Research article

Antioxidant and antihyperlipidaemic activity of protocatechuic acid on streptozotocin-diabetic rats

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Oxidative stress in diabetes co-exists with a reduction in the antioxidant status, which can further increase the deleterious effects of free radicals. Hyperlipidaemia is one of the major risk factors of cardiovascular complications in diabetes. A study was undertaken to evaluate the antioxidant and antihyperlipidaemic activity of protocatechuic acid (PCA) on streptozotocin (STZ)-induced diabetic rats. The levels of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) were increased and the level of enzymatic and non-enzymatic antioxidants decreased except vitamin E. Lipid profile increased in diabetic rats, whereas HDL-C level decreased. These alterations reverted to near control levels after the diabetic rats were treated with PCA. Histopathological studies also revealed the protective effects of PCA on liver and kidney. These findings suggest that PCA treatment exerts a therapeutic property by decreasing the oxidative stress and lipid profile. The effect of PCA was comparable to glibenclamide, a well-known hypoglycaemic drug.

Keywords: diabetes, antioxidant, oxidative stress, hyperlipidaemia, protocatechuic acid

Introduction

Under normal physiological conditions, a wide range of antioxidant defences protect against the adverse effects of free radical production *in vivo*.[1] In diabetes hyperglycaemia, protein glycation and glucose auto-oxidation have been suggested to induce free radical generation.[2] At the same time, disturbances in antidiabetic defence systems including scavenging enzymes like superoxide dismutase (SOD), glutathione reductase and deficiencies of antioxidants like vitamins C and E have been reported.[3] Diabetes mellitus is a major risk factor for the development of cardiovascular complications, and cardiovascular disease now accounts for 80% of all diabetic mortality. During diabetes, a profound alteration in the concentration and composition of lipid occurs. Lipid-lowering therapy in diabetes is effective in reducing the risk of vascular complications.[4]

In recent years, much attention has been focused on the protective properties of exogenous antioxidants in biological systems, and on the mechanisms of their action. Scientific interest in phenolic compounds in plants has been stimulated due to their anti-inflammatory, antimutagenic, and anticarcinogenic properties.[5]

The simple phenolic protocatechuic acid (Fig. 1) is one of the major benzoic acid derivatives from vegetables and fruits and also occurs naturally in many Chinese herbal medicines such as *Salvia miltiorrhiza*

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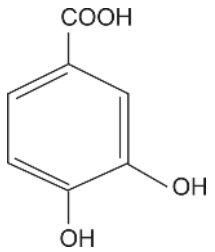


Figure 1 Structure of protocatechuic acid (PCA)

(Danshen)[6] and *Hibiscus sabdariffa* L.[7] Studies with laboratory animals demonstrated protocatechuic acid to be an efficacious anticarcinogen, inhibiting chemical-induced carcinogenesis in the liver.[8] The mechanism of its action is mostly associated with antioxidant activity, including inhibition of the generation, as well as scavenging, of free radicals and up-regulating enzymes which participate in their neutralization. In a recent *in vivo* study, as a result of its antioxidant properties and its ability to block oxidative stress signal transduction, PCA was shown to protect against hepatic damage induced by *tert*-butyl hydroperoxide in rats.[7]

However, no scientific investigation has so far been conducted on the antioxidant and antihyperlipidaemic activity of protocatechuic acid, on diabetic rats and thus the present investigation sets out to study the anti-oxidant and antihyperlipidaemic activity of proto-catechuic acid and other related biochemical parameters in normal and streptozotocin (STZ)-induced diabetic rats. The effect produced by the drug was compared with that of glibenclamide a standard drug.

Materials and methods

Animals

Male albino (9-week-old) rats of Wistar strain with a body weight ranging from 180–200 g, were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and were maintained in an air-conditioned room (25 ± 1ºC) with a 12-h light/12-h dark cycle. Feed and water were provided *ad libitum* to all the animals. The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No.160/1999/CPCSEA, Proposal number: 492, Annamalai University, Annamalainagar).

Chemicals

Streptozotocin and protocatechuic acid were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals used in this study were of analytical grade obtained from E. Merck or HIMEDIA, Mumbai, India.

Experimental induction of diabetes

The animals were rendered diabetic by a single intraperitoneal injection of streptozotocin (40 mg/kg body weight) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. Streptozotocin-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycaemic mortality. Streptozotocin-injected animals exhibited hyperglycaemia within a few days. Diabetes in streptozotocin rats was confirmed by measuring the blood glucose (by glucose oxidase method) 96 h after injection with streptozotocin. The animals with blood glucose above 235 mg/dl were considered to be diabetic and used for the experiment.

Experimental design

The animals were randomly divided into seven groups of six animals each as given below. Protocatechuic acid and glibenclamide were administered orally once a day in the morning for 45 days.

Group I Normal (saline only).

Group II Normal + protocatechuic acid (200 mg/kg body weight/day in saline).

Group III Diabetic control (saline only).

Group IV Diabetic + protocatechuic acid (50 mg/kg body weight/day in saline).

Group V Diabetic + protocatechuic acid (100 mg/kg body weight/day in saline).

Group VI Diabetic + protocatechuic acid (200 mg/kg body weight/day in saline).

Group VII Diabetic + glibenclamide (600 μg/kg body weight/day in saline).

Biochemical estimations

Thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) were estimated by the methods of Niehaus and Samuelson,[9] and Jiang *et al.*,[10] respectively. The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured by the methods of Kakkar *et al.*,[11] Sinha,[12] and Rotruck *et al.*,[13] respectively. The non-enzymic antioxidants reduced glutathione (GSH), vitamin C and vitamin E were estimated by the methods of Ellman,[14] Roe and Kuether,[15] and Baker *et al.*,[16] respectively. Total lipids were extracted from the liver and kidney tissues according to the method of Folch *et al.*[17] Total cholesterol (TC) was estimated by the method of Allain *et al.*[18] High density lipoprotein-C (HDL-C) was estimated by the method of Izzo *et al.* [19] Very low density lipoprotein-C (VLDL-C)

and low density lipoprotein-C (LDL-C) were calculated by the method of Friedewald *et al*.[20] Triglycerides (TG) were estimated by the method of McGowan *et al*.[21] Free fatty acid content was estimated by the method of Falholt *et al*.[22] Phospholipid was estimated by the method of Silversmit and Davis.[23]

Statistical analysis

Values are given as mean ± SD for six rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS v10 (SPSS, Chicago, IL, USA). The limit of statistical significance was set at $P = 0.05$.

Results

Table 1 shows the effect of oral administration of protocatechuic acid on the level of plasma glucose and insulin at three different doses (50, 100, 200 mg/kg body

Table 1 Effect of protocatechuic acid (PCA) on body weight and glucose in normal and STZ-diabetic rats

Groups	Changes in blood glucose (mg/dl) Day 0	After 45 days	Insulin (μU/ml)
Control	82.93 ± 3.52[a]	83.39 ± 6.23[a]	15.48 ± 1.66[a]
Diabetic control	245.04 ± 15.87[b,c]	265.42 ± 16.94[b]	6.44 ± 0.57[b]
Diabetes + PCA (100 mg/kg body weight)	238.15 ± 10.05[b]	108.33 ± 8.06[d]	11.76 ± 0.82[d,e]
Diabetes + glibenclamide (600 μg/kg body weight)	247.17 ± 14.86[b,c]	93.99 ± 6.55[f]	13.66 ± 1.06[a,e]

Values are mean ± SD of six rats.
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 2 Effect of protocatechuic acid on TBARS and lipid hydroperoxides in the plasma and tissues of STZ-diabetic and control rats

Groups	Plasma (mmole/dl)		Liver (mmole/100 g wet tissue)		Kidney (mmole/100 g wet tissue)	
	TBARS	Lipid hydro-peroxides	TBARS	Lipid hydro-peroxides	TBARS	Lipid hydro-peroxides
Control	0.133 ± 0.01[a]	9.77 ± 0.59[a]	0.56 ± 0.03[a]	82.66 ± 6.32[a,c]	1.73 ± 0.07[a]	62.24 ± 5.07[a]
Control + PCA (200 mg/kg body weight)	0.121 ± 0.01[a]	9.69 ± 0.91[a]	0.52 ± 0.03[a]	80.83 ± 7.42[c]	1.66 ± 0.08[a]	60.55 ± 4.44[a]
Diabetic control	0.381 ± 0.03[b]	25.98 ± 1.72[b]	3.07 ± 0.26[b]	110.90 ± 10.91[b]	3.75 ± 0.24[b]	159.09 ± 10.54[b]
Diabetes + PCA (50 mg/kg body weight)	0.281 ± 0.02[c]	20.16 ± 1.19[c]	2.69 ± 0.17[c]	93.82 ± 5.42[d]	3.26 ± 0.27[c]	122.66 ± 7.77[c]
Diabetes + PCA (100 mg/kg body weight)	0.158 ± 0.01[d]	12.61 ± 0.71[d]	1.14 ± 0.11[d]	89.14 ± 3.81[a,c]	2.04 ± 0.10[d]	99.02 ± 7.88[d]
Diabetes + PCA (200 mg/kg body weight)	0.232 ± 0.01[e]	15.49 ± 1.63[e]	2.04 ± 0.17[e]	90.90 ± 8.44[a,d]	2.61 ± 0.18[e]	113.39 ± 10.40[e]
Diabetes + glibenclamide (600 μg /kg body weight)	0.138 ± 0.01[a]	10.13 ± 0.54[a]	0.94 ± 0.06[f]	84.42 ± 5.06[a,c]	1.85 ± 0.10[a]	85.98 ± 6.75[f]

Values are mean ± SD of six rats from each group.
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 3 Effect of protocatechuic acid on vitamin C, vitamin E and GSH in the plasma, liver and kidney of STZ-diabetic and control rats

Groups	Vitamin C (mg/dl)	Vitamin E (mg/dl)	GSH		
			Plasma (mg/dl)	Liver (mg/100 g of tissue)	Kidney (mg/100 g of tissue)
Control	1.87 ± 0.05[a]	1.60 ± 0.078[a]	39.50 ± 2.32[a]	121.06 ± 9.99[a,c]	117.50 ± 11.05[a]
Control + PCA (200 mg/kg body weight)	1.92 ± 0.06[a]	1.50 ± 0.15[a]	40.70 ± 2.57[a]	127.29 ± 9.06[c]	122.13 ± 10.02[a]
Diabetic control	0.77 ± 0.03[b]	3.74 ± 0.26[b]	19.73 ± 0.81[b]	73.77 ± 7.28[b]	74.31 ± 6.24[b]
Diabetes + PCA (50 mg/kg body weight)	0.95 ± 0.04[c]	3.12 ± 0.29[c]	22.82 ± 1.93[c]	93.33 ± 5.83[d]	87.46 ± 5.56[c]
Diabetes + PCA (100 mg/kg body weight)	1.13 ± 0.04[d]	2.01 ± 0.20[d]	30.15 ± 2.42[d]	108.80 ± 10.07[e]	99.02 ± 7.49[d,e]
Diabetes + PCA (200 mg/kg body weight)	1.05 ± 0.08[e]	3.23 ± 0.30[c]	27.62 ± 1.57[e]	97.06 ± 8.29[d]	93.33 ± 7.29[c,d]
Diabetes + glibenclamide (600 μg/kg body weight)	1.28 ± 0.05[f]	1.76 ± 0.10[a,d]	35.20 ± 2.10[f]	113.42 ± 9.34[a,e]	104.89 ± 8.11[e]

Values are mean ± SD of six rats from each group.
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

weight) in normal and STZ-induced diabetic rats. Plasma glucose level increased in diabetic rats and administration of protocatechuic acid significantly decreased the plasma glucose level. Plasma insulin level was decreased in diabetic rats whereas treatment with protocatechuic acid shows an increase in the level of plasma insulin.

Table 2 shows the effect of protocatechuic acid on TBARS and lipid hydroperoxides in the plasma and tissues of control and STZ-diabetic rats. The levels of TBARS and hydroperoxides in the plasma and tissues increased in STZ diabetic rats, and administration of protocatechuic acid and glibenclamide showed a significant decrease in the levels of TBARS and hydroperoxides.

Table 3 shows the effect of protocatechuic acid on vitamin C and vitamin E in the plasma and GSH in the plasma, liver and kidney of control and STZ-diabetic animals. In diabetic rats, the level of vitamin E increased significantly while vitamin C decreased in the plasma. Oral administration of protocatechuic acid and glibenclamide decreased vitamin E and increased vitamin C levels. The levels of GSH in the plasma and tissues decreased in diabetic rats, and the levels increased significantly in protocatechuic acid and glibenclamide treated groups.

Tables 4 and 5 show the effect of protocatechuic acid on enzymatic antioxidant activities in the erythrocytes and tissues of control and STZ-diabetic rats. The activities of superoxide dismutase, catalase and glutathione peroxidase significantly decreased in the erythrocytes and tissues of diabetic rats. protocatechuic acid and glibenclamide administration resulted in a significant increase in the activities of enzymatic antioxidants in the erythrocytes and tissues of diabetic rats.

Table 6 shows the effect of protocatechuic acid on the plasma lipid profiles of control and STZ-diabetic rats. A significant elevation of plasma TC, LDL-C, VLDL-C and TG and reduction in HDL-C were observed in diabetic rats. Oral administration of protocatechuic acid

Table 4 Effect of protocatechuic acid on enzymatic antioxidant activities in the erythrocytes of STZ-diabetic and control rats

Groups	SOD (U*/mg Hb)	CAT (U#/mg Hb)	GPx (U+/mg Hb)
Control	10.15 ± 0.50[a]	149.95 ± 4.26[a]	14.27 ± 1.17[a]
Control + PCA (200 mg/kg body weight)	10.31 ± 0.51[a]	153.03 ± 6.38[a]	14.76 ± 1.35[a]
Diabetic control	3.59 ± 0.27[b]	76.61 ± 5.21[b]	6.33 ± 0.55[b]
Diabetes + PCA (50 mg/kg body weight)	5.69 ± 0.39[c]	107.87 ± 6.41[c]	9.47 ± 0.65[c]
Diabetes + PCA (100 mg/kg body weight)	7.85 ± 0.56[d,e]	132.25 ± 12.63[d]	11.72 ± 0.61[d,f]
Diabetes + PCA (200 mg/kg body weight)	7.28 ± 0.38[d]	120.11 ± 9.32[e]	11.03 ± 0.66[d]
Diabetes + glibenclamide (600 μg/kg body weight)	8.47 ± 0.59[e]	142.69 ± 8.66[a]	12.35 ± 0.78[f]

U*, enzyme concentration required to inhibit the NBT to 50% reduction in 1 min.
U#, μmole of H_2O_2 consumed/min.
U+, μg of GSH utilized/min.
Values are mean ± SD of six rats from each group.
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 5 Effect of protocatechuic acid on enzymatic antioxidant activities in the liver and kidney of STZ-diabetic and control rats

Groups	SOD (U*/mg protein)		CAT (U#/mg protein)		GPx (U+/mg protein)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	7.25 ± 0.29[a]	16.40 ± 1.47[a]	72.84 ± 6.39[a]	36.34 ± 1.80[a]	8.96 ± 0.41[a]	8.48 ± 0.76[a]
Control + PCA (200 mg/kg body weight)	7.23 ± 0.39[a]	16.78 ± 0.74[a]	71.25 ± 3.78[a,e]	33.37 ± 2.58[c]	8.78 ± 0.57[a]	8.65 ± 0.65[a]
Diabetic control	4.61 ± 0.43[b]	6.86 ± 0.52[b]	40.35 ± 4.09[b]	19.76 ± 1.06[b]	6.55 ± 0.62[b]	4.48 ± 0.29[b]
Diabetes + PCA (50 mg/kg body weight)	5.60 ± 0.38[c]	12.89 ± 1.07[c]	59.48 ± 3.87[c]	25.29 ± 2.49[d]	7.60 ± 0.71[c]	6.80 ± 0.44[c]
Diabetes + PCA (100 mg/kg body weight)	6.60 ± 0.59[d]	15.62 ± 0.76[a]	65.88 ± 5.73[d,e]	30.02 ± 2.52[e]	8.52 ± 0.66[a,d]	7.56 ± 0.33[d]
Diabetes + PCA (200 mg/kg body weight)	6.14 ±0.56[c,d]	13.94 ± 0.99[c]	60.75 ± 5.55[c,d]	28.69 ± 2.50[e]	7.83 ± 0.46[c,d]	6.86 ± 0.48[c]
Diabetes + glibenclamide (600 μg/kg body weight)	7.53 ± 0.66[a]	16.39 ± 1.47[a]	68.33 ± 4.38[a,e]	34.02 ± 2.52[c]	8.43 ± 0.70[a,d]	7.86 ± 0.34[d]

U*, enzyme concentration required to inhibit the NBT to 50% reduction in 1 min.
U#, μmole of H_2O_2 consumed/min.
U+, μg of GSH utilized/min.
Values are mean ± SD of six rats from each group.

Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 6 Effect of protocatechuic acid on lipid profiles of plasma in the STZ-diabetic and control rats

Groups	Plasma (mg/dl)				
	Total cholesterol	HDL-C	LDL-C	VLDL-C	Triglycerides
Control	85.76 ± 1.78^{a,f}	45.54 ± 2.47^{a}	28.77 ± 2.86^{a}	10.77 ± 0.76^{a}	54.88 ± 3.56^{a}
Control + PCA (200 mg/kg body weight)	80.33 ± 1.93^{a}	46.37 ± 2.56^{a}	23.81 ± 2.64^{a}	10.14 ±0.84^{a}	50.72 ± 4.23^{a}
Diabetic control	160.68 ± 7.64^{b}	24.73 ± 1.16^{b}	108.79 ± 6.91^{b}	27.16 ± 2.10^{b}	135.80 ± 10.52^{b}
Diabetes + PCA (50 mg/kg body weight)	143.17 ± 7.26^{c}	33.16 ± 1.61^{c}	86.44 ± 7.36^{c}	23.56 ± 1.07^{c}	117.81 ± 5.37^{c}
Diabetes + PCA (100 mg/kg body weight)	112.33 ± 3.59^{d}	38.35 ± 1.52^{d}	60.94 ± 4.55^{d}	13.04 ± 0.69^{d}	65.24 ± 3.49^{d}
Diabetes + PCA (200 mg/kg body weight)	129.87 ± 7.29^{e}	34.71 ± 2.73^{c}	77.90 ± 8.00^{e}	17.26 ± 1.48^{e}	86.30 ± 7.40^{e}
Diabetes + glibenclamide (600 μg/kg body weight)	91.60 ± 1.52^{f}	42.52 ± 1.57^{e}	36.45 ± 2.19^{f}	12.59 ± 1.38^{d}	62.97 ± 4.12^{d}

Values are mean ± SD of six rats from each group.
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 7 Effect of protocatechuic acid on lipid profiles of liver in the STZ-diabetic and control rats

Groups	Liver (mg/g of tissue)			
	Total cholesterol	Triglycerides	Phospholipids	Free fatty acids
Control	4.74 ± 0.40^{a}	3.69 ± 0.20^{a,f}	22.39 ± 2.26^{a}	7.64 ± 0.39^{a}
Control + PCA (200 mg/kg body weight)	4.65 ± 0.38^{a}	3.40 ± 0.24^{a}	20.26 ± 1.50^{a}	7.86 ± 0.63^{a}
Diabetic control	7.25 ± 0.30^{b}	7.10 ± 0.26^{b}	55.29 ± 3.73^{b}	17.06 ± 1.34^{b}
Diabetes + PCA (50 mg/kg body weight)	6.41 ± 0.38^{c}	5.21 ± 0.33^{c}	47.55 ± 2.89^{c}	14.18 ± 1.06^{c}
Diabetes + PCA (100 mg/kg body weight)	5.22 ± 0.36^{d}	4.15 ± 0.26^{d}	32.83 ± 3.04^{d}	10.34 ± 0.93^{d}
Diabetes + PCA (200 mg/kg body weight)	5.85 ± 0.20^{e}	4.77 ± 0.23^{e}	42.13 ± 3.33^{e}	11.38 ± 1.21^{d}
Diabetes + glibenclamide (600 μg/kg body weight)	4.87 ± 0.30^{a,d}	3.89 ± 0.33^{f}	29.95 ± 1.76^{d}	8.22 ± 0.72^{a}

Values are mean ± SD of six rats from each group.
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 8 Effect of protocatechuic acid on lipid profiles in the kidney of STZ-diabetic and control rats

Groups	Kidney (mg/g of tissue)			
	Total cholesterol	Triglycerides	Phospholipids	Free fatty acids
Control	5.40 ± 0.24^{a}	6.74 ± 0.51^{a}	13.68 ± 1.45^{a,f}	6.14 ± 0.58^{a}
Control + PCA (200 mg/kg body weight)	4.40 ± 0.32^{c}	6.29 ± 0.47^{a}	12.35 ± 1.03^{a}	6.04 ± 0.40^{a}
Diabetic control	10.73 ± 0.64^{b}	16.67 ± 1.27^{b}	35.49 ± 2.83^{b}	17.95 ± 1.73^{b}
Diabetes + PCA (50 mg/kg body weight)	9.38 ± 0.48^{d}	12.83 ± 0.90^{c}	24.97 ± 1.32^{c}	13.58 ± 1.27^{c}
Diabetes + PCA (100 mg/kg body weight)	7.25 ± 0.32^{e}	8.64 ± 0.58^{d}	16.79 ± 0.99^{d}	10.58 ± 0.38^{d}
Diabetes + PCA (200 mg/kg body weight)	8.80 ± 0.59^{f}	10.31 ± 0.51^{e}	21.15 ± 1.29^{e}	11.24 ± 1.06^{d}
Diabetes + glibenclamide (600 μg /kg body weight)	6.78 ± 0.27^{e}	7.94 ± 0.56^{d}	14.57 ± 1.19^{f}	7.52 ± 0.35^{e}

Values are mean ± SD of six rats from each group.
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

significantly decreased the levels of TC, LDL-C, VLDL-C and TG, while significant elevation in HDL-C level was observed in diabetic rats.

Tables 7 and 8 show the effect of protocatechuic acid on tissue lipid profiles in the control and STZ-diabetic rats. The levels of total cholesterol, triglycerides, phospholipids, free fatty acids increased significantly in the tissues of diabetic rats. Oral administration of protocatechuic acid and glibenclamide significantly decreased these parameters in diabetic rats.

Figures 2 and 3 show histopathological studies on the effect of protocatechuic acid in the liver and kidney of STZ-diabetic rats. In diabetic rats, liver and kidney show abnormal conditions, while the diabetic rats treated with protocatechuic acid brought the liver and kidney to near normal levels.

Discussion

Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms.[24] Hence, in the present study, we observed an increase in the level of plasma glucose and decrease in the level of insulin. The decrease in the plasma glucose in diabetic rats treated with protocatechuic acid might be due to elevated secretion of insulin from the existing β-cells, which, in turn, increases the utilization of glucose by the tissues. However, the standard drug glibenclamide showed a better reduction of blood glucose than protocatechuic acid.

Free radicals may play an important role in the causation and complications of diabetes mellitus.[25] The increased free radicals produced may react with polyunsaturated fatty acids in cell membrane leading to lipid peroxidation, and it will, in turn, result in elevated free radical production. Free radicals react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation.[26] In our study, the lipid peroxidation markers (TBARS and HP) were elevated in diabetic rats as reported earlier.[27] The increase in

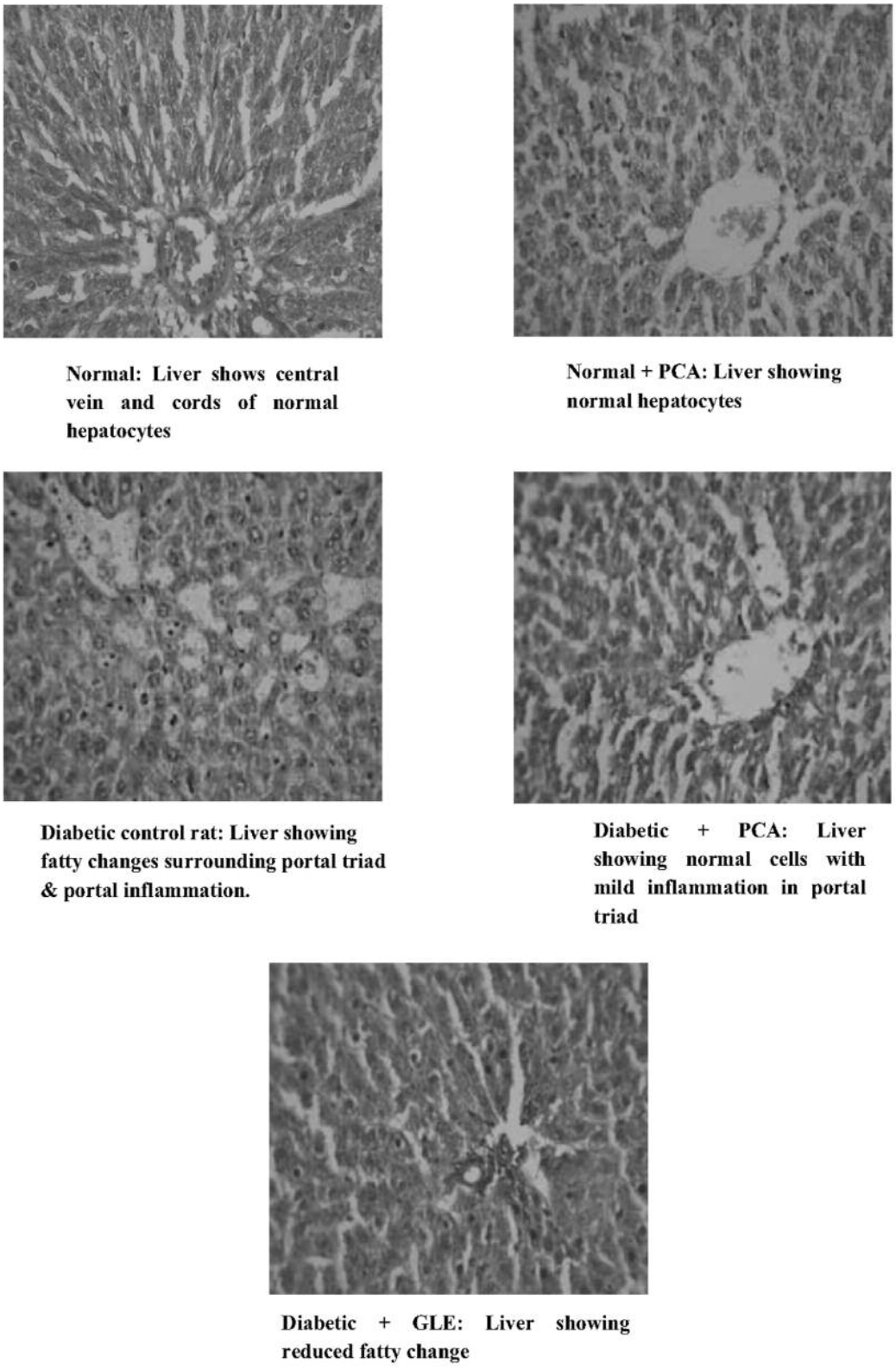
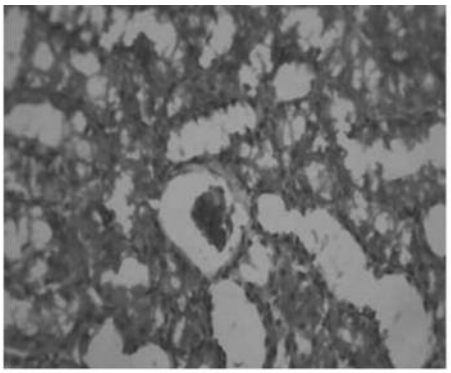
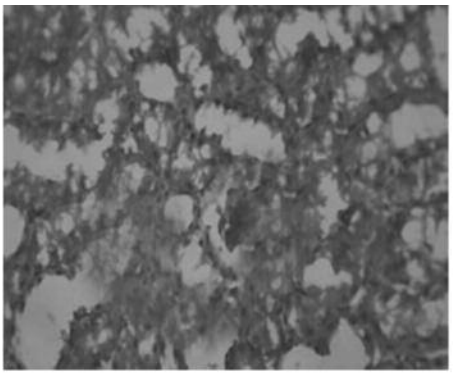


Figure 2 Histopathology – liver (haematoxylin & eosin ×40)

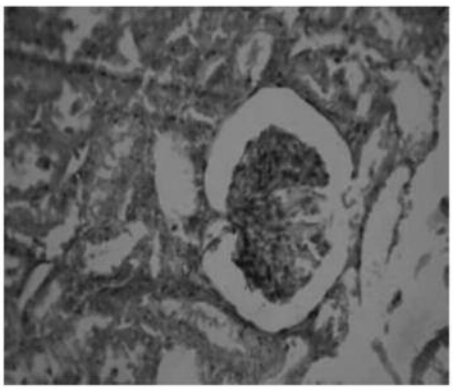
lipid peroxidation might be a reflection of a decrease in enzymatic and non-enzymatic antioxidants of defence systems.[28] Treatment with protocatechuic acid and glibenclamide brought back lipid peroxidation markers to near normal levels which could be as a result of improved glycaemic control.



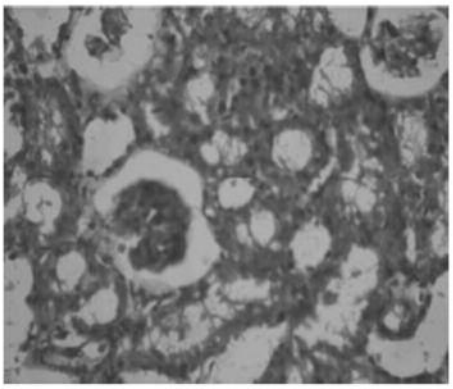
Normal rat: Kidney shows normal glomeruli and tubules.



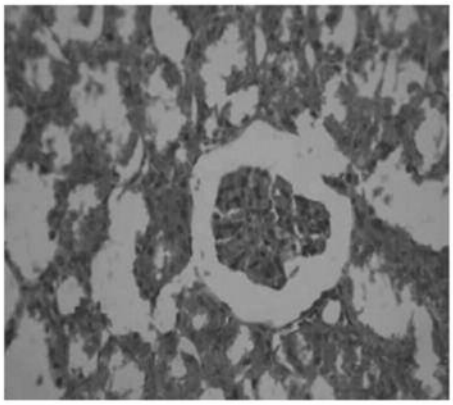
Normal + PCA: Kidney shows normal glomeruli & tubules



Diabetic rat: Kidney shows messangial capillary proliferation of glomeruli and fatty infiltration of



Diabetic + PCA: Kidney shows mild fatty infiltration and mild dilation of tubules



Diabetic + GLE: Kidney shows normal tubules with congested glomeruli.

Figure 3 Histopathology – kidney (haematoxylin & eosin ×40)

Endogenous antioxidant enzymes (SOD, CAT and GPx) are involved in the detoxification of deleterious oxygen radicals.[29] In our study, the activities of SOD, CAT and GPx decreased in diabetic rats as reported earlier,[30] which could be due to increased utilization for scavenging free radicals. Treatment with protocatechuic acid and glibenclamide has brought towards normality the activities of these enzymatic antioxidants, which could be a result of decreased lipid peroxidation and/or decreased utilization of antioxidants. Moreover, protocatechuic acid has good antioxidant activity as reported earlier.[31]

Apart from the enzymatic antioxidants, non-enzymatic antioxidants such as vitamin C, vitamin E and GSH play an excellent role in preventing the cells from oxidative threats. Vitamin C is a hydrophilic antioxidant and disappears faster than other antioxidants on exposure to reactive oxygen species. The decreased level of ascorbic acid in diabetic rats may be due to increased utilization of antioxidants against increased reactive oxygen species. The reduction in vitamin C levels may also due to decrease in glutathione level, since glutathione is required for the recycling of ascorbic acid.[32] In our study, vitamin C levels decreased in diabetic rats as reported earlier.[33] Treatment with protocatechuic acid and glibenclamide brought vitamin C to near normal levels which could be due to decreased utilization.

Vitamin E is used in combating free radicals and if vitamin C is present, vitamin E levels are preserved. So, the increase may also be due to decreased levels of vitamin C or the storage of vitamin E by diabetic rats.[34] In our study, vitamin E was increased in diabetic rats as reported earlier.[35] Treatment with protocatechuic acid and glibenclamide brought vitamin E to near normal levels which could be as a result of decreased membrane damage as evidenced by decreased lipid peroxidation.

GSH functions as a free radical scavenger and is involved in the repair of free radical caused biological damage.[36] GSH is required for the recycling of vitamin C[37] and acts as a substrate for GPx both of which are involved in preventing the deleterious effect of oxygen radicals.[38] The observed decrease in the concentration of GSH in the diabetic condition is consistent with an earlier report.[39] Treatment with protocatechuic acid and glibenclamide increased GSH levels in the plasma and tissues of diabetic rats which could be due to decreased lipid peroxidation.

Diabetes is associated with profound alterations in the plasma lipid, triglycerides and lipoprotein profile and with an increased risk of coronary heart disease.[40] Lowering the plasma lipid levels through dietary or drug therapy appears to be associated with a decrease in the risk of vascular disease.[41] In the present study, we observed higher levels of cholesterol in the plasma and tissues of diabetic rats. Administration of protocatechuic acid to diabetic rats decreased the levels of cholesterol. Normally, circulating LDL-C undergoes re-uptake in the liver via specific receptors and is cleared from the circulation.[42] This increased LDL-C concentration in the plasma of diabetic rats might be due to the defect in LDL-C receptor either through failure in its production and/or function. A greater increase of LDL-C and VLDL-C may also cause a greater decrease of HDL-C as there is a reciprocal relationship between the concentration of LDL-C and HDL-C. Decreased HDL-C may also be due to diminished lecithin cholesterol acyl transferase activity. In our study, the diabetic rats treated with protocatechuic acid showed an elevation in HDL-C and reduction in LDL-C and VLDL-C. Thus, protocatechuic acid could alleviate the risk of cardiovascular diseases. Hypertriglyceridaemia is a common finding in patients with diabetes mellitus and is responsible for vascular complications.[43] Braun and Severson[44] have reported that deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of triglycerides in diabetes. Lopez-Virella *et al*.[45] reported that treatment of diabetes with insulin served to lower plasma triglyceride levels by returning lipoprotein lipase activity to normal. Thus decreased TG level following protocatechuic acid treatment might be due to the increased insulin secretion, which, in turn, increases lipoprotein lipase activity. The abnormal high concentration of serum lipids in diabetic subjects is mainly due to the increase in the mobilization of free fatty acids from fat deposits,[46] since insulin is required for the inhibition of hormone-sensitive lipase. Diabetic rats treated with protocatechuic acid had decreased free fatty acid, which might be due to the increased insulin secretion which, in turn, inhibits hormone-sensitive lipase. Phospholipids are vital components of biomembranes rich in poly-unsaturated fatty acids, which are susceptible substrates for free radicals, such as $O_2^{\bullet -}$ and $HO^{\bullet}$ radicals. These phospholipids are important for the maintenance of cellular integrity, microviscosity and survival. The level of phospholipids increased in diabetic rats, which decreased on treatment with protocatechuic acid. Of the three different doses used, the lower dose of protocatechuic acid (50 mg/kg body weight) was ineffective, because its concentration might not have been high enough to counteract the free radicals generated by STZ. The higher concentration of protocatechuic acid (200 mg/kg body weight) might have resulted in the production of by-

products that are interfering with the antioxidant activity and, consequently, decreasing its effect. Hence, 100 mg/kg body weight protocatechuic acid seems to be optimum for quenching free radicals.

Histopathological examination of diabetic liver showed fatty changes surrounding portal triad and portal inflammation. The treatment with protocatechuic acid showed normal hepatocytes with mild inflammation in the portal triad, whereas glibenclamide treated rats showed normal hepatocytes.

Histopathological studies of kidney showed mesangial capillary proliferation of glomeruli and fatty infiltration of tubules in the diabetic rats, which may be associated with membrane damage, caused by hyperglycaemia mediated-oxidative stress and altered fatty acid composition. Treatment with protocatechuic acid showed mild fatty infiltration, mild dilatation of tubules and glibenclamide-treated rats showed normal tubules with congested glomeruli. Thus, treatment with protocatechuic acid and glibenclamide brought the changes in diabetic-treated rats to near normality, which could be associated with decreased membrane damage as evidenced by improved glycaemic control.

Conclusions

This study shows that protocatechuic acid is having antioxidant and antihyperlipidaemic effects on diabetic rats, and the effect of protocatechuic acid is comparable to the standard drug, glibenclamide. Further studies on the metabolism and pharmacokinetics of protocatechuic acid are warranted.

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