Original Article

Evaluation of the Antidiabetic and Antilipaemic Activities of the Hydroalcoholic Extract of Phoenix Dactylifera Palm Leaves and Its Fractions in Alloxan-Induced Diabetic Rats

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Abstract

Background: **The antidiabetic and antilipaemic effects of Phoenix dactylifera leaf extract (PDE) and its fractions were investigated in various rat models.**

Methods: **Diabetes was induced in male Wistar rats by alloxan monohydrate. Diabetic animals were randomly divided into 8 groups (1 diabetic control and 7 treated groups). Diabetic control animals received saline (5 mL/kg) orally, whereas the treatment groups received different doses of PDE (100, 200, and 400 mg/kg), PDE fractions (50, 100, and 200 mg/kg), or glibenclamide (4 mg/kg) orally once a day for 14 days. Blood was withdrawn for glucose determination on the 1st, 6th, 10th, and 14th days. The rats were fasted overnight and then sacrificed on the 14th day; blood was collected for biochemical evaluation, including the levels of blood glucose, plasma insulin, serum triglyceride, and cholesterol.**

Results: **Subacute administration of PDE or its fractions in alloxan-induced diabetic rats significantly reduced blood glucose ($P < 0.01$). Water intake, serum triglyceride, and cholesterol also decreased in treated animals compared with the control group ($P < 0.01$). Plasma insulin level increased in the treated groups relative to the control group ($P < 0.01$).**

Conclusion: **The results suggested that PDE exhibits antidiabetic and antilipaemic effects in alloxan-induced diabetic rats.**

Keywords: *antidiabetics, antilipaemics, antioxidants, diabetes metabolism, plant extracts*

Introduction

Diabetes mellitus (DM) is a common disorder associated with markedly increased morbidity and mortality rates. DM, which affects a large number of people around the globe, can be defined as a group of metabolic diseases characterised by chronic hyperglycaemia that results from defects in insulin secretion, insulin action, or both, and causes impaired function in carbohydrate, lipid, and protein metabolism. Pharmacological treatment of DM is based on oral hypoglycaemic agents and insulin, but these clinical approaches either do not succeed in restoring normoglycaemia in most patients or fail after a variable period. Moreover, continuous use of synthetic antidiabetic drugs causes side effects and toxicity (1,2). Therefore, there is a need for natural and non-toxic antidiabetic drugs for diabetic therapy. Some studies have demonstrated the hypoglycaemic effects of different parts of various species of palm. It has been shown that an aqueous extract from the leaves of the European fan palm, *Chamaerops humilis*, decreased plasma glucose levels as well as total cholesterol and triglycerides levels (3). Additionally, hypoglycaemic compounds have been found in the roots of *Arocomia aculeata* in many studies (4–6); this coincides well with its traditional use in the treatment for diabetes among indigenous peoples in Mexico. Palm oil tocotrienol-rich fractions were found to reduce the blood glucose level and improve dyslipidaemia in streptozotocin-induced diabetic rats (7); whereas the alcoholic extract of palm seeds decreased the blood glucose and lipid concentration in male diabetic rats (8). However, a clinical trial showed that there was no significant difference in plasma glucose levels after eating

dates versus sugar cubes in patients with type 1 diabetes mellitus. Thus, eating dates in diabetic patients is not preferable to eating sugar cubes (9). A biochemical study showed that date sugar has high alpha-glucosidase, alpha-amylase, and angiotensin I-converting enzyme (ACE) inhibitory activities that correlate with its high total phenolic content and antioxidant activity (10). It has been shown that these agents have beneficial effects in the management of hypertension in type 2 diabetes mellitus (10–11).

The fruit of the plant, date, is used as a food and a beverage, as well as a remedy against various complaints in traditional medicine (8). Its leaves are also used as a folk remedy in the United States and southwest Iran to reduce blood glucose level in diabetes (8–12).

Because there have been no studies assessing the antidiabetic effect of *P. dactylifera* leaves on diabetic rats, the present study evaluates the antidiabetic and antilipaemic effects of hydroalcoholic extract from *P. dactylifera* leaves (PDE) and its fractions on normoglycaemic, glucose-induced hyperglycaemic, and alloxan-induced diabetic rats.

Materials and Methods

Animals

This study was carried out in Ahwaz Jundi Shapoor University of Medical Sciences in 2009. Male Wistar rats weighing 150–180 g were obtained from the animal house of Ahwaz Jundi Shapoor University of Medical Sciences. The rats were fed on conventional diets and water *ad libitum* and maintained under standard conditions of humidity, temperature (20–24 °C), and light–dark cycle (12-hour light:12-hour dark). The rats were randomly assigned to control and treatment groups consisting of 6 rats per group. All animal experiments were carried out in accordance with Ahwaz Jundi Shapour University of Medical Sciences Ethical Committee acts.

Preparation of PDE and its fractions

P. dactylifera leaves (barhee palm) were collected July–September 2009 from Ahwaz City (Khozestan Province, Iran); they were authenticated by Associate Professor Alemzadeh Ansari N (Department of Horticulture, Faculty of Agriculture, Shahid Chamran University, Iran) and S.S. Marashi (Institute of Date Research and Tropical Fruits, Ahvaz, Iran). The voucher specimen (No. Irandate, 10-005) is deposited in the Herbarium, Jundi Shapour University of Medical Sciences. The collected leaves were dried in shade and then powdered. The powder was macerated in 70% ethanol (1:10, w/v) for 3 days with occasional shaking. The combined ethanolic extract was filtered through cheesecloth and centrifuged at 3000 g for 15 minutes. The supernatant was concentrated and lyophilised for preservation (yield = 8.2%) and then stored at 4°C until use.

Fractionation was carried out with solvents of increasing polarity to obtain organic and aqueous fractions. Twenty grams of PDE were dissolved in about 50 mL of distilled water. The same volume of chloroform was added with vigorous shaking. The chloroform layer (lower) was collected thrice and evaporated in a rotary evaporator to give the chloroform fraction, ChCl3 Fr (yield = 16%). The other layer (upper) was again taken into a separating funnel, ethyl acetate was added, and it was separated and then evaporated in rotary evaporator to give the ethyl acetate fraction, EtOAc Fr (yield=6%). The remaining lower layer was collected and evaporated to obtain the aqueous fraction, Aq Fr (yield=11%) (13).

Acute toxicity assessment

To assess acute toxicity, the extract was orally administered in graded doses (1, 2, 4, 6, and 8 g/kg) to 5 treatment groups, while the control group received saline (5 mL/kg). All treated animals were closely observed for any abnormal or toxic manifestations and mortality up to 48 hours.

Preliminary phytochemical screening

PDE was subjected to qualitative chemical screening to identify the various major classes of active chemical constituents, namely tannins, steroid, terpenoids, saponins, flavonoids, and alkaloid.

Test For Tannins: 500 mg of extract was stirred with about 10 mL of distilled water and then filtered. Four drops (0.3 ml) of 1% ferric chloride solution were added to 2 mL of the filtrate. The occurrence of a blue–black, green, or blue–green precipitate indicated the presence of tannins (14).

Liebermann–Burchard Test For Steroids: To 0.2 g of extract, 2 mL of acetic acid was added, the solution was cooled in ice, and then concentrated H_2SO_4 was carefully added. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring, i.e., a glycone portion of cardiac glycoside (15).

Test For Terpenoids: 100 mg of extract was dissolved in ethanol. Then, 1 mL of acetic anhydride was added, followed by the addition

of concentrated hydrogen sulphate. A change in colour from pink to violet showed the presence of terpenoids (15).

Test For Saponins: 1 g of extract was boiled with 5 mL of distilled water and filtered. Then, 3 mL of distilled water was added to the filtrate, and the mixture was shaken vigorously for about 5 minutes. Frothing that persisted upon warming was taken as evidence of the presence of saponins (15).

Shinoda's Test For Flavonoids: 500 mg of extract was dissolved in ethanol, warmed, and then filtered. Three magnesium chips were then added to the filtrate followed by few drops of concentrated hydrochloric acid. Colour changing from pink, orange, or red to purple indicated the presence of flavonoids (14).

Ferric Chloride Test For Flavonoids: 500 mg of extract was boiled with distilled water and then filtered. Four drops (0.3 ml) of 10% ferric chloride solution were then added to 2 mL of the filtrate. A green-blue or violet colour indicated the presence of a phenolic hydroxyl group (14).

Lead Ethanoate Test For Flavonoids: 100 mg of the extract was dissolved in water and filtered. Then, 3 mL of lead ethanoate solution was then added to 5 mL of each of the filtrate. The appearance of a buff-coloured precipitate indicated the presence of flavonoids (14).

Test For Alkaloids: 100 mg of the extract was stirred with 5 mL of 1% aqueous hydrochloric acid in a waterbath and subsequently filtered. Then, 1 mL filtrate was taken individually into 2 test tubes. To the first portion, 4 drops of Dragendorff's reagent were added; the occurrence of an orange–red precipitate was taken as positive. To the second portion, Mayer's reagent was added, and the appearance of a buff-coloured precipitate indicated the presence of alkaloids (15).

Induction of Diabetes

A freshly prepared solution of alloxan monohydrate in normal saline solution was injected intra-peritoneally (150 mg/kg) to rats that had fasted overnight. After 1 hour, the animals were allowed to feed ad libitum. Their blood glucose level was checked before and 1 week after alloxan injection with a one-touch glucometer. The animals were considered diabetic when the blood glucose level reached 250 mg/dL of blood (16). Water intake was measured within 3 consecutive days (11^{th}–14^{th} day). Animals were placed in metabolic cages with food and water given ad libitum. Water intake was measured for the entire 72 hours.

Determination of blood glucose, plasma insulin, serum cholesterol, and triglyceride levels

Blood samples (20 µl) were obtained from the tail tip of fasted rats, and blood glucose levels were determined using a one-touch glucometer (Elegance, Frankenberg, Germany). Plasma insulin concentrations were measured using Insulin-EIA Test (DiaPlus Inc, USA). Serum triglyceride (TG) and cholesterol levels were analysed using commercial kits (Roche Diagnostics GmbH, Mannheim, Germany) with a Hitachi autoanalyser.

Determination of PDE hypoglycaemic activity upon acute administration

The hypoglycaemic effect of the extract after a single oral administration was tested in normoglycaemic, glucose-induced hyperglycaemic, and diabetic rats. Each group was further divided into 4 treatment groups: 1 negative control group given normal saline (0.154 mM NaCl solution), 1 positive control group given glibenclamide (4 mg/kg), and 3 test groups given PDE (100, 200, and 400 mg/kg; all suspended in the same vehicle).

Firstly, the fasting blood sugar level of each rat was determined at the beginning of the experiment after overnight fasting with free access to water. Then, the designated treatments were administered orally. For the normoglycaemic and diabetic rats, blood glucose levels were determined at 0, 30, 60, and 120 minutes after the treatments. For the glucose-induced hyperglycaemic group, animals received test samples and 30 minutes later, they were given glucose (2 g/kg b.w.) orally. Blood glucose concentrations were measured just before (0 minute) and 30, 60, and 120 minutes after the oral administration of glucose.

Determination of hypoglycaemic activity of PDE and its fractions upon subacute administration in alloxan-induced diabetic rats

The hypoglycaemic effect of the extract and its fractions ($ChCl_3$ Fr, EtOAc Fr, Aq Fr) in diabetic rats was tested. PDE (100, 200, and 400 mg/kg) and its fractions (50, 100, and 200 mg/kg each) were administered orally, once per day for 14 consecutive days. The blood glucose levels were determined on the 1^{st}, 6^{th}, 10^{th}, and 14^{th} days after the administration of the test samples, and the rats' body weights were also monitored on the same days. On the 14^{th} day, all animals were sacrificed. The blood was withdrawn for measurement of plasma insulin, serum cholesterol, and triglyceride levels.

Statistical analysis

All results were expressed as mean ± SEM for each group. Statistical analysis was performed using one-way analysis of variance (ANOVA). Tukey's test was used for multiple comparisons. The values were considered to be significantly different when $P < 0.05$.

Results

Phytochemical analysis and acute toxicity assessment

Phytochemical screening of the PDE indicated the presence of flavonoids, phenols, steroids, and saponins (Table 1). The extract was rich in flavonoids. PDE administration up to 8 g/kg did not show any toxicity to the rats.

Hypoglycaemic activity of PDE upon acute administration in normal, glucose-induced hyperglycaemic, and alloxan-induced diabetic rats

The extract at 400 mg/kg showed significant hypoglycaemic activity in glucose-induced hyperglycaemic rats ($P < 0.05$), while no significant effect was observed in normal and alloxan-induced diabetic rats (Table 2).

Hypoglycaemic activity of PDE and its fractions upon subacute administration in alloxan-induced diabetic rats

A significant antidiabetic effect of PDE at 400mg/kg was observed starting from the 6th day onwards ($P < 0.05$), and from 10th days onwards for 200 mg/kg of PDE; however, administration of extract at 100 mg/kg had not the antidiabetic effect. Dose-dependent antidiabetic activity experiments demonstrated that the extract possessed a remarkable hypoglycaemic effect at 400 mg/kg in diabetic rats ($P < 0.05$). PDE fractions at two doses, 100 and 200mg/kg, also showed a hypoglycaemic effect ($P < 0.05$), with chloroform fraction at 200 mg/kg being the most effective in reducing glucose level (Table 3).

Effects of PDE and its fractions on body weight and water intake in alloxan-induced diabetic rats

Table 4 presents the variations in body weight and water intake of the diabetic control and diabetic treatment groups after 14 days. On the 14th day, alloxan significantly reduced body weight of the diabetic control rats as compared with the 1st day ($P < 0.05$).The extract at 200 and 400 mg/kg and its fractions at 200 mg/kg attenuated this weight loss. PDE at 200 and 400 mg/kg and its fractions (aqueous, chloroform, and ethyl acetate) at 200 mg/kg also significantly decreased water intake compared with the diabetic control rats ($P < 0.05$). The extract at 400 mg/kg demonstrated a significant beneficial effect on water intake when compared with the reference drug glibenclamide.

Effects of PDE and its fractions on plasma insulin and serum lipid levels in alloxan-induced diabetic rats

As shown in Table 5, subacute administration of PDE at 200 and 400 mg/kg and its fractions (aqueous, chloroform, and ethyl acetate) at 200 mg/kg increased plasma insulin levels in the treated groups compared with the diabetic control group ($P < 0.01$). Administration of PDE and its fractions for 14 days also decreased the

Table 1: Phytochemical analysis of the hydroalcoholic extract of *P. dactylifera* leaves

Chemical group	Results
Tannins	-
Flavonoids	+
Saponins	+
Alkaloids	-
Steroids	+
Phenols	+
Terpenoids	-

Positive sign (+) indicates presence, negative sign (-) indicates absence.

Table 2: Acute hypoglycaemic effect of the hydroalcoholic extract of *P. dactylifera* on normoglycaemic, glucose-induced hyperglycaemic, and alloxan-induced diabetic rats

Group	Dose (mg/kg)	Mean blood glucose concentration ± SEM, mg/dL (% inhibition)			
		0 min	30 min	60 min	
Normoglycaemic					
Control	-	86.7 ± 5.7	97.3 ± 7.1 (-12.0)	97.3 ± 6.6 (-12.0)	75.0 ± 6.1 (12.8)
Glyben	4	80.0 ± 1.8	78.6 ± 1.3 (2.5)	75.4 ± 0.8 (6.3)	71.2 ± 0.3[α] (11.2)
PDE	100	88.2 ± 1.8	92.3 ± 2.1 (-4.0)	86.7 ± 1.5 (2.2)	85.8 ± 1.3 (3.4)
	200	87.3 ± 6.2	93.3 ± 7.9 (-6.0)	88.7 ± 6.4 (-1.0)	89.0 ± 6.1 (-2.2)
	400	85.3 ± 3.2	91.2 ± 4.1 (-7.0)	90.0 ± 2.6 (-5.0)	87.2 ± 2.5 (-2.3)
Glucose-induced hyperglycaemic					
Control	-	84.6 ± 4.2	131.1 ± 7.9 (-44.0)	118.3 ± 9.4 (-59.0)	113.0 ± 7.2 (-65.5)
PDE	100	91.5 ± 2.3	136.0 ± 3.1 (-43.0)	125.5 ± 1.8 (-37.0)	115.5 ± 1.2 (-26.0)
	200	88.2 ± 4.42	134.2 ± 6.1 (-52.0)	120.0 ± 7.4 (-36.0)	116.5 ± 7.0 (-31.0)
	400	83.6 ± 3.5	130.8 ± 5.7 (-56.0)	107.3 ± 6.6 (-29.0)	87.1 ± 8.2[α] (-4.8)
Diabetic					
Control	-	384.5 ± 14.9	406.8 ± 13.3 (-5.5)	398.3 ± 7.4 (-3.6)	287.0 ± 7.2 (24.5)
Glyben	4	394.5 ± 32.2	347.0 ± 9.9 (11.9)	347.0 ± 8.9 (11.5)	126.0 ± 5.2 (69.1)
PDE	100	360.0 ± 16.5	338.2 ± 11.0 (6.2)	332.0 ± 11.0 (7.8)	310.5 ± 9.5 (13.9)
	200	389.8 ± 27.9	389.3 ± 26.7 (1.0)	402.5 ± 23.6 (-3.0)	335.3 ± 3.3 (25.5)
	400	381.8 ± 21.8	397.8 ± 20.7 (-4.0)	412.8 ± 21.4 (-8.0)	209.3 ± 9.5 (46.0)

[α] $P < 0.05$ indicates significant difference in comparison with control group using one-way ANOVA followed by Tukey's test.
Abbreviation: Glyben = glibenclamide, PDE = hydroalcoholic extract of *P. dactylifera*

Table 3: Subacute hypoglycaemic effect of the hydroalcoholic extract of *P. dactylifera* and its fractions (aqueous, chloroform, and ethyl acetate) on alloxan-induced diabetic rats

Group	Dose (mg/kg)	Mean blood glucose concentration ± SEM, mg/dL (% inhibition)			
		1st day	6th day	10th day	14th day
Control	-	378.8 ± 22.4	368.2 ± 16.4	371.6 ± 12.3	360.2 ± 14.2
Glyben	4	342.4 ± 14.2	203.6 ± 11.4$^{\alpha}$ (40.3)	195.0 ± 5.8$^{\alpha}$ (43.0)	155.6 ± 4.2$^{\alpha,\beta}$ (55.0)
PDE	100	400.8 ± 20.9	350.0 ± 20.5 (12.5)	321.0 ± 18.0 (20.0)	275.4 ± 15.4 (31.7)
	200	374.4 ± 21.0	267.0 ± 16.5 (28.7)	180.3 ± 16.3$^{\alpha}$ (51.9)	158.0 ± 13.7$^{\alpha,\beta}$ (57.7)
	400	422.0 ± 23.9	180.2 ± 13.4$^{\alpha}$ (57.3)	131.2 ± 6.4$^{\alpha}$ (68.8)	108.2 ± 5.7$^{\alpha,\beta}$ (74.4)
Aq Fr	50	328.0 ± 19.0	296.0 ± 11.2 (9.8)	276.8 ± 8.2 (15.9)	272.6 ± 3.6 (17.1)
	100	352.7 ± 14.4	301.0 ± 17.2 (14.4)	267.5 ± 14.3 (24.2)	213.3 ± 12.0$^{\alpha}$ (39.5)
	200	455.8 ± 23.9	245.4 ± 14.2$^{\alpha}$ (46.2)	153.6 ± 11.5$^{\alpha}$ (66.4)	104.0 ± 5.2$^{\alpha,\beta}$ (77.2)
ChCl3 Fr	50	395.3 ± 15.5	370.0 ± 16.1 (7.4)	333.3 ± 24.4 (15.7)	292.7 ± 22.6 (26.1)
	100	380.7 ± 15.1	286.7 ± 10.3 (24.8)	183.8 ± 7.7$^{\alpha}$ (27.5)	145.2 ± 6.9$^{\alpha,\beta}$ (48.3)
	200	471.8 ± 28.3	222.0 ± 11.2$^{\alpha}$ (52.8)	126.4 ± 11.9$^{\alpha}$ (70.0)	93.0± 4.6$^{\alpha,\beta}$ (80.2)
EtOAc Fr	50	318.3 ± 11.4	296.5 ± 13.1 (6.9)	280.8 ± 9.2 (12.0)	251.3 ± 14.1 (21.1)
	100	357.9 ± 13.4	297.5 ± 11.5 (16.7)	214.2 ± 6.9 (41.1)	185.2 ± 4.6$^{\alpha}$ (48.8)
	200	421.6 ± 19.4	274.3 ± 16.5$^{\alpha}$ (35.0)	214.2 ± 17.1$^{\alpha}$ (49.2)	142.4 ± 6.2$^{\alpha,\beta}$ (66.3)

$^{\alpha}$ $P < 0.05$ indicates significant difference in comparison with 1st day while $^{\beta}$ $P < 0.05$ indicates significant difference in comparison with 6th and 14th day using one-way ANOVA followed by Tukey's test. Abbreviation: Glyben = glibenclamide, PDE = hydroalcoholic extract of *P. dactylifera*, Aq Fr = aqueous fraction, ChCl3 Fr = chloroform fraction, EtOAc Fr = ethyl acetate fraction

serum levels of cholesterol and triglyceride in the treated groups compared with the diabetic control animals ($P < 0.01$).

Discussion

In the present study, treatment with PDE and its fractions had significant antihyperglycaemic and antilipaemic effects. Considering the increase in plasma insulin concentration that was detected, the antihyperglycaemic activity of this extract may (at least in part) occur via the release of insulin from the pancreas. In our study, we observed that PDE and its fractions decreased blood glucose in alloxan-induced diabetic rats. The mechanism of action of the extract and its fractions could be similar to that of hypoglycaemic sulphonylureas, which promote insulin secretion by closure of K^+–ATP (adenosine 5-triphosphate) channels. This results in membrane depolarisation and increased Ca^{2+} influx, and it is a key initial step in insulin secretion. In this context, a number of other plants have also been reported to have antihyperglycaemic and insulin-stimulatory effects (18,19). Because alloxan is known to destroy

Table 4: Effect of the hydroalcoholic extract of *P. dactylifera* and its fractions (aqueous, chloroform, and ethyl acetate) on body weight and water intake in alloxan-induced diabetic rats

Group	Dose (mg/kg)	Body weight (g)		Water intake (mL/day)
		Initial 1st day	Final 14th day	
Control	-	208.0 ± 6.8	183.8 ± 4.2[α]	223.5 ± 15.3
Glyben	4	193.0 ± 3.4	198.0 ± 2.2	80.3 ± 3.2[α]
PDE	100	186.8 ± 6.6	161.5 ± 7.7[α]	210.0 ± 11.8
	200	201.4 ± 9.9	204.4 ± 13.2	71.2 ± 4.2[α]
	400	213.8 ± 7.1	209.5 ± 8.3	52.2 ± 3.2[α]
Aq Fr	200	180.2 ± 8.5	185.3 ± 6.5	48.6 ± 2.8[α]
ChCl3 Fr	200	172.8 ± 4.3	175.4 ± 6.1	50.0 ± 3.3[α]
EtOAc Fr	200	204.9 ± 11.2	210.0 ± 12.3	63.7 ± 5.1[α]

[α] $P < 0.05$ indicates significant difference in comparison with initial body weight using one-way ANOVA followed by Tukey's test. Abbreviation: Glyben = glibenclamide, PDE = hydroalcoholic extract of *P. dactylifera*, Aq Fr = aqueous fraction, ChCl3 Fr = chloroform fraction, EtOAc Fr = ethyl acetate fraction

Table 5: Effect of the hydroalcoholic extract of *P. dactylifera* and its fractions (aqueous, chloroform, and ethyl acetate) on plasma insulin level, serum cholesterol and triglyceride levels in alloxan-induced diabetic ratss

Chemical group	Dose (mg/kg)	Insulin (µIU/mL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Control	-	3.5 ± 0.2	121.2 ± 3.1	131.0 ± 6.3
Glyben	4	6.1 ± 0.4[α]	90.2 ± 2.9[α]	96.0 ± 2.4[α]
PDE	100	3.8 ± 0.3	116.5 ± 3.2	135.2 ± 5.5
	200	5.1 ± 0.4[α]	98.0 ± 1.5[α]	105.0 ± 3.4[α]
	400	7.2 ± 0.4[α]	88.2 ± 1.2[α]	72.6 ± 2.2[α]
Aq Fr	200	6.5 ± 0.4[α]	93.8 ± 2.5[α]	90.6 ± 1.8[α]
ChCl3 Fr	200	5.4 ± 0.2[α]	90.2 ± 2.3[α]	83.6 ± 1.8[α]
EtOAc Fr	200	7.2 ± 0.4[α]	89.7 ± 3.1[α]	75.3 ± 2.4[α]

[α] $P < 0.01$ indicates significant difference in comparison with control group using one-way ANOVA followed by Tukey's test. Abbreviation: Glyben = glibenclamide, PDE = hydroalcoholic extract of *P. dactylifera*, Aq Fr = aqueous fraction, ChCl3 Fr = chloroform fraction, EtOAc Fr = ethyl acetate fraction

pancreatic β-cells, the present findings appear to be in consonance with an earlier suggestion by Jackson and Bressler (20) that sulphonylureas have an extra-pancreatic, antihyperglycaemic mechanism of action secondary to their insulin-secreting effect and the attendant glucose uptake into (and utilisation by) tissues.

The antidiabetic effect of PDE and its fractions also may be due to the effect of active flavonoids, phenols, steroids, and saponins; these compounds may scavenge free radicals liberated by alloxan in diabetic rats (21,22). Hypoglycaemic effects have been reported for some plants that contain flavonoids (22,23).

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lipolysis because it inhibits the activity of hormone-sensitive lipases in adipose tissue and suppresses the release of free fatty acids. In diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids into circulation. Increased fatty acid concentrations also increased the

β-oxidation of fatty acids, producing more acetyl-CoA and cholesterol in diabetics. The hypocholesterolaemic activity of PDE and its fractions after subchronic administration may be due to a number of mechanisms, including a) stimulation of cholesterol-7-alpha-hydroxylase (CYP7A1), which converts cholesterol into bile acids; b) inhibition of HMG-CoA reductase; and/or c) inhibition of cholesterol absorption from the intestines due to the formation of complexes with compounds such as glycosides and saponins (24–26). A reduction in triglyceride levels may be due to decreased lipogenesis and increased lipolytic activity by activation of the hormone-sensitive lipase (27) or lipogenic enzymes (28), and/or activation of lipoprotein lipase (29), as is observed in antidiabetic plants such as Ormodica charantia (29), Artemisia herba alba (27), and *Ceasalpinea bondecella* (30), which exhibit hypolipidaemic activity. Furthermore, several plant constituents, including flavonoids, are known to decrease triglyceride level (31).

Our results suggest that the extract and its fractions have insulin-like activity. It is possible that the hypolipidaemic effects of the extract may be related to this effect. In the present study, acute toxicity was tested up to the high concentration of 8 g/kg (20 times more than the therapeutic dose). Even at this dose, the extract did not exhibit any sign of toxicity. The main purpose of a preliminary acute toxicity study is to provide some idea of conspicuous behavioural changes and/or death, and PDE did not exhibit any toxic symptoms in this limited toxicity evaluation in male rats. A review of the literature shows that studies have not been carried out on this plant with regard to other pharmacological properties or phytochemistry.

Dehydration and loss of body weight have been associated with diabetes mellitus (32). In diabetic rats, increased water intake and decreased body weight were observed. This indicates a polydipsic condition and loss of weight due to excessive breakdown of tissue proteins (33). The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats (34) as well as proteins, which might lead to muscle wasting (35). Oral administration of PDE and its fractions for 14 consecutive days to diabetic rats decreased their water intake and improved body weight. These effects could be due to better control of the hyperglycaemic state in diabetic rats. Decreased fasting blood sugar improves body weight in alloxan-induced diabetic rats (36,37). PDE caused hypoglycaemia in alloxan-induced diabetic rats, thus validating the traditional use of *P. dactylifera* leaves in southwest Iran for the treatment of diabetes. As many antidiabetic drugs do not correct dyslipidaemia, the observed hypocholesterolaemic and hypotriglyceridaemic effects of the extract in alloxan-induced diabetic rats unveils PDE potential in the management of diabetes because the extract may also reverse dyslipidaemia associated with diabetes and prevent cardiovascular disease complications (38).

Conclusion

The findings of the current study showed that PDE and its fractions have a hypoglycaemic effect in alloxan-induced diabetic rats. In addition, they were highly effective in managing the complications of diabetes mellitus such as hyperlipidemia and weight loss. The antidiabetic effects of PDE and its fractions may be mediated through an increase in insulin secretion, the stimulation of glucose uptake and glycogen synthesis by cells, and/or protection of pancreatic β-cells from alloxan- and glucose-induced oxidative stress.

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Authors' Contributions

Conception and design, drafting of the article, final approval of the article: SAM, MKGN
Obtaining of funding, analysis and interpretation of the data, critical revision of the article: SAM
Provision of study materials, collection and assembly of data, administrative, technical, or logistic support: KJ, MJ, HB

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