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Mulberry leaf polyphenol extracts reduced hepatic lipid accumulation involving regulation of adenosine monophosphate activated protein kinase and lipogenic enzymes

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ABSTRACT

Fat accumulation in the liver increases the risk of developing progressive liver injury. It can induce all the symptoms of metabolic syndrome, which is associated with many additional health problems, including increased risk of obesity, hypertension, insulin resistance, and non-alcoholic fatty liver disease (NAFLD). Therefore, prevention and treatment of fat accumulation in the liver are relevant to health promotion. Mulberry leaf polyphenol extracts (MLPE) have been known to modulate serum fasting glucose, lipid and antiatherosclerosis. However, the effect of MLPE on regulating hepatic lipid metabolism is unclear. This study evaluated the effects and mechanisms of MLPE in reducing hepatic lipid accumulation in cell culture. We found MLPE could regulate hepatic lipid accumulation. Further, numerous lipogenic enzymes, such as FAS (fatty acid synthetase), ACC (acetyl-CoA carboxylase), HMGCR (HMG-CoA reductase) and associated-lipogenic transcriptional factors (SREBP1 and SREBP2) were suppressed by MLPE. Our results show MLPE is able to reduce hepatic lipid accumulation through activation of the AMPK (AMP-activating protein kinase) signaling pathway. It may have potential therapeutic implications for human NFALD.

1. Introduction

Unburnt energy is conserved in the form of fat in adipose tissue, leading to obesity and obesity-associated fatty liver disease (FLD) (Evans, Barish, & Wang, 2004). Obesity is closely associated with diseases such as non-alcoholic fatty liver disease (NAFLD), hypertension, hyperlipidemia, arteriosclerosis and cancer (Kopelman, 2000; Tilg & Moschen, 2006; Wellen & Hotamisligil, 2005). Obesity seems to be an important risk factor for non-alcoholic fatty liver disease (Ruderman, Chis-

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holm, Pi-Sunyer, & Schneider, 1998). Therefore, prevention and treatment of NAFLD are relevant to health promotion.

Cryptogenic cirrhosis has been estimated to account for 5–30% of end-stage liver disease, and it has been asserted many of these cases of cirrhosis and the associated hepatocellular carcinoma represent the progression of NAFLD (Adams, Angulo, & Lindor, 2005). The underlying cause of fat accumulation in NAFLD is mostly due to the synthesis of fatty acids and inhibition of fatty acid oxidation (Reddy & Rao, 2006). Differentiated adipocytes store fatty acids in the form of triglycerols (TG) in their cytoplasm, with involvement of various enzymes such as FAS (Weissman, 1999). Activation of FAS expression through modulation of SREBP-1 has been reported in human breast cancer (Magana & Osborne, 1996). The hepatic TG content in human is significantly correlated with plasma TG level and fat mass (Qureshi & Abrams, 2007). TG is synthesized in the liver, secreted into the blood stream and transported to the peripheral organs, including the adipose tissue (Duval, Muller, & Kersten, 2007; Muller, Lindman, Brantsaeter, & Pedersen, 2003). High intake of saturated fatty acids is associated with a high level of serum cholesterol (Steinberg, 1995). It is believed circulating concentrations of lipids and free fatty acids are increased after high-fat feeding, and the hyperlipidemia as well as the elevated plasma low-density lipoprotein (LDL) may result in initiation of atherosclerosis (Carling, 2004; Hardie, Hawley, & Scott, 2006; Rusinol et al., 2000).

Mulberry leaf has been used in traditional medicine for antidiabetes, antihyperlipidemics, reducing high blood pressure, high cholesterol and neutral fat (Assy et al., 2000; Steinberg, Parthasarathy, Carew, Khoo, & Witztum, 1989). The literature indicates mulberry leaf possesses anticancer effects and inhibits hyperglycaemia (Zhou et al., 2001). Several studies have reported mulberry leaf has potential antioxidant activity (Kim et al., 1999; Arabshahi-Delouee & Urooj, 2007; Katsube et al., 2006). Our previous studies have shown that mulberry leaf extract (MLE) is rich in polyphenols and can effectively inhibit vascular smooth muscle cells (VSMC) proliferation and migration (Chan et al., 2010; Yang et al., 2011). It contains several functional components, including flavonoids, which are known to be powerful polyphenols and antioxidants (Oliaro-Bosso et al., 2009).

This study investigated the hepatic hypolipidemia effect of MLPE. The results showed MLPE attenuated OA-induced hepatic lipid accumulation by activating of AMPK signals in hepatic cells.

2. Materials and methods

2.1. Materials

The mulberry leaves were collected in Dadu Township, located in central Taiwan. The 3-(4,5-dimethylthiazol-zyl)-2,5-diphenylterazolium bromide (MTT), oleic acid, and statin were purchased from Sigma to Aldrich (St. Louis, MO, USA). GSH peroxidase (GPx), superoxidase (SOD) and SREBP antibodies were obtained from Santa Cruz Biotechnology (CA, U.S.A.). Anti-pThr172-AMPK and anti-AMPK antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-β-actin and anti-catalase antibodies were purchased from Sigma to Aldrich.

2.2. Preparation of MLE and MLPE

Fresh mulberry leaves (100 g) were harvested and immediately dried at 50 °C in an oven. The dried leaves were heated in 1500 mL of deionized water. Following filtration, we removed the residue. The suspension was stored at −80 °C overnight and lyophilized to obtain MLE particles. For preparation of the polyphenol 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 lyophilized under reduced pressure at room temperature. The powder was then resuspended in 500 mL of 50 °C distilled water, followed by extraction with 180 mL of ethyl acetate three times, redissolved in 250 mL of distilled water, stored at 70 °C overnight, and lyophilized.

2.3. Total phenolic content assay

Total phenolic compound content in each extract was spectrophotometrically determined in accordance with the Folin–Ciocalteu procedure by reading the absorbance at 725 nm against a methanol blank (Lakenbrink, Lapczynski, Maiwald, & Engelhardt, 2000). Briefly, samples (20 μl, water added to 1.6 ml) were introduced into test tubes, and then 100 μl of Folin–Ciocalteu reagent and 300 μl of sodium carbonate (20%) were added. The tubes' contents were mixed and incubated at 40 °C for 40 min. Absorption at 725 nm was measured. The total phenolic contents were expressed as milligrams per gram of MLPE for gallic acid (GA) and rutin.

2.4. Total polysaccharide content assay

We used the phenol–sulfuric acid method (Siddiqui et al., 2009) to measure the polysaccharide content. The MLE was diluted in deionized water, and the dissolved extracts were filtered through a 0.22 μm filter (MILLEXHA) prior to treatment. Briefly, 100 μl of MLE, 100 μl of phenol (5%), and 500 μl of H_2SO_4 (95.5%) were mixed and then incubated at room temperature for 15 min. The absorbance at 490 nm was used to determine the amount of carbohydrate in the sample. Different concentrations of glucose (0, 10, 30, 50, 70, and 90 μg/ml) were used as standards.

2.5. Lipid content assay

Lipid content was measured by the acid hydrolysis method. The MLE (1 g) were mixed with hydrochloric acid (20 ml) in a conical flask, then heated in a water bath at 70–80 °C for about 50 min. After heating, the mixture was cooled to room temperature, and then 10 mL of ethanol and 20 ml of ethyl ether were added to a separatory funnel. The separatory funnel was vigorously shaken to ensure complete mixing of the two liquid phases. Then, the two liquid phases were allowed to separate for at least 2 h until the layers were clearly separated. The lower solvent was collected in a new beaker, and the upper solvent (ethyl ether) was collected in a conical flask. Ethyl ether (20 ml) was added to the lower solvent and poured into a separatory funnel, which was shaken vigorously to ensure complete