Biomedicine & Pharmacotherapy 86 (2017) 605–611

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Effects of a standardized extract of *Rheum turkestanicum* Janischew root on diabetic changes in the kidney, liver and heart of streptozotocin-induced diabetic rats

Azar Hosseini[a], Hamid Mollazadeh[b], Mohammad Sadegh Amiri[c], Hamid Reza Sadeghnia[d], Ahmad Ghorbani[a,*]

[a] *Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran*
[b] *Department of Physiology and Pharmacology, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran*
[c] *Department of Biology, Payame Noor University, Tehran, Iran*
[d] *Department of Pharmacology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

ARTICLE INFO

Article history:
Received 30 October 2016
Received in revised form 12 December 2016
Accepted 15 December 2016

Keywords:
Diabetes
Kidney
Lipid
Malondialdehyde
Rheum turkestanicum

ABSTRACT

Numerus studies highlighted benefits of natural flavonoids in the management of diabetes. The present study was aimed to investigate the effects of a high flavonoids containing extract of *Rheum turkestanicum* on diabetic changes in different tissues. Male Wistar rats were divided into normal and streptozotocin-induced diabetic groups received saline or hydroalcoholic extract of *R. turkestanicum* root (100, 200 and 300 mg/kg) through orogastric gavage for 4 weeks. Serum glucose, HbA1c, lipids, creatinine, uric acid, liver enzymes (alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase), and cardiac enzymes (lactate dehydrogenase and creatine phosphokinase) in diabetic control group were significantly higher compared to normal control group ($p < 0.001$). The extract significantly reduced these factors, increased body weight, and improved both glucosurea and proteinuria. Lipid peroxidation was high in the liver of diabetic rats compared to normal rats ($p < 0.001$) and reverted toward control values by *R. turkestanicum*. Also, the extract significantly protected the liver, kidney, and heart of diabetic rats against histopathological changes. In conclusion, *R. turkestanicum* inhibited the development of nephropathy, liver injury, and myocardial damage in diabetes by lowering the serum levels of glucose and lipids, and by inhibiting oxidative stress mediated lipid peroxidation.

1. Introduction

Diabetes mellitus is a chronic metabolic disease, which occurs either when pancreatic beta-cells are not able to produce and secrete sufficient insulin (type 1 diabetes) or when the body is not able to effectively use the insulin (type 2 diabetes). According to the World Health Organization, in 2014 the global prevalence of diabetes was approximately 9% among adults and in 2030 diabetes will be the 7th leading cause of mortality in the world. Over time, diabetes leads to micro vascular complications such as retinopathy and nephropathy, and macro vascular complications such as heart attacks and stroke [1,2]. Despite advances in the understanding the pathogenesis of diabetes and in the control of blood glucose with insulin and oral hypoglycemic drugs, the morbidity and mortality of the disease are still high. Therefore, studies for finding new antidiabetic agents are continued. In this regard, development of antidiabetic drugs from medicinal plants with a history of folk-usage in treating diabetes is of great interest. Numerous experimental and clinical studies have documented beneficial effects of medicinal plants for managing diabetes [3,4].

Recent experimental studies have shown that some medicinal plants in genus of *Rheum* (family: Polygonaceae) have anti-hyperglycemic activity and assist in the prevention of the complications of diabetes [5–7]. Plants in genus of *Rheum* are rich sources of flavonoids and numerus studies highlighted benefits of natural flavonoids in the management of blood glucose and diabetic complications [8–10]. *Rheum turkestanicum* Janisch is a plant in genus of *Rheum* that grows widely in central Asia and also

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; DTNB, 2,20-dinitro-5,50-dithiodibenzoic acid; FBG, fasting blood glucose; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein.

* Corresponding author at: Pharmacological Research Center of Medicinal Plants, Faculty of Medicine, Mashhad University of Medical Sciences, Iran.
E-mail address: ghorbania@mums.ac.ir (A. Ghorbani).

http://dx.doi.org/10.1016/j.biopha.2016.12.059
0753-3322/

in north-east of Iran. Traditionally, people in north-east of Iran use the root of this plant for treatment of hypertension, diabetes, and cancer [11]. In spite of the traditional use of *R. turkestanicum* as an antidiabetic remedy, there is no experimental evidence for anti-diabetic effect this plant up till now. Therefore, the present study was aimed to investigate the possible beneficial effects of *R. turkestanicum* root on diabetic changes in the kidney, liver and heart in streptozotocin induced hyperglycemic rats.

2. Materials and methods

2.1. Chemicals and reagents

Ethylenediaminetetraacetic acid and 2,20-dinitro-5,50-dithiodibenzoic acid (DTNB) were purchased from Sigma (USA). Streptozotocin was obtained from Enzo Life Sciences (USA). Diethyl ether, 2-thiobarbituric acid, trichloroacetic acid, phosphoric acid, tris (hydroxymethyl) aminomethane, and Folin-Ciocalteu's phenol reagent were purchased from Merck (Germany).

2.2. Plant material and extraction

The roots of *R. turkestanicum* were collected from Kalat region (Razavi Khorasan Province, Iran) and identified at the herbarium of Ferdowsi University of Mashhad (voucher specimen number 42,082). The hydroalcoholic extract of *R. turkestanicum* was prepared by maceration method as described previously [12]. Briefly, the air-dried powder of the roots was suspended in 70% ethanol (10 mL per g of root) and maintained for 72 h at 40 °C with gentle shaking. In order to remove the solvent, the extract was dried on a water bath and the yield was kept at −20 °C until use.

2.3. Determination of the total phenol content in the extract

Total phenolic content of *R. turkestanicum* root extract was determined by the Folin-Ciocalteu method. Samples of extract (50 μL of 10 mg/ml) or gallic acid were transferred to test tubes containing 4 mL distilled water and 250 μL Folin-Ciocalteau reagent. Then, 1.25 mL sodium carbonate solution (1 M) was added to each tube and the volume of tubes was completed to 10 mL with distilled water. The mixtures were incubated at room temperature in the darkness for 40 min. Absorbance of samples was measured at 715 nm and calibration curve was constructed for gallic acid.

2.4. Animals

Adult male Wistar rats (220–270 g) were purchased from Laboratory Animals Research Center, Mashhad University of Medical Sciences. They were housed in a temperature-controlled environment (22 ± 4 °C) with a 12 h dark/light cycle. The animals had free access to normal laboratory chow and tap water *ad libitum*. All experimental procedures were carried out in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of Mashhad University of Medical Sciences.

2.5. Experimental design

Thirty rats were randomly divided into the following 5 experimental groups, each group including 6 animals: group 1 included normal control rats; group 2 comprised diabetic control animals; groups 3, 4 and 5 included diabetic animals which received the extract of *R. turkestanicum* at doses of 100, 200 and 300 mg/kg, respectively. Considering body surface area for dose translation, doses 100, 200 and 300 mg/kg of *R.turkestanicum* in rat approximately are comparable to the doses of 16, 32 and 48 mg/kg in human, respectively [13].

Diabetes was induced by a single dose of streptozotocin (65 mg/kg, ip). Development of hyperglycemia was confirmed by measuring fasting blood glucose (FBG) 3 days after streptozotocin injection. Rats with FBG level of 200 mg/dL or higher were considered to be diabetic. Treatments with the extract were started 3 days after streptozotocin injection and lasted for 4 weeks. The extract was suspended in water and administrated through orogastric gavage once daily, and animals in control groups received saline as vehicle. Body weight was recorded before treatment and at the end of experiment. Also, 24-h urine samples were collected using metabolic cages to measure glucose and protein excretion. At the end of the 4th week, the animals were fasted 12 h, anesthetized with diethyl ether, and blood samples were taken out by cardiac puncture for biochemical measurements. The left kidney, heart and liver samples were homogenized in cold KCl solution (1.5%, pH 7) to give a 10% homogenate suspension and used for organs' biochemical analysis.

2.6. Biochemical analysis

Fasting blood samples were used for analysis of biochemical parameters reflecting metabolic status and function of liver, kidney and heart. The level of glucose, HbA1c, triglyceride, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) was measured with commercially available standard kits to monitor glycemic and lipid status. Markers of liver functions were alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Concentration of creatinine was evaluated as marker of kidney function. The activity of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) was measured as indexes of cardiac damage. The level of HbA1c was determined by chromatography. The levels of glucose and protein in urine were measured by glucose oxidase assay and turbidimetry method, respectively.

2.7. Lipid peroxidation in the left kidney, heart and liver

The lipid peroxidation level in the left kidney, heart and liver was evaluated by measured malondialdehyde (MDA). The MDA is end product of lipid peroxidation and reacts with thiobarbituric acid to produce a red-colored complex with peak absorbance at 532 nm. In our experiment, 3 mL phosphoric acid (1%) and 1 mL thiobarbituric acid (0.6%) were added to 0.5 mL of tissue homogenate and the mixture was heated for 45 min in a boiling water bath. Then, 4 mL of *n*-butanol was added to the mixture, vortexed for 1 min, and centrifuged at 20,000 rpm for 20 min. The organic layer was transferred to a fresh tube and its absorbance was measured at 532 nm. Concentration of MDA was calculated using its standard curve and the content of MDA was expressed as nano moles per gram of tissue [14,15].

2.8. Histological examination

The heart, liver and right kidney of control and treated groups were fixed in 10% formalin. The tissues samples were dehydrated with ethanol, cleared in xylene, and embedded in paraffin. The samples were then cut into sections of 5 μm in thickness and stained with haematoxylin and eosin dye. Histopathological changes were examined under phase contrast microscope (Leica DMRB, India) and photographs were taken using a Canon PowerShot S70 digital camera (Canon, Japan).