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Original article

Antihyperlipidemic effect of coumarin in experimental type 2 diabetic rats

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ABSTRACT

The present study was aimed to examine the hypolipidemic effect of coumarin on streptozotocin–nicotinamide induced type 2 diabetic rats. Diabetes mellitus was induced by single intraperitoneal injection of 45 mg/kg streptozotocin, 15 min after the intraperitoneal administration of 110 mg/kg nicotinamide. Streptozotocin–nicotinamide induced diabetic rats showed a significant increase in the levels of plasma and tissue (liver and kidney) lipids (total cholesterol, triglycerides, free fatty acids, phospholipids), LDL, VLDL and a significant decrease in the levels of HDL were observed. A significant increase in the activity of HMG-CoA reductase in tissues and significant decrease in the activities of LPL and LCAT in plasma were observed in type 2 diabetic rats. After oral administration of coumarin to diabetic rats, were found to alleviate the lipid profiles and lipid metabolizing enzymes. Conclusively, oral treatment of coumarin exhibited in a marked antihyperlipidemic effect against diabetes mellitus.

1. Introduction

Diabetes mellitus is currently one of the most severe and incurable chronic diseases and is characterized by hyperglycemia with derangement in carbohydrate, protein and lipid metabolism. The abnormalities of lipid profiles are involved in alterations of regulatory process, mainly defects in insulin secretion and action which may cause hypercholesterolemia and hypertriglyceridemia [1].

Type 2 diabetes mellitus is a major compliant in the world and the people having complications like hypertension, atherosclerosis and microcirculatory disorders [2]. During diabetes mellitus, liver plays pivotal role in glucose and lipid homeostasis such as oxidation and metabolic conversion of free fatty acids, cholesterol and phospholipid synthesis and the secretion of plasma lipoproteins [3]. Dyslipidemia affects people in diabetes by the alteration in triglycerides, LDL-C and HDL-C levels which may produce cardiovascular and cerebrovascular diseases.

Medicinal plants have been found to be useful to manage hyperlipidemia and recommended in the complementary and alternative system of medicine for treatment of diabetes mellitus [4]. Medicinal plants and their active phytochemicals exhibit delay the development of diabetic complications and correct the metabolic abnormalities.

Coumarin (1,2-benzopyrone; Fig. 1), the parent molecule of its derivative, is the simple compound of large class of naturally-obtaining phenolic substances. It was first isolated from tonka beans and is found naturally from human dietary fruits and vegetables such as cassia, lavender, cinnamon, melilot, green tea, peppermint, celery, bilberry, honey, carrots and other foodstuffs [5]. Coumarin is known to have antioxidant potential like vitamin E (a-tocopherol) and has lipid-lowering effect [6,7]. Coumarin is also a potent chemopreventative agent that reduces nephrotoxocity [8] and inhibits mammary tumor formation in rats [9]. In our previous studies, we have reported that administration of coumarin has antidiabetic [10], antioxidant effects [11,12], and also reduce the changes in glycoprotein components [13] in streptozotocin–nicotinamide induced diabetic rats. To our knowledge, there is no available experimental evidence on antihyperlipidemic effect of comarin in streptozotocin–nicotinamide induced type 2 diabetes. Therefore, the present work was undertaken to evaluate the antihyperlipidemic effect of coumarin in experimental type 2 diabetic rats.

Abbreviations: HDL, high-density lipoproteins; HMG-CoA, 3-hydroxy 3-methylglutaryl coenzyme A; LDL, low density lipoproteins; LPL, lipoprotein lipase; LCAT, lecithin cholesterol acyl transferase; VLDL, very low-density lipoproteins.

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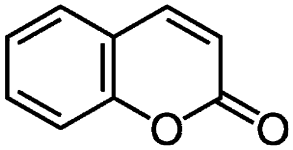

Fig. 1. Structure of coumarin.

2. Materials and methods

2.1. Animals

Healthy adult male albino Wistar rats (weighing 200–220 g) were used for this investigation. The animals were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University. The animals were maintained under standard conditions (12 h light/dark cycle) and temperature (28 ± 2 °C) with relative humidity (45–55%). The animals were fed with a balanced commercial diet (Hindustan Lever Ltd, Mumbai, India) and purified drinking water *ad libitum*. All the experimental procedures conducted after the approval of Animal Ethical Committee (Reg. No. 160/1999/CPCSEA; vide No.565, 2008) Annamalai University. The care and handling of the animals were carried out in the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

2.2. Drugs and chemicals

Streptozotocin and coumarin was purchased from the Sigma Chemical Co., St. Louis, MO, USA. Nicotinamide was purchased from Himedia, Mumbai, India. All the chemicals and solvents were of analytical grade and procured from Himedia and SD fine chemicals Ltd., Mumbai, India.

2.3. Induction of experimental diabetes

Streptozotocin was dissolved in 0.1 M cold citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg), 15 minutes after the intraperitoneal administration of 110 mg/kg of nicotinamide [14]. Hyperglycemia was confirmed by the elevation of plasma glucose in rats determined at 72 hrs and the animals, which have plasma glucose more than 250 mg/dL were considered as diabetic and used for this antihyperlipidemic study.

2.4. Experimental protocol

The experimental rats were randomly divided into the following four groups, each group containing six animals.

Group 1	Normal control (vehicle treated)
Group 2	Normal rats received coumarin (100 mg/ kg body weight) dissolved in corn oil for 45 days [10]
Group 3	Diabetic control
Group 4	Diabetic rats received coumarin (100 mg/ kg body weight) dissolved in corn oil for 45 days [10]

After 45 days the experimental period was completed, the animals were anaesthetized and sacrificed by cervical dislocation. The anticoagulant agent of ethylene diamine tetra acetic acid was taken in the test tubes and blood samples were collected. The tubes were centrifuged at 20 mins (2000 × g) for the separation of plasma and used for estimation of lipid parameters. Liver and kidneys were quickly excised off, washed in ice-cold saline and patted dried. The tissues were used for the extraction of lipids.

2.5. Lipid extraction

The extractions of plasma and tissue lipids were done by the method of Folch et al. [15] using chloroform: methanol mixture (2:1, v/v). The known weight of tissues were rinsed in cold physiological saline and dried by the use of filter paper. The test samples were homogenized in cold chloroform–methanol mixture (2:1, v/v) and the contents were extracted after 24 h. This process was repeated for four times. The non-lipid components were washed by using 0.7% of potassium chloride (0.1 N). The supernatant was pooled and made up to a specified volume with chloroform and aliquot of this extract was then used for the estimation of cholesterol, free fatty acids, triaceylglycerol and phospholipids.

2.6. Estimation of lipids

The total cholesterol was estimated from the lipid extract and plasma by using the method of Zlatkis et al. [16]. To 0.1 mL of the plasma/lipid extract was evaporated to dryness and 5 mL of ferric chloride acetic acid reagent and 3 mL of concentrated sulfuric acid was added. After 20 min, the colour developed was read at 560 nm in spectrophotometer. Foster and Dunn's [17] method was used for the estimation of triglycerides levels. An aliquot 0.5 mL of plasma/ lipid extract was evaporated to dryness and added 0.1 mL of methanol followed by 4 mL of isopropanol and 0.4 g of alumina was added, shaken well for 15 min, and centrifuged. 2 mL of supernatant was treated with 0.6 mL of the saponification reagent (5 g of potassium hydroxide in 60 mL of distilled water and 40 mL of isopropanol) and 0.5 mL of acetyl acetone reagent (40 mL of isopropanol and 0.75 mL of acetyl acetone in 60 mL of distilled water) were mixed and then saponified by kept in a water bath at 65 °C for 1 h. The absorbance was read spectrophotometrically at 405 nm.

Non-esterified free fatty acids were estimated by the method of Falholt et al. [18]. 0.1 mL of lipid extract was evaporated to dryness. To the residue dissolved in 1 mL of phosphate buffer (33 mM, pH 6.4), 6 mL of chloroform–heptane–methanol solvent (5:5:1), and 2.5 mL of copper reagent (10 mL of copper solution was mixed with 10 mL of triethanolamine and 6 mL of sodium hydroxide and made up to 100 mL. Add 33 g of sodium chloride) were added. The tubes were shaken vigorously, treated with 200 mg of activated silicic acid and kept aside for 30 min and centrifuged (3000 × g). To 3 mL copper layer was transferred to another tube containing 0.5 mL of diphenyl carbazide and mixed carefully. The absorbance was read spectrophotometrically at 550 nm. The phospholipid estimation was done by the method of Zilversmit and Davis [19]. An aliquot (0.5 mL) of the lipid extract was evaporated to dryness, add 1 mL of 5 N sulfuric acid and kept in a digestion rack till the appearance of light brown colour. Add 2–3 drops of concentrated nitric acid and digested to a colorless solution. After cooling, 1 mL of water was added and heated in a boiling water bath for 5 min. Then, 1.0 mL of 2.5% ammonium molybdate and 0.1 mL of amino naphthol sulphonic acid (500 mg of amino naphthol sulphonic acid was dissolved in a mixture of 195 mL of 15% sodium bisulphate and 5 mL of 20% sodium sulphite solution) were added. The volumes of the tubes were made up to 5 mL with distilled water and the absorbance was measured spectrophotometrically at 660 nm with in 10 min.

2.7. Determination of lipoprotein fractions

HDL-C fraction was separated by the method of Burnstein et al. [20] and the cholesterol content was determined by method of Zlakis et al.[16]. 1 mL of plasma was added to 0.18 mL of heparin-manganese chloride reagent and mixed. The solution was allowed to stand at 4 °C for 30 min and then centrifuged in a refrigerated centrifuge at 1800 g for 30 min. The supernatant of the HDL-C