

## D-pinitol attenuates the impaired activities of hepatic key enzymes in carbohydrate metabolism of streptozotocin-induced diabetic rats

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**Abstract.** During diabetes mellitus, endogenous hepatic glucose production is increased as a result of impaired activities of the key enzymes of carbohydrate metabolism, which leads to the condition known as hyperglycemia. D-pinitol, a bioactive constituent isolated from soybeans, has been shown to reduce hyperglycemia in experimental diabetes. We therefore designed this study to investigate the effect of oral administration of D-pinitol (50 mg/kg b.w. for 30 days) on the activities of key enzymes in carbohydrate and glycogen metabolism in the liver tissues of streptozotocin-induced diabetic rats. The efficacy was compared with glyclazide, a standard hypoglycemic drug. Oral administration of D-pinitol to diabetic group of rats showed a marked decrease in the levels of blood glucose, glycosylated hemoglobin and an increase in plasma insulin and body weight. The activities of the hepatic enzymes such as hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, glycogen synthase and hepatic glycogen content were significantly ( $p < 0.05$ ) increased whereas the activities of glucose-6-phosphatase, fructose-1,6-bisphosphatase, lactate dehydrogenase and glycogen phosphorylase were significantly ( $p < 0.05$ ) decreased in diabetic rats treated with D-pinitol. The results suggest that alterations in the activities of key metabolic enzymes of carbohydrate metabolism could be one of the biochemical rationale by which D-pinitol attenuates the hyperglycemic effect in diabetic rats.

**Key words:** D-pinitol — Diabetes mellitus — Insulin — Liver — Streptozotocin

### Introduction

Diabetes mellitus, a common metabolic disorder, is characterized mainly by chronic hyperglycemia resulting from defects in insulin secretion and/or its action. This eventually leads to improper regulation of carbohydrate, protein and lipid metabolism that ultimately contributes to a key factor in the development and the progression of micro and macrovascular complications (Adisakwattana et al. 2005). The prevalence of diabetes has been increased alarmingly worldwide and the total number of people with diabetes is expected to double approximately from 171 million in 2000 to 366 million in 2030 (Wild et al. 2004). The “top three” countries with largest number of diabetic people are India, China and USA. The prevalence of diabetes in India is expected to increase from current 40.9 to 69.9 million by the year 2025 unless urgent preventive steps are taken (Huizinga and Rothman 2006).

Liver plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose *via* glycogenesis and the release of glucose through glycogenolysis and gluconeogenesis. Maintaining the blood glucose levels within a narrow range requires the regulation of two major metabolic pathways, gluconeogenesis and glycogenolysis, which produce glucose in the liver. In addition, the key enzymes in opposing metabolic pathways such as glycolysis and glycogenesis must also be regulated in order for net flux in the appropriate direction to be achieved. There are several regulatory enzymes which play key roles in this process and are part of substrate cycles in which cycling between the substrates and products of the enzymes occurs (Nordlie et al. 1999). Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances by altering carbohydrate metabolizing enzymes and leads to diabetes (Bailey 2000).

The underlying goal of all diabetes treatment and management is to maintain normoglycemia. A number of antidiabetic

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regimens are available commercially, but most of them produce undesirable side effects like severe hypoglycemia, weight gain, hepatotoxicity and gastrointestinal disturbances after long-term consumption. Therefore, the search for more effective and safer treatment regimes is underway. Thus, it is necessary to continue to search for new and if possible more efficacious drugs and the vast reserves of phytochemicals may be an ideal target.

D-pinitol, methyl ether of D-chiro-inositol, found in large quantities in soy foods and legumes – for example, about 1% dry weight of soybean meal is D-pinitol (Phillips et al. 1982). It is an active low-molecular cyclitol isolated from the seed coat, cotyledon and embryo axis of soybean seeds (Kuo et al. 1997). D-pinitol has been suggested to possess multi-functional properties including anti-inflammatory activity (Singh et al. 2001), prevention of cardiovascular diseases (Kim et al. 2005) and reduces allergic airway inflammation and hyperresponsiveness (Lee et al. 2007).

Further, D-pinitol is an active ingredient of *Talisapatra*, a traditional Ayurvedic medicine and has been shown to exhibit antidiabetic activities. However, the mechanism by which D-pinitol elicits antihyperglycemic property is unknown; Bates et al. (2000) suggested that D-pinitol and D-chiro-inositol are structurally similar to the inositol phosphates that influence insulin signaling *via* phosphatidylinositol-3-kinase. Narayanan et al. (1987) also reported that administration of D-pinitol declined plasma glucose level in normal and diabetic mice. However, there is no such systematic study to explore the antihyperglycemic properties of D-pinitol.

Hence, the present study was aimed to investigate the antihyperglycemic effects of D-pinitol by determining the activities of key enzymes involved in the metabolism of carbohydrate in the liver tissues of streptozotocin (STZ)-induced diabetic rats and the efficacy of D-pinitol was compared with a standard oral hypoglycemic drug, glyclazide.

## Materials and Methods

### Chemicals

D-pinitol and STZ were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade available commercially.

### Animals and diet

All the experiments were conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (Approval No. 01/017/08). Groups of six-week-old, male, Wistar albino rats weighing 160–180 g, procured from Tamilnadu Veterinary and Animal Sciences University (Chennai, India), were used in this study.

The rats were maintained under standard laboratory conditions at  $25 \pm 2^\circ\text{C}$ , relative humidity  $50 \pm 15\%$  and normal photoperiod (12 h dark/12 h light). Throughout the experimental period, the rats were fed with balanced commercial pellet diet supplied by Hindustan Lever Ltd. (Bangalore, India). Diet and water were provided *ad libitum*.

### Experimental induction of diabetes

The rats were fasted overnight and experimental diabetes was induced by single intraperitoneal injection of STZ (50 mg/kg b.w.) dissolved in freshly prepared 0.1 mol/l cold citrate buffer of pH 4.5 (Rakieten et al. 1963). Because STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, the STZ-treated rats were provided with 10% glucose solution after 6 h for the next 24 h to prevent hypoglycemia. After a week time for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration,  $>250$  mg/dl) that exhibited glycosuria and hyperglycemia were selected for the experiment.

### Dosage fixation studies

The suitable optimum dosage was assessed by administration of graded doses of D-pinitol (25, 50, 75 and 100 mg/kg b.w.) at different time periods (15, 21, 30 and 45 days) to the control and diabetic groups of rats. The dosage was adjusted every week according to the change in body weight to maintain similar dose *per kilogram* of body weight of rat over the entire period of study for each group. Body weight, food intake, morphological and behavioral changes were monitored periodically to assess the signs of toxicity of D-pinitol, if any. At the end of the experimental period, the animals were sacrificed and the blood was collected with and without anti-coagulant. The estimation of blood glucose, glycosylated hemoglobin, blood urea, serum creatinine and the assay of pathophysiological enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were carried out. The optimum dosage was fixed as the minimum possible dosage of D-pinitol, which elicits no toxicological effect, maximum efficacy indicated by the normal levels of blood glucose and glycosylated hemoglobin, maximum safety indicated by normal levels of urea and creatinine and optimized activities of AST, ALT, ALP and LDH. Based on the results obtained in the above-mentioned physiological and biochemical parameters (data not shown), the optimal dosage of D-pinitol for the treatment of STZ-induced diabetic rats was fixed as 50 mg/kg b.w. (single oral dose/day) for a period of 30 days.

### Experimental design

The rats were divided into four groups, each group comprises of six rats: group 1 – control rats; group 2 – STZ-induced

diabetic rats; group 3 – diabetic rats treated with D-pinitol (50 mg/kg b.w./rat/day) orally for 30 days; group 4 – diabetic rats treated with glyclazide (5 mg/kg b.w./rat/day) orally for 30 days (Pulido et al. 1997).

During the experimental period, body weight, respiratory changes, distress, abnormal locomotion and catalepsy of all the rats were monitored at regular intervals and the blood glucose level was estimated twice a week. At the end of the experimental period, the rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. The blood was collected with and without EDTA (ethylenediaminetetraacetic acid) for the separation of plasma and serum, respectively.

#### Glucose tolerance test

After 30 days of treatment, a fasting blood sample was collected from all the groups of rats. To perform Oral glucose tolerance test, blood samples were also collected at 30, 60, 90 and 120 min intervals after an oral administration of glucose at a concentration of 2 g/kg b.w. (Joy and Kuttan 1999) and blood glucose level was determined by the method of Sasaki et al. (1972) using o-toluidine reagent.

#### Biochemical estimations

Blood glucose level was determined by the method of Sasaki et al. (1972) using o-toluidine reagent. The presence of urine glucose was assessed in the fresh urine using glucose indicator sticks (Boehringer Mannheim, Germany). Hemoglobin was determined based on the formation of cyanmethemoglobin by the method of Drabkin and Austin (1932). The glycosylated hemoglobin was determined by the method of Nayak and Pattabiraman (1981). Plasma insulin was determined using ELISA kit (for rats) supplied by Lincoplex Ltd. (USA). A portion of the liver tissue was dissected out, washed with ice-cold saline immediately and homogenized in 0.1 mol/l Tris-HCl buffer (pH 7.4) for the assay of key enzymes of carbohydrate metabolism. The homogenate was centrifuged at 10,000 rpm to remove the debris and the supernatant was used as an enzyme source for the determination of the activities of hexokinase (Brandstrup et al. 1957), pyruvate kinase (Pogson and Denton 1967), LDH (King 1959), glucose-6-phosphatase (Koide and Oda 1959), fructose-1,6-bisphosphatase (Gancedo and Gancedo 1971), glucose-6-phosphate dehydrogenase (Ells and Kirkman 1961), glycogen synthase (Leloir and Goldemberg 1962) and glycogen phosphorylase (Cornblath et al. 1963). Another portion of wet liver tissue was used for the determination of glycogen content (Morales et al. 1973).

#### Statistical analysis

The values were expressed as mean  $\pm$  SEM for six rats in each group. All data were analyzed with SPSS 15.0 software.

Hypothesis testing method included one way analysis of variance (ANOVA) followed by *post hoc* testing performed with LSD (least significant difference) test. Values of  $p < 0.05$  were considered as significant.

#### Results

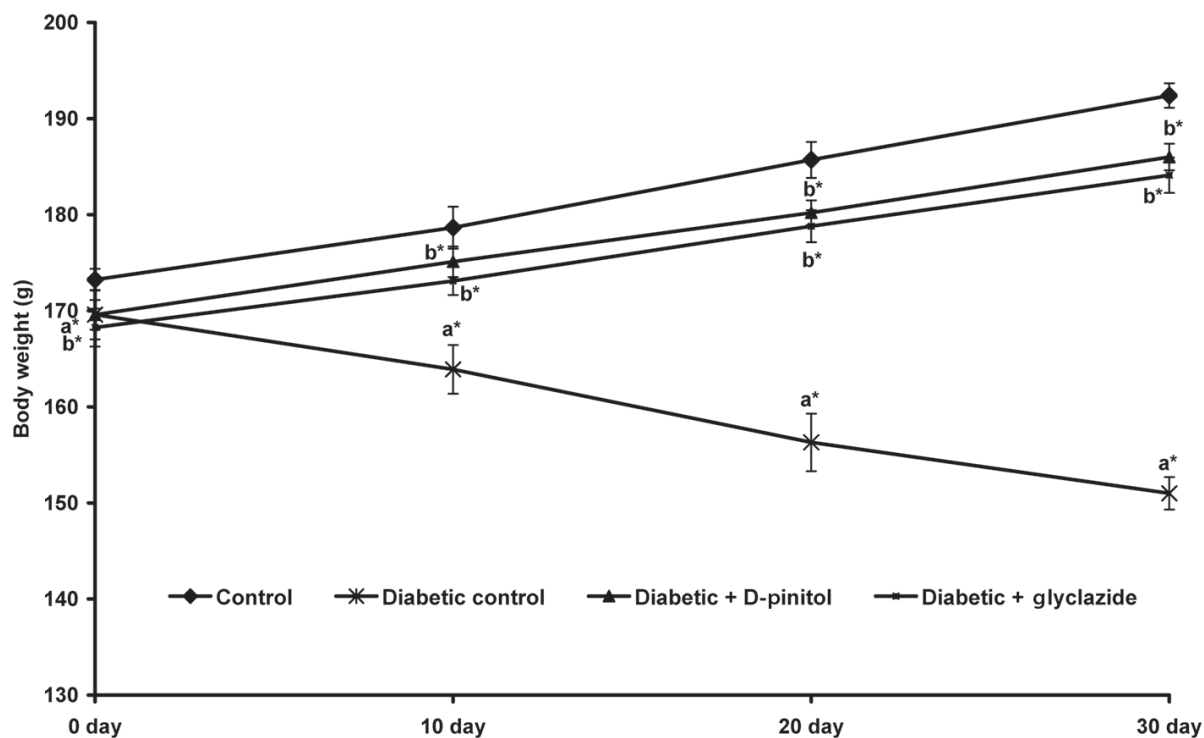
Figure 1 shows the changes in body weight in control and experimental groups of rats. A gradual increase in body weight was observed in the control group of rats. The body weight was significantly ( $p < 0.05$ ) decreased in STZ-induced diabetic rats when compared to control rats. Oral administration of D-pinitol as well as glyclazide to STZ-induced diabetic rats significantly ( $p < 0.05$ ) increased the body weight to near normalcy.

Figure 2 shows the changes on glucose tolerance curve in control and experimental groups of rats. The blood glucose level in the control rats increased to a peak value at 60 min after glucose load and decreased to near normal level at 120 min. In STZ-induced diabetic rats the peak increase in blood glucose concentration was observed after 60 min and remained high over the next 60 min. Oral administration of D-pinitol as well as glyclazide on STZ-induced diabetic rats showed significant ( $p < 0.05$ ) decrease in blood glucose concentration at 60 and 120 min when compared with diabetic control suggesting the glucose lowering properties of D-pinitol as well as glyclazide.

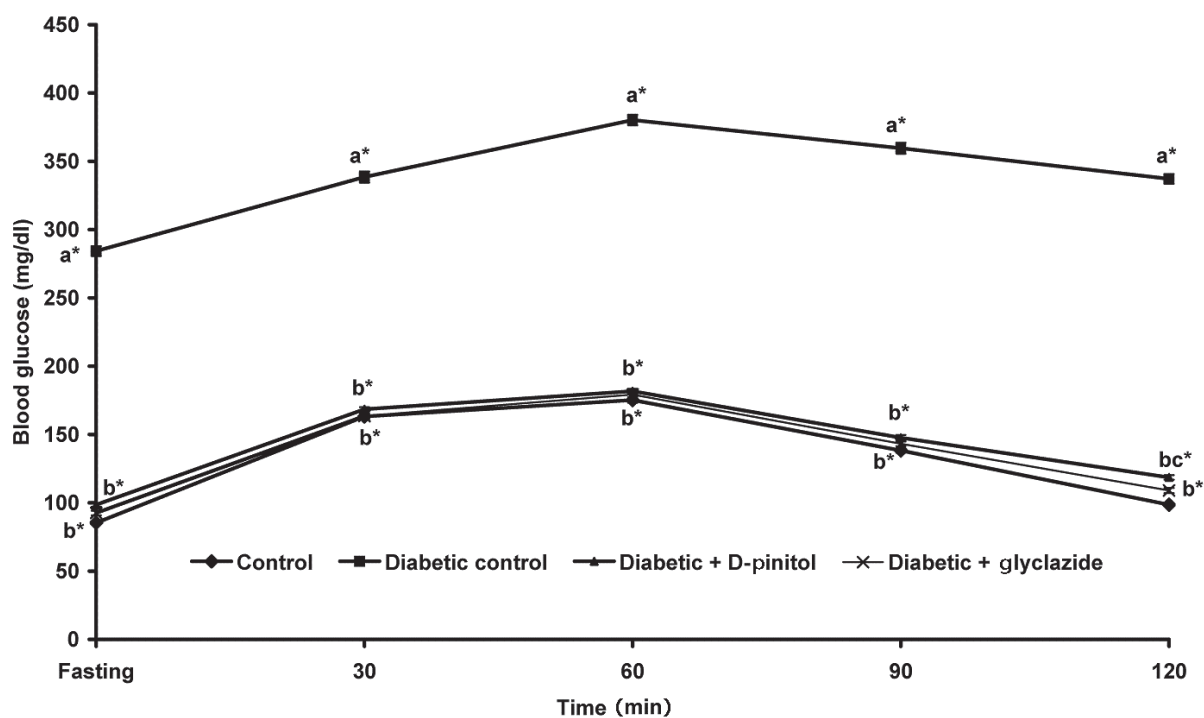
The control rats were normoglycemic throughout the course of study (Table 1). The blood glucose level was significantly ( $p < 0.05$ ) increased and the plasma insulin content was significantly ( $p < 0.05$ ) decreased in STZ-induced diabetic rats. Oral administration of D-pinitol significantly ( $p < 0.05$ ) reduced the blood glucose level and increased the plasma insulin level in STZ-induced diabetic rats to near normalcy and the results were comparable to glyclazide.

The levels of hemoglobin and glycosylated hemoglobin of control and experimental groups of rats were also depicted in Table 1. The STZ-induced diabetic rats showed a significant ( $p < 0.05$ ) decrease in the level of hemoglobin and a significant ( $p < 0.05$ ) increase in the level of glycosylated hemoglobin. Oral administration of D-pinitol reversed these changes in hemoglobin and glycosylated hemoglobin levels to near normalcy.

Table 2 shows the activities of hexokinase, pyruvate kinase, LDH, glucose-6-phosphatase, fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase in the liver tissues of control and experimental groups of rats. The activities of hexokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase were decreased significantly ( $p < 0.05$ ), while the activities of LDH, glucose-6-phosphatase and fructose-1,6-bisphosphatase were increased significantly ( $p < 0.05$ ) in



**Figure 1.** Changes in body weight of control and experimental groups of rats. Values are given as mean  $\pm$  SEM for groups of six rats in each. Values are statistically significant at \*  $p < 0.05$  when compared with <sup>a</sup> control rats, <sup>b</sup> diabetic rats, <sup>c</sup> STZ + glyclazide.



**Figure 2.** Glucose tolerance test curve of control and experimental groups of rats. Values are given as mean  $\pm$  SEM for groups of six rats in each. Values are statistically significant at \*  $p < 0.05$  when compared with <sup>a</sup> control rats, <sup>b</sup> diabetic rats, <sup>c</sup> STZ + glyclazide.

**Table 1.** Levels of fasting blood glucose, plasma insulin, hemoglobin, glycosylated hemoglobin and urine sugar in control and experimental groups of rats

Parameters	Control	Diabetic control	Diabetic + D-pinitol	Diabetic + glyclazide
Blood glucose (mg/dl)	87.38 ± 0.61	298.43 ± 1.45 <sup>a,*</sup>	98.65 ± 1.43 <sup>b,c,*</sup>	94.77 ± 1.83 <sup>b,*</sup>
Plasma insulin (μU/ml)	14.84 ± 0.15	6.42 ± 0.22 <sup>a,*</sup>	10.58 ± 0.17 <sup>b,c,*</sup>	11.22 ± 0.39 <sup>b,*</sup>
Hemoglobin (g/dl)	13.31 ± 0.68	8.4 ± 0.51 <sup>a,*</sup>	10.62 ± 0.37 <sup>b,*</sup>	11.32 ± 0.67 <sup>b,*</sup>
Glycosylated hemoglobin (%)	6.05 ± 0.05	12.83 ± 0.09 <sup>a,*</sup>	7.29 ± 0.03 <sup>b,*</sup>	6.86 ± 0.03 <sup>b,*</sup>
Urine sugar	–	+++	–	–

Results are mean ± SEM ( $n = 6$ ). One way ANOVA followed by *post hoc* test LSD. Values are statistically significant at \*  $p < 0.05$ , when compared with <sup>a</sup> control rats, <sup>b</sup> diabetic rats, <sup>c</sup> STZ + glyclazide; +++ indicates more than 2% sugar.

**Table 2.** Activities of carbohydrate metabolizing enzymes in the liver of control and experimental groups of rats

Parameters	Control	Diabetic control	Diabetic + D-pinitol	Diabetic + glyclazide
Hexokinase	260.39 ± 0.96	129.62 ± 1.55 <sup>a,*</sup>	227.71 ± 0.64 <sup>b,c,*</sup>	242.59 ± 0.94 <sup>b,*</sup>
Pyruvate kinase	195.51 ± 1.99	102.22 ± 1.17 <sup>a,*</sup>	168.70 ± 1.37 <sup>b,c,*</sup>	173.55 ± 1.87 <sup>b,*</sup>
Lactate dehydrogenase	254.87 ± 2.18	450.49 ± 2.97 <sup>a,*</sup>	290.46 ± 2.23 <sup>b,c,*</sup>	276.12 ± 2.12 <sup>b,*</sup>
Glucose-6-phosphatase	966.31 ± 2.84	1964.43 ± 4.82 <sup>a,*</sup>	1141.49 ± 3.31 <sup>b,c,*</sup>	1040.30 ± 2.99 <sup>b,*</sup>
Fructose-1,6-bisphosphatase	498.53 ± 1.90	754.95 ± 3.59 <sup>a,*</sup>	536.79 ± 2.91 <sup>b,c,*</sup>	511.13 ± 1.23 <sup>b,*</sup>
Glucose-6-phosphate dehydrogenase	551.37 ± 2.41	284.06 ± 1.85 <sup>a,*</sup>	492.82 ± 2.72 <sup>b,c,*</sup>	517.25 ± 2.32 <sup>b,*</sup>

Results are mean ± SEM ( $n = 6$ ). One way ANOVA followed by *post hoc* test LSD. Values are statistically significant at \*  $p < 0.05$ , when compared with <sup>a</sup> control rats, <sup>b</sup> diabetic rats, <sup>c</sup> STZ + glyclazide. Units are expressed as: μmol of glucose-6-phosphate formed *per h*/mg of protein for hexokinase, mU/mg of protein for pyruvate kinase, μmol of pyruvate formed *per h*/mg of protein for lactate dehydrogenase, μmol of Pi liberated *per h*/mg of protein for glucose-6-phosphatase and fructose-1,6-bisphosphatase and μmol of NADPH *per min*/mg of protein for glucose-6-phosphate dehydrogenase.

**Table 3.** Glycogen content and activities of glycogen synthase and glycogen phosphorylase in the liver of control and experimental groups of rats

Groups	Glycogen	Glycogen synthase	Glycogen phosphorylase
Control	50.39 ± 1.37	811.37 ± 2.93	655.34 ± 2.84
Diabetic control	21.37 ± 0.92 <sup>a,*</sup>	503.33 ± 2.87 <sup>a,*</sup>	895.86 ± 3.83 <sup>a,*</sup>
Diabetic + D-pinitol	43.75 ± 1.22 <sup>b,c,*</sup>	787.11 ± 2.48 <sup>b,*</sup>	704.37 ± 2.64 <sup>b,c,*</sup>
Diabetic + glyclazide	45.94 ± 1.92 <sup>b,*</sup>	792.67 ± 2.32 <sup>b,*</sup>	681.62 ± 2.01 <sup>b,*</sup>

Results are mean ± SEM ( $n = 6$ ). One way ANOVA followed by *post hoc* test LSD. Values are statistically significant at \*  $p < 0.05$ , when compared with <sup>a</sup> control rats, <sup>b</sup> diabetic rats, <sup>c</sup> STZ + glyclazide. Units are expressed as: mg/g wet tissue for glycogen, μmol of UDP formed *per h*/mg protein for glycogen synthase and μmol Pi liberated *per h*/mg protein for glycogen phosphorylase.

STZ-induced diabetic rats when compared to control group of rats. Oral administration of D-pinitol as well as glyclazide reversed the activities of these carbohydrate metabolizing enzymes to near control levels.

Table 3 shows the changes in the level of liver glycogen content and in the activities of glycogen synthase and glycogen phosphorylase of control and experimental groups of rats. There was a significant ( $p < 0.05$ ) decrease in level of glycogen content and in the activity of liver glycogen synthase of STZ-induced diabetic rats when compared to control group of rats, whereas there was a significant ( $p < 0.05$ ) increase in the activ-

ity of glycogen phosphorylase in the STZ-induced diabetic rats. Oral administration of D-pinitol as well as glyclazide brought back the glycogen content and activities of glycogen synthase and glycogen phosphorylase to near normalcy.

## Discussion

STZ selectively destroys the insulin secreting pancreatic β-cells, leaving the less active β-cells and thus, resulting in diabetic state (Szkudelski 2001). STZ-induced diabetes is

characterized by severe weight loss which was observed in our present study. The loss in weight in diabetic rats might be the result of degradation of structural proteins due to unavailability of carbohydrate as energy source (Pepato et al. 1996). A significant improvement in the body weight of STZ-induced diabetic rats was observed in D-pinitol administered rats. This may be due to the ability of D-pinitol to enhance glucose metabolism and thus, improves body weight in STZ-induced diabetic rats.

STZ-induced diabetes causes a notable reduction in insulin release by the destruction of the pancreatic  $\beta$ -cells and thereby induces hyperglycemia (Schein et al. 1973). There was a significant increase in the level of plasma insulin in D-pinitol administered diabetic rats. Though the exact mechanism of action of D-pinitol is unknown, it could be due to the increased pancreatic secretion of insulin from the existing remnant  $\beta$ -cells. The fundamental mechanism underlying hyperglycemia in diabetes involves the overproduction of glucose by excessive hepatic glycogenolysis and gluconeogenesis and decreased utilization by the tissues (Yamamoto et al. 1981). Reports have shown that the level of blood glucose was elevated in STZ-induced diabetic rats. Consequently, in the present study, we have also observed a marked elevation in blood glucose level of STZ-induced diabetic rats and are normalized by the oral treatment with D-pinitol.

The decreased level of total hemoglobin in diabetic rats is mainly due to the increased formation of glycosylated hemoglobin. Glycosylated hemoglobin was found to increase in uncontrolled diabetes and the increase is directly proportional to the fasting blood glucose level for about 3 months (Sen et al. 2005). Measurement of glycosylated hemoglobin remains the gold standard biochemical marker for the assessment of glycemic control in patients with diabetes (Fonseca 2003). During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin (Kumar et al. 2005). Oral administration of D-pinitol as well as glyclazide to diabetic rats reduced the formation of glycosylated hemoglobin by virtue of its normoglycemic activity. Since the level of glycosylated hemoglobin has been shown to provide an index of blood glucose concentration (Haller et al. 2004), the decreased level of glycosylated hemoglobin and the increased level of hemoglobin in D-pinitol administered diabetic rats show the antihyperglycemic activity of D-pinitol.

Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes. One such enzyme is hexokinase that catalyses the conversion of glucose to glucose-6-phosphate and plays a central role in the maintenance of glucose homeostasis (Laakso et al. 1995). In the liver, hexokinase is an important regulatory enzyme

in the oxidation of glucose (O'Doherty et al. 1999). Being an insulin-dependent enzyme, the hepatic hexokinase activity of diabetic rats is almost entirely inhibited or inactivated due to the absence of insulin (Gupta et al. 1999). This impairment results in a marked reduction in the rate of glucose oxidation *via* glycolysis, which ultimately leads to the accumulation of glucose in blood, a state known as hyperglycemia. Oral administration of D-pinitol to STZ-induced diabetic rats resulted in a significant reversal in the activity of hexokinase. The increased plasma insulin and decreased glucose levels in STZ-induced diabetic rats administered with D-pinitol result in increased hepatic hexokinase activity thereby increased the oxidation of glucose.

Pyruvate kinase is a ubiquitously expressed key glycolytic enzyme that catalyzes the conversion of phosphoenolpyruvate to pyruvate with the generation of ATP and the altered expression could be expected to impair the glucose metabolism and energy production. This reaction is irreversible under physiological conditions and has been considered as critical for the regulation of metabolic flux in the second part of glycolysis. This central position is evident from the regulatory properties of pyruvate kinase, a typical allosteric protein (Wang et al. 2002). The observed decrease in the activity of pyruvate kinase in the liver of STZ-induced diabetic rats readily accounts for the decreased utilization of glucose (glycolysis) and increased production of glucose (gluconeogenesis) by liver indicating that these two pathways are altered in diabetes (Taylor and Agius 1988). Oral administration of D-pinitol to STZ-induced diabetic rats resulted in a significant increase in the activity of pyruvate kinase. This reversal to near normalcy might be due to the restored endocrine status of pancreas.

LDH catalyzes the conversion of pyruvate to lactate in anaerobic glycolysis, which is subsequently converted to glucose in gluconeogenic flux. In diabetic condition, an increased activity of LDH was observed (Lemieux et al. 1984). The LDH system reflects the  $\text{NAD}^+/\text{NADH}$  ratio indicated by the lactate/pyruvate ratio of hepatocyte cytosol (Williamson et al. 1967). Oral administration of D-pinitol as well as glyclazide to diabetic groups of rats showed a significant reduction in the LDH activity, probably due to the regulation of  $\text{NAD}^+/\text{NADH}$  ratio by the oxidation of glucose.

Glucose-6-phosphatase, a crucial gluconeogenic enzyme, is mainly found as an integral protein in the lumen of the endoplasmic reticulum of hepatocytes that catalyzes the dephosphorylation of glucose-6-phosphate to glucose in the liver (Roden and Bernroider 2003) and it is transported out of the liver to increase blood glucose concentration. Fructose-1,6-bisphosphatase is another gluconeogenic enzyme that catalyzes one of the irreversible steps in gluconeogenesis and serves as a site for the regulation of gluconeogenesis (Pilkis and Claus 1991). STZ increases the expression and activities

of glucose-6-phosphatase and fructose-1,6-bisphosphatase in the liver of diabetic rats (Massillon et al. 1996). Activation of these gluconeogenic enzymes is due to the state of insulin deficiency, because under normal conditions, insulin functions as a suppressor of glucose-6-phosphatase activity (Chen et al. 2000). In our study, oral administration of D-pinitol reversed the glucose-6-phosphatase and fructose-1,6-bisphosphatase activities in STZ-induced diabetic rats. The reduction in glucose-6-phosphatase and fructose-1,6-bisphosphatase can lead to decrease in gluconeogenesis and blood glucose concentration.

Glucose-6-phosphate dehydrogenase is the first rate-limiting enzyme of the pentose phosphate pathway, which results in the production of ribose-5-phosphate and the reducing equivalent, NADPH (Xu et al. 2005). The activity of glucose-6-phosphate dehydrogenase is found to be decreased in diabetic conditions (West 2002). Further, the oral treatment of D-pinitol to STZ-induced diabetic rats significantly increased the activity of liver glucose-6-phosphate dehydrogenase through increased secretion of insulin. It seems to increase the influx of glucose into the pentose monophosphate shunt in an effort to decrease high blood glucose level and results in an increased production of the reducing agent, NADPH, with concomitant decrease in oxidative stress.

Glycogen is the primary intracellular storable form of glucose and its level in various tissues are a direct indication of insulin activity as insulin promotes the deposition of intracellular glycogen by stimulating glycogen synthase and inhibiting glycogen phosphorylase activities. The decreased hepatic glycogen content in STZ-induced diabetes is due to the marked decrease in insulin level, since STZ causes selective destruction of  $\beta$ -cells and the liver also depends on insulin for the entry of glucose (Whitton and Hems 1975). Thus assessment of glycogen level serves as a marker for the evaluation of antidiabetic activity. Moreover, glycogen synthase and glycogen phosphorylase are the two key regulatory enzymes that catalyze glycogenesis and glycogenolysis, respectively. In 1990, Ferrannini and his colleagues reported that liver glycogen content was reduced significantly in the STZ-induced diabetic rats and the same was also observed in our study. Further, the decreased glycogen content in diabetic condition is due to the increased activity of glycogen phosphorylase and decreased activity of glycogen synthase (Roesler and Khandelwal 1986). The oral administration of D-pinitol to STZ-induced diabetic rats significantly increased the glycogen content by stimulating the glycogen synthase activity and inhibiting the glycogen phosphorylase activity in the liver by stimulating the remnant  $\beta$ -cells to release insulin.

The results of the present study show that D-pinitol exerts its antidiabetogenic effects by regulating the activities of key enzymes involved in carbohydrate metabolism. Further

studies are in progress to elucidate the exact mechanism of action of D-pinitol in experimental diabetes.

The present study revealed that D-pinitol has a beneficial antihyperglycemic effect on normalizing the blood glucose level as well as the hepatic key metabolic enzymes associated with carbohydrate and glycogen metabolism in STZ-induced experimental diabetic rats. This suggests the possible biochemical rationale through which glucose homeostasis are regulated by D-pinitol. Thus, eliciting the anti-hyperglycemic activity of D-pinitol and it may be a useful therapy in the management of diabetes mellitus.

**Acknowledgement.** The research fellowship of the University Grant Commission, New Delhi, India, to the first author is gratefully acknowledged.

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Received: December 5, 2008

Final version accepted: February 9, 2009