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Mulberry leaf polyphenol extract improves obesity by inducing adipocyte apoptosis and inhibiting preadipocyte differentiation and hepatic lipogenesis

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ABSTRACT

This study investigated the effects of mulberry leaf extract (MLE) and mulberry leaf polyphenol extract (MLPE) on lipid accumulation in 3T3-L1 cells and obesity in mice fed a high-fat diet (HFD). MLPE was extracted from mulberry leaves using ethanol, and those polyphenolic compounds that can be analysed by HPLC. Both MLE and MLPE efficiently suppressed the expression of SREBP-1c and PPAR-γ proteins and the target genes A-FABP and FAS, whereas both of these compounds increased phosphorylation of AMPK in vivo and in vitro. Treatment of quercetin, caffeic acid, hydroxyflavin and hesperetin, the main ingredients of MLPE, also inhibited the differentiation of 3T3-L1 preadipocytes. In addition, orally administering MLE significantly reduced body weight gain and lipid accumulation in the liver and serum/hepatic triglyceride and total cholesterol levels compared with those in the HFD group. Therefore, the mulberry leaf may be used as a dietary supplement in patients with certain diseases with obesity involvement.

1. Introduction

The prevalence of obesity has rapidly increased in the past several decades because of changes in lifestyle factors such as diet (Kopelman, 2000). A high-fat diet (HFD) is considered a major risk factor for metabolic syndrome, which is characterized by obesity, hyperlipidaemia, hypertension, cardiovascular disease, diabetes, renal disease, respiratory complications, osteoarthritis, nonalcoholic fatty liver disease (NAFLD), and certain types of cancer (Harvey, Lashinger, & Hursting, 2011). Obesity is caused by excessive lipid accumulation in the body, which

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Abbreviations: MLE, mulberry leaf extract; MLPE, mulberry leaf polyphenolic extract; HFD, high-fat diet; HPLC, high-performance liquid chromatography; NAFLD, non-alcoholic fatty liver disease; FAS, fatty acid synthase; SREBPs, sterol regulatory element binding proteins; PPAR-γ, peroxisome proliferator activated receptors-gamma; A-FABP, adipocyte-specific fatty acid binding protein; FAS, fatty acid synthase; AMPK, adenosine monophosphate-activated protein kinase; ACC, acetyl-Coenzyme A carboxylase; HMGCR, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol; GLU, glucose; BUN, blood urea nitrogen; CRE, creatinine; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase

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results from increased adipose tissue mass and dysregulation of lipid metabolism. Adipocytes are the major cellular component of adipose tissue. Many studies have reported treating obesity by reducing the differentiation of fibroblastic preadipocytes to mature adipocytes (adipogenesis), inhibiting lipogenesis, increasing lipolysis, and inducing the apoptosis of adipocytes (Prins & O'Rahilly, 1997). Obesity has become a major public health problem worldwide and is a crucial concern in the field of preventive medicine.

Identifying the molecular basis for controlling adipogenesis is a potential strategy for obesity prevention and treatment, because adipocyte differentiation plays a key role in the growth of adipose tissue mass (Wang et al., 2014). Adipocyte differentiation is a multistep process that is regulated by a network of transcription factors and adipogenesis-related genes. Representatives of two transcription factor families, namely, the nuclear hormone receptor peroxisome proliferator-activated receptors (PPARs) and sterol regulatory element-binding proteins (SREBPs), have been implicated in this process. During adipogenesis, PPARγ activates the expression of lipid-metabolizing enzymes, such as adipocyte-specific fatty acid-binding protein (A-FABP; also designated aP2 or FABP4) and fatty acid synthetase (FAS), leading to morphological changes and lipid accumulation in cells (Farmer, 2006). Two SREBP isoforms, SREBP-1c and -2, are expressed in the liver. SREBP-1c preferentially regulates fatty acid synthesis by activating the expression of genes such as acetyl-coenzyme A carboxylase 1 (ACC1) and FAS. SREBP-2 regulates the transcription of cholesterol-related genes, including 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), which encodes the rate-limiting enzyme of cholesterol biosynthesis. In addition, adenosine monophosphate-activated protein kinase (AMPK) is also a potential molecular candidate involved in the control of adipocyte differentiation (Lin, Ribar, & Means, 2011). AMPK regulates the expression of SREBPs and their target genes (e.g., A-FABP and FAS) (Ceddia, 2013).

Mulberry (*Morus alba* L.; Moraceae family) is cultivated in Asian countries (e.g., China, Korea, Japan, and Taiwan), and its leaves contain various phytochemical constituents, such as flavonoid and polyphenol compounds. These leaves possess medicinal benefits and have antibacterial, antihypertensive, antihypoglycaemic, and antiatherosclerotic effects and consequently have been used as a remedy for several decades (Yang, Tan, Chen, & Kang, 2014c). In Taiwan, mulberry leaf preparations are commonly used in commercial beverages (mulberry tea) and health foods. Water extracts of mulberry leaves have been shown to exhibit a variety of biological functions, including antidiabetic, antiobesity, anti-inflammatory, antioxidative, antiatherosclerotic, antimicrobial, antitumor, and antihypertensive actions (Lee et al., 2011; Lim, Lee, Kim, Yang, & Lim, 2013; Yang, Wang, & Li, 2014a; Zhang et al., 2014). Consistently, our previous studies have indicated that mulberry leaves have not only anticholesteremic effects (Chan et al., 2013) but also cardiovascular and hepatoprotective properties (Chan et al., 2010; Yang, Lee, Ou, Chang, & Wang, 2012). Furthermore, recent scientific evidence has demonstrated that the dried powder, water extract, and ethanol extract of mulberry leaves show several entirely distinct, new functions such as antiplatelet (D. S. Kim et al., 2014), antiosteoporotic (Sungkamanee, Wattanathorn, Muchimapura, & Thukham-mee, 2014), antiaging (Zheng et al., 2014), neuroprotective (Bauomy, 2014), and anti-cancer stem cell (S. Park, Kim, & Kim, 2012) properties. Altogether, increasing evidence suggests a beneficial role of mulberry leaves in preventing obesity, although the underlying mechanisms must be elucidated. Hence, in this study, we investigated protein expression in the livers of hyperlipidaemic mice (in vivo) and 3T3-L1 cells (in vitro) treated with mulberry leaf extracts to elucidate the mechanisms underlying the lipid-lowering effects of mulberry leaves. Two mulberry leaf extracts, mulberry leaf extract (MLE) and mulberry leaf polyphenol extract (MLPE), had multiple antiadipogenic effects in these model systems. Adding quercetin, caffeic acid, hydroxyflavin and hesperetin, the main ingredients of MLPE, also inhibited adipocyte differentiation and induced apoptosis in 3T3-L1 preadipocytes.

2. Materials and methods

2.1. *Preparation of MLE and MLPE*

Fresh mulberry leaves (200 g) were harvested and immediately dried at 50 °C. The dried leaves were heated in 3000 mL of deionized water. After filtration, we removed the residue. The suspension was stored at –80 °C overnight and then evaporated with a freeze-dryer. The dried powder remaining was an aqueous fraction of mulberry leaves (MLE). According to the previous paper, ethanol is reported to be a better solvent to extract phenolic compounds from mulberry leaves compared with other solvents (Jeszka-Skowron et al., 2014; Kim, Chung, Jung, Wee, & Kwon, 2013; Park, Lee, Lee, & Kim, 2013; Yang, Park, & Lim, 2014b). For preparation of the polyphenol-rich extract of mulberry leaves (MLPE), 100 g dried powder of mulberry leaves was merged in 300 mL of ethanol and heated at 50 °C for 3 h. The extract was filtered and thereafter concentrated by evaporation under reduced pressure at room temperature. The powder was then resuspended in 500 mL of distilled water, followed by extraction with 180 mL of ethyl acetate three times, redissolved in 250 mL of distilled water, stored at –80 °C overnight, and lyophilized. MLE and MLPE were filtrated by 0.22 μm filter before use in cell culture.

2.2. *HPLC analysis*

HPLC was performed with a Hitachi HPLC system (Hitachi, Danbury, CT, USA) which consisted of a pump (L-6200A), an ultraviolet detector (L-4250) and the Hitachi D-7000 HPLC System Manager program. A reported procedure was used for analysing the phenolic acids, which contained column, Mightysil RP-18 GP 250 (Kanto, Tokyo, Japan); mobile phase solvent A, acetic acid/water (2:98, v/v), and solvent B, 0.5% acetic acid in water/acetonitrile (50:50, v/v). The flow rate was 1 mL/min. The gradient for the separation was 100% solvent A at 0 min, 70% solvent A and 30% solvent B at 5 min, 65% solvent A and 35% solvent B at 50 min, 60% solvent A and 40% solvent B at 55 min, 0% solvent A and 100% solvent B at 60 min, followed by a 5 min post-run with HPLC grade water. Phenolic acids were detected at 260 nm.

2.3. *Cell culture*

Mouse embryo 3T3-L1 cells (BCRC 60159) were obtained from the Bioresource Collection and Research Center (BCRC, Food