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Original Research

Mulberry ethanol extract attenuates hepatic steatosis and insulin resistance in high-fat diet–fed mice

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ABSTRACT

Nonalcoholic fatty liver disease is one of the most common complications of obesity. Mulberry is an important source of phytochemicals, such as anthocyanins, polyphenols and flavonoids, which are related to its antioxidant activity. In this study, we developed a hypothesis that mulberry exerted beneficial effects on metabolic disorders and evaluated the influence of the mulberry ethanol extract (MEE) on high-fat diet–induced hepatic steatosis and insulin resistance in mice. Thirty-six male C57BL/6J mice were assigned into 3 groups and fed either a low-fat diet or a high-fat diet with or without supplementation with MEE. Our results showed that administration of MEE reduced diet-induced body weight gain, improved high-fat diet–induced hepatic steatosis and adipose hypertrophy, alleviated insulin resistance, and improved glucose homeostasis. Analysis of hepatic gene expression indicated that MEE treatment changed the expression profile of genes involved in lipid and cholesterol metabolism. In conclusion, the present study demonstrated that MEE supplementation protected mice from high-fat diet–induced obesity, hepatic steatosis, and insulin resistance. Moreover, the protective effects of MEE were associated with the induction of fatty acid oxidation and decreased fatty acid and cholesterol biosynthesis.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease and is characterized by excessive lipid accumulation in hepatocytes. It includes a pathological spectrum that ranges from benign simple steatosis to more severe nonalcoholic steatohepatitis, liver fibrosis, cirrhosis, and even hepatocellular carcinoma [1]. NAFLD is usually closely associated with insulin resistance and is often regarded as the hepatic manifestation of the metabolic syndrome [2]. The pathogenesis of NAFLD is complex, and the underlying mechanisms remain largely unknown. Various genetic and environmental factors (eg, insulin resistance, oxidative stress, lipid peroxidation,

Abbreviations: ANOVA, analysis of variance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; H&E, hematoxylin and eosin; HDL-C, high-density lipoprotein cholesterol; HFD, mice fed high-fat diet; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-IS, homeostasis model assessment of insulin sensitivity; LDL-C, low-density lipoprotein cholesterol; LFD, mice fed low-fat diet; MEE, mulberry ethanol extract; NAFLD, nonalcoholic fatty liver disease; NPY, neuropeptide Y; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; TG, triglyceride; TC, total cholesterol.

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inflammation, and hepatic fibrosis and apoptosis) appear to promote hepatic lipid deposition, contributing to the occurrence of NAFLD [1,3,4]. There are currently no effective pharmacological treatments for NAFLD, and the only proven strategy for amelioration of NAFLD is lifestyle modification, for example, weight loss through healthy diet and exercise. Thus, the beneficial effects of dietary supplements on NAFLD are gaining increasing attention due to their minimal adverse effects [5]. Recently, anti-NAFLD effects of a wide range of plant-derived bioactive substances, including antioxidants, anti-inflammatory compounds, lipid-lowering agents, and insulin sensitizers, have been evaluated in human and animal studies [6,7].

Natural bioactive substances present in fruits and vegetables, including phenolic compounds, flavonoids, and anthocyanins, have received growing attention due to their antioxidant properties, and they are increasingly recognized as a potential strategy for reducing the risk of obesity and obesity-related metabolic syndrome [5,6,8,9]. Mulberry, which is a natural source of phenolics, flavonoids, anthocyanins, and many other antioxidants, has traditionally been used in foods and medicines because of its pharmacological effects [8,10,11]. As one of the most important constituents of the mulberry fruit, anthocyanins are a major subgroup of water-soluble bioactive compounds of the polyphenol class and are found in vegetables and fruits. Numerous studies have shown that anthocyanins can decrease hepatic lipid accumulation and exert anti-inflammatory, anticarcinogenic, and antihyperlipidemic effects [12–15]. Moreover, Chang et al [16] reported that mulberry anthocyanins inhibited oleic acid–induced hepatic lipid accumulation in HepG2 cells, suggesting that mulberry might be a potential candidate for the treatment of NAFLD. However, the benefits of mulberry in animal models with NAFLD have not yet been clearly elucidated. Although the beneficial effects of mulberry possibly arise from the presence of anthocyanins, increasing evidence supports a key role of flavonoids and polyphenols, which are also found in mulberry, in the protection against obesity-associated metabolic disorders [9,17].

We developed a research hypothesis that mulberry exerted beneficial effects on metabolic disorders, for example, NAFLD. The main objective of the present study was to evaluate the influence of mulberry ethanol extract (MEE) on NAFLD in a murine model and to investigate the potential mechanisms by which MEE might protect against NAFLD.

2. Methods and materials

2.1. Preparation of MEE

Fresh mulberry (*Morus nigra L.*) was purchased from a local market in Hangzhou. MEE was obtained using a previously described method with modifications [18]. Mulberry fruits were homogenized with 80% aqueous ethanol and 0.1% trifluoroacetic acid. The homogenates was maintained at 4°C for 24 hours and then centrifuged at 4000*g* for 40 minutes. The resulting supernatants were filtered through 0.45-μm pore size filter (Millipore, Bedford, MA, USA), and the solvent was removed using a vacuum rotary evaporator. Then, the concentrated extract was extracted with ethyl acetate. Subsequently, the aqueous layer was collected and evaporated again. Finally, the aqueous solution was lyophilized to a dry powder and stored at –80°C until use. Total polyphenol and total flavonoid content was determined using previously described methods [19,20] and expressed as milligram gallic acid and rutin equivalents and per gram of MEE, respectively. High-performance liquid chromatography analysis was performed to separate the anthocyanins contained in MEE. The mobile phase consisted of solvent A (0.1% aqueous formic acid) and solvent B (acetonitrile 0.1% formic acid). The injection volume and flow rate were 0.8 mL/min and 10 μL, respectively. A linear gradient from 5% B to 30% B in 30 minutes was programmed. The anthocyanin compounds were measured at the wavelength of 520 nm. Results of the analysis for the MEE are shown in Table 1.

2.2. Animals and treatments

Four-week-old male C57BL/6J mice (n = 36; JOINN Laboratories, Suzhou, China) were housed 4 animals per cage in a temperature-controlled environment (23°C ± 3°C) with a standard 12-hour light-dark cycle. The animals had free access to water and food. After acclimation for 1 week, the animals were randomly divided into 3 groups (n = 12): LFD group, mice were fed low-fat diet; HFD group, mice were fed high-fat diet; and MEE group, mice were fed high-fat diet. The ingredients of the purified low-fat and high-fat diets are presented in Table 2. The mice in MEE group were given 100 mg/(kg d) of MEE by oral gavage for 14 weeks, and the mice in LFD and HFD groups received an equal volume of saline. The body weight and food intake were monitored weekly. At the end of the experiment, the mice were fasted for 12 hours and killed by decapitation. The whole blood was drawn and centrifuged at 3000 rpm for 10 minutes to obtain serum samples. The hearts, kidneys, livers, spleens, and fat pads were collected, weighted, and stored at –80°C before use. All procedures in this experiment were approved by the Committee on the Ethics of Animal Experiments of Zhejiang University (permission no.: ZJU201550501).

2.3. Measurement of serum parameters

The serum concentrations of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol

Table 1 – Chemical characterization of MEE

Component	Content
Cyanidin-3-O-glucoside	228.15 ± 15.42 mg/g
Cyanidin-3-rutinoside	121.65 ± 7.13 mg/g
Pelargonidin-3-glucoside	19.26 ± 0.97 mg/g
Total polyphenols	29.02 ± 3.18 mg GAE/g
Total flavonoids	36.94 ± 8.19 mg RE/g
Total sugar	40.29 ± 6.27 mg/g

Values are presented as means ± SEM (n = 3). GAE, gallic acid; RE, rutin.