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Effect of tangeretin, a polymethoxylated flavone on glucose metabolism in streptozotocin-induced diabetic rats

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ABSTRACT

The present study was designed to evaluate the antihyperglycemic potential of tangeretin on the activities of key enzymes of carbohydrate and glycogen metabolism in control and streptozotocin induced diabetic rats. The daily oral administration of tangeretin (100 mg/kg body weight) to diabetic rats for 30 days resulted in a significant reduction in the levels of plasma glucose, glycosylated hemoglobin (HbA1c) and increase in the levels of insulin and hemoglobin. The altered activities of the key enzymes of carbohydrate metabolism such as hexokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, glucose-6-phosphate dehydrogenase, glycogen synthase and glycogen phosphorylase in liver of diabetic rats were significantly reverted to near normal levels by the administration of tangeretin. Further, tangeretin administration to diabetic rats improved hepatic glycogen content suggesting the antihyperglycemic potential of tangeretin in diabetic rats. The effect produced by tangeretin on various parameters was comparable to that of glibenclamide – a standard oral hypoglycemic drug. Thus, these results show that tangeretin modulates the activities of hepatic enzymes via enhanced secretion of insulin and decreases the blood glucose in streptozotocin induced diabetic rats by its antioxidant potential.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide that is caused by an absolute or relative lack of insulin and or reduced insulin activity, which results in hyperglycemy and abnormalities in carbohydrate, protein, and fat metabolism (Kamtchouing et al. 2006; Fatima et al. 2010). The World Health Organization (WHO) estimates that more than 220 million people worldwide have diabetes, and this number is liable to double by 2030 (WHO 2009). The chronic hyperglycaemia of diabetes is associated with damage, dysfunction and failure of various organs such as kidneys, retina, heart, liver, peripheral and central nervous system (Shanmugam et al. 2011).

Recently, much attention has been focused on screening of products from natural sources, such as flavonoids, that may be beneficial for reducing the risk for metabolic syndrome (Sabu et al. 2002; Jung et al. 2004), because products from plant sources are usually considered to be less toxic and have fewer side effects than products from synthetic sources.

Tangeretin is a polymethoxylated flavones abundant in the citrus fruit rinds, including mandarin orange, Poncirus trifoliate Raf. Tangeretin plays an important role in preventing various diseases including cancer, oxidative stress, and inflammation (Yoon et al. 2011; Xu et al. 2008). In addition to its anti-oxidant effects, tangeretin has been reported to inhibit the growth of hepatocytes both *in vitro* and *in vivo* via inhibition of mTOR/p70S6 kinase (Cheng et al. 2011). Regarding tangeretin mediated effects on neuronal disease, a number of studies have shown that tangeretin reduces dopaminergic neurotoxin-induced neuronal injury and prevents tunicamycin-induced cell death in mice through an increase in glucose-regulated protein (GRP)78 and heme oxygenase (HO)-1 expression in renal tubular epithelium (Takano et al., 2007). A recent study investigating the anti-inflammatory effects of citrus fruit peels showed that tangeretin had a beneficial effect on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 macrophage cells, which may provide protection against disease resulting from excessive NO production (Choi et al. 2007).

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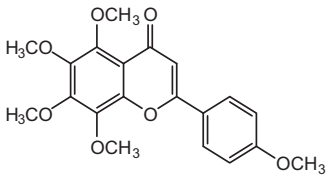

Fig. 1. Tangeretin.

Considering the wide variety of pharmacological action of tangeretin, the present study was undertaken to explore the tangeretin on key hepatic enzyme in streptozotocin induced diabetic rats. The effect of tangeretin was compared with conventional antidiabetic agent glibenclamide. The chemical structure of tangeretin was given in Fig. 1.

Materials and methods

Sources of chemicals

All fine chemicals including streptozotocin were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of good quality and analytical grade and obtained from Himedia, Mumbai, India.

Animals

Male albino Wistar rats weighing 180–200 g m body weight were procured from the Central Animal House Facility, University of Madras, Taramani Campus, Chennai, Tamil Nadu, India. They were maintained at an ambient temperature of 25 ± 2 °C and 12/12 h of light/dark cycle. Animals were given standard commercial rat chow and water *ad libitum* and housed under standard environmental conditions throughout the study. The experiments were conducted according to the ethical norms approved by the Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines.

Experimental induction of diabetes

Diabetes was induced in overnight fasted experimental rats by single intraperitoneal injection of streptozotocin (60 mg/kg bodyweight) dissolved in freshly prepared 0.2 ml of 0.1 mol/l citrate buffer, pH 4.5. Streptozotocin injected animals were allowed to drink 20% glucose solution overnight to overcome the initial drug-induced hypoglycemic mortality. Control rats were injected with the vehicle (0.2 ml of 0.1 mol/l citrate buffer, pH 4.5) alone. After 96 h, plasma glucose was determined and those rats with fasting blood glucose greater than 300 mg/dl were used in the present study. In diabetic rats, the treatment was started six weeks after the onset of diabetes because, in many studies, the duration of diabetes required to induce cardiac dysfunction in experimental rats has been found to be six weeks (Paulson 1997).

Oral glucose tolerance test (OGTT) was performed according to the method of Du Vigneaud and Karr (1925). 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 body weight) 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 kit method of Trinder (1969).

Experimental design

The animals were randomly divided into seven groups of six animals in each (30 diabetic surviving and 12 normal).

Group I: Control animals (normal healthy control rats received intra gastrically 0.5 ml of 0.9% saline for 30 days.

Group II: Drug control (normal healthy control rats received intra gastrically tangeretin (100 mg/kg b.w.) dissolved in 0.5 ml of 0.9% saline for 30 days.

Group III: Diabetic control rats.

Group IV: Diabetic rats received intra gastrically tangeretin (25 mg/kg b.w) dissolved in 0.5 ml of 0.9% saline for 30 days.

Group V: Diabetic rats received intra gastrically tangeretin (50 mg/kg b.w) dissolved in 0.5 ml of 0.9% saline for 30 days.

Group VI: Diabetic rats received intra gastrically tangeretin (100 mg/kg b.w) dissolved in 0.5 ml of 0.9% saline for 30 days.

Group VII: Diabetic rats received intra gastrically glibenclamide (5 mg/kg b.w) dissolved in 0.5 ml of 0.9% saline for 30 days.

At the end of the treatment period (75 days), the rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. The blood was collected with or without EDTA for plasma or serum separation, respectively. The liver tissue was dissected out, washed in ice-cold saline, and weighed. Tissue was minced and homogenized (10%, w/v) with 0.1 M Tris–HCl buffer (pH 7.4) and centrifuged (3000 × *g* for 10 min). The resulting supernatant was used for enzyme assays. Body weights of all the animals were recorded prior to the treatment and sacrifice. Food and water intake of all groups of animals were monitored on a daily basis for 30 days at a fixed time. Fixed amount of rat chow and fluid was given to each rat and replenished the next day.

Biochemical analysis

The level of plasma glucose was estimated spectrophotometrically using commercial diagnostic kit (Agappe Diagnostics Pvt. Ltd., Kerala, India) Trinder (1969). Plasma insulin level was assayed by enzyme linked immunosorbent assay kit (ELISA) (Boehringer Mannheim kit). Hemoglobin and HbA1C was estimated by diagnostic kit (Agappe Diagnostic Pvt. Ltd., India) Bisse and Abragam, (1985). The estimation of protein was carried out by the method of Lowry et al. (1951). Hepatic hexokinase activity was assayed by the method of Brandstrup et al. (1957). Glucose-6-phosphate dehydrogenase was assayed by the method of Ellis and Kirkman (1961). Glucose-6-phosphatase was assayed by the method of Koide and Oda (1959). Fructose-1,6-bisphosphatase activity was measured by the method of Gancedo and Gancedo (1971), pyruvate kinase activity was estimated by the method of Pogson and Denton (1967), lactate dehydrogenase activity was estimated by the method of King (1965), glycogen synthase activity was estimated by the method of Leloir and Goldemberg (1962), glycogen phosphorylase activity was estimated by the method of Cornblath et al. (1963). Another portion of wet liver glycogen content was estimated by the method of Morales et al. (1973).

Histopathological studies

Pancreatic tissues were harvested from the sacrificed animals and were fixed in 10% neutral buffered formalin solution, dehydrated in ethanol and embedded in paraffin. Sections of 5 μm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin dye and mounted in neutral deparaffinated xylene medium for microscopic observations.