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p-Coumaric acid regulates blood glucose and antioxidant levels in streptozotocin induced diabetic rats

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ABSTRACT

Antihyperglycemic agents from natural and synthetic sources have been found to successfully manage diabetes. This study was to hypothesis that the oral administration of p-coumaric acid regulates blood glucose and antioxidant levels in streptozotocin (STZ) induced diabetic rats. The STZ induced diabetic rats exhibited increased level of blood glucose, lowered the level of insulin, and decreased the activities of enzymatic and non enzymatic antioxidants in blood as compared with normal rats. Oral administration of p-coumaric acid (100 mg/kg b.wt) was given for a period of 30 days. In our study we recognized that the administration of p-coumaric acid could be lowered blood glucose and improve the level of insulin, enzymatic and non enzymatic antioxidants activities in circulation. The histopathological study of pancreas, liver and kidney revealed the protective role of p-coumaric acid. We could not observe any significant changes in all the biochemical and histopathological studied in normal rats treated with p-coumaric acid. The overall results suggest that the p-coumaric acid possesses potential antihyperglycemic and antioxidant activity in STZ-induced diabetic rats.

Keywords: *p*-coumaric acid, antihyperglycemic, streptozotocin.

INTRODUCTION

Type 2 diabetes mellitus (DM) consists of a range of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion this leads to chronic hyperglycemia [1]. During DM persistent hyperglycemia causes an increased production of reactive oxygen species (ROS) via auto-oxidation of glucose and non-enzymatic protein glycation which may lead to disruption of cellular functions and oxidative damage to membranes [2]. An increase in ROS is an impairment of antioxidant defense system or an insufficient capacity to repair oxidative damage [3].

Insulin affects many sites of mammalian lipid metabolism. It stimulates synthesis of fatty acid in liver adipose tissue and in the intestine. The insulin has also been reported to increase the cholesterol synthesis [4]. The activity of lipoprotein lipase in white adipose is also increased. From this point of view the assessment of various lipid fractions and lipid peroxide in the cases of DM may be help in the prognosis of patients and in preventing the possibilities of complications or secondary disorders [5]. Peroxidation of lipid membrane has been related to the pathogenesis of many degenerative diseases, such as atherosclerosis, oxidative damage to DNA aging, carcinogenesis, sickle cell disease and etc. [6].

In 2013, there were 382 million people with diabetes, and this number is expected to rise to 592 million by 2035 [7]. In India, it is estimated that 61.3 million people with diabetes aged 20-79 years live (2011 estimates). This number is

expected to increase to 101.2 million by 2030 [8]. In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively.

Streptozotocin (STZ) is a broad spectrum antibiotic and alkylating genotoxic agent which possesses antibacterial, tumoricidal, carcinogenic and diabetogenic properties [9;10]. Induction of experimental diabetes in rats using streptozotocin is very simple to do and provides a convenient model to study the activity of hypoglycemic agents [11;12]. Furthermore, herbal supplements and other choice of medicines have progressively increase to use for the treatment of diabetic disorders [13]. Plant derived polyphenolic compounds possess a wide range of pharmacological properties and the study of their mechanism of action has been the subject of considerable interest in recent years [14]. World Health Organization (WHO) has given sufficient stress in utilizing traditional plants and plant products for diabetes, since they are non-toxic, efficient, with less or no side effect. There is an inverse association between dietary phenolic compound intake and mortality from various diseases. 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 [15]. *p*-Coumaric acid (3-[4-hydroxyphenyl]-2-propenoic acid) is a phenolic compound, abundantly present in pineapple. *p*-Coumaric acid is a ubiquitous plant metabolite possess antioxidant [16], antiinflammatory, anticancer [17], and hepatoprotective effect [18].

In the present study, we assess the effect of *p*-coumaric acid on the levels of glucose, insulin and the activities of enzymatic and non enzymatic antioxidants and also the changes occurs in the histopathology of pancreas, liver and kidney tissues of experimental rats.

EXPERIMENTAL SECTION

1.1. 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 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).

1.2. Chemicals

p-Coumaric acid and Streptozotocin (STZ) were purchased from Sigma Chemical Co (St. Louis, Mo. USA). All other chemicals and solvents were of analytical grade and purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India.

1.3. Experimental induction of type 2 diabetes in rats

Diabetes was induced in overnight-fasted experimental animals by a single intrapertonial (i.p) injection of STZ (40 mg/kg b.w.), dissolved in citrate buffer (0.1M, pH 4.5) [19]. 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 h, plasma glucose was determined and those rats with fasting blood glucose greater than 250 mg/dl were used in the present study.

1.4. 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 intra gastric 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.)

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. The pancreas, liver and kidney tissues were dissected and collected in ice-cold formalin for histopathological estimations**.**

1.5. Biochemical analysis

Plasma glucose level was estimated by the method of trinder using a commercial kit [23]. Plasma insulin was assayed by ELISA kit (Boeheringer-Manneheim Kit, Manneheim, Germany). Thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) by the method of Niehius and Samuelsson [45]. SOD [20], CAT [21]), GPx [22].

1.6. Histopathological examination

Pancreas, liver and kidney were excised, fixed in 10% neutral formalin, dehydrated in graded alcohol (80-100%), cleared in xylene, and embedded in paraf-169 fin. Then, the tissues were sliced into 5 μm pieces using microtome, deparaffinated in xylene, passed through from 80% to 100% alcohol and attained with hematoxylin and eosin for Olympus BX 40 photo-172 microscope assessments.

1.7. Statistical analysis

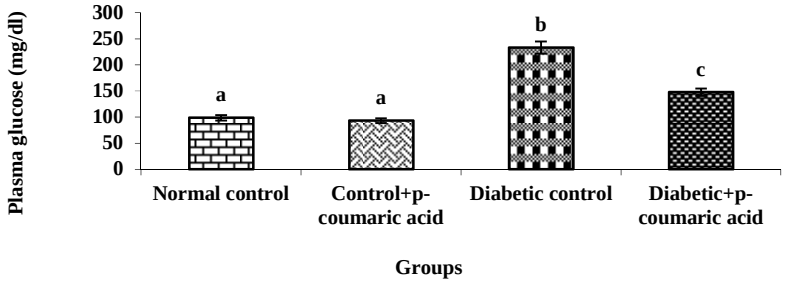
All the data were expressed as mean ± SD of number of experiments (n= 6). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS Version 15 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Duncan's multiple range test (DMRT). Values are considered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

RESULTS

1.7.1.Effect of *p*-coumaric acid on plasma glucose and insulin levels

Figure 1 and 2 describes the levels of plasma glucose and insulin in different experimental groups. Diabetic rats exhibited increased levels of plasma glucose with a decrease in insulin level when compared to normal control rats. Oral administration of *p*-coumaric acid to diabetic rats improved the glycemic status with a significant increase in plasma insulin level.

Figure-1. The changes in the plasma glucose of control and experimental rats



1.7.2.Effect of *p*-coumaric acid on enzymatic antioxidants in normal and diabetic rats

Figure 3 and 5 represents the activities of antioxidant enzymes (SOD, CAT and GPx) in erythrocytes 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 in the antioxidant status.

Figure-2. The changes in the plasma insulin of control and experimental rats

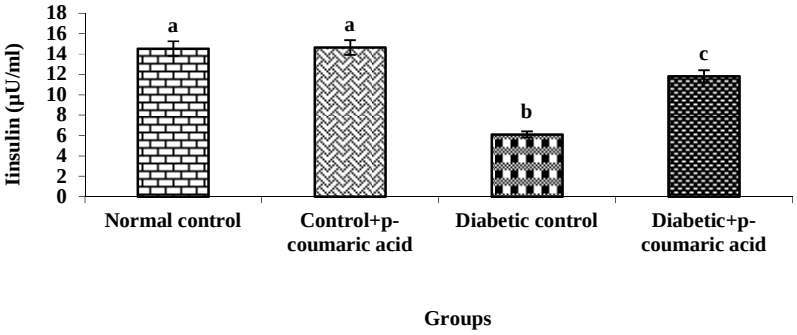


Figure-3. The changes on the activity of SOD in erythrocytes of control and experimental rats

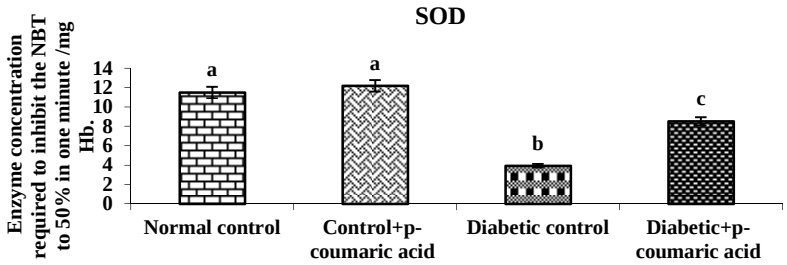
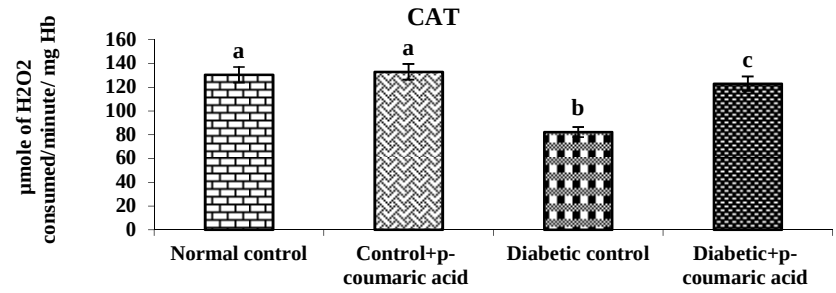


Figure-4. The changes on CAT in erythrocytes of control and experimental rats



1.7.3. Effect of *p*-coumaric acid on non-enzymatic antioxidants in normal and diabetic rats

Figure 5 and 6 shows the effect of *p*-coumaric acid on vitamin C, vitamin E and GSH in plasma of control and diabetic rats. In diabetic rats the level of vitamin E, vitamin C and GSH levels were decreased in the circulation. Oral administration of *p*-coumaric acid increased the vitamin E, vitamin C and GSH levels when compared with diabetic rars.

Figure-5. The changes on GPx in erythrocytes of control and experimental rats

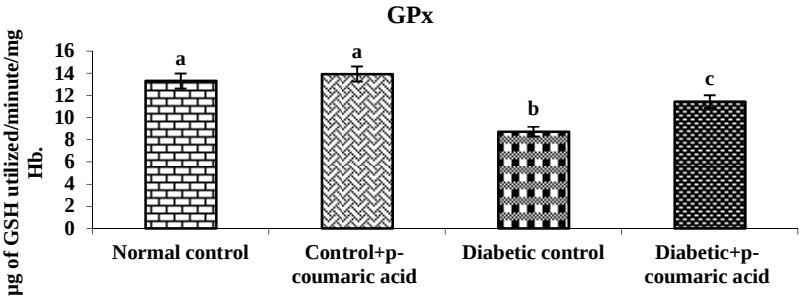


Figure-5. The changes on vitamin-C and E in plasma of control and experimental rats

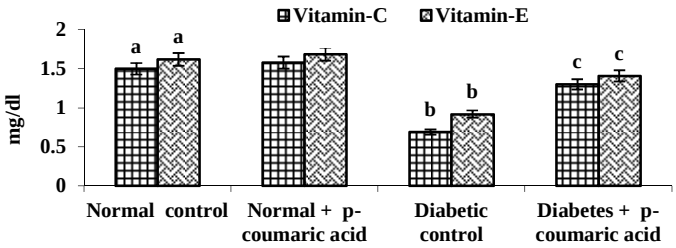
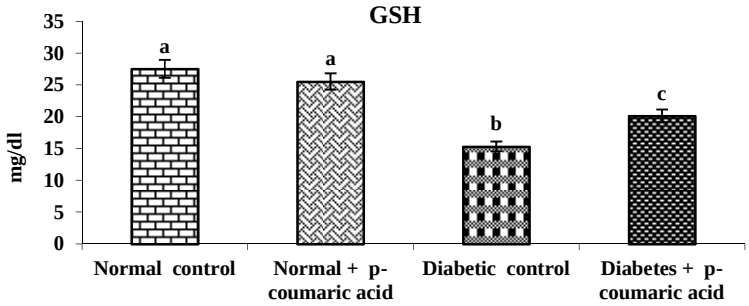


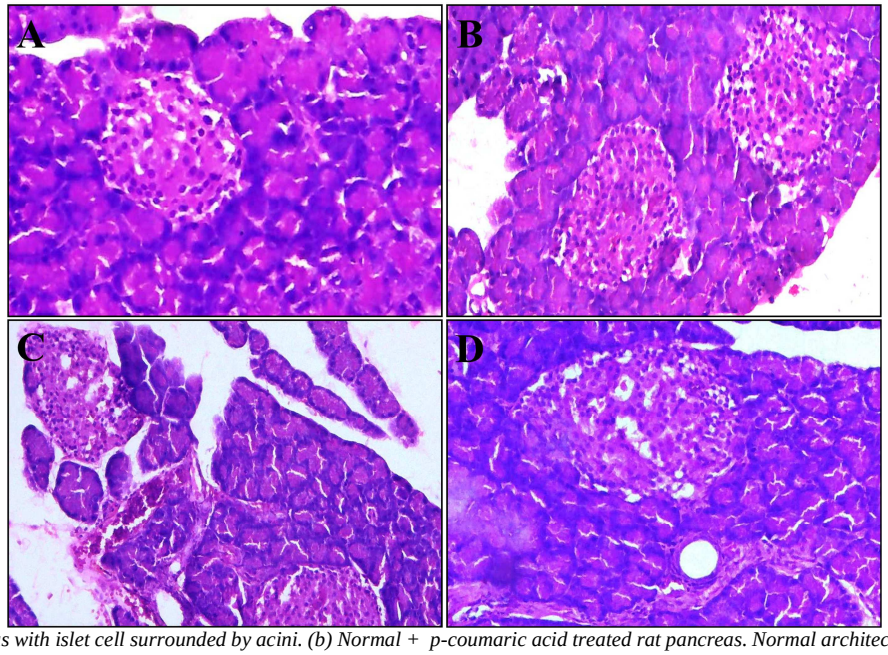
Figure-6. The changes on GSH in plasma of control and experimental rats



1.7.4. Effect of *p*-coumaric acid on histopathology of pancreas, liver and kidney

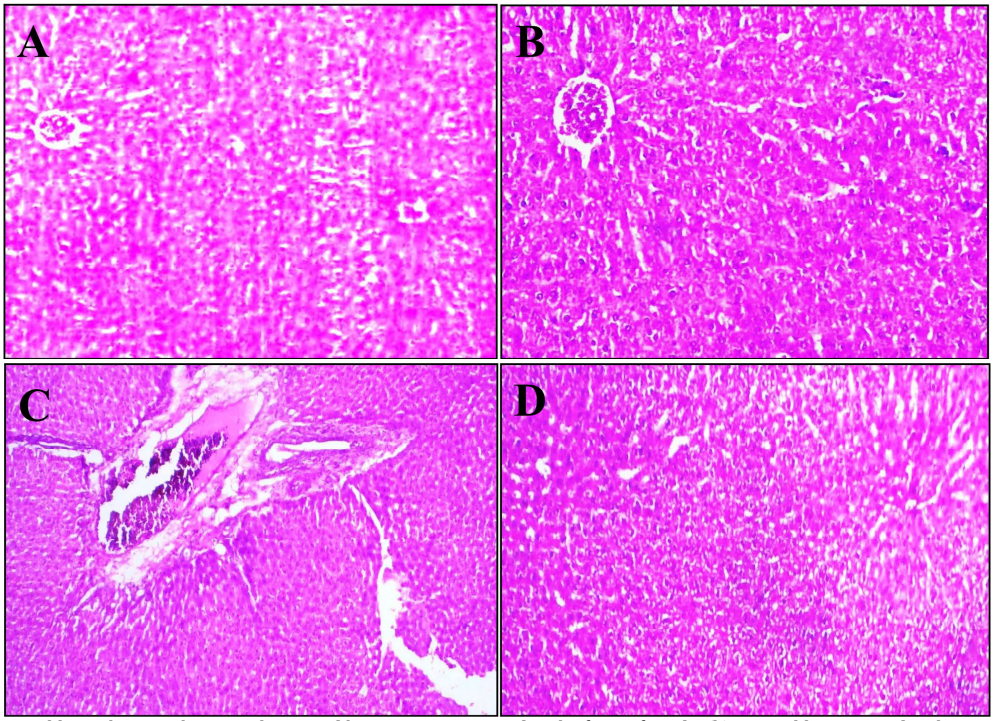
Figure-7 to 9 shows histopathological observation of pancreas, liver and kidney in experimental rats after 30 days of treatment with *p*-coumaric acid (100 mg/kg b.wt). H&E staining Histopathology studies of pancreas of STZ induced diabetic rat displayed reduction of the Islets of langerhans, damaged or reduced the size of β-cells and extensive necrosis changes followed by fibrosis and atrophy. STZ induced diabetic rat treated with p-coumaric acid restored the necrotic and fibrotic changes and raised the number of β-cells. Histopathology studies of liver normal rats shows central vein surrounded by normal hepatocytes. Diabetic control rats shows central vein, surrounded by dilated sinusoids with focal fatty changes of hepatocytes. *p*-Coumaric acid treated rats present central vein surrounded by hepatocytes. Histopathology studies of kidney Shows normal glomerulus with normal tubules. Diabetic rat shows congested glomeruli with lymphocytic in the interstium. *p*-Coumaric acid treated rats present glomeruli surrounded by normal and fin dilated tubules.

Figure-7. Histopathological changes in pancreas of normal and experimental rats. All the sections are in H&E 10X



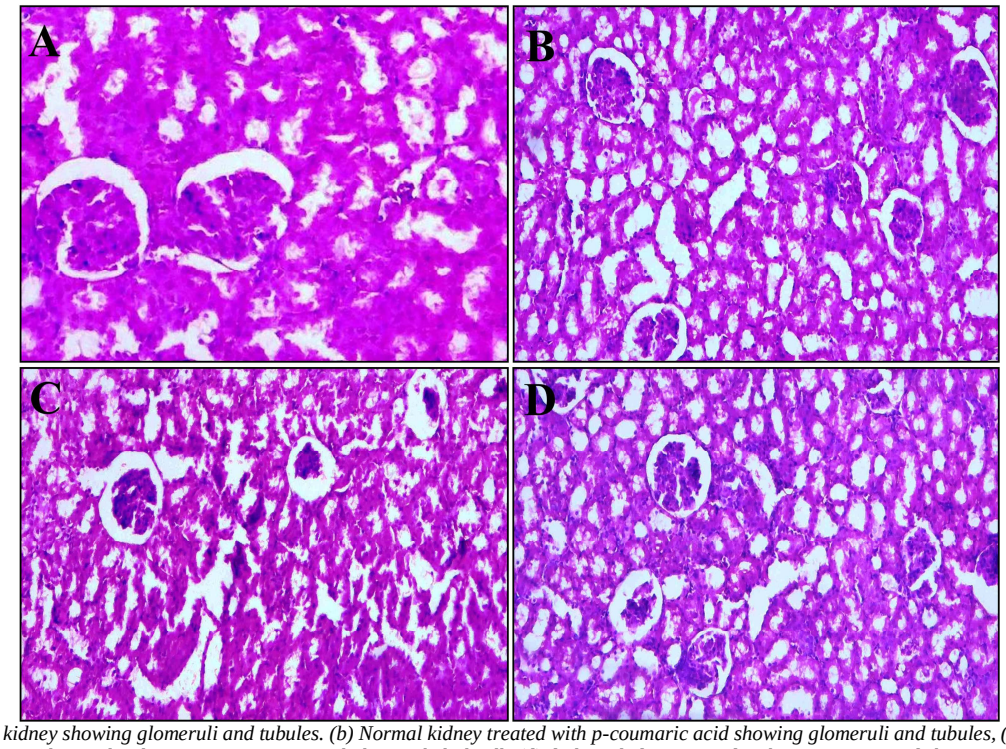
(a) Normal pancreas with islet cell surrounded by acini. (b) Normal + p-coumaric acid treated rat pancreas. Normal architecture of pancreatic cells. (c) Diabetic rat pancreas shows atrophic acini with fatty infiltration. (d) Diabetic + p-coumaric acid treated rat pancreas. Preservation of islet cells with few atrophic acini.

Figure-8. Histopathological changes of liver in normal and experimental rats. All the sections are in H&E 10X.



(a) Normal control liver showing the central vein and hepatocytes arranged in the form of cords. (b) Normal liver treated with p-coumaric acid showing normal histology, (c) diabetic liver showing feathery degeneration, micro and macrovesicular fatty changes, periportal fibrosis and vascular congestion, (d) diabetic liver treated with p-coumaric acid changes with the central vein congestion.

Figure-9. Histopathological changes of kidney in normal and experimental rats. All the sections are in H&E 10X



(a) Normal kidney showing glomeruli and tubules. (b) Normal kidney treated with p-coumaric acid showing glomeruli and tubules, (c) diabetic kidney showing Glomerulosclerosis, vacuoation in tubular epithelial cells (d) diabetic kidney treated with p-coumaric acid showing near normal appearance of glomeruli and tubules.

DISCUSSION

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 [23].

STZ causes a massive reduction in insulin release, by the destruction of the β-cell of the islets of langerhans and thereby induces hyperglycemia [24]. In diabetes mellitus, insulin is not or insufficiently synthesized, developing hyperglycemia with biochemical changes in glucose and lipid metabolism leading to an increased production of reactive oxygen species [25]. Fasting plasma glucose of diabetic rats was significantly increased than normal rats. In *p*-coumaric acid treated diabetic rats, significant decrease in blood glucose levels and significant increase in plasma insulin levels were observed in our earlier report [26]. No change was observed in the blood glucose levels of normal and *p*-coumaric acid treated with normal rats.

Phenolic acids such as coumarin, cinnamic acid etc., stimulate the secretion of insulin in β-cells of pancreas [27;28]. Normal mechanism of glucose induced insulin secretion begins with its transport into the β-cell by the GLUT-2 glucose transporter. Glucose phosphorylation by glucokinase is the rate limiting step that controls glucose-regulated insulin secretion. Further metabolism of glucose-6-phosphate via gycolysis generates ATP, which inhibits the activity of and ATP-sensitive K^+ channel. Inhibition of this K^+ channel induces β-cell membrane depolarization, which open voltage-dependent calcium channels (leading to an influx of calcium), and simulates insulin secretion. The decrease in blood glucose and increase the level of insulin in diabetic rats treated with *p*-coumaric acid might be due to enhanced insulin secretion and increase the utilization of glucose.

The antioxidant enzyme system is closely integrated and the alterations of any one enzyme may have its effect on other enzymes [29]. Free radical scavenging enzymes such as SOD, CAT and GPx are the first line of cellular defense against oxidative injury which is involved in the disposal of superoxide anions and H_2O_2 [30].

The enzymatic antioxidants are playing a vital role in preventing cells from being exposed to oxidative damage. SOD is capable of reducing the super oxide radical in H_2O_2. The other enzymatic antioxidant CAT catalyzes the reduction of hydrogen peroxides and protects the tissues against reactive hydroxyl radicals [31]. 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 [32]. CAT, SOD and GPx activities were bought to near normal indicating the efficacy of *p*-coumaric acid in attenuating the oxidative stress in liver of diabetic rats. Previous studies have also shown that phenolic compounds had free radical scavenging properties and reduced the oxidative stress associated with diabetes mellitus [33]. Hence, a compound that could prevent the generation of these oxygen free radicals or increase the free radical scavenging enzymes may be effective in STZ-induced diabetes. In our study the enzymatic antioxidant activities such as SOD, CAT and GPx were decreased in diabetic rats and diabetic rats treated with *p*-coumaric acid shows a significant increase in these enzyme activities. The results suggest that *p*-coumaric acid possess free radical scavenging activity, which could exert a beneficial effect against pathological alterations caused by free radicals.

Non-enzymatic antioxidants such as vitamin C and Vitamin E and GSH, play an excellent role in preventing the cells from oxidative stress. Vitamin C (ascorbic acid) is a hydrophilic antioxidant and disappears faster than other antioxidants on exposure to ROS [34]. The decreased level of ascorbic acid in diabetic rats may be due to increased utilization of antioxidants against increased ROS. The reduction in Vitamin C levels may also due to decrease in glutathione level, since glutathione is required for the recycling of ascorbic acid [35]. In our study, Vitamin C was decreased in diabetic rats as reported earlier [36]. Treatment with *p*-coumaric acid to diabetic rats reversed Vitamin C to near normal level which acts as strong superoxide radical and singlet oxygen quencher.

Vitamin E is one of the major chain breaking lipophilic antioxidants within the cell membrane where it protects membrane fatty acids from LPO. Vitamin E quenches the singlet oxygen and is converted to vitamin E radical. Vitamin E also reacts with lipid peroxides to terminate the radical chain reaction in the membrane lipids [37]. The decreased level of α-tocopherol observed in the diabetic rats may be due to increased utilization of vitamin E in scavenging the oxyradicals generated by high levels of glucose (or) might be due to decreased vitamin C concentration because there is a well established synergism between vitamin E and vitamin C [38]. Decreased vitamin E levels have also been found in diabetic patients with increased lipid peroxidation products [39]. Upon the treatment with *p*-coumaric acid to diabetic rats increased Vitamin E to near normal rats which may act as an inhibitor for further free radical induced lipid peroxidation.

GSH is a major endogenous antioxidant which counterbalance free radical mediated damage. It is involved in the maintenance of normal cell structure and function, probably through its redox and detoxification reaction [40]. GSH functions as a free radical scavenger and in the repair of free radical caused biological damage [41]. GSH is required for the recycling of vitamin C and acts as a substrate for GPx and GST that are involved in preventing the deleterious effect of oxygen radicals [42;34]. STZ-diabetic rats exhibited a decreased level of GSH which might be due to increased utilization for scavenging free radicals and increased consumption by GPx and GST. Treatment with *p*-coumaric acid significantly improved GSH level in plasma and tissues of diabetic rats which could be due to decreased lipid peroxidation. Thus, *p*-coumaric acid exerts potential antioxidant property, as evidenced by increased antioxidants status.

Histopathological examination of diabetic pancreas expressed island of islet cells with fatty infiltration. Administration of *p*-coumaric acid showed conserved islet cells with acini. Diabetic liver expressed central vein, surrounded by dilated simusoids with focal fatty change of hepotocytes. Administration of *p*-coumaric acid expressed central vein surrounded by hepatocytes. Diabetic kidney showed congested glomeruli with lymphocytic infilterate in the interstium. *p*-Coumaric acid treated diabetic kidney showed normal glomeruli free dilated tubules. Histopathological observations such as pancreas, liver and kidney are represented on Figure 7 to 9.

CONCLUSION

It could be concluded that treatment with *p*-coumaric acid (100 mg/kg b.wt) exhibited preventive effects on altered levels of blood glucose and insulin. It is also prevented enzymatic and non enzymatic antioxidants. The possible mechanism for the observed preventive effects of *p*-coumaric acid is due to its hypoglycemic and free radical scavenging properties. According to our study *p*-coumaric acid may be beneficial for protection against diabetes and its complication.

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