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RESEARCH ARTICLE

Effect of *p*-Coumaric Acid on Biochemical Parameters in Streptozotocin-Induced Diabetic Rats

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Abstract

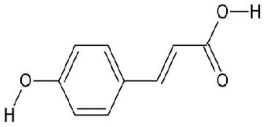
The present study investigated the effect of *p*-coumaric acid in streptozotocin (STZ) induced diabetic rats. Streptozotocin (45 mg/kg/i.p) is used to induce diabetes in rats. Albino Wistar rats were divided in to 4 groups, *p*-coumaric acid (100 mg/kg/day) was dissolved in 10% propylene glycol and administered to rats for 45 d. Diabetic rats had elevated levels of glucose, glycosylated hemoglobin, glucose-6-phosphatase and fructose-1,6-bisphosphatase and decreased levels of insulin, c-peptide, total protein, hemoglobin, glycogen and hexokinase when compared with normal control rats. Treatment with *p*-coumaric acid showed near normal levels of the above parameters when compared with STZ-induced diabetic rats. Our present study suggests that *p*-coumaric acid may be included in diabetes mellitus treatment regimens as a drug with good antidiabetic actions but no toxic manifestations.

Keywords: *p*-coumaric acid, streptozotocin, diabetes mellitus, glycosylated hemoglobin, antidiabetic.

Introduction

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. The complications include macrovascular complications (myocardial infarction, heart failure, ischemic stroke), as well as the microvascular complications (retinopathy, neuropathy and nephropathy) (LeRoith, 2009). It is the most common endocrine disorder and estimated that more than 200 million people worldwide will have diabetes and 300 million will subsequently have the disease by 2025 (WHO, 1999). Streptozotocin (STZ) is frequently used to induce diabetes mellitus in experimental animals. It possesses antibacterial, antitumor, mutagenic and diabetogenic activities and shown to inhibit DNA synthesis in bacteria and mammalian cells (Bhuyan, 1970). STZ is cytotoxic to pancreatic β-cells; its effect can be seen within 1 h after administration. Cytotoxic effects of STZ depend upon DNA alkylation by site-specific action with DNA bases and by free-radical generation during STZ metabolism (Bolzan and Bianchi, 2002). Types of DNA lesions formed by STZ include mono adducts, single and double stranded breaks and alkali-labile sites. Severe DNA damage by STZ results in cell death by apoptosis or necrosis. The disease become a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable (Djrolo *et al.,* 1998).

Fig. 1. Structure of *p*-coumaric acid.



Currently, the available therapy for diabetes includes insulin and various oral anti-diabetic agents such as sulfonylureas, metformin, etc. These drugs are used as monotherapy or in combination to achieve better glycemia control. Synthetic antidiabetic agent can produce serious side effects. Treatment of diabetes without any side effects is still a challenge to the medical systems. This leads to increasing demands for natural products with antidiabetic activity with less side effects (Moller, 2001). Hydroxy-derivatives of cinnamic acid as *p*-coumaric acid (Fig. 1) are found in a number of edible plants ingested by animals and humans (Evans *et al.,* 1997; Olsen *et al.,* 2010). *p*-Coumaric acid is present in a plenty of foods, such as grapes, white and red wine, tomato, spinach, coffee, carrot and garlic (Alamed *et al.,* 2009). *p*-Coumaric acid has attracted substantial attention due to its several pharmacological and biological actions, such as antioxidant (radical scavenging) (Evans *et al.,* 1996), chemoprotective (Hudson *et al.,* 2000), neuroprotective (Vauzour *et al.,* 2010), cardioprotective (Abdel-Wahab *et al.,* 2003), anti-microbial (Cho *et al.,* 1998), anti-cancer (Janicke *et al.,* 2005), anti-ulcer activity (Barros *et al.,* 2008). Keeping the above facts in view, this study evaluated antidiabetic effect of *p*-coumaric acid on the STZ-induced diabetes in rats.

Materials and methods

Experimental animals: Male albino Wistar rats (170-200 g) used in this study were obtained from Venkateswara Enterprises, Bangalore. The rats were housed in polypropylene cages (47×34×20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22°C and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolizable energy of 3,600 kcal. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC) of Muthaymmal College of Arts and Science, Rasipuram (MCAS/Ph.D./02/2012-2013).

Chemicals: Streptozotocin was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. *p*-coumaric acid, anthrone, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used in the study were of analytical grade. Glucose, total protein, hemoglobin and glycosylated hemoglobin kits were purchased from Agappe Diagnostics, Kerala, India.

Induction of experimental diabetes: Streptozotocin was used to induce diabetes mellitus in normoglycemic male albino Wistar rats. A freshly prepared solution of STZ (45 mg/kg body weight) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 mL/kg body weight in overnight fasted rats (Pari and Venkateswaran, 2002). Rats having a fasting plasma glucose value of above 13.89 mmol/L (250 mg/dL) were included in the study as diabetic rats (cam *et al.,* 2003).

Experimental design: In the experiment, a total of 36 rats (24 diabetic surviving rats, 12 control rats) were used in the study. The rats were divided into 6 groups of 6 rats in each group. *p*-Coumaric acid was dissolved in 10% propylene glycol and administrated to rats orally using an intragastric tube daily for a period of 45 d.

Group 1: Normal control rats
Group 2: Normal + p-coumaric acid (100 mg/kg)
Group 3: Diabetic control rats
Group 4: Diabetic + p-coumaric acid (25 mg/kg)
Group 5: Diabetic + p-coumaric acid (50 mg/kg)
Group 6: Diabetic + p-coumaric acid (100 mg/kg)

At the end of the treatment period, all rats were anaesthetized with pentobarbital sodium (35 mg/kg) and sacrificed by cervical decapitation. Blood collected using potassium oxalate and sodium fluoride as anticoagulant and plasma was separated. The tissues (muscle, liver and kidney) were dissected out, washed in ice-cold saline, and patted dry. The tissues were weighed and homogenized and 10% tissue homogenate was used for estimation of various biochemical parameters.

Biochemical estimations: Plasma glucose was estimated by the method of Trinder (1969). Plasma insulin and C-peptide were determined by the method of Kann and Rosenthal (1979) using an immunoenzymatic assay kit. Total proteins levels were estimated by Doumas *et al.* (1971) method using a commercial kit obtained from Agappe Diagnostics, Kerala, India. Glycogen was estimated by the method of Wieland (1963). Glycosylated hemoglobin was estimated by the method of Tietz (1999) and hemoglobin in blood was estimated by the method of Samuel (1989) using kit from Agappe diagnostics. Hexokinase was assayed by the method of Brandstrup *et al.* (1957). Glucose-6-phosphatase was assayed by the method of Koide and Oda (1959). Fructose-1, 6-bisphosphatase was assayed by the method of Gancedo and Gancedo (1971).

Statistical analysis: Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Sciences (SPSS) software package version 9.05. P values <0.05 were considered significant.

Results

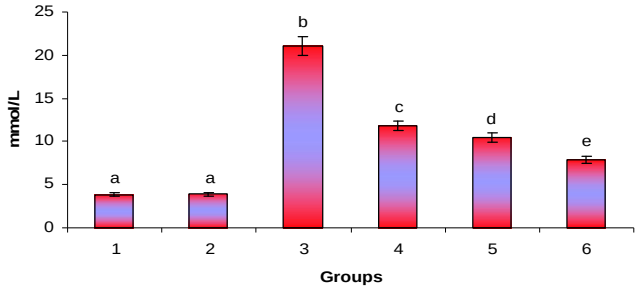
Effect of p-coumaric acid on the levels of glucose: The level of plasma glucose of diabetic and control rats is presented in Fig. 2. Streptozotocin (45 mg/kg body weight) induced diabetic rats had an elevated level of plasma glucose at the end of experimental period (45 d). Oral administration of *p*-coumaric acid at three different doses (25, 50 and 100 mg/kg body weight) significantly decreased the level of plasma glucose in STZ-induced diabetic rats. *p*-coumaric acid at a dose of 100 mg/kg exhibited a maximum plasma glucose lowering effect (22.01 mmol/L) in diabetic rats than the other two doses (25 and 50 mg/kg body weight) at the end of the experiment. Hence, further studies were carried out with 100 mg/kg of *p*-coumaric acid alone in normal and STZ-induced diabetic rats.

Effect of p-coumaric acid on insulin, c-peptide and total protein: The levels of insulin, c-peptide and total protein in diabetic and control rats are showed in Fig. 3. STZ-induced diabetic rats showed significant decreased levels of insulin, c-peptide and total protein. The lowered levels of insulin, c-peptide and total protein in STZ-induced diabetic rats were increased to near normal levels due to *p*-coumaric acid treatment.

Effect of p-coumaric acid on liver and muscle glycogen: The effect of *p*-coumaric acid on glycogen content in liver and muscle of normal and experimental rats are presented in Fig. 4.

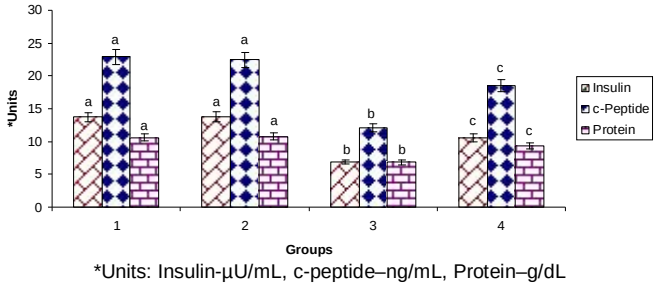
STZ-induced diabetic rats showed a significant reduction in liver and muscle glycogen. Treatment with *p*-coumaric acid significantly increased the concentration of liver and muscle glycogen in STZ-induced diabetic rats.

Fig. 2. Effect of *p*-coumaric acid on the levels of plasma glucose in normal and STZ-induced diabetic rats.



Each value is mean ± S.D. for six rats in each group. Columns not sharing a common superscript (a-e) differ significantly with each other (P<0.05, DMRT).

Fig. 3. Effect of *p*-coumaric acid on the levels of plasma insulin, c-peptide and total protein in normal and STZ-induced diabetic rats.



*Units: Insulin-µU/mL, c-peptide–ng/mL, Protein–g/dL

Fig. 4. Effect of *p*-coumaric acid on the levels of muscle and liver glycogen in normal and STZ-induced diabetic rats.

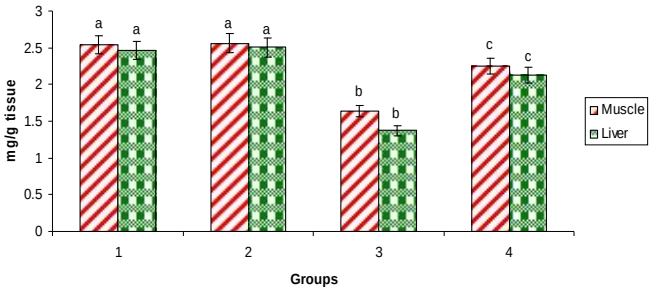
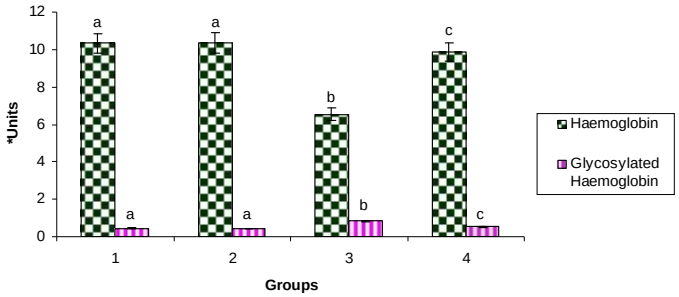


Fig. 5. Effect of *p*-coumaric acid on the levels of blood haemoglobin and glycosylated hemoglobin in normal and STZ-induced diabetic rats.

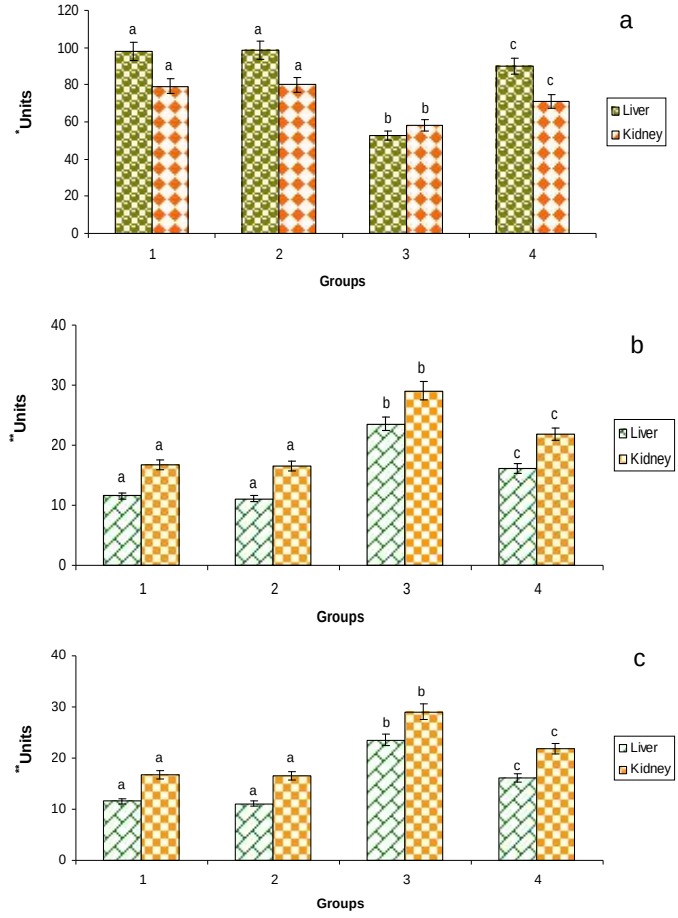


*Units: Hemoglobin–g%, Glycosylated hemoglobin–mg/dL

Effect of p-coumaric acid on total hemoglobin and glycosylated hemoglobin: The concentration of total hemoglobin and glycosylated hemoglobin in normal and STZ-induced diabetic rats are shown in Fig. 5. Rats induced with STZ, showed a significant increase in the levels of glycosylated hemoglobin with significant decrease in total hemoglobin levels. *p*-Coumaric acid treated rats showed significant decreased levels of glycosylated hemoglobin and increased level of hemoglobin when compared with diabetic control rats.

Effect of p-coumaric acid on carbohydrate metabolic enzymes: The levels of carbohydrate metabolic enzymes in liver and kidney of diabetic and control rats are presented in Fig. 6a, b, c. The activity of hexokinase in liver and kidney decreased markedly whereas the activity of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney increased significantly in STZ-induced diabetic rats. Treatment with *p*-coumaric acid in diabetic rats increased the hexokinase activity and decreased the glucose-6-phosphatase and fructose-1,6-bisphophatase activity.

Fig. 6a-c. Effect of *p*-coumaric acid on the levels of hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase of liver and kidney in normal and STZ-induced diabetic rats.



*Unit-mmoles of glucose phosphorylated/h/mg protein.
**Unit-µmoles inorganic phosphorous liberated/min/mg protein.

Discussion

Streptozotocin (STZ) induced (45 mg/kg body weight) hyperglycemia has been described as a useful experimental model to study the effect of hypoglycemic agents. The mechanism by which STZ brings about its diabetic state includes selective destruction of pancreatic β-cells which make cells less active, leading to poor sensitivity of insulin for glucose uptake by tissues and hyperglycemia (Burns and Gold, 2007). STZ destroy pancreatic β-cells in rats leading to insufficient insulin secretion causing type 2 diabetic model. Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus (Luzi, 1998). STZ-induced diabetes leads to β-cell necrosis that is caused due to diabetic oxidative stress. As a result of β-cell necrosis, insulin deficiency predominates resulting in repression of glycolytic enzyme and expresssion of gluconeogenic enzyme which promotes gluconeogenesis in liver and decreased utilization of glucose by the peripheral tissues contributes to hyperglycemia (Giugliano *et al.,* 1996). The elevated blood glucose level observed in the diabetic rats was significantly decreased in *p*-coumaric acid treated rats suggesting insulin secretory effect of *p*-coumaric acid from the remnant β-cells. In the present study, we have observed a significant decrease in the levels of insulin and c-peptide in STZ-induced diabetic rats. Insulin and c-peptide are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations (Steiner, 1978). The measurement of both c-peptide and insulin levels have been reported to be a valuable index of insulin secretion rather than insulin alone. In diabetes mellitus, insulin deficiency is manifested in a number of biochemical and physiological alterations. Insulin is synthesized from its precursor proinsulin. C-peptide promotes insulin action at low hormone concentration and inhibits it at high hormone levels suggesting a modulatory effect by c-peptide on insulin signaling (Grunberger *et al.,* 2001). C-peptide has insulino-mimetic effects on its own by activating insulin receptor and increases glycogen synthesis and amino acid uptake. Oral administration of *p*-coumaric acid improves the insulin secretion and c-peptide level which showed the insulin secretory effect of *p*-coumaric acid.

STZ-induced diabetic rats showed decreased levels of total protein. This might be due to microproteinuria and is due to increased protein catabolism. Excessive breakdown of body protein in conjunction with either inadequate supply or defective utilization of in diabetic rat may be accompanied by hypoalbuminemia (Almdal and Vilstrup, 1998). The decrease in total protein concentration in serum of diabetic rats may be due to decreased amino acid uptake (Garber, 1980), decreased concentration of variety of essential amino acids (Brosnan *et al.,* 1984), increased conversion rate of glycogenic amino acids to CO_2 and H_2O (Mortimore and Mandon, 1970) and reduction in protein synthesis secondary to a decreased amount and availability of mRNA (Peavy *et al.,* 1985). Treatment with *p*-coumaric acid increased the levels of total protein. These data suggest that *p*-coumaric acid might have the ability to increase amino acid uptake.

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues, especially hepatic and skeletal muscle, are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. Glycogen deposition in muscle and liver is a physiological response in mammals due to increase in blood glucose concentration that occurs after a meal (Ferrer *et al.,* 2003). Diabetes mellitus impairs the normal capacity of the liver to synthesize glycogen and low content may be attributed to lack of insulin. Oral administration of *p*-coumaric acid significantly increased liver and muscle glycogen content which indicates better insulin activity in the diabetic rats. Glycosylated hemoglobin is a standard biochemical marker in assessment of diabetes. In diabetes mellitus, due to persistent hyperglycemia, the excess blood glucose reacts with hemoglobin in a non-enzymatic process to form glycosylated hemoglobin. Since the glycation rate is directly proportional to blood glucose concentration, level of glycosylated hemoglobin indicates (Monnier, 1982) glycemic control in the diabetic state. Estimation of hemoglobin is a well established parameter useful in the management and prognosis of the disorder (Chang and Nobel, 1979). In the present study, administration of *p*-coumaric acid significantly reduced the elevated glycosylated hemoglobin levels and increased the hemoglobin levels in STZ-induced diabetic rats further substantiating its potential in long term glycemic control of diabetes mellitus. Liver is the primary site of endogenous glucose production with a minor contribution from the kidneys through gluconeogenesis or via glycogenolysis (Meyer *et al.,* 2004). Glycolysis and gluconeogenesis are the two primary complementary events balancing the glucose load in our body, which is characterized by partial or total deficiency of insulin and plays a pivotal role during the disarray of glucose metabolism leading to elevated systemic glucose. Insulin prevents hyperglycemia, in part, by suppressing hepatic gluconeogenesis and glycogenolysis and facilitating hepatic glycogen synthesis.

Glucokinase is a 50 KD enzyme that catalyzes the ATP-dependent phosphorylation of glucose to glucose-6-phosphate as the first and rate-limiting step in glucose utilization (Kang *et al.,* 2006). Among the defects noted in carbohydrate metabolism, low glucokinase activity probably plays a prominent role which decreases the rate of glucose utilization. The restoration of glucokinase activity might be involved in normalization of glucose levels (Matschinsky *et al.,* 2006). It also acts as a glucose sensor in pancreatic β-cells leads to glucose-induced insulin secretion (Nakamura *et al.,* 2009).

In the present study, a decreased glucokinase activity in diabetic rats may be production of glycated proteins. An increase in the activity of glucokinase in the liver and kidney as observed in the diabetic animals administered with *p*-coumaric acid which protects the hepatic tissues against STZ-induced diabetic condition. Glucose-6-phosphatase, a key enzyme in the homeostatic regulation of blood glucose and is critical in providing glucose to other organs during diabetes, prolonged fasting or starvation (Kurosaki *et al.,* 2003). Decreased glucose-6-phosphatase activity leads to a severe metabolic disorder mainly characterized by hypoglycaemia and its activity is stimulated by c-AMP and repressed by insulin. Insulin deficiency achieved in experimental diabetic rat increases glucose-6-phosphatase activity, which in turn increase blood glucose. Our result demonstrated that the administration of *p*-coumaric acid diminishes the activity of glucose-6-phosphatase in STZ-induced diabetic rats. The reduction in the activity of glucose-6-phosphatase leads to decreased gluconeogenesis and thereby reducing the endogenous production of glucose. Fructose-1,6-bisphosphatase is a key regulatory enzyme of the hepatic gluconeogenesis and appeared as a target for efficient and safe glycemic control in diabetes (Prabakaran and Ashokkumar, 2012). In this study, we found that its activity significantly increased in the liver and kidney of the diabetic rat possibly because of insulin deficiency. Administration of *p*-coumaric acid enhanced the reversal of high fructose-1,6-bisphosphatase activity in diabetic rats.

Conclusion

The results of the present study clearly indicate that *p*-coumaric acid have a glucose lowering effect on STZ-induced diabetic rats. It was also found to be highly effective in managing the complications associated with diabetes mellitus, such as insulin maintenance, protein metabolism and carbohydrate metabolizing enzymes. Therefore *p*-coumaric acid shows therapeutic promise as a protective agent against the development and possible related cardiovascular complications in diabetes mellitus.

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