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Cissus quadrangularis extract (CQR-300) inhibits lipid accumulation by downregulating adipogenesis and lipogenesis in 3T3-L1 cells

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ABSTRACT

The objective of this study was to evaluate the anti-obesity activity and the action mechanism of *Cissus quadrangularis* extracts (CQR-300) in 3T3-L1 adipocytes. *Cissus quadrangularis* was extracted with hot water, resulting in CQR-300. The anti-obesity activity of CQR-300 in 3T3-L1 adipocytes was examined by Oil-red O staining. Possible mechanisms of CQR-300 in 3T3-L1 adipocytes were determined by real-time PCR and western blot. Treatment with CQR-300 inhibited lipid accumulation without showing cytotoxicity to 3T3-L1 adipocytes. Furthermore, CQR-300 decreased adipogenesis/lipogenesis-related mRNA expression levels of fatty acid binding protein (aP2), fatty acid synthase (FAS), lipoprotein lipase (LPL), stearoyl-CoA desaturase-1 (SCD-1), and acetyl-CoA carboxylase (ACC). CQR-300 also down-regulated expression levels of adipogenesis/lipogenesis-associated proteins, including peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein α (C/EBPα), sterol regulatory element binding protein-1c (SREBP-1c), and FAS. It's also up-regulated the expression level of phosphorylated-AMPK (p-AMPK). Collectively, these results suggested that CQR-300 might have an anti-obesity effect by its ability to decrease expression levels of adipogenesis/lipogenesis-related genes and proteins.

1. Introduction

Obesity is a serious world-wide health problem. Its occurrence has been increasing in both developed and developing countries [1]. It has been estimated that worldwide overweight and obese population is about 6 billion. This figure is expected to increase to over 10 billion by 2025 [2]. Obesity is a multi-factorial disease. Diet with high calories, genetics, lifestyle, culture, and environment all play important roles in the development of obesity [3]. It can lead to metabolic syndromes such as cardiovascular disease, dyslipidemia, atherosclerosis, stroke, hypertension, dyslipidemia, type II diabetes mellitus, and various types of cancer [1,4,3]. Recently, people have recognized the need to prevent obesity and manage weight [4]. Obesity is characterized by increased adipose tissue mass associated with increased fat cell number and size [5]. Adipocytes are important cellular components of fatty tissues. Excessive amounts of lipid (triglycerides) can accumulate in adipose tissues accompanied by increased expression levels of adipogenesis/lipogenesis and enhanced the body weight gain [6]. Obesity and its related diseases are closely related to adipocyte differentiation and fat accumulation [7]. 3T3-L1 cells are widely used in anti-obesity studies to investigate key molecular markers of adipocyte differentiation [4,8].

Cissus quadrangularis Linn has been used as a common medicinal plant in Africa and Asia for more than a century [9]. Its stem and leaf have been used in food preparation and raw drug in India for treating various diseases [10]. Several reports have demonstrated the anti-obesity effect of *C. quadrangularis* in animals and humans with lipase, amylase, and α-glucosidase inhibition activities [9,11,12].

However, the mechanisms involved in the effect of *C. quadrangularis* on adipocytes related to adipogenesis and lipogenesis have not been reported yet. Therefore, the objective of the present study was to examine the effect of *C. quadrangularis* extract (CQR-300) on adipocytes differentiation and lipid accumulation and its regulatory mechanisms in 3T3-L1 adipocytes.

Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; aP2, fatty acid binding protein (aP2); BCS, bovine calf serum; C/EBPα, CCAAT/enhancer-binding protein α; CQR-300, *Cissus quadrangularis* extract; DMEM, Dulbecco's modified Eagle's medium; FAS, fatty acid synthase; FAS-α, fatty-acid synthase; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LPL, lipoprotein lipase; MDI, medium dependent interface; MTT, 3-(4, 5-dimetylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; ORO, Oil-red O; p-AMPK, phosphorylated-AMPK; PPARγ, peroxisome proliferator-activated receptor γ; RIPD, radioimmunoprecipitation assay buffer; SCD-1, stearoyl-CoA desaturase-1; SREBP-1c, sterol regulatory element binding protein-1c; TG, triglycerides

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Table 1
Sequences and condition of primers used in real-time quantitative PCR.

Gene	Primer (5′ → 3′)
aP2	F: 5′-CCAATGAGCAAGTGGCAAGA-3′ R: 5′-GATGCCAGGCTCCAGGATAG-3′
FAS	F: 5′-TGGTGGGTTTGGTGAATTGTC-3′ R: 5′-GCTTGTCCTGCTCTAACTGGAAGT-3′
LPL	F: 5′-GGCCAGATTCATCAACTGGAT-3′ R: 5′-GCTCCAAGGCTGTACCCTAAG-3′
ACC	F: 5′-GCGTCGGGTAGATCCAGTT-3′ R: 5′-CTCAGTGGGGCTTAGCTCTG-3′
SCD-1	F: 5′-CATCGCCTGCTCTACCCTTT-3′ R: 5′-GAACTGCGCTTGGAAACCTG-3′
GAPDH	F: 5′-AACTTTGGCATTGTGGAAGG-3′ R: 5′-ACACATTGGGGGTAGGAACA-3′

2. Materials and methods

2.1. CQR-300 preparation

The CQR-300 was provided from Gateway Health Alliance, INC (Fairfield, CA, USA). The stems and leaves of *Cissus quadrangularis* were washed and extracted by aqueous water for 3 times at 100 °C for 3 h and then filtered. The filtered extract was concentrated at 60 °C for 3 h with vacuum evaporator and then dried. The CQR-300 was dissolved in dimethyl sulfoxide (DMSO) for *in vitro* study.

2.2. Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM), Bovine Calf Serum (BCS), Fetal Bovine Serum (FBS), and penicillin-streptomycin were purchased from Gibco BRL (Grand Island, NY, USA). 3-isobutylmethylxanthine, insulin, and dexamethasone were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Oil-red O (ORO), 3-(4, 5-dimetylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), and isopropanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyclonal antibodies against α-actin, PPARγ, C/EBPα, AMPK, p-AMPK, SREBP-1c, and FAS-α were obtained from Cell signaling (Danvers, MA, USA). Horseradish peroxidase (HRP)-linked anti-rabbit IgG and HRP-linked anti-mouse IgG were purchased from Bio-Rad (CA, USA).

2.3. 3T3-L1 cell culture and differentiation

3T3-L1 mouse preadipocytes were purchased from the American Type Culture Collection (Rockville, MD, USA) and cultured in DMEM supplemented with 10% BCS and 1% penicillin-streptomycin at 37 °C under a humidified atmosphere with 5% CO_2. For differentiation of 3T3-L1 preadipocytes to mature adipocytes, full confluent 3T3-L1 preadipocytes (defined as Day 2) were incubated in differentiation medium containing DMEM, 10% fetal bovine serum, 0.5 mM 3-isobutylmethylxanthine, 5 μg/ml insulin, and 1 μM dexamethasone (Wako Pure Chemical Industries Ltd., Osaka, Japan). After two days (Day 4) of culture, cells were switched to DMEM supplemented with 10% FBS and 5 μg/ml of insulin. The medium was changed every two days. These cells were fully differentiated into mature adipocytes on Day 7.

2.4. Cell viability assay

Effects of CQR-300 on cell viability of 3T3-L1 adipocytes were analyzed by MTT assay. Briefly, cells were incubated with various concentrations (50–200 μg/ml) of CQR-300 in DMEM containing 10% FBS for 24 h. Sterile (filtered) MTT solution (5 mg/ml) in phosphate-buffered saline (PBS) was added to cells to reach a final concentration of 0.5 mg/ml. After 4 h of incubation, unreacted MTT reagent was removed and insoluble formazan crystals were dissolved in DMSO. Absorbance at 595 nm was measured using a microplate reader (Tecan, Mannedorf, Switzerland).

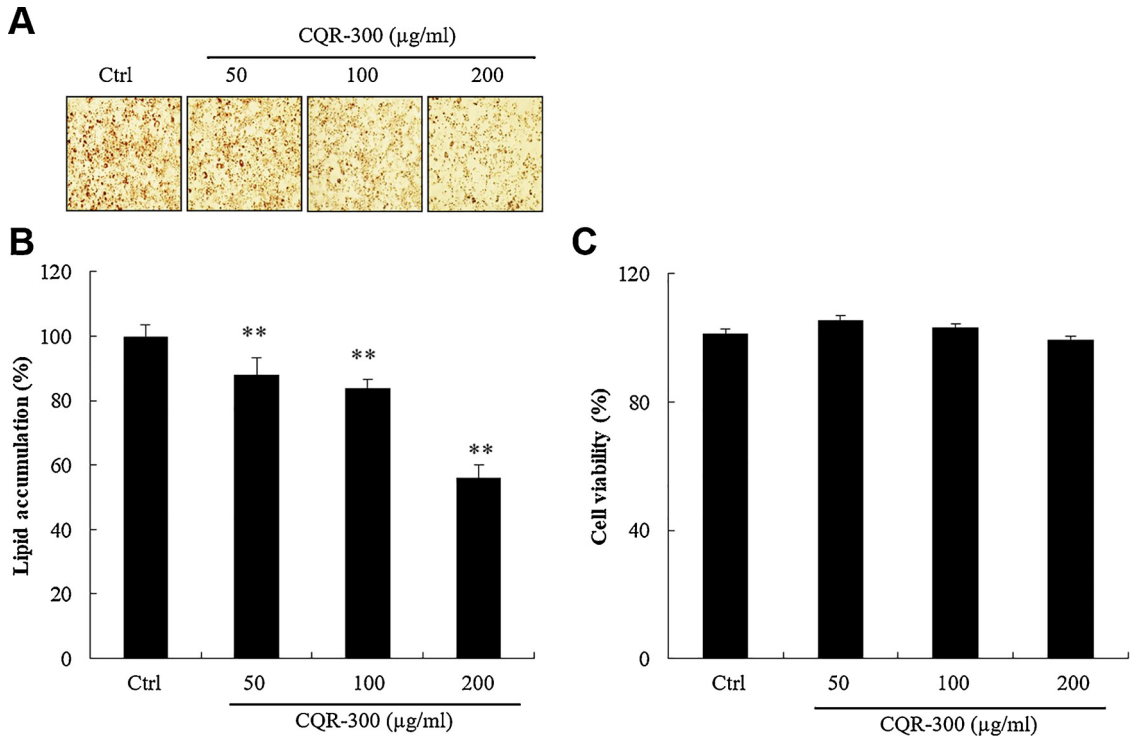

Fig. 1. CQR-300 inhibits adipogeneic differentiation in 3T3-L1 cells. 3T3-L1 preadipocytes were induced to differentiate into adipocytes by indicated MDI solution in the presence or absence of CQR-300. (A) Cell viability after treatment with CQR-300 was determined by MTT assay. (B) Lipid droplets were photographed after Oil Red O staining. (C) Stained lipids were quantified by measuring the absorbance at 490 nm. Data are expressed as means ± SE. $^{**}p < 0.01$ compared to the control.