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FREE RADICAL SCAVENGING ACTIVITY OF LEAF AND CALLUS EXTRACT OF *PERGULARIA DAEMIA* (FORSSK.) CHIOV. IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

The GPx activity was significantly lower in diabetic rats treated with the various leaf extract of *P. daemia* ($P< 0.05$) compared to control diabetic rats. GSH concentration was significantly lower in diabetic control rats compared to the treated diabetic and normal rats ($P< 0.05$). The GST activity was significantly lower in diabetic rats treated with the various leaf extract of *P. daemia* ($P< 0.05$) compared to control diabetic rats. The activities of SOD, CAT, and TBARS were significantly lower in diabetic rats treated with leaf extracts compared to control diabetic rats ($P< 0.05$). The HPx level was significantly higher in diabetic control rats compared to the treated diabetic and normal rats ($P< 0.05$). There was no significant difference among the normal and control groups. A significantly elevated level was observed in diabetic control rats and those treated with ethanol callus extract when compared with diabetic rats treated with the ethanolic, aqueous, chloroform leaf extract and the normal groups. However, the enzymatic antioxidant in liver of diabetic rats treated with the ethanolic callus extract was higher than that of other extracts.

Keywords: *P. daemia*, liver, chloroform.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease which may be suspected or recognized clinically by the onset of one or more of the characteristic symptoms such as polyuria, polydipsia, polyphagia and unsolved weight loss [1, 2]. The high concentration of blood glucose and other biochemical abnormalities result from a deficiency of β-cells of the endocrine

pancreas and/or from a sub sensitivity to insulin in target cells [3, 4]. A worldwide survey reported that the estimated incidence of diabetes and projection for year 2030 is 350 million [5, 6]. The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered [7]. Defects in carbohydrate machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism pose an over exertion on the endocrine system leading to the deterioration of endocrine control [8]. Continuing deterioration of endocrine control exacerbates the metabolic disturbances by altering carbohydrate metabolic enzymes and leads primarily to hyperglycemia [9, 10].

Pergularia daemia is said to have more magical application than medical application as it posses diverse healing potential for a wide range of illnesses. Some of the Folklore people use this plant to treat Jaundice, expectorants and also in infantile diarrhea. The leaf latex is locally used as pain killers and for relief from toothache. The sap expressed from the leaves are held to cure sore eyes in Ghana. The plant reduces the incidence of convulsion and asthma. It is used to regulate the menstrual cycle and intestinal functions. The root is useful in treating leprosy, mental disorders, anemia and piles [11]. The present study was undertaken to study the effect of differeny plant leaf extract of *P. daemia* plant on antioxidant enzymes in liver of streptozotocin induced diabetic rats.

MATERIAL AND METHODS

EXPLANT COLLECTION

Pergularia daemia was collected from the Southern parts of Pudukkottai district, Tamil Nadu, India, and planted in the J.J. College Botanical Garden. The plants were raised in pots containing mixture of soil and farmyard manure in the ratio of 1:1. Small disease free tender leaf were collected from 5-6 months grown plants, cut into 0.5-1.0 cm segments and used as explants for callus induction.

MEDIA PREPARATION, CALLUS INDUCTION AND ITS PROLIFERATION

Murashige and Skoog medium [12] supplemented with auxins viz., 2,4-D, NAA, IAA and cytokinins viz., BAP and Kin alone at different concentrations was used for callus initation. The cultures were incubated under fluorescent lights with 1500-2000 lux for 16 h at 25±1°C and 80±10 relative humidity. Each experiment had 20 replicates and was repeated thrice. The proliferated callus culture were sub cultured and maintained for 45-50 days on the same medium supplemented with the same growth regulator.

PREPARATION OF EXTRACTS

The leaves and calli were dried under shade, coarsely powdered, and extracted with chloroform (60-80°C) followed by alcohol, and then water using Soxhlet apparatus. The extracts so collected were distilled off on a Water bath at atmospheric pressure and the last traces of the solvents were removed in vacuo [13].

ANIMALS

In the present study healthy, matured male albino rats (wistar strain) were used. Rats weighing 180-230g were obtained from the Periyar College of Pharmaceutical Sciences, Tiruchirapalli, Tamil Nadu, India and kept in plastic animal cages with 12 h light and dark cycle in the institutional animal house. The animals were fed with standard rodent diet and provided water *ad libitum*. After one week of acclimatization the animals were used for the further experiments. Approval from the Institutional Animal Ethical Committee for the usage of animals in the experiments was obtained as per the Indian CPCSEA guidelines.

CHEMICALS

Streptozotocin (STZ) and glibenclamide were purchased from Sigma Aldrich, St. Louis, MO, USA. All other chemicals and solvents used were of Analytical Grade obtained from E-Merck and Himedia, Mumbai, India.

ACUTE TOXICITY STUDY

Acute toxicity studies were carried out using Acute Toxic Class Method as per OECD-423 Guideliness [14]. Chloroform leaf extract, ethanol leaf extract, aqueous leaf extract and ethanol leaf callus extract of *P. daema* were administered at a starting dose of 2000 mg/kg b.w of orally to 4 male rats. The animals were observed for mortality and behavioral changes during 48 h.

EXPERIMENTAL INDUCTION OF DIABETES

Diabetes was induced in a group of rats after an overnight fast by single intraperitoneal injection of STZ, which was freshly dissolved in 0.1M citrate buffer (pH 4.5). The dose was 40 mg/kg b.w. STZ treated animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. After 48 h of STZ administration, the blood glucose ranges above 200-300 mg/dl was considered as diabetic rats and used for the experiment.

EXPERIMENTAL DESIGN

In the experiment, a total of 162 rats were used, randomly divided into 27 groups of 6 animals each and treatments continued in an aqueous solution daily using an intragastric tube for 21 days.

Group-I	:	Normal rats received 3% gum acacia
Group-II, III, IV	:	Leaf chloroform extract (100, 200, 300 mg/kg b.w.)
Group-V, VI, VII	:	Leaf ethanol extract (100, 200, 300 mg/kg b.w.)
Group-VIII, IX, X	:	Leaf aqueous extract (100, 200, 300 mg/kg b.w.)
Group-XI, XII, XIII	:	Leaf ethanol callus extract (100, 200, 300 mg/kg b.w.)
Group-XIV	:	Streptozotocin (STZ) 40 mg/kg b.w. (Diabetic control)
Group-XV, XVI, XVII	:	STZ+Leaf chloroform extract (100, 200, 300 mg/kg b.w.)
Group-XVIII, XIX, XX	:	STZ+Leaf ethanol extract (100, 200, 300 mg/kg b.w.)
Group-XXI, XXII, XXIII	:	STZ+Leaf aqueous extract (100, 200, 300 mg/kg b.w.)
Group-XXIV, XXV, XXVI	:	STZ+Leaf ethanol callus extract (100, 200, 300 mg/kg b.w.)
Group-XXVII	:	STZ+Glibenclamide (600 µg/kg b.w.)

BIOCHEMICAL ASSAYS

After the termination of the experiment, all the animals were anaesthetized using ketamine chloride (24 mg/kg b.w.) and sacrificed by cervical dislocation after an overnight fast. Blood was collected and tissues (liver) were immediately removed, blotted and kept at -20°C until use. After the separation of plasma, the buffy coat was removed and the packed erythrocytes were washed thrice with cold physiological saline. A known volume of the erythrocyte was lysed with cold hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 2000 rpm for 10 min and the supernatant was used for antioxidant activity. Glutathione peroxidase was measured by the method of Rotruck *et al.* [15] reduced glutathione was estimated by the method of Ellman [16], glutathione-S-transferase was determined by the method of Habig *et al.* [17], superoxide dismutase assayed by the method of Kakkar *et al.* [18], catalase was determined by the method of Sinha [19], hydroperoxides were estimated by the method of Jiang *et al.* [20], thiobarbituric acid reactive substances was estimated by the method of Niehaus and Samuelson [21].

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS Software Package, version 11.5. The values were analyzed by One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple

Range Test (DMRT). All these results were expressed as mean±SD for six rats in each group, p-values <0.05 were considered as significant [22].

RESULTS AND DISCUSSION

Callus was initiated from stem and leaf explants of *P. daemia* on basal MS medium supplemented with 2,4-D, α-NAA, BAP, KIN and IBA at different concentrations 1.0 - 3.0 mg/l. The maximum induction rate was observed at 2,4-D 2.0 mg/l combination with 0.5mg/l BAP and 1.0 mg/l α-NAA.

The acute toxicity study revealed the non-toxic nature of the chloroform, ethanol, aqueous and ethanol callus extracts at the tested concentrations. No lethal toxic reactions were observed until the end of the experiment.

The glutathione peroxidase (GPx) levels were found significantly lower in the liver of diabetic animals (Table 1). It was 3.49 (µmole of glutathione utilized/min). In normal animals it was 9.10 µmole. But the GPx level was higher in diabetic animals treated with 300 mg/kg of all the extracts. The amount of GPx varied from 7.89 µmole to 4.66 µmole. The highest figure was with the ethanol callus extract treated groups (7.89 µmole), with the ethanol extract treated group the figure was 6.05 µmole and 5.15 µmole with the aqueous extract treated groups and 5.57 µmole in the group treated with glibenclamide at the rate of 600 µg/kg. The GPx rate was lowest with the group treated with the chloroform leaf extract (4.66 µmole).

The reduced glutathione (GSH), levels were found significantly lower in the liver of diabetic animals it was 25.12 mg/100mg tissue. With normal animal it was 67.79 mg/100mg tissue. But the GSH levels were higher in diabetic animals treated with 300 mg/kg of all the extracts. The levels were 50.10 mg/100 mg tissue in animals treated chloroform leaf extract, 58.25 mg/100 mg tissue in those treated with ethanol extract and 52.46 mg/100 mg tissue in the aqueous extract, 60.04 mg/100 mg tissue in the ethanol callus extract treated group. In the group treated with glibenclamide at the rate of 600 µg/kg it was 59.09 mg/100 mg tissue (Table 1).

Table 1 Changes in the level of glutathione peroxidase (GPx) and reduced glutathione (GSH) in liver of normal and experimental animals

Control & Treatment		Groups	Glutathione peroxidase (µmole of glutathione utilized/min)	Reduced glutathione (mg/100 mg tissue)
Normal		I	9.10±0.41	67.79±2.95
Chloroform	100 mg/kg	II	7.05±0.31	67.45±1.48
	200 mg/kg	III	8.65±0.37	67.14±1.36
	300 mg/kg	1V	7.30±0.28	67.79±1.59
Ethanol	100 mg/kg	V	7.15±0.49	67. 79± 2.61
	200 mg/kg	VI	7.65±1.39	68.44±2.55
	300 mg/kg	VII	8.35±1.86	66.71±2.83
Aqueous	100 mg/kg	VIII	7.12±0.12	68.01±2.42
	200 mg/kg	IX	7.94±0.95	67.24±1.86
	300 mg/kg	X	8.87±1.15	67.78±1.55
Ethanol callus	100 mg/kg	XI	7.47±1.05	66.74±1.19
	200 mg/kg	XII	7.80±1.90	66.16±1.13
	300 mg/kg	XIII	7.16±1.41	67.21±1.65
Diabetic (STZ)	40 mg/kg	XIV	3.49±0.12*	25.12±2.98*
STZ + Chloroform	100 mg/kg	XV	4.05±0.51#	45.65±0.75#
	200 mg/kg	XVI	4.52±0.56#	48.91±0.25#
	300 mg/kg	XVII	4.66±0.59#	50.10±0.13#
STZ + Ethanol	100 mg/kg	XVIII	5.21±0.24#	57.32±1.35#
	200 mg/kg	XIX	5.51±0.65#	57.88±1.41#
	300 mg/kg	XX	6.05±0.21#	58.25±3.14#
STZ + Aqueous	100 mg/kg	XXI	4.75±0.61#	50.47±1.47#
	200 mg/kg	XXII	5.11±0.31#	52.33±0.12#
	300 mg/kg	XXIII	5.15±0.33#	52.46±0.10#
STZ + Ethanol callus	100 mg/kg	XXIV	6.13±0.99#	58.98±2.25#
	200 mg/kg	XXV	7.45±0.16#	59.40±2.23#
	300 mg/kg	XXVI	7.89±0.51#	60.04±1.19#
STZ + Glibenclamide	600 µg/kg	XXVII	5.57±0.19#	59.09±1.01#

Values are expressed as mean±SD (n=6).

Diabetic control is compared with normal; *Values are statistically significant at P^{*} <0.05 compared to normal.

Treated groups are compared with diabetic control; # Values are statistically significant at $P^{\#}$ <0.05 compared to diabetic control.

The glutathione-S-transferase (GST), levels were found significantly lower in the liver from diabetic animals. It was 1.77 (µmole of CDNB-GSH conjugate formed/min/mg/protein). With normal animals it was 7.97 µmole on 21st day (Table 2). The level of GST is increased

in animals treated with all the three extracts. With chloroform leaf extract treated animals it was 3.01 µmole. The corresponding figure with animals treated with the ethanol extract was 4.55 µmole, with the aqueous extract treated group 3.22 µmole, while with the ethanol callus extract treated groups it was 6.02 µmole. In animals treated with glibenclamide at the rate of 600 µg/kg the corresponding figure was 3.89 µmole of CDNB-GSH conjugate formed/min/mg/protein on the 21st day.

Table 2 Changes in the level of glutathione-S-transferase (GST), superoxide dismutase (SOD) in liver of normal and experimental animals

Control & Treatment		Groups	Glutathione-S-transferase (µmole of CDNB-GSH conjugate formed/min/mg/ protein)	Superoxide dismutase (U_A^A /min/ mg of protein) The amount of enzyme required to inhibit 50% NBT reduction
Normal		I	7.97±0.86	14.84±0.12
Chloroform	100 mg/kg	II	6.81±0.09	12.86±0.17
	200 mg/kg	III	6.95±0.44	11.42±0.04
	300 mg/kg	1V	7.67±0.89	15.44±0.33
Ethanol	100 mg/kg	V	7.78±0.07	17.83±0.11
	200 mg/kg	VI	8.85±0.96	13.69±0.97
	300 mg/kg	VII	6.86±0.86	16.15±0.63
Aqueous	100 mg/kg	VIII	7.82±0.08	15.88±0.21
	200 mg/kg	IX	7.87±0.26	12.46±0.39
	300 mg/kg	X	7.99±0.36	11.76±0.74
Ethanol callus	100 mg/kg	XI	6.12±0.75	12.74±0.14
	200 mg/kg	XII	7.43±0.36	15.16±0.65
	300 mg/kg	XIII	7.85±0.87	12.21±0.13
Diabetic (STZ)	40 mg/kg	XIV	1.77±0.65*	4.99±0.87*
STZ + Chloroform	100 mg/kg	XV	2.97±0.41#	5.04±0.99#
	200 mg/kg	XVI	2.99±0.15#	5.35±0.48#
	300 mg/kg	XVII	3.01±0.16#	5.59±0.62#
STZ + Ethanol	100 mg/kg	XVIII	3.52±0.29#	6.78±0.44#
	200 mg/kg	XIX	4.45±0.39#	7.13±0.52#
	300 mg/kg	XX	4.55±0.32#	7.55±0.03#
STZ + Aqueous	100 mg/kg	XXI	3.12±0.87#	5.87±0.55#
	200 mg/kg	XXII	3.15±0.74#	6.02±0.52#
	300 mg/kg	XXIII	3.22±0.91#	6.16±0.36#
STZ + Ethanol callus	100 mg/kg	XXIV	5.71±0.54#	8.09±0.54#
	200 mg/kg	XXV	5.85±0.13#	8.92±0.17#
	300 mg/kg	XXVI	6.02±0.21#	9.05±0.36#
STZ + Glibenclamide	600 µg/kg	XXVII	3.89±0.12#	7.81±0.09#

The superoxide dismutase (SOD), levels were found lower in liver from diabetic animals. It was 4.99 U^A/mg protein, ($^{A-}$ The amount of enzyme required to inhibit 50% NBT reduction). With normal animals it was14.84 U^A/mg protein on 21st day (Table 24 & fig. 50.1). The levels of SOD increased in animals treated with all the extracts at the rate of 300 mg/kg. In the chloroform leaf extract treated animals it was 5.59 U^A/mg protein; in the groups treated with ethanol extract animals it was 7.55 U^A/mg protein; in the groups treated with aqueous extract it was 6.16 U^A/mg protein. In groups treated with ethanol callus extract it was 9.05 U^A/mg protein and 7.81 U^A/mg protein, in the group treated with glibenclamide at the rate of 600 µg/kg (Table 2).

Table 3 Changes in the level of catalase in liver of normal and experimental animals

Control & Treatment		Groups	Catalase (^{B} (unit ^{B}/mg protein) µmoles of H_2O_2 utilized/min
Normal		I	88.55±5.81
Chloroform	100 mg/kg	II	91.22±4.46
	200 mg/kg	III	86.78±4.34
	300 mg/kg	1V	88.55±5.81
Ethanol	100 mg/kg	V	88.36±3.35
	200 mg/kg	VI	85.96±2.21
	300 mg/kg	VII	89.53±2.23
Aqueous	100 mg/kg	VIII	89.40±2.35
	200 mg/kg	IX	88.61±2.67
	300 mg/kg	X	86.56±2.31
Ethanol callus	100 mg/kg	XI	85.21±2.49
	200 mg/kg	XII	85.67±2.18
	300 mg/kg	XIII	83.21±2.85
Diabetic (STZ)	40 mg/kg	XIV	42.12±1.15*
STZ + Chloroform	100 mg/kg	XV	48.30±1.82$^{\#}$
	200 mg/kg	XVI	51.52±1.35$^{\#}$
	300 mg/kg	XVII	55.58±1.21$^{\#}$
STZ + Ethanol	100 mg/kg	XVIII	64.3±3.71$^{\#}$
	200 mg/kg	XIX	65.47±2.23$^{\#}$
	300 mg/kg	XX	67.80±2.31$^{\#}$
STZ + Aqueous	100 mg/kg	XXI	58.45±1.83$^{\#}$
	200 mg/kg	XXII	60.2±5.04$^{\#}$
	300 mg/kg	XXIII	61.02±2.09$^{\#}$
STZ + Ethanol callus	100 mg/kg	XXIV	69.61±2.41$^{\#}$
	200 mg/kg	XXV	70.02±1.20$^{\#}$
	300 mg/kg	XXVI	79.32±4.39$^{\#}$
STZ + Glibenclamide	600 µg/kg	XXVII	74.11±0.46$^{\#}$

Values are expressed as mean±SD (n=6).

Diabetic control is compared with normal; *Values are statistically significant at P^{*} <0.05 compared to normal.
Treated groups are compared with diabetic control; # Values are statistically significant at $P^{\#}$ <0.05 compared to diabetic control.

The catalase level was found lower in liver from diabetic animals. It was 42.12 $unit^{B}$/mg protein (^{B} - µmoles of H_2O_2 utilized/min). In normal animals the rate was almost more than double 88.55 $unit^{B}$/mg protein on 21st day (Table 3). The levels of catalase increased in leaf extracts treated animals. The figure was 55.58 $unit^{B}$/mg protein in the chloroform leaf extract treated groups. In the ethanol treated group it was 67.80 $unit^{B}$/mg protein; in the aqueous extract treated group it was 61.02 $unit^{B}$/mg protein; whereas in ethanol callus extract treated groups it was as high as 79.32 $unit^{B}$/mg protein. In case of animals treated with glibenclamide 600 µg/kg the corresponding figure 74.11 $unit^{B}$/mg protein.

Values are expressed as mean±SD (n=6).
Diabetic control is compared with normal; *Values are statistically significant at P^{*} <0.05 compared to normal.
Treated groups are compared with diabetic control; # Values are statistically significant at $P^{\#}$ <0.05 compared to diabetic control.

The hydroperoxides (HPx) level were higher in liver from diabetic animals 90.94 µmole/mg of protein. In normal animal the figure was 66.11 µmole/mg of protein. But the hydroperoxides level decreased in diabetic animals treated with 300 mg/kg each of the three extract. It was 85.42 µmole/mg of protein of in the case of animals treated with chloroform leaf extract; in the ethanol extract treated groups it was 79.12; in the aqueous extract treated groups 81.01 and 73.28 in the ethanol callus extract treated groups. In the group treated with glibenclamide at the rate of 600 µg/kg it was 89.17 µmole/mg of protein (Table 4).

The tissue thiobarbituric acid reactive substances (TBARS), level was found higher in liver from diabetic animals. It was 4.25 nmol/mg of protein. With the normal animals it was 1.67 nmol/mg of protein on 21st day (Table 4). The levels of TBARS decreased in the experimental groups. The values were 4.15 nmol/mg of protein in the chloroform leaf extract treated groups; it was 3.28 nmol/mg of protein in the ethanol extract treated groups; in the aqueous extract treated groups it was 3.86 nmol/mg of protein. Where as in the ethanol callus

extract treated groups it was 5.16 nmol/mg of protein. In the glibenclamide treated groups the levels of TBARS was as low as 3.85 nmol/mg of protein.

Table 4 Changes in the level of hydro peroxides (HPx) and tissue thiobarbituric acid reactive substances (TBARS) in liver of normal and experimental animals

Control & Treatment		Groups	Hydro peroxides (µmole/mg of protein)	Tissue thiobarbituric acid reactive substances (nmole/mg of protein)
Normal		I	66.11±4.02	1.67±0.02
Chloroform	100 mg/kg	II	55.18±3.54	1.34±0.05
	200 mg/kg	III	57.8±3.61	1.32±0.04
	300 mg/kg	1V	52.54±3.59	1.62±1.02
Ethanol	100 mg/kg	V	65.97±3.87	1.44±0.18
	200 mg/kg	VI	57.8±4.70	1.36±0.14
	300 mg/kg	VII	58.82±4.20	1.86±0.13
Aqueous	100 mg/kg	VIII	66.3±4.05	1.56±0.10
	200 mg/kg	IX	64.76±3.99	1.75±0.04
	300 mg/kg	X	53.76±4.86	1.86±0.02
Ethanol callus	100 mg/kg	XI	55.40±4.64	1.59±0.32
	200 mg/kg	XII	55.85±4.66	1.15±0.15
	300 mg/kg	XIII	55.37±4.39	1.18±0.26
Diabetic (STZ)	40 mg/kg	XIV	90.94±6.85*	4.25±0.31*
STZ + Chloroform	100 mg/kg	XV	89.17±5.82#	4.18±0.13#
	200 mg/kg	XVI	87.29±5.79#	4.16±0.08#
	300 mg/kg	XVII	85.42±4.32#	4.15±0.25#
STZ + Ethanol	100 mg/kg	XVIII	80.40±5.97#	3.75±0.12#
	200 mg/kg	XIX	79.96±5.86#	3.62 ±0.37#
	300 mg/kg	XX	79.12±5.75#	3.28±0.21#
STZ + Aqueous	100 mg/kg	XXI	83.87±2.04#	4.08±0.33#
	200 mg/kg	XXII	82.05±4.12#	4.01±0.15#
	300 mg/kg	XXIII	81.01±4.55#	3.86±0.19#
STZ + Ethanol callus	100 mg/kg	XXIV	76.09±4.02#	3.18±0.64#
	200 mg/kg	XXV	74.25±5.05#	2.35±0.14#
	300 mg/kg	XXVI	73.28±2.91#	5.16±0.09#
STZ + Glibenclamide	600 µg/kg	XXVII	89.17±3.82#	3.85±0.04#

Values are expressed as mean±SD (n=6).

Diabetic control is compared with normal; *Values are statistically significant at P^{*} <0.05 compared to normal.

Treated groups are compared with diabetic control; # Values are statistically significant at $P^{\#}$ <0.05 compared to diabetic control.

Carbonylation of proteins is a feature of irreversible oxidative damage, often leading to a loss of protein function, which is considered a widespread indicator of severe oxidative damage and disease-derived protein dysfunction. When moderately carbonylated proteins are degraded by the proteasomal system, heavily carbonylated proteins tend to form high molecular weight aggregates which are resistant to degradation and accumulates as damaged or unfolded proteins. STZ-induced oxidative damage in proteins was revealed by the increased content of carbonylated proteins in the tissue [23]. The treatment of STZ-injected animals with *P. daemia* extract lowered the proteins oxidant damage in rat liver tissues.

Oxidative stress in diabetes coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of the free radicals. The endogenous antioxidant system may counteract the ROS and reduce the oxidative stress with the enzymatic antioxidants SOD, CAT and GPx. SOD protects tissues against oxygen free radicals by catalysing the removal of superoxide radical, converting it into H_2O_2 and molecular oxygen, which damage both the cell membrane and other biological structures [24]. Catalase is a haem-protein, which is responsible for the detoxification of significant amounts of H_2O_2 [25].

The liver of diabetic animals showed decrease in free radical and reactive oxygen scavenging activity of the key antioxidant enzymes GPx, GST and GSH. Reduced antioxidant activity results in over accumulation of O_2^- and H_2O_2, which further generate OH^- diabetic kidney damage [26]. *P. daemia* administration increased the activities of antioxidants in the kidney of diabetic rats. This may be due to the excess production of antioxidants due to *P. daemia* administration and further protection from toxic effects of free radical intermediates, so it is concluded that the *P. daemia* extract is very effective in diabetes and that the effects could be mediated through the pancreatic antioxidant without side effects.

Lipid peroxidation is a characteristic of diabetes mellitus. Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated fatty acids. Under physiological conditions, the concentrations of lipid peroxides in the tissues are low. Karpen *et al.* [27] reported elevated levels of lipid peroxides in the plasma of diabetic rats. Lipid peroxide-mediated tissue damage resulted in the development of both type I and II diabetes.

Low levels of lipid peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation, thus leading to cellular infiltration and islet cell damage in type I diabetes [28]. The most commonly used indicators of lipid peroxidation are TBARS products. The increased lipid peroxidation in the

tissues of diabetic animals may be due to the observed increase in the concentration of TBARS [29]. In the present study, there was an increase in the concentration of TBARS and hydroperoxides were observed in the liver and kidney of diabetic rats. The increase in the level of TBARS and hydroperoxides suggests enhanced LPO leading to tissue injury and failure of the antioxidant defence mechanism to prevent the formation of excess free radicals. The administration of *P. daemia* and glibenclamide resulted in significant reduction of TBARS and hydroperoxides levels. This reduction may be due to the availability of antioxidants. Thus *P. daemia* leaf extract offered protection against oxidative stress by scavenging the free radicals that cause injuries.

CONCLUSION

In conclusion, the results of the present study indicated that leaf and callus extract of *P. daemia* has a beneficial effect on oxidative stress and improved antioxidant effect in liver on STZ-induced diabetic rats.

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