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Biochemical evaluation of antidiabetic properties of *Pithecellobium dulce* fruits studied in streptozotocin induced experimental diabetic rats

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ABSTRACT

Diabetes mellitus is a multisystemic metabolic disorder as old as mankind that has reached epidemic proportions worldwide. Though drugs are plenty for the treatment of diabetes, none is found to be ideal due to undesirable side effects and diminution after prolonged use. Hence, search for novel drugs, especially from plant origin continues. *Pithecellobium dulce* Benth, (Leguminosae) commonly known as Manila Tamarind is found to exhibit a wide range of pharmacological properties. Based on folkloric use, the present study was designed to evaluate the antidiabetic potential of *Pithecellobium dulcee fruits* in STZ-induced experimental diabetes in rats. Phytochemical analysis of the fruit extract revealed the presence of alkaloids, flavonoids, glycosides, saponins, phytosterols, and triterpenoids. Oral administration of *Pithecellobium dulce fruit* extract (300 mg/kg b.w. /day) to diabetic rats for 30 days significantly reduced the levels of blood glucose, glycosylated hemoglobin, urea and creatinine. The altered levels of serum aminotransferases and alkaline phosphatase were normalized upon treatment with the fruit extract. The observed decrease in the levels of plasma protein, plasma insulin, and hemoglobin in the diabetic rats were elevated to near normal by the extract treatment. The level of glycogen content was improved upon treatment with the extract. The results of the present study indicate that the fruit extract is nontoxic and possess antidiabetic nature.

Keywords: *Pithecellobium dulce* fruit, Diabetes, Streptozotocin, Antidiabetic potential.

1. Introduction

Diabetes mellitus is a multifactorial, multisystemic endocrine disorder characterized by persistent hyperglycemia resulting from the defects in insulin secretion, action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development and progression of diabetes mellitus. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency [1].

Estimates of the current and future burden of diabetes are important in order to allocate community as well as health resources, to emphasize the role of lifestyle, and encourage measures to counteract trends for increasing prevalence [2]. Ageing and the changes that are associated with urbanization, globalization and development are increasingly adding to the burden of diabetes in all countries.

The International Diabetes Federation (IDF) estimated the global burden of diabetes was 366 million in 2011 and it would rise to 552 million by 2030. Despite of newer and successful treatment strategies, new diagnostic devices, strict glycemic targets, better treatment guidelines and increased awareness of the disease, baseline glycosylated hemoglobin remains relatively high in subjects diagnosed and treated with diabetes [3]. The search continues for an ideal anti-diabetic drug that will not only normalize blood glucose but also provide beta cell rest and possibly restoration of beta cell function. The development of anti-diabetic drugs is riddled with fundamental challenges.

Currently available antidiabetic medications such as sulfonylureas, biguanides, α- glucosidase inhibitors, thiazolidinediones and insulin are often associated with undesirable side-effects or diminution in response after prolonged use.

The main side effects are weight gain and hypoglycemia with sulfonylureas, gastrointestinal (GI) disturbances with metformin, weight gain, GI disturbances and liver injury with thiazolidinediones, GI disturbances, weight gain and hypersensitivity reactions with meglitinides and flatulence, diarrhea and abdominal bloating with alpha-glucosidase inhibitors [4] . Thus, it is indispensable that we continue to look for new and, if possible, more efficacious drugs, and the vast reserves of phytotherapy may be an ideal target.

Herbal medicines are popular remedies for a number of diseases and used by a vast majority of the world's population. Since pre historic times, herbs were the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century. Further, most of our marketed medicines are distillations, combinations, reproductions or variations of substances which are abundantly found in nature. Our forefathers recommended some of the substances, which are abundantly found in nature long before their pharmacological actions were demonstrated and understood by scientific validations [5]. In the traditional system of Indian medicine, formulation with extracts of plant parts is used as the drug of choice as antidiabetic, antiulcerative, hepatoprotective and lipid-lowering agents. Several medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases without proper knowledge of their function. Although phytotherapy continues to be used in several countries, few plants have received scientific or medical scrutiny. One such medicinal plant, which lacks scientific evidence for its folklore use is *Pithecellobium dulce*.

Pithecellobium dulce Benth. (Leguminosae) is a small to medium sized, evergreen, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also useful in dermatitis and eye inflammation. The leaves have been reported to possess astringent, emollient, abortifacient and antidiabetic properties. A steroid saponin, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported from the seeds [6, 7, 8]. Quercetin kaempferol, dulcitol and afzelin have been reported from the leaves [9]. Roots have been reported to possess estrogenic activity [10]. Studies on alkylated resins from seed oil have been reported [11]. Recently, it is reported *P. dulce* possess antiulcer activity [12]. The fruits of P. dulce have been consumed as a dietary supplement for its high nutritive and medicinal value. The edible fruit has been widely used traditionally to combat gastric problems and found to be non-toxic in nature. The fruit extract was found to be rich in phenolic compounds and revealed the presence of flavonoids–quercitrin, rutin, kaempferol, naringin and daidzein [13].

In the absence of systemic studies in the literature, the present study is designed with an aim to explore the antidiabetic effect of edible part of *P. dulce* fruits on streptozotocin induced experimental diabetes in rats.

2. Materials and Methods

2.1. Plant Material

Fresh, Mature *Pithecellobium dulce* fruits were collected from Tirunelveli District, Tamil Nadu. The plants were identified and authenticated by a taxonomist and an exemplar specimen was deposited at the Department of Botany, University of Madras, Chennai.

2.2. Preparation of Plant extract

The edible part of fruits were selectively removed and dried at room temperature and powdered in an electrical grinder, which was then stored in an airtight container at 5 °C until further use. The powdered edible fruit was delipidated with petroleum ether (60–80 °C) for overnight. It was then filtered and soxhlation was performed with 95% Ethanol. Ethanol was evaporated in a rotary evaporator at 40–50 °C under reduced pressure (yield 17.6g).

2.3. Experimental Animals

Male albino Wistar rats (150-180 g) were purchased from Tamilnadu Veterinary and Animal Sciences University, MADAVARAM, Chennai. The rats were housed in polypropylene cages lined with husk and maintained in centralized Animal house Facility. The husk was renewed every 24 hours. The rats were fed with commercial pelleted rats chow (VRK Nutritional Solutions, Maharashtra, India) and had free access to water. The experimental rats were maintained in a controlled environment (12:12 hours light/dark cycle) and temperature (30±2 °C). The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines for the investigation of experimental pain in conscious rats. The rats were acclimatized for one week before starting the experiments. (IAEC NO. 17/01/2012)

2.4. Preliminary Phytochemical Screening

The ethanolic extract of *Pithecellobium dulce* fruits were subjected to preliminary phytochemical screening [14, 15].

2.5. Induction of Diabetes Mellitus

Experimental diabetes was induced in overnight fasted rats by single intraperitoneal injection of streptozotocin (45 mg/kg b.w) dissolved in freshly prepared 0.1M of cold citrate buffer (pH 4.5) [16]. Since, STZ is capable of inducing fatal hypoglycemia due to massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6 h of STZ administration for the next 24 h to overcome drug induced hypoglycemia [17]. Neither death nor any other adverse effect was observed. After a week time, for the development and aggravation of diabetes, rats with moderate diabetes (i.e. fasting blood glucose concentration, >250 mg/dl) that exhibited hyperglycemia and glycosuria were selected for further experimentation.

2.6. Experimental Design

The rats were grouped into 4 groups, comprising of 6 rats in each group as follows:

Group I: Control rats (water and food *ad libitum*).

Group II: Streptozotocin induced diabetic Rats.

Group III: Diabetic rats treated with *P. dulce* fruit extract (300 mg/Kg body weight/rat/day) in aqueous solution orally for 30 days.

Group IV: Diabetic rats treated with gliclazide (5 mg/kg body weight/day) in aqueous solution orally for 30 days.

During the experimental period, body weight and blood glucose levels of all the rats were determined at regular intervals. At the end of the experimental period, the rats were fasted overnight, anaesthetized, and sacrificed by cervical decapitation. The blood was collected with and without anticoagulant for plasma and serum separation respectively.

2.7. Oral Glucose Tolerance Test

At the end of the experimental period, fasting blood samples were taken from all the groups of rats to perform oral glucose tolerance test. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after an oral administration of glucose solution at a dosage of 2 g kg^{-1} body weight. All the blood samples were collected with EDTA for the estimation of glucose by O-toluidine method [18].

2.8. Biochemical parameters

Fasting blood glucose level was estimated by using O-toluidine reagent [18]. Plasma insulin was assayed using the Ultra-sensitive ELISA kit for rat insulin (Linco Research, St Charles, MO, USA). Hemoglobin and glycosylated hemoglobin (HbA1c) levels were estimated [19, 20]. Blood urea, serum creatinine and uric acid were also assessed [21, 22, 23].

Urine sugar was detected using urine strips. Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) were assayed [24, 25]. The liver and muscle tissues were dissected out and washed with ice-cold saline for determination of glycogen content as described by Morales *et al* [26].

2.9. Statistical analysis

All the grouped data were statistically evaluated with SPSS 16.0 software. Hypothesis testing methods included one-way analysis of variance followed by least significant difference test. A value of $p<0.05$ was considered to indicate statistical significance. All results are expressed as mean ± SEM for six rats in each group.

3. Results

Phytochemical analysis of the fruit extract revealed the presence of alkaloids, flavonoids, glycosides, saponins, phytosterols, and triterpenoids (Table 1). Acute toxicity studies using graded doses of the fruit extract revealed no signs and symptoms such as restlessness, respiratory distress, diarrhoea, convulsions, and coma (data not shown).

Table 1: Phytochemical screening of *P. dulce fruit* extract

Phytoconstituents	Inference
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	+
Tannins	+
Phytosterol	+
Triterpenoids	+
Anthraquinones	-

Fig 1 shows the changes in body weight gain in control and experimental groups of rats. The body weight of control group of rats was gradually increased throughout the experimental period. The body weight was significantly ($p<0.05$) decreased in STZ-induced diabetic rats when compared to control rats. Oral administration of fruit extract as well as gliclazide to STZ-induced diabetic rats significantly ($p<0.05$) increased the body weight to near normalcy.

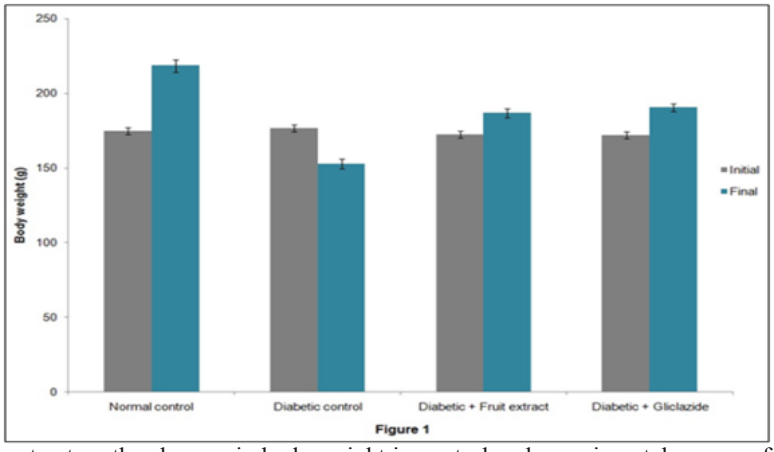

Fig 1: Effect of *P. dulce* fruit extract on the changes in body weight in control and experimental groups of rats. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. P<0.05. The results were compared with @Control rats, #Diabetic rats.

Fig 2 shows the changes on glucose tolerance curve in control and experimental groups of rats. The blood glucose level in the control rats was increased to a peak value at 60 min after oral glucose load and decreased to near normal level at 120 min. In STZ-induced diabetic rats the peak increase in blood glucose concentration was observed after 60 min and remained high over the next 60 min. Oral administration of fruit extract as well as gliclazide on STZ-induced diabetic rats showed significant ($p < 0.05$) decrease in blood glucose concentration at 60 and 120 min when compared with diabetic control suggesting the glucose lowering properties of the fruit extract as well as gliclazide.

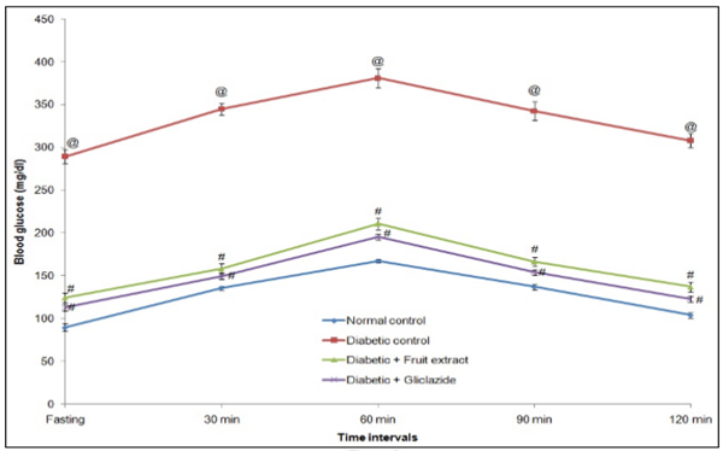

Fig 2: Effect of *P. dulce* fruit extract on the blood glucose level in control and experimental groups of rats receiving an oral glucose challenge. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. P<0.05. The results were compared with @Control rats, #Diabetic rats.

The glycogen content in the liver and muscle tissues is presented in Fig 3. There was a significant decrease in glycogen content in both the liver and muscle tissues of STZ-diabetic rats compared with control rats. Oral administration of fruit extract and gliclazide to diabetic rats for 30 days significantly improved glycogen content in both the liver and muscle tissues compared with untreated diabetic rats.

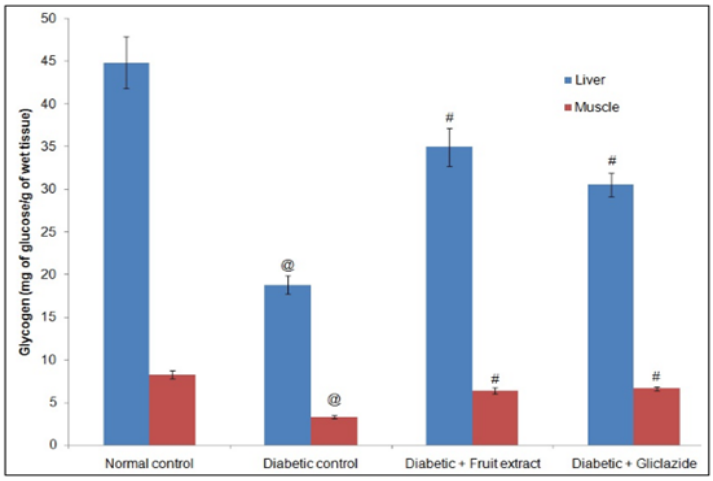

Fig 3: The glycogen content in the liver and muscle tissues. : Effect of *P. dulce* fruit extract on the liver and muscle glycogen content. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. P<0.05. The results were compared with @Control rats, #Diabetic rats

The levels of blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin, and urine sugar of control and experimental groups of rats are shown in Table 2. Control rats did not show any significant variation in the blood glucose throughout the experimental period. Administration of STZ led to hyperglycemia, which was maintained over the experimental period.

Table 2: Effect of *Pithecellobium dulce* fruit extract on the levels of fasting blood glucose (FBG), plasma insulin, hemoglobin, glycosylated hemoglobin (HbA1c), and urine sugar in experimental diabetic rats.

Groups	FBG	Plasma insulin	Hemoglobin	HbA1c	Urine sugar
Control	78.62 ± 3.30	1.07 ± 0.065	14.75 ± 0.27	5.62 ± 0.24	Nil
Diabetic control	304.91 ± 12.17$^{@}$	0.39 ± 0.030$^{@}$	11.67 ± 0.46$^{@}$	11.56 ± 0.44$^{@}$	+++
Diabetic + Fruit extract	131.67 ± 6.94$^{\#}$	0.76 ± 0.044$^{\#}$	13.05 ± 0.21$^{\#}$	7.64 ± 0.32$^{\#}$	Nil
Diabetic + Gliclazide	122.95 ± 5.58$^{\#}$	0.81 ± 0.046$^{\#}$	13.68 ± 0.26$^{\#}$	7.29 ± 0.28$^{\#}$	Nil

Units: mg/dl for fasting blood glucose, ng/ml for plasma insulin, g/dl for hemoglobin, % hemoglobin for HbA1c, +++ indicates more than 2% sugar. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. P<0.05. The results were compared with $^{@}$Control rats, $^{\#}$Diabetic rats

In experimental rats, the levels of blood glucose, glycosylated hemoglobin was increased and the levels of hemoglobin, plasma insulin were decreased when compared with the control group of rats. Upon oral administration of fruit extract and gliclazide, these levels were found to be similar to those of normal rats and the effect was more distinct in the group of rats treated with fruit extract. Urine sugar present in diabetic rats was found to be absent in the rats treated with fruit extract and gliclazide. Table 3 shows the levels of total proteins, blood urea, serum uric acid and serum creatinine of control and experimental groups of rats. The level of total protein was found to be decreased in STZ induced diabetic rats. The levels of blood urea, serum uric acid and serum creatinine were found to be elevated in STZ induced diabetic rats. These biochemical markers were reverted back to near normalcy upon the oral administration of the fruit extract.

Table 3: Effect of *Pithecellobium dulce* fruit extract on the levels of plasma protein, blood urea, serum uric acid and serum creatinine in experimental diabetic rats.

Groups	Plasma protein	Blood urea	Serum uric acid	Serum creatinine
Control	8.13 ± 0.30	23.90 ± 1.60	2.61 ± 0.14	0.64 ± 0.038
Diabetic control	5.70 ± 0.21@	49.09 ± 2.62@	5.98 ± 0.24@	1.32 ± 0.050@
Diabetic + Fruit extract	7.07 ± 0.24#	28.39 ± 1.75#	3.32 ± 0.24#	0.77 ± 0.036#
Diabetic + Gliclazide	6.86 ± 0.28#	33.18 ± 1.68#	3.10 ± 0.18#	0.85 ± 0.050#

Units: g/dl for plasma protein, mg/dl for blood urea, serum uric acid and serum creatinine. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. P<0.05. The results were compared with @Control rats, #Diabetic rats.

The activities of AST, ALT and ALP in the serum of control and experimental groups were presented in Table 4. A significant ($p<0.05$) elevation in the levels of AST, ALT and ALP were noted in serum of STZ induced diabetic rats. Oral administration of fruit extract brought down the activity of AST, ALT and ALP to near normal in serum of diabetic rats.

Table 4: Effect of *Pithecellobium dulce* fruit extract on the levels of activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in serum of experimental diabetic rats.

Groups	AST	ALT	ALP
Control	63.58 ± 2.50	18.77 ± 0.87	76.57 ± 4.29
Diabetic control	136.52 ± 4.84@	53.25 ± 4.03@	161.43 ± 5.86@
Diabetic + Fruit extract	89.79 ± 3.99#	27.34 ± 1.85#	103.88 ± 8.19#
Diabetic + Gliclazide	96.54 ± 4.52#	26.13 ± 2.43#	115.98 ± 3.87#

Enzyme activities are expressed as: AST and ALT - µmoles of pyruvate/h/mg of protein, ALP - µmoles of phenol liberated/min/mg of protein. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. P<0.05. The results were compared with @Control rats, #Diabetic rats.

4. Discussion

Diabetes is a metabolic disorder that afflicts a major proportion of the population globally. The treatment of diabetes mellitus is based on the variable use and combination of diet, antidiabetic oral agents (metformin, sulphonylureas, acarbose and thiazolidinediones) and insulin or its analogs, depending on the type and severity of diabetes. The conventional therapies for diabetes have many shortcomings like undesirable side effects and high rate of secondary complications. In general, there is very little scientific knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain active ingredients such as flavonoids, alkaloids, glycosides, terpenoids, etc., which possess antidiabetic effects [27, 28].

Phytochemical analysis of the fruit extract revealed the presence of flavonoids, alkaloids, glycosides, polyphenols, tannins, saponins, phytosterols and triterpenes in the fruit extract. Phytocompound-based strategies play a pivotal role in the prevention and treatment of diabetes [29]. Polyphenolic compounds such as flavonoids contribute to increased plasma antioxidant capacity, decreased oxidative stress markers and reduced total and LDL cholesterol [30]. Growing evidence indicates that various dietary polyphenols may influence carbohydrate metabolism at many levels [31].

Phytochemicals include compounds with various biological properties which allow plants to cope up with environmental challenges including exposure to radiation and toxins [32]. They are bioactive compounds (secondary metabolites) found in plants that works with nutrients and dietary fibres to protect against diseases [33]. The presence of the biologically active ingredients in the fruit extract may account for the observed pharmacological actions.

Animal models of diabetes such as genetically derived, nutrition induced, and chemically induced have been used extensively in diabetes research. Alloxan and streptozotocin are widely used chemicals in the diabetic research to induce diabetes in experimental animals. However, STZ induced diabetes exhibits many features similar to human diabetes [34]. Streptozotocin is specifically cytotoxic to β-cells of the pancreas. The mechanism behind its action is that STZ is preferentially up taken by pancreatic beta cell via GLUT2 transporter and causes DNA alkylation followed by the activation of poly ADP ribose polymerase leading to depletion of NAD^+ and ATP. Enhanced ATP dephosphorylation after STZ treatment supplies substrate for xanthine oxidase resulting in the formation of superoxide radicals and also nitric oxide moiety is liberated. As a net result, destruction of β-cells occurs by necrosis [35]. However, the dose of streptozotocin required for inducing diabetes depends on the animal species, route of administration, dosage, duration and nutritional status.

According to the administered dose of streptozotocin, features similar to either type 1, or type 2 diabetes can be induced [36]. The intraperitoneal administration of single low dose of STZ (45 mg/kg) induces moderate diabetes by selectively destroying the pancreatic beta cells [37]. Hence, streptozotocin-induced

experimental diabetes in rats was chosen in the present study.

STZ-induced diabetes is characterized by a severe loss of body weight, which is due to increased catabolism of tissue proteins and due to unavailability of carbohydrate as energy source as a result of deficient insulin secretion leading to significant reduction in the body weight gain of diabetic rats, which was observed in the present study [38]. A significant increase in the body weight observed in diabetic rats treated with fruit extract indicates the beneficial effect of the extract in controlling muscle wasting.

The oral glucose tolerance test (OGTT) measures the body's ability to utilize glucose, the body's main source of energy. An oral glucose tolerance test is a perceptive measure of early abnormalities in glucose regulation than fasting plasma glucose [39]. Impaired glucose tolerance due to pancreatic dysfunction results in the defective utilization of glucose by the tissues and increased hepatic gluconeogenesis [40]. The impaired glucose tolerance observed in STZ induced diabetic group of rats were altered to near normal by the treatment with fruit extract which indicates improved glucose homeostasis.

Hyperglycemia is considered as a main factor in the development and progression of the complications of diabetes mellitus [41]. STZ induced diabetes leads to beta cell necrosis resulting in insulin deficiency and decreased utilization of glucose by the peripheral tissues contributes to hyperglycemia [42]. Also, hyperglycemia and renal glycosuria are the most critical abnormalities in diabetes. Therefore, the hypoglycemic effect and consequent decrease in urine sugar excretion have been considered as one of the essential characteristics of anti-diabetic agents [43]. In the present study, oral administration of fruit extract to diabetic rats showed the absence of urine sugar due to the normalization of blood glucose levels. The reduction in blood glucose level accompanied with the absence of glucose in urine (glycosuria) indicates the hypoglycemic nature of the fruit extract.

Glycated hemoglobin or βN-1-deoxyfructosyl-hemoglobin (HbA1c) is the product of a nonenzymatic reaction of glycation, namely a condensation between the aldehydic group of glucose and the amino group of the terminal valine in the β-chain of hemoglobin A_0. The amount of HbA1c is strictly related to blood glucose concentration. Considering the average life span of red cells, the HbA1c value should mimic the mean glycemic value of the previous 2–3 months. The American Diabetes Association (ADA) recommends HbA1c determination in patients with diabetes mellitus on therapy in order to monitor the glycometabolic status in the medium–long term and thus reduce the risk of vascular complications [44]. During diabetes, the excess of glucose present in the blood reacts irreversibly with amino groups of lysine residues in Hb to form HbA1c. Diabetic rats showed higher levels of HbA1c indicating their poor glycemic control. Oral administration of fruit extract to diabetic rats decreased the levels of glycosylated hemoglobin by virtue of its hypoglycemic activity. This normalization of HbA1c indicates decreased glycation of proteins and confirms the anti- diabetic potential of fruit extract.

Chemically induced diabetes causes a notable reduction in insulin release by the destruction of the pancreatic β-cells and thereby induces hyperglycemia [45]. In the present study, the decreased plasma insulin content was elevated upon treatment with the fruit extract. The possible mechanism by which *P. dulce* fruit extract brings about its antidiabetic action may be by potentiating the insulin effect through the stimulation of insulin release from remnant pancreatic β cells or its release from the bound form. In the above context a number of other plants have also shown similar mechanism [46, 47, 48].

Defect in insulin action/secretion leads to defective amino acid/protein metabolism, which may be a more crucial factor than hyperglycemia in the etiology of some diabetic complications [49]. Experimentally induced diabetes in rat model indicates several alterations of amino acid metabolism, which may be attributed to increased muscle proteolysis, reduced protein synthesis, an energy-dependent process in the liver, and stimulated hepatic gluconeogenesis utilizing gluconeogenic amino acids [50]. This readily accounts for observed decrease in the total protein content in STZ-induced rats. Administration of fruit extract to diabetic rats significantly inhibits proteolysis caused by insulin deficiency and improves total protein level.

The amount of urea in the blood is increased with concomitant decrease in plasma protein levels in experimental diabetes as a result of increased breakdown of plasma and tissue proteins due to negative nitrogen balance. Further, the supraphysiological concentration of glucose in diabetic state causes severe derangement in protein metabolism that result in the development of negative nitrogen balance. This in turn elevates urea and creatinine levels [51] which acts as biochemical diagnostic markers for assessing renal impairment and drug-induced toxicity [52]. Serum creatinine concentration is often used as a variable not only to assess impairment of kidney function but also as clinical end point to detect treatment related toxic effects of compounds on the kidney in experimental animals [53]. The observed alteration in the levels of blood urea and serum creatinine in group of diabetic rats reverted to near normalcy by treatment with fruit extract, indicating renal protective nature of the extract during glucose toxicity.

The level of purines is elevated due to accelerated muscle wasting. These accumulated purines are the main source for the production of uric acid by the activity of xanthine oxidase [54]. This accumulated purines evidence the increased oxidative stress which is closely related to diabetes and its vascular complications. In the present study, the increased levels of serum uric acid observed in diabetic rats were restored to near normalcy by the administration of fruit extract indicating the free radical scavenging activity of fruit extract.

Glycogen is a storage form of carbohydrates in vertebrates. The excess glucose is converted into glycogen and stored as an energy fuel in tissues, predominantly in the liver and skeletal muscle [55]. During diabetes, there is a decrease in liver weight due to enhanced catabolic processes such as glycogenolysis, lipolysis and proteolysis [56] and therefore the quantification of glycogen, the primary intracellular storage form of glucose in liver can be considered as an important indicator of diabetes mellitus. A significant decline in the glycogen level was observed in diabetic group of rats. Oral treatment with fruit extract as well as gliclazide to diabetic rats restored the level of glycogen indicating the improved glucose homeostasis.

Transaminases such as ALT and AST are the intracellular cytosolic enzymes that have leaked into the circulation and serve as a marker of tissue injury chiefly hepatocyte as well as renal injury. ALP acts as a marker of biliary function and cholestasis. It is hypothesized that elevation in the levels of serum ALT, AST and ALP are considered as predictors of diabetes. The cytosolic enzymes AST, ALT and membrane bound ALP are the physiological markers normally present in low levels in serum and their activities elevated during tissue damage. A rise in ALT activity indicates the hepatocellular damage followed by cardiac tissue damage and is usually accompanied by a rise in AST activity. Further, ALP is a

marker of biliary function and cholestasis. The observed increase in activities of these enzymes in the serum of diabetic rats may be due to the leakage of these enzymes from the liver cytosol into blood stream as a consequence of the hepatic tissue damage [57]. The reversal of AST, ALT and ALP activities in fruit extract treated diabetic rats towards near normalcy indicate the nontoxic as well as hepatoprotective nature of the fruit extract.

5. Conclusion

The results of the present study clearly indicate that *Pithecellobium dulce* fruit extract possess significant antidiabetic activity which is evidenced from improved OGTT, HbA1c, and glycogen content. Also, the effect of the fruit extract on biochemical alterations indicates the nontoxic as well as beneficial effect of fruits in maintaining the normoglycemia. The possible mechanism of the antidiabetic action of *Pithecellobium dulce* may be through inhibitory effect of glucose absorption, increased incorporation of circulating glucose as hepatic glycogen and enhanced secretion of insulin. The phytochemicals present in the fruit extract may account for the observed pharmacological actions. The results of the present study also provide a scientific rationale for the use of *Pithecellobium dulce* fruit in the traditional medicine system.

6. Disclosure of Interest

The authors declare that they have no conflict of interest.

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