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Effect of an aqueous extract of *Phaseolus vulgaris* on plasma insulin and hepatic key enzymes of glucose metabolism in experimental diabetes

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Oral administration of 200 mg/kg of aqueous extract of *Phaseolus vulgaris* pods (PPEt) to diabetic animals for 45 days resulted in a significant decrease in blood glucose, glycosylated haemoglobin and significant increase in total haemoglobin and plasma insulin. Similarly oral administration of PPEt to normal animals resulted in a significant hypoglycemic effect. The activities of hepatic hexokinase, glucose 6-phosphatase, fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase, a lipogenic enzyme, were measured in the liver of normal and experimental animals. The activities of the lipogenic enzyme and hexokinase were significantly decreased, whereas the activities of gluconeogenic enzymes were significantly increased in the diabetic liver. Both PPEt and glibenclamide reversed the activities of these enzymes to near normal levels. PPEt was more effective than glibenclamide. The results indicate that the administration of PPEt to diabetic animals normalizes blood glucose and causes a marked improvement of altered carbohydrate metabolic enzymes during diabetes.

1. Introduction

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It represents a heterogeneous group of disorders having hyperglycemia, which is due to impaired carbohydrate (glucose) utilization resulting from a defective or deficient insulin secretory response [1]. The therapy of non-insulin dependent diabetes mellitus (NIDDM) presently relies upon compounds from two chemical classes, sulphonylureas and biguanides [2]. Chronic treatment with sulphonylurea concentrations may desensitize β-cells and may inhibit insulin biosynthesis [3].

In traditional practices medicinal plants are used to control diabetes mellitus in many countries. Some of the plants used by the population, as antidiabetic remedies are edible plants. *Phaseolus vulgaris* L. (Leguminosae), commonly known as Kidney bean, is a food item of mass consumption in Asia and Eastern countries. Various parts of the plant have been extensively used in Ayurvedic and Unani practice in the Indian subcontinent for the treatment of diabetes mellitus [4]. In 1995, Roman-Ramos et al., showed that the aqueous extract of *Phaseolus vulgaris* pods possessed antihyperglycemic activity [5]. *Phaseolus vulgaris* is also reported to contain nearly 50 mg of flavanoids per 100 g [6]. Recently we have proved the antioxidant effect and hypolipidemic effect of *Phaseolus vulgaris* pods in diabetic rats [7, 8].

To our knowledge, no other biochemical investigations have been carried out in streptozotocin diabetic rats on plasma insulin, hexokinase, glucose-6-phosphatase and fructose1,6-bisphosphatase and glucose-6-phosphate dehydrogenase. The present investigation was carried out to study the mechanism responsible for the antihyperglycemic effect of *Phaseolus vulgaris*. The effects of *Phaseolus*

Table 1: Effect of PPEt on the levels of blood glucose and plasma insulin in normal and experimental rats

Group	Blood glucose (mg/dl)		Plasma insulin (μU/ml)	
	30 days	45 days	30 days	45 days
1. Normal	77.60 ± 3.90^a	80.30 ± 4.42^a	14.20 ± 0.58^a	14.60 ± 0.60^a
2. Normal + PPEt	70.70 ± 3.60^a	67.90 ± 3.77^a	15.30 ± 0.54^a	15.85 ± 0.69^b
3. Diabetic Control	270.70 ± 23.60^b	278.54 ± 22.50^b	6.96 ± 0.33^b	6.89 ± 0.32^b
4. Diabetic + PPEt (50 mg/kg)	248.30 ± 17.60^b	233.60 ± 21.30^b	7.30 ± 0.28^b	7.42 ± 0.30^b
5. Diabetic + PPEt (100 mg/kg)	182.60 ± 15.60^b	161.50 ± 13.61^c	8.12 ± 0.43^c	8.92 ± 0.41^c
6. Diabetic + PPEt (200 mg/kg)	102.40 ± 5.30^c	89.80 ± 3.40^d	9.95 ± 0.42^d	11.00 ± 0.37^d
7. Diabetic + glibenclamide	118.70 ± 7.95^c	97.60 ± 6.90^d	8.40 ± 0.45^d	10.65 ± 0.30^d

Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT). Duncan Procedure; Ranges for the level: 2.89; 3.04; 3.14; 3.20; 3.25.

Table 2: Changes in the body weight, total haemoglobin, glycosylated haemoglobin and urine sugar of normal and experimental rats

Group	Changes in body weight (g)		Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb)	Urine sugar
	Initial	Final			
1. Normal	180.60 ± 6.5	191.50 ± 5.3^{NS}	12.25 ± 0.40^{a}	0.250 ± 0.018^{a}	Nil
2. Normal + PPEt	184.50 ± 5.0	188.30 ± 4.6^{NS}	12.81 ± 0.43^{a}	0.230 ± 0.016^{a}	Nil
3. Diabetic control	182.30 ± 7.2	$158.60 \pm 4.8^{*}$	6.89 ± 0.32^{b}	0.900 ± 0.053^{b}	+ + +
4. Diabetic + PPEt (200 mg/kg)	178.60 ± 4.5	$194.30 \pm 6.4^{*}$	11.00 ± 0.37^{c}	0.390 ± 0.020^{c}	Nil
5. Diabetic + glibenclamide (600 µg/kg)	181.70 ± 5.3	$192.10 \pm 5.2^{*}$	10.65 ± 0.30^{d}	0.432 ± 0.024^{d}	Trace

Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT). Duncan Procedure; Ranges for the level: 2.89; 3.04; 3.14; 3.20; 3.25.* – values are statistically significant at $p < 0.001$ as compared with their initial weights. NS – Not significant as compared with their initial weights. (+ + +) – indicates more than 2% sugar.

vulgaris pods are compared with the hypoglycemic sulphonylurea derivative glibenclamide as standard therapy. 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.

2. Investigations and results

2.1. Effect of PPEt on blood glucose and plasma insulin

Table 1 demonstrates the levels of blood glucose and plasma insulin in normal and experimental animals after 30 and 45 days. The level of blood glucose was significantly increased whereas the level of plasma insulin was significantly decreased in diabetic rats. The administration of PPEt at the dose of 200 mg/kg body weight showed a highly significant effect compared to 50 mg and 100 mg/kg body weight. In the PPEt treated groups, although a significant antihyperglycaemic effect was evident on 30^{th} day of treatment, the decrease in blood sugar was highly significant on 45^{th} day in the group treated with 200 mg/kg body weight. PPEt was more effective than glibenclamide.

As the effect of PPEt at a dose of 200 mg/kg body weight for 45 days was more effective, the dose was selected for further biochemical studies.

2.2. Effect of PPEt on haemoglobin, glycosylated haemoglobin, change in body weight and urine sugar

Table 2 shows the level of total haemoglobin, glycosylated haemoglobin, change in body weight and urine sugar of different experimental groups. The diabetic rats showed a significant decrease in the level of total haemoglobin and a significant increase in the level of glycosylated haemoglobin. The administration of PPEt and glibenclamide to diabetic rats restored the changes in the level of body weight, total haemoglobin and glycosylated haemoglobin to almost control levels. In the case of normal rats, the level of haemoglobin and glycosylated haemoglobin remains unaltered.

2.3. Effect of PPEt on hepatic enzymes

The changes in the activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase and fructose-1,6-bisphosphatase are shown in Table 3. The activities of hexokinase and glucose-6-phosphate dehydrogenase were significantly decreased whereas the activities of gluconeogenic enzymes namely glucose-6-phosphatase and fructose-1,6-bisphosphatase were significantly increased in the diabetic rats. Both PPEt and glibenclamide treatment to diabetic rats significantly reversed the changes in the hepatic enzymes. The administration of PPEt and glibenclamide to normal rats showed a significant effect on hepatic enzymes when compared to other groups. The effect of PPEt was more prominent when compared with glibenclamide.

3. Discussion

Persistent hyperglycemia is a major contributor to alterations in enzymes of glucose and fatty acid metabolism in experimental diabetes that leads to diabetic complications such as neuropathy and macrovascular diseases [9].

The capacity of PPEt to decrease the elevated blood sugar to normal levels is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. The possible mechanism by which PPEt exerts its hypoglycemic action in diabetic rats may be by potentiating the plasma insulin effect by increasing either the pancreatic secretion of insulin from the existing β-cells or its release from bound form as it is evidenced by the significant increase in the level of insulin by PPEt in diabetic rats (Table 1). Administration of PPEt to normal rats showed a significant decrease in the level of blood glucose and an increase in the level of plasma insulin

Table 3: Changes in the activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase and fructose-1,6-bisphosphatase in normal and experimental rats

Group	Hexokinase (Units A)	Glucose-6-phosphate dehydrogenase (Units B)	Glucose-6-phosphatase (Units C)	Fructose-1,6-bisphosphatase (Units D)
1. Normal	145.6 ± 11.8^{a}	4.06 ± 0.18^{a}	0.183 ± 0.013^{a}	0.373 ± 0.021^{a}
2. Normal + PPEt	150.7 ± 7.8^{a}	4.23 ± 0.14^{a}	0.154 ± 0.011^{b}	0.306 ± 0.019^{b}
3. Diabetic control	101.5 ± 10.2^{b}	2.76 ± 0.10^{b}	0.265 ± 0.020^{c}	0.576 ± 0.031^{c}
4. Diabetic + PPEt (200 mg/kg)	124.7 ± 6.3^{c}	3.60 ± 0.16^{c}	0.193 ± 0.015^{ad}	0.421 ± 0.022^{de}
5. Diabetic + glibenclamide (600 µg/kg)	119.6 ± 5.9^{d}	3.42 ± 0.13^{d}	0.212 ± 0.017^{d}	0.469 ± 0.028^{e}

Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT). Duncan Procedure; Ranges for the level: 2.89; 3.04; 3.14; 3.20; 3.25. A – µmol of glucose phosphorylated/min/g protein. B – $\times 10^{-4}$ mIU/mg protein. C – µmol of Pi liberated/min/mg protein. D – µmol of Pi liberated/h/mg protein.

(Table 1). This clearly shows that PPEt has a better effect on the secretion of insulin from pancreatic β-cells. The possible mechanism of action of the extract could be correlated with the reminiscent effect of the hypoglycaemic sulphonylureas which promote insulin secretion by closure of K^+ – ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion.

Flavonoids are reported to regenerate the damaged pancreatic β-cells in diabetic animals [10]. As the *Phaseolus vulgaris* pods are reported to be rich in flavonoids [6], the flavonoids present in PPEt, may be responsible for stimulating insulin secretion from existing β-cells of diabetic rats and thereby mediating its antihyperglycaemic effect.

The decreased level of total haemoglobin in diabetic animals is mainly due to the increased formation of glycosylated haemoglobin. Since during diabetes, the excess glucose present in the blood reacts with haemoglobin to form glycosylated haemoglobin [11, 12, 23, 24]. Administration of PPEt to diabetic rats significantly increased the level of total haemoglobin and significantly decreased the level of glycosylated haemoglobin and this may be due to the decreased level of blood glucose.

The body weight was decreased in diabetic rats [13]. Administration of PPEt to diabetic rats significantly reversed the loss in body weight that seems to be due to its ability to reduce hyperglycemia and increased plasma insulin thereby promoting the synthesis of fat.

Hexokinase is the prime enzyme in the glycolytic pathway, which is insulin dependent and plays an important role in the maintenance of glucose homeostasis. The activity of hexokinase decreased significantly in the liver of diabetic rats [12]. Administration of PPEt to diabetic rats resulted in a significant reversal in the activity of hexokinase. The increased activity of hexokinase causes the increase in glycolysis and utilization of glucose for energy production. Administration of PPEt have been observed to decrease the concentration of blood glucose in streptozotocin diabetic rats, which may be due to the increased level of insulin, since the administration of PPEt to diabetic rats showed a significant increase in the level of plasma insulin. The decrease in the concentration of blood glucose in diabetic rats given PPEt may also be as a result of increased liver hexokinase activity there by increased glycolysis.

Significant reversal of the enzyme glucose-6-phosphate dehydrogenase in diabetic rat liver by PPEt treatment suggests that the hydrogen shuttle systems and the redox state of the cell becomes more oxidised which results in the increased formation of NADPH for increased utilization in lipogenesis and, inturn, activating the enzyme, as NADPH is a strong inhibitor of glucose-6-phosphate dehydrogenase.

Glucose-6-phosphatase and fructose-1,6-bisphosphatase are regulatory enzymes of the gluconeogenic pathway. Activation of gluconeogenic enzymes in diabetes is due to a state of insulin deficiency. Under normal conditions insulin function as a suppressor of gluconeogenic enzymes [14]. Administration of PPEt and glibenclamide to diabetic rats significantly decreased the activities of gluconeogenic enzymes in diabetic rats. The level of plasma insulin was found to increase significantly in diabetic rats treated with PPEt, which may be a consequence for the significant reduction in the level of gluconeogenic enzymes. The reduction in the activities of gluconeogenic enzymes can result in the decreased concentration of glucose in blood.

Administration of PPEt to normal rats also resulted in a significant increase in the activity of hexokinase and a significant decrease in the activities of gluconeogenic enzymes, which may be a result for the significant decrease in the level of blood glucose.

Phaseolus vulgaris exerts a significant antihyperglycemic effect in streptozotocin induced experimental diabetes. A sequential metabolic correlation between increased glycolysis, decreased gluconeogenesis, increased hydrogen shuttle reactions and normoglycemia stimulated by PPEt suggests the possible biochemical mechanism through which glucose homeostasis is regulated.

These results suggest that the antihyperglycaemic activity of *Phaseolus vulgaris* is due to insulin-releasing and insulin-like activity. Further studies are necessary to identify the nature of the active principles and their mechanism of action on insulin – secreting cells.

4. Experimental

4.1. Animals

Male albino Wistar rats of body weight 170–200 g bred in the Central Animal House, Rajah Muthiah Medical College were used in this study. The animals were fed with normal laboratory pellet diet and water *ad libitum*. The ethical committee, Annamalai University, approved the use of animals in the present study.

4.2. Drugs and chemicals

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

4.3. Plant material

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

4.4. Preparation of plant extracts

132 g of dried pods of *Phaseolus vulgaris* were extracted with 1.0 l of water by the method of continuous hot extraction and evaporated to dryness in a rotavapor 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 [5].

4.5. Induction of experimental diabetes

A freshly prepared solution of streptozotocin (45 mg/kg) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg [15]. After 48 h of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with blood glucose of 200–300 mg/dl) were taken for the experiment.

4.6. Experimental design

In the experiment a total of 42 rats (30 diabetic surviving rats, 12 normal rats) were used. The rats were divided into 7 groups of 6 rats each. Group 1: Normal untreated rats. Group 2: Normal rats given *Phaseolus vulgaris* pod extract (PPEt) (200 mg/kg body weight) [5, 7, 8] in aqueous solution daily using an intragastric tube for 45 days. Group 3: Diabetic control rats. Group 4: Diabetic rats given PPEt (50 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days. Group 5: Diabetic rats given PPEt (100 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days. Group 6: Diabetic rats given PPEt (200 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days. Group 7: Diabetic rats given glibenclamide (600 μg/kg body weight) [16] in aqueous solution daily using an intragastric tube for 45 days.

After 30 days and 45 days, the animals were deprived of food overnight and blood was collected through sino-ocular puncture in a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose and plasma insulin. At the end of 45 days, after the estimation of blood glucose and plasma insulin, the animals were sacrificed by decapitation. Blood was collected for the estimation of haemoglobin and

glycosylated haemoglobin. Liver was dissected out, washed in ice cold saline, patted dry and weighed.

4.7. Analytical methods

Fasting blood glucose was estimated by the *o*-toluidine method [17]. Haemoglobin was estimated by the cyanmethaemoglobin method [18]. Glycosylated haemoglobin was estimated by the method of Sudhakar Nayak and Pattabiraman [19] modified by Bannon [20]. Plasma insulin level was estimated by an ELISA method using a Boehringer-Mannheim GMBH kit, Germany.

Hexokinase (E.C 2.7.1.1), Glucose-6-phosphatase (E.C 3.1.3.9) and Fructose-1,6-bisphosphatase (E.C 3.1.3.11) were assayed according to the method of Brandstrup et al., [21], Baginsky et al. [22] and Gancedo and Gancedo [23] and the inorganic phosphate (Pi) liberated was estimated by the method of Fiske and Subbarow [24]. Glucose-6-phosphate dehydrogenase (E.C 1.1.1.49) was determined by the method of Ellis and Kirkman [25].

4.8. Statistical analysis

Statistical analysis was done by analysis of variance (ANOVA) followed by Duncans Multiple Range Test (DMRT) [26]. Values were considered statistically significant when $p < 0.05$.

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