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Original article

Antihyperglycemic effect of syringic acid on attenuating the key enzymes of carbohydrate metabolism in experimental diabetic rats

Subramani Srinivasan[a,*], Jayachandran Muthukumaran[b], Udaiyar Muruganathan[a], Rantham Subramaniyam Venkatesan[b], Abdulkadhar Mohamed Jalaludeen[c]

[a] Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608002, Tamilnadu, India
[b] Postgraduate and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, G.B. Nagar, Kalavai, Vellore District 632506, Tamil Nadu, India
[c] Department of Animal and Food Bioscience, College of Natural Science, Konkuk University, Danwoldong, Chungju, South Korea

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ABSTRACT

Diabetes mellitus is one of the most common endocrine entities, which coexist with defect in carbohydrate metabolism. The Indian traditional system of medicine prescribed plant phytochemical therapies for diseases including diabetes mellitus. The present study was aimed to evaluate the therapeutic potential of syringic acid (SA) by assaying the activities of key enzymes of carbohydrate metabolism in experimental diabetic rats. Diabetes was induced into male albino Wistar rats by intraperitoneal administration of alloxan (150 mg/kg). SA was administered to diabetic rats intragastrically at 25, 50 and 100 mg/kg b.w daily once for 30 days. The levels of plasma glucose, insulin, hemoglobin (Hb), glycated hemoglobin (HbA_{1c}) and glycogen, levels of carbohydrate metabolic enzymes, liver and kidney markers were evaluated. Oral administration of SA (50 mg/kg) for 30 days, dose dependently improved the glycemic status in diabetic rats. The levels of insulin, Hb and glycogen increased with significant decrease in glucose and HbA_{1c} levels in SA treated rats. The altered activities of carbohydrate metabolic enzymes, hepatic and renal marker were restored to near normal. Histopathological analysis of pancreas revealed that treatment with SA reduced the pancreatic damage induced by alloxan and stimulated β-cell regeneration in diabetic rats. The present findings suggest the antihyperglycemic effect of SA and its therapeutic potential for the management of diabetes.

1. Introduction

Diabetes mellitus, a modern-day epidemic, is precisely recognized as a global public health concern and is characterized by chronic hyperglycemia due to abnormal insulin secretion or insulin receptor events affecting metabolism of carbohydrate, protein, and fats [1]. In diabetes the activities of key enzymes of carbohydrate metabolism were markedly altered due to impaired insulin secretion and action thus producing hyperglycemia. The epidemiological studies strongly support the impression that hyperglycemia leads to long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels, and creates a huge economic burden related to the management of diabetic complications [2].

The most recent global estimates posit that there were 285 million people living with diabetes worldwide in 2010 and that this number is projected to reach 438 million by 2030 [3]. Carbohydrates from various dietary sources are the primary exogenous source of glucose. Glucose is the main fuel for energy requirement of the body. Therefore, a continuous supply of glucose is necessary to ensure proper function and survival of all organs. Glucose homeostasis depends on the balance between their formation and utilization by major peripheral tissues namely liver. This process is usually severely altered during diabetes. Glucose over- or under-utilization is of critical importance during diabetes, since it leads to the accumulation of cellular glucose and glucose toxicity, ultimately contributing to the severe complications associated with diabetes [4]. The enzymes that regulates hepatic glucose metabolism are potential targets for controlling endogenous glucose production and thereby blood glucose levels in diabetes.

Alloxan is known for its selective pancreatic islet β-cells cytotoxicity and has been extensively used to induce a hyperglycemic state in rats. Despite remarkable advances made in the management of diabetes by the use of synthetic drugs, there has been a renewed interest phytochemicals identified from traditional medicinal plants. They are presenting an exciting opportunity for

* Corresponding author. Tel.: +91 04144 239343; fax: +91 04144 239141.
E-mail addresses: sivaseenu77@gmail.com, seenu77@gmail.com (S. Srinivasan).

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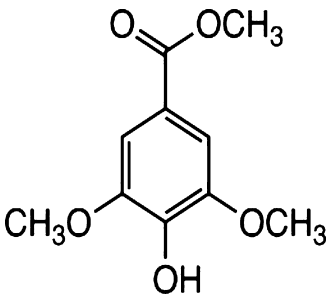

Fig. 1. Chemical structure of syringic acid.

the development of new types of therapeutics. This has accelerated the global effort to harness and harvest those medicinal plants that bear substantial amount of potential phytochemicals showing multiple beneficial effects in combating diabetes and diabetes related complications [5]. Phenolic acids are secondary metabolites, which are commonly found in many vegetables and fruits. Many epidemiological studies have found that the consumption of foods and drinks with high phenolic content is associated with the prevention of many diseases including diabetes [6]. Syringic acid (SA) (4-hydroxy-3, 5-dimethoxybenzoic acid; Fig. 1) is an active phenolic compound isolated from *Alpiniacalcarata Roscoe*. The leaves of *A. Roscoe* are a well-known traditional Indian ayurveda medicine and have been used to treat diabetes. SA shows strong antioxidant, antiproliferative [7], anti-endotoxic, [8], anti-cancer activity [9] and hepatoprotective activity [10].

The objective of the present study is to determine the antihyperglycemic property of SA in assessing the activities of key enzymes of carbohydrate metabolism in experimental diabetes. These findings will contribute to the understanding of the mechanisms of action of this phenolic compound and may shed light on novel targets for the development of new hypoglycemic drugs.

2. Materials and methods

2.1. Chemicals

Alloxan and SA were purchased from Sigma Chemical Co. (St. Louis, Mo. USA). All other chemicals and solvents were of analytical grade and purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India.

2.2. Animals

Male albino Wistar rats (200–220 g) were breed in the Central Animal House, Adhiparasakthi College of Arts and Science, Kalavai, Tamilnadu, used in this study. The rats had free access to water and a commercial standard pelleted diet (Lipton India Ltd., Mumbai, India). The animals were housed in standard polypropylene cages and maintained under controlled room temperature ($22 \pm 2\,^{\circ}C$) and humidity ($55 \pm 5\%$) with 12:12 h light and dark cycle. The animal experiment was designed and performed in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (registration No. 1282/ac/09/CPCSEA).

2.3. Induction of diabetes

Diabetes was induced in 12 h fasted experimental rats by a single intraperitoneal injection of alloxan (150 mg/kg b.w) dissolved in freshly prepared sterile normal saline. Alloxan injected animals were allowed to drink 20% glucose solution overnight to overcome the initial drug-induced hypoglycemic mortality. Control rats were injected with same volume of saline alone. The diabetic state was assessed by measuring non-fasting plasma glucose concentration 72 h after alloxan treatment. The rats with a plasma glucose level above 250 mg/dL, as well as with polyuria, polydipsia, and polyphagia were selected for the experiment.

2.4. Experimental design

The animals were randomly divided into six groups of six animals in each group (24 diabetic surviving and 12 normal). SA was dissolved in vehicle solution of 0.9% saline and administered daily once to experimental rats:

- group I: normal control (vehicle treated);
- group II: normal rats received SA (100 mg/kg b.w) intragastrically dissolved in 1 mL of 0.9% saline for 30 days;
- group III: diabetic control;
- group IV: diabetic rats received SA (25 mg/kg b.w) intragastrically dissolved in 1 mL of 0.9% saline for 30 days;
- group V: diabetic rats received SA (50 mg/kg b.w) intragastrically dissolved in 1 mL of 0.9% saline for 30 days;
- group VI: diabetic rats received SA (100 mg/kg b.w) intragastrically dissolved in 1 mL of 0.9% saline for 30 days.

The initial and final body weight of the rats in each group was recorded. At the end of the experimental period, the animals were fasted overnight, anesthetized using ketamine hydrochloride (24 mg/kg b.w, intramuscular injection) and sacrificed by cervical decapitation. Blood samples collected in dry test tubes were allowed to coagulate at ambient temperature for 30 min centrifugation at $2000 \times g$ for 10 min used for the estimation of serum ALT, AST and ALP. Blood samples were collected in tubes containing potassium oxalate and sodium fluoride (3:1) mixture used for the estimation of plasma glucose and insulin. Hemoglobin (Hb) and glycosylated hemoglobin (HbA_{1c}) levels were estimated in whole blood samples. Liver and kidney was immediately dissected, washed in ice-cold saline to remove the blood.

2.5. Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed according to the method of [11] after overnight fasting, "0" minute blood sample (0.2 mL) was taken from control and experimental rats. Without delay, a glucose solution (2 g/kg b.w) was administered by oral gavage. Blood samples were taken at 30, 60, 90 and 120 min after glucose administration. Blood samples were collected with potassium oxalate and sodium fluoride and glucose levels were determined by the method of Trinder [12].

2.6. Biochemical estimations

Plasma glucose was estimated by using a commercial kit (Sigma Diagnostics Pvt. Ltd., Baroda, India) by the method of Trinder [12]. Hemoglobin (Hb) and glycosylated hemoglobin (HbA_{1c}) were estimated by diagnostic kit (AgappeDiagnostic Pvt. Ltd., India) [13], respectively. The plasma was separated and used for the assay of insulin using RIA assay kit (for rats) supplied by Linco Research, Inc. (USA). A portion of the liver and kidney tissues were dissected out washed with ice-cold saline immediately and were homogenized in 0.1 M Tris–HCl buffer (pH 7.4) for the assay of key enzymes of carbohydrate metabolism. The homogenate was centrifuged at $2000 \times g$ to remove the debris and the supernatant was used as enzyme source for the assays of glucokinase [14], glucose-6-phosphate dehydrogenase [15], glucose-6-phosphatase [16] and fructose-1,6-bisphosphatase [17]. Another portion of wet liver tissue was used for the estimation of glycogen content [18]. The activities of serum