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Antioxidant effect of tetrahydrocurcumin in streptozotocin–nicotinamide induced diabetic rats

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Abstract

Oxidative stress has been suggested to be a contributory factor in development and complication of diabetes. In the present study, we have investigated the effect of tetrahydrocurcumin (THC), one of the active metabolites of curcumin on antioxidants status in streptozotocin–nicotinamide induced diabetic rats. Oral administration of THC at 80 mg/kg body weight of diabetic rats for 45 days resulted in significant reduction in blood glucose and significant increase in plasma insulin levels. In addition, THC caused significant increase in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, reduced glutathione, vitamin C and vitamin E in liver and kidney of diabetic rats with significant decrease in thiobarbituric acid reactive substances (TBARS) and hydroperoxides formation in liver and kidney, suggesting its role in protection against lipid peroxidation induced membrane damage. These biochemical observations were supplemented by histopathological examination of liver and kidney section. The antidiabetic and antioxidant effects of THC are more potent than those of curcumin at the same dose. Results of the present study indicated that THC showed antioxidant effect in addition to its antidiabetic effect in type 2 diabetic rats.

Keywords: Antioxidants; Tetrahydrocurcumin; Curcumin; Lipid peroxidation

Introduction

Type 2 diabetes is associated with increased oxidative stress (Mc Coll et al., 1997). Free radicals are continually produced in the body as the result of normal metabolic processes and interaction with environmental stimuli. Under physiological conditions, a wide range of antioxidant defenses protects against adverse effects of free radical production *in vivo* (Halliwell and Gutteridge, 1989). Oxidative stress results from an imbalance between radical production and reduced activity of antioxidant defenses or both these phenomena. Hyperglycemia causes release of tissue damaging reactive oxygen species (ROS) balance between radical production and protective antioxidant defense (Signorini et al., 2002; Halliwell and Gudtteridge, 1990). It has been proposed that streptozotocin (STZ) acts as a diabetogenic agent owing to its ability to destroy pancreatic β-cells, possibly by a free radical mechanism (Halliwell and Gutteridge, 1994; Simmons, 1984). The level of lipid peroxidation in cell is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenger systems, the levels of which are altered in diabetes (Wohaieb and Godin, 1987). Moreover, disturbances of antioxidant defense systems in diabetes were shown: alteration in antioxidant enzyme (Strain, 1991), impaired glutathione metabolism (Mc Lennan et al., 1991) and decreased ascorbic acid (Jennings et al., 1987). In recent years, considerable focus has been given to an intensive search for novel type of antioxidants from numerous plant materials (Srivastava et al., 1993). Management of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs possess undesirable side effects (Kameswara Rao and Appa Rao, 2001). Plants with antidiabetic activities provide useful sources for the development of drugs in the treatment of diabetes mellitus. Phytochemicals isolated from plant source are used for the prevention and treatment of cancer, heart disease, diabetes and high blood pressure etc. (Mary et al., 2002).

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Curcuma longa is commonly used in the treatment of diabetes by ayurvedic physicians. Curcumin is a biologically active component isolated from the rhizome of *C. longa* that possess anti-hyperglycemic activity (Arun and Nalini, 2002), hypolipidemic action (Suresh Babu and Srinivasan, 1997) and anti-renal lesion effect (Suresh Babu and Srinivasan, 1998). The use of curcumin is recommended for prevention of advanced glycated endproducts (AGE) accumulation and the associated complications of diabetes (Sajithlal et al., 1998).

Tetrahydrocurcumin (THC) (Fig. 1) is one of the major colourless metabolite of curcumin. THC has been reported to exhibit the same physiological and pharmacological properties of curcumin (Majeed et al., 1995; Sugiyama et al., 1996). Curcumin is rapidly metabolized during absorption from the intestine, yielding THC (Ravindranath and Chandrasekara, 1999), which has shown the strongest antioxidant activity among all curcuminoids (Osawa et al., 1995). Several studies in experimental animals indicated that THC also prevent(s) cancer (Lin and Lin-Shiau, 2001), protect(s) against inflammation (Nakamura, 1998; Hong et al., 2004), atherosclerotic lesions (Naito et al., 2002) and hepatotoxicity (Pari and Murugan, 2004). In our previous study, we have demonstrated the antidiabetic effect of THC in STZ induced diabetic rats (Pari and Murugan, 2005).

To our knowledge, so far no other biochemical investigations has been carried out on the effect of THC in tissue antioxidant status of experimental diabetic rats. The present investigation was carried out to study the effect of THC on tissue lipid peroxides and antioxidants in rats with STZ and nicotinamide induced diabetes.

Materials and methods

Animals

Adult male albino Wistar rats (8 weeks), weighing 180 to 200 g bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used. All animal experiments were approved by the ethical committee (Vide. No: 284, 2005), Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The animals were housed in polycarbonate cages in a room with a 12 h day–night cycle, temperature of 24±2 °C, humidity of 45% to 64%. During the whole experimental period, animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Drugs and chemicals

THC was a gift provided by the Sabinsa Corporation, USA. Curcumin was purchased from Sigma chemicals company, St Louis, USA. All other chemicals and biochemicals were of analytical grade.

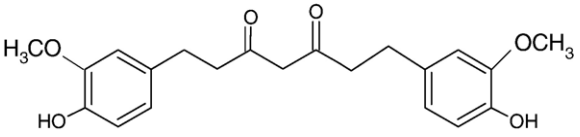

Fig. 1. Tetrahydrocurcumin.

Induction of diabetes

Non-insulin dependent diabetes mellitus was induced (Masiello et al., 1998) in overnight fasted rats by a single intraperitoneal injection (i.p) of 65 mg/kg body weight STZ, 15 min after the i.p administration of 110 mg/kg body weight of nicotinamide. STZ was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl will be used for the study.

Experimental design

In the experiment, a total of 24 rats (18 diabetic surviving rats, 6 normal rats) were used. The rats were divided into four groups of six each, after the induction of STZ diabetes. The experimental period was 45 days. Group I: Normal rats. Group II: Diabetic control rats. Group III: Diabetic rats given THC (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days. Group IV: Diabetic rats given curcumin (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Arun and Nalini, 2002).

At the end of 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose. Plasma was separated for the estimation of insulin. Liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood.

Preparation of tissue homogenate

The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris–HCl buffer, pH 7.5. After centrifugation at 10,000 ×*g* for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances (TBARS) and hydroperoxides. For the determinations of vitamin E level the liver and kidney tissues were weighed and lipids were extracted from tissues by the method of Folch et al. (1957) using chloroform–methanol mixture ($CHCl_3$: MeOH)(2:1; v/v). The extract used for the estimation of vitamin E.

For the estimation of non-enzymic and enzymic antioxidants, tissue was minced and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged for 10 min and the resulting supernatant was used for enzyme assays.

Analytical procedure

Measurement of blood glucose and plasma insulin

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) (Lott and Turner, 1975). Plasma insulin was assayed by ELISA using a Boehringer–Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany).