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Effect of pterostilbene on hepatic key enzymes of glucose metabolism in streptozotocin- and nicotinamide-induced diabetic rats

L. Pari *, M. Amarnath Satheesh

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar, Tamil Nadu-608 002, India

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Abstract

The purpose of this study was to investigate the effect of pterostilbene and its effect on key enzymes of glucose metabolism. Diabetic rats were orally administered with pterostilbene (10, 20, 40 mg/kg) for 2, 4 and 6 weeks on glucose was determined. Administration of pterostilbene at 40 mg/kg significantly decreases plasma glucose. Based on these data, the higher dose, 40 mg/kg pterostilbene, was selected for further evaluation. Oral administration of pterostilbene for 6 weeks on glucose, insulin levels and hepatic enzymes in normal and streptozotocin (STZ)–nicotinamide-induced diabetic rats. A significant decrease in glucose and significant increase in plasma insulin levels were observed in normal and diabetic rats treated with pterostilbene. Treatment with pterostilbene resulted in a significant reduction of glycosylated hemoglobin and an increase in total hemoglobin level. The activities of the hepatic enzymes such as hexokinase was significantly increased whereas glucose-6-phosphatase, fructose-1,6-bisphosphatase were significantly decreased by the administration of pterostilbene in diabetic rats. A comparison was made between the action of pterostilbene and the antidiabetic drug – metformin.

Keywords: Pterostilbene; Antidiabetic; Carbohydrate key enzymes; Experimental diabetes

Introduction

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Baquer et al., 1998). Globally, the estimated incidence of diabetes and projection for year 2010, as given by International Diabetes Federation (IDF) is 239 million (Gandhi, 2001). Defects in carbohydrate machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an over exertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbate the metabolic disturbances by altering carbohydrate metabolic enzymes and leads primarily to hyperglycemia (Tiwari and Madhusudana, 2002). Hyperglycemia increases oxygen-reactive species generation and reduces the protective capabilities of antioxidant defense systems (Halliwell and Gutteridge, 1989). Chronic hyperglycemia is the primer of a series of cascade reactions causing the over production of free radicals and increasing evidences indicate that these contributes to the development of diabetic complications such as blindness, cardiac and kidney disease (Bortoli et al., 1997).

Pterocarpus marsupium is one of the traditional medicinal plants that has been used for many years in the treatment of diabetes mellitus (Warrier et al., 1995). The *Pterocarpus marsupium* Roxb. (Leguminosae) is popularly known as 'Indian kino'. The heartwood of this plant is primarily used in the treatment of diabetes in Ayurvedic system of medicine (Satyavathi et al., 1987). *P. marsupium* has also been reported to prevent the hyperglycemia and insulin resistance in fructose diet-induced diabetes model (Grover et al., 2005). Pterostilbene is a naturally methoxylated analogue of resveratrol (Rimando et al., 2002), found in extract of the heartwood of *P. marsupium* (Maurya et al., 1984) and also in *Pterocarpus santalinm* (Sehadri, 1972) and *Vitis vinifera* leaves (Langcake et al., 1979). An aqueous extract of heartwood of *P. marsupium* has been

* Corresponding author. Tel.: +91 4144 238343; fax: +91 4144 238145.
E-mail address: paribalaji@gmail.com (L. Pari).

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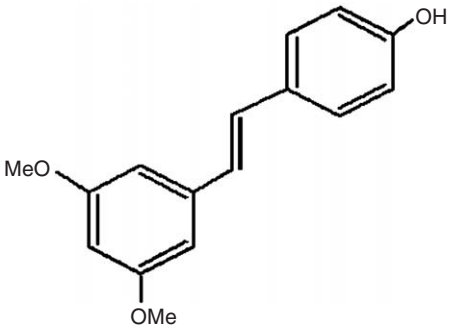
tested clinically and found to be effective in non-insulin-dependent diabetes mellitus (ICMR, 1998).



Structure of Pterostilbene

Pterostilbene has been reported to significantly lower the blood glucose level in hyperglycemic rats (Manickam et al., 1997). Although, previous workers have assessed the anti-hyperglycemic effect of pterostilbene by determining the blood glucose level in experimental animals, the study was of very short duration (maximum 3 days).

Metformin is now widely used as one of the mainstays in the management of type 2 diabetes. Metformin can be used either as initial therapy or as an additional drug with other antidiabetic agents (Fonseca et al., 2000). The present investigation is carried out to study the underlying mechanism that was responsible for the antidiabetic action of pterostilbene. In addition, we also described the dose-dependent response of pterostilbene in streptozotocin and nicotinamide induced type 2 diabetic rats. The effects of pterostilbene were compared with metformin.

Materials and methods

Animals

Male Albino Wistar rats weighing 200–220 g bodyweight were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University. All animal experiments in this study were approved by the institutional ethical committee Annamalai University (Vide. No. 158, 2003). Animals were fed with a standard pellet (Lipton India Ltd) and water ad libitum. They were maintained at a temperature of 27–29 °C and at a photoperiod of 12 h day/night cycle. Animals described as fasted were deprived of food for 16 h but had free access to water.

Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical Company, St Louis, MO, USA. Pterostilbene was received as gift sample from Sabinsa Corporation, USA. All the other chemicals and reagents used were of analytical grade.

Induction of diabetes

Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Non-insulin-dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 65 mg/kg streptozotocin, 15 min after the i.p administration of 110 mg/kg of nicotinamide (Masiello et al., 1998). Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h. The animals with blood glucose concentration more than 250 mg/dl will be used for the study.

Experimental design

In the experiment, a total of 42 rats (30 diabetic surviving rats, 12 normal rats) were used. The rats were divided into five groups of 6 each, after the induction of streptozotocin diabetes.

Group I:	Normal control (vehicle-treated).
Group II:	Normal rats received pterostilbene (40 mg/kg bodyweight) in 1 ml of 0.5% methylcellulose suspension (Klimes et al., 1998) for 6 weeks.
Group III:	Diabetic control
Group IV:	Diabetic rats received pterostilbene (10 mg/kg bodyweight) in 1 ml of 0.5% methylcellulose suspension for 6 weeks.
Group V:	Diabetic rats received pterostilbene (20 mg/kg bodyweight) in 1 ml of 0.5% methylcellulose suspension for 6 weeks.
Group VI:	Diabetic rats received pterostilbene (40 mg/kg bodyweight) in 1 ml of 0.5% methylcellulose suspension for 6 weeks.
Group VII:	Diabetic rats received metformin (500 mg/kg bodyweight) in 1 ml of saline (Soon and Tan, 2000) for 6 weeks.

Sample collection

At the end of 2 and 4 weeks, fasting blood samples were collected in fresh vials containing sodium fluoride and potassium oxalate as a anticoagulant agent. Plasma was separated for the estimation of glucose and insulin. At the end of the 6th week period, the samples were collected for glucose, insulin, hemoglobin and glycosylated Hb. Liver was dissected out, washed in ice-cold saline, patted dry and weighed.

Biochemical investigation

The level of plasma glucose was estimated spectrophotometrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India). Plasma insulin level was assayed by enzyme-linked immunosorbent assay kit (ELISA) (Boehringer Mannheim kit). Haemoglobin was estimated by cyanmethaemoglobin method (Drabkin and Austin, 1932). Glycosylated hemoglobin was estimated by the method of Sudhakar and Pattabiraman (1981) was modified by Bannon (1982).

The activity of glucose-6-phosphatase and fructose-1,6-phosphatase were assayed according to the methods of Koide and Oda (1959) and Gancedo and Gancedo (1971) respectively,