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Antioxidant and antidiabetic activity of *Phoenix pusilla* Gaertn. unripe fruit extract in streptozotocin-induced sprague dawley rats

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ABSTRACT

The fruit of *Phoenix pusilla* Garetn. has been used in herbal medicines, as it is sweet, sour, cooling and laxative, cardiotonic, aphrodisiac, carminative and roborant. The objective of the present study was to investigate the antioxidant and antidiabetic effect of ethanolic extract of unripe fruit of *Phoenix pusilla* in streptozotocin-induced diabetic rats. The extract was analyzed for the presence of various phytoconstituents like tannins, flavonoids, vitamin C, vitamin E, protein, carbohydrates, lipids and phenolic compounds. Streptozotocin (40 mg/kg body weight, i.p.) was administered to induce diabetes in adult rats. The extract (100 and 200 mg/kg) and glibenclamide (6 mg/kg) were administered orally for 21 days to evaluate antioxidant and antidiabetic activity. Blood glucose, serum total cholesterol and triglycerides levels were estimated. Carbohydrate and lipid metabolizing enzymes glucose-6-phosphatase, fructose-1,6-diphosphatase, glycolytic enzymes like hexokinase and liver-function enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), production of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALP), renal-function markers like urea and creatinine level were also measured. Histopathology of pancreas was studied. The results indicated that extract normalized the blood, liver, renal and pancreatic functions in streptozotocin-induced diabetic rats. Hence it can be concluded that the extract possesses antioxidant and antidiabetic activity. The findings support the conventional usage of Phoenix pusilla unripe fruit in treating diabetes.

Keywords: Hyperglycemia, Phytochemicals, Extraction, Diabetes, Cholesterol, Histopathology.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) level that result from defects in insulin secretion, or its action, or both. Diabetes mellitus, commonly referred to as diabetes, was first identified as a disease associated with "sweet urine," and excessive muscle loss in the ancient world. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine. Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas. Insulin lowers blood glucose level [1-3].

Diabetic complications arise partly from glycosylation damage to structural and functional proteins and reflect chronic failure to maintain blood glucose homeostasis. Other complications such as diabetic nephropathy, diabetic retinopathy, diabetic neuropathy and diabetic cardiomyopathy prevail as a result of hyperglycemia. Non-insulin dependent diabetes mellitus accounts for over 85% of diabetes mellitus worldwide and is associated with a high incidence of morbidity and mortality [4]. There is an estimated 143 million people worldwide suffering from the disease and this is almost five times the estimate ten years ago. It has been predicted that the number may probably double by the year 2030. With an estimated 50.8 million people living with diabetes, India has the world's largest diabetes population, followed by China with 43.2 million [5].

Fruits and vegetables have been implicated in preventing or reducing the risk of coronary heart disease, [6, 7] cancer [8, 9] and other chronic diseases [10]. For these reasons, recommendations to increase the dietary intakes of fruits and vegetables have been suggested even for diabetes by many world authorities [11].

Phoenix pusilla Gaertn., (Family: Arecaceae) a multipurpose palm species closely related to the date palm, is commonly known as the small date palm in India [12]. It grows wild in dry areas in India at

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low elevations. The fruit is 20–22 mm long, oblong, terete and fleshy. It is initially green, red at maturity, and black at ripening. The seeds are oblong and ventrally grooved [13]. Its flowering season starts in November and runs through January. Clusters of edible orange-red fruits turn into black drupes in the months of July and August [14]. Its fruit is used in herbal medicines, as it is cooling and laxative, cardiotonic, aphrodisiac, carminative and roborant. The fruit is also used for hyperdipsia, burning sensation, fevers, consumption, cardiac debility, seminal weakness, gasteropathy and general debility [15].

Previous study has reported α-amylase and α-glucosidase enzyme inhibitory activity [16]. Though ethno-medicinally well recognized, until now there is no published scientific reporting, where pharmacological investigations related to antidiabetic activity of this plant have shown. Hence in the present study, *Phoenix pusilla* unripe fruit ethanol (PFE) powdery crude extract was investigated for its antioxidant snf antidiabetic activity *in vivo* in streptozotocin-induced diabetic rat model and the same was compared with glibenclamide, a standard hypoglycemic drug.

MATERIALS AND METHODS

Drug and chemicals

Streptozotocin was obtained from Sigma chemicals, Bangalore, India. Kits to estimate glucose, triglyceride (TG), total cholesterol (TC), superoxide dismutase (SOD), glutathione peroxidase (GPx), thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), serum glucose-6-phosephatase(G-6-P), fructose-1,6-diphosphatase (F-1,6-diP), hexokinase, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALP), urea and creatinine were purchased from Accurex Biomedical Pvt. Ltd., Mumbai. All other chemicals used were of analytical grade.

Plant material and extraction

The plant material (unripe fruits of *Phoenix pusilla*) used for the investigation was obtained from in and around Vellore District, Tamilnadu and authenticated by Prof. Jayaraman, Plant Anatomy Research Center, Chennai, India. The shade-dried unripe fruits were pulverized to get coarse powder. The coarse powder was extracted with 80% ethanol in soxhlet apparatus. The extract was evaporated to dryness in a rotary vacuum evaporator. The residue, *Phoenix pusilla* unripe fruit ethanol (PFE) powdery crude extract was stored in air-tight container until used for the experiment.

Phytochemical screening

Preliminary phytochemical analysis was carried out by employing standard phytochemical procedures to identify the various primary and secondary metabolites.

Experimental animals

Male Sprague Dawley rats (160-180 g) were used for the study. Animals were housed in groups of 6 animals/cage in polypropylene cages in a well-ventilated room (air cycles: 15/min; recycle ratio (70:30)) under an ambient temperature of 22±3°C and 40–65% relative humidity, with a 12-h light/dark artificial light cycle. They were provided with rodent feed (M/s. Provimi Animal Nutrition India Pvt. Ltd, India) and purified water *ad libitum*. The Institutional Animal Ethics Committee (IAEC), Sri Ramachandra University, Chennai, India approved the study.

Acute oral toxicity study

Acute oral toxicity study was performed according to the OECD test guideline 423 – Acute toxic class method [17]. Young healthy adult Sprague Dawley female rats (120-180 g) were divided into two groups of 3 animals each. PFE extract was administered once orally via gastric intubation at a dose level of 2000 mg/kg b. wt. Lethality and abnormal clinical signs were observed on the day of dosing and thereafter for 14 days. Body weight was recorded before dosing and thereafter once in a week till completion of the experiment. Gross pathological changes were also observed at the end of experiment.

Induction of diabetes

Diabetes was induced in rats by single intraperitoneal injection of streptozotocin (STZ) in citrate buffer (pH 4.5) at a dose of 40 mg/kg b. wt. After 72 h of induction, the animals which showed fasting blood glucose ≥ 250 mg/dl were considered diabetic and selected for the study. The rats were divided into five groups of six rats each. The PFE extract suspended in 0.5% CMC was administered orally for 21 days.

Group I: Normal control (saline)
Group II: STZ (40 mg/kg b. wt. i.p.)
Group III: STZ + glibenclamide (GBC) (6 mg/kg b. wt. p.o.)
Group IV: STZ + PFE extract Low Dose (100 mg/kg b. wt. p.o.)
Group V: STZ + PFE extract High Dose (200 mg/kg b. wt. p.o.)

Biochemical analysis and histopathology

Blood samples were withdrawn by retro-orbital puncture under light ether anaesthesia on the 0th, 7th, 14th and 21st day of the study for estimating blood glucose and body weight. At the end of 21 days treatment, the animals were euthanized and the levels of TG and TC, AST, ALT, ALP, urea and creatinine were determined using commercial kits following the manufacturer standard protocols in a semi-auto analyzer. Activity of superoxide dismutase (SOD) [18], GPx [19], reduced glutathione (GSH) [20], TBARS [21], glucose 6-phosphatase [22], fructose 1, 6-bisphosphatase [23], hexokinase [24], AST and ALT [25], ALP [26], urea [27] and creatinine [28], were assayed using standard procedures. Histological structures of liver and pancreatic sections were examined using a light microscopy (Motic DMB1-2MP, China).

Statistical analysis

Data were expressed as Mean ± SEM of six replicates and subjected to one-way ANOVA followed by Tukey's multiple comparison tests using Graph Pad Prism 5.03 (Graph Pad Software, San Diego, CA, USA). Values were considered statistically significant at $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS

Acute oral toxicity

No treatment-related deaths, abnormal clinical signs or remarkable body weight changes were observed in all the experimental animals. No gross pathological observation was recorded in all the

experimental animals. From the above tested condition, LD_{50} of the test drug was found to be greater than 2000 mg/kg b. wt., and was found to be safe when administered once orally to fasted female Sprague Dawley rats.

Effect of PFE extract on body weight

Degeneration of muscle tissue, conversion of glycogen to glucose and weight loss is a serious problem in management of diabetes mellitus [29]. There was significant ($p<0.001$) decrease in body weight of STZ-induced diabetic control group as compared to normal control group. Body weight increased significantly ($p<0.001$) after treatment with 200 mg/kg PFE extract when compared with diabetic control. Progress in weight gain of animals in extract-treated group was observed till the end of the study (Table-1).

Effects of PFE extract on blood glucose levels

The effect of repeated oral administration (21 days) of PFE extract on blood glucose levels in STZ-induced diabetic rats is represented in Table-2. In diabetic control group, significant increase ($p<0.001$) in blood glucose level compared to the normal control group animals was observed and blood glucose levels was on the increase on the 7th, 14th and 21st day. PFE extract at 100 mg/kg (p<0.01) and 200 mg/kg showed maximum ($p<0.001$) blood glucose reducing effect from the 14th day in diabetic rats as compared to diabetic control rats.

Table 1: Effect of PFE extract on body weight

Group	Body weight (g)			
	Day 0	Day 7	Day 14	Day 21
Normal	168.6±4.615	175.8±4.970	182.2±4.324	188.6±4.506
STZ (40 mg/kg)	133.2±5.848$^{###}$	127.4±5.177$^{###}$	120.6±4.037$^{###}$	116.8±4.970$^{###}$
STZ + GBC (6 mg/kg)	139.6±4.278	144.8±3.493	153.0±4.950***	162.2±3.114***
STZ + PFE (100 mg/kg)	138.0±6.595	143.2±6.458	148.4±4.722**	151.8±5.168***
STZ + PFE (200 mg/kg)	140.2±4.817	147.6±6.107	151.6±5.128***	158.6±6.066***

All values are Mean ± SEM for six rats. $^{###}$ indicates $p<0.001$ vs. group I; **, *** indicates $p<0.01$, $p<0.001$ respectively vs. group II

Table 2: Effect of PFE extract on blood glucose levels

Group	Blood glucose level (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
Normal	90.06±3.661	92.98±4.162	93.30±4.370	92.86±2.666
STZ (40 mg/kg)	261.9±3.969$^{###}$	263.3±4.525$^{###}$	265.3±4.927$^{###}$	270.8±3.553$^{###}$
STZ + GBC (6 mg/kg)	260.4±5.459	172.2±5.913***	129.8±4.920***	94.64±3.542***
STZ + PFE (100 mg/kg)	264.3±7.268	241.5±5.854*	211.0±4.950***	184.5±4.742***
STZ + PFE (200 mg/kg)	266.5±9.203	234.8±4.826***	177.9±5.094***	145.6±4.726***

All values are Mean ± SEM for six rats. $^{###}$ indicates $p<0.001$ vs. group I; *, *** indicates $p<0.05$, $p<0.001$ respectively vs. group II

Table 3: Effect of PFE on lipid profile

Group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)
Normal	69.07±5.217	42.96±4.153
STZ (40 mg/kg)	105.50±5.045$^{###}$	147.60±6.923$^{###}$
STZ + GBC (6 mg/kg)	72.16±5.885***	34.99±4.752***
STZ + PFE (100 mg/kg)	76.90±4.553**	41.26±4.217***
STZ + PFE (200 mg/kg)	68.92±3.275***	32.12±2.923***

All values are Mean ± SEM for six rats. $^{###}$ indicates $p<0.001$ vs. group I; **, *** indicates $p<0.01$, $p<0.001$ respectively vs. group II

Effect of PFE extract on lipid profile

A significant ($p<0.001$) increase in the levels of total cholesterol (TC) and triglyceride (TG), were observed in STZ-induced diabetic rats as compared with normal control group. Daily oral treatment with PFE extract 100 and 200 mg/kg reversed the STZ-induced changes in lipid profile. There was significant ($p<0.001$) decrease in the levels of TC and TG compared to diabetic control. PFE extract at a dose of 200 mg/kg was more effective than 100 mg/kg in correcting the lipid levels (Table-3).

Effect of PFE extract on glucose metabolizing enzymes

The level of serum hexokinase decreased, while the level of G-6-P and F-1, 6-diP increased in STZ-diabetic rats as compared to normal rats. Oral administration of PFE extract (200 mg/kg), decreased ($p<0.01$) the activity of G-6-P and F-1,6-diP enzyme and increase the activity of hexokinase enzyme significantly ($p<0.001$) when compared to diabetic control rats (Table-4).

Effect of PFE extract on antioxidant enzymes

STZ induced diabetic rats had decreased GSH, SOD, GPx, enzyme levels in liver as compared to control. Administration of PFE extract to the diabetic increased the levels of GSH, SOD, and GPx. STZ diabetic rats were found to exhibit significant ($p<0.001$) increase in TBARS level in liver as compared to normal rats. Treatment with PFE extract produced significant ($p<0.001$) decrease in TBARS compared to diabetic rats (Table-5).

Effect of PFE extract on renal and hepatic markers

The efficacy of PFE extract on renal markers was analyzed on the 21st day (Table-6). At 200 mg/kg extract, there was a significant reduction ($p<0.01$) in the urea and significant ($p<0.001$) reduction in creatinine levels when compared with the diabetic control group. Table-7 represents the effect of PFE extract on hepatic markers: serum AST, ALT and ALP. These pathophysiological indices in diabetic rats were significantly ($p<0.001$) elevated as compared with normal rats. Oral administration of PFE extract to diabetic rats significantly ($p<0.001$) normalized the altered ALP and AST levels in comparison with diabetic rats. Levels of ALT in 200 mg/kg PFE treated rats significantly ($p<0.001$) reduced compared to diabetic rats.

Table 4: Effect of PFE extract on glucose metabolizing enzymes

Group	Glucose-6-phosphatase (U^a/mg protein)	Fructose-1,6-diphosphatase (U^b/mg protein)	Hexokinase (U^c/mg protein)
Normal	0.260±0.011	0.488±0.03	0.48±0.044
STZ (40 mg/kg)	$0.543±0.036^{\#\#}$	$0.917±0.039^{\#\#\#}$	$0.18±0.030^{\#\#\#}$
STZ + GBC (6 mg/kg)	$0.211±0.092^{***}$	$0.515±0.045^{***}$	$0.46±0.038^{***}$
STZ + PFE (100 mg/kg)	$0.360±0.033^{ns}$	$0.727±0.045^{ns}$	$0.28±0.024^{ns}$
STZ + PFE (200 mg/kg)	$0.292±0.022^{**}$	$0.657±0.065^{**}$	$0.36±0.025^{***}$

All values are Mean ± SEM for six rats. $^{\#\#}$, $^{\#\#\#}$ indicates $p<0.01$, $p<0.001$ respectively vs. group I; **, *** indicates $p<0.01$, $p<0.001$ respectively vs. group II; ns = no significance; a=U-µmol of Pi liberated/minute/mg of protein; b=U-µmol of Pi liberated/hour/mg protein; c=U-µmol of inorganic phosphate liberated/minute for hexokinase

Table 5: Effect of PFE extract on in vivo anitoxidant parameters

Group	GSH^a (mg/100g of tissue)	SOD (U/mg of protein)	GPx (U/mg of protein)	TBARS (mmoles/100g of tissue)
Normal	133.0±10.960	11.66±0.327	13.31±1.218	1.35±0.201
STZ (40 mg/kg)	$60.09±6.151^{\#\#\#}$	$6.60±0.337^{\#\#\#}$	$4.49±0.112^{\#\#\#}$	$3.87±0.071^{\#\#\#}$
STZ + GBC (6 mg/kg)	$125.5±10.210^{***}$	$12.19±0.172^{***}$	$9.03±0.885^{**}$	$2.63±0.259^{**}$
STZ+ PFE (100 mg/kg)	$117.10±10.160^{**}$	$9.68±0.709^{***}$	$7.58±0.775^{ns}$	$1.71±0.374^{***}$
STZ + PFE (200 mg/kg)	$108.80±8.846^{**}$	$10.49±0.373^{***}$	$8.22±0.81^{*}$	$1.69±0.123^{***}$

All values are Mean ± SEM for six rats. $^{\#\#\#}$ indicates $p<0.001$ vs. group I; *, **, *** indicates $p<0.05$, $p<0.01$, $p<0.001$ respectively vs. group II; ns = no significance

Table 6: Effect of PFE extract on renal markers

Group	Urea (mg/dl)	Creatinine (mg/dl)
Normal	28.58±2.987	1.25±0.098
STZ (40 mg/kg)	$46.82±2.271^{\#\#\#}$	$2.93±0.169^{\#\#\#}$
STZ + GBC (6 mg/kg)	$26.60±2.769^{***}$	$1.41±0.097^{***}$
STZ + PFE (100 mg/kg)	$34.01±2.955^{*}$	$0.36±0.033^{***}$
STZ + PFE (200 mg/kg)	$30.95±2.709^{**}$	$0.29±0.021^{***}$

All values are Mean ± SEM for six rats. $^{\#\#\#}$ indicates $p<0.001$ vs. group I; *, **, *** indicates $p<0.05$, $p<0.01$, $p<0.001$ respectively vs. group II.

Table 7: Effect of PFE extract on hepatic markers

Group	ALP[a] (IU/L)	AST[b] (IU/L)	ALT[b] (IU/L)
Normal	81.23±8.767	76.14±7.970	23.20±2.350
STZ (40 mg/kg)	153.60±6.141###	147.20±9.580###	116.70±7.527###
STZ + GBC (6 mg/kg)	78.13±8.415***	81.69±8.703***	26.33±2.722***
STZ + PFE(100 mg/kg)	110.60±12.070*	79.38±8.526***	40.83±5.092***
STZ + PFE(200 mg/kg)	91.81±10.910***	81.64±8.612***	31.53±3.419***

All values are Mean ± SEM for six rats. ### indicates p<0.001 vs. group I; *, *** indicates p<0.05, p<0.001 respectively vs. group II; a-µmole of phenol liberated/min; b-µmol of pyruvate liberated/hour

Histopathological studies

Histopathology of pancreas was studied in normal rats, diabetic rats, glibenclamide-treated rats and extract-treated rats. The normal histological pancreas section shows normal acini and normal cellular population in islets of Langerhans. There is no damage to islets. In STZ-induced diabetic rats, partial destruction of pancreatic islets was noticed. Histopathology of diabetic rats treated with standard drug glibenclamide (6 mg/kg) shows normal cellular population size of islets of Langerhans. Histopathology of diabetic rats treated with PFE (100 mg/kg) shows slight improvement in cellular population size of islets of Langerhans. Histopathology of diabetic rats treated with PFE (200 mg/kg) showed restoration of the pancreas to normal.

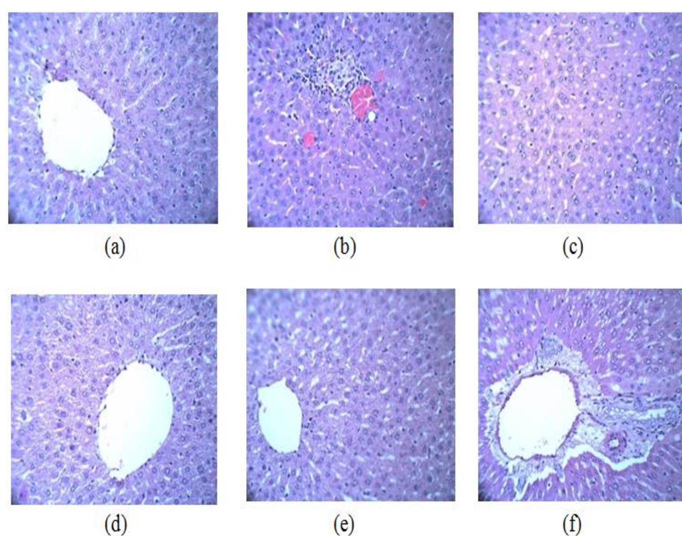

Figure 1: Photomicrograph of rat liver tissue of (a) normal group, showing normal portal triad, bile duct, hepatic artery along with normal hepatocytes with a hepatic vein; (b) diabetic group, showing infiltration of inflammatory cells with congestion in hepatic vein; (c) diabetic group, showing large and binucleated hepatocytes; (d) glibenclamide-treated group, showing normal central vein, hepatocytes with sinusoids; (e) 100 mg/kg PFE-treated group, showing normal hepatocytes with normal central vein; (f) 200 mg/kg PFE-treated group, showing normal hepatocytes, hepatic artery, hepatic vein, bile duct, sinusoids and decreased periportal inflammation.

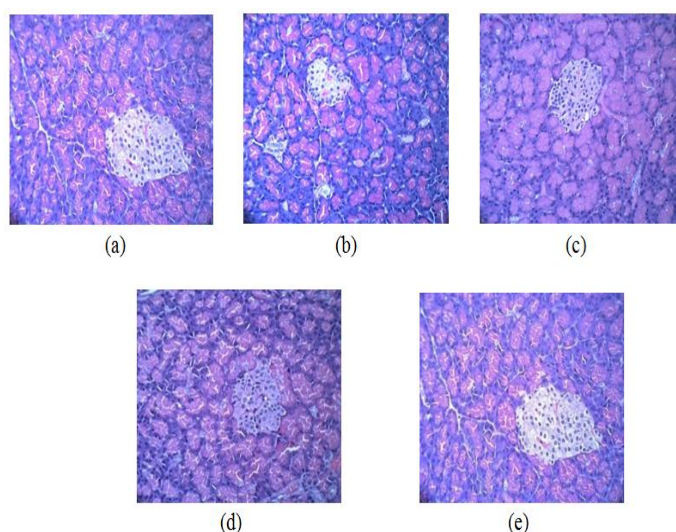

Figure 2: Photomicrograph of rat pancreas tissue in (a) normal group, showing intact, big and round clusters of islet cells surrounded by exocrine acini of pancreas; (b) diabetic group, showing shrunken islets of Langerhans displaying degenerative changes in β-cells leading to decrease in the number of functioning β-cells; (c) glibenclamide-treated group, showing increase in the number of functioning β-cells and acinar cells around the islets though seem to be in normal proportion; (d) 100 mg/kg PFE-treated group and (e) 200 mg/kg PFE-treated group showing restoration of normal pancreas histology.

DISCUSSION

This study was undertaken to evaluate the antioxidant and antidiabetic activity of PFE extract in diabetic rats. To assess the antidiabetic potential of plant extract, streptozotocin-induced diabetic rats is a very good model to study. Streptozotocin induced diabetes leads to significant hyperglycemia, hypoinsulinemia, weight loss accompanied by altered lipid profile, carbohydrate and lipid metabolizing enzymes levels. Streptozotocin prevents DNA synthesis by inhibiting many of the enzymes involved in DNA synthesis [30].

Administration of STZ selectively destroys the β-cells of the islets of Langerhans [31]. The destruction of β-cells cause the marked decrease in insulin levels [32]. In this study the results indicate that fruit extract of PFE was able to decrease the level of blood glucose in STZ-induced diabetic rats. The antihyperglycemic action of this extract is possible through the insulinomimetic action or by other mechanisms such as stimulation of glucose uptake by peripheral tissues, inhibition of endogenous glucose production, or activation of gluconeogenesis in liver and muscles, as similar mechanisms have been reported for plant extracts with antidiabetic activity [33].

In STZ-induced diabetic rats, body weight decreased due to lipolysis, muscle destruction and degeneration of structural proteins as a consequence of insulin insufficiency [34, 35]. Treatment with PFE extract and glibenclamide improved body weight of diabetic rats, which could be due to increased insulin secretion. From Table-1 it is clear that diabetic rats treated with PFE extracts showed an increase in body weight which proves that the PFE extracts restored the muscle and tissue proteins.

Another possible mechanism may be attributed to the rich fiber content of PFE. Dietary fibers play a major role in lowering the blood glucose level by slowing the rate of carbohydrate absorption from intestine and are hence beneficial for diabetes, especially type II diabetics [36]. Under normal conditions, the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hypertriglyceridemia. Dietary fibers lower the cholesterol and triglyceride levels [37]. Therefore, the significant control of levels of serum lipids in the treated groups may be attributed to the rich fiber content in *Phoenix pusilla*. The altered lipid profiles were reaches to near normal level after the treatment of PFE extract and glibenclamide in STZ induced diabetic rats. This lipid lowering action may be due to proper stabilization of glucose level and increase in insulin level after the administration of test extract, which may normalize the disturbed lipid metabolism in diabetic rats. Therefore, hypolipidemic effect of PFE in diabetic rats supports its ability to prevent the cardio vascular diseases (CVD) associated with diabetes.

The important pathways which regulate glucose and insulin levels are glucose-regulated insulin secretion, hepatic glucose synthesis and insulin-mediated glucose uptake in the tissues. Gluconeogenesis and glycolysis are the two main metabolic pathways in liver that regulate glucose level in blood. Gluconeogenic pathway is mainly regulated by the enzymes like glucose-6-phosphatase and fructose-1, 6-disphosphatase. Glucose-6-phosphate is converted to glucose mediated by the enzyme glucose-6-phosphatase and also lipogenesis process that is the conversion of carbohydrate or protein to fat [38]. Fructose-1, 6-diphosphatase mediate the conversion of fructose-1,6-diphosphate to fructose-6-phosphate, which determines whether or not a tissue is capable of resynthesizing glycogen from pyruvate triose phosphates [39]. Glucose- 6-phosphatase and fructose-1, 6-disphosphatase enzymes levels were significantly increased in STZ-induced diabetic rats. PFE extract decreased the activity of these two enzymes in the liver of diabetic rats.

Hexokinase is the prime enzyme catalyzing glucose phosphorylation. The first step in glycolysis is severely impaired during diabetes. Impairment of hexokinase activity suggests impaired oxidation of glucose via glycolysis leading to its accumulation resulting in hyperglycemia [40]. In the present study, hexokinase activity was found to be decreased in diabetic rats which may be due to insulin deficiency. Treatment with PFE extract elevated the activity of hexokinase in liver. PFE extract may stimulate insulin secretion, which may activate hexokinase, thereby increasing utilization of glucose leading to decreased blood sugar levels.

Consequently, the levels of GSH, SOD and GPx were improved by the application of PFE extract on diabetic rats. Earlier reports indicate that STZ-induced diabetic animals may exhibit most of the diabetic problem mediated through oxidative stress [41]. GSH, SOD and GPx are the three effective scavenging enzymes that remove free radicals *in vivo* [42]. They also play an important role in restoring antioxidant activities in the tissue of diabetic animals [43]. In our study, GSH, SOD and GPx enzymes decreased in STZ-induced diabetic rats, which may be due to inactivation caused by free radicals. An increased level of these antioxidants enzymes with PFE extract may be due to free radical scavenging activity of the extract, which may exert a beneficial effect against pathological alterations caused by reactive oxygen species.

The impaired activities of ALP, AST and ALT levels are associated with hepatic damage or the changes in the permeability of hepatocyte membrane due to oxidative stress. Elevated levels of liver-function enzymes like ALT and AST in serum are not only used for the identification of liver damage but also as a marker for the hepatic insulin resistance. A strong correlation exists between serum ALT level and insulin resistance but not for the AST, and it has been indicated as a predictor of Type 2 diabetes in human subjects [44]. Oral administration of the PFE extracts to STZ-induced diabetic rats decreased the activity of these hepato-specific enzymes indicating its non toxic nature.

Put together, the study shows that administration of PFE extract to STZ-induced diabetic rats restore the body weight of animals and the results were comparable to glibenclamide-treated animals. Since steroids, phenols, polysaccharides and tannins present in plant extracts are known for their antidiabetic activity [45], on the basis of phytochemicals, it may be concluded that synergestic effect of these chemical constituents may be responsible for the collective antioxidant and antidiabetic activity of the extract.

CONCLUSION

Ethanolic extract of *Phoenix pusilla* unripe fruit at a dose of 100 and 200 mg/kg displayed antioxidant and antidiabetic activity in streptozotocin-induced diabetic rats. The results were comparable to reference drug glibenclamide. Activity was due to normalization of carbohydrate and lipid metabolizing enzymes and lipid profile. The extract possesses pancreas regenerating ability and normalized the functions of both pancreas and liver. Therefore, this medicinal plant could be considered as a potential and alternative approach for the treatment of diabetes. However, further pharmacological investigation is in progress to evaluate the precise mechanism of its anti-diabetic action.

Conflict of interest

There are no conflicts of interest.

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