMPK levels were marginally higher in the groups treated orally with CA than the HFHSD; however, the differences were not statistically significant.

Discussion

HFHSD contains high proportions of sugar and saturated fats, which accumulate in fat pads across body and promotes excessive WAT development [28]. HFHSD results in an increase in body weight and serum comorbidity factors. CA is considered useful in the treatment of obesity [29, 30]. Abas et al. [31] reported that long-term treatment of obese rats with CA reversed plasma glucose and lipid levels as well as the tricarboxylic acid cycle and amino acid metabolic disorders, returning them to normal states. Based on biochemical analysis, they concluded that CA exerts anti-obesity effects and modulates specific metabolic pathways. Available evidence shows that CA can inhibit body weight gain, lower plasma glucose levels, and reduce oxidative stress [32]. The potential of CA as an anti-obesity agent has been proven by the fact that it suppresses lipid levels and enhances sensitivity to leptin and insulin. At the molecular level, CA can also increase levels of enzymatic antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase, and reverse the expression of palmitoyltransferase-1 and uncoupling protein-2. In an animal model of obesity, CA increased the activities of SOD, GSH, and catalase, thereby improving the enzyme anti-oxidant system [18, 20]. Therefore, it can be deduced that CA alleviates obesity-driven oxidative stress, and suppress body weight gain by promoting fatty acid oxidation [18]. The anti-obesity effect of CA seems to be due to the effect of madecassic acid. It has been reported that madecassic acid reduces triacylglycerol levels, suppresses

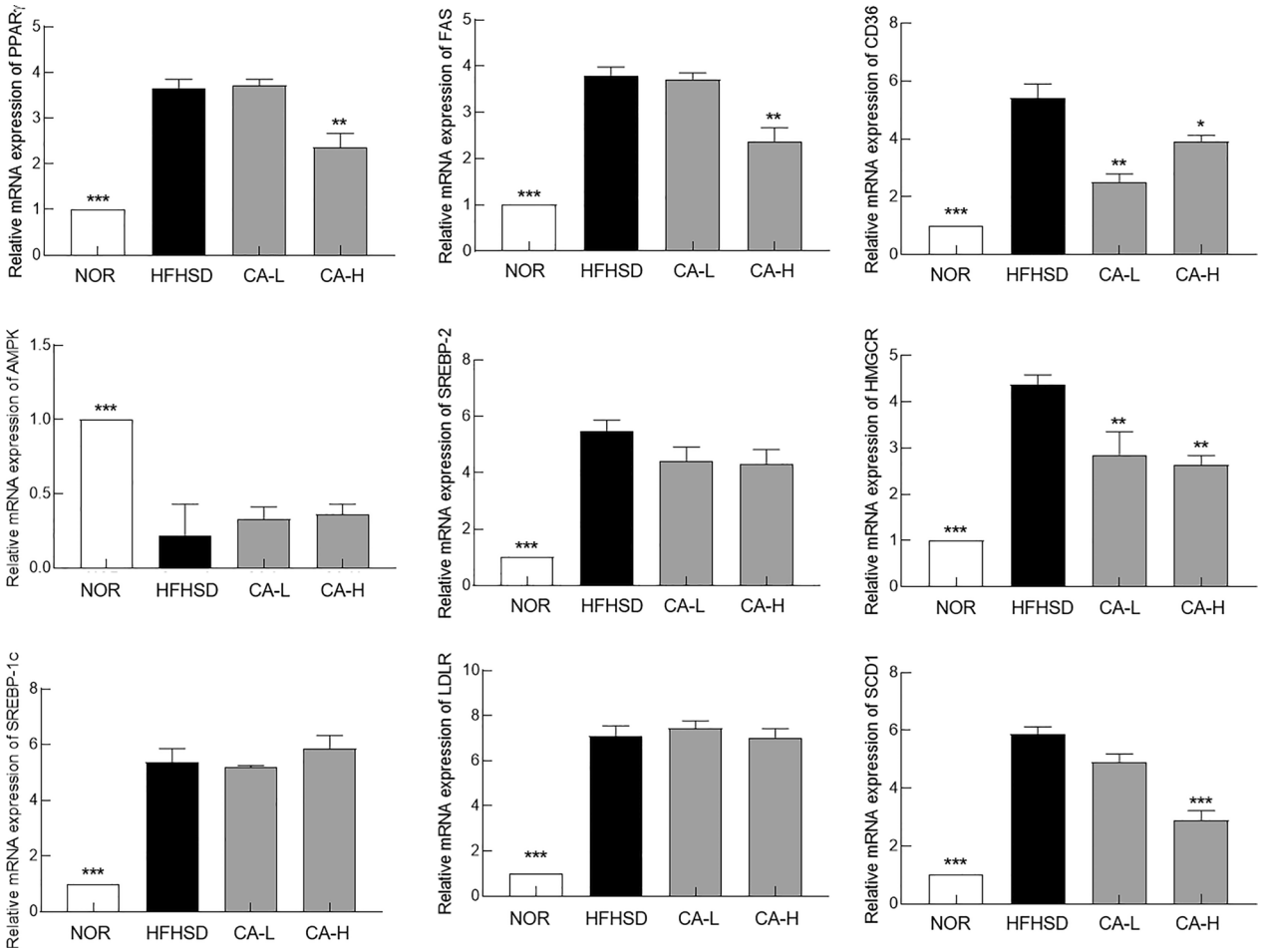

Fig. 5 Relative mRNA expression in liver tissues of mice with high-fat, high-sugar diet (HFHSD)-induced obesity that were orally treated with *Centella asiatica* (CA) for 12 weeks. Values are the means ± standard error of mean (SEM) for eight mice. The asterisk indicates a significant difference compared to HFHSD (* $p < 0.05$, *** $p < 0.001$). The mRNA levels of the target genes were normalized to the expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the results are presented as the fold changes relative to the NOR. *NOR* normal diet, *HFHSD (Control)* HFHSD, *CA-L* HFHSD+CA 300 mg/kg, *CA-H* HFHSD+CA 600 mg/kg, *PPARγ* peroxisome proliferator activated receptor gamma, *FAS* fatty acid synthase, *CD36* cluster of differentiation 36, *AMPK* 5′adenosine monophosphate activated protein kinase, *SREBP-2* sterol regulatory element-binding protein 2, *HMGCR* 3- hydroxyl-3-methylglutaryl CoA reductase, *SREBP-1C* sterol regulatory element-binding protein 1C, *LDLR* low-density lipoprotein receptor, *SCD1* stearoyl CoA desaturase 1

lipogenesis in mesenteric fat, promote epididymal lipolysis and fatty acid oxidation [33]. In the present study, CA treatment significantly attenuated HFHSD-induced increases in body weight ($p < 0.01$) and serum glucose and triacylglycerol levels ($p < 0.05$). This study confirms the results of previous studies, and our results suggest that CA can attenuate diet-induced hyperlipidemia and hyperglycemia.

Recent studies have shown that CA affects pre-adipocyte differentiation and lipid accumulation by regulating the expression of adipogenic beta-oxidation and lipolysis metabolism-related genes, which are early transcription factors involved in pre-adipocyte differentiation [33, 34]. Previous studies reported the effect of CA on pre-adipocyte differentiation and found that adipocyte accumulation decreased in a dose-dependent manner when pre-adipocytes were treated with CA [18, 35, 36].

We stained the liver tissue (Fig. 4A) and WAT (Fig. 4B) with H&E, and viewed under a microscope. Then to understand the mechanisms involved in the anti-obesity effects of CA in liver tissue and WAT, we evaluated the expression of cholesterol homeostasis- and lipid metabolism-related genes in liver tissues (Fig. 5) and the diameter and macrovesicular area of adipocyte in WAT (Fig. 4C). We confirmed that CA treatment significantly decreased WAT weight in mice with HFHSD-induced obesity ($p < 0.05$). Moreover, CA treatment markedly reduced hepatic steatosis and adipocyte hypertrophy

in these mice. Furthermore, the adipocyte diameter and macrovesicular area of eWAT decreased with CA treatment compared to those in the HFHSD ($p < 0.05$). Another important finding of our study was that CA may affect cholesterol homeostasis and lipid metabolism in mice with HFHSD-induced obesity. Although consistent results were not produced, CA 300 mg/kg treatment prevented weight gain more than CA 600 mg/kg treatment, and showed better effects on some anti-obesity indicators in liver tissue and WAT. It is thought that CA does not have an anti-obesity effect on a dose-dependent manner and, like other herbal medicines [37, 38], has an appropriate dose for anti-obesity. In this study, the expression levels of cholesterol homeostasis- and lipid metabolism-related genes in the liver revealed that CA treatment significantly attenuated the mRNA expression levels of PPARγ, FAS, CD36, HMGCR, and SCD1 in mice with HFHSD-induced obesity ($p < 0.05$). CA treatment upregulates lipid oxidation-related genes and downregulates transcription factors that regulate adipocyte differentiation. Therefore, we hypothesized that CA contributes to the inhibition of preadipocyte differentiation by regulating cholesterol homeostasis- and lipid metabolism-related genes. It is likely that the anti-obesity effects of CA treatment are due to the regulation of gene expression in the liver.

CA treatment markedly ameliorated body fat accumulation in mice with HFHSD-induced obesity and reduced their body weight gain, serum glucose, triacylglycerol, WAT weight, and adipocyte size. Furthermore, CA exerts anti-obesity effect by lowering body fat accumulation via regulating the expression of cholesterol homeostasis- and lipid metabolism-related genes in the liver. Thus, we conclude that CA has beneficial lipid-lowering capacity and may be a useful agent for preventing obesity. Additional investigations are required to determine the chemical identities of the bioactive constituents of CA (Additional file 1).

Abbreviations

AMPK	5′Adenosine monophosphate activated protein kinase
CA	*Centella asiatica*
CD36	Cluster of differentiation 36
eWAT	Epididymal white adipose tissue
FAS	Fatty acid synthase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GPX	Glutathione peroxidase
H&E	Hematoxylin and eosin
HFHSD	High-sugar diet
HMGCR	3-Hydroxyl-3-methylglutaryl CoA reductase
iWAT	Inguinal white adipose tissue
LDLR	Low-density lipoprotein receptor
mWAT	Mesenteric white adipose tissue
PPARγ	Peroxisome proliferator activated receptor gamma
SCD1	Stearoyl CoA desaturase 1
SEM	Standard error of mean
SOD	Superoxide dismutase
SREBP-1C	Sterol regulatory element-binding protein 1C
SREBP-2	Sterol regulatory element-binding protein 2
WAT	White adipose tissue

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-023-00846-7.

Additional file 1. Relative weight of organs in mice with high-fat, high-sugar diet (HFHSD)-induced obesity that were treated orally with *Centella asiatica* (CA) for 12 weeks.

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Author contributions
Conceptualization, HJS and EYJ; formal analysis, YBC and YA; Investigation, DS, SB and YHH; methodology, HJS, DS and EYJ; writing-original draft, YBC, YA, DD, SB and YHH; writing-editing. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Competing interests
The authors declare that they have no competing interests.

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