

Regulation of Lipid Metabolism and Cholesterol Homeostasis Genes Underlies the Anti-Obesity Effects of *Centella asiatica* in Mice

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ABSTRACT

To elucidate the mechanisms underlying the anti-obesity properties of *Centella asiatica* (CA), this study evaluated body weight, serum profiles, white adipose tissue (WAT) mass, histological characteristics, and the expression of genes associated with cholesterol homeostasis and lipid metabolism. These parameters were assessed in mice with obesity induced by a high-fat, high-sugar diet (HFHSD) following oral administration of CA for 12 weeks. Male C57BL/6J mice, aged eight weeks, were divided into four experimental cohorts (8 mice/group): NOR, normal diet; HFHSD (Control), HFHSD; CA-L, HFHSD + CA 300 mg/kg; and CA-H, HFHSD + CA 600 mg/kg. A suspension of powdered CA leaves was administered via oral gavage. Treatment with CA significantly mitigated the HFHSD-induced elevations in body weight gain, serum glucose, triacylglycerol concentrations, and WAT mass ($p < 0.05$). In comparison to the HFHSD control group, both the diameter of adipocytes and the macrovesicular area within the epididymal WAT were significantly reduced following CA intervention ($p < 0.05$). Furthermore, the mRNA expression levels of peroxisome proliferator-activated receptor gamma (PPAR γ), fatty acid synthase (FAS), cluster of differentiation 36 (CD36), 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR), and stearoyl CoA desaturase 1 (SCD 1) exhibited significant downregulation in the CA-H group relative to the HFHSD group ($p < 0.05$). Therefore, CA demonstrates anti-obesity efficacy by suppressing body fat accumulation through the modulation of gene expression, highlighting its potential as a lipid-lowering therapeutic agent.

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

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Introduction

Characterized by the excessive accumulation of body fat, obesity is primarily caused by a dysregulation between energy intake and expenditure [1, 2]. This pathological condition elevates the susceptibility to a range of comorbidities, including diabetes, stroke, cardiovascular diseases, arthritis, systemic inflammation, and various malignancies [3]. While numerous pharmacological agents have been evaluated for obesity treatment since the 1930s, the majority have demonstrated only modest efficacy in weight reduction, and several have been withdrawn from the market due to adverse side effects [4, 5]. Consequently, complementary and alternative medicinal approaches have garnered growing interest due to the suboptimal outcomes and associated adverse events of conventional clinical interventions for weight management [6, 7]. Furthermore, epidemiological research has advocated for the utilization of botanical therapies to mitigate obesity risk [8].

Plants serve as natural pharmacological resources that typically present minimal to no toxic or adverse effects. They produce numerous chemical compounds designed for diverse biological functions, such as defense mechanisms against fungi, insects, and pathogens. Often referred to as medicinal herbs, these botanical sources

have been identified and utilized within traditional medicinal practices since antiquity [9]. A multitude of *in vitro* and *in vivo* studies have investigated these medicinal plants and their bioactive constituents to validate their therapeutic efficacy and elucidate their underlying mechanisms of action [10, 11].

Centella asiatica (CA) has attracted substantial scientific interest regarding both its phytochemical composition and biological characteristics [12, 13]. As a perennial herbaceous plant within the Apiaceae family, CA is an important medicinal botanical cultivated predominantly in the wetland regions of India, China, Malaysia, Sri Lanka, Indonesia, Madagascar, and Korea. The Indian pharmacopoeia documents CA as having diverse therapeutic benefits for conditions including psoriasis, diarrhea, fever, varicose ulcers, eczema, leprosy, lupus, and amenorrhea. Contemporary investigations have highlighted numerous biological properties of CA, attributing its efficacy to a variety of antioxidant, anti-inflammatory, antimicrobial, neuroprotective, cognitive-enhancing, and antidepressant constituents [13-16]. Additionally, recent literature indicates that CA is efficacious in addressing endocrine disorders, notably obesity, by enhancing specific metabolic pathways and exerting adiposuppressive effects [17, 18]. Over recent decades, multiple hypotheses have been proposed regarding the specific mechanisms driving the anti-obesity effects of CA [19, 20]; nevertheless, further empirical data are required to substantiate its expanding clinical application.

In an obesogenic state, the primary mechanisms governing lipid metabolism are regulated through insulin signaling, adipogenesis, adipocyte differentiation, lipolysis, and the β -oxidation of free fatty acids (FFAs). Lipolysis involves the catabolism of triglycerides into FFAs and glycerol. This process is facilitated by lipases, which serve as direct transcriptional targets for peroxisome proliferator-activated receptor gamma (PPAR γ) [21]. The surplus FFAs generated via lipolysis can enter systemic circulation and accumulate within the bloodstream or hepatic tissue, subsequently driving the upregulation of proteins essential for *de novo* lipogenesis, including sterol regulatory element-binding protein (SREBP) and fatty acid synthase (FAS) [22]. Conversely, cellular lipid accumulation can be diminished through the upregulation of genes responsible for FFA β -oxidation within hepatic and adipose tissues [23]. Consequently, the modulation of these molecular factors represents an optimal therapeutic approach for the prevention or mitigation of complications associated with obesity. Moreover, the application of natural compounds as therapeutic modalities for the treatment and prophylaxis of obesity has garnered widespread popularity [24].

The activation of 5'-adenosine monophosphate-activated protein kinase (AMPK) initiates advantageous physiological responses, notably the reduction of adipose deposition. Conversely, the knockdown of AMPK leads to the upregulation of FAS and SREBP, both of which are critical enzymes mediating lipid synthesis [25]. However, the specific mechanistic impact of CA on adipogenesis and lipolysis within the white adipose tissue (WAT) of obese murine models remains largely unexplored. Therefore, the present *in vivo* study was designed to investigate the ameliorative properties of CA to elucidate the underlying mechanisms responsible for its anti-obesity effects, thereby offering scientific substantiation for the use of CA as a prophylactic supplement against obesity. Specifically, this investigation evaluated body weight, serum biochemical profiles, WAT mass, histological changes, and the gene expression related to lipid metabolism and cholesterol homeostasis in obese mice subjected to oral CA administration.

Materials and Methods

Preparation of Centella asiatica

ASKBASE Co. (Seoul, Republic of Korea) supplied the foliage of CA, which had been harvested from Jeju (Republic of Korea) at the onset of summer. Prior to harvesting, these plants underwent a 45-day growth period within a controlled, chemical-free greenhouse environment. Once gathered, the raw botanical material underwent immediate dehydration for 24 h using a forced-convection oven set to 60 °C (VS-1202D4N; Vision Bionex, Bucheon, Korea). A commercial food processor was utilized to mill the dried leaves, and the resulting powder was filtered using a 25-mesh screen to ensure all collected particles were smaller than 0.70 mm. Appropriate masses of this refined powder were then dispersed into distilled water. To ensure a homogenous mixture prior to oral administration, the aqueous preparation underwent a 30 min sonication process within a 37 °C water bath.

Animals

Male C57BL/6J mice (eight weeks of age) were supplied by Orient Bio located in Seongnam, Korea. Ethical clearance for all handling and termination procedures was granted by the Animal Care and Use Committee at

Korea University under protocol number KUIACUC-2021-0098. Following standard institutional welfare protocols, each animal was isolated in a stainless steel enclosure. The housing facility operated under stringently controlled atmospheric variables, specifically a 12/12 h light-dark cycle, ambient temperature maintained at 24 ± 1 °C, and relative humidity kept between 50–60%. Following an initial 7-day adaptation phase, the 32 rodents were divided into four equal cohorts (8 mice/group) via random selection. Over the subsequent 12 weeks, the subjects consumed either a standard basal diet or an obesogenic high-fat, high-sugar diet (HFHSD, formulated with 45 kcal% fat and 32 kcal% sucrose). The experimental strata were defined as: a control group on the standard diet (Group 1, NOR); an untreated disease model on the HFHSD (Group 2, HFHSD); a low-dose intervention group receiving the HFHSD plus 300 mg/kg of the CA suspension (Group 3, CA-L); and a high-dose intervention group receiving the HFHSD plus 600 mg/kg of the CA suspension (Group 4, CA-H). Detailed nutritional breakdowns for both the standard and HFHSD regimens are provided in **Table 1**. Selection of the specific therapeutic doses (300 mg/kg and 600 mg/kg) was guided by established literature verifying both the toxicological safety and functional efficacy of these concentrations [17, 26, 27]. The daily treatment regimen consisted of 200 µl of the designated liquid mixture delivered directly into the stomach of each mouse using a ball-tipped oral gavage needle.

Table 1. Nutritional makeup of the baseline normal diet (ND) compared with the obesogenic high-fat, high-sugar formulation (HFHSD, comprising 45 kcal% fat and 32 kcal% sucrose)

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

Both dietary intake and mass fluctuations for all animals were documented on a weekly schedule. Upon reaching the 12-week endpoint, the subjects underwent a 12 h fasting window before being humanely euthanized via carbon dioxide inhalation. Venous blood was drawn directly from the inferior vena cava. To isolate the serum fraction, these blood samples were spun in a centrifuge at 3000g for 15 min under refrigerated conditions (4 °C). A FUJI DRI-CHEM 3500 clinical chemistry analyzer (Fuji Photo Film, Osaka, Japan) was utilized to quantify systemic concentrations of total cholesterol, triacylglycerols, and glucose. Following exsanguination, discrete white adipose tissue depots—specifically the epididymal (eWAT), mesenteric (mWAT), and inguinal (iWAT) compartments—were rapidly excised and their masses recorded. These fat pads were subsequently cleansed in phosphate-buffered saline and cryopreserved at -80 °C pending downstream evaluations.

Histological analysis

Specimens of eWAT and hepatic tissue underwent a 42 h fixation process submerged in a 10% neutral-buffered formalin solution. After fixation, the biomaterials were transferred into histological cassettes and subjected to a triple-wash sequence using phosphate-buffered saline. The clearing phase involved immersing the tissues in xylene twice for 30 min intervals, which was followed by three sequential 1 h paraffin embedding steps. Once firmly set in paraffin blocks, the specimens were sectioned into slices with a thickness of 5 µm. Traditional hematoxylin and eosin (H&E) staining was applied to these tissue slices, allowing for cellular observation via

optical microscopy (Leica, Wetzlar, Germany). Representative images of the cellular architecture were captured using a 200× magnification objective. To quantitatively assess adipocyte dimensions and the extent of macrovesicular vacuolation, the digitized micrographs were analyzed employing ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Quantitative real-time polymerase chain reaction (PCR)

Hepatic total RNA was isolated by deploying Trizol reagent (Gibco-BRL, Grand Island, NY, USA) in strict accordance with the protocols supplied by the vendor. The resulting RNA yield then served as a template for cDNA synthesis, catalyzed by Moloney murine leukemia virus reverse transcriptase. To quantify the transcription rates of specific target genes, this newly generated cDNA was analyzed on an Excyler 96 Real-Time Quantitative Thermal Block system (Bioneer) powered by the AccuPower GreenStar qPCR PreMix (Bioneer, Daejeon, Korea). A comprehensive inventory of the oligonucleotide primers deployed during these assays is documented in **Table 2**. The amplification protocol commenced with an initial 5 min denaturation hold at 95 °C. This was succeeded by 50 amplification cycles comprising a 10 s denaturation at 95 °C, a 40 s annealing phase at 60 °C, and a 10 s extension step at 72 °C. Absolute transcript abundance (expressed as copies per µl) was extrapolated from a predetermined standard curve. To account for variances in input, target gene expression was standardized against the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All final expression data are delineated as fold-difference magnitudes relative to the baseline NOR cohort.

Table 2. Inventory of oligonucleotide primers utilized for the real-time PCR assays

Gene	Primer sequence (5'-3')	
	Forward	Reverse
GAPDH	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG
PPARγ	GTAAGTGTCTGGTTCAGAAAGTGCC	ATCTCCGCCAACAGCTTCTCTCT
FAS	CTGCGATTCTCTGGCTGTGAA	CAACAACCATAGGCGATTCTCTGG
CD36	GGACATTGAGATTCTTTTCTCTCTG	GCAAAGGCATTGGCTGGAAGAAC
AMPK	GGTGTACGGAAGGCCAAAATGGC	CAGGATTCTTCTTCGTACACGC
SREBP-2	AGAAAGAGCGGTGGAGTCTTGTG	GAACTGCTGGAGAATGGTGAGG
HMGCR	GCTCGTCTACAGAACTCCACG	GCTTCAGCAGTGCTTTCTCCGT
SREBP-1 C	CGACTACATCCGCTTCTTGACAG	CCTCCATAGACACATCTGTGCC
LDLR	GAATCTACTGGTCCGACCTGTC	CTGTCCAGTAGATGTTGCGGTG
SCD1	GCAAGCTCTACACCTGCCTCTT	CGTGCCTTGTAAGTTCTGTGGC

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PPAR γ , 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

Statistical analyses

All quantitative findings are reported as the central mean alongside the standard error of the mean (SEM). For the computational evaluation of the data, SPSS analytical software (version 19.0; International Business Machines, Armonk, NY, USA) was employed. To detect significant variance across multiple cohorts simultaneously, a one-way analysis of variance (ANOVA) was implemented, seamlessly coupled with Tukey's post-hoc diagnostic for paired group comparisons. When specifically comparing the HFHSD control directly against individual experimental parameters, an independent Student's t-test was utilized. Analytical outcomes yielding a p-value strictly below 0.05 were classified as statistically significant.

Results and Discussion

Dynamics of body mass and feed intake

Throughout the 12-week regimen, no adverse clinical indicators—such as mortality, diarrhea, erratic locomotion, or piloerection—were detected during the administration of *Centella asiatica*. The impact of CA on weight gain in a murine model of diet-induced obesity is depicted in **Figure 1**. Initial body weights were established at 22.70 g (NOR), 22.71 g (HFHSD), 22.80 g (CA-L), and 22.76 g (CA-H). The high-fat, high-sugar diet fundamentally altered weight gain trajectories, resulting in consistently higher mass accretion in the HFHSD group compared to the standard diet cohort throughout the experimental duration (12th week: NOR 4.32 g vs. HFHSD 20.00 g, $p <$

0.001). Daily feed consumption was recorded as 2.48 g (NOR), 2.72 g (HFHSD), 2.58 g (CA-L), and 2.55 g (CA-H) (data omitted). Total feed ingestion remained statistically uniform across all groups. CA intervention exhibited a clear trend toward dampening the weight gain induced by the HFHSD. Notably, despite similar levels of food intake, the low-dose CA group (CA-L) experienced a statistically significant suppression in weight gain starting at week 10 compared to the HFHSD group (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, $p < 0.001$).

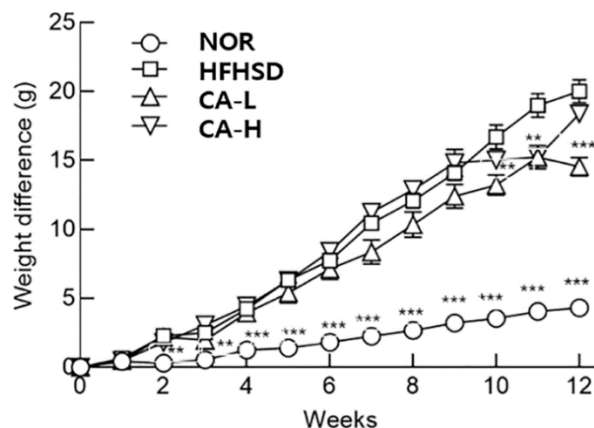


Figure 1. Weight gain profiles in mice subjected to a high-fat, high-sugar diet (HFHSD) and oral *Centella asiatica* therapy for 12 weeks. Data are expressed as mean $\pm$ standard error of the mean (SEM) ($n = 8$ per group). Asterisks denote statistical differences relative to the HFHSD group ($p < 0.01$, $*p < 0.001$). NOR, normal diet; HFHSD (Control), HFHSD; CA-L, HFHSD + CA 300 mg/kg; CA-H, HFHSD + CA 600 mg/kg.

Biochemical serum profiles

Figure 2 illustrates the modifications in circulating glucose, triacylglycerol, and total cholesterol levels following CA intervention in the HFHSD-induced obese mice. The HFHSD protocol resulted in marked elevations 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 total cholesterol (NOR 45.38 mg/dl vs. HFHSD 86.80 mg/dl, $p < 0.001$). CA administration effectively mitigated the HFHSD-driven increases in both serum glucose and triacylglycerol. Specifically, the high-dose CA cohort (CA-H) displayed significantly lower concentrations 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, $p < 0.05$) relative to the untreated HFHSD control. No significant variations in total serum cholesterol were observed between the HFHSD control and the CA-treated groups.

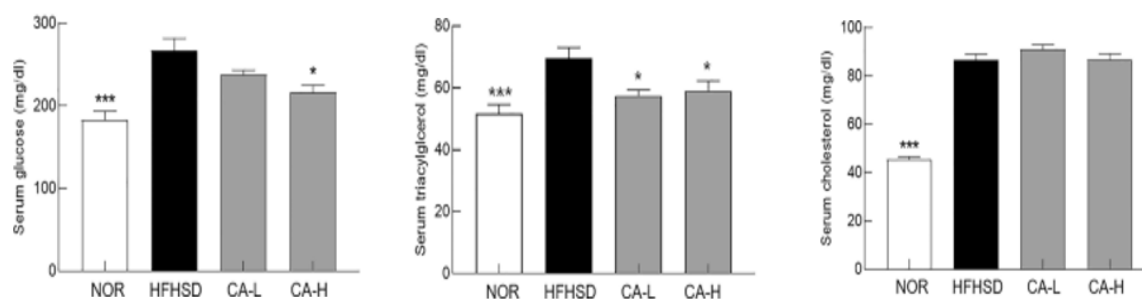


Figure 2. Serum concentrations of glucose, triacylglycerol, and cholesterol in HFHSD-induced obese mice treated with *Centella asiatica* for 12 weeks. Values reflect the mean $\pm$ SEM ($n = 8$). Asterisks indicate significant divergence from the HFHSD control ($p < 0.05$, $*p < 0.001$). NOR, normal diet; HFHSD (Control), HFHSD; CA-L, HFHSD + CA 300 mg/kg; CA-H, HFHSD + CA 600 mg/kg.

White adipose tissue (WAT) distribution

To assess how CA intervention influences fat deposition, the relative masses of iWAT, mWAT, and eWAT were evaluated (**Figure 3**). Consistent with expected physiological outcomes, all assessed WAT depot weights were substantially lower in the normal diet cohort than in the HFHSD group (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, $p < 0.001$). While mWAT mass remained largely unaffected by CA treatment, a trend toward volume reduction in iWAT and eWAT was observed. CA administration significantly decreased iWAT relative weight in the obese mice (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, $p < 0.01$). Additionally, the CA-L group exhibited a significant reduction in eWAT relative weight compared to the HFHSD group (CA-L 48.65 mg/g vs. HFHSD 57.76 mg/g, $p < 0.01$).

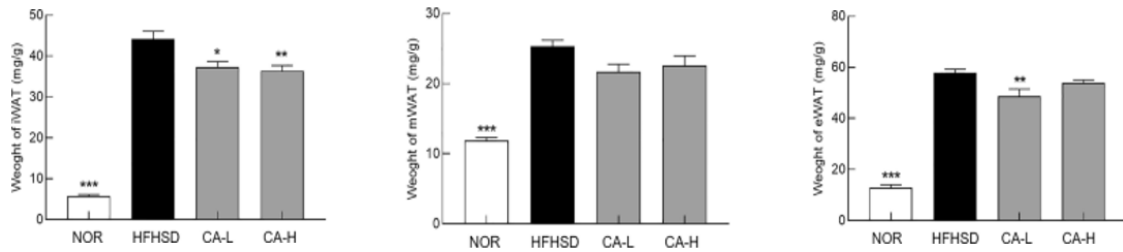


Figure 3. Relative weights of white adipose tissue (WAT) depots in HFHSD-induced obese mice treated with *Centella asiatica* for 12 weeks. Data are presented as mean $\pm$ SEM ($n = 8$). Asterisks denote statistical significance versus the HFHSD group ($p < 0.05$, $p < 0.01$, $*p < 0.001$). NOR, normal 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.

Histopathological evaluation

As shown in **Figure 4a**, liver tissue from the HFHSD group displayed large macrovascular adipocytes in contrast to the normal-diet control, though CA administration visibly ameliorated this hepatic steatosis. Adipocyte hypertrophy in eWAT was similarly pronounced in the HFHSD-fed animals (**Figure 4b**). CA intervention successfully curtailed this hypertrophy compared to the untreated HFHSD state (**Figure 4c**). A marked, statistically significant enlargement in eWAT adipocyte diameter occurred in the HFHSD group (NOR 39.50 μm vs. HFHSD 104.83 μm , $p < 0.001$), alongside a significant expansion in the macrovesicular area (NOR 100% vs. HFHSD 841.51%, $p < 0.001$). CA treatment mitigated these increases; specifically, the CA-H group showed a significantly lower eWAT adipocyte diameter (CA-H 80.50 μm vs. HFHSD 104.83 μm , $p < 0.001$). Furthermore, the macrovesicular eWAT area was significantly diminished in both treated groups relative to the HFHSD control (CA-L 428.54% vs. HFHSD 841.51%, $p < 0.05$; CA-H 458.84% vs. HFHSD 841.51%, $p < 0.05$).

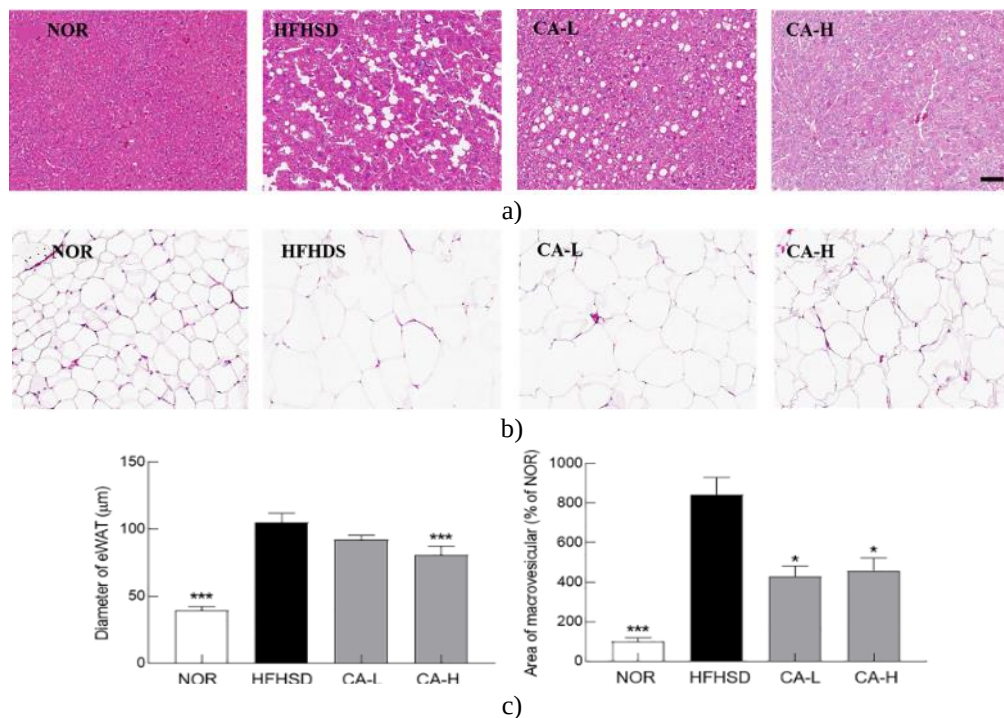


Figure 4. Microscopic assessment of liver and epididymal white adipose tissue (eWAT) in HFHSD-induced obese mice after 12 weeks of CA treatment. (a) Hepatic and (b) eWAT tissues were stained with H&E and

visualized at $\times 200$ magnification (scale bar = 100 μm). (c) Morphometric quantification of adipocyte diameter and macrovesicular area in eWAT using ImageJ. Values represent mean $\pm$ SEM (n = 8). Asterisks indicate significance relative to the HFHSD group ($p < 0.05$, $*p < 0.001$). NOR, normal diet; HFHSD (Control), HFHSD; CA-L, HFHSD + CA 300 mg/kg; CA-H, HFHSD + CA 600 mg/kg.

Analysis of mRNA expression profiles

To determine how *Centella asiatica* influences genes linked to lipid metabolism and cholesterol homeostasis, we assessed hepatic mRNA expression via quantitative real-time PCR (**Figure 5**). Mice suffering from obesity induced by a high-fat, high-sugar diet exhibited markedly elevated mRNA levels of PPAR γ , FAS, CD36, SREBP-2, HMGCR, SREBP-1 C, LDLR, and SCD1 relative to the normal diet control group ($p < 0.001$). Oral administration of CA to the HFHSD-induced obese mice led to a dose-dependent downregulation in the mRNA expression of PPAR γ , FAS, CD36, HMGCR, and SCD1. Specifically, the high-dose CA (CA-H) cohort demonstrated a significant reduction in these transcripts compared to the untreated HFHSD group (PPAR γ , 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, $p < 0.001$). While oral CA therapy resulted in a marginal, non-significant decline in SREBP-2 expression relative to the HFHSD control, it had no observable impact on the expression of SREBP-1 C or LDLR in the obese mice.

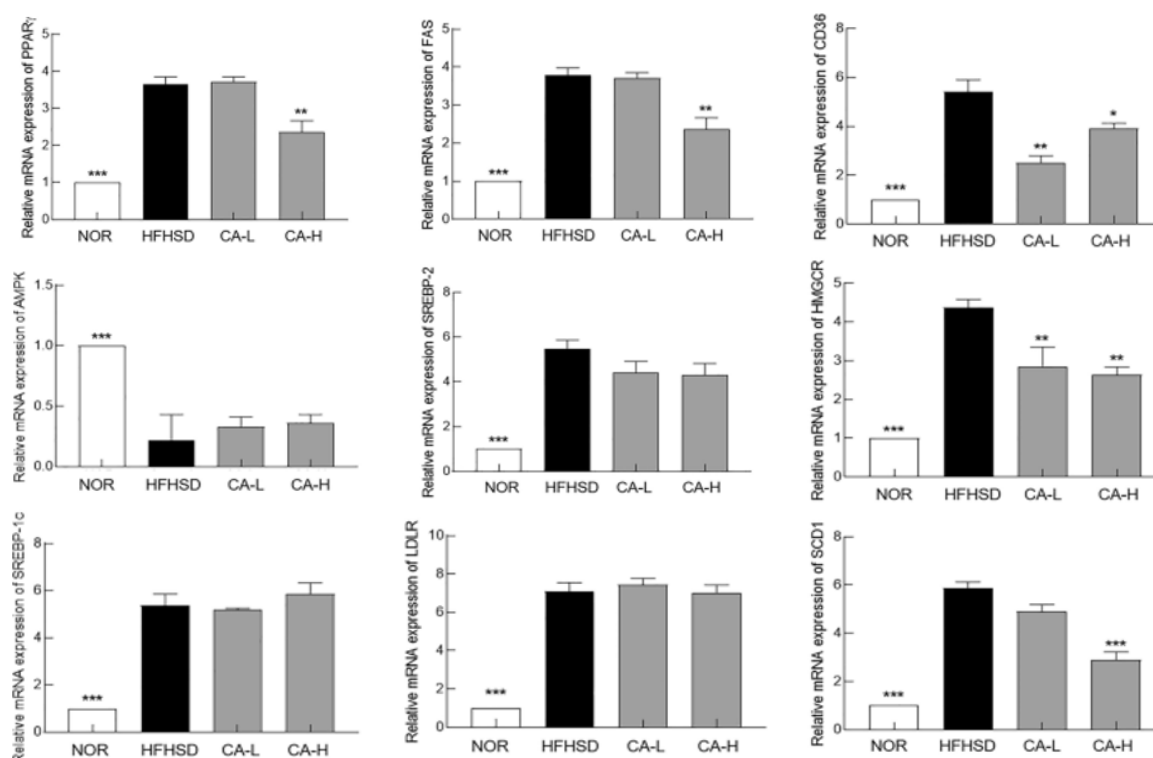


Figure 5. Comparative mRNA expression profiles in the hepatic tissues of HFHSD-induced obese mice following a 12-week oral administration of *Centella asiatica*. Data are expressed as the mean $\pm$ standard error of the mean (SEM) for eight mice per group. Asterisks denote statistical significance relative to the HFHSD control group (* $p < 0.05$, *** $p < 0.001$). Target gene expression was normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH), with findings displayed as fold alterations compared to the NOR group.

Abbreviations: NOR, normal diet; HFHSD (Control), high-fat high-sugar diet; CA-L, HFHSD+CA 300 mg/kg; CA-H, HFHSD+CA 600 mg/kg; PPAR γ , 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.

Furthermore, we assessed the activity of AMPK, a critical regulator of cellular energy balance that stimulates fatty acid transport and oxidation during energy depletion. The AMPK concentration was substantially diminished in

the HFHSD-induced obese mice relative to those consuming a standard diet (NOR 1.00 vs. HFHSD 0.22, $p < 0.001$). Although the cohorts receiving oral CA demonstrated slightly elevated AMPK levels compared to the untreated HFHSD group, these variations did not achieve statistical significance.

A high-fat, high-sugar diet is rich in saturated lipids and carbohydrates, which leads to systemic fat deposition and stimulates disproportionate white adipose tissue expansion [28]. Consequently, this dietary pattern drives substantial weight gain and elevates serum risk markers associated with metabolic comorbidities. Previous literature recognizes CA as a beneficial therapeutic option for obesity management [29, 30]. Research by Abas *et al.* [31] demonstrated that chronic CA administration in an obese rat model effectively normalized plasma lipid and glucose profiles, while also correcting disruptions in amino acid metabolism and the tricarboxylic acid cycle. Based on their biochemical evaluations, they concluded that CA possesses pronounced anti-obesity properties through the modulation of targeted metabolic cascades. Additional evidence indicates that CA can curtail weight gain, decrease circulating glucose, and mitigate oxidative damage [32]. Its efficacy as an anti-obesity intervention is further supported by its capacity to lower lipid concentrations while simultaneously improving insulin and leptin sensitivity. At the molecular level, CA enhances the availability of antioxidant enzymes—including catalase, glutathione peroxidase (GPX), and superoxide dismutase (SOD)—and normalizes the expression profiles of uncoupling protein-2 and palmitoyltransferase-1. In corresponding animal models, the application of CA boosted catalase, glutathione (GSH), and SOD activity, thereby fortifying the endogenous enzymatic antioxidant defense network [18, 20]. This supports the conclusion that CA mitigates oxidative stress associated with obesity and limits weight gain through the enhancement of fatty acid oxidation [18]. The underlying mechanism for CA's weight-reducing benefits is likely attributed to madecassic acid, which has been shown to lower triacylglycerol concentrations, inhibit lipogenesis in mesenteric adipose depots, and stimulate both fatty acid oxidation and lipolysis in epididymal fat [33]. Within our current investigation, the administration of CA markedly restricted HFHSD-driven weight gain ($p < 0.01$) and suppressed elevations in serum triacylglycerol and glucose concentrations ($p < 0.05$). These findings align with earlier research, reinforcing the premise that CA is capable of mitigating diet-induced hyperglycemia and hyperlipidemia.

Contemporary research highlights that CA influences lipid deposition and pre-adipocyte maturation by modulating the expression of genes linked to lipolysis and adipogenic beta-oxidation, which serve as foundational transcription factors in pre-adipocyte development [33, 34]. Earlier experiments examining CA's impact on this differentiation process demonstrated that treating pre-adipocytes with CA led to a dose-dependent reduction in adipocyte accumulation [18, 35, 36].

Hepatic samples (**Figure 4a**) and WAT sections (**Figure 4b**) underwent hematoxylin and eosin staining for subsequent microscopic evaluation. To elucidate the mechanisms driving CA's anti-obesity effects in these tissues, we measured the transcript levels of genes regulating lipid metabolism and cholesterol homeostasis in the liver (**Figure 5**), alongside analyzing adipocyte diameter and macrovesicular area within the WAT (**Figure 4c**). Our data confirmed that administering CA significantly diminished WAT mass in the HFHSD-fed obese model ($p < 0.05$). Additionally, this treatment visibly alleviated adipocyte hypertrophy and hepatic steatosis. Compared to the untreated HFHSD controls, CA-treated mice exhibited a notable reduction in both the macrovesicular area and the diameter of epididymal WAT (eWAT) adipocytes ($p < 0.05$). A further critical observation from our analysis is CA's potential to modulate lipid metabolism and cholesterol balance in diet-induced obesity. While dose-response outcomes were somewhat variable, the 300 mg/kg CA dosage proved more effective at curtailing weight gain than the 600 mg/kg dosage, while also yielding superior improvements across specific obesity-related biomarkers in the WAT and liver. This suggests that CA's efficacy against obesity does not strictly follow a linear dose-dependent trajectory; rather, analogous to certain other botanical therapies [37, 38], it possesses an optimal therapeutic window. In the present study, our hepatic gene expression profiling indicated that CA administration significantly suppressed the mRNA levels of PPAR γ , FAS, CD36, HMGCR, and SCD1 in the obese mice ($p < 0.05$). By downregulating the transcription factors responsible for adipocyte maturation and simultaneously upregulating genes associated with lipid oxidation, CA appears to impede pre-adipocyte differentiation via the control of lipid- and cholesterol-regulating genetic pathways. Consequently, it is highly probable that CA's anti-obesity attributes stem primarily from its modulation of hepatic gene expression.

Conclusion

Ultimately, the application of CA substantially mitigated fat deposition in the HFHSD-induced obesity model, simultaneously restricting increases in overall body weight, serum glucose, triacylglycerol, WAT mass, and adipocyte dimensions. Furthermore, CA limits systemic adiposity by fine-tuning the hepatic expression of genes integral to lipid metabolism and cholesterol homeostasis. We therefore conclude that CA possesses significant lipid-reducing properties, rendering it a promising candidate for obesity prevention. Future research is warranted to definitively characterize the specific bioactive compounds present within CA.

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Conflict of Interest: None

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Ethics Statement: None

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