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Assessment of the Antidiabetic Potential of the Ethanolic Extract of Date Palm (*Phoenix Dactylifera*) Seed in Alloxan-Induced Diabetic Rats

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Abstract

Background: Reports exist of the allopathic use of dates as a hypoglycemic, antioxidant and anti-diabetic agent. Date palm seed have also been found to be of nutritional and medicinal value.

Aim: this study was aimed at evaluating the anti-diabetic potential of the ethanolic extract of date palm seed in alloxan-induced diabetic rats.

Methodology: Proximate composition of date palm seed was determined using standard methods. Ethanolic extract of date palm seed was prepared by crude extraction protocol. The total flavonoids, phenolic contents and total antioxidant capacity of the seed extract was determined. Also, the scavenging activity of the extract using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO) and the reducing power was also evaluated. Thirty wistar rats were randomly divided into five groups of six rats each. Group A was the control group, group B received date palm seed extract only (200 mg/kg bw) i.p, group C, D and E were the diabetic groups that received alloxan (150 mg/kg bw) intra peritoneally; group C was the diabetic control group, group D and group E were treated with glibenclamide (5 mg/kg bw) and date palm seed extract (400 mg/kg bw) intraperitoneally for fourteen days. At the end of the experiment, the lipid profile parameters (HDL, LDL, VLDL, cholesterol and triglyceride) levels were determined from serum samples of the animals. Levels of glucose and antioxidant parameters (SOD, CAT, GSH and MDA) were also analysed from the serum samples of the experimental animals.

Results: Results of proximate analysis revealed fiber to be highest (61.75%), carbohydrate (20.95%), fat (8.55%), moisture (3.40%), ash (2.79%) and protein (2.63%). The total flavonoid and phenolic content of date palm seed was found to be 45.28 ± 0.32 mg/100 g and 28.22 ± 0.43 mg/100 g respectively while the total antioxidant capacity was 30.11 ± 0.21 mg/100 g. There was a significant ($P<0.05$) decrease in the levels of LDL, VLDL, cholesterol, triglyceride and blood glucose with no significant ($P>0.05$) increase in the HDL of the diabetic treated groups as compared to the diabetic control group. A significant ($P<0.05$) increase was observed in the SOD, CAT, GSH levels while there was a significant ($P<0.05$) decrease in the MDA level of the diabetic group treated with date palm seed extract as compared to the diabetic control group.

Conclusion: This study suggests the anti-diabetic potential of date palm seed extract which might be due to its hypolipidemic, hypoglycemic and antioxidant properties.

Keywords *Phoenix dactylifera*; Alloxan; Anti-diabetic; Antioxidant; Lipid profile

Introduction

Diabetes mellitus has been defined as a group of metabolic diseases associated with chronic hyperglycemia attributed to anomalies in the secretion and/or action of insulin which results in abnormalities in the metabolism of carbohydrate, lipid and protein [1,2]. Abnormalities in lipids are prevalent in diabetic patients and dyslipidemia is regarded as one of the main risk factor for cardiovascular disease observed in type 2 diabetes [3,4]. Diabetes mellitus has been found to be linked to increased oxidative stress and consequently an alteration in the body's antioxidant defense system [5]. Alloxan is a urea derivative commonly used for the induction of diabetes in experimental studies because of its selective destruction of the pancreatic beta islets which is responsible for the production of insulin [6,7].

Various drugs have been employed in combatting the different complications and the abnormalities associated with diabetes mellitus but due to the nefarious side effects of those drugs, hence there is a dire need to exploit other natural therapeutic options with fewer side effects for the amelioration of those abnormalities associated with diabetes towards a better management and prognosis. In this light, one major current area of research is in medicinal plants as those plants continue to provide significant therapeutic agents both in modern medicine and allopathy. Many indigenous medicinal plants and extracts from various parts of these plants like root, shoot, seeds etc. have been exploited in the management of diabetes [8,9] due to the various active phytochemicals present in these plants.

The date palm (*Phoenix dactylifera* L.) is a palm extensively cultivated for its edible fruit, and is a member of the Palmae family [10]. Date seeds constitute 5.6-15.0% of the total fruit weight and have been found to contain variable quantities of nutrients and mineral elements [11]. The seed has also been used locally to relieve tooth aches, fever, oedema [12] and has also been found to possess some pharmacological properties such as antioxidant [13]; hepatoprotective [14] and neuroprotective effects [15]. The date pulp also contains considerable contents of phytochemicals like sterols, flavonoids, phenolics, carotenoids etc. [16]. There is still little study on the potential of date plam seeds as a potential anti-diabetic agent and this study will provide a greater insight on the potential of the seed as an anti-diabetic agent. This study was aimed at evaluating the potential of date palm seed as an anti-diabetic agent by evaluating the hypolipidemic, hypoglycemic and antioxidant effect of the ethanolic extract of the seed in non-diabetic and diabetic rats.

Methodology

Plant collection and identification

Date palm and its seeds were obtained from Bodija market, Oyo State, Nigeria. The dried plant sample (*Phoenix dactylifera*) and its seed were identified and authenticated in University of Lagos Herbarium, with the Voucher Ndndnumber LUH7552. The seeds were separated from its fruits, air-dried and grounded into powdery form using mechanical grinder which was then stored in air tight container prior to the commencement of the study.

Proximate composition determination

The dry matter, moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fiber contents of the powdered seeds were determined in accordance to the methods described by AOAC [17]. The carbohydrate content was expressed as a percentage of the net difference between the sum of other nutrients and the total percentage composition.

Chemicals and reagents

Alloxan monohydrate and the standard drug glibenclamide were obtained at a licenced pharmacy store in Ile-Ife, Osun State. Assay kits were purchased from Randox diagnostics. All other chemicals used were of analytical grade.

Preparation of ethanolic extract of *P. dactylifera* seed

Phoenix dactylifera seeds were soaked for 48 hrs in a solvent (70% ethanol and 30% distilled water), filtered and then extracted using a rotary evaporator. The extract was later freeze dried for 24 hours to obtain the crude extract which was dissolved in normal saline and used for this study.

Evaluation of the *in vitro* antioxidant property of *P. dactylifera* seed extract

Estimation of the total phenolic content: The amount of total phenol content was determined by Folin-Ciocateu reagent method [18] using gallic acid as a standard following the method of Slinkard and Singleton [19]. A 0.5 ml sample of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 minutes. After this, 2.5 ml sodium carbonate solution (7.5% w/v) was added and further incubated for 30 minutes at room temperature. The absorbance of the solution was measured at 760 nm. The concentration of total phenol was expressed as gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used as a reference value.

Determination of flavonoids: This was done according to the method described by McDonald et al. [18]. 10 g of the extracts was extracted repeatedly with100 ml of 80% aqueous methanol at room temperature. The initial weight of the filter paper was taken (W1). The whole solution was filtered through Whatman-filter paper No42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath. The final weight of the paper was then taken (W2).

Total antioxidant capacity determination: The total antioxidant capacity of the extract was determined using the method of Prieto et al. [20]. A sample of the extract (0.3 ml) was mixed with 3 ml of TAC reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm.

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) Free Radical scavenging activity assay: The free radical scavenging activity of the extract, based on the scavenging of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by Cuendet et al. [21]. An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (20, 40, 60, 80, 100 µg/ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the expression:

$$\% \text{ inhibition} = (A_0\text{-}A_1) \times 100/A_0$$

Where A_0 is the absorption of the blank sample and A_1 is the absorption of the extract

Nitric oxide scavenging activity assay: 4ml sample of plant extract or standard solution of different concentrations (20, 40, 60, 80, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 Mm in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30°C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was measured at 550 nm [22]. Gallic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation:

$$(A_0\text{-}A_1)/A_0 \times 100.$$

Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract or standard.

Reducing power assay: Various concentrations of the extracts (20-100 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The

mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000rpm for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract [23].

% Increase in reducing power = $(A_{test} / A_{blank}) -1 \times 100$

The concentration in mg/100 g of sample values required scavenging 50% (SC_{50}) of DPPH radicals, NO and the reducing power was extrapolated from the graph of percentage inhibition in µg/ml against concentration using gallic acid as positive control.

Experimental design

Experimental Animals: A total of thirty female Wister Albino rats weighing about 102-163 g were procured from animal house University of Lagos, Idi-Araba, Lagos State and used for the study. The animals were maintained for five weeks and were housed in well ventilated, standard clean cages made of plastic and wire gauze. Wood shavings were used as beddings to keep each compartment dry. Here, normal standard ambient conditions of temperature between 28-31°C, relative humidity between 50%-60% and a photoperiodicity of 12 hours natural light and 12 hours dark were maintained. The animals were allowed to acclimatize for two weeks for proper adaptation to their new environment and were weighed weekly. They had access to pelletized feed and tap water ad libitum. All the experimental procedures were carried out in accordance to the guidelines of the Institutional Animal Ethics Committee (IAEC). All the ethical and humanity considerations as well as euthanasia of the animals were considered and performed. All animals were allowed free access to feed and water throughout the period of the study and the treatment spanned over a period of 14 days. Experimental design and treatment: The animals were divided into five groups of six animals each. Diabetes induction was done by single intra peritoneal injection of Alloxan monohydrate (150 mg/kg) per body weight into rats in groups C, D and E. The hyperglycemia was confirmed after 72 hours by the elevation of blood glucose and the behavioral changes (excess thirst and frequent urination).The rats with blood glucose level more than 250 mg/dl after day 3 of Alloxan injection were considered as diabetic rats and were used for the study. The experimental design of the animals into groups is as follows:-

Group A, normal healthy control; this rats served as the control group

Group B received *P. dactylifera* seed extract only (200 mg/kg body weight, intraperitoneally)

Group C, served as the diabetic control, received alloxan (150 mg/kg, intraperitoneally)

Group D, diabetic followed by treatment with glibenclamide (5 mg/kg body weight, intraperitoneally).

Group E, diabetic + followed by intraperitoneal administration of *P. dactylifera* seed extract (400 mg/kg body weight)

The treatment commenced after day 3 of diabetes induction and was considered as day 1 of treatment. Body weights of the animals were measured weekly using a weighing balance. The blood glucose levels of the animals were determined before the administration of extract and after the seed extract administration with a commercially available glucometer kit (ACCU-CHEK blood glucose monitor). On the 14th day after commencement of treatment, the rats were anaesthetized with chloroform and sacrificed by cervical dislocation. Blood was collected into fresh vials and separated in a refrigerated centrifuge at 5000 rpm for 5mins. Serum was separated with micropipette and stored until used for further biochemical analysis.

Assay for the lipid profile levels from serum samples of experimental animals

Serum lipid profile; triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low density lipoprotein were estimated colorimetrically using assay kit method from RANDOX diagnostics according to manufactured instructions [24].

Evaluation of the in vivo antioxidant activity of *P. dactylifera* seed extract in serum samples of experimental animals

Determination of superoxide dismutase (SOD) activity: Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine and this was determined by the increase in absorbance at 480nm as described by Sun and Zigma [25]. The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. Σ= 4020M^{-1} cm^{-1}

Catalase activity determination: Catalase activity was determined according to Sinha [26]. It was assayed colorimetrically at 620 nm and expressed as µmoles of H_2O_2 consumed/min/mg protein at 25°C. The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate and 0.4 ml of 2 M H2O2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Σ = 40 M^{-1} cm^{-1}

Reduced glutathione determination: The reduced glutathione (GSH) content of blood tissue as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay [27]. To the serum, 10% TCA was added, centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

Σ = 1.34 x 104 M^{-1} cm^{-1}

Malondialdehyde determination: Malondialdehyde (MDA), an index of lipid peroxidation was determined using the method of Buege and Aust [28]. 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24 N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100oC for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of 1.56×10^5 $M^{-1}CM^{-1}$.

Data and statistical analysis

Results were expressed as Mean ± SD of three determinations and the statistical significance was evaluated by one way analysis of variance (ANOVA) and individual comparisons were obtained by Dunnett's multiple comparisons test. A value of $P<0.05$ was considered to indicate significant difference between groups.

Results

Proximate composition of *P. dactylifera* seed

The result of the proximate composition of *Phoenix dactylifera* seed in percentage as shown in Table 1 revealed that fiber was present in the highest amount (61.75 ± 0.61), followed by Carbohydrate (20.95 ± 0.47), Crude fat (8.55 ± 0.07), Moisture content (3.40 ± 0.04), Ash (2.79 ± 0.04), and Protein (2.63 ± 0.29).

Proximate composition	Phoenix dactylifera seed (%)
Carbohydrate	20.95 ± 0.47
Protein	2.63 ± 0.29
Crude fiber	61.75 ± 0.61
Ash	2.79 ± 0.04
Moisture	3.40 ± 0.04
Crude fat	8.55 ± 0.07
Results are expressed as Mean ± SD values of three determinations	

Table 1: Proximate analysis of the date palm seed (Phoenix dactylifera).

Total flavonoid, phenol and antioxidant capacity of *Phoenix dactylifera* seed extract

The total antioxidant capacity of the date palm seed extract as shown in Figure 1 was found to be (30.11 ± 0.21) mg/100 g, while the total flavonoid and total phenol contents were found to be (45.28 ± 0.32) mg/100 g and (28.22 ± 0.43) mg/100 g respectively.

DPPH, NO free radical scavenging activity and reducing power

The results of the DPPH, NO free radical scavenging activity and reducing power of date seed extract and gallic acid (positive control) is shown in Table 2. Date seed extract was found to have for DPPH a Sc_{50} of 46 mg/100 g while that of gallic acid is 52 mg/100 g and for NO; a SC_{50} of 34 mg/100 g while that of gallic acid is 41 mg/100 g. The Sc_{50} for the reducing power of date seed extract is 0.39 while that of gallic acid is 0.52.

Effect of *P. dactylifera* seed extract on the body weights and blood glucose levels of diabetic rats

There was a decrease in the body weight of diabetic rats as compared to the control (results not shown) but treatment with date palm seed extract resulted in a slight increase in the body weight of the animals two weeks after treatment began. The result of the effect of date palm seed on the blood glucose level of diabetic rats as shown in Table 3 revealed that diabetic rats treated with 400 mg/kg *P. dactylifera* seed extract and the standard drug resulted in a very significant ($P<0.05$) decrease in the blood glucose level when compared with the diabetic untreated group.

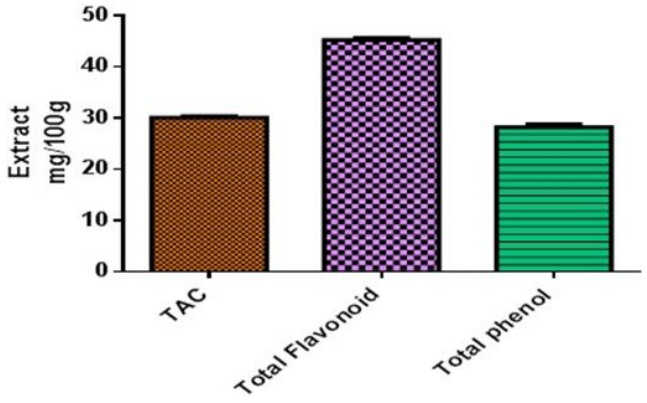

Figure 1: Bar chart showing total antioxidant capacity (TAC), flavonoid and phenol content of date seed extract in mg/100 g. (Results are expressed as mean values of three determinations ± SD).

% inhibition in µg/ml	Date seed extract (mg/100 g)	Gallic acid
DPPH		
20	32.55 ± 0.78	38.88 ± 0.27
40	42.77 ± 0.31	49.85 ± 0.27
60	55.03 ± 0.63	78.06 ± 0.32
80	60.38 ± 0.63	87.98 ± 0.43
100	65.26 ± 0.79	90.62 ± 0.32
NITRIC OXIDE		
20	23.47 ± 0.30	24.40 ± 0.38
40	31.31 ± 0.13	35.65 ± 0.45
60	36.84 ± 0.21	47.40 ± 0.25
80	41.78 ± 0.13	58.39 ± 0.19
100	50.98 ± 0.13	70.72 ± 0.45
REDUCING POWER		
20	0.36 ± 0.01	0.37 ± 0.03
40	0.38 ± 0.03	0.48 ± 0.03
60	0.39 ± 0.01	0.57 ± 0.01
80	0.40 ± 0.03	0.61 ± 0.03
100	0.55 ± 0.02	0.80 ± 0.02

Table 2: DPPH, NO Scavenging activity and reducing power of date seed extracts.

Groups	Treatment	Blood glucose before induction (mg/dL)	72 hours after induction (mg/dL)	14th day treatment began (mg/dL)
A	Control	88.66 ± 10.26	84.66 ± 6.02a	61 ± 16.26a
B	Extract only	95 ± 17.08	92.33 ± 10.96a	75.33 ± 8.42b
C	Diabetic control	107 ± 8.54	442.66 ± 9.29a	444.33 ± 6.02a
D	Diabetic+Glibenclamide	93.33 ± 13.2	481 ± 9.54a	314.33 ± 14.85b
E	Diabetic+Extract	46.33 ± 5.03	426.66 ± 8.26a	388 ± 4.58b
Values a, b differ significantly at P<0.05				

Table 3: Effect of Phoenix dactilifera (date palm) seed extract on the blood glucose levels in Alloxan-induced diabetic rats.

Effect of *Phoenix dactylifera* (date palm) seed extract on the serum lipid profile (HDL, LDL, VLDL, triglycerides and cholesterol) levels in diabetic animals.

As shown in Table 4, there was a significant (P<0.05) decrease in the serum levels of Cholesterol, LDL, VLDL, and Triglycerides in all the treated groups when compared to the positive control and diabetic control group. However, the HDL level in the diabetic control group was found to be significantly (P<0.05) reduced as compared to the normal control rats. Treatment with standard drug (glibenclamide) resulted in a significant (P<0.05) increase in the HDL level while there was no significant (P>0.05) increase in the HDL levels of diabetic rats treated with *P. dactylifera* seed extract as compared to the diabetic control rats.

GROUPS	CHOL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TRIG (mg/dL)	VLDL (mg/dL)
A	102.61 ± 6.16	71.33 ± 0.618	58.45 ± 0.25	94.76 ± 0.45	18.80 ± 0.47
B	98.52 ± 5.79	31.73 ± 0.80****	35.37 ± 0.86****	12.20 ± 0.65****	22.38 ± 0.62**
C	85.27 ± 3.74	44.61 ± 0.67****	30.78 ± 1.35****	100.60 ± 1.83**	21.33 ± 0.39**
D	115.12 ± 5.87	63.31 ± 1.17***	51.10 ± 0.94**	96.68 ± 0.89	19.64 ± 0.62
E	71.78 ± 4.53**	36.26 ± 0.51****	45.27 ± 1.16***	73.73 ± 0.75****	14.54 ± 0.15***
Data are represented as Mean ± SD and are significantly different at <0.05 *Statistically different compared to positive control (Group A) at (P<0.05)					

Table 4: Effect of Phoenix dactylifera seed extract on serum Cholesterol, HDL, LDL, VLDL and Triglycerides in alloxan induced diabetic rats.

Groups	Catalase (CAT) (µmol/ml)	Malondialdehyde (MDA) (µmol/ml)	Superoxide Dismutase (SOD) (µmol/ml)	GSH
A (Positive Control)	10.30 ± 0.11	1.90 ± 0.44	1.52 ± 0.15	17.72 ± 0.385
B (Date seed extract treated group.)	7.9 ± 10.03****	2.47 ± 0.96**	1.48 ± 0.005	19.42 ± 0.23**
C (Diabetic control group)	8.95 ± 0.0****	4.26 ± 0.08****	1.41 ± 0.0***	15.17 ± 0.61*
D (Alloxan-induced Glibenclamide treated group)	9.24 ± 0.03***	3.15 ± 0.16**	1.51 ± 0.01	20.58 ± 0.310***
E (Alloxan-induced date seed extract treated group)	9.54 ± 0.02***	3.59 ± 0.20***	1.55 ± 0.0	23.83 ± 0.3100****
Data are represented as Mean ± SD and are significantly different at <0.05 *Statistically different compared to positive control (Group A) at (P<0.05)				

Table 5: Effect of Phoenix dactylifera (date palm) seed on antioxidant parameters of experimental animals.

Effect of *Phoenix dactylifera* seed extract on the levels of antioxidant parameters (SOD, CAT, GSH, MDA) in diabetic animals

As shown in the Table 5, there was a significant (P<0.05) decrease in the catalase and GSH level in the diabetic control group, compared to the positive control group. However, there was a significant (P<0.05) increase in the Catalase, superoxide dismutase (SOD) and reduced glutathione (GSH) level of the diabetic rats treated with date palm seed extract as compared to the diabetic control group. A significant (P<0.05) increase in the level of MDA was observed in the diabetic control rats as compared to the normal control animals. However, treatment with the date seed extract resulted in a significant (P<0.05) decrease in the MDA level as compared to the diabetic control group.

Discussion

The anti-diabetic potential of *P. dactylifera* seed extract was evaluated by assessing its hypoglycemic, hypolipidemic and antioxidant activity in alloxan induced diabetic rats.

The result of the proximate analysis of date seed shows that the seed contains 61.71% Crude fiber, 20.95% Carbohydrate, 8.55% crude fat, 3.40% Moisture, 2.79% ash and 2.63% Protein. This result indicates that the seed contains a reasonable amount of carbohydrate which serves as a good source of energy to the body. The low moisture content of the seed indicates an enhanced shelf life [29]. The seed of date palm is also a good source of dietary fibre with every 100 g of dates providing 34% of the daily recommended amount of dietary fibre [30] which is 25 g/day [31]. Dietary fibre is considered good for one's health and is claimed to have a pre-emptive effect against many diseases such as diabetes, obesity, hyperlipidaemia, coronary heart disease etc. [32] Fibre cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Dietary fiber has a positive effect in the management of diabetes by controlling post-prandial hyperglycemia. This it does by delaying gastric emptying or by increasing the viscosity of gastro-intestinal tract content thereby suppressing digestion of carbohydrate and delaying its absorption. Fibre aids and speeds up the excretion of waste and toxins from the body, preventing them from sitting in the intestine or bowel for too long, which could cause a build-up and lead to several diseases [33].

P. dactylifera seed extract scavenged DPPH, NO radicals in an efficient way and has a relatively high reducing power. This might be due to the high antioxidant capacity of the seed extract. Results from the total flavonoid, phenol and antioxidant capacity of *Phoenix dactylifera* shows that the seed is relatively rich in flavonoids and phenolic compounds. In addition, the antioxidant activity of date palm can be attributed to its antioxidant compounds, such as ascorbic acid, vitamin E, carotenoids and selenium [34,35] and also to flavonoids, tannins and other phenolic constituents [35,37].

It has been reported that flavonoids and phenolics possess hydrogen donating capabilities and act as an antioxidant [38] by mopping out free radical scavengers that prevent oxidative cell damage. These activities could be attributed to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation [39]. The evaluation of the total antioxidant capacity (TAC) is an appropriate tool to determine the additive antioxidant properties of date seed [40]. Date-pits have the potential to be used as a supplement for antioxidants in nutraceutical, pharmaceutical, and medicinal products [15].

Alloxan is a popular drug used to induce diabetes in an experimental set up. This is because of its metabolites -dialuric is capable of forming superoxide radicals. These radicals are scavenged and dismutated to hydrogen peroxide with a concurrent large increase in the concentration of calcium in the cytosolwhich consequently leads to the destruction of the pancreatic beta cells with accompanying hyperglycemia [7]. Induction of diabetes by alloxan causes loss of body weight due to the increased muscle wasting and loss of tissue proteins. Due to destruction of β-cells, the decrease in body weight and increase in food and water intake were commonly observed in diabetes and this may be due to metabolic changes caused by lack or deficiency of insulin [41]. In diabetic rats, reduction in body weight changes observed might be the result of degradation of structural proteins due to deficiency of carbohydrate for the energy metabolism [42]. A slight increase in the body weight of diabetic animals treated with date palm seed extract after 14 days was observed when compared to the diabetic control group which signifies the reversal of gluconeogenesis. The slight increase might be due to the short duration of this study. Further, this antidiabetic activity indicates that *Phoenix dactylifera* may stimulate insulin secretion from the remaining β-cells or regenerated β-cells.

There was a significant (P<0.05) decrease in the blood glucose levels of the diabetic rats treated with *P. dactylifera* seed extract and glibenclamide as compared to the diabetic control group. This might be due to the ability of the seed extract potentiate the pancreatic secretion of insulin from β-cells of islets, in a way similar to glibenclamide. The reference drug- glibenclamide is an ATP-sensitive K+ channel blocker used to treat non-insulin-dependent diabetes [43]. Another reason which might be adduced to the hypoglycemic ability of the seed extract is its phytochemical constituents. A major phytochemical constituent of the extract that have been reported is flavonoids, with different types such as; flavans, anthocyanidins and isoflavones. Bioflavonoids are well known for their multi-directional biological activities including hypoglycemic effects [7]. Also, the mechanisms of actions could be either by increasing the tissue utilization of glucose [44] by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues [45].

Various plant seeds like nutmeg (*Myristica fragrans*) [46]; *Moringa oleifera* [47] have also been reported as potential anti-diabetic agents due to the fact that some possess hypoglycemic properties and some were reported to possess hypolipidemic effects like fruit pulp of baobab (*Adansonia digitata* L.) [48]; root extract of banana (*Musa balbisiana*) [49].

In diabetic conditions, there is increase in blood glucose level which is usually accompanied by an increase in serum cholesterol, TAGs, LDL and decrease in HDL [50]. Under normal conditions insulin triggers the enzyme lipoprotein lipase, which hydrolyzes triglycerides. However in diabetic state, lipoprotein lipase is not triggered due to insulin deficiency resulting in hypertriglyceridemia. This was confirmed in our studies as the diabetic control rats had a significantly (P<0.05) increased triglycerides, LDL, VLDL and cholesterol levels. Alteration of serum lipid profile is known to occur in diabetes and this is likely to increase the risk of coronary heart disease [51].

As revealed in the results shown in Table 3, administration of date seed extract produced significant beneficial effects in the lipid profile of the treated diabetic rats, by significantly (P<0.05) reducing total cholesterol, low density lipoprotein, triacylglycerol and very low density lipoprotein. The findings is similar to the findings reported by Yusufoglu et al. [52] that administration of date extracts reversed the

changes in plasma lipoproteins of diabetic rats and significantly improved their values towards near normal levels except the reduction of HDL in this study unlike other studies where HDL level increased after administration of date extract. This might be due to the short time duration of this study. The hypolipidemic effect of *P.dactylifera* seeds in diabetic rats might be attributed to the relatively high amounts of fibre that the seed contains. Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer [53]. Another reason might also be the potential of the extract to potentiate the pancreatic secretion of insulin from β-cells of islets which enables insulin to activate lipoprotein lipase for the breakdown of lipids. Moreover, flavonoids in the seed might also play a role in boosting the activity of lecithin acyl transferase (LCAT), which regulates blood lipids [54].

Oxidative stress (OS) is found to be increased in patients with diabetes mellitus [55]. Evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of diabetes [56]. Reactive oxygen species (ROS) such as H2O2, superoxide (O2-) and hydroxyl (.OH) radicals are responsible for OS at the time of diseased condition, as supported by increased cellular accumulation of lipid peroxides and depletion of endogenous antioxidants [57]. OS in diabetes co-exists with a reduction in the antioxidant status, which can increase the deleterious effects of free radicals. In diabetes, OS may occur due to auto oxidation of glucose, shifts in redox balances, decreased tissue concentrations of low molecular weight antioxidants, such as reduced glutathione (GSH) and impaired activities of antioxidant defense enzymes such as SOD, CAT, and increase MDA level in the body [58].

Reactive oxygen species (ROS) generated in the cells is scavenged by antioxidant enzymes [59]. Moreover, diabetes also induced changes in the tissue content and activity of the antioxidant enzymes [60]. Markers of oxidative stress, such as ROS and reduced levels of antioxidants, have been found in the blood and renal tissues in both human and experimental diabetes [61]. Oxygen free radicals react with all biological substances; however, the most susceptible ones are polyunsaturated fatty acids. Reactions with these cell membrane constituents lead to lipid peroxidation (LPO) [62]. Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors [63]. In our study, serum Malondialdeyhde (MDA) (as an indicator of LPO) levels in the diabetic control group was found to be significantly ($P<0.05$) higher than other groups indicating increased free radical generation in diabetic and there was a significant ($P<0.05$) decrease in the MDA levels of rats treated with *P. dactylifera* seed extract which is also in alignment with the research carried out by Marghoob and Abdelmarouf in 2016 [64]. The primary ROS produced in the course of oxygen metabolism is superoxide which is a highly reactive, cytotoxic ROS. Superoxide is dismutated to a far less reactive product, hydrogen peroxide (H_2O_2), by a family of metalloenzymes known as superoxide dismutase (SOD) [65]. There was a significant ($P<0.05$) increase in the values obtained for superoxide dismutase, GSH and catalase in the diabetic group treated with *P. dactylifera* seed extract as compared to the diabetic control group which suggests an *in vivo* antioxidant activity of *P. dactylifera* seed. This might be attributed to the presence of considerable amounts of active flavonoids, phenols, steroids, and saponins resident in the seed as those compounds may scavenge free radicals liberated by alloxan in diabetic rats [66,67]. Moreover, since the seed contains a high amount of fiber, fiber might also play a role in the enhanced *in-vivo* antioxidant effect observed because it includes various types of complex carbohydrates that can influence the absorption and metabolism of carbohydrate and fat, leading to a lowered glucose level and consequently; a reduced auto-oxidation of glucose that generates more free radicals and thereby conserving and enhancing the endogeneous antioxidant parameters [48].

Conclusion

This study has confirmed the anti-diabetic potential of the ethanolic extract of *P. dactylifera* seed which might be attributed to its hypolipidemic, hypoglycemic and antioxidant properties of the seed as was observed in alloxan-induced diabetic rats treated with the seed extract. However, further studies on the isolation of candidate lead compounds which might be responsible for its anti-diabetic potential and its precise mechanism of action and its effects on the pancreas is warranted. Nonetheless, it is recommended that the seed be incorporated as a food supplement and nutraceutical in the management of diabetes as it could possible alleviate some other biochemical anomalies associated with diabetes mellitus.

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