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Antihyperglycemic effect of p-Coumaric acid on Streptozotocin induced Diabetic Rats

KEYWORDS: Diabetes mellitus, p-Coumaric acid, streptozotocin.

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ABSTRACT *The present study was to investigate the possible protective effects of p-coumaric acid against streptozotocin (STZ) induced diabetic rats. Diabetes mellitus was induced in male albino Wistar rats by a single dose of STZ intraperitoneally 40 mg/kg b.w. and treated 30 days with p-coumaric acid (100 mg/kg b.w.). Body weight, glucose, insulin, hemoglobin (Hb), glycosylated hemoglobin (HbA^{1C}), lipid peroxidation products and enzymic antioxidants were investigated. Administration of p-coumaric acid showed significant decreased fasting blood glucose and increased insulin level as compared with diabetic rats. The treatment also results in significantly increased levels of Hb and decreased HbA^{1C}. The activities of enzymes antioxidant increased by the administration of p-coumaric acid in liver and kidney tissues of diabetic rats. The overall results suggest that p-coumaric acid may effectively possess potential antihyperglycemic and antioxidant activity in STZ-induced diabetic rats by scavenging of free radicals and reducing the risk of diabetic complications.*

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous group of disorders characterized by hyperglycemia resulting from insulin resistance and/or abnormal insulin secretion. Defects in carbohydrate metabolic machinery pose an over exertion on the endocrine system leading to the disruption of endocrine control. It exacerbates the metabolic disturbances by altering carbohydrate metabolic enzymes which leads to hyperglycemia (Anandaprabu et al 2012). According to International Diabetes Foundation (IDF), the prevalence of type 2 diabetes mellitus (T2DM) affects 366 million people affect worldwide in 2011and is expected to rise 552 million by the year 2030 (Whiting et al 2011). India is home to the largest number of people with diabetes in the world, 40.9 million diabetic cases in 2007 and these numbers are predicted to 69.9 million by the year 2025 (Tharkar et al 2010).

Treatment of T2DM patients with sulfonylureas and biguanides is associated with side effects (Bolen et al 2007). Naturally occurring phytochemicals with antidiabetic activities are relatively non-toxic, inexpensive and accessible in an ingestive form. At present, there is special interest on natural antioxidants derived from edible plants with antioxidant and anti-diabetic properties and fewer side effects for the management of lifestyle-related diseases, which are caused by an imbalance in energy homeostasis (Khan et al 2012). There are evidences suggesting that phytochemicals having antioxidant properties are associated with a lower risk of mortality (Tang et al 2006). The antioxidant activity of plants may be due to their phenolic compounds, flavonoids and carotenoids.

There is an inverse association between dietary phenolic compound intake and mortality from various diseases (Yoon et al 2013). Phenolic compounds are a group of phenolic acids that are widely distributed in whole grains, fruits, pears, vegetables and beverages such as tea, coffee, wine and chocolate. p-Coumaric acid is a phenolic compound, abundantly present in pineapple widely distributed in plants and form a part of human diet (Scalbert and Williamson 2000). p-Coumaric acid is a ubiquitous plant metabolite possess antioxidant (Abdel-Wahab et al 2003) anti-inflammatory, anticancer (Yoon et al 2013) and hepatoprotective effect (Vetrikumaran et al 2011).

We investigate this study to evaluate the effect of p-coumaric acid on body weight, glucose, insulin, hemoglobin (Hb), glycosylated hemoglobin (HbA^{1C}), lipid peroxidation products and antioxidants in streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS

Animals

All the experiments were carried out with male albino Wistar rats weighing 180–220g, obtained from the Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India. They were housed in polypropylene cages (47 cm×34 cm×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 experiment was carried out according to the guidelines of the Committee for the (http://icmr.nic.in/bioethics/ final_CPCSEA.pdf/ http://icmr.nic.in/bioethics/INSA_guidelines.pdf) Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Animal Ethical Committee of Annamalai University (Approval no. 1075; dated 17/04/2014).

Chemicals

p-Coumaric acid and STZ were purchased from Sigma Chemical Co (St.Louis, Mo. USA). All other chemicals were of analytical grade.

Experimental induction of type 2 diabetes in rats

Diabetes was induced in overnight fasted experimental an-

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imals by a single intraperitoneal injections of STZ (40 mg/ kg b.w.) dissolved in citrate buffer (0.1M, pH 4.5) (Murali et al 2013). The 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 citrate buffer alone. After 72 hours, plasma glucose was determined and those rats with fasting blood glucose greater than 250 mg/dl were used in this study.

Experimental design

In this study, a total of 24 rats were divided in to four groups of six rats each. p- Coumaric"acid"dissolved in 0.2% dimethyl sulfoxide (DMSO) and administered to rats orally using an intragastric tube daily for a period of 30 days.

Group 1: Normal control (vehicle treated; DMSO: 1ml/kg b.w.)
Group 2: Normal + p-Coumaric acid (100 mg/kg b.w.)
Group 3: Diabetic control (40 mg/kg b.w.)
Group 4: Diabetic + p-Coumaric acid (100 mg/kg b.w.)

The initial and final body weight of the rats in each group was recorded. At the end of the experimental period, rats were fasted overnight and sacrificed by cervical dislocation. Blood samples were collected in tubes containing potassium oxalate and sodium fluoride (3:1) mixture used for the estimation of plasma glucose and insulin. Hb and HbA_{1c} levels were estimated in whole blood samples. The liver and kidney tissues were dissected and collected in ice-cold saline for various estimations**.**

Biochemical analysis

Plasma glucose by the method of Trinder, (1969). Plasma insulin was assayed by ELISA kit (Boeheringer-Manneheim Kit, Manneheim, Germany). Hb and HbA_{1c} (Bisse and Abragam 1999). Thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) in liver and kidney tissues by the method of Niehius and Samuelsson (1968) and Jiang et al (1992), respectively. SOD (Kakkar et al 1989), CAT (Sinha (1972), GPx (Rotruck et al 1973).

Statistical analysis

All the data were expressed as mean $\pm$SD of number of experiments (n= 6). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS Version 15 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Duncan's multiple range test (DMRT). Values are considered statistically significant when $P<0.05$. Duncan, (1957).

RESULTS

The changes in the body weight of control and experimental rats are represented in Table 1. Body weight significantly ($p<0.05$) decreased in diabetic rats compared with control rats. Diabetic rats treated with p-coumaric acid 100 mg/ kg b.w significantly ($p<0.05$) increased body weight when compared with diabetic control rats.

Table 2 shows the levels of plasma glucose and insulin in different experimental groups. Diabetic rats exhibited increased levels of plasma glucose with a decrease in insulin when compared to normal control rats. Administration of p-coumaric acid to diabetic rats improved the glycemic status with a significant increase in plasma insulin level.

Table 2 shows the levels of Hb and HbA_{1c} in normal and diabetic rats. Decreased Hb and an increased HbA_{1c} were observed in diabetic rats. These levels improved towards near normal in rats treatment with p-coumaric acid.

Table 3 shows the levels of TBARS and HP in liver and kidney tissues of experimental rats. Diabetic rats exhibited increased levels of TBARS and HP when compared to normal control rats. Administration of p-coumaric acid to diabetic rats significantly decreased lipid peroxidation markers in liver and kidney tissues.

Table 4 represents the activities of antioxidant enzymes (SOD, CAT and GPx) in hepatic and renal tissues of experimental animals. A fall in the activities of antioxidant enzymes were observed in diabetic rats when compared to normal control. The administration of p-coumaric acid to diabetic rats significantly improved the activities of the above enzymes.

DISCUSSION

Diabetes mellitus is a heterogeneous group of disorders characterized by high blood glucose levels. The pancreatic β-cell and its secretary product insulin are central in the pathophysiology of diabetes (Kahn, 2001). In type 2 or non-insulin-dependent diabetes mellitus, muscle and fat cells are 'resistant' to the actions of insulin and compensatory mechanisms that are activated in the β-cell to secrete more insulin are not sufficient to maintain blood glucose levels within a normal physiological range. STZ-induced hyperglycemia has been described as an utilizable experimental model to study the activity of hypoglycemic agents (Gandhi et al 2011). It interferes with cellular metabolic oxidative mechanisms. STZ selectively destroys insulin producing β-cells of pancreas by inducting high levels of DNA strand breaks in the cells, causing activation of poly (ADP-ribose) polymerase (PARP), resulting in reduction of cellular NAD^+ and cell death lead to diabetes (Bolzan and Bianchi, 2003). In this investigation, diabetic rats showed significant decrease in plasma glucose level after treatment with p-coumaric acid. This could be due to the potential of the pancreatic secretion of insulin from regenerated β -cells by inhibiting ATP sensitive K^+ channels like glibenclamide (Ambika et al 2013). Previous studies showed that phenolic compounds acted on ATP sensitive K^+ channels and regulated blood glucose (Adisakwattana et al 2005). This was followed by a significant increase in body weight of the diabetic treated rats. This is a reverse to diabetic state characterized by a severe loss in body weight due to loss or degradation of structural protein. Rise in insulin level upon treatment with p-coumaric acid in diabetic rats resulted in improved glycemic control, which has been prevented the loss of body weight. Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation.

The decreased level of total hemoglobin in diabetic rats is mainly due to the increased formation of HbA^{1C}. In diabetes mellitus, the excess glucose present in the blood reacts with hemoglobin to form HbA^{1c}. The amount of HbA_{1C} increase is directly proportional to the blood glucose level (Nain et al 2012). Administrations of p-coumaric acid to diabetic rats reduced the glycosylation of hemoglobin by virtue of its normal glycaemic activity and thus increase the levels of hemoglobin in diabetic rats.

STZ, is produced by Streptomyces achromogenes, has been widely used for inducing diabetes in the experimental rats through its toxic effects on pancreatic β-cells. The cytotoxic action of STZ is associated with the generation of ROS causing oxidative damage that culminates in β-cell destruction through the induction of apoptosis and suppression of insulin biosynthesis (Zhang et al 2010). It

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is thought to be mediated by the inhibition of free radical scavenger-enzymes thereby enhancing the production of the superoxide radical which can damage pancreatic β-cells. Insulin secretion is impaired during diabetes and this may evoke lipid peroxidation in biological systems (Pari and Suman 2010). Enhanced levels of TBARS and HP observed in the liver of diabetic rats indicate excessive formation of free radicals and activation of lipid peroxidative system. Present study shows the administration of p-coumaric acid inhibits production of liver peroxides. This indicates the anti-lipid peroxidative potential of p-coumaric acid.

SOD, CAT, and GPx are enzymatic antioxidants that play a vital role in preventing cells from being exposed to oxidative damage. SOD is capable of reducing the super oxide radical in hydrogen peroxide (H_2O_2). The other enzymatic antioxidant CAT catalyzes the reduction of hydrogen peroxides and protects the tissues against reactive hydroxyl radicals (Eliza et al 2010). When cell has increased levels of SOD without a proportional increase in peroxidases (GPx), cells face a peroxide overload challenge. Peroxide can react with transitional metals and generate the radical hydroxyl, which is the most harmful radical. In diabetes mellitus, high glucose can inactivate antioxidant enzymes SOD, CAT and GPx by glycating these proteins thus producing induced oxidative stress, which in turn, cause lipid peroxidation (Kennedy and Lyons 1997). CAT, SOD and GPx activities were bought to near normal indicating the efficacy of p-coumaric acid in attenuating the oxidative stress in diabetic liver. Previous studies have also shown that phenolic compounds had free radical scavenging properties and reduced the oxidative stress associated with diabetes mellitus (kusirisin et al 2009). From this study we concluded that the administration of p-coumaric acid significant results in renovation of the plasma glucose, insulin, HbA^{1C}, lipid peroxidative products and enzymatic antioxidant status in diabetic rats by enhancing the antioxidant activity of tissues in STZ induced diabetic rats.

Table-1: Effect of p-coumaric acid on changes in the body weight of control and experimental rats.

Groups	Body weight (g)	
	Initial	Final
Normal control	185.20+10.23	198+10.50 a
Control+p-Coumaric acid	183.00+11.13	206+08.54 a
Diabetic control	210.50+13.94	148+12.54b
Diabetic+p-coumaric acid	215.20+12.85	189+11.82c

Values are mean ± SD for 6 rats in each group. In each rows, means with different superscript letter differ significantly at $p < 0.05$ (DMRT).

Table-2: Effect of p-coumaric acid on changes in the levels of plasma glucose, insulin, Hb and HbA^{1C} in control and experimental rats.

Groups	Plasma glucose (mg/dl)	Plasma insulin (µU/ml)	Total hemoglobin (g/dl)	Glycosylated hemoglobin (Hb%)
Normal control	98.56+6.29 a	14.52+0.88 a	13.32+0.73a	5.48+0.32a
Control+p-coumaric acid	93.20+6.17 a	14.63+0.84 a	13.40+0.80 a	5.13+0.22 a
Diabetic control	233.36+14.18 b	06.11+0.42 b	7.88+0.50 b	13.81+0.96 b
Diabetic+p-coumaric acid	147.72+9.11 c	11.80+0.73 c	11.27+0.71 c	7.63+0.46 c

Values are mean ± SD for 6 rats in each group. In each column, different superscript letters mean significant differences at $p < 0.05$ (DMRT).

Table-3: Effect of p-coumaric acid on changes in the levels of TBARS and hydroperoxides in tissues of control and experimental rats

Groups	TBARS (mM/100 g)		Hydroperoxides (mM/100 g)	
	Liver	Kidney	Liver	Kidney
Normal control	0.92+ 0.05a	1.43+0.07a	61.88+3.41a	59.91+3.31a
Control+p-coumaric acid	0.85+ 0.06a	1.35+0.08a	61.20+3.72a	59.03+3.59a
Diabetic control	2.39+ 0.15b	3.21+0.21b	109.12+7.24b	148.19+9.84b
Diabetic+p-coumaric acid	1.52+ 0.09c	2.10+0.12c	83.73+5.00c	70.44+4.20c

Values are mean ± SD for 6 rats in each group. In each rows, means with different superscript letter differ significantly at $p < 0.05$ (DMRT).

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Table-4: Effect of p-coumaric acid on changes in the activities of SOD, CAT and GPx in tissues of control and experimental rats.

Groups	SOD (Units/mg)		CAT (Units/mg)		GPx (Units/mg)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal control	8.52±0.47 [a]	15.43±0.93 [a]	72.24±4.62 [a]	60.32±3.33 [a]	09.71±0.53 [a]	8.70±0.48 [a]
Control+*p*-coumaric acid	8.83±0.53 [a]	15.97±0.60 [a]	73.30±4.86 [a]	61.79±3.55 [a]	10.13±0.56 [a]	8.78±0.55 [a]
Diabetic control	4.51±0.38 [b]	8.22±0.79 [b]	42.73±3.83 [b]	39.45±2.62 [b]	5.38±0.32 [b]	4.38±0.29 [b]
Diabetic+*p*-coumaric acid	7.09±0.30 [c]	11.88±0.72 [c]	61.18±4.06 [c]	47.97±3.07 [c]	8.29±0.47 [c]	6.43±0.39 [c]

Values are mean ± SD for 6 rats in each group. In each rows, means with different superscript letter differ significantly at $p < 0.05$ (DMRT).

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