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The antioxidant role of pterostilbene in streptozotocin–nicotinamide-induced type 2 diabetes mellitus in Wistar rats

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Abstract

The antioxidant effect of pterostilbene on streptozotocin–nicotinamide-induced diabetic rats has been assessed. The activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione was significantly decreased in liver and kidney of diabetic animals when compared with normal control. There were significant improvements in these activities after treatment with pterostilbene at a dose of 40 mg kg^{-1} for six weeks. The increased levels of lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS) in liver and kidney of diabetic rats were also normalized by treatment with pterostilbene. Chronic treatment of pterostilbene remarkably reduced the pathological changes observed in liver and kidney of diabetic rats. These results indicated the antioxidant property of pterostilbene.

Introduction

Diabetes mellitus is characterized by derangement in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or of insulin action (O'Brien & Granner 1996). Reactive oxygen species (ROS) can easily initiate the peroxidation of the membrane lipids, leading to the accumulation of lipid peroxides. The peroxidation products by themselves and their secondary oxidation products react with biological substrates such as protein, amines and deoxyribonucleic acid (DNA) (Kehrer 1993). Oxygen derived free radicals (OFRs) have been implicated in the pathophysiology of various diseases including diabetes mellitus. Oxidative stress plays an important role in chronic complications of diabetes and is postulated to be associated with increased lipid peroxidation (Elangovan et al 2000).

An increase in the production of free radicals can result in hyperglycaemia-induced enhancement in glucose autoxidation, protein glycation, and subsequent oxidative degradation of glycated proteins (Singal et al 2001). Cellular defence mechanisms, such as antioxidant materials and antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), play a fundamental role in protecting the cell against reactive free radicals and other oxidant species (Adaramoye & Adeyemi 2006). Therefore, antioxidants with free radical scavenging activity may have great relevance in the prevention and therapy of disease in which oxidants or free radicals are implicated (Soares et al 1997). In this respect, polyphenolic compounds, such as flavonoids and phenolic acids commonly found in plants, have been reported to have multiple biological effects including antioxidant activity and may be of use (Kahkonen et al 1999).

Pterostilbene (Figure 1) was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium* (Maurya et al 2004). The water stored in tumblers made out of the heartwood of *P. marsupium* is used as a traditional therapy for patients with diabetes mellitus (Maheswari et al 1980). An aqueous extract of heartwood of *P. marsupium* has been tested clinically and found to be effective in non-insulin dependent diabetes mellitus patients (Indian Council of Medical Research 1998). When pterostilbene and marsupin, two of the major phenolic constituents in an aqueous decoction of the heartwood of *P. marsupium*, were administered to streptozotocin-induced hyperglycaemic rats, there was a significant decrease in plasma glucose (Manickam et al 1997).

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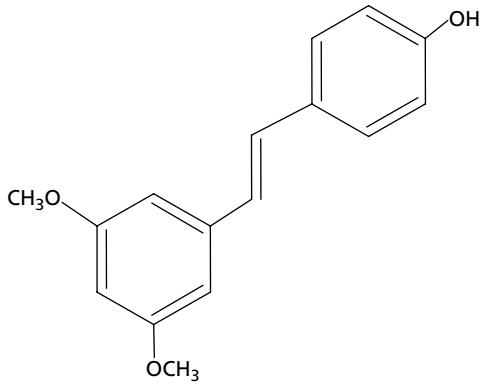

Figure 1 Structure of pterostilbene.

The streptozotocin–nicotinamide type 2 model shares a number of features with human type 2 diabetes, and is characterized by moderate stable hyperglycaemia, glucose intolerance, altered but significant glucose-stimulated insulin secretion, in-vivo and in-vitro. After streptozotocin and nicotinamide administration, a partial loss of β-cell mass occurs by necrosis and/or apoptosis, induced by the relatively specific cytotoxic effect of streptozotocin, which is only partially counteracted by nicotinamide. The residual β cells (approximately 60% of the original mass) are most likely those which escaped from irreversible damage and maintained the differentiation of mature β cells (Novelli et al 2001).

Previously, we found that pterostilbene ($40\,mg\,kg^{-1}$) effectively reduced the blood glucose in diabetic rats (Pari & Amarnath Satheesh 2006). However, no scientific studies have established the antioxidant effects of pterostilbene in experimental type 2 diabetes. We have evaluated the antioxidant activity of pterostilbene against streptozotocin–nicotinamide-induced diabetic rats.

Materials and Methods

Animals

Male albino Wistar rats (200–220 g) were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. They were housed in an animal room under controlled conditions on a 12-h light:dark cycle. They all received a standard pellet diet (Lipton India Ltd, India) and water was freely available. The animals used in this study were cared for as per the principles and guidelines of the Institutional Animal Ethical Committee of Annamalai University, Annamalainagar (Vide No. 158, 2003).

Chemicals

Streptozotocin was purchased from Sigma Chemical Company (St Louis, MO). Pterostilbene was received as a gift sample from Sabinsa Corporation, USA. Nicotinamide was purchased from Himedia, Mumbai, India. All the other chemicals and reagents used were of analytical grade.

Experimental induction of type 2 diabetes

Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Type 2 diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of $65\,mg\,kg^{-1}$ streptozotocin, 15 min after the intraperitoneal administration of $110\,mg\,kg^{-1}$ nicotinamide (Masiello et al 1998). Hyperglycaemia was confirmed by the elevated glucose levels in plasma, determined at 72 h. Animals with a blood glucose concentration of more than $250\,mg\,dL^{-1}$ were used for the study.

Experimental design

A total of 40 rats (24 diabetic surviving rats, 16 normal rats) were used. The rats were divided into five groups of eight each, after the induction of type 2 diabetes. Two rats from each group were randomly selected and used for histopathological studies.

The groups were treated as follows: Group 1, normal control (vehicle treated); Group 2, normal rats received pterostilbene ($40\,mg\,kg^{-1}$) in 1 mL 0.5% methylcellulose suspension (Klimes et al 1998) for six weeks; Group 3, diabetic control; Group 4, diabetic rats received pterostilbene ($40\,mg\,kg^{-1}$) in 1 mL 0.5% methylcellulose suspension for six weeks; Group 5, diabetic rats received metformin ($500\,mg\,kg^{-1}$) in 1 mL saline (Soon & Tan 2000) for six weeks.

At the end of six weeks, all the rats were killed by cervical decapitation after an overnight fast. Plasma was separated for the estimation of glucose. Tissues (liver and kidney) were dissected out, washed in ice-cold saline and stored at −20°C until used. The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris-HCl buffer, pH 7.5. After centrifugation at $2000\,rev\,min^{-1}$ for 10 min, the clear supernatant was used for biochemical assays.

Analytical methods

Fasting plasma glucose was estimated by the Trinder (1969) method. Thiobarbituric acid reactive substance (TBARS) was estimated by the method of Fraga et al (1988). Lipid hydroperoxide (HP) was determined by the method of Jiang et al (1992). Reduced glutathione (GSH) was estimated by the method of Ellman (1959). The levels of GSH in tissues were expressed as mg/100 g tissue. Superoxide dismutase (SOD) was assayed by the method of Kakkar et al (1984), in which one unit of activity was defined as the enzyme reaction which gave 50% inhibition of nitroblue tetrazolium reduction in one minute. Catalase was assayed by the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2); the chromic acetate thus produced was measured spectrophotometrically at 610 nm. The activity was expressed as $\mu M\ H_2O_2$ consumed min^{-1} (mg protein)$^{-1}$ (Sinha 1972). The activity of glutathione peroxidase (GPx) and glutathione S-transferase (GST) was assayed according to the method described by Rotruck et al (1984) and Habig et al (1974), respectively. The GPx activity was expressed as μg GSH consumed min^{-1} (mg protein)$^{-1}$. The activity of GST was expressed as mM GSH-CDNB conjugate

formed min^{-1} (mg protein)$^{-1}$ using an extinction coefficient of 9.6 mM^{-1} cm^{-1}. Protein content in tissue homogenate was measured by the method of Lowry et al (1951).

Histopathological studies

The liver and kidney samples were fixed for 48 h in 10% formalin and dehydrated by passing successfully in different mixtures of ethyl alcohol–water, cleaned in xylene and embedded in paraffin. Sections of liver and kidney (5–6 μm thick) were prepared and then stained with haematoxylin and eosin dye (H&E), mounted in neutral deparaffinated xylene and examined using a light microscope.

Statistical analysis

The data for various biochemical parameters were analysed using one-way analysis of variance and the group means were compared by Duncan's multiple range test (Duncan 1957). Values were considered statistically significant when $P < 0.05$.

Results

Table 1 demonstrates the level of plasma glucose in normal and experimental rats. Fasting plasma glucose levels were significantly increased (327%) in diabetic control rats when compared with normal control rats. Pterostilbene (40 mg kg^{-1}, p.o.) administered to diabetic rats significantly decreased the plasma glucose levels (56.5%) when compared with diabetic control rats at the end of the study. However, oral administration of pterostilbene to normal rats did not show any significant effect on fasting plasma glucose levels.

Table 1 Changes in the levels of plasma glucose in normal and experimental rats

Groups	Glucose (mg dL^{-1})	
	Initial	Final
Normal control	72.51 ± 6.96	74.50 ± 6.30[a]
Normal + pterostilbene (40 mg kg^{-1})	80.48 ± 7.06	67.48 ± 6.67[a]
Diabetic control	310.24 ± 27.07	394.19 ± 32.67[b]
Diabetic + pterostilbene (40 mg kg^{-1})	279.58 ± 23.66	121.50 ± 10.84[e]
Diabetic + metformin (500 mg kg^{-1})	288.49 ± 24.52	140.89 ± 12.09[f]

Values are mean ± s.d. for six rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Table 2 represents the concentration of TBARS and HP in tissues of normal and experimental animals. In diabetic control rats, the concentration of liver and kidney thiobarbituric acid reactive substances (TBARS) increased significantly (133.3% and 50%, respectively) on comparison with normal control rats. A significant increase in liver and kidney HP was also observed in diabetic tissues (36.7% and 54.12%, respectively). Treatment of diabetic rats with pterostilbene significantly decreased TBARS (61.5% and 33.3%) and HP (27.7% and 28.3%) concentration in liver and kidney, respectively.

The content of GSH in tissues of normal and experimental animals is shown in Table 3. There was a significant decrease in the concentration of GSH (52.7% and 44.5%) in liver and kidney of diabetic control rats compared with normal control rats. Oral administration of pterostilbene to diabetic rats led to a significant increase in the liver and kidney levels of GSH (91.4% and 53%) when compared with diabetic control rats.

Tables 4 and 5 show the activity of SOD, catalase, GPx and GST in the tissues of normal and experimental groups. In diabetic rats, the activity of superoxide dismutase (SOD), catalase, GPx and GST were significantly decreased in liver (SOD 36%, catalyse 43.3%, GPx 36.3% and GST 49%), kidney (SOD 30%, catalyse 28%, GPx 51% and GST 55.5%) when compared with normal control rats. Pterostilbene-treated diabetic rats exhibited a significant increase in the activity of these enzymic antioxidants in liver ((SOD 48.2%, catalyse 57.1%, GPx 36.2% and GST 82.9 %) and kidney (SOD 30.7%, catalyse 20.4%, GPx 78.3% and GST 104.2%) when compared with diabetic control rats.

Histopathological observations

The histopathological changes in control and experimental rats are shown in Figure 2. Pathological changes of liver included hepatic nuclear condensation of the portal triad with inflammation and sinusoidal dilation in diabetic control rats. These pathological changes were reduced in rats treated with pterostilbene and metformin, with mild inflammation and mild sinusoidal dilatation. Normal rats treated with pterostilbene showed no inflammation but showed sinusoidal dilation. Diabetic control rat kidney showed glomeruli mesangial capillary proliferation with tubular epithelial damage. These changes were reduced in pterostilbene- and metformin-treated diabetic rats. Tissues of normal

Table 2 Changes in the concentration of TBARS and HP in liver and kidney of normal and experimental animals

Groups	TBARS (mmol/100 g tissue)		HP (mmol/100 g tissue)	
	Liver	Kidney	Liver	Kidney
Normal control	0.90 ± 0.07[a]	1.60 ± 0.12[a]	77.36 ± 5.89[a]	54.21 ± 4.13[a]
Normal + pterostilbene (40 mg kg^{-1})	0.84 ± 0.06[a]	1.53 ± 0.12[a]	74.90 ± 5.63[a]	50.49 ± 3.84[a]
Diabetic control	2.10 ± 0.16[b]	2.40 ± 0.18[b]	105.27 ± 8.02[b]	83.55 ± 6.36[b]
Diabetic + pterostilbene (40 mg kg^{-1})	1.30 ± 0.10[c]	1.80 ± 0.14[c]	82.42 ± 6.28[c]	65.13 ± 4.96[c]
Diabetic + metformin (500 mg kg^{-1})	1.48 ± 0.11[d]	1.93 ± 0.15[c]	88.69 ± 6.75[d]	69.38 ± 5.28[c]

Values are mean ± s.d. for six rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Table 3 Changes in the concentration of reduced glutathione (GSH) in liver and kidney of normal and experimental animals

Groups	GSH (mg/100 mg tissue)	
	Liver	Kidney
Normal control	45.22 ± 3.44^{a}	33.49 ± 2.55^{a}
Normal + pterostilbene (40 mg kg^{-1})	47.57 ± 3.70^{a}	36.13 ± 2.75^{a}
Diabetic control	21.35 ± 1.63^{b}	18.59 ± 1.42^{b}
Diabetic + pterostilbene (40 mg kg^{-1})	40.86 ± 3.11^{c}	28.46 ± 2.17^{c}
Diabetic + metformin (500 mg kg^{-1})	38.25 ± 2.91^{c}	26.06 ± 1.98^{c}

Values are mean ± s.d. for six rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

rats treated with pterostilbene revealed near normal appearance with no significant changes. Administration of pterostilbene did not alter the normal function or morphology of normal rats.

Discussion

Lipid peroxidation is one of the characteristic features of chronic diabetes. Lipid peroxide mediated tissue damage has been observed in the development of type 1 and type 2 diabetes mellitus. Oxidative stress is increased in diabetes because of multiple factors. Dominant among these factors is glucose autoxidation leading to the production of free radicals. Other factors include cellular oxidation/reduction imbalances and reduction in antioxidant defences (including decreased cellular antioxidant levels and a reduction in the activity of enzymes that dispose of free radicals) (Rahimi et al 2005).

Our previous study showed that pterostilbene produced a marked decrease in blood glucose in diabetic rats after six weeks of treatment (Pari & Amarnath Satheesh 2006). This study confirmed that pterostilbene (40 mg kg^{-1}) significantly lowered the glucose level in diabetic rats. Our finding was in agreement with the results of Manickam et al (1997). The antidiabetic effect of pterostilbene might have been due to increased release of insulin from the existing β-cells of the pancreas or increased utilization of glucose in peripheral tissues (Pari & Amarnath Satheesh 2006).

Patients and experimental diabetes have increased levels of lipid peroxidation products (Atalay & Laaksonen 2002; Rajasekaran et al 2005). Free radicals can damage DNA and oxidatively modify lipids, and have been implicated in glycation and protein-modifying reactions that contribute to tissue damage in diabetes (Arai et al 1987). In this context, a marked increase in the concentration of TBARS and HP was observed in liver and kidney of streptozotocin–nicotinamide-induced diabetic rats.

Administration of pterostilbene significantly decreased the levels of lipid peroxidation products in diabetic rats. The antioxidative activity of pterostilbene was first demonstrated in-vitro by its inhibition of methyl linoleate oxidation (Charvet-Faury et al 1998). Recently, pterostilbene was reported to scavenge DPPH free radical as well as reduce lipid peroxidation in rat liver microsomes and in cultured human fibroblast (Stivala et al 2001). Pterostilbene has been shown to effectively scavenge peroxyl radicals ($ROO^{\bullet}$) and reduce singlet-oxygen induced peroxidation at levels similar to those of resveratrol (Rimando et al 2002). In fact, pterostilbene, which has a free hydroxyl group in the 4′ position, exerts a significant antioxidant activity. The trans isomery and the double bond in the stilbenic skeleton play a role in its antioxidant property (Stivala et al 2001). This indicated that pterostilbene has the capability to inhibit oxidative damage of hepatic and renal tissues.

Table 4 Changes in activity of catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in liver of normal and experimental animals

Groups	Catalase (U (mg protein)$^{-1}$)	SOD (U (mg protein)$^{-1}$)	GPx (U (mg protein)$^{-1}$)	GST (U (mg protein)$^{-1}$)
Normal control	75.26 ± 5.73^{a}	6.10 ± 0.46^{a}	6.60 ± 0.50^{a}	6.08 ± 0.46^{ac}
Normal + pterostilbene (40 mg kg^{-1})	78.55 ± 5.98^{a}	6.36 ± 0.48^{a}	6.78 ± 0.52^{a}	6.32 ± 0.47^{a}
Diabetic control	42.66 ± 3.25^{b}	3.90 ± 0.30^{b}	4.20 ± 0.32^{b}	3.10 ± 0.24^{b}
Diabetic + pterostilbene (40 mg kg^{-1})	67.03 ± 5.10^{c}	5.78 ± 0.44^{cd}	5.72 ± 0.44^{c}	5.66 ± 0.43^{c}
Diabetic + metformin (500 mg kg^{-1})	61.68 ± 4.70^{c}	5.40 ± 0.41^{d}	5.04 ± 0.38^{d}	5.02 ± 0.38^{d}

Values are mean ± s.d. for six rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Table 5 Changes in activity of catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in kidney of normal and experimental animals

Groups	Catalase (U (mg protein)$^{-1}$)	SOD (U (mg protein)$^{-1}$)	GPx (U (mg protein)$^{-1}$)	GST (U (mg protein)$^{-1}$)
Normal control	32.52 ± 2.48^{a}	13.20 ± 1.01^{a}	4.70 ± 0.36^{a}	5.40 ± 0.41^{a}
Normal + pterostilbene (40 mg kg^{-1})	33.30 ± 2.54^{a}	14.03 ± 1.07^{a}	5.00 ± 0.38^{a}	5.60 ± 0.43^{a}
Diabetic control	23.39 ± 1.78^{b}	9.24 ± 0.70^{b}	2.30 ± 0.18^{b}	2.40 ± 0.18^{b}
Diabetic + pterostilbene (40 mg kg^{-1})	28.15 ± 2.14^{c}	12.08 ± 0.92^{c}	4.10 ± 0.31^{c}	4.90 ± 0.37^{c}
Diabetic + metformin (500 mg kg^{-1})	25.10 ± 1.91^{c}	10.97 ± 0.84^{d}	3.70 ± 0.28^{d}	4.10 ± 0.31^{d}

Values are mean ± s.d. for six rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

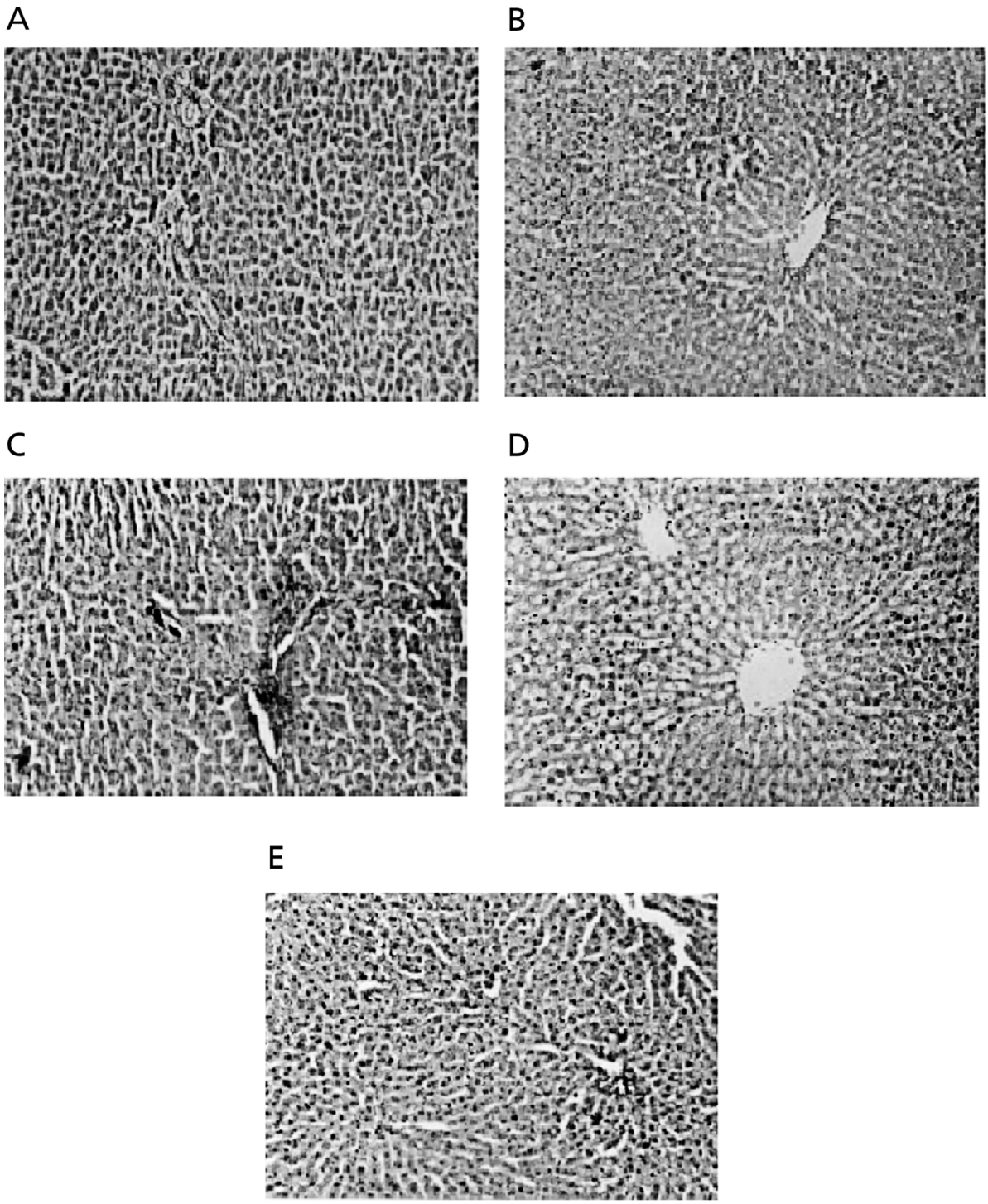

Figure 2 Histopathological changes in control and experimental rats. All the sections are in H&E 100×. A. Control rat liver. Normal architecture of liver. B. Normal + pterostilbene (40 mg kg^{-1})-treated rat liver. Normal appearance of liver cells. C. Diabetic rat liver. Hepatocytic nuclear condensation portal triad with inflammation, sinusoidal dilation. D. Diabetic + pterostilbene-treated rat liver. Normal hepatocytes with mild inflammation. E. Diabetic + metformin treated rat liver. Normal hepatocytes with mild inflammation and mild sinusoidal dilation. F. Control rat kidney. Normal architecture of liver. G. Normal + pterostilbene (40 mg kg^{-1})-treated rat kidney. Normal glomeruli with tubules. H. Diabetic rat kidney. Glomeruli mesangial capillary proliferation with tubular epithelial damage. I. Diabetic + pterostilbene-treated rat kidney. Normal tubules with focal glomerular changes. J. Diabetic + pterostilbene treated rat kidney. Normal tubules with focal glomerular changes.

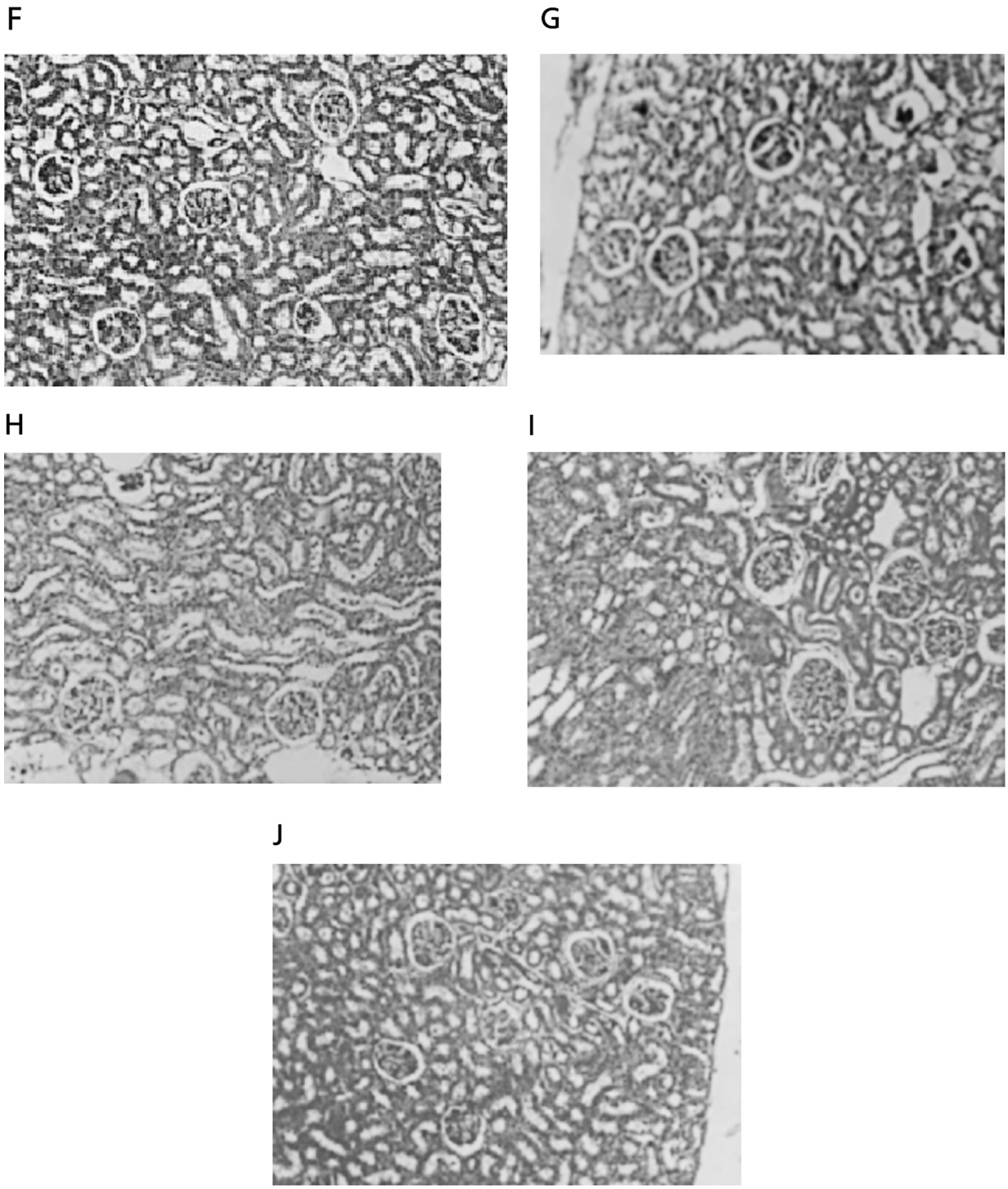

Figure 2 Continued.

SOD and catalase are the two major scavenging enzymes that remove toxic free radicals in-vivo. Reduced activity of SOD and catalase in liver and kidney tissues has been observed during type 2 diabetes and this activity may result in a number of deleterious effects due to the accumulation of $O_2^{\bullet-}$ and H_2O_2 (Searle & Wilson 1980). Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of oxidative damage. Therefore removing $O_2^{\bullet-}$ and $^{\bullet}OH$ is probably one of the most effective defences against disease (Ananthan et al 2004). Treatment with

pterostilbene increased the activity of SOD and catalase in diabetic rats when compared with diabetic control rats. This showed that pterostilbene possessed free radical scavenging activity, which could exert a beneficial action against pathologic alterations caused by the presence of $O_2^{\bullet-}$ and $^{\bullet}OH$.

GSH is the most important biomolecule which participates in the elimination of reactive intermediates by reducing hydroperoxides in the presence of GPx. GSH also functions as a free radical scavenger and in the repair of radical-caused biological damage (Meister 1984; Nicotera & Orrenius 1986). Decreased glutathione levels in type 2 diabetes have been considered to be an indicator of increased oxidative stress (McLennan et al 1991). The decrease in the GSH level represented an increased utilization in trapping the oxy radicals.

GPx and GST catalyse the reduction of hydrogen peroxide and hydroperoxides to non-toxic products (Bruce et al 1982). Previous studies revealed that the activity of GPx and GST was significantly decreased in diabetic rat tissues (Latha et al 2004; Dias et al 2005). The decreased activity of these enzymes results in the involvement of deleterious oxidative changes due to the accumulation of toxic products.

The histopathological changes we observed in diabetic control rats could have been due to the increased production of highly reactive intermediates during oxidative stress. Normally the radicals are detoxified by endogenous GSH, but when present in excess it can deplete GSH stores, allowing the reactive intermediate to react with and destroy hepatic and renal cells (Blum & Fridovich 1985). These pathological changes were reduced in diabetic rats treated with pterostilbene and metformin. This might have been due to the antioxidant nature of the pterostilbene (Stivala et al 2001) and suppression of free radical generation by metformin (Ouslimani et al 2005).

As the alterations produced in the antioxidant activity indicated the involvement of deleterious oxidative changes, the increased activity of the components of the antioxidant defence system would therefore be important in protection against radical damage. Pterostilbene administration effectively scavenged the free radicals and decreased the oxidative stress in diabetic rats. This suggested that pterostilbene plays an antioxidant role in addition to its antidiabetic activity in the diabetic state.

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