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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 (eight mice/group): NOR, normal diet; Control, HFHSD; CA-L, HFHSD+CA 300 mg/kg; CA-H, HFHSD+CA 600 mg/kg. CA treatment significantly attenuated HFHSD-induced increase in body weight gain, serum glucose, serum triacylglycerol, and WAT weight ($p < 0.05$). Compared to that in Control group, 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 group compared to the Control group ($p < 0.05$). CA exerts anti-obesity effects by lowering body fat accumulation via regulating gene expression in the liver and thus, is a potential lipid-lowering agent.

Keywords: *Centella asiatica*; body weight; obesity; adipocyte

1. 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]. Obesity is the second leading preventable cause of mortality globally, and its incidence has been increasing alarmingly among both adults and children [4]. 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 [5,6]. Alternative and complementary medicine has gained increasing attention because of the unsatisfactory results and adverse reactions of medical intervention in obesity management [7,8]. Epidemiological studies have recommended the use of plants to reduce the risk of obesity [9].

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

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traditional medicine since prehistoric times [10]. Medicinal plants can be used in alternative and complementary medicine, and the pharmacological activity of medicinal phytochemicals forms the scientific basis for their use in modern medicine. Numerous animal- and cell-based studies have been performed on medicinal plants and their active components to confirm their efficacy and mechanisms of action [11,12].

Centella asiatica (CA) has been receiving widespread attention from researchers interested in both its phytochemical and biological properties [13,14]. 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 [15]. Commonly known as Indian pennywort or mandukparni or jalbrahmi, CA has long been used in Ayurvedic medicine in India. The pharmacological effects of CA have been recorded in the historic "Sushruta Samhita," an ancient Indian medical text [16,17]. 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 [14,18-20]. CA contains pharmacological components such as triterpenoids (madecassoside, asiaticoside, madecassic acid, and asiatic acid), flavonoids (quercetin, kaempferol, catechin, rutin, apigenin, naringin, castiliferol, and castilicetin), volatile mono-and sesquiterpenes (caryophyllene, farnesol, and elemene), and other compounds (vellarine and hydrocotyline) [17]. In particular, asiaticoside and madecassoside, the most abundant pentacyclic triterpenoids, and their corresponding aglycones, asiatic acid and madecassic acid, respectively, have received substantial research attention and are recognized as the main pharmacological components of CA [21,22].

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 [17,23]. There have been several claims regarding the underlying mechanisms involved in the anti-obesity effects of CA over the past few decades [21,22]; however, more scientific data are needed to justify its ever-increasing use. Thus, in this study, we investigated its ameliorative effects *in vivo* to understand the 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, white adipose tissue (WAT) weight, histological analysis, and the expression of cholesterol homeostasis- and lipid metabolism-related genes in obese mice treated orally with CA.

2. Materials and Methods

2.1. Preparation of Centella asiatica (CA)

CA used in this study was provided by ASKBASE Co. (Jeju, 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 hours in a forced-convection oven (VS-1202D4N; Vision Bionex, Bucheon, Republic of Korea). The dried CA was pulverized using a food processor (HMF-3000S; Hanil, Seoul, Republic of Korea) and passed through a 25-mesh sieve to collect particles less than 0.70 mm.

2.2. Animals

Eight-week-old, C57BL/6J male mice were procured from Orient Bio (Seongnam, Republic of 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°C, humidity: 50–60%, and light-dark cycle: 12/12 hour). The mice were fed a commercial rodent diet (Samyang Co., Seoul, Republic of Korea) with the following composition (grams per kilogram of diet): moisture, 80; protein, 230; fat, 35; fiber, 50; and carbohydrate, 600. Food and water were provided ad libitum. After one week acclimatization, 32 mice were randomly assigned to four groups (eight mice/group) and fed with either a normal diet or high-fat high-sugar diet (HFHSD, Research Diets #D 08020201, 45 kcal% fat and 32 kcal% sucrose)

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for 12 weeks as follows: Group 1: mice fed with normal diet (NOR group), Group 2: mice fed with HFHSD (Control group), Group 3: mice fed with HFHSD along with 300 mg/kg CA extract treatment (CA-L group), Group 4: mice fed with HFHSD along with 600 mg/kg CA extract treatment (CA-H group). The composition of the experimental diets (normal diet and HFHSD) is shown in Table 1. CA (300 mg/kg or 600 mg/kg) in saline or vehicle was administered 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 hours and anatomized under diethyl ether anesthesia. Blood samples were collected from inferior vena cava. Serum was separated by centrifugation at 3,000 *g* for 15 minutes at 4°C. Serum glucose, triacylglycerol, and cholesterol levels were measured using FUJI DRI-CHEM 3500 (Fuji Photo Film, Osaka, Japan). After collecting blood, the liver, kidneys, spleen, heart, and WAT (inguinal WAT, iWAT; mesenteric WAT, mWAT; epididymal WAT, eWAT) were removed and weighed immediately. Their weights are expressed in relative terms (mg/100 g of body weight). They were then rinsed with phosphate-buffered saline, and stored at −80°C until analysis.

Table 1. Composition of normal diet (ND) and high-fat, high-sugar diet (HFHSD).

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
Choline bitartrate	0.2	0.2
Sucrose	10	45.1
Coconut oil	-	25.3
Total	100.28	109.18

2.3. Histological analysis

Liver and eWAT were fixed in 10% neutral formalin for 42 hours. The tissues were placed in cassettes, washed in phosphate-buffered saline with three changes, cleared in xylene for 30 minutes with two changes, and embedded in paraffin for 1 hour 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).

2.4. 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 were as follows (forward and reverse, respectively): glyceraldehyde-3-phosphate dehydrogenase (GAPDH), CAT CAC TGC CAC CCA GAA GAC TG and ATG CCA GTG AGC TTC CCG TTC AG; peroxisome proliferator activated receptor gamma (PPARγ), GTA CTG TCG GTT TCA GAA GTG CC and ATC TCC GCC AAC AGC TTC TCC T; fatty

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acid synthase (FAS), CTG CGA TTC TCC TGG CTG TGA A and CAA CAA CCA TAG GCG ATT TCT GG; cluster of differentiation 36 (CD36), GGA CAT TGA GAT TCT TTT CCT CTG and GCA AAG GCA TTG GCT GGA AGA AC; 5'adenosine monophosphate activated protein kinase (AMPK), GGT GTA CGG AAG GCA AAA TGG C and CAG GAT TCT TCC TTC GTA CAC GC; sterol regulatory element binding protein 2 (SREBP-2), AGA AAG AGC GGT GGA GTC CTT G and GAA CTG CTG GAG AAT GGT GAG G; 3- hydroxyl-3-methylglutaryl CoA reductase (HMGCR), GCT CGT CTA CAG AAA CTC CAC G and GCT TCA GCA GTG CTT TCT CCG T; sterol regulatory element binding protein 1C (SREBP-1C), CGA CTA CAT CCG CTT CTT GCA G and CCT CCA TAG ACA CAT CTG TGC C; low-density lipoprotein receptor (LDLR) GAA TCT ACT GGT CCG ACC TGT C and CTG TCC AGT AGA TGT TGC GGT G; stearoyl CoA desaturase 1 (SCD1), GCA AGC TCT ACA CCT GCC TCT T and CGT GCC TTG TAA GTT CTG TGG C. The PCR included the following steps: denaturing at 95°C for 5 minutes followed by 50 cycles of 95°C for 10 seconds, 60°C for 40 seconds, and 72°C for 10 seconds. Transcript concentrations were calculated as copies per µl using a standard curve. The mRNA expression was normalized to that of *GAPDH*, and the results are presented as fold-changes relative to NOR group.

2.5. Statistical analysis

Each result is expressed as mean ± standard error of mean (SEM). Statistical analyses were performed using the Statistical Package for Social Sciences (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 Control group and others were evaluated using Student's *t*-test. Results with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of CA on 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 ± 0.05 g (NOR group), 22.71 ± 0.14 g (Control group), 22.80 ±0.10 g (CA-L group), and 22.76 ± 0.21 g (CA-H group). 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 (12^{th} week, NOR group 4.32 ± 0.47 g vs Control group 20.00 ± 0.84 g, $p < 0.001$). The average daily food consumption was 2.48 ± 0.06 mg/day (NOR group), 2.72 ± 0.06 mg/day (Control group), 2.58 ± 0.01 mg/day (CA-L group), and 2.55 ± 0.04 mg/day (CA-H group) (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 group was suppressed significantly from week 10 compared to the Control group (10^{th} week, CA-L group 13.22 ± 0.73g vs Control group 16.70 ± 0.87 g, $p < 0.01$; 11^{th} week, CA-L group 15.24 ± 0.83 g vs Control group 18.98 ±0.86 g, $p < 0.01$, 12^{th} week, CA-L group 14.56 ± 0.68 g vs Control group 20.00 ± 0.84 g, $p < 0.001$).

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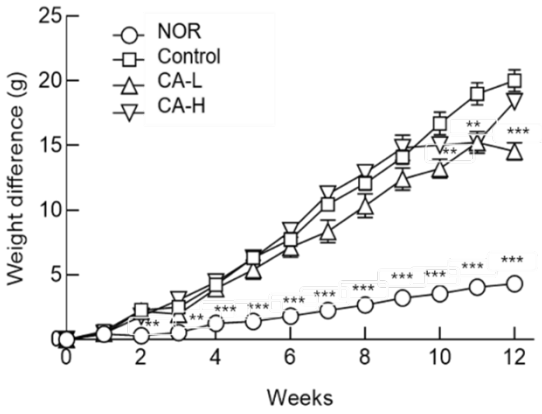

Figure 1. Body weight gains in mice with high-fat, high-sugar diet (HFHSD)-induced obesity treated orally with *Centella asiatica* (CA) for 12 weeks. Values are the means ± standard error of mean (SEM) for eight mice. The asterisk indicates a significant difference compared to Control (**p < 0.01, ***p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

3.2. Effects of CA on 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 Figure 2. HFHSD induced significant increases in serum glucose (NOR group 183.38 ± 10.55 mg/dl vs Control group 266.83 ± 15.09 mg/dl, $p < 0.001$), triacylglycerol (NOR group 92.00 ± 6.48 mg/dl vs Control group 139.20 ± 15.34 mg/dl, $p < 0.001$), and cholesterol levels (NOR group 45.38 ± 1.13 mg/dl vs Control group 86.80 ± 2.24 mg/dl, $p < 0.001$). CA treatment attenuated HFHSD-induced increase in serum glucose and triacylglycerol levels. In particular, CA-H group had significantly lower both of serum glucose (CA-H group 216.86 ± 8.16 mg/dl vs Control group 266.83 ± 15.09 mg/dl, $p < 0.05$) and triacylglycerol (CA-H group 115.20 ± 2.36 mg/dl vs Control group 139.20 ± 15.34 mg/dl, $p < 0.05$) than the Control group. However, there were no significant differences in serum cholesterol levels between the Control group and groups treated with CA.

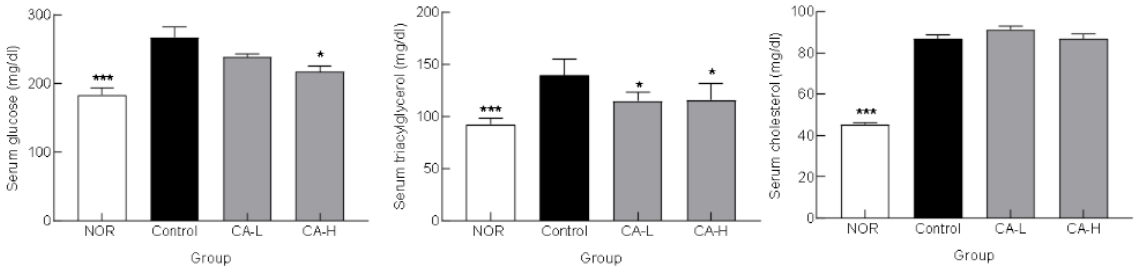

Figure 2. Serum glucose, triacylglycerol, and cholesterol in mice with high-fat, high-sugar diet (HFHSD)-induced obesity orally treated with *Centella asiatica* (CA) for 12 weeks. Values are the means ± standard error of mean (SEM) for 8 mice. The asterisk indicates a significant difference compared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

3.3. Effects of CA on relative weights of organs and WAT

Table 2 shows the relative weights of the liver, kidneys, spleen, and heart in mice with HFHSD-induced obesity that were orally treated with CA for 12 weeks. No statistically significant differences were observed in the relative weights of the liver, kidneys, and spleen between groups. However, HFHSD induced a significant decrease in relative weight of heart in relation to normal diet (NOR

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group 6.94 ± 1.13 mg/g vs Control group 4.69 ± 0.53 mg/g, $p < 0.001$). There were no significant differences in organ weights between the Control group and groups treated with CA.

Table 2. 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.

Group	Organ weight (mg/g)			
	Liver	Kidneys	Spleen	Heart
NOR	38.48±1.69	10.29±2.38	2.14±0.23	6.94±1.13***
Control	38.70±3.00	8.82±0.54	1.98±0.13	4.69±0.53
CA-L	38.79±2.79	10.12±0.37	2.07±0.24	4.77±0.86
CA-H	36.41±2.92	9.09±0.60	1.88±0.13	4.39±0.65

Values represent the means ± standard error of the mean (SEM) of eight mice. The asterisk indicates a significant difference compared to the Control group (*** $P < 0.001$). NOR, normal diet; Control, HFHSD; CA-L, HFHSD+CA 300 mg/kg; CA-H, HFHSD+CA 600 mg/kg CA.

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 group 5.74 ± 0.31 mg/g vs Control group 44.14 ± 1.95 mg/g, $p < 0.001$; mWAT, NOR group 11.89 ± 0.44 mg/g vs Control group 25.33 ± 0.88 mg/g, $p < 0.001$; eWAT, NOR group 12.93 ± 1.07 mg/g vs Control group 57.76 ± 1.19 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 group 37.30 ± 1.36 mg/g vs Control group 44.14 ± 1.95 mg/g, $p < 0.05$; CA-H group 36.36 ± 1.34 mg/g vs Control group 44.14 ± 1.95 mg/g, $p < 0.01$) and relative weight of eWAT in CA-L group was significantly lower than that in the Control group (CA-L group 48.65 ± 2.88 mg/g vs Control group 57.76 ± 1.53 mg/g, $p < 0.01$).

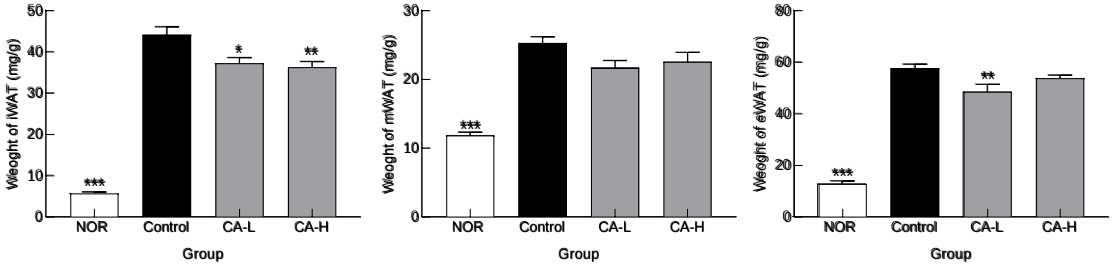

Figure 3. Relative weight of white adipose tissue (WAT) in mice with high-fat, high-sugar diet (HFHSD)-induced obesity that were orally treated with *Centella asiatica* (CA) for 12 weeks. Values are the means ± standard error of mean (SEM) for eight mice. The asterisk indicates a significant difference compared to Control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). NOR, normal diet; 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.

3.4. Effects of CA on microphotographic observations in liver and eWAT

Figure 4 shows microphotographic observations in the liver and eWAT in mice with HFHSD-induced obesity that were orally treated with CA for 12 weeks. As illustrated in Figure 4A, large macrovascular adipocytes were observed in the liver tissue of the Control group compared with those in the NOR group. 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 (Figure 4B). In the groups treated orally with CA, we observed a marked reduction in adipocyte hypertrophy compared to the Control group. The

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histological analysis of eWAT in HFHSD-induced obese mice treated orally with CA is shown in Figure 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 group 39.50 ± 2.63 μm vs Control group 104.83 ± 6.84 μm, $p < 0.001$). HFHSD also caused a significant increase in the macrovesicular area of eWAT compared to the normal diet (NOR group 100 ± 18.40% vs Control group 841.51 ± 88.70%, $p < 0.001$). CA treatment attenuated HFHSD-induced increase in adipocyte size in eWAT. In particular, CA-H group had significantly smaller adipocyte diameter in eWAT than the Control group (CA-H group 80.50 ± 6.80 um vs Control group 104.83 ± 6.84 um, $p < 0.001$). Compared to that in the Control group, macrovesicular area of eWAT was decreased following CA treatment (CA-L group 428.54 ± 53.60% vs Control group 841.51 ± 88.70%, $p < 0.05$; CA-H group 458.84 ± 53.60% vs Control group 841.51 ± 88.70%, $p < 0.05$).

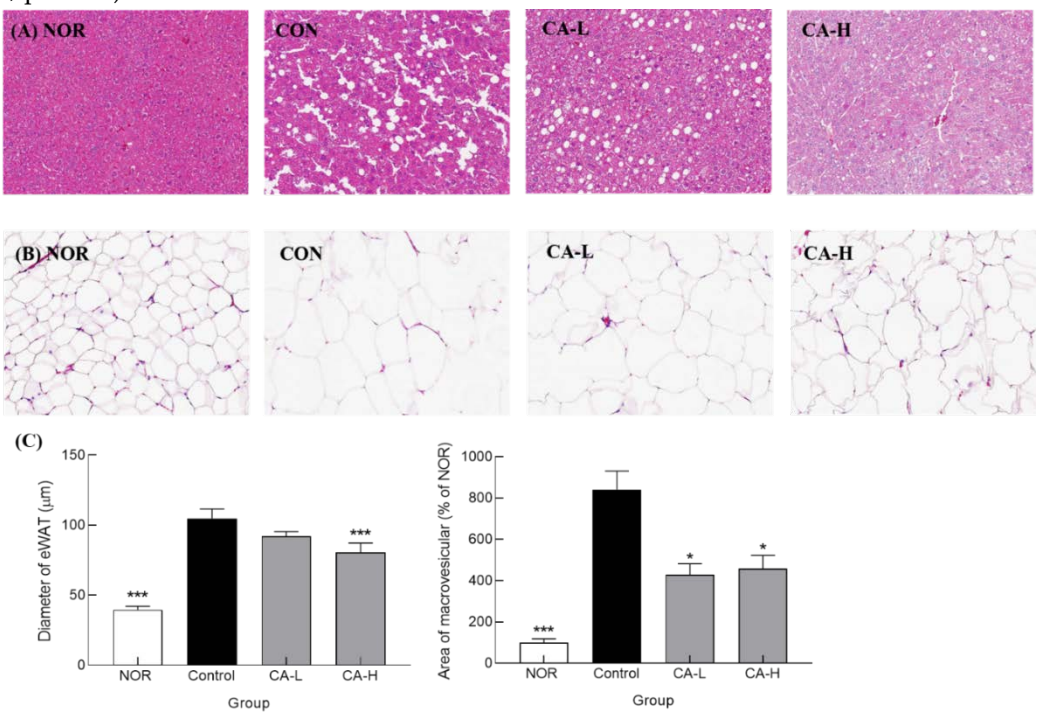

Figure 4. Microphotographic observation in mice with high-fat, high-sugar diet (HFHSD)-induced obesity that were orally treated with *Centella asiatica* (CA) for 12 weeks. The liver tissue (A) and epididymal white adipose tissue (eWAT) (B) were stained with hematoxylin and eosin (H&E), and viewed under a microscope (×200). 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 Control group (* $p < 0.05$, *** $p < 0.001$). NOR, normal diet; control, HFHSD; CA-L, HFHSD+CA 300 mg/kg; CA-H, HFHSD+CA 600 mg/kg.

3.5. Effects of CA on liver 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. Figure 5 shows the relative mRNA expression in liver tissues of mice with HFHSD-induced obesity that were orally treated with CA for 12 weeks. The mRNA expression levels of PPARγ, FAS, CD36, SREBP-2, HMGCR, SREBP-1C, 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 group, the mRNA expression levels of PPARγ, FAS, CD36, HMGCR, and SCD 1 were significantly decreased compared to those in the Control group (PPARγ, CA-H group 2.36 ± 0.90 vs Control group 3.66 ± 0.19, $p < 0.01$; FAS, CA-H group 2.36 ± 0.90 vs Control group 3.78 ± 0.20, $p < 0.01$; CD36, CA-H group 3.90 ± 0.22 vs Control

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group 5.41 ± 0.49, $p < 0.05$; HMGCR, CA-H group 2.64 ± 0.20 vs Control group 4.36 ± 2.85, $p < 0.01$; SCD1, CA-H group 2.90 ± 0.32 vs Control group 5.87 ± 0.25, $p < 0.001$). During CA treatment, SREBP-2 expression was slightly reduced in the groups treated orally with CA compared to that in the Control group, without significant differences. In this study, we did not observe any effect of CA treatment on SREBP-1C or LDLR in mice with HFHSD-induced obesity.

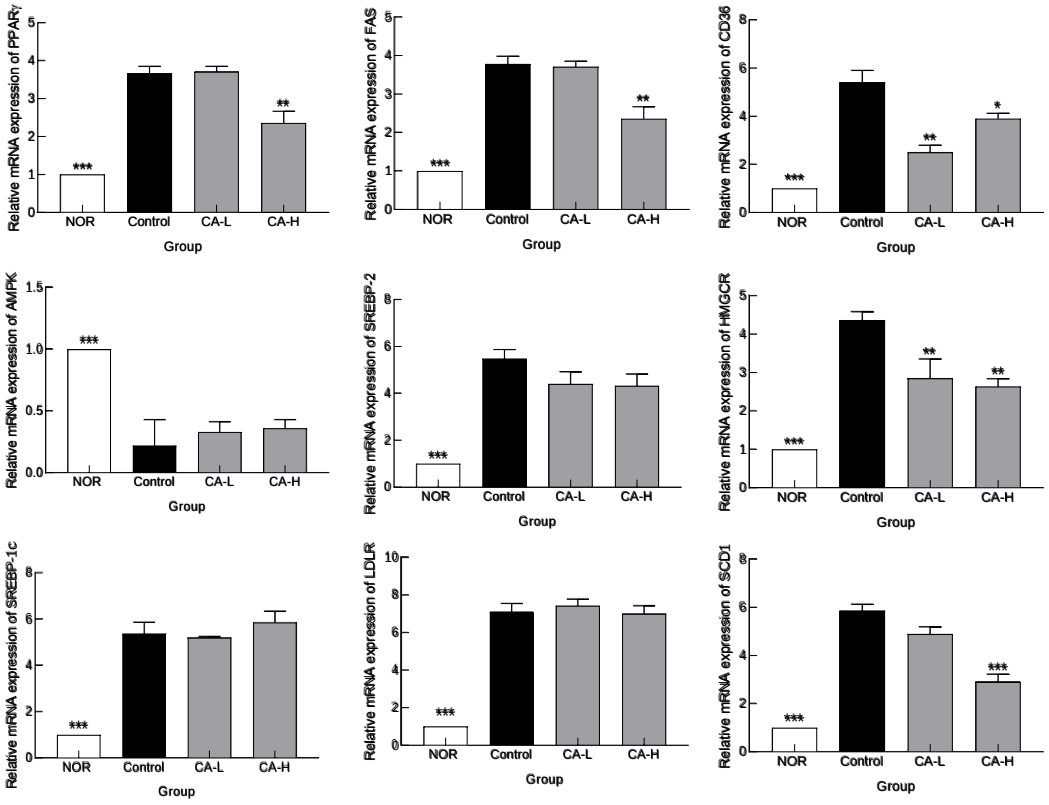

Figure 5. Relative mRNA expression in liver tissues of mice with high-fat, high-sugar diet (HFHSD)-induced obesity that were orally treated with *Centella asiatica* (CA) for 12 weeks. Values are the means ± standard error of mean (SEM) for eight mice. The asterisk indicates a significant difference compared to Control group (* $p < 0.05$, *** $p < 0.001$). 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 group. NOR, normal diet; 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.

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 group 1.00 ± 0.00 vs Control group 0.22 ± 0.21, $p < 0.001$). AMPK levels were marginally higher in the groups treated orally with CA than the Control group; however, the differences were not statistically significant.

4. Discussion

This study assessed the mechanisms underlying the effects of CA in reducing body fat through body weight, serum levels, WAT weight, histological analysis, and the expression of cholesterol

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homeostasis- and lipid metabolism-related gene in mice with HFHSD-induced obesity that were orally treated with CA for 12 weeks. The HFHSD results in an increase in body weight and serum comorbidity factors. HFHSD contains high proportions of sugar and saturated fats, which accumulate in fat pads across body and promotes excessive WAT development [24]. CA is considered useful in the treatment of obesity [25,26]. Abas et al. [27] 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 [16]. 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 [18,19]. 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 [22,23]. Therefore, it can be deduced that CA alleviates obesity-driven oxidative stress, and suppress body weight gain by promoting fatty acid oxidation [23]. 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 lipogenesis in mesenteric fat, promotes epididymal lipolysis and fatty acid oxidation [28]. In the present study, CA treatment significantly attenuated HFHSD-induced increases in body weight (Figure 1, $p < 0.01$) and serum glucose and triacylglycerol levels (Figure 2, $p < 0.05$). This study confirms the results of previous studies, and our results suggest that CA can attenuate diet-induced hyperlipidemia and hyperglycemia.

Lipid homeostasis is maintained by the balance between lipogenesis and fat burning. A major source of hepatic lipids is de novo fatty acid synthesis, in which the lipogenic transcription factor, SREBP, plays an important role [29]. PPAR-γ, a major gene regulating preadipocyte differentiation is expressed and the cell cycle is arrested again by the adipocyte induction complex [30]. Furthermore, SREBP and PPAR-γ induces the expression of SCD-1 and FAS, the enzymes involved in adipocyte differentiation and lipid synthesis, thus that intracellular lipids are accumulated and preadipocyte differentiation is completed [31]. It has been shown that reducing excessive accumulation of triacylglycerol levels, which is the cause of obesity, is useful for treating this condition and can be achieved by modulating the expression of the main factors involved in lipid precursor differentiation [32]. 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 [28,33]. 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 extract of CA [23,34,35]. We confirmed that CA treatment significantly decreased WAT weight in mice with HFHSD-induced obesity (Figure 3, $p < 0.05$). Moreover, CA treatment markedly reduced hepatic steatosis and adipocyte hypertrophy in these mice (Figure 4A and 4B). Furthermore, the adipocyte diameter and macrovesicular area of eWAT decreased with CA treatment compared to those in the Control group (Figure 4C, $p < 0.05$). Another important finding of our study was that CA may affect cholesterol homeostasis and lipid metabolism in mice with HFHSD-induced obesity. 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 (Figure 5, $p < 0.05$). 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.

5. Conclusion

CA treatment markedly ameliorated body fat accumulation in mice with HFHSD-induced obesity and reduced their body weight gain, serum glucose, serum 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. These findings demonstrate that CA has the potential to prevent obesity and related complications. 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.

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