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Anti-Diabetic Activity Of Aqueous Extract Of *Pithecellobium Dulce* Benth Fruit Peel On Streptozotocin Induced Diabetic Rats

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ABSTRACT

The decoction of the *Pithecellobium dulce* fruit peel has been used for the control of diabetes mellitus in the traditional method adopted by the local people of northwest region of Tamil Nadu, India. The present study was carried out to investigate the anti-diabetic potential of aqueous extract of *Pithecellobium dulce* fruit peel by its oral administration (200 mg/kg) to streptozotocin-induced diabetic rats. The levels of blood glucose, urine sugar, glycosylated hemoglobin, glucose-6-phosphatase, fructose-1,6-bisphosphatase, total cholesterol, triglycerides, aspartate transaminase, alanine transaminase, alkaline phosphatase and reduced glutathione were increased significantly whereas the levels of plasma insulin, hexokinase, protein, liver glycogen, reduced glutathione, superoxide dismutase, catalase and glutathione peroxidase were decreased in streptozotocin-induced diabetic rats and it was normalized after treatment of aqueous extract. Glibenclamide was used as the standard drug. These outcomes suggest that the aqueous extract possesses anti-diabetic activity and supports the traditional use of the *Pithecellobium dulce* fruit peel decoction as hypoglycemic agent.

Indexing terms/Keywords

Pithecellobium dulce fruit peel; anti-diabetic activity; streptozotocin; glibenclamide

Academic Discipline And Sub-Disciplines

Chemistry, Phytopharmacology, Herbal drugs

SUBJECT CLASSIFICATION

Pharmacological studies of herbal extract

TYPE (METHOD/APPROACH)

Animal model and Biological parameters

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic disease in human beings caused by inherited and/or acquired deficiency in the production of insulin by the pancreas. Persistent diabetes affects carbohydrate, protein, fat and lipid metabolism and leads to dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (ADA, 2009). Out of several drugs in clinical practice for the treatment of diabetes mellitus, the plant based drugs find extensive applications due to the minimal adverse effects even if consumed for a prolonged period. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered to be one of the promising sources for a new drug or may yield better prospects to make a new drug.

The plant, *Pithecellobium dulce* Benth belongs to Mimosaceae family. The biological potential of the plant is thoroughly investigated. The root extracts are abortifacient (Banarjee, 2005). The leaf extracts possess anti-diabetic (Sugumaran et al., 2009), anti-hyperlipidemic (Sundarrajan et al., 2010), anti-oxidant (Sugumaran et al., 2008a), antitubercular (Shanmugakumaran et al., 2006), CNS depressant (Sugumaran et al., 2008b) and neuropharmacological potentials (Mule et al., 2011). The fruits are anti-inflammatory (Bhargva Krishna et al., 1970), anti-oxidant (Megala and Geetha, 2010), and nephroprotective (Pal et al., 2012). The seeds have antimicrobial (Khan et al., 1997, Bautista et al., 2003; Barrera et al., 2003; Ali et al., 2001) and protease inhibition potential (Delgado et al., 2004). The bark of *Pithecellobium dulce* also possesses antimicrobial (Singh et al., 2010) and anti-venom activities (Pithayanukul et al., 2005). The aril powder has been proposed as a supplement in baby foods (Rao et al., 2011). The antioxidant and antibacterial activity (Sukantha et al., 2011) and the wound healing potential (Sukantha et al., 2014) of extracts *Pithecellobium dulce* fruit peel have been reported.

The plant *Pithecellobium dulce* Benth has been used for the control of diabetes mellitus by the local people of northwest region of Tamil Nadu, India. A survey taken with the people of the age group between 50-60 years has shown that 5 % of the people in that area have known the usage of this plant for the treatment of madumega characterized by sugar in urine. As of today, the fruit is widely used for the control of blood glucose level. A few groups of people chew raw fruit peel as well as drink the decoction for the same purpose. Literature revealed that, there is no scientific documentation for the

usage of fruit peel for the treatment or control of diabetes mellitus but the ethnomedical use revealed that leaves and seeds have been used for the same. Based on the ethnomedicinal knowledge of its use in the control of diabetes, the fruit peel of *P. dulce* was taken up for pharmacological investigation. Herein we report the anti-diabetic activity of aqueous extract of *Pithecellobium dulce* fruit peel in streptozotocin-induced type 2 diabetic rats.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant material

The plant *Pithecellobium dulce* was collected from Namakkal district, Tamil Nadu, India. The plant was taxonomically identified and authenticated by Botanical Survey of India, Coimbatore (Tamil Nadu) and a voucher specimen was deposited in our laboratory for future reference (BSI/SRC/5/23/2011-12/Tech.752).

2.2 Extraction of fruit peel of *Pithecellobium dulce*

Air dried pieces of fruit peel of *Pithecellobium dulce* (1 Kg) were thoroughly percolated and extracted with distilled water for a period of (2 x 6) hours. The aqueous extract of *Pithecellobium dulce* fruit peel (PDFPAQ) obtained was filtered and concentrated under reduced pressure to yield a brown residue.

2.3 Animals

Male albino Wistar rats (150-200 g) were purchased from TANUVAS, Madavaram, Chennai. The laboratory animal protocol used for this study was approved by the Institutional Animals Ethics Committee, KMCH College of Pharmacy, Coimbatore, India (KMCRET/Ph.D/09/2011).

2.4 Preparation of Diabetic Rats

Diabetes was induced in overnight fasted adult Wistar albino male rats by a single intraperitoneal injection of 60 mg/kg streptozotocin, dissolved in 0.1 M citrate buffer (pH 4.5). Hyperglycemia was confirmed by the elevated glucose levels (above 250 mg/dl) in plasma, determined at 72 h and then on day 7 after injection. Six rats injected with 2% gum acacia alone served as control.

2.5 Experimental design

After successful induction of experimental diabetes, the rats were divided into four groups each comprising a minimum of six rats. These were: Group 1, control rats; Group 2, Diabetic control rats; Group 3, Diabetic rats administered with PDFPAQ (200 mg/kg bw) in aqueous solution orally for 30 days; and Group 4, Diabetic rats administered with glibenclamide (5 mg/ kg bw) in aqueous solution orally for 30 days (Kaleem et al., 2006). Body weight and blood glucose level measurements were conducted periodically. At the end of the experimental period, rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. The blood was collected with or without EDTA (Ethylene diamine tetraacetic acid) for plasma or serum separation, respectively.

2.6 Biochemical assays

Glucose levels were estimated by a commercially available glucose kit based on the glucose oxidase method (Span Diagnostics Ltd., Surat, India). The glycosylated hemoglobin was estimated by the method of Nayak and Pattabiraman (1981). Plasma insulin was estimated using ELISA assay kit (for rats) supplied by Linco Research Inc. (Stat Diagnostics, Mumbai). The serum total protein was estimated as per the method of Lowry et al. (1951). Liver was immediately dissected, washed in ice-cold saline to remove the blood and homogenized in 0.1 M Tris–HCl buffer, pH 7.4. The supernatant was used for enzyme activity assays. Hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase were assayed by the methods of Trinder (1969), King (1965) and Gancedo and Gancedo (1971) respectively. Assay of glycogen (Morales et al., 1973), AST and ALT (Reitmann and Frankel, 1957), ALP (King and Armstrong, 1934), serum total cholesterol (Parekh and Jung, 1970), serum triglycerides (Foster and Dunn, 1973), total reduced glutathione (Sedlak and Lindsay, 1968, modified according to the method of Moron et al., 1979), superoxide dismutase (Misra and Fridovich, 1972), catalase (Takahara et al., 1960) and glutathione peroxidase (Rortruck et al., 1973) were performed as per the standard protocols.

2.7 Statistical analysis

All the grouped data were statistically evaluated with SPSS 16.0 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by the least significant difference (LSD) test; p values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as the mean ± S.D. for six animals in each group.

3. RESULTS

Table 1 shows the blood glucose, glycosylated hemoglobin and urine sugar level of control and experimental group of rats. The blood glucose, glycosylated hemoglobin and urine sugar level in the control rats were significantly increased in diabetic rats. Treatment with PDFPAQ extract as well as glibenclamide to diabetic rats elicited significant ($p < 0.05$) decreases in blood glucose, glycosylated hemoglobin and urine sugar level when compared with diabetic control rats.

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Table 1 Effects of aqueous extract of *P. dulce* fruit peel on blood glucose, HbA_{1c} and urine sugar in control and experimental rats

GROUPS	BLOOD GLUCOSE MG/DL	HBA_{1C} (% HB)	URINE SUGAR
Control	87.3 ± 3.8	5.5 ± 0.3	Nil
Diabetic control	298.5 ± 7.7	10.8 ± 0.4	+++
Diabetic + PDFPAQ (200 mg/kg)	89.7 ± 1.8	6.8 ± 0.3	Nil
Diabetic + glibenclamide (5 mg/kg)	99.3 ± 3.3	6.7 ± 0.4	Nil
Each value is mean ± SD of six rats in each group; $p<0.05$ compared to control			

Table 2 summarizes the levels of initial and final body weight, and plasma insulin in the control and experimental groups of rats. A significant decrease in body weight and insulin level was observed in STZ (Streptozotocin)-diabetic rats and it was normalized after treatment with PDFPAQ extract and glibenclamide.

Table 2 Effects of aqueous extract of *P. dulce* fruit peel on body weight and insulin level in control and experimental rats

GROUPS	INITIAL BODY WEIGHT (G)	FINAL BODY WEIGHT (G)	PLASMA INSULIN (µU/ML)
Control	187.2 ± 7.8	208.8 ± 6.8	16.0 ± 0.7
Diabetic control	197.3 ± 6.9	155.3 ± 7.4	6.3 ± 0.3
Diabetic + PDFPAQ (200 mg/kg)	195.8 ± 6.1	192.7 ± 5.1	14.3 ± 0.7
Diabetic + glibenclamide (5 mg/kg)	193.8 ± 10.4	195.5 ± 9.5	14.7 ± 0.7
Each value is mean ± SD of six rats in each group; $p<0.05$ compared to control			

Table 3 summarizes the levels of hexokinase, glucose-6-phosphatase and fructose-6-phosphatase in the control and experimental groups of rats. A significant decrease in hepatic hexokinase level and concomitant increase in glucose-6-phosphatase and fructose-1,6-bisphosphatase level was observed in STZ-diabetic rats and it was normalized after treatment with PDFPAQ extract and glibenclamide.

Table 3 Effects of aqueous extract of *P. dulce* fruit peel on hexokinase, glucose-6-phosphatase and fructose-6-phosphatase in control and experimental rats

GROUPS	HEXOKINASE	GLUCOSE-6-PHOSPHATASE	FRUCTOSE1,6-BISPHOSPHATASE
Control	242.0 ± 5.6	1016.8 ± 40.2	478.8 ± 17.0

Diabetic control	135.5 ± 3.5	1609.5 ± 26.1	756.5 ± 29.3
Diabetic + PDFPAQ (200 mg / kg)	231.2 ± 9.0	1089.5 ± 37.1	522.33 ± 16.1
Diabetic + glibenclamide (5 mg / kg)	231.7 ± 6.3	1086.8 ± 40.3	508.3 ± 2.6
Each value is mean ± SD of six rats in each group; p<0.05 compared to control µmoles of glucose phosphorylated/min/mg protein for Hexokinase µmoles of Pi liberated/min/mg protein for Glucose-6-phosphatase µmoles of Pi liberated/min/mg protein for Fructose-1, 6-bisphosphatase			

Table 4 summarizes the levels of liver glycogen, serum protein, total cholesterol and triglycerides level in the control and experimental groups of rats. A significant decrease in liver glycogen, protein level and concomitant increase in total cholesterol and triglycerides level was observed in STZ-diabetic rats and it was normalized after treatment with PDFPAQ extract and glibenclamide.

Table 4 Effects of aqueous extract of *P. dulce* fruit peel on Liver glycogen, Serum protein, Total cholesterol and Triglycerides level in control and experimental rats

GROUPS	LIVER GLYCOGEN (MG/G WET TISSUE)	SERUM PROTEIN (G/DL)	TOTAL CHOLESTEROL (G/DL)	TRIGLYCERIDES (G/DL)
Control	51.2 ± 2.4	7.9 ± 0.4	99.7 ± 4.1	83.0 ± 4.6
Diabetic control	21.0 ± 1.0	4.7 ± 0.2	178.7 ± 7.4	155.5 ± 7.9
Diabetic + PDFPAQ (200 mg/kg)	45.7 ± 2.2	7.0 ± 0.4	103.7 ± 4.6	95.7 ± 3.7
Diabetic + glibenclamide (5 mg/kg)	47.5 ± 1.7	7.0 ± 0.4	106.0 ± 5.1	89.7 ± 4.5
Each value is mean ± SD of six rats in each group; p<0.05 compared to control				

Table 5 summarizes the levels of AST, ALT and ASP in the control and experimental groups of rats. A significant increase in hepatic enzymes AST, ALT and ASP level was observed in STZ-diabetic rats and it was normalized after treatment with PDFPAQ extract and glibenclamide.

Table 5 Effects of aqueous extract of *P. dulce* fruit peel on AST, ALT and ALP level in control and experimental rats

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	73.3 ± 3.5	67.3 ± 0.9	108.3 ± 4.7
Diabetic control	166.8 ± 8.2	138.5 ± 6.5	223.5 ± 11.2

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Diabetic + PDFPAQ (200 mg/kg)	75.7 ± 2.5	72.7 ± 2.3	115.2 ± 4.5
Diabetic + glibenclamide (5 mg/kg)	75.8 ± 3.1	70.3 ± 2.6	118.0 ± 5.6
Each value is mean ± SD of six rats in each group; p<0.05 compared to control			

Table 6 summarizes the levels of the level of glutathione (GSH) and antioxidant enzymes SOD (Superoxide dismutase), CAT (Catalase) and GPx (Glutathione peroxidase) in liver tissue in the control and experimental groups of rats. A significant decrease in the level of glutathione and antioxidant enzymes in liver tissue was observed in STZ-diabetic rats and it was normalized after treatment with PDFPAQ extract and glibenclamide.

Table 6 Effects of aqueous extract of *P.dulce* fruit peel on the level of glutathione and antioxidant enzymes in liver tissue of control and experimental rats

GROUPS	PLASMA GSH (MG/DL)	LIVER GSH (MG/100 G WET TISSUE)	SOD (U/MG PROTEIN)	CAT (U/MG PROTEIN)	GPX (U/MG PROTEIN)
Control	30.0 ± 1.4	46.0 ± 1.4	10.7 ± 0.5	53.2 ± 2.0	17.5 ± 0.9
Diabetic control	20.0 ± 0.8	21.5 ± 0.9	5.7 ± 0.3	21.3 ± 1.0	10.0 ± 0.5
Diabetic + PDFPAQ (200 mg/kg)	27.7 ± 1.6	45.2 ± 1.3	10.5 ± 0.5	44.8 ± 2.0	15.5 ± 0.8
Diabetic + glibenclamide (5 mg/kg)	27.3 ± 1.6	44.8 ± 1.7	9.7 ± 0.5	48.2 ± 2.0	15.7 ± 0.8
Each value is mean ± SD of six rats in each group; p<0.05 compared to control					

4.DISCUSSION

Diabetes mellitus is characterized by hyperglycemia which involves the overproduction (excessive glycogenolysis and gluconeogenesis) and reduced utilization of glucose by the tissues (Kameshwara Rao, 2003). In the present study, we observed an increased level of blood glucose in diabetic rats, while the oral administration of PDFPAQ extract and glibenclamide significantly reduced the blood glucose level (Table 1). This result clearly indicates the presence of hypoglycemic agents in the PDFPAQ extract. The hypoglycemic potential of PDFPAQ extract is found to be higher than the standard drug glibenclamide. Several plants were found to possess hypoglycemic potential by reducing the blood glucose level by the oral administration of plant extracts in the STZ induced diabetic rats (Alarcon-Aguilar *et al.*, 1998; Osadebe *et al.*, 2010; Devi and Latha, 2013).

Urine analysis of diabetic rats shows that the oral administration of PDFPAQ extract and glibenclamide for 30 days completely eliminates the urine glucose (Table 1).

In diabetic condition, various proteins, including hemoglobin undergo non-enzymatic glycation and consequently increase the level of glycosylated hemoglobin, which is directly proportional to the fasting blood glucose level (Kumar et al., 2005). Oral administration of PDFPAQ extract for 30 days suppresses the glycation process and reduces the level of glycosylated hemoglobin in diabetic rats, similar to that of glibenclamide treated rats (Table 1).

Streptozotocin has been extensively used to induce diabetes mellitus in animals by progressive destruction of selective pancreatic insulin islet β cell. In the present study, it was observed that oral administration of PDFPAQ extract could reverse the aforesaid diabetic effect, possibly due to an insulin-like effect of components of PDFPAQ extract on peripheral tissues, either by promoting glucose uptake, or by inhibiting hepatic gluconeogenesis (Henry, 1997; Kumar et al., 1996). This could be due to the potentiating effect of PDFPAQ extract, by stimulating pancreatic secretion of insulin or its action to release the bound insulin from regenerated β cells by inhibiting ATP sensitive K^+ channels like standard drug

glibenclamide (Sharma et al., 2014). A number of compounds have also been shown to exert hypoglycemic activity through stimulation of insulin release (Palsamy et al., 2008). Table 2 reveals the potential recovery of insulin by the oral administration of PDFPAQ extract, comparable to that of glibenclamide, a standard hypoglycemic drug (Kim et al., 2007). The initial body weight of diabetic rats was reduced due to the increased catabolism of glycogen in muscle and liver instead of being stored. Oral administration of PDFPAQ extract and glibenclamide recovered the body weight nearly to the normal level compared to diabetic control (Table 2) by stimulating insulin release.

In the deficiency of insulin, the insulin-dependent and insulin-sensitive enzyme hexokinase, is inhibited or inactivated in the liver of diabetic rat (Gupta et al., 1997). Decreased activity of hexokinase results in the depletion of liver and muscle glycogen (Murray et al., 2000). Oral administration of PDFPAQ extract and glibenclamide to diabetic rats resulted in an increased activity of hexokinase in liver, by the activation of glycolysis, which, in turn, increased the utilization of glucose by restored insulin secretion in the treated rats (Table 3).

The hepatic glucose-6-phosphatase catalyses the glucose production and it plays a vital role in the maintenance of blood glucose homeostasis. Increased activity of glucose-6-phosphatase stimulates lipogenesis in diabetic rats by providing hydrogen to $NADP^+$ and forms NADPH and enhances synthesis of fats from carbohydrates (Bopanna et al., 1997) and increases the levels of glucose in blood (Venkateswaran and Pari, 2002). The present study demonstrates that hepatic glucose-6-phosphatase activity in diabetic rats was significantly higher than that of normal rats and the oral feeding of PDFPAQ extract and glibenclamide markedly lowered its activity and decreased the serum glucose released into the blood stream (Table 3).

Fructose 1-6-phosphatase is an important regulatory enzyme of the gluconeogenic pathway and catalyzes the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate. The activity of fructose 1-6-phosphatase is increased in diabetic condition and results in a decrease of the glycolytic flux (Baquer et al., 1998). The oral administration of PDFPAQ extract and glibenclamide reduces the activities of the gluconeogenic enzyme in diabetic rats (Table 3), by potentiating the effect of insulin release from β-cells of the islets of Langerhans which might enhance glucose utilization (Saravanan *et al.*, 2009).

Glycogen content was reduced drastically in diabetic animals (Ikino et al., 1989) due to insulin deficiency (Gannon and Nuttall, 1997) because of the damage of Langerhans caused by STZ. The oral administration of PDFPAQ extract and glibenclamide stimulates the secretion/release of insulin and thereby recovers the storage of glycogen (Table 4).

Diabetes mellitus shows profound changes in circulating amino acids and hepatic amino acid uptake (Felig et al., 1977). The significant decrease of serum protein in STZ-induced diabetic rats could be attributed to increased protein catabolism. The oral administration of PDFPAQ extract and glibenclamide have significantly inhibited proteolysis caused by insulin deficiency and thus restored the serum protein levels to near normal levels. Hyperglycemia coexists with hypercholesterolemia and hypertriglyceridemia in STZ induced diabetic rats (Pushparaj et al., 2000), which leads to secondary complications such as atherosclerosis and coronary heart disease (Ananthan et al., 2003) due to the excess production of free fatty acids in diabetic condition. The oral administration of PDFPAQ extract and glibenclamide stimulates the secretion/release of insulin and thereby controls the synthesis of free fatty acids and cholesterol (Table 4).

Measurement of enzymic activities of aminotransferases (AST and ALT) and alkaline phosphatase (ALP) is of clinical and toxocological importance, since they were directly associated with the conversion of amino acids to keto acids. Hence, the changes in their activities are indicative of tissue damage by toxicants or in disease conditions. Hepatocellular disorders result in extremely elevated transaminase levels (Hultcrantz et al., 1986). The increase in the activities of plasma AST and ALT indicated that diabetes may induce hepatic dysfunctions due to liver necrosis (Larcan et al., 1979). Oral administration of PDFPAQ extract and glibenclamide significantly reduces the activity of these enzymes when compared to the diabetic group. Recovery of plasma AST, ALT and ALP levels of diabetic rats towards normal shows that the PDFPAQ extract has no adverse effect on functions of liver, bone and intestinal tissues (Table 5).

In diabetic condition there is a marked increase of production of free radicals or impaired antioxidant defense mechanism (Wolff and Dean, 1987; Jiang et al., 1990). STZ induces a significant oxidative stress (Adewole et al., 2007) due to the glucose oxidation, free radical generation, and nitric oxide donor property of STZ (Mohamed et al., 1999) and reduces the activity of antioxidant enzymes such as, catalase (CAT), glutatahione peroxidase (GPx), superoxide dismutase (SOD) and non-enzymatic antioxidant reduced glutathione (GSH), which play a vital role in scavenging the toxic intermediates of incomplete oxidation. In the present study, reduced level of SOD, CAT, GPx and GSH levels were observed in STZ-induced diabetic rats when compared to control rats. Oral administration of PDFPAQ extract and glibenclamide significantly increases the activity of SOD, CAT, GPx and GSH levels in diabetic rats (Table 6). The PDFPAQ extract's restorative action of the altered antioxidant enzymes and glutathione in STZ-induced diabetic rats indicates its free radical scavenging potential.

5. CONCLUSIONS

The present study clearly indicates that the aqueous extract of *Pithecellobium dulce* fruit peel possesses the ability to control blood glucose level in diabetic rats. Its hypoglycemic and free radical scavenging potential is used to prevent diabetic-associated complications. The present investigation supports the traditional use of *Pithecellobium dulce* fruit peel in the treatment or control of diabetes. This study is limited to its animal model design and further investigations are to be carried over to infer clinical correlations to humans. Further phytochemical, biochemical and pharmacological studies are underway to indicate the precise mechanism, efficacy and promises for the therapeutic use.

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