Journal of Functional Foods 46 (2018) 90–100

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

The protective effects of mangiferin on metabolic and organs functions in the adolescent rat model of alcohol abuse

Ce Chu, Mengran Li, Jianheng Li, Chenyan Zhou*

College of Pharmaceutical Sciences, Key Laboratory of Pharmaceutical Quality Control of Hebei Province, Hebei University, 180 WuSi Road, Lianchi District, Baoding 071000, China

ARTICLE INFO

Keywords:
Mangiferin
Alcohol abuse
Adolescent
Glial fibrillary acidic protein
Insulin receptor substrate-2
Growth differentiation factor-9

ABSTRACT

This study was carried out to investigate the beneficial effects and potential mechanism of mangiferin (MG) against the disorders of metabolic and organs functions induced by alcohol abuse (40% alcohol, 10 mL/kg day^{-1} BW, ig) in adolescent rats. MG administration (100 mg/kg·day^{-1} BW, ig) can effectively prevent the above disorders. The mechanisms of potential effect of MG were related to scavenged free radicals, increased the levels of serum calcium, estradiol, testosterone and bone density, and up-regulated brain GFAP, testes IRS-2 and ovaries GDF-9 expression levels. In addition, lipid accumulation and blood glucose level were effectively regulated by MG, which are also important for adolescent metabolic and organs functions. These systems and balances in vivo were not isolated from each other, but interrelated and influenced each other. These results suggested that MG can be a novel phytochemicals for preventing the disorders of metabolic and organs functions induced by alcohol abuse.

1. Introduction

Adolescence, characterized by physiological, psychological and behavioral changes, is a vulnerable period for the morbidity of high-risk drinking and alcohol abuse (Spear & Swartzwelder, 2014). According to the date from World Health Organization on Alcohol and Health, the annual consumption reached 6.2 L of alcohol per person aged 15 years or older, which implies consumption of 13.5 g of pure alcohol every day (Orellana et al., 2017). Recent projections indicate that alcohol consumption will continue to rise in the next few decades, and the rate of adolescents is the highest among the rising consumption rates (Eade, Youngentob, & Youngentob, 2016). At the same time, adolescent alcohol abuse (AAAs) can lead to the disorders of metabolic and organs functions, such as brain shrinkage, reproductive system injury, diabetes mellitus, abnormal calcium balance, metabolic syndrome, alcoholic liver diseases and hypertension (Picot et al., 2017; Qu et al., 2017; Zou et al., 2017). Thus, AAAs has been a widespread problem, and has attracted increasing attention for its ever-increasing health risk (Crews, Vetreno, Broadwater, & Robinson, 2016). Despite a significant progress in AAAs therapy, clinicians still expect effective and safer medicines (Spear, 2015).

Mangiferin (MG), a natural C-glucosyl xanthone, isolated from various parts of plant families such as Liliaceae, Anacardiaceae, Mangiferaceae, Iridaceae and Gentianaceae, and is known to modulate several biological targets in inflammation, hyperlipidemia, metabolic disorders and cancer (Irondi, Oboh, & Akindahunsi, 2016; Lobo et al., 2017; Suchal et al., 2017; Zhou, Li, et al., 2015; Zhou, et al., 2015). MG has many applications in medicine and clinic, and no clinical evidence for the side effects of MG has yet been found (Bulugonda et al., 2017). In the preliminary experiments, we have found that MG can ameliorate the disorders of metabolic and organs functions in the AAAs rats. However, the MG protective mechanisms are multifactorial, and remain not completely understood at present. Therefore, the main purpose of this study was aimed to elucidate the potential mechanisms of MG by calcium balance, cerebral cortex, reproductive system, oxidative stress, lipid metabolism and glucose metabolism in the AAAs rats.

2. Materials and methods

2.1. Animals and ethical statement

Seventy two 3-week-old Sprague–Dawley Rats (50–70 g), with the equal number of male and female, were obtained from the Vital River Laboratory Animal Technology (Beijing, China, certification number: 11400700205565). The rats were housed in departmental animal house in controlled temperature (21 ± 2 °C), relative humidity (60 ± 5%) with 12 h light/dark cycle (lights on 7:00–19:00 h). All rats were acclimatized one week prior to the experiment. During the entire

* Corresponding author.
E-mail address: xuefanone@163.com (C. Zhou).

https://doi.org/10.1016/j.jff.2018.04.056
Received 14 August 2017; Received in revised form 26 April 2018; Accepted 26 April 2018

experimental period, rats had free access to tap water and food pellets *ad libitum*.

In the experiment, all rats were treated in accordance with the Guide for the care and use of Laboratory Animals of the National Institutes of Health (publication no. 85-23, revised 1996) and followed the guidelines of the Animal Welfare Act (SFS1998:56). The experimental protocol was approved by the Ethic and research Committee at Hebei University (Baoding, Hebei, China). All efforts were made to minimize animal suffering and social isolation, as well as to reduce the number of animals used.

2.2. Experimental design

After a week of acclimatization, all rats (n = 72) were randomly divided into 3 groups according to average body weight, sex in half. The animal experiment period was 4 weeks. All rats were treated by gavage daily throughout the animal experiment. Group 1 (normal control group, NC, n = 24): The NC rats were administered 0.9% normal saline (NS) solution (10 mL/kg day^{-1} BW, ig) in the morning (at 9:00 a.m.), and then given 0.9% NS (10 mL/kg day^{-1} BW, ig) in the afternoon (at 3:00 p.m.). Group 2 (alcohol control group, AC, n = 24): The AC rats were administered 0.9% normal saline (NS) solution (10 mL/kg day^{-1} BW, ig) in the morning (at 9:00 a.m.), and then given 40% alcohol (56% Red Star wine, China, was diluted with water; 10 mL/kg day^{-1} BW, ig) in the afternoon (at 3:00 p.m.). Group 3 (mangiferin administration group, DA, n = 24): The DA rats were administered with 20 mg/mL MG (10 mL/kg day^{-1} BW, ig) in the morning (at 9:00 a.m.), and then given 40% alcohol (10 mL/kg day^{-1} BW, ig) in the afternoon (at 3:00 p.m.). The dosage regimen for MG was selected on the basis of previous reports (Bhatt, Sebastian, & Joshi, 2017; Guo et al., 2011; Wang et al., 2017; Zhou, Li, et al., 2015; Zhou, et al., 2015) and our preliminary studies. MG (≥98%, HPLC; Chengdu Mann Stewart Biological Technology Co. Ltd., China) was suspended in 0.9% NS solution to obtain doses of 100 mg/kg day^{-1} BW for gavage administration, thus MG content in the 0.9% NS solution was 20 mg/mL. All rats were given a normal diet (AIN-93G growth purified diet) throughout the experiment. The macronutrient composition of the AIN-93G diet contained 15.91% fat, 20.50% protein and 63.59% carbohydrate, and the total energy was 3.96 Kcal/g diet. Food intake was measured every three days, water uptake was monitored every two days and body weight was measured weekly for individual rats. Meantime, we conducted a mid-term detection at the end of 2 weeks, and 8 rats were randomly selected in each group, male and female in half. At the end of 4 weeks, all rats were sacrificed. The detailed experimental design was shown in Supplementary Fig. 1.

2.3. Sample collection

After withholding food for 12 h, all rats were anaesthetized via intraperitoneal injection with 3% pentobarbital (40 mg/kg; ip), taking randomly one animal at a time of each one of three groups. Blood was collected via abdominal aorta, centrifuged at 4000 rpm and stored in −80 °C till analysis. The rats were sacrificed promptly by cervical vertebra dislocation. Then, main organs (brain, testes, ovaries, bone, liver, kidney, lung, heart, spleen and pancreas) and adipose tissues (abdominal fat, perirenal fat, epididymal fat, subcutaneous fat and brown fat) were excised, washed with ice-chilled normal saline and weighed, snap frozen in liquid nitrogen, and stored at −80 °C for subsequent assays. The organs and adipose tissues weights were expressed with the relative weights (Relative weights (g/100 g BW) = (organs or adipose tissues weight/body weight) × 100). In addition, portions of the main organs were fixed in 10% neutral formalin solution for histological observation.

2.4. Measurement of serum parameters

Serum testosterone (T) and estradiol (E_2) were measured by enzyme-linked immunosorbent assays (ELISA) method; serum calcium was measured by microplate method; serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL),γ-glutamyl transpeptidase (GGT), cholinesterase (CHE) and alkaline phosphatase (ALP), total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C), superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione (GSH) were measured by colorimetric enzymatic method. All these biochemical indexes were measured using commercial assay kits according to the manufacturer's protocols (Nanjing Jiancheng Bioengineering Research Institute, China).

2.5. Oral glucose tolerance test and insulin measurements

According to the dynamic observation, the oral glucose tolerance tests (OGTT) were carried out in the 2 and 4 weeks, respectively. Firstly, the rats received daily treatment in the morning. After 35 min, the rats were further administered with 50% glucose (2 g/kg BW) by oral gavage. Blood were obtained by severing the tip of the tail at 0 (before), 15, 30, 60, 90 and 120 min after glucose administration. Blood glucose levels were evaluated by a glucometer (EZIII, China). The rats had free access to drinking water throughout the OGTT. Blood glucose levels and areas under the curves (AUC) for OGTT were calculated to measure glucose tolerance. In addition, serum glucose was analyzed by glucose oxidase method using semiautomatic analyzer, and serum insulin was determined by ELISA Kit (Nanjing Jiancheng Bioengineering Research Institute, China) at the end of the experiment.

2.6. Measurement of SOD, MDA and GSH levels in liver and brain

The tissues (liver and brain) were homogenized with cold normal saline (NS) at 1:10 (1 g in 10 mL) ratio using Ultra-Turrax (IKA, Germany) (Wang, Zhang, Bai, Wang, & Du, 2016). After centrifuged (4 °C, 12,500*g*, 10 min), the supernatant was collected. Then, SOD, MDA and GSH levels were detected according with the instructions of the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.7. Measurement of TG, TC, LDL-C, HDL-C levels and histopathological observations in liver

For hepatic lipid measurements, liver sample (100 mg) was homogenized in 2 mL of chloroform / methanol (2:1, v/v) using Ultra-Turrax (IKA, Germany), and 1 mL ice-cold 0.9% NS was added to each sample. After standing for 12 h, the homogenate were then centrifuged, and the organic layer was removed and dried under nitrogen gas (Park et al., 2014). The resulting pellet was redissolved in phosphate-buffered saline containing 1% Triton X-100, then TG, TC, LDL-C and HDL-C levels were detected according with the instructions of the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Portions of liver were fixed in 10% neutral formalin solution, and the rest of liver was snap frozen and stored at −80 °C. Fixed liver tissue was divided for paraffin embedding and cryostat sectioning. The paraffin sections (4 μm, Thermo Fisher Scientific, America) were stained with hematoxylin and eosin (H&E), and examined and evaluated by light microscopy (ZEISS, Germany) at 200× and Image Pro Plus 6.0. Cryostat sections (Leica CM1100) were stained with oil red O to visualize hepatic steatosis.

2.8. Histopathological observations of testicle, ovary, brain and bone

Portions of testicle, ovary, brain, bone, kidney and spleen were fixed