Original Article

Antioxidant effect of *Phaseolus vulgaris* in streptozotocin-induced diabetic rats

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The antioxidant effect of an aqueous extract of *Phaseolus vulgaris* pods, an indigenous plant used in Ayurvedic medicine in India, was studied in rats with streptozotocin-induced diabetes. Oral administration of *Phaseolus vulgaris* pod extract (PPEt; 200 mg/kg body weight) for 45 days resulted in a significant reduction in thiobarbituric acid reactive substances and hydroperoxides. The extract also causes a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the liver and kidneys of rats with streptozotocin-induced diabetes. These results clearly show the antioxidant property of PPEt. The effect of PPEt at 200 mg/kg body weight was more effective than glibenclamide.

Key words: aqueous extract, enzymic antioxidants, *Phaseolus vulgaris*, streptozotocin-induced diabetes.

Introduction

Diabetes mellitus type 2 is associated with increased oxidative stress.[1] Free radicals, lipid peroxides and oxidation of low-density lipoproteins (LDL) have been suggested to have a role in the increased risk of cardiovascular disease associated with diabetes mellitus type 2. In diabetes, impaired glucose metabolism may lead to an increase in hydroxyl radical production. Free radicals may also be formed via the auto-oxidation of unsaturated lipids in plasma and membrane lipids. The free radicals produced may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation.[2]

The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenger systems.[3,4] The levels of these defense mechanisms are altered in diabetes and therefore, the ineffective scavenging of free radicals plays a crucial role in determining tissue injury.[5]

Phaseolus vulgaris L. (Leguminosae), commonly known as kidney bean, is a food item of mass consumption in Asian and Eastern countries. Various parts of the plant have been extensively used in Ayurvedic and Unani medicine in the Indian subcontinent for the treatment of diabetes mellitus.[6] In 1995, Roman-Ramos *et al.* showed that the aqueous extract of *Phaseolus vulgaris* pods possessed antihyperglycemic activity.[7] *Phaseolus vulgaris* was also reported to contain nearly 50 mg of flavonoids per 100 g.[8] Recently, we have proved the insulin-stimulatory effect of *Phaseolus vulgaris* pods from existing β-cells in diabetic rats.[9]

To our knowledge, no other biochemical investigations had been carried out on tissue antioxidant status in experimental diabetic rats. The present investigation was carried out to study the effect of *Phaseolus vulgaris* pod extract on tissue lipid peroxides and enzymatic antioxidants in rats with streptozotocin-induced diabetes. As *Phaseolus vulgaris* is consumed widely in various parts of the world, the demonstration of beneficial effects of the species would have considerable practical significance.

Materials and methods

Animals

Male albino Wistar rats weighing 170–200 g were used in this study. They were bred in the Central Animal House, Rajah Muthiah Medical College, Annamalainagar, India. The animals were fed *ad libitum* with a normal laboratory pellet diet (Hindustan Lever, Bangalore, India) and water.

Drugs and chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company (St Louis, MO, USA). The chemicals were of analytical grade.

Plant material

Phaseolus vulgaris was purchased from the local market in Chidambaram, Cuddalore District, Tamil Nadu, India. The plant was identified at the herbarium of the Botany Directorate in Annamalai University. A voucher specimen (No. 2387) was deposited in the Botany Department of Annamalai University.

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Preparation of plant extract

A total of 132 g of dried pods of *Phaseolus vulgaris* were extracted with 1.0 L of water by the method of continuous hot extraction. The extract was evaporated to dryness in a rotavapor (Air Blow Equipment, Chennai, India) at 40–50°C under reduced pressure. A semisolid material was obtained (15–20 g). It was stored at 0–4°C until used. When needed, the residual extract was suspended in distilled water and used in the study.[7]

Induction of experimental diabetes

A freshly prepared solution of streptozotocin (45 mg/kg) in 0.1 mol/L citrate buffer, pH 4.5, was injected intraperitoneally in a volume of 1 mL/kg.[10] After 48 h of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with a blood glucose of 200–300 mg/dL) were taken for the experiment.

Experimental procedure

In the experiment a total of 40 rats (30 diabetic surviving rats, 10 normal rats) were used. The rats were divided into four groups of 10 rats each: group 1, normal rats; group 2, diabetic control; group 3, diabetic rats given *Phaseolus vulgaris* pod extract (PPEt) daily for 45 days (200 mg/kg body weight in aqueous solution administered with an intragastric tube);[7] and group 4, diabetic rats given glibenclamide daily for 45 days (600 µg/kg body weight in aqueous solution administered with an intragastric tube).[11]

After 45 days, the animals were deprived of food overnight and killed by decapitation. Blood was collected for the estimation of glucose. The liver and kidneys were dissected out, washed in ice-cold saline, patted dry and weighed.

Analytical methods

Fasting blood glucose was estimated by the O-toluidine method.[12] Thiobarbituric acid reactive substances (TBARS) were estimated by the method of Fraga *et al.*[13] Hydroperoxide was determined by the method of Jiang *et al.*[14] Glutathione was estimated by the method of Ellman.[15] The activity of superoxide dismutase (SOD) was assayed by the method of Kakkar *et al.*[16] Catalase was carried out according to the method described by Sinha.[17] The activities of glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were assayed according to the method described by Rotruck *et al.*[18] and Habig *et al.*[19] Protein content in tissue homogenate was measured by the method of Lowry *et al.*[20]

Statistical analysis

The data for various biochemical parameters were analysed using ANOVA and the group means were compared by Duncan's multiple range test. Values were considered statistically significant when $P < 0.05$.

Results

Table 1 demonstrates the level of blood glucose in normal and experimental animals. There was a significant elevation in blood glucose in diabetic rats compared to control rats. Administration of PPEt and glibenclamide significantly decreased the level of blood glucose in treated diabetic rats compared to untreated diabetic rats. *Phaseolus vulgaris* pod extract was more effective than glibenclamide.

Table 2 shows the concentration of TBARS and hydroperoxides in tissues of normal and experimental animals. There was a significant elevation in tissue TBARS and hydroperoxides during diabetes compared to the corresponding control group. Administration of PPEt and glibenclamide significantly decreased the level of TBARS and hydroperoxides in rats with streptozotocin-induced diabetes.

Table 3 shows the content of reduced glutathione (GSH) in tissues of normal and experimental groups. There was a significant decrease in the concentration of GSH in tissues during diabetes compared to the corresponding control groups. Administration of PPEt and glibenclamide increased the content of GSH in the liver and kidneys of diabetic rats. *Phaseolus vulgaris* pod extract was more effective than glibenclamide.

Tables 4 and 5 illustrate the activities of SOD, catalase, GPx and GST in the liver and kidneys of normal and experimental groups. During diabetes there was a significant reduction in the activities of SOD, catalase, GPx and GST in tissues, such as liver and kidney. Administration of PPEt and glibenclamide increased the activity of SOD, catalase, GPx and GST in diabetic rats. The effect of PPEt was more prominent compared with glibenclamide.

Discussion

Lipid peroxidation is one of the characteristic features of chronic diabetes. Tissue antioxidant status is suggested to be an important factor in the development of diabetic complications.[21] Low levels of lipoxygenase peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases it may initiate uncontrolled lipid peroxidation leading to cellular infiltration and islet cell damage in type 1 diabetes.[22]

The increased susceptibility of the tissues of diabetic animals to lipid peroxidation may be due to the observed increased concentration of TBARS and hydroperoxides in the liver and kidneys of diabetic rats.[23] An increase in lipid peroxide concentration in the liver and kidneys of diabetic animals has been observed.[24] Administration of PPEt and glibenclamide significantly decreased the level of TBARS and hydroperoxides in rats with streptozotocin-induced diabetes.

Table 1. Effect of *Phaseolus vulgaris* pod extract on blood glucose in normal and experimental groups

Group	Blood glucose (mg/dL)
Normal	77.05 ± 4.87^{a}
Diabetic control	266.10 ± 21.00^{b}
Diabetic + PPEt (200 mg/kg)	89.80 ± 3.40^{ac}
Diabetic + glibenclamide (600 µg/kg)	96.00 ± 6.70^{c}

Values are given as mean ± SD from six rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test). Duncan procedure ranges for the level: 2.95; 3.09; 3.20. PPEt, *Phaseolus vulgaris* pod extract.

Table 2. Effect of *Phaseolus vulgaris* pod extract on the concentration of TBARS and hydroperoxides in liver and kidney of normal and experimental rats

Group	TBARS (mmol/L per 100 g tissue)		Hydroperoxides (mmol/L per 100 g tissue)	
	Liver	Kidney	Liver	Kidney
Normal	0.829 ± 0.08^{a}	1.280 ± 0.114^{a}	68.35 ± 3.25^{a}	55.43 ± 2.34^{a}
Diabetic control	1.770 ± 0.13^{b}	2.150 ± 0.12^{b}	94.95 ± 4.94^{b}	73.46 ± 5.50^{b}
Diabetic + PPEt (200 mg/kg)	1.200 ± 0.09^{c}	1.510 ± 0.07^{c}	81.20 ± 3.40^{c}	64.41 ± 3.81^{c}
Diabetic + glibenclamide (600 µg/kg)	1.320 ± 0.09^{d}	1.750 ± 0.08^{d}	84.26 ± 4.10^{d}	68.00 ± 4.3^{d}

Values are given as mean ± SD from six rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test). Duncan procedure ranges for the level: 2.95; 3.09; 3.20. PPEt, *Phaseolus vulgaris* pod extract; TBARS, thiobarbituric acid reactive substances.

Table 3. Effect of *Phaseolus vulgaris* pod extract on the level of reduced glutathione in liver and kidney of normal and experimental rats

Group	Glutathione (mg/100 g tissue)	
	Liver	Kidney
Normal	50.20 ± 4.36^{a}	34.58 ± 1.97^{a}
Diabetic control	25.30 ± 2.24^{b}	21.48 ± 1.45^{b}
Diabetic + PPEt (200 mg/kg)	42.00 ± 2.90^{c}	29.80 ± 1.92^{c}
Diabetic + glibenclamide (600 µg/kg)	37.10 ± 2.78^{d}	27.30 ± 1.39^{d}

Values are given as mean ± SD from six rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test). Duncan procedure ranges for the level: 2.95; 3.09; 3.20. PPEt, *Phaseolus vulgaris* pod extract.

Table 4. Effect of *Phaseolus vulgaris* pod extract on the activities of SOD, catalase, GPx and GST in liver of normal and experimental rats

Group	SOD (U/mg protein)†	Catalase (U/mg protein)‡	GPx (U/mg protein)§	GST (U/mg protein)¶
Normal	9.20 ± 0.55^{a}	83.20 ± 5.80^{a}	9.42 ± 0.76^{a}	7.05 ± 0.56^{a}
Diabetic control	3.65 ± 0.18^{a}	42.30 ± 2.52^{b}	5.43 ± 0.36^{a}	3.20 ± 0.22^{b}
Diabetic + PPEt (200 mg/kg)	5.91 ± 0.21^{c}	68.53 ± 3.39^{c}	7.89 ± 0.45^{c}	5.70 ± 0.34^{c}
Diabetic + glibenclamide (600 µg/kg)	5.10 ± 0.17^{d}	62.58 ± 2.87^{d}	7.09 ± 0.32^{d}	4.83 ± 0.21^{d}

†One unit of activity was taken as the enzyme reaction which gave 50% inhibition of nitroblue tetrazolium (NBT) reduction in 1 min. ‡Hydrogen peroxide consumed (µmol/min). §Glutathione consumed (µg/min). ¶CDNB-GSH conjugate formed (µmol/min). Values are given as mean ± SD from six rats in each group. Values not sharing a common superscript letter ($^{a–d}$) differ significantly at $P < 0.05$ (Duncan's multiple range test). Duncan procedure ranges for the level: 2.95; 3.09; 3.20. CDNB-GSH, 1-chloro-2,4-dimtrobenzedene-reduced glutathione; GPx, glutathione peroxidase; GST, glutathione-S-transferase; PPEt, *Phaseolus vulgaris* pod extract; SOD, superoxide dismutase.

Table 5. Effect of PPEt on the activities of SOD, catalase, GPx and GST in kidney of normal and experimental rats

Group	SOD (U/mg protein)†	Catalase (U/mg protein)‡	GPx (U/mg protein)§	GST (U/mg protein)¶
Normal	14.3 ± 0.66^{a}	43.0 ± 2.40^{a}	7.37 ± 0.57^{a}	6.02 ± 0.42^{a}
Diabetic control	8.21 ± 0.37^{b}	25.7 ± 1.19^{b}	4.50 ± 0.23^{a}	2.40 ± 0.22^{b}
Diabetic + PPEt (200 mg/kg)	11.6 ± 0.44^{c}	36.5 ± 1.66^{c}	6.10 ± 0.32^{c}	4.70 ± 0.25^{c}
Diabetic + glibenclamide (600 µg/kg)	10.7 ± 0.51^{d}	30.33 ± 1.17^{d}	5.59 ± 0.33^{d}	4.04 ± 0.19^{d}

†One unit of activity was taken as the enzyme reaction which gave 50% inhibition of nitroblue tetrazolium (NBT) reduction in 1 minute. ‡Hydrogen peroxide consumed (µmol/min). §Glutathione consumed (µg/min). ¶CDNB-GSH conjugate (formed µmol/min).Values are given as mean ± SD from six rats in each group. Values not sharing a common superscript letter ($^{a–d}$) differ significantly at $P < 0.05$ (Duncan's multiple range test). Duncan procedure ranges for the level: 2.95; 3.09; 3.20. CDNB-GSH, 1-chloro-2,4-dimtrobenzedene-reduced glutathione; GPx, glutathione peroxidase; GST, glutathione-S-transferase; PPEt, *Phaseolus vulgaris* pod extract; SOD, superoxide dismutase.

We observed a decrease in GSH in the liver and kidneys during diabetes. GSH is the most important biomolecule against chemically induced toxicity and can participate in the elimination of reactive intermediates by reducing hydroperoxides in the presence of GPx.[25,26] The decrease in the GSH level represents increased utilization due to oxidative stress.[27] Administration of PPEt and glibenclamide increased the content of GSH in the liver and kidneys of diabetic rats.

Superoxide dismutase is an important defense enzyme that catalyses the dismutation of superoxide radicals.[28] Catalase is a hemoprotein that catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals.[29] Therefore, the observed reduction in the activity of these enzymes (SOD, catalase) may result in a number of deleterious effects due to the accumulation of superoxide anion radicals and hydrogen peroxide. Administration of PPEt and glibenclamide increased the activities of SOD and catalase in diabetic rats.

The activities of GPx and GST are observed to decrease significantly in diabetic rats. Glutathione peroxidase, an enzyme with selenium, and GST catalyse the reduction of hydrogen peroxide and hydroperoxides to non-toxic products.[30] The depletion in the activity of these enzymes may result in deleterious oxidative changes due to the accumulation of toxic products. In this context, other workers also reported a decrease in the activities of these antioxidant enzymes (SOD, catalase, GPx and GST) in the liver and kidneys of diabetic rats.[23,27] As the alterations produced in the antioxidant activities indicate the involvement of deleterious oxidative changes, increased activities of the components of this defense system would therefore be important in protection against radical damage. Administration of PPEt and glibenclamide increased the activities of GPx and GST in the liver and kidneys of diabetic rats.

The overexpression of these antioxidant enzymes in diabetic rats treated with PPEt implies that this potential oxidant defense is reactivated by the active principles of PPEt. This results in an increase in the capacity of detoxification through enhanced scavenging of oxy radicals.

In conclusion, the present investigation shows that PPEt possesses an antioxidant activity that may contribute to its protective action on lipid peroxidation and to enhancing its effect on cellular antioxidant defense. This activity contributes to the protection against oxidative damage in streptozotocin-induced diabetes.

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