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Polyphenol-enriched extract of *Rosa rugosa* Thunb regulates lipid metabolism in diabetic rats by activation of AMPK pathway

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ABSTRACT

This study was designed to investigate the mechanism of polyphenol-enriched extract of *Rosa rugosa* Thunb (RPE) in the control of dyslipidemia in diabetic rats. RPE was tested at three dosages (37.5 mg/kg, 75 mg/kg and 150 mg/kg) in the rat dyslipidemia model established with high fat diet feeding in combination with STZ injection (30 mg/kg). The RPE effect was evaluated after 4 weeks of treatment. In the RPE-treated rats, hepatic total cholesterol (TC) and triglyceride (TG) were significantly reduced, lipoprotein lipase (LPL) and liver lipase (HL) were significantly increased. The levels of alanine transaminase (ALT) and aspartate transaminase (AST) were decreased in the serum. Those effects of RPE were observed primarily at the mediate and high dosages. Expression of FGF21 was increased in the liver tissue and hepatic cell line 1c1c7 by RPE. The signals of p-AMPK, p-ACC, ACC, p-SIRT, and PGC-1α were significantly induced in the liver by RPE. The results suggest that RPE may improve hepatic steatosis and liver function by induction of AMPK signaling activity in the control of dyslipidemia.

1. Introduction

Prevalence of type 2 diabetes, a chronic metabolic disease, has increased rapidly worldwide in recent years [1]. Type 2 diabetes caused 1.5 million of deaths in 2012 primarily through diabetic complications in multiple organs including cardiovascular and cerebrovascular diseases, etc [2]. Control of diabetes and its complications has been a challenge in the health care system in many countries worldwide [3–6]. Obesity and aging are the major epidemic factors under the increased prevalence of type 2 diabetes. In both conditions, energy surplus is the common risk factor of type 2 diabetes [7]. Hepatic steatosis is a result of energy surplus in hepatocytes, known as fatty liver. As a result of lipid disorder in hepatocytes, the steatosis contributes to insulin resistance in type 2 diabetes. Fatty liver is considered a target in the control of type 2 diabetes. However, there is no effective medicine in the treatment of hepatic steatosis. Herbal medicines have an advantage in the treatment of chronic diseases with good safety, low toxicity, and less gastrointestinal responses. Application of herbal medicine is gradually increased worldwide in recent years [8–10].

Rosa rugosa Thunb, a member of Rosaceae, has a long history in the treatment of liver diseases and diabetes in the traditional Chinese medicine and Uygur medicine [11,12]. Xinjiang is one of the main cultivation areas of China's *Rosa rugosa* Thunb, which yields a higher content of active ingredient under the unique local climate. In previous studies, we have showed that a polyphenol-enriched extract of *Rosa rugosa* Thunb (RPE) from Xinjiang has activities in the treatment of diabetes in rat model established with high fat diet feeding in combination of STZ injection. The activity was observed with a reduction in TC, TG and free fatty acids in the plasma of rats [13], which suggests an activity of RPE in the control of hyperlipidemia [14–17]. The activity is consistent with the lipid-lowing activity of *Rosa rugosa* Thunb in rodents [18–20]. However, mechanism of the activity remains unknown for RPE. In this study, we investigated the mechanism of RPE activity with a focus on AMPK signaling activity in the liver of obese and diabetic rats.

2. Materials and methods

2.1. Materials

The antibodies to β-actin, p-ACC, ACC, p-AMPK, p-SIRT1 were obtained from the Cell Signaling Technology (USA). The antibodies to

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PGC-1α and FGF21 were purchased from Abcam (UK). The secondary antibody was obtained from Boster Biological Technology Co., Ltd (China). ECL was purchased from GE Healthcare (UK). BCA protein assay kit was brought from Thermo Scientific (USA). The kit of total cholesterol (TC), triglyceride (TG), alanine transaminase (ALT), aspartate transaminase (AST) lipoprotein lipase (LPL) and hepatic lipase (HL) were purchased from Nanjing Jiancheng Institute of Biotechnology (China). The mouse hepatocyte cell line Hepa-1c1c7 (1c1c7) was purchased from the American Type Culture Collection (ATCC) (CRL-2026™, Manassas, VA 20110).

2.2. Preparation of Rosa rugosa Thunb extract

Flowers of *Rosa rugosa* Thunb were collected from Hetian district, Xinjiang, China. The voucher specimen (Numb. 01397) was deposited in Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China. The flowers were air-dried and stored at room temperature prior use. The polyphenol-enriched extract was made according to the protocol described in a published study [13]. The polyphenol-enriched extract of *Rosa rugosa* Thunb was named RPE in this study.

2.3. Animals and treatment

A total of 60 male SD rats were divided into six groups in the experiment. After one week of quarantine on the regular chow diet (Research Diet, D12450B), fifty rats were fed on the high fat diet (HFD, Research Diet, D12492) for 6 weeks to establish diet-induced obesity (DIO). The rest 10 rats were kept on the chow diet as the normal control (NC). The DIO rats were injected once with STZ (30 mg/kg) to induce diabetes. After establishment of hyperglycemia for 2 weeks, the diabetic rats were orally treated with RPE at three dosages for 4 weeks between 9–12 weeks on HFD. The diabetic rats were divided into 5 groups: DC (diabetic control), DX (diabetic + 120 mg/kg xuezhikang), DL (Diabetic low dose at 37.5 mg/kg RPE), DM (diabetic mediate dose at 75 mg/kg RPE), DH (diabetic high dose at 150 mg/kg RPE). The experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Xinjiang Medical University. (Approved number: 2015006).

2.4. Biochemical analysis

The level of alanine transaminase (ALT) and aspartate transaminase (AST) were determined in the serum and liver of rats using the assay kits (Nanjing Jiancheng Institute of Biotechnology, China). The concentration of total cholesterol (TC), triglyceride (TG), lipoprotein lipase (LPL) and hepatic lipase (HL) were measured in liver tissues using the corresponding assay kits (Nanjing Jiancheng Institute of Biotechnology, China). The assays were conducted based on the instructions of the kits commercially available.

2.5. Cell culture

The mouse hepatoma cell line Hepa-1c1c7 (1c1c7) was maintained in Dulbecco's modified eagle's mediate (DMEM) supplemented with 10% fetal bovine serum at 37 °C in a 5% CO_2 incubator. The cells were treated with RPE after serum-starvation overnight in DMEM mediate containing 0.25% fatty acid-free bovine serum albumin.

2.6. Promoter assay

HEK293 cells were transfected with plasmid DNA of the FGF21-Luciferase reporter [21] and an internal control Renilla luciferase using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). The transfection experiment was performed based on the instructions of Lipofectamine 2000 kit. The reporter assay was conducted using the dual luciferase substrate system (E1501; Promega, Madison, WI), and the result was normalized with the internal control Renilla luciferase. Each experiment was repeated at least three times with consistent results.

2.7. mRNA expression

FGF21 mRNA expression was determined by quantative RT-PCR (qRT-PCR). Total RNA was prepared from the cells using TRIzol reagent (Invitrogen, Carlsbad, CA). FGF21 mRNA was determined with the SYBR green primer (forward 5 - CCTCTAGGTTTCTTTGCCAACAG - 3; reverse 5 - AAGCTGCAGGCCTCAGGAT - 3). The mRNA value was normalized to ribosome 18S RNA. SYBR Green Master Mix (4309155, Applied Biosystems) was used in the assay with the ABI 7900 machine.

2.8. Western blotting

The liver tissue was homogenized in a lysate buffer and the supernatant was collected as a protein extract after centrifugation. The protein concentration was determined using the BCA kit (Thermo Scientific, USA). Equal amount of protein from each rat was loaded onto SDS-PAGE to resolve the proteins by molecular size, and the proteins were then transferred to a PVDF membrane. The membrane was blocked with 5% skim milk for 1 h. The primary antibodies to β-actin, p-ACC, ACC, p-SIR, PGC-1α, p-AMPK and FGF21 were used to detect the corresponding signals overnight at 4 °C in 5% BSA buffer. The membranes were blotted with the secondary antibody for 1 h and the specific bands were detected with the enhanced chemiluminescent reagent (ECL).

2.9. Statistical analysis

The data are presented as the mean ± SE. The statistical difference between groups was evaluated in one-way ANOVA analysis and Student's *T*-test. GraphPad Prism (version 5.01) was used in the statistical analysis. $P < .05$ was considered significant.

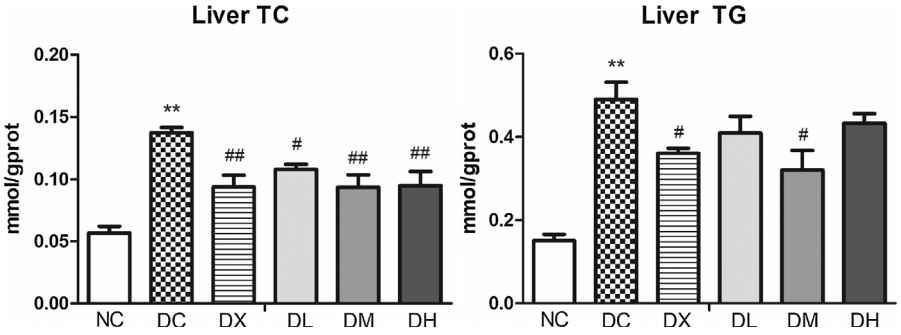

Fig. 1. Effect of RPE on total cholesterol (TC) and triglyceride (TG) in the liver of rats.
Values are presented as the mean ± SE (n = 10).
*p < .05, compared to the NC; **p < .01, compared to the NC; #p < .05, compared to the DC; ##p < .01, compared to the DC.
NC (normal control); DC (diabetic control); DX (diabetic + xuezhikang); DL (diabetic low dose at 37.5 mg/kg RPE); DM (diabetic mediate dose at 75 mg/kg RPE); DH (diabetic high dose at 150 mg/kg RPE).