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Original Article

Mulberry Leaf Extracts prevent obesity-induced NAFLD with regulating adipocytokines, inflammation and oxidative stress

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ABSTRACT

Mulberry (*Morus alba*) leaf has been used in Chinese medicine as the remedy for hyperlipidemia and metabolic disorders. Recent report indicated Mulberry leaf extract (MLE) attenuated dyslipidemia and lipid accumulation in high fat diet (HFD)-fed mice. Non-alcoholic fatty liver (NAFLD) is generally considered as the liver component of metabolic syndrome. The hepatic lipid infiltration induces oxidative stress, and is associated with interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) which are regulated by the leptin and adiponectin. MLE could prevent obesity-related NAFLD via downregulating the lipogenesis enzymes while upregulating the lipolysis markers. Treatment of MLE, especially at 2%, enhanced the expression of superoxide dismutase (SOD) and clenched the oxidative stress of liver. MLE decreased the plasma level of leptin but increased adiponectin. The advantage of MLE is supposed mainly attributed to chlorogenic acid derivative. We suggest MLE, with promising outcome of research, could be nutraceutical to prevent obesity and related NAFLD.

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1. Introduction

Overweight and obesity is associated with a great diversity of diseases involving cardiovascular and metabolic systems [1]. There is a metabolic link between the expanded body fat, high triacylglycerol (TG), high low density lipoprotein cholesterol (LDL-C), and low high-density lipoprotein cholesterol (HDL-C), which leads to impaired metabolic regulation of adipose and flux of free fatty acids (FFA) [2].

Liver plays an essential role of modulating plasma lipid level through LDL clearance and HDL recruitment. However, the lipid uptake must affect the hepatic fat composition and burden the liver function. Hence the regulation of hepatic lipid metabolism should be emphasized to prevent dyslipidemia and accompanying illness. Expressions of fatty acid synthase (FAS) and 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase, the important enzymes regulating TG and cholesterol synthesis, are indicated markers of lipogenesis [3]. As well, 1-acylglycerol-3-phosphate acyltransferase (AGPAT) is involved in the synthesis of glycerophospholipid [4]. On the other hand, the expressions of carnitine palmitoyltransferase-1 (CPT-1) and peroxisome proliferator-activated receptor α (PPARα) are critically associated with the process of lipolysis [5].

Non-alcoholic fatty liver (NAFLD) is generally considered to be the liver component of metabolic syndrome, which is frequently accompanied with obesity, dyslipidemia, and insulin resistance [6]. The degree of fat infiltration of liver is related to the subsequent development of necrosis, inflammation, cirrhosis, and the propensity to progress to hepatocellular carcinoma [7]. Increased fat mass and associated fat gene expression enhance lipogenesis and oxidative stress [8]. The oxidative stress, disrupted nitric oxide (NO) signaling, and mitochondrial dysfunction are proposed to be pivotal events that accelerate steatosis and initiate the progression to hepatitis and fibrosis [9]. Oxidative stress could be eliminated by endogenous antioxidative enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), or the exogenous application of antioxidants [10].

The fatty liver index often accompanied with elevation of aspartate transaminase (AST) and alanine transaminase (ALT) [11], is independently associated with inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [12]. Leptin and adiponectin are adipokines produced by white adipose tissue. Leptin upregulates TNF-α and IL-6, and is associated with insulin resistance and type 2 diabetes mellitus. In contrast, adiponectin has anti-inflammatory properties and downregulates the expression and release of proinflammatory immune mediators [13].

Mulberry leaf (the leaf of *Morus alba*), commonly used as the silkworm diet, has been used in Chinese medicine for antidiabetes, antihyperlipidemics, and prevention of coronary artery disease. It contains a lot of components including flavonoid, which is known as a powerful polyphenol and antioxidant [14]. Our previous study suggested mulberry leaf extract (MLE) and its polyphenols possessed antiatherogenesis effect via Inhibiting LDL oxidation and foam cell formation [15]. A recent report indicated that MLE attenuated the dyslipidemia and lipid accumulation in high fat diet (HFD)-fed mice; the mulberry leaf polyphenols induced adipocyte apoptosis and inhibited preadipocyte differentiation [16].

In the present study, we aim to investigate if MLE improves the oxidative stress, inflammation, and ratio of adipokines, thus prevents obesity-induced metabolic disturbance and NAFLD.

2. Material and methods

2.1. Preparation of MLE and chemical analysis

Fresh mulberry leaves (100 g) were harvested and immediately dried at 50 °C. The dried leaves were heated in 1500 mL of deionized water. After filtration, the residue was removed, and the suspension was stored at −80 °C overnight and then evaporated with a freeze-dryer. The dried powder remained was an aqueous fraction of mulberry leaves (MLE), which was used in the following animal experiment. The polyphenols of mulberry leaf was extracted as in the previous report (Yang et al., 2011) [15], and then analyzed for its chemical composition. In our previous report, the composition analysis revealed that it contains neochlorogenic acid (35.5%), cryptochlorogenic (31.7%), chlorogenic (23.8%), rutin (9.2%), isoquercitrin (5.6%), astragalin acid (5.3%), nicotiflorin (3.5%), and protocatechuic acid (1.3%) [17].

2.2. Animal experiment

The animal experimental project was approved by the Animal Model Experimental Ethics Committee of Chung-Shan Medical University, and was in accordance with the recommendation in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Briefly, male Wistar rats (weight 220 ± 10 g) were obtained from LuxBiotech Co., Taiwan. The rats were acclimated in laboratory conditions (23 ± 2 °C, 60 ± 5% relative humidity, and 12 h light/dark cycle), and fed a basic chow consisting of 12% fat. After one week-adaptation, the Wistar rats were randomly grouped. Each group (n = 8) was fed a unique diet for 14 wks and weighed every 2 wks. The groups and their corresponding meals were (1) control, normal diet; (2) HFD, normal diet supplemented 2% cholesterol and 20% lard oil; (3) HFD + 0.5% MLE; (4) HFD + 1% MLE; (5) HFD + 2% MLE. All the formulas of MLE were added from 4 wk. In the end of the experiment, blood and livers were collected from rats fasted for 12–14 h and then sacrificed. The blood was collected by EDTA tubes and centrifuged at 3000 rpm or 10 min at 4 °C. The supernatant plasma was transferred into new tubes for determination of serum biomarkers. The livers were quickly frozen in liquid nitrogen for the extraction of liver lipids or freshly cut into pieces for H-E stain.

2.3. Serum biochemical markers

The serum sample was collected using ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged at 3000 rpm (1400 g) for 10 min at 4 °C. Concentrations of glucose, TGs, total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), FFA, aspartate aminotransferase (AST), alanine

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