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Attenuation of oxidative stress and alteration of hepatic tissue ultrastructure by D-pinitol in streptozotocin-induced diabetic rats

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Abstract

The present study was aimed to investigate the effect of D-pinitol on hyperglycaemia mediated oxidative stress by analysing the hepatic antioxidant competence, pro-inflammatory cytokines and ultrastructural changes in liver tissues of streptozotocin-induced diabetic rats. Oral administration of D-pinitol (50 mg/kg b.w.) resulted in significant ($p < 0.05$) attenuation in blood glucose, glycosylated haemoglobin and pro-inflammatory markers such as TNF-α, IL-1β, IL-6, NF-κB p65 unit and NO and significant ($p < 0.05$) elevation in the plasma insulin level. In addition, D-pinitol instigated a significant escalation in the levels of hepatic tissue non-enzymatic antioxidants and the activities enzymatic antioxidants of diabetic rats with significant ($p < 0.05$) decrease in lipid peroxides and hydroperoxides formation, thus demonstrating the protective role of D-pinitol on the hepatic tissues from oxidative stress-induced liver damage. These biochemical observations were complemented by histological and ultrastructural examination of liver section. Thus, the present study demonstrates the hepatoprotective nature of D-pinitol by attenuating hyperglycaemia-mediated pro-inflammatory cytokines and oxidative stress.

Keywords: *D-pinitol, diabetes mellitus, streptozotocin, lipid peroxidation, oxidative stress, pro-inflammatory cytokines*

Introduction

Diabetes mellitus is a major endocrine metabolic disorder, affecting ~170 million people worldwide and is characterized by chronic hyperglycaemia resulting from impaired insulin secretion or insulin action or post-receptor events leading to impairment in the carbohydrate, protein and lipid metabolism. The onset of diabetes mellitus is insidious until the development of clinical symptoms, thus hyperglycaemia advances progressively. The supraphysiological level of blood glucose leads to augmented generation of reactive oxygen species by autoxidation of glucose, decline in tissue concentration of non-enzymatic antioxidants, impaired activities of antioxidant enzymes [1,2] and cellular dysfunction that may become irreversible over time, the process commonly known as glucose toxicity [3,4]. The hyperglycaemia-mediated reactive oxygen species is a by-product of cellular processes such as mitochondrial respiration. It damages the DNA, proteins and lipids and further activates stress response pathways. Various reports implicated the role of these pathways, resulting in insulin resistance and insufficient insulin secretion. Thus, the production of reactive oxygen species is more in diabetes state than in normal state [5,6] and is related to glycemic levels [7].

A disturbance in the equilibrium between enhancement of reactive oxygen metabolites and the rate at which they are scavenged by enzymatic and non-enzymatic antioxidants is designated as oxidative stress [8]. In the absence of an appropriate compensatory response from the endogenous antioxidant network and the overwhelming production of reactive oxygen species results in cellular damage. Moreover,

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there is a widespread acceptance for the probable role of free radicals in instigating various secondary complications of diabetes, such as nephropathy, retinopathy, neuropathy and macro- and microvascular damage [8–10]. Thus, oxidative stress is an important factor in the aetiology and pathogenesis of diabetes. Several mechanisms, which have been reported both in experimental diabetes and diabetic patients, seem to be involved in the genesis of this oxidative stress.

The underlying goal of diabetes treatment and management is to restrain oxidative stress. The antioxidants instigate their action on oxidative stress by free radical scavenging mechanism, in turn attenuating the adverse effects of chronic hyperglycaemia. A classic example for an antioxidant is vitamin E, which hampers the reactive oxygen species by scavenging the free radicals [11]. Efforts to discover a potent antioxidant from the vast reserves of phytochemicals as a useful drug candidate to combat diabetes and diabetic complications are going on relentlessly.

D-pinitol, a 3-methoxy analogue of D-chiroinositol, was identified as an active principle in soy foods and legumes [12–14]. The mature and dried soybean seeds contain up to 1% D-pinitol [15]. D-pinitol functions as an osmolyte by improving the tolerance to drought stress or heat stress [16,17] and is involved in reducing the negative effects of osmotic stress and increasing the tolerance of plant tissues to water deficiencies [18]. In addition, D-pinitol has been suggested to possess multifunctional properties, including feeding stimulant [19], anti-inflammatory [20], cardioprotective [21], anti-hyperlipidemic [22] and creatine retention promotion properties [23]. Recently, we have reported the anti-hyperglycaemic nature of D-pinitol by determining its modulatory effects on the activities of hepatic key carbohydrate metabolizing enzymes [24] and its antioxidant nature by assessing its pancreatic tissue protective nature on amelioration of oxidative stress in streptozotocin-induced experimental diabetes [25].

Liver is the principal organ in maintenance of systemic glucose homeostasis, oxidative stress and detoxifying processes, as well as free radical reactions. Liver was exposed to oxidative stress in addition to pancreatic β-cells in streptozotocin-induced diabetic rats. Thus, in many diseases the biomarkers of oxidative stress are imminent in liver at an early stage [26]. Hence, the present study was aimed to investigate the hepatic tissue protective nature of D-pinitol by amelioration of liver oxidative stress and pro-inflammatory cytokines.

Materials and methods

Chemicals

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

Animals and diet

All the experimental procedures complied 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). Male Wistar rats weighing 160–180 g, procured from Tamilnadu Veterinary and Animal Sciences University, Chennai, India, were used in this study. The rats were housed in standard laboratory conditions 20–25°C, relative humidity 50±15% and under a 12 h light–dark cycle. The animals were acclimatized to the laboratory conditions 2 week prior to the commencement of experiments. Throughout the experimental period, the rats were fed with balanced commercial pellet diet (Hindustan Lever Ltd., Bangalore, India) with a composition of 5% fat, 21% protein, 55% nitrogen-free extract and 4% fibre (w/w) with adequate mineral and vitamin levels for the animals. Diet and water were provided *ad libitum*.

Induction of experimental diabetes

The overnight fasted rats received a single intraperitoneal injection of streptozotocin (50 mg/kg body weight) dissolved in freshly prepared 0.1 M cold citrate buffer, pH 4.5 [27]. As streptozotocin is capable of inducing fatal hypoglycaemia as a result of massive pancreatic insulin release, the streptozotocin-treated rats were provided 10% glucose solution after 6 h for the next 24 h to prevent hypoglycaemia [28]. After a week in time for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration, >14 mM) were selected for the experiment.

Experimental design

The rats were divided into four groups, each group comprised of six rats, as follows; Group 1 served as Control rats; Group 2 served as Streptozotocin-induced diabetic rats; Group 3 served as Diabetic rats treated with D-pinitol (50 mg/kg body weight/rat/day) orally for 30 days; and Group 4 served as Diabetic rats treated with glyclazide (5 mg/kg body weight/rat/day) orally for 30 days.

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 anti-coagulant.

Biochemical estimations

Fasting blood glucose level was determined by glucose oxidase peroxidase diagnostic enzyme kit (Span Diagnostic Chemicals, India) and plasma insulin was determined using ELISA kit (for rats) supplied by Lincoplex Ltd. (St. Charles; MO, USA). Both the parameters were performed according to the manufacturer's instructions. The glycosylated haemoglobin was determined by the method of Nayak and Pattabiraman [29].

Determination of proinflammatory cytokines

The levels of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 in plasma were determined by using specific ELISA kits (Biosource, CA). The analyses were performed according to instructions of the manufacturer. The concentration of pro-inflammatory cytokines was determined spectrophotometrically at 450 nm. Standard plots were constructed by using standard cytokines and the concentrations for unknown samples were calculated from the standard plot. The level of NF-κB p65 unit was determined in the nuclear fraction of hepatic tissues by using ActivELISA (Imgenex, San Diego, CA) kit. The serum NO level was indirectly estimated by determining the nitrite levels in serum using a colourimetric method based on the Griess reaction [30].

Preparation of liver homogenate

A portion of the liver tissue was dissected out, rinsed with ice-cold saline and homogenized in 0.1 M Tris-HCl buffer (pH 7.4) with a Teflon homogenizer at 4°C. The homogenate was centrifuged at 12000 $\times$ g to remove the debris and the supernatant was used for the estimations. The protein content in the tissue homogenate was estimated by the method of Lowry et al. [31].

Assessment of antioxidant status

The levels of liver non-enzymatic antioxidants such as vitamin C [32], vitamin E [33] and reduced glutathione [34] were estimated. Further, the levels of lipid peroxides [35] and hydroperoxides [36] were determined in the liver homogenate. The activities of liver enzymatic antioxidants such as superoxide dismutase [37], catalase [38], glutathione peroxidase [39] and glutathione-S-transferase [40] were determined in the control and experimental groups of rats.

Histological study

A portion of the liver was fixed in 10% formalin for a week at room temperature. Then the specimens were dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin wax. The blocks were then sectioned to 5 μm thick using a rotary microtome. Sections were stained by haematoxylin and eosin and photomicrographs were obtained under light microscope.

Transmission electron microscopic study

A portion of the liver (~1 mm^3) were excised from the control and experimental groups of rats and fixed in 3% glutaraldehyde in sodium phosphate buffer (0.2 M, pH 7.4) for 3 h at 4°C. Tissue samples were washed with the same buffer, post-fixed in 1% osmium tetroxide and sodium phosphate buffer (0.2 M, pH 7.4) for 1 h at 4°C. The samples were again washed with the same buffer for 3 h at 4°C, dehydrated with graded series of ethanol and embedded in Araldite. Thin sections were cut with a LKBUM4 ultramicrotome using a diamond knife, mounted on a copper grid and stained with 2% uranyl acetate and Reynolds lead citrate. The grids were examined under a Philips EM201C transmission electron microscope.

Statistical analysis

The results were expressed as mean $\pm$ SEM of six rats per group and the statistical significance was evaluated by one-way analysis of variance (ANOVA) using the SPSS/16.0 software followed by the *post-hoc* test LSD. Values were considered statistically significant at $p < 0.05$.

Results

Effect of D-pinitol administration on the blood glucose, plasma insulin and glycosylated haemoglobin levels

Figure 1 summarizes the levels of fasting blood glucose, plasma insulin and glycosylated haemoglobin of the control and experimental groups of rats. The control rats were normoglycaemic throughout the course of study. The streptozotocin-induced diabetic group of rats showed a significant ($p < 0.05$) elevation in the levels of blood glucose and glycosylated haemoglobin with a concomitant decline in the level of plasma insulin. Oral administration of D-pinitol as well as glyclazide to diabetic groups of rats reversed these biochemical changes to near normalcy.

Attenuation of proinflammatory cytokines by D-pinitol

The level of TNF-α, IL-1β and IL-6 of control and experimental groups of rats is depicted in Figures 2A–C. There was a significant ($p < 0.05$) elevation in the levels of TNF-α, IL-1β and IL-6 in streptozotocin-induced diabetic group of rats when compared to control group of rats. However, the diabetic rats treated with D-pinitol

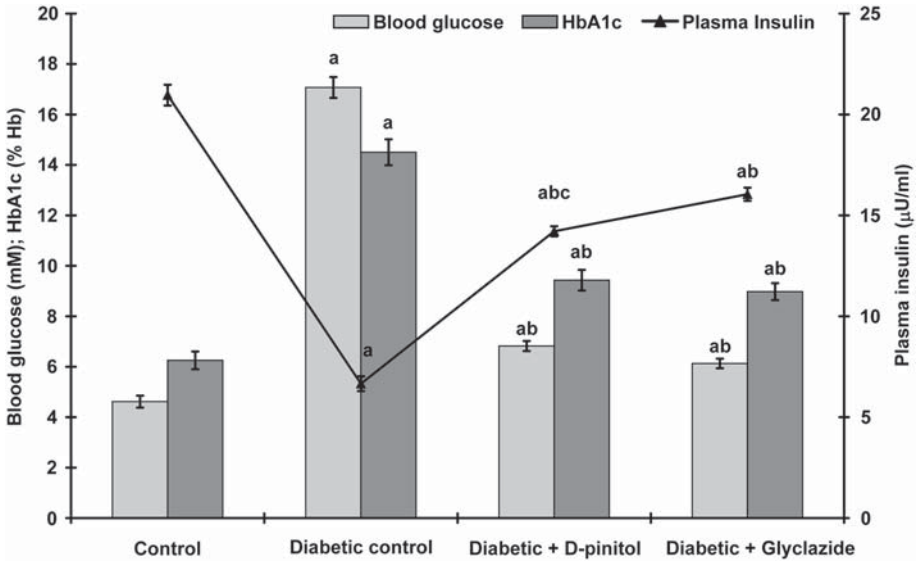

Figure 1. Levels of fasting blood glucose, plasma insulin and glycosylated haemoglobin in control and experimental groups of rats. Results are mean ± SEM ($n = 6$). One-way ANOVA followed by *post-hoc* test LSD. Values are statistically significant at $^{*}p < 0.05$, when compared with [a]Control rats, [b]Diabetic control, [c]Diabetic + Glyclazide.

as well as glyclazide showed a notable decline in the levels of these pro-inflammatory cytokines.

The hepatic NF-κB p65 unit and serum NO level in control and experimental groups of rats is demonstrated in Figures 3A and B. The levels of hepatic NF-κB p65 unit and serum NO were significantly ($p < 0.05$) escalated in diabetic group of rats when compared with control group of rats. Conversely, these levels were significantly ($p < 0.05$) lowered in diabetic rats treated with D-pinitol as well as glyclazide.

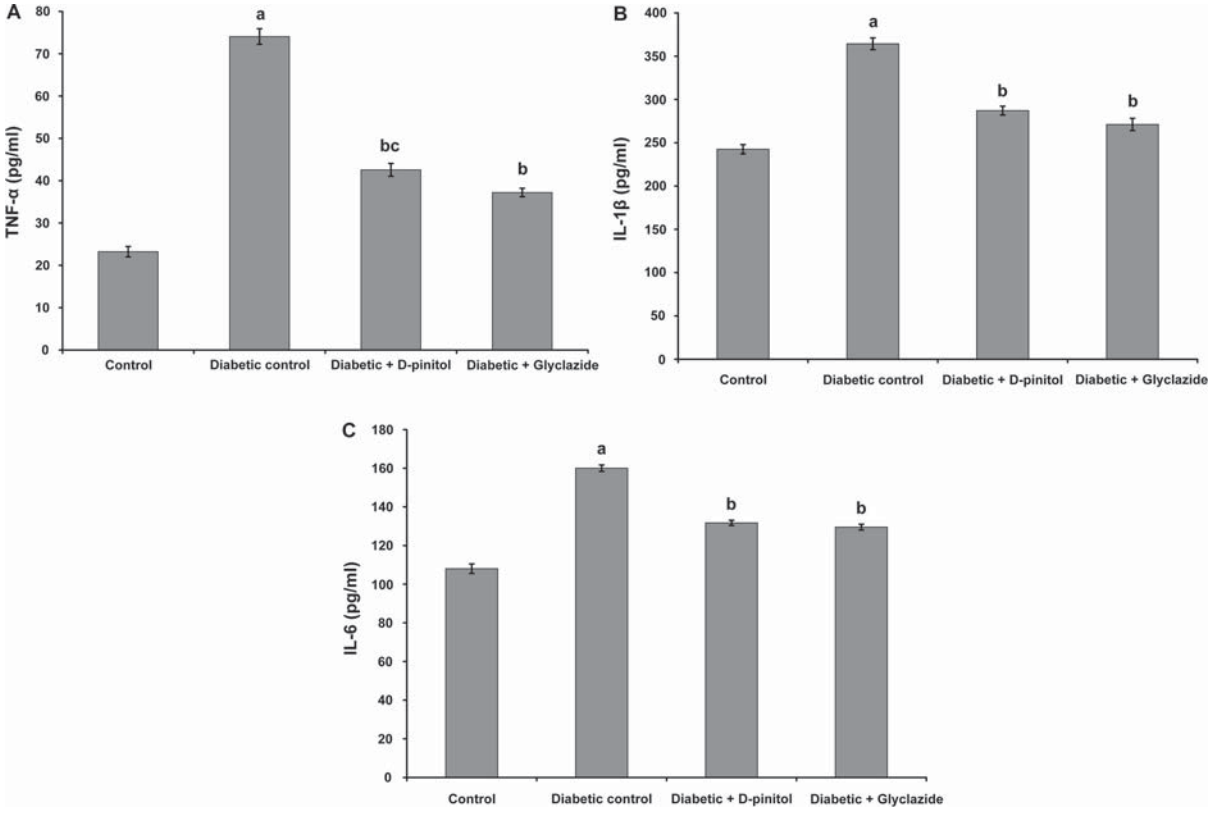

Figure 2. Levels of plasma (A) TNF-α, (B) IL-1β and (C) IL-6 in control and experimental groups of rats. Results are mean ± SEM ($n = 6$). One-way ANOVA followed by *post-hoc* test LSD. Values are statistically significant at $^{*}p < 0.05$, when compared with [a]Control rats, [b]Diabetic control, [c]Diabetic + Glyclazide.

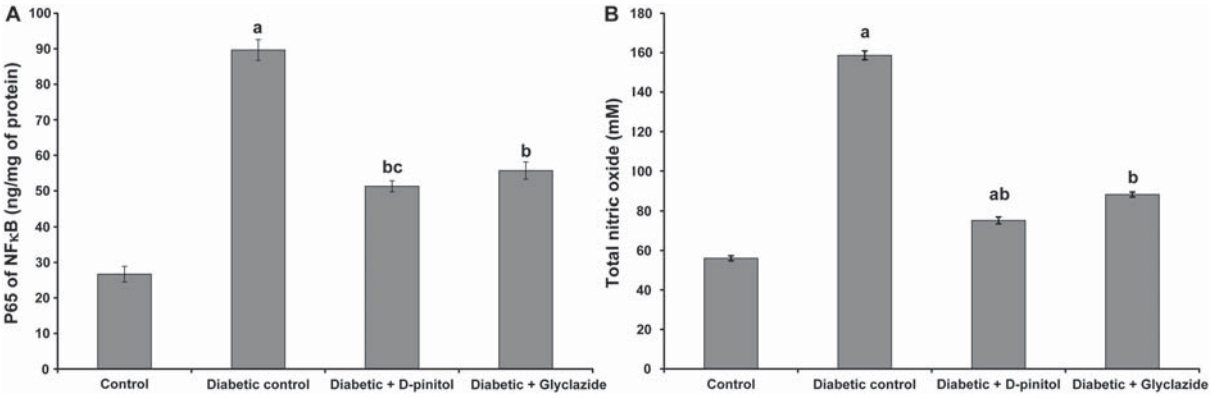

Figure 3. Levels of (A) hepatic p65 unit of NF-κB and (B) serum NO in control and experimental groups of rats. Results are mean ± SEM ($n = 6$). One-way ANOVA followed by *post-hoc* test LSD. Values are statistically significant at $^{*}p < 0.05$, when compared with [a]Control rats, [b]Diabetic control, [c]Diabetic + Glyclazide.

Amelioration of antioxidant status by D-pinitol

The levels of lipid peroxides and hydroperoxides were significantly ($p < 0.05$) elevated in liver tissues of diabetic group of rats compared to control rats. The oral administration of D-pinitol as well as glyclazide to streptozotocin-induced diabetic group of rats showed a marked decline in the levels of lipid peroxides and hydroperoxides (Figures 4A and B).

The levels of hepatic non-enzymatic antioxidants such as vitamin C, vitamin E and reduced glutathione in control and experimental groups of rats are depicted in Table I. There was a significant ($p < 0.05$) decline in levels of non-enzymatic antioxidants in hepatic tissues of streptozotocin-induced diabetic rats when compared to the control group of rats. A significant ($p < 0.05$) elevation in the levels of non-enzymatic antioxidants was observed on oral administration of D-pinitol as well as glyclazide to diabetic groups of rats.

Table II exemplifies the activities of enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in control and experimental groups of rats. The activities of these enzymatic antioxidants were significantly ($p < 0.05$) declined in streptozotocin-induced diabetic group of rats when compared to the control group of rats. However, oral administration of D-pinitol as well as glyclazide to diabetic groups of rats markedly ($p < 0.05$) reversed the activities of these enzymatic antioxidants.

Alteration in histology and ultrastructure of liver by D-pinitol

Figures 5A–D depict the histological observation of the liver of control and experimental groups of rats. The liver of control rat (Figure 5A) shows concentric arrangement of the hepatocytes around the central vein. Hepatic tissues of diabetic rats show fibrosis in the portal area and microvesicular vacuolization, granular degeneration and distortion in the arrangement of cells around the central vein. The capillaries in the liver tissues enlarge leading to generation of spaces between the hepatocytes, which in turn results

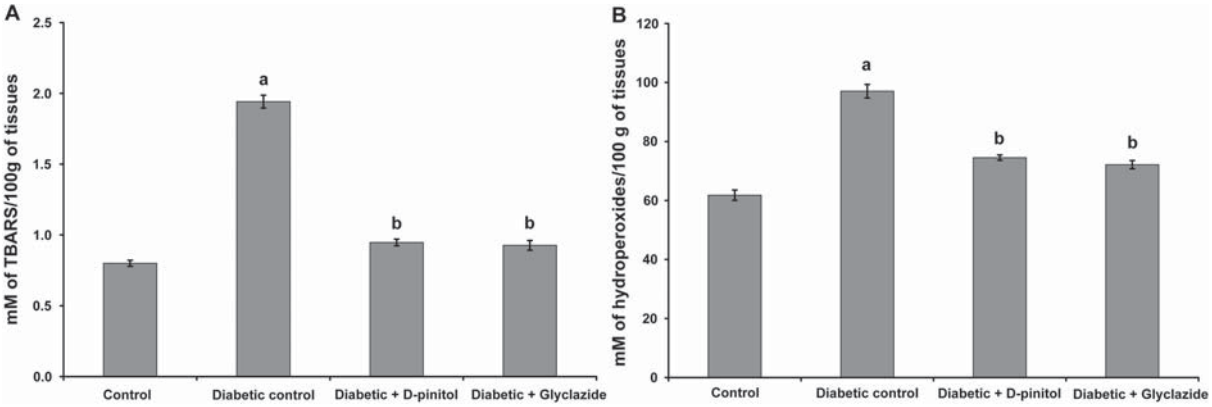

Figure 4. Levels of (A) lipid peroxide and (B) hydroperoxide in liver tissues of control and experimental groups of rats. Results are mean ± SEM ($n = 6$). One-way ANOVA followed by *post-hoc* test LSD. Values are statistically significant at $^{*}p < 0.05$, when compared with [a]Control rats, [b]Diabetic control, [c]Diabetic + Glyclazide.

Table I. Levels of non-enzymatic antioxidants such as vitamin C, vitamin E and reduced glutathione in the liver tissues of control and experimental groups of rats.

Groups	Vitamin C (mg/dl)	Vitamin E (mg/dl)	Reduced glutathione (mg/dl)
Control	1.70±0.06	1.92±0.05	47.85±1.13
Diabetic Control	0.54±0.03[a]	0.95±0.15[a]	24.56±0.96[a]
Diabetic+D-pinitol	1.35±0.05[b]	1.56±0.04[bc]	30.75±0.27[b]
Diabetic+Glyclazide	1.17±0.09[b]	1.08±0.06[b]	29.38±0.73[b]

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 [a]Control rats, [b]Diabetic rats, [c]Diabetic+Glyclazide.

in the loss of concentric arrangement of the hepatocytes in the diabetic group of rats (Figure 5B). The diabetic rats treated with D-pinitol as well as glyclazide (Figures 5C and D) showed a normal architecture when compared to the control group of rats.

Figures 6A–D exemplifies the ultrastructural changes in the hepatocytes of liver of control and experimental group of rats. The liver cells of control rats showed a normal cellular architecture (Figure 6A). The streptozotocin-induced diabetic rats showed a major amendment in the structural veracity of intracellular organelles such as pyknotic nuclei, increased lipid droplet accumulation, reduced mitochondria and decreased glycogen content (Figure 6B). The oral administration of D-pinitol as well as glyclazide normalized these ultrastructural changes to normal architecture (Figures 6C and D).

Discussion

Streptozotocin, an antibiotic produced by *Streptomyces achromogenes*, is an analogue of N-acetylglucosamine and is thought to specifically damage pancreatic β-cells by alkylating DNA and activating immune mechanisms [41]. Release of nitric oxide, increased glycation of pancreatic proteins and an increased production of reactive oxygen species has been proposed as a possible cause of streptozotocin-induced pancreatic β-cell damage [42,43]. Further, the cytotoxic nature of streptozotocin is arbitrated by the generation of excessive oxidants and elicits toxic effects on the pancreas, liver and kidneys [44]. The oxidative stress causes cellular damage by DNA-modification, lipid peroxidation and reaction with thiol-groups [45,46]. The peroxidation of membrane phospholipids leads to alteration in permeability and loss of membrane integrity [47].

The foremost insulin-responsive and major metabolic tissues are liver, skeletal muscle and adipose tissue. In these tissues insulin restrains numerous physiological functions, such as glucose uptake, intracellular glucose metabolism, lipid metabolism and protein synthesis at the transcriptional and translational level [48]. In hepatic tissues the expression of key gluconeogenic enzymes is inhibited due to the decreased insulin secretion or increased insulin resistance, thereby leading to elevated levels of hepatic glucose production [49]. The chronic elevation of blood glucose leads to major complications of diabetes [50,51]. The elevated level of blood glucose in streptozotocin-induced diabetic rats is due to the augmentation of hepatic glucose collectively with reduced glucose utilization. This elevation in blood glucose level was reverted to near normal levels by the administration of D-pinitol. Glucose is the key physiological regulator of insulin secretion. The decline in plasma insulin levels in streptozotocin-induced diabetic rats was significantly elevated to near normal levels by the administration of D-pinitol. The elevation in plasma insulin level might be due to the stimulation of remnant β-cells to synthesize and secrete more insulin for normalizing the supraphysiological glucose, which in turn reduces the glycosylated haemoglobin level. Moreover, these results suggest the anti-hyperglycemic nature of D-pinitol.

Table II. Activities of enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the hepatic tissues of control and experimental groups of rats.

Groups	Superoxide dismutase	Catalase	Glutathione peroxidase	Glutathione-S-transferase
Control	10.49±0.25	82.84±1.15	9.56±0.34	7.78±0.18
Diabetic Control	4.42±0.26[a]	42.90±1.21[a]	4.58±0.26[a]	4.07±0.97[a]
Diabetic+D-pinitol	7.37±0.21[b]	68.83±1.53[bc]	7.59±0.28[b]	6.48±0.17[b]
Diabetic+Glyclazide	6.66±0.27[b]	74.24±1.18[b]	8.05±0.22[b]	6.33±0.33[b]

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 [a]Control rats, [b]Diabetic rats, [c]Diabetic+Glyclazide.
Activity is expressed as: 50% of inhibition of epinephrine auto-oxidation/min for superoxide dismutase; µmol of hydrogen peroxide decomposed/min/mg of protein for catalase; µmol of glutathione oxidized/min/mg of protein for glutathione peroxidase; units/min/mg of protein for glutathione-S-transferase.

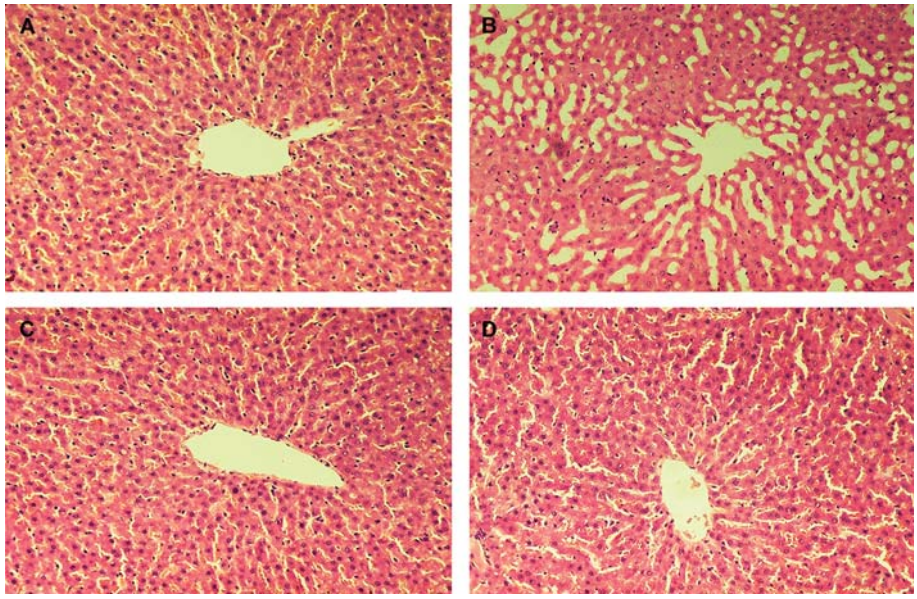

Figure 5. Light micrographs of haematoxylin and eosin staining of liver tissues of control and experimental groups of rats. Photomicrographs of (A) Control, (B) Diabetic control, (C) Diabetic + D-pinitol and (D) Diabetic + Glyclazide stained by haematoxylin and eosin at 200× magnification.

Pro-inflammatory cytokines are key mediators in the pathophysiology of acute and chronic liver diseases [52]. Pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β, may be a causative factor in the development of diabetes and/or its complications by restraining insulin synthesis and secretion [53–55]. Elevated levels of IL-6 and TNF-α are allied with worsening of glycemic control and dyslipidemia, which further contributes to the dysfunction of metabolic status in diabetics [56].

TNF-α is predominantly produced in macrophages and adipocytes. It causes cellular damage by various mechanisms including elevation in the production of reactive oxygen species and impairment of cellular components such as proteins, lipids and DNA [57,58]. In addition, it impairs insulin action on consumption

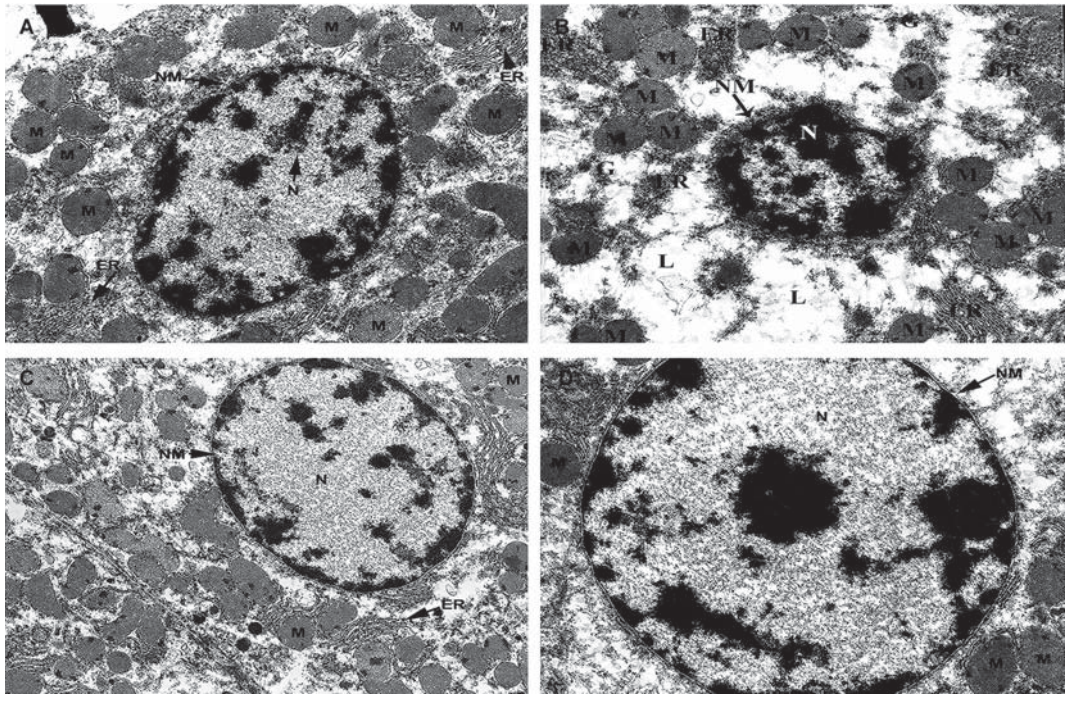

Figure 6. Transmission electron micrographs of liver tissues of control and experimental groups of rats. Transmission electron micrographs of (A) Control, (B) Diabetic control, (C) Diabetic + D-pinitol and (D) Diabetic + Glyclazide showed at 15 000× magnification. Nucleus (N), Nuclear membrane (NM), Mitochondria (M), Endoplasmic reticulum (ER), Glycogen (G) and Lipid droplets (L).

of peripheral glucose and output of hepatic glucose [59]. TNF-α is involved in the production of other cytokines, such as IL-1β and IL-6. IL-1β and TNF-α are multi-functional cytokines involved in inflammation, cell growth and apoptosis [60,61]. The signal transduction cascades elicited after exposure to IL-1β and TNF-α culminate in a nuclear response characterized by the activation of several key transcriptional regulators, including NF-κB [62]. IL-1β along with TNF-α restrains glucose induced insulin secretion [63,64]. IL-6 is known to enhance insulin resistance by acting on insulin signalling pathway in hepatocytes [65]. The circulating levels of TNF-α and IL-6 are elevated in diabetics [66]. In the present study, oral administration of D-pinitol as well as glyclazide to diabetic group of rats produced a significant reduction in pro-inflammatory cytokines concentration. The decline in pro-inflammatory cytokines levels in diabetic rats might be one of the ameliorative ways in which D-pinitol recovers the diabetic state.

The elevated level of nitric oxide reacts with superoxide to produce peroxynitrite, a strong oxidant, which in turn elevates lipid peroxidation [67]. The level of lipid peroxidation in the cell is controlled by various cellular defense mechanisms consisting of enzymatic and non-enzymatic scavenger systems, the levels of which are altered in diabetes [2]. The formation of peroxides along with impaired antioxidant enzyme and autoxidation of glucose contributes to the pathogenesis of diabetes mellitus by oxidative stress besides the reactive oxygen species [46]. Further, the escalated levels of reactive oxygen species in streptozotocin-induced diabetic rats elevated the levels of lipid peroxidation and hydroperoxides by oxidative deterioration of polyunsaturated fatty acids, leading to extensive membrane damage. Thus, lipid peroxidation is a key marker of oxidative stress. The oral administration of D-pinitol for 30 days significantly attenuated the increased levels of lipid peroxidation and hydroperoxides to near normal levels. Therefore, from the results of the present study the anti-lipid peroxidative potential of D-pinitol in streptozotocin-induced diabetic rats is substantiated.

The possible sources of oxidative stress in diabetics include augmented mitochondrial production of superoxide anion, non-enzymatic glycation of proteins [68], decline in tissue concentration of non-enzymatic antioxidants and impaired activities of antioxidant enzymes [1,2]. The levels of non-enzymatic antioxidants such as vitamin E, vitamin C and reduced glutathione are significantly declined in streptozotocin-induced diabetic rats. Vitamin E is a fat-soluble vitamin and is well known to quench lipid peroxides and vitamin C quenches singlet molecular oxygen [69,70]. The decline in vitamin E and vitamin C might be due to the increased utilization against free radicals produced in elevated amounts in diabetic state or due to decline in major non-protein thiol like reduced glutathione, as reduced glutathione is requisite for recycling of vitamin C. Reduced glutathione is the first line of defense against pro-oxidant status and serves as a co-substrate for glutathione peroxidase activity and as a co-factor for many enzymes [71]. Glutathione level is found to be reduced in the liver of chemically-induced diabetic rats [72]. The levels of these non-enzymatic antioxidants were significantly elevated by the oral administration of D-pinitol to the diabetic group of rats. Further, suggesting the free radical scavenging activity of D-pinitol, this in turn may contribute to its antioxidant potential.

Hyperglycaemia is accompanied by increased generation of free radicals such as superoxide radicals, hydrogen peroxide and hydroxyl radicals by glucose autoxidation [8,46,73] or impairment in antioxidant defense [74,75]. These radicals are readily deactivated by cellular antioxidants such as superoxide dismutase, catalase and glutathione peroxidase to protect the cells from oxidative damage. However, these enzymatic antioxidant defenses can be overwhelmed during diabetic state. The rapid production of superoxide radicals and the antioxidant capacity of the cell unable to keep up with the production lead to oxidative damage.

Superoxide dismutase, an important enzyme, converts superoxide anion radicals into hydrogen peroxide, thereby reducing the probability of superoxide anion interacting with nitric oxide to form reactive peroxynitrite. The decrease in superoxide dismutase activity in diabetic rats could result from inactivation by hydrogen peroxide or by glycosylation of the enzyme, which have been reported to occur in diabetes [76].

Catalase, located in peroxisomes, decomposes the hydrogen peroxide produced by superoxide dismutase to water and molecular oxygen. Decrease in catalase activity in hepatic tissues of streptozotocin-induced diabetic group of rats could result from inactivation by increased endogenous production of superoxide radical and glycation of the enzyme [77].

Glutathione peroxidase is an enzyme with selenium and plays a primary role in minimizing oxidative damage. Glutathione peroxidase protects the cells from lipid peroxidation and reduces the loss of membrane integrity by metabolizing hydrogen peroxide to water by using reduced glutathione as a hydrogen donor. Glutathione is oxidized to glutathione disulphide by glutathione peroxidase, which is converted back to glutathione by glutathione reductase in an NADPH-consuming process [78]. The activity of glutathione peroxidase is declined due to the inactivation and glycation of the enzyme in streptozotocin-induced diabetic rats. Oral administration of D-pinitol to the streptozotocin-induced diabetic group of rats brought back the activities of these enzymatic antioxidants to near normal levels. Thus, the free radical scavenging

potential of D-pinitol is elicited by its antioxidant nature.

Moreover, our results are in concurrence with the results of Wolff (1993) [6], who reported the elevation in the levels of lipid peroxides and a decline in the antioxidant status in diabetes mellitus. Further, results of the present study demonstrate the hepatic tissue protective role of D-pinitol by the amelioration of the antioxidant status and inhibition of lipid peroxidation in diabetic group of rats. This might be due to the antioxidant nature of D-pinitol. In conclusion, the present investigation shows that D-pinitol possesses antioxidant activity that may contribute to a novel protective action against liver damage by reducing lipid peroxidation and enhancing its effect on cellular antioxidant defense. This activity contributes to the protection against oxidative damage in streptozotocin-induced diabetes. In addition, the histological and ultrastructural observation made on the liver tissues further corroborate that D-pinitol has hepatoprotective nature.

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