

Review Article

Aqueous and Methanolic Extracts of Palm Date Seeds and Fruits (*Phoenix dactylifera*) Protects against Diabetic Nephropathy in Type II Diabetic Rats

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Abstract

Objective: The present study examined the effects of aqueous and methanolic extracts of palm dates (fruits and seeds) on diabetic nephropathy in a rat model of type 2 DM.

Methods: Sixty male albino rats subdivided into a) normal control (NC) group, b) diabetic control (DM) group, c) aqueous fruit extract (AFE) group, d) methanolic fruit extract (MFE) group, e) aqueous seed extract (ASE) group and f) methanolic seed extract (ASE) group. Treatment with both extracts done for 15 weeks after induction of type 2 DM. by the end of experiment fasting blood glucose, serum lipid profile, creatinine, urea, uric acid, urinary albumin excretion, kidney tissue MDA, catalase, SOD, GSH were measured and kidney histopathological examination was done.

Results: Diabetic untreated rats showed significant hyperglycemia and hyperlipidemia and deterioration in kidney functions and morphology with enhanced oxidative stress in kidney tissues. On other hand, rats treated with either aqueous or methanolic fruit or seed extracts showed significant improvement in all studied parameters compared to DM group (p<0.05). However, the effects of fruit extracts especially aqueous extract were more powerful than seed extracts.

Conclusion: Aqueous and methanolic fruit and seed extracts of palm date protect kidneys from diabetic nephropathy in rats which might be due to their antioxidant properties.

Keywords: *Phoenix dactylifera*; Fruits; Seeds; Extracts; Diabetic nephropathy; Rats

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease manifested by hyperglycaemia and caused by either lack of insulin (type 1 DM) or deficiency of its action (type 2 DM) [1]. One of the serious sequelae of DM is diabetic nephropathy (DN) that accounts for the high mortality rate in diabetic patients from end stage renal disease (ESRD) [2,3]. Diabetic nephropathy (DN) affects about 15%–25% of type I diabetes patients [4] and 30%–40% of type II DM patients [5]. In DN, the excessive deposition of extracellular matrix (ECM) in kidney results in glomerular mesangial expansion and tubulointerstitial fibrosis [6,7]. Pathophysiological mechanisms underlying DN include excessive formation of advanced glycation end products (AGEs) and reactive oxygen species (ROS), upregulation of transforming growth factor-beta 1 (TGF- β 1), renin-angiotensin system (RAS) and polyol pathway [8].

Phoenix dactylifera L. tree and its products have been used traditionally in treatment of many pathological conditions [9]. The palm date fruits are composed of a fleshy pericarp and seed [10]. The date seeds represent about 15% of the total weight of the date fruits [11]. The fleshy fruit part is delicious and highly nutritious [12] and can be consumed at any of the three major stages of maturity [9]. The extracts of palm date fruits and seeds showed strong antioxidant properties in many *in vitro* studies [9,13] and *in vivo* studies [10,14,15]. Recently, El Arem et al. [16] demonstrated the beneficial effect of aqueous palm fruit extract (ADE) against the oxidative stress and hepatotoxicity induced by trichloroacetic acid (TCA) and Saafi-Ben Salah et al. [17] and El Arem et al. [18] demonstrated the beneficial effect for palm date extracts against nephrotoxicity induced by dichloroacetic acid and dimethoate respectively. Moreover, we demonstrated in a recent study by our group that palm date extracts ameliorates the hepatic dysfunctions in type 2

DM [19]. We hypothesized that the beneficial antioxidant components of palm dates could protect the kidney from diabetic nephropathy. So, this study aimed to investigate the effects aqueous and methanolic fruit and seed extracts palm date on diabetic nephropathy in a rat model of type 2 DM and their underlying mechanisms.

Material and Methods

Extraction of Palm date fruit and seeds

Palm date (*Phoenix dactylifera* L.) was collected in September 2013 from Sharkyia Governorate at tamr stage and stored in a refrigerator at 4°C and finally identified by Botany department, Faculty of Science, Zagazig University. We removed the flesh of date from the pits and crushed and cut 100 g of fruits into small pieces with a sharp knife and 100 g of seeds were ground into a fine powder. Fruits and seeds were defatted by using n-hexane then extracted three times with 500 ml methanol at room temperature for 24 h by a magnetic stirrer. To obtain methanol crude extracts, the extracts were filtered and centrifuged at 6000 radius centrifugation force (RCF) for 30 min at 3°C, then the supernatant was concentrated under low pressure at 40°C for 1 to 2 h using a rotary

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evaporator. These extracts were stored in dark glass bottles at -4°C. Also, we soaked the dry date palm fruits in water (1:5 w/v) at 40°C then stirred for 24 h at a temperature of 4°C. This mixture was centrifuged at 6000 RCF for 20 min and the precipitate was discarded and the supernatant was lyophilized and stored at 4°C for further analysis [20,21].

HPLC-PDA analysis determined the composition of major phenolic substances in the extracts at 280 and 340 nm. We identified and quantified the phenolics and flavonoids contents of in the extracts by measuring their retention times and UV spectra with that of standards.

Experimental animals

The present study involved sixty male Sprague Dawely rats weighing 180-200 g (aged 3-4 months). Rats were bred and housed in the animal house of the "Research Center, Faculty of Science, Zagazig University, Egypt". They were kept in glass cages under controlled environmental conditions with a 12 h light/dark cycle and have free access to the tape water. Experimental procedures and techniques were done according to the international guidelines for animal ethics and care. The study was approved by local ethical committee of Zagazig, Faculty of Science, Egypt.

Induction of diabetic nephropathy animal model

The rat model of type 2 DM was induced according previous technique done by our group [19]. Briefly, rats feed on a high-fat diet "(22.5% hydrogenated vegetable oil, 22.5% milk powder, 51.5% soybean ground, 2% corn starch, 1% sucrose and 0.5% vitamins and minerals)" for two weeks. Then a dose of 35 mg/kg of freshly prepared streptozocin (STZ) (Sigma chemical Company, Saint Louis, MO, USA) was injected in tail vein after overnight fasting. Usually, glucose was detected in urine (by glucose strips) within 2 days of STZ injection. If rats did not develop glucosuria within 2 days, we repeated the STZ injection for a second time. Then, we measured blood glucose and rats having blood glucose levels more than 250 mg/dl (in two successive samples) were enrolled in the present study. Then urinary albumin excretion was measured weekly to confirm development of microalbuminuria (early sign of nephropathy). Most of rats developed microalbuminuria by the end of 10-12 weeks.

Preparation of plant extracts

Methanolic extract: One hundred grams (100 g) of the fresh blended fruit or seed of *P. dactylifera* were extracted exhaustively with 1 L of methanol and the mixture sieved and the remaining methanol in the extract was evaporated to get the concentrated crude extract. The extract was reconstituted in distilled water at a concentration of 1 mg/ ml and stored in the refrigerator until needed.

Aqueous extract: One hundred grams (100 g) of the fresh blended fruit or seed of *P. dactylifera* were extracted exhaustively with 1 L of distilled and the mixture sieved and the remaining water in the extract was evaporated to get the concentrated crude extract. The extract was reconstituted in distilled water at a concentration of 1 mg/ml and kept in the refrigerator until needed.

Study design

Rats were randomly allocated into 6 groups (10 rats each); a) normal control (NC) group; normal rats, b) diabetic control (DC) group; diabetic rats received saline *via* gastric gavage for 15 weeks c) DM+AFE group: diabetic rats received aqueous fruit extract (4 ml/ kg b.w. daily) for 15 weeks, d) DM+MFE group: diabetic rats received methanolic fruit extract (5 ml/kg b.w. daily) for 15 weeks, e) DM+ASE

group: diabetic rats received aqueous seed extract (10 ml/kg b.w. daily) for 15 weeks and f) DM+MSE group: diabetic rats received methanolic seed extract (5 ml/kg b.w. daily) for 15 weeks [22]. All extracts were given via gastric gavage.

Collection of urine and blood samples and harvesting kidney specimens

Rats were weighted and placed in metabolic cages for 24 h urine collection at the end of experiment (15 weeks after treatment). Urine samples were kept at -20°C till further analyses. Also, blood samples were collected by Pasteur pipette from the ophthalmic venous plexus under light halothane anaesthesia. The collected blood samples were centrifuged at 1000 rpm and sera were kept at -20°C till the time of biochemical analysis. Finally, rats were sacrificed by an overdose of Na⁺-thiopental (75 mg/kg b.w., i.p.). Then the rat abdomen was opened and a cannula was inserted into the abdominal aorta to perfuse the kidneys with phosphate-buffered saline (PBS). The kidneys were removed rapidly and each kidney was cut into two equal halves by a scalpel. One half of the kidney was rapidly placed in 10% neutral buffered formalin (pH 7.4) for histopathological examination and the other half was stored at -20°C for biochemical assay of oxidative stress markers.

Assessment of serum creatinine, urea, uric acid, triglycerides, cholesterol, HDL, LDL and urinary albumin excretion

Serum creatinine, urea, uric acid, TGs, HDL, LDL and UAE were measured by commercially available kits according to manufacturer instructions. Kits were purchased from Diamond Diagnostics, Egypt (serum creatinine), Stanbio Lab., Texas, USA (urea and uric acid), and Biomed Diagnostics- EGY- Chem., Egypt (triglycerides, cholesterol, HDL and LDL). Assay of urinary albumin excretion was achieved using specific kits (Fortress Diagnostics Limited Unit 2C Antrim Technology Park, Antrim BT41 1QS, United Kingdom).

Measurement of lipid peroxidations marker (MDA) and antioxidant (GSH, SOD and catalase enzyme) in kidney tissues

The half of the kidney stored at -20°C was weighed and homogenized in 5-10 ml cold buffer (50 mM potassium phosphate, pH 7.5, 1 mM EDTA) per gram tissue, using mortar and pestle then centrifuged at 4,000 rpm for 15 min at 4°C and the supernatant was kept at -20°C until analysis of the markers of oxidative stress. Malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase enzyme were measured in kidney homogenates by a colorimetric kit (Bio-Diagnostics, Dokki, Giza, Egypt). The concentrations of MDA, GSH, catalase activity and SOD activity were measured in nmol/g kidney tissues, mg/g kidney tissue, U/g kidney tissues and U/g kidney tissues respectively.

Histopathological examination of kidney tissues

The kidney half fixed in 10% formalin (pH 7.4) was embedded in paraffin and sections (3-4 μ m thick) were prepared. Slides were stained with hematoxylin and eosin (H&E) and PAS stains. The sections were observed on an Olympus BX51 light microscope in a blind fashion. Pictures were obtained by a PC-driven digital camera (Olympus E-620). Morphometric analysis of image was done by the computer software (Cell*, Olympus Soft Imaging Solution GmbH). The morphologic changes in glomeruli, arterioles and tubular epithelium were semi quantitatively evaluated according to previous studies [23,24].

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Statistical analysis

All statistical analyzes were done by SPSS version 14. To measure the statistical significant difference among groups, ANOVA with Scheffe's post-hoc test were used. Statistical significance was considered when P value ≤ 0.05 . Data were presented as mean \pm standard deviation (SD).

Results

Results of HPLC analyses of palm date aqueous and methanolic fruit and seed extracts

Gummy yellowish extract with a concentration of 40 g/100 g fruits was yielded from fruit extracts, while palm seed yielded dark brown extract with final concentration of 1.5 g/100 g. Eight compounds were detected by HPLC analysis for fruit methanolic and aqueous extracts. The compounds, from high to low concentration, were p-coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, sinapic acid, quercitin-3-o-glycoside, apiginin-c-glycoside and luteoline -7-o-glycoside respectively (Figures 1a and 1b). On other hand, five compounds were detected by HPLC analysis for seed methanolic and aqueous extracts. From high to low concentration, they were caffeic acid, gallic acid, vanillic acid, quercitin and p-coumaric acid respectively (Figures 1c and 1d). Moreover, the concentration of these substances in aqueous extracts was greater than in methanolic extracts in both fruits and seeds (Figures 1b and 1d).

Results of animal survival and body weight in different groups

By the end of experiment 10 rats were survived in NC group, 4 rats in DM group, 8 rats in DM+AFE and DM+MFE groups, 6 rats in DM+ASE group and 5 rats in DM+MSE group. Meyer-Kaplan curve showed that survival rate was greater in aqueous extracts than methanolic extracts and in fruits than seed extracts (Figure 2). DC group showed significant increase in body weight compared to NC group (p<0.05). Also, compared to diabetic group, DM+AFE, DM+MFE, DM+ASE and DM+MSE group showed significant reduction in body weight (p<0.05). Moreover, we did not found any statistical significant difference in body weight among these groups (Table 1).

Results of fasting blood glucose (mg/dl) and lipid profile (serum TC, TGs, LDL and HDL) in different groups

DC group showed significant increase in fasting blood glucose compared to NC group (p<0.001) and treatment with seed and fruit extracts caused significant decrease in fasting blood glucose compared to diabetic group (p<0.05). Also, fruit extracts (AFE, MFE) groups showed more significant decrease in fasting blood glucose compared to seed extracts (ASE, MSE) (p<0.05) and aqueous extracts showed more significant reduction in fasting blood glucose than methanolic extracts (p<0.05) (Table 1).

DM group showed significant increase in serum TGs, TC and LDL with significant decrease in serum HDL compared to NC group (p<0.05) and this significant elevation was significantly attenuated in treated groups (AFE, MFE, ASE and MSE) compared to DM group (p<0.05). There were no statistical significant difference among all treated groups except in TC between AFE group and MSE group (p<0.05). On the other hand, HDL showed significant decrease in DM group compared to NC group and this attenuation was improved in all treated groups (p<0.05) (Table 1).

Results of markers of kidney functions (serum creatinine, urea, uric acid and urinary albumin excretion) in different groups

Diabetic rats showed significant increase in serum creatinine, urea and uric acid and urinary albumin excretion (UAE) compared to normal rats (p<0.05) and these parameters significantly attenuated in all treated groups (DM+AFE, DM+MFE, DM+ASE and DM+MSE) compared to DM groups (p<0.05). However, the degree of attenuation of these parameters was significantly higher in fruit extract groups (AFE and MFE) than seed extract groups (AFE and MFE) (p<0.05) and in aqueous extracts (AFE and ASE) groups than methanolic extract (MFE and MSE) groups (p<0.05) (Table 2).

Results of oxidative stress markers (MDA, GSH, SOD and catalase enzyme activities) in kidney tissues in different groups

Kidney tissues obtained from diabetic rats showed significant increase in MDA concentration compared to those obtained from normal rats (p<0.001) (Figure 3a), while the activities of catalase and SOD enzymes as well as the concentration of GSH showed significant decrease in kidney tissues obtained from diabetic rats compared to those obtained from normal rats (p<0.001) (Figures 3b-3d). These parameters showed significant improvement in all treated groups (DM+AFE, DM+MFE, DM+ASE and DM+MSE) compared to DM groups (p<0.05). However, the degree of improvement was significantly higher in fruit extract groups (AFE and MFE) than seed extract groups (ASE and MSE) (p<0.05) and in aqueous extracts (AFE and ASE) groups than methanolic extract (MFE and MSE) groups (p<0.05).

Results of histopathological damage score in different groups

The damage score showed significant increase in glomerular sclerosis, arterial lesions and tubulointerstitial sclerosis in diabetic rats compared to normal rats (p<0.001) (Figure 4a). The degree of renal damage was significantly improved in all treated groups (DM+AFE, DM+MFE, DM+ASE and DM+MSE) compared to DM groups (p<0.001). However, the degree of improvement was significantly higher in fruit extract groups (AFE and MFE) than seed extract groups (AFE and MFE) (p<0.001), in AFE group than MFE group and in MSE group than ASE group (p<0.05). Kidneys obtained from NC group showed normal glomerular and tubular structure with normal vascular architecture (Figure 4b), while kidney obtained from DM group showed segmental glomerular sclerosis with formation of synechiae with parietal epithelial cells and periglomerular sclerosis, lost tubular cell brush border and arterial injury in the form of medial or intimal hyperplasia with luminal narrowing (Figures 4c and 4d), the degree of glomerular sclerosis and tubular injury was reduced in all treated groups (Figures 4e-4h).

Discussion

The main findings of the present study can be summarized as follow a) diabetic nephropathy in type 2 DM was associated with dyslipidemia, impairment of kidney functions and morphology and enhanced redox state in kidney tissues b) pretreatment with either fruit (aqueous or methanolic) extracts or seed (aqueous or methanolic) extracts caused improvement in dyslipidemia, kidney functions and morphology and oxidative stress state in kidney tissue and c) fruit extracts offer powerful protective effects than seed extracts and aqueous showed more protective effects than seed extracts.

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	NC group (n=10)	DM group (n=4)	DM+AFE group (n=8)	DM+MFE group (n=8)	DM+ASE group (n=6)	DM+MSE group (n=5)
Body weight (g)	176.93 ± 17.1	248.34 ± 15.9 [*]	194.60 ± 27.2#	196.29 ± 10.5#	189.86 ± 7.2#	196.57 ± 15.5#
Fasting blood glucose (mg/dl)	86.28 ± 6.77	464.71 ± 43.96*	125.00 ± 13.6 [#]	154.71 ± 17.4*#	173.14 ± 13.71*#\$	197.71 ± 9.44*#\$&
Serum cholesterol (mg/ dl)	135.14 ± 18.31	218.7 ± 25.52 [*]	147.46 ± 18.63 [#]	140.2 ± 10.19 [#]	141.2 ± 11.2#	133.1 ± 16.0 ^{#\$}
Serum triglycerides (mg/dl)	102.5 ± 11.32	177.8 ± 22.6 [*]	122.8 ± 17.7#	112.1 ± 17.2#	109.5 ± 13.8 [#]	105.5 ± 11.5 [#]
Serum HDL (mg/dl)	51.0 ± 4.0	31.28 ± 9.8*	41.5 ± 6.6	45.2 ± 7.47	47.4 ± 7.27	41.9 ± 13.2
Serum LDL (mg/dl)	63.6 ± 10.8	151.2 ± 13.77 [*]	81.3 ± 10.6 [#]	72.5 ± 5.92 [#]	74.4 ± 19.2 [#]	63.3 ± 6.7 ^{#\$}

All results are expressed as mean \pm SD. One way ANOVA with Scheffe's post hoc test (significance at $p \le 0.05$). significant vs. NC group, #significant vs. DM group, significant vs. DM+AFE group and significant vs. DM+MFE and \in significant vs. group treated with ASE. NC: Normal Control, DC: Diabetic Control, DM+AFE: diabetic rats treated with aqueous Fruit extract, DM+MFE: diabetic rats treated with methanolic fruit extract, DM+ASE: diabetic rats treated with aqueous seed extract, DM+MSE: diabetic rats treated with methanolic seed extract.

Table 1: Body weight (g), fasting blood glucose (mg/dl) and Lipid profile (serum cholesterol (mg/dl), serum triglycerides (mg/dl), serum HDL (mg/dl) and serum LDL (mg/dl)) in different studied groups.

The present study demonstrated that type 2 DM was characterized by significant increase in body weight, fasting blood sugar, serum TC, TGs, LDL with significant decrease in animal survival and serum HDL. Previous studies reported similar findings such as Wang et al. [25], Khanra et al. [26] and Srimaroeng et al. [27]. Activation of hormone sensitive lipase in DM (normally inhibited by insulin) which increases the mobilization of free fatty acids from the peripheral fat deposits to blood stream might explain hyperlipidemia demonstrated in the present study. The elevated levels of serum lipids in DM cause the risk of development of diabetic nephropathy [28,29]. The significant increase in animal survival, body weight in type 2 diabetic rats in the present study might be due to the consumption of a diet rich in energy (saturated fats) and its deposition in different body fat stores [30] and reduction in energy expenditure [31]. On other hand, treatment with fruit and seed aqueous and methanolic extracts caused significant improvement in animal survival, body weight, fasting blood sugar, serum TC, TG, LDL and HDL. In agreement with these findings, Zangiabadi et al. [22] demonstrated that aqueous date extract improved body weight and fasting blood glucose in rat model of diabetic peripheral neuropathy. These findings suggest that consumption of palm date fruit and seed extracts could improve the body weight, hyperglycemia and hyperlipidemia in type 2 diabetes mellitus. Improvement in body weight in palm date extracts treated groups might be a reflection for improvement of hyperglycemia and hyperlipidemia.

Development of diabetic nephropathy in the present study was evidenced by significant increase in serum creatinine, uric acid, urea and urinary albumin excretion (UAE). In consistence with these findings,

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Figure 3: Oxidative stress markers in kidney tissues from different groups a) Malondialdhyde (MDA) (nmol/g kidney tissue), b) CAT enzyme activity (U/g kidney tissue), c) SOD enzyme activity (U/g kidney tissue) and d) reduced glutathione (GSH) concentration (mg/g kidney tissue). 'significant vs. NC group, *significant vs. DM+AFE group, *significant vs. DM+MFE and € significant vs. group treated with ASE. NC: Normal Control, DC: Diabetic Control, DM+AFE: diabetic rats treated with aqueous Fruit extract, DM+MFE: diabetic rats treated with methanolic fruit extract, DM+ASE: diabetic rats treated with aqueous seed extract.

	Serum creatinine (mg/dl)	Serum urea (mg/dl)	Serum uric acid (mg/dl)	Urinary albumin excretion (UAE) (mg/24 h)
NC group (n=10)	0.58 ± 0.066	23.71 ± 3.14	3.37 ± 0.13	19.57 ± 1.71
DM group (n=4)	2.92 ± 0.23 [*]	71.57 ± 2.14 [*]	6.67 ± 0.20 [*]	398.4 ± 14.19*
DM+AFE group (n=8)	1.44 ± 0.097*#	34.14 ± 3.71 ^{*#}	4.1 ± 0.14 ^{*#}	125.7 ± 5.52*#
DM+MFE group (n=8)	1.92 ± 0.17 ^{*#\$}	50.57 ± 3.69*#\$	4.82 ± 0.26*#\$	282.2 ± 11.35 ^{*#s}
DM+ASE group (n=6)	2.34 ± 0.09*#\$&	57.42 ± 1.51 ^{*#\$&}	5.47 ± 0.19*#\$&	307.10 ± 13.8*#\$&
DM+MSE group (n=5)	2.77 ± 0.11 ^{*\$&€}	65.0 ± 1.63*#\$&€	6.21 ± 0.13 ^{*#\$&€}	336.14 ± 7.92 ^{*#\$&€}

All results are expressed as mean \pm SD. One way ANOVA with Scheffe's post hoc test (significance at $p \le 0.05$). significant vs. NC group, #significant vs. DM group, significant vs. DM+AFE group and significant vs. DM+MFE and \in significant vs. group treated with ASE. NC: Normal Control, DC: Diabetic Control, DM+AFE: diabetic rats treated with aqueous Fruit extract, DM+MFE: diabetic rats treated with methanolic fruit extract, DM+ASE: diabetic rats treated with aqueous seed extract, DM+MSE: diabetic rats treated with methanolic seed extract.

Table 2: Kidney function parameters (serum creatinine (mg/dl), urea (mg/dl), uric acid (mg/dl) and urinary albumin excretion (UAE) (mg/24 h)) in different studied groups.

previous studies demonstrated significant increase in plasma creatinine and BUN [32-34] and serum urea levels [35] in diabetic rats compared to normal. The elevation of serum creatinine, uric acid, urea and UAE are reflection to impairment of kidney functions with development of diabetic nephropathy and loss of glomerular membrane permeability [36]. Histopathopathological examination for kidneys obtained from diabetic rats revealed significant increase in glomerular damage (segmental glomerular sclerosis and the formation of synechiae with parietal epithelial cells with periglomerular sclerosis), loss of tubular epithelial cell brush border and arterial injury in the form of medial or intimal hyperplasia with luminal narrowing. In agreement with these findings Khanra et al. [26] demonstrated thickening of glomerular and tubular basement membrane of kidneys in type 2 diabetic rats and Tervaert et al. [37] demonstrated mesangial expansion in type 2 diabetic rat's kidney which might influence membrane permeability. Treatment with fruit and seed aqueous and methanolic extracts caused significant improvement in kidney functions and morphology as evidenced by significant decrease in serum creatinine, urea, uric acid and urinary albumin excretion as well as significant reduction in glomerular and tubulointerstitial damage scores compared to diabetic untreated rats. These findings suggest renoprotective effects for fruit and seed aqueous and methanolic extracts against diabetic nephropathy in rats. Moreover, the effects of fruit extracts were more powerful than seed extracts and aqueous extracts were more powerful than methanolic extracts. This could be explained by the difference in compounds present in each type of extract as well as the concentration these compounds in the extracts. In the present study, fruit extracts yielded 8 compounds (p-coumaric acid, caffeic acid and ferulic acid have the highest concentration) while seed extracts yielded 5 compounds (caffeic acid and gallic acid have the highest concentration). Also,

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Figure 4: Effects of aqueous and methanolic fruit and seed extracts on renal morphology (a) 'significant vs. NC group, *significant vs. DM group, \$significant vs. DM+MFE and † significant vs. group treated with ASE. Kidney specimens showing b) normal glomerulus (arrow) and normal tubular cell brush border (arrow) (NC group), c) glomerular damage, segmental glomerular sclerosis and the formation of synechiae with parietal epithelial cells with periglomerular sclerosis (arrow) and lost tubular cell brush border (arrow) (DM group), d) arterial injury in the form of medial or intimal hyperplasia with luminal narrowing (arrow) and loss of brush border (arrow) (DM group), e) segmental glomerular sclerosis (arrow) in <25% together with loss of tubular brush border (arrow) (DM+AFE group), f) an artery with average wall thickness (arrow) together with focal loss of tubular brush border (arrow) (DM+AFE group), f) an artery with average wall thickness (arrow) together with focal loss of tubular brush border (arrow) (DM+AFE group), f) an artery with average wall thickness (arrow) together with focal loss of tubular brush border (arrow) (DM+AFE group), f) an artery with average wall thickness (arrow) (DM+ASE group) and h) an artery with wall hyperplasia (arrow) together with focal loss of tubular brush border (arrow) (DM+AFE group), g) segmental glomerular sclerosis (arrow) in <25% together with loss of tubular brush border (arrow) (DM+ASE group) and h) an artery with wall hyperplasia (arrow) together with focal loss of tubular brush border (arrow) and inter-tubular inflammatory cells (arrow) (DM+MSE group) (PAS X400). NC: Normal Control, DC: Diabetic Control, DM+AFE: diabetic rats treated with aqueous Fruit extract, DM+MFE: diabetic rats treated with methanolic fruit extract, DM+ASE: diabetic rats treated with aqueous seed extract.

the concentrations of these compounds in both extracts were great in aqueous extracts than methanolic extracts. In line with agreement with El Arem et al. [18] attributed the nephroprotective effect of the aqueous extracts of palm date against the renal damage induced by dichloroacetic acid to its richness in antioxidant compounds such as ferulic, caffeic and *p*-coumaric acids.

Improvement of renal functions and morphology by palm dates in the present study might be due to control of hyperglycemia and hyperlipidemia in this animal model and due to the compounds present in palm date extracts such as quercitin and p-coumaric acid. In consistence with this hypothesis, Mallikarjun et al. [38] demonstrated that quercetin reduced the excretion of urinary albumin excretion by 40% at the 4th week in diabetic rats. In consistence with the findings of the present study, El Arem et al. [18] demonstrated that aqueous date extract (ADE) significantly improved the plasma levels of creatinine, urea and uric acid as well as kidney morphology in a rat model of DCA-induced nephropathy. Also, Al-Qarawi et al. [39] reported that administration of the extracts of the flesh and pits of palm date caused significant improvement in serum creatinine and urea concentrations and the proximal tubular damage in a gentamicin-induced nephrotoxicity in rats. Moreover, Saafi-Ben Salah et al. [17] showed that aqueous extract of Deglet Nour cultivar has improved serum creatinine and urea and attenuated the histopathological damage induced by dimethoate in kidney of rats. Also, Ali and Abdu [40] showed that the aqueous date extract of Ajwa cultivar has improved the histopathological change induced by Ochratoxin (A) in renal distal and proximal tubules.

Reactive oxygen species (ROS) and oxidative stress play a crucial role in diabetic pathophysiology [41] and play important role in high glucose-induced renal injury [42,43]. The present study demonstrated enhanced redox status within the kidney tissues of type 2 diabetic rats. This is evidenced by significant increase in MDA (product and marker of peroxidations of cell membrane lipids) with significant depletion in the endogenous antioxidants such as GSH, SOD and catalase enzyme activities in kidney tissues of diabetic untreated rats. Treatment with fruit and seed aqueous and methanolic extracts caused significant reduction in MDA in kidney tissues with significant increase in the endogenous antioxidants such as GSH, SOD and catalase enzymes activities. Moreover, the effect of aqueous fruit extract was more powerful compared to other types of extracts. Previous studies reported similar findings for palm date extracts. For instance, in a rat model of trichloroacetic acid (TCA)-induced hepatoxicity, El Arem et al. [16] demonstrated that aqueous palm fruit extract attenuated enhanced redox state in liver as evidenced by significant reduction in hepatic lipid peroxidation products (MDA) with improvement of antioxidant enzyme e.g. catalase, SOD and glutathione peroxidase activities. Al-Qarawi et al. [39] suggested that the nephroprotective action of extracts of the flesh and pits of Phoenix dactylifera in gentamicin-induced nephrotoxicity attributed to the antioxidant components of date such as melatonin, vitamin E and ascorbic acid. Also, Saafi-Ben Salah et al. [17] showed that date aqueous extract of Deglet Nour cultivar has attenuated lipid peroxidation and improved antioxidant defense system in dimethoate-induced nephrotoxicity. The protective effect of aqueous date extract to their high amounts of antioxidant substances such as polyphenols, trace elements, and vitamin C [13,44-46].

Conclusion

We demonstrated in the present study renoprotective effects for palm date fruit and seeds aqueous and methanolic extracts against diabetic nephropathy in rats of type 2 DM. Moreover, the nephroprotective effects of fruit extracts were more powerful than seed extracts. This nephroprotective action might be due to improvement of glucose and lipid homeostasis and the antioxidants properties of its contents such as p-coumaric acid, ferulic acid, caffeic, quercetin and chlorogenic acid.

Conflict of Interest

All authors declare that there was no conflict of interest.

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