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Naringin enhances reverse cholesterol transport in high fat/low streptozocin induced diabetic rats

Solomon Oladapo Rotimi*, Isaacson Bababode Adelani, Goodness Esther Bankole, Oluwakemi Anuoluwapo Rotimi

Department of Biochemistry and Molecular Biology Research Laboratory, Covenant University, Ota, Nigeria

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ABSTRACT

Naringin, a citrus-derived flavonoid with antihyperglycemic, antihyperlipidemic, and antioxidant properties, is reported to be a useful nutraceutical in the management of diabetes and its complications. This study investigated the mechanism of antiatherogenic properties of naringin in type 2 diabetes (T2DM) using high fat-low streptozocin rat model of T2DM. Rats were treated daily with 50, 100 and 200 mg/kg naringin orally for 21 days. Levels of biomarkers of T2DM, lipid profile and activity of paraoxonase (PON) were assayed spectrophotometrically. The levels of expression of hepatic 3-hydroxy-3-methyl-glutaryl-CoA reductase (*Hmgcr*), scavenger receptor class B member 1 (*Scarb1*), aryl hydrocarbon receptor (*Ahr*), hepatic Lipase (*Lipc*), and lecithin-cholesterol acyltransferase (*Lcat*) were assessed using relative reverse transcriptase polymerase chain reaction technique. Naringin treatment resulted in a dose-dependent significant ($p < 0.05$) decrease in the levels of plasma cholesterol and triglyceride from 84.84 ± 1.62 to 55.59 ± 1.50 mg/dL and 123.03 ± 15.11 to 55.00 ± 0.86 mg/dL, respectively, at 200 mg/kg naringin. In the liver, *Scarb1* and *Ahr* were significantly ($p < 0.05$) upregulated at 200 mg/kg naringin while *Lipc* and *Lcat* were significantly ($p < 0.05$) upregulated by 50 mg/kg naringin. T2DM-induced decrease in PON activities in the plasma, liver and HDL was significantly ($p < 0.05$) reversed by 200 mg/kg naringin treatment. These genes play critical roles in reverse cholesterol transport and hence our results showed that the antiatherogenic property of naringin in T2DM involves enhancement of reverse cholesterol transport and PON activity.

1. Introduction

Type 2 diabetes mellitus (T2DM) is the most prevalent form of diabetes and it is characterized by hyperglycemia associated with insulin resistance and impairment in insulin secretion with concomitant alteration in the intermediary metabolism of carbohydrate, protein and lipid [1,2]. The prevalence of T2DM has continued to be on the rise as a result of increase in obesity, which has been attributed to change in lifestyle and diet. Hence, the developing countries are expected to experience the greatest increase in the morbidity and mortality of this disease by the year 2025 [3].

T2DM is known to be associated with major complications like atherosclerotic coronary heart disease, cardiomyopathy, stroke and nephropathy. These complications are often responsible for increased mortality due to T2DM and they are associated with dyslipidemia and hypertension [2]. For example, atherosclerotic coronary heart disease is promoted by lipoprotein abnormalities which involve elevated very low density lipoproteins (VLDL) cholesterol and low high density lipoprotein (HDL) cholesterol [4]. Although the pathogenesis of atherosclerosis is complex, its development is dependent on the oxidation of LDL. This oxidation is prevented by paraoxonases, which are antioxidant protein component of lipoproteins, and are considered as better predictors of atherosclerotic risk than HDL in diabetes [5,6].

Although T2DM is a chronic disease, its management often involves control of both short-term and long-term diabetes-related problems [7]. Hence, the reduction of hyperglycemia and the risk of long-term complications is the target of any effective treatment regimen.

Although, there are a number of anti-diabetic pharmacological agents; they are however, limited by their unwanted side effects [8]. Thus, there has been a growing interest in nutraceuticals and functional

Abbreviations: T2DM, type 2 diabetes mellitus; PON, paraoxonase; *Hmgcr*, hepatic 3-hydroxy-3-methylglutaryl-CoA reductase; *Scarb1*, scavenger receptor class B member 1; *Ahr*, aryl hydrocarbon receptor; *Lipc*, hepatic Lipase; *Lcat*, lecithin-cholesterol acyltransferase; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoprotein; STZ, streptozotocin; HFD, high fat diet; DPP-IV, dipeptidyl peptidase-4; ELISA, enzyme linked immunosorbent assay; CPT, carnitine palmitoyl transferase; ACE, angiotensin converting enzyme; RT-PCR, reverse transcriptase polymerase chain reaction; GSP, gene specific primers

* Corresponding author at: Department of Biochemistry, Covenant University, Ota, Nigeria.
E-mail address: Ola.rotimi@covenantuniversity.edu.ng (S.O. Rotimi).

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food that can be useful in the management of T2DM and its associated complications.

Citrus is one of such functional foods that has been proposed to be beneficial in the management of diabetes mellitus and naringin is one of its active phytochemicals [9]. Naringin (PubChem CID: 442,428), a flavone glycoside, and aglycone of naringenin that gives grapefruit its bitter taste, has been reported for its antihyperglycemic, antioxidant and antihyperlipidemic properties [9,10]. For instance, Xulu and Oroma Owira [11] showed that naringin could ameliorate dyslipidemia in type I diabetic rats while Choi et al [10] further demonstrated the antioxidant effect of this flavonoid in rats fed with high cholesterol diet. Subsequently, the potential of naringin to improveT2DM in rats was demonstrated by Parmar et al. [12] using *in silico*, in vitro and in vivo inhibition of a biomarker of T2DM. Recently, Pari and Chandramohan [13] showed that the anti- T2DM property of naringin is due to the modulation of key carbohydrate metabolism enzymes in type 2 diabetic rat model.

However, more detailed studies are still needed to understand the mechanism by which naringin improves T2DM and prevents its associated complications. This present study therefore seeks to investigate the effects of naringin on a high fat fed/streptozotocin-induced T2DM rat model.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) was a product of Sigma-Aldrich (St. Loius, MO, USA) while Metformin was a product of Swipha Pharma, Lagos, Nigeria. Naringin was a product of Human Kang Biotechnology Company, Human Province, China. RNA later and RNA extraction spin column kits were products of Aidlab Biotechnologies Co. Ltd (Beijing, China) while TransGen Easy Script one-step RT-PCR kit was a product of TransGen Biotech Co. Ltd (Beijing, China). All other chemicals were products of Sigma-Aldrich (St. Loius, MO, USA).

2.2. Animals

Thirty male inbred albino rats weighing between 150 and 200 g were used for this study. The rats were housed in specific pathogen-free polypropylene cages under normal temperature (22 ± 2 °C) with 12-h light and dark cycle. The animals were allowed to acclimatize for three weeks before commencement of the experiment. The experiment was approved by the Covenant University Ethical Committee (CU/BIOSC-RECU/BIO/2015/004) and carried out according to the guidelines of the committee.

2.3. Experimental design

The rats were randomly divided into six groups of five rats each. Five of the groups were maintained on a high fat diet (HFD), which contained 45% fat (Table 1), throughout the period of the experiment (9 weeks) while the last group was maintained on normal pellet diet and served as the normal control. After 4 weeks, the HFD fed groups were given a low dose (35 mg/kg body weight) of STZ intraperitoneally while the normal control group was administered the vehicle (0.1 mL/kg body weight of 0.1 M citrate buffer), as described by Zhang et al. [14]. Another dose of STZ was administered at week 6 of the experiment, after which fasting blood glucose was checked and naringin was then administered orally for 21 days. The rats were grouped as shown below;

Group A: Diabetic Control: Rats fed HFD

Group B: Diabetic rats treated with 50 mg/kg naringin: Rats fed HFD

Group C: Diabetic rats treated with 100 mg/kg naringin: Rats fed HFD

Group D: Diabetic rats treated with 200 mg/kg naringin: Rats fed HFD

Table 1
Composition of diet.

Component	Level (g/100 g) in diet	
	Normal diet	High fat diet
Fish meal	25	25
Sucrose	10	10
Corn starch	49.5	4.5
Vegetable oil	5	5
Salt/mineral mix*	5.5	5.5
Cellulose	5	5
Tallow	–	45

* Salt/mineral mix contains the following (in g/100 g): calcium phosphate, 49.50; sodium powder, 11.80; potassium sulfate, 5.20; sodium chloride, 7.40; magnesium oxide, 2.40; potassium citrate, 22.40; ferric citrate, 0.60; manganous carbonate, 0.35; cupric carbonate, 0.03; zinc carbonate, 0.16; chromium potassium sulfate, 0.055; potassium iodate, 0.001; sodium selenate, 0.001; choline chloride, 0.50; thiamine HCl, 0.06; riboflavin, 0.06; niacin, 0.30; calcium pantothenate, 0.16; biotin, 0.01; vitamin B12, 0.10; vitamin D3, 0.025; vitamin E acetate, 1.00; pyridoxine, 0.07; folic acid, 0.02; vitamin A acetate, 0.08.

Group E: Diabetic rats treated with 50 mg/kg metformin: Rats fed HFD

Group F: Normal Control: Rats fed normal diet

Twenty-four hours after the last dose of naringin, the rats were euthanized under light ether anesthesia. Blood was collected from the anaesthetized animals by cardiac puncture while liver and kidney were also excised for biochemical and molecular analysis. The blood and organs were processed as previously described by Rotimi et al. [15], while portions of the liver were stabilized in RNAlater® for RNA analysis.

2.4. Biochemical analysis

Glucose, bicarbonate, α-amylase and α-hydroxyl butyrate dehydrogenase were determined spectrophotometrically in the plasma using commercially available kits (BioSino Biotechnology & Science Inc., Changping District Beijing, China) while insulin and dipeptidyl peptidase-4 (DPP-IV) were determined using enzyme linked immunosorbent assay (ELISA) kits (Hangzhou Eastbiopharm Co., Ltd. Hangzhou, China).

2.5. Plasma lipid profiles

Plasma cholesterol and triacylglycerols were determined spectrophotometrically using commercially available kits according to manufacturer's instructions. HDL and HDL_3 were obtained from the plasma using the dextran sulfate – $MgCl_2$ precipitation method as described by Rifai et al. [16]. The supernatant obtained after centrifugation contained the HDL and HDL_3, while the precipitate contained VLDL and $VLDL_3$ respectively. Free fatty acid was determined spectrophotometrically as described by Rotimi et al. [17].

2.6. Liver lipid profiles

Lipids were extracted from the liver according to the method of Folch et al. [18] and aliquots of the extract were used for determining cholesterol and triacylglycerol concentrations as previously described by Rotimi et al. [19].

2.7. Determination of paraoxonase activity

Paraoxonase was determined in the plasma, HDL, HDL_3, VLDL, $VLDL_3$ and liver homogenate as described by Afolabi et al. [20]. Briefly, phenylacetate was prepared freshly in 100 mM Tris-acetate buffer pH