ABSTRACT

Diabetes Mellitus is a chronic widely spread metabolic disorder. Chronic hyperglycemia in diabetes is associated with oxidative stress mediated tissue damage. The present study was designed to investigate the effect of p-coumaric acid on lipid peroxidative products (thiobarbituric acid reactive substance (TBARS) and lipid hydroperoxide), antioxidant status and histopathological examinations in streptozotocin (STZ)-induced diabetic rats. STZ induction (45 mg/kg/i.p) caused a hyperglycemic state that lead to various physiological and biochemical alterations. 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 days. Diabetic rats had elevated levels of TBARS and hydroperoxide and decreased antioxidant activities (both enzymatic and non-enzymatic) when compared with normal control rats. Treatment with p-coumaric acid shows the decreased level of lipid peroxides and increased levels of antioxidants in STZ-induced diabetic rats. These results demonstrated that p-coumaric acid has strong antidiabetic effects thus; it may have beneficial properties in the prevention of diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a widespread disease that is associated with high morbidity and health care costs (Mednieks, et al., 2009). DM is a generalized common chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion and/or action (Mitrovic, et al., 2006; Unger and Parkin, 2010) and certain abnormalities in carbohydrate, fat, electrolyte, and protein metabolism (Kumar and Clark, 2002). Prolonged periods of hyperglycemia lead to glucose degradation within living tissues, causing an accumulation of glucose within organ cells, which result in various micro and macrovascular complications of DM such as coronary heart disease, retinopathy, nephropathies, and neuropathies (Chaiyawat, et al., 2010). World Health Organization (WHO) estimates that more than 220 million people worldwide have diabetes and this number is likely to double by 2030 (Aragao, et al., 2010). According to the Diabetes atlas 2009, India has the largest number of people with diabetes in the world, with an estimated 50.8 million people diabetic and this may rise to 87 million by the year 2030 (IDA, 2009).

Streptozotocin (STZ), an antibiotic produced by Streptomyces achromogenes, is frequently used to induce DM in experimental animals through its toxic effects on pancreatic β-cells (Kim, et al., 2003). It is taken into the pancreatic β-cells by glucose transporter 2 (GLUT-2) (Vessal, et al., 2003). The cytotoxic action of STZ is associated with the generation of ROS causing oxidative damage (Szkudelski, 2001). Substances with antioxidant properties and free radical scavenging properties may help in the regeneration of β-cells and protect the pancreatic islets against the cytotoxic effects of STZ (Coskun, et al., 2005).

Oxidative stress caused by the production of free radical is a recognized participant in the development and progression of diabetes and its complications (Ceriello, 2000). These free radicals also destroy pancreatic β-cells that produce and secrete insulin (Laybutt, et al., 2002). Most of the diabetic complications are due to generation of reactive oxygen species (ROS), which lead to endothelium dysfunction (Potenza, et al., 2009). There are many protective enzymes against ROS, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Soto, et al., 2003).
The pharmacological agents currently used for the treatment of type 2 diabetes include sulfonylurea, biguanide, thiazolidinedione and α-glycosidase inhibitors. These agents, however, have restricted usage due to several undesirable side effects and fail to significantly alter the course of diabetic complications (Rang and Dale, 1991). The high prevalence of diabetes as well as its long term complications has led to an ongoing search for hypoglycemic agents from natural sources (Nicasio, et al., 2005). Herbal remedies have been used since ancient times for the treatment of diabetes mellitus. About 90% of the world population in rural areas of developing countries relies solely on traditional medicines for their primary health care (Hassan, et al., 2010).

*p*-Coumaric acid (4-hydroxyphenyl-2-propenoic acid) is a phenolic acid widely distributed in plants and form a part of human diet (Fig 1) (Scalbert and Williamson, 2000). The main dietary phenolic acids sources are fruits and beverages such as tea, coffee, wine, chocolate, and beer (King and Young, 1999). The mechanisms of antioxidant effect of phenolics include binding of metal ions, scavenging of reactive oxygen species (ROS); reactive nitrogen species (RNS); or their precursors, up-regulation of endogenous antioxidant enzymes, or the repair of oxidative damage to biomolecules (Ursini, et al., 1999). The antioxidant activity of natural phenolic acids depends on the number and relative position of the hydroxyl groups on the ring, which give reducing properties and hydrogen donating abilities (Rice-Evans, et al., 1996). Recent interest in food phenolics has increased owing to their roles as antioxidants and scavengers of free radicals and their implication in the prevention of many pathologic diseases such as cardiovascular disease (Ursini, et al., 1999) and certain types of cancer (Hudson, et al., 2000). The aim of the present study is to investigate the anti-diabetic effect of *p*-coumaric acid on lipid peroxidation and antioxidant status in STZ-induced diabetic rats.

![Figure 1: Structure of *p*-coumaric acid](image)

**MATERIALS AND METHODS**

**Animals and diet**

Male albino Wistar rats (170-200 g) were obtained from Venkateswaran Enterprises, Bangalore and used in this study. They were housed in polypropylene cages (47x34x20 cm) lined with husk, renewed every 24 hours under a 12:12 hours 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 metabolisable energy of 3,600 kcal. The experiment was carried out in accordance with 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 Muthayammal College of Arts & Science, Rasipuram (MCAS/Ph.D.,/02/2012-2013).

**Chemicals**

*p*-Coumaric acid, reduced nicotinamide adenine dinucleotide (NADH), and phenazine methosulphate (PMS) were purchased from Sigma Chemical Company, St. Louis, MO, USA. Streptozotocin (STZ), thiobarbituric acid (TBA), 1, 1’, 3 , 3’ tetramethoxy propane, butylated hydroxy toluene (BHT), xylenol orange, dithionitro bis benzoic acid (DTNB), ascorbic acid, 2, 2’ dipyridyl, p-phenylene diamine and sodium azide were obtained from Himedia laboratory, Mumbai, India. All other chemicals were obtained under analytical grade.

**Induction of experimental diabetes**

Streptozotocin (STZ) 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).

**Experimental design**

In the experiment, a total of 24 rats (12 diabetic surviving rats, 12 control rats) were used in the study. The rats were divided into 4 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 days.

- 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 (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 (pancreas, 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
The level of plasma TBARS was estimated by the method Yagi (1987), and the tissue TBARS was estimated by the method of Fraga, et al. (1988). The levels of lipid hydroperoxides (HP) were estimated by the method of Jiang, et al. (1992). The activity of SOD, catalase and GPx was assayed according to the procedures of Kakkar, et al. (1984), Sinha (1972) and Rotruck, et al. (1973), respectively. The level of ceruloplasmin was estimated by the method of Ravin (1961). The level of GSH was estimated by the method of Ellman (1959). The level of vitamin C and E was estimated by the methods of Omaye, et al. (1979) and Baker, et al. (1980), respectively.

Histopathology
Tissues (liver and kidney) obtained from all experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the tissues were processed by embedding in paraffin. Then, the tissues were sectioned and stained with hematoxylin and eosin (H&E) and examined under high power microscope (400x) and photomicrographs were taken.

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 16.00. P values <0.05 were considered significant.

RESULTS
Effect of p-coumaric acid on TBARS and hydroperoxides in plasma and tissues
The levels of TBARS and hydroperoxides in plasma and tissue of diabetic and control rats are presented in Tables 1 & 2. STZ-induced diabetic rats had elevated levels of TBARS and hydroperoxides in plasma and tissue. Treatment with p-coumaric acid (100 mg/kg for 45 days) in STZ-induced diabetic rats showed reversal of these parameters to near normal levels.

Table 1: Effect of p-coumaric acid (PCA) on the levels of thiobarbituric acid reactive substances (TBARS) in plasma and tissues in normal and streptozotocin (STZ)-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma TBARS (nmol/ml)</th>
<th>Tissue TBARS (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pancreas</td>
<td>Liver</td>
</tr>
<tr>
<td>Normal control</td>
<td>2.16 ± 0.11a</td>
<td>0.32 ± 0.01a</td>
</tr>
<tr>
<td>Normal + PCA (100 mg/kg)</td>
<td>2.12 ± 0.10b</td>
<td>0.30 ± 0.01a</td>
</tr>
<tr>
<td>Diabetic control (45 mg/kg)</td>
<td>3.73 ± 0.19c</td>
<td>0.67 ± 0.03a</td>
</tr>
<tr>
<td>Diabetic + PCA (100 mg/kg)</td>
<td>2.69 ± 0.13b</td>
<td>0.40 ± 0.02a</td>
</tr>
</tbody>
</table>

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

Table 2: Effect of p-coumaric acid (PCA) on the levels of lipid hydroperoxide (HP) in plasma and tissues in normal and streptozotocin (STZ)-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma HP (nmol/ml)</th>
<th>Tissue HP (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pancreas</td>
<td>Liver</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.84 ± 0.04a</td>
<td>0.18 ± 0.01a</td>
</tr>
<tr>
<td>Normal + PCA (100 mg/kg)</td>
<td>0.81 ± 0.03a</td>
<td>0.18 ± 0.01a</td>
</tr>
<tr>
<td>Diabetic control (45 mg/kg)</td>
<td>1.67 ± 0.08a</td>
<td>0.35 ± 0.02a</td>
</tr>
<tr>
<td>Diabetic + PCA (100 mg/kg)</td>
<td>1.02 ± 0.04a</td>
<td>0.22 ± 0.01a</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-c) differ significantly with each other (P<0.05, DMRT).
Effect of p-coumaric acid on antioxidants in plasma and tissues
The activities of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the pancreas, liver and kidney of normal and STZ-induced diabetic animals were shown in Tables 3, 4 & 5. STZ-induced diabetic rats showed significant reduction in the activity of SOD, CAT and GPx. Oral administration of p-coumaric acid exerted a significant effect on the antioxidants in STZ-induced diabetic rats.

Table 3: Effect of p-coumaric acid (PCA) on the activities of superoxide dismutase (SOD) in normal and streptozotocin (STZ)-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pancreas</td>
</tr>
<tr>
<td>Normal control</td>
<td>6.40 ± 0.32a</td>
</tr>
<tr>
<td>Normal + PCA (100 mg/kg)</td>
<td>6.40 ± 0.32a</td>
</tr>
<tr>
<td>Diabetic control (45 mg/kg)</td>
<td>4.11 ± 0.20b</td>
</tr>
<tr>
<td>Diabetic + PCA (100 mg/kg)</td>
<td>5.80 ± 0.29c</td>
</tr>
</tbody>
</table>

U – Enzyme concentration required to inhibit the chromogen produced by 50% in one minute.
Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-c) differ significantly with each other (P<0.05, DMRT).

Table 4: Effect of p-coumaric acid (PCA) on the activities of catalase in normal and streptozotocin (STZ)-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pancreas</td>
</tr>
<tr>
<td>Normal control</td>
<td>10.98 ± 0.54a</td>
</tr>
<tr>
<td>Normal + PCA (100 mg/kg)</td>
<td>11.03 ± 0.55a</td>
</tr>
<tr>
<td>Diabetic control (45 mg/kg)</td>
<td>7.19 ± 0.33b</td>
</tr>
<tr>
<td>Diabetic + PCA (100 mg/kg)</td>
<td>10.01 ± 0.50c</td>
</tr>
</tbody>
</table>

U – μmol of hydrogen peroxide consumed per minute.
Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-c) differ significantly with each other (P<0.05, DMRT).

Table 5: Effect of p-coumaric acid on the activities of glutathione peroxidase (GPx) in normal and streptozotocin (STZ)-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GPx (U/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pancreas</td>
</tr>
<tr>
<td>Normal control</td>
<td>8.49 ± 0.42a</td>
</tr>
<tr>
<td>Normal + PCA (100 mg/kg)</td>
<td>8.53 ± 0.43a</td>
</tr>
<tr>
<td>Diabetic control (45 mg/kg)</td>
<td>4.09 ± 0.20b</td>
</tr>
<tr>
<td>Diabetic + PCA (100 mg/kg)</td>
<td>7.18 ± 0.36c</td>
</tr>
</tbody>
</table>

U = μg glutathione consumed.
Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-c) differ significantly with each other (P<0.05, DMRT).
The activity of non-enzymatic antioxidants such as reduced glutathione (GSH), ceruloplasmin, vitamin C and vitamin E in the plasma, pancreas, liver and kidney of normal and STZ-induced diabetic rats were shown in Tables 6, 7, 8 & 9. The levels of ceruloplasmin, GSH, vitamin C and E were found to be significantly reduced in plasma, pancreas, liver and kidney of diabetic rats. Oral administration of p-coumaric acid significantly increased the levels of ceruloplasmin, GSH, vitamin C and E in STZ-induced diabetic rats.

Table 6: Effect of p-coumaric acid on the activities of plasma ceruloplasmin and reduced glutathione (GSH) in normal and streptozocin (STZ)-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ceruloplasmin (nmol/ml)</th>
<th>GSH (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.33 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal + PCA (100 mg/kg)</td>
<td>1.34 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic control (45 mg/kg)</td>
<td>0.89 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + PCA (100 mg/kg)</td>
<td>1.22 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.56 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

Table 7: Effect of p-coumaric acid on the activities of reduced glutathione (GSH) in normal and streptozocin (STZ)-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pancreas</td>
</tr>
<tr>
<td>Normal control</td>
<td>17.57 ± 0.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal + PCA (100 mg/kg)</td>
<td>17.63 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic control (45 mg/kg)</td>
<td>12.77 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + PCA (100 mg/kg)</td>
<td>15.92 ± 0.78&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-c) differ significantly with each other (P<0.05, DMRT).
Effect of p-coumaric acid on histopathology of diabetic rat liver and kidney

The effects of p-coumaric acid on histopathology of diabetic rat liver are shown in Fig 2. Normal control rats showed normal hepatic structure. Normal rats treated with p-coumaric acid (100 mg/kg) showed portal triad and normal architecture of the hepatic tissue. STZ-induced diabetic control rats showed dilation of hepatic sinusoids and inflammatory cells. Rats treated with p-coumaric acid (100 mg/kg) showed near normal appearance with few inflammatory cells.

Table 8: Effect of p-coumaric acid on the levels of vitamin C in normal and streptozotocin (STZ)-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma (µmol/ml)</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.08 ± 0.004a</td>
</tr>
<tr>
<td>Normal + PCA (100 mg/kg)</td>
<td>0.09 ± 0.005a</td>
</tr>
<tr>
<td>Diabetic control (45 mg/kg)</td>
<td>0.02 ± 0.001b</td>
</tr>
<tr>
<td>Diabetic + PCA (100 mg/kg)</td>
<td>0.07 ± 0.004a</td>
</tr>
</tbody>
</table>

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

Table 9: Effect of p-coumaric acid on the levels of vitamin E in normal and streptozotocin (STZ)-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma (µmol/ml)</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.02 ± 0.002a</td>
</tr>
<tr>
<td>Normal + PCA (100 mg/kg)</td>
<td>0.02 ± 0.002a</td>
</tr>
<tr>
<td>Diabetic control (45 mg/kg)</td>
<td>0.01 ± 0.001b</td>
</tr>
<tr>
<td>Diabetic + PCA (100 mg/kg)</td>
<td>0.02 ± 0.002c</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-c) differ significantly with each other (P<0.05, DMRT).
The effects of $p$-coumaric acid on histopathology of diabetic rat kidney are shown in Fig 3. Normal control rats showed normal architecture of mesangial cells and glomeruli. Normal rats treated with $p$-coumaric acid (100 mg/kg) showed normal glomeruli and tubules. STZ-induced diabetic control rats showed severe epithelial atrophy and mesangial cell proliferation and cloudy swelling of the tubules. Rats treated with $p$-coumaric acid (100 mg/kg) showed showing mild glomerular changes with normal tubules.

**DISCUSSION**
Streptozotocin (STZ) is a nitrosourea analogue, preferentially uptaken by pancreatic β-cells via GLUT-2 glucose transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD⁺ and ATP. Enhanced ATP dephosphorylation after STZ treatment provides substrate for xanthine oxidase resulting in the formation of superoxide radicals. Further, nitric oxide moiety is liberated from STZ leading to the destruction of β-cells by necrosis (Szkudelski, 2001). Hence, STZ-induced experimental diabetic animal model is chosen for the present study.

Oxidative stress in diabetes coexisted with a reduction in the antioxidant capacity, which could increase the deleterious effects of free radicals. The accumulation of free radical observed in diabetic rats is attributed to chronic hyperglycemia that alters antioxidant defense system (Hong, et al., 2004). Free radicals may also be formed through the auto-oxidation of unsaturated lipids in plasma and membrane lipids. They may react with polyunsaturated fatty acids in cell membrane leading to lipid peroxidation (Lery, et al., 1999).

Lipid peroxidation is one of the characteristic features of diabetes mellitus. The decline of antioxidant defense mechanisms associated with an excess of free radicals lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance (Maritim, et al., 2003). Lipid peroxidation results in damage to the cell membrane, caused by ROS (Das, et al., 2000). Measurement of plasma thiobarbituric acid reactive substances (TBARS) was used as an index of lipid peroxidation and it helps to assess the extent of tissue damage (Gutteridge, 1995). Lipid peroxides and hydroperoxides are the oxidative stress markers that are elevated as a result of the toxic effect of reactive oxygen species (ROS) produced during chronic hyperglycemia (Evans, et al., 2002). Several studies have reported an increase in TBARS and hydroperoxides in plasma and tissues in experimental diabetes mellitus (Pari and Venkateswaran, 2002). Oral administration of p-coumaric acid (100 mg/kg for a period of 45 days) significantly decreases the levels of TBARS and hydroperoxides in plasma and tissues. This shows that p-coumaric acid has the ability to protect organism from lipid peroxidation and reduces the risk of tissue damage.

Cells and tissues contain an array of antioxidant machinery, which averts the buildup of ROS and maintain the redox balance. Chronic oxidative stress is associated with decrease in the antioxidant competence, which can further increase the deleterious effects of free radicals (Atli, et al., 2004). Antioxidant enzymes such as SOD, CAT and GPx, form the first line of defense against ROS in the organism, play an important role in scavenging the toxic intermediate of incomplete oxidation.

Superoxide dismutase is an antioxidative defense enzyme, protects from oxygen free radicals by catalyzing the removal of superoxide radical, which damage the membrane and biological structures. SOD destroys the superoxide radical through dismutation and generates hydrogen peroxide, which is consecutively reduced by the activities of catalase or glutathione peroxidase. Several studies have reported a reduced activity of SOD in experimental diabetes (Meghana, et al., 2007; Bagri, et al., 2009). Thus the reduced activity of SOD could be the result of superoxide anion over accumulation in the cell (Raha and Robinson, 2000), and its inactivation by hydrogen peroxide (Ravi, et al., 2004) or by glycation of the enzyme (Meghana, et al., 2007).

Catalase, another enzymatic antioxidant predominantly present in peroxisomes, ameliorated the deleterious effect of hydrogen peroxide, which was produced by SOD, into water and non-reactive oxygen species. It restrained the generation of hydroxyl radical and protected the cells from oxidative damage. In diabetic conditions, the uncontrolled production of hydrogen peroxide due to the auto-oxidation of glucose, protein glycation and lipid oxidation led to a marked decline in the catalase activity (Rajasekaran, et al., 2005; Farombi and Ige, 2007). Recently, the decreased activity of catalase in the protection of pancreatic β-cells from oxidative stress during diabetic conditions has been reported (Rajasekaran, et al., 2005).

Another antioxidant enzyme, glutathione peroxidase (GPx) was a selenium-containing tetrameric glycoprotein plays a primary role in minimizing oxidative damage. GPx is involved in the detoxification of hydrogen peroxide into water and molecular oxygen. During diabetic conditions, the activity of glutathione peroxidase is decreased as a result of radical-induced inactivation and glycation of the enzyme (Zhang and Tan 2000).

Reduced activities of these antioxidant enzyme in pancreas, liver and kidney was observed in diabetic rats and this activity may resulting a number of deleterious effects due to accumulation of superoxide anion (O) and hydrogen peroxide (H₂O₂), which in turn generate hydroxyl radicals (OH), resulting in initiation and propagation of LPO. Treatment with p-coumaric acid showed a significant increase in SOD, CAT and GPx activities of the diabetic rats. This means that p-coumaric acid can reduce the potential glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes.
Reduced glutathione, a ubiquitous tripeptide thiol, is an important intracellular metabolite. It acts as an antioxidant and provides secondary line of defence against intracellular free radicals and peroxides generated by oxidative stress (Mohammed, 2008). Reduced state of cell is maintained by high level of GSH/GSSG ratio. When the level of GSSG, the oxidized form of GSH increased in the presence of persistently elevated ROS then the redox state of cell get affected and it may result in the development of diabetic complications. Measurement of intracellular GSH/GSSG ratio may provide valuable information about the redox status of the cell (Veerapan, et al., 2004). Diabetic rats showed the decreased levels of GSH and this may be due to the decrease of GSH synthesis or an increase of its degradation induced by STZ oxidative stress. Treatment with p-coumaric acid showed a significant increase in the levels of GSH. This may be due to the increase in GSH biosynthesis or reduce the oxidative stress leading to less degradation of GSH.

Ceruloplasmin is also a sialoglycoprotein. Removal of ceruloplasmin from blood is triggered by the loss of sialic acid units (Osaki, et al., 1966). Increase in sialic acid level may resialate the desialated, ceruloplasmin which may also be responsible for the decreased removal and thereby increasing the level of ceruloplasmin in hyperlipidemic patients with or without diabetes (Kaviarasan, et al., 2005). The level of ceruloplasmin was significantly decreased in diabetic rats, which may facilitate the scavenging action on peroxyl radicals. Administration of p-coumaric acid showed significant reversal of ceruloplasmin level in plasma of diabetic rats.

Vitamin E is the most ancient antioxidant in the lipid phase (Ingold, et al., 1987). Apart from enzymic antioxidants, non-enzymic antioxidants play a vital role in protecting cells from oxidative changes. Vitamin E neutralizes the free radicals, preventing the chain reaction that contributes to oxidative damage (Sun, et al., 1999). It has the potential to quench lipid peroxidation and protects cellular structure from the attack of free radicals (Bertoni-Freddari, et al., 1995). The decreased level of vitamin E observed in diabetic rat (Murugan and Pari, 2006).

Vitamin C (ascorbic acid) is the most widely cited form of water-soluble antioxidant. It prevents oxidative damage to the cell membrane induced by aqueous radicals. Vitamin C can act as a co-oxidant by regenerating α-tocopherol from the α-tocopheroxyl radical produced during scavenging of free radicals. It is also possible that the correction of hyperglycemia has a sparing effect on vitamin C, and this increases the potential to recycle vitamin E (Chan, 1993; Stah and Sies, 1997). We also observed significant decrease in the levels of vitamin C in diabetic rats could be due to increased utilization of vitamin C as an antioxidant defense against reactive oxygen species (Ceriello, et al., 1998). Oral administration of p-coumaric acid improved the level of vitamin C and E level in plasma and tissues of STZ-induced diabetic rats. This may be due to the antioxidant properties of the plant phenolics.

Histopathology of liver in control rats showed normal hepatic structure. Diabetic control liver showed dilation of hepatic sinusoids and inflammatory cells. Diabetic rats treated with p-coumaric acid showed mild sinusoidal dilation and few inflammatory cells. Normal rats treated with p-coumaric acid showed portal triad and normal architecture of the hepatic tissue.

Histopathology of kidney in control rats revealed normal architecture of the mesangial cells and glomeruli. Diabetic control rats showed severe epithelial atrophy and mesangial cell proliferation and cloudy swelling of the tubules. Diabetic rats treated with p-coumaric acid showed mild glomerular changes with normal tubules. Normal rats treated with p-coumaric acid showed normal glomeruli and tubules. This could be due to free radical scavenging, antioxidant and membrane stabilizing properties of p-coumaric acid. The liver and kidney exhibits numerous morphological and functional alterations during diabetes.

CONCLUSION
The results of the present investigation indicate that p-coumaric acid ameliorates hyperglycemia mediated oxidative stress in STZ-induced diabetic rats, which was evidenced by improved glycemic as well as antioxidant status. Thus, we can conclude that p-coumaric acid is a promising adjuvant agent in diabetes mellitus therapy.

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