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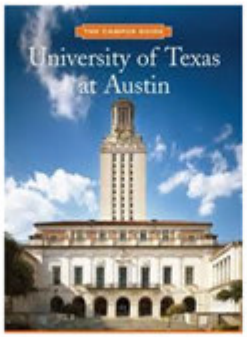

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RESCUE OF INFLAMMATORY RENAL DAMAGE BY MEDICINAL PLANT EXTRACTS IN DIABETIC RATS

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ABSTRACT

Diabetic nephropathy (DN) is the most critical diabetic complication that leads to renal failure. The purpose of this research was to explore the anti-diabetic effectiveness of *Cucumis melo* var. *flexuosus* and/or *Phoenix dactylifera* fruit aqueous extracts and their mechanisms in alleviating nephropathy in hyperglycemic rats. Diabetes was promoted in rats by injection (i.p.) of a single dose of streptozotocin (STZ). *C. flexuosus* and *P. dactylifera* extracts (CFE and PDE respectively) were ingested to diabetic rats for thirty consecutive days. The data revealed that intake of either plant extract or their combination to diabetic rats, significantly diminished the serum glucose and raised the serum insulin concentration. The plant extracts significantly ameliorated the increases in relative kidney weights as well as in renal tumor necrosis factor (TNF-α), serum C –reactive protein (CRP) and renal vascular endothelial factor (VEGF). Serum kidney function markers (creatinine, uric acid and urea) were also decreased in diabetic rats treated with the plant extracts. Histopathological observation was also carried out to confirm the biochemical results. This study has proven that the current plant extracts have potential hypoglycemic effect and could attenuate diabetic inflammatory renal damage in rats. The combination of the two extracts was synergistically the effective one. This result may help in exploring a novel therapy to manage diabetes and its complications.

KEY WORDS: ***Phoenix dactylifera*, *Cucumis melo var. flexuosus*, *inflammatory molecules, renal damage.***

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INTRODUCTION

Diabetes mellitus ranks among the top causes of death worldwide. This syndrome is identified by impaired of glucose regulation, resulting from deficiency in insulin release, insulin function or both, causing defect in glucose degradation and other energy-producing elements, including fats and proteins[1]. Modification in metabolism leads to microvascular problems, including nephropathy[2]. Diabetic nephropathy (DN) is the principle reason of progressive kidney damage, leading to dialysis or transplantation[3]. DN has been shown to develop in 20–50% of diabetic subjects and has become a leading reason of renal failing globally[3]. DN is discriminated by structural and functional aberrations[4]. Patients with DN have a gradual drop in glomerular function[5]. Studies on DN, revealed that chronic inflammatory stress participates in its development and progression[6]. NF-kB related inflammatory reactions has been shown to have a fundamental role in the pathogenesis of DN[7]. Generation of inflammatory molecules, like growth factors, cytokines and chemokines has been proven in DM[4]. Plant based therapy is presently used for controlling diabetes due to its capability to suppress the incidence and/or progression of its complexity. Cucurbitaceous is a family for many medicinal plants cultivated in equatorial regions[8]. Some reports have demonstrated that many cucurbits have potential pharmacological properties versus complexity of diabetic [9-10]. *Cucumis melo var. flexuosus* (known as snake melon) is one of cucurbit plants[11]. *C. melo* var. *flexuosus* leaf extract has been found to possess antidiabetic, anti-inflammatory, antioxidant and anti-apoptosis capacities[12]. *Phoenix dactylifera* Linn. (date palm, family *Arecaceae*) fruits are broadly consumed worldwide for their beneficial nutritive importance. The fruit water extract has many medicinal activities, such as anti-mutagenic, anti-inflammatory, antioxidant and antidiabetic activities [13-14]. The anti-inflammatory effect attributes to its active components namely flavonoids, steroids, saponins and phenolic compounds[15]. It has reported that date can stimulate insulin release and suppress glucose absorption[16]. Although the antidiabetic impacts of both *C. melo var. flexuosus* and *P. dactylifera* were studied, the exact mode of actions of their extracts in management diabetic renal damage are still unexplored. The goal of this work was to investigate the key mechanism (s) of CFE and /or PDE in protecting against diabetes promoted renal damage in diabetic rats.

MATERIALS AND METHODS

Chemicals

All chemicals utilized in this study were highly pure, obtained from Sigma and Merck companies, USA.

Plants

C. melo var. *flexuosus* and *P. dactylifera* fruits were bought from the market. The plants were described by a taxonomist in the Department of Biological Science, king Abdulaziz University, Jeddah, Saudi Arabia.

Procedure of C. flexuosus fruit extraction

C. *melo* var. *flexuosus* fruits were cut into pieces after removing the seeds and then dried at 40 °C for 48 hours. 500 g of the dried fruits were crushed and soaked in 4 L bi-distilled water and then heated at 100 °C for half hour. The aqueous phase was gotten by centrifugation for 20 min at 5000x*g* and then freeze dried.

Procedure of *P. dactylifera* fruit extraction

P. dactylifera L. fruits (400 g) were blended with 4 L bi-distilled water, utilizing an electric blender. The commixture was left for 24 h, and then centrifuged for 25 min. at 4000x*g*. The liquid phase was gathered and then freeze dried.

Animals

Male Wistar rats (180-200 g) were used for this investigation. The rats were bought from the Experimental Animal Care Center, King Fahad Medical Research Center, King Abdulaziz University (Jeddah, KSA). Animals were maintained at control circumstances at 22–25 °C and supplied with balanced diet and water *ad libitum*. Animal care was performed following the roles supplied by the Experimental Animal Care Committee (Reference No 1007-16) of Faculty of Science, Jeddah University.

Experimental design

Diabetes was promoted in diabetic rat groups by injecting a single dose (45 mg/ kg b.w. i. p.) of streptozotocin (STZC, Sigma, USA), prepared in 0.05M citrate buffer (pH 4.5)[17]. The non-diabetic normal group were injected with an equivalent amount of citrate buffer. Blood glucose was monitored utilizing a glucometer, at the tenth day after STZC administration; rats with fasting blood glucose above 220 mg/dl were selected as diabetic. The rats were categorized into five groups (n= 10), group 1, control rats; group 2, diabetic rats; group

3, diabetic rats ingested with CFE (400mg /kg)[9]; group 4, diabetic rats ingested with PDE (400mg/kg)[18]; group 5, diabetic rats ingested with the combination of CFE and PDE (400 mg/ kg each). Lyophilized CFE and PDE were solved in distilled water and given orally to animals for a month, forty days after promotion of diabetes. The combination of the two plant extracts was dissolved as a mixture in distilled water before administration. Each rat was weighed at the beginning and the end of the experiment to estimate the change in body mass. Finally, the rats were fasted for 12-14 hours and the blood samples were withdrawn for serum separation and used for biochemical investigations. After withdrawing the blood, all rats were sacrificed under light anesthesia and the kidney samples were collected and washed in phosphate buffered saline, blotted on a filter paper and weighed then used for biochemical and histopathological tissue analysis.

Estimation of diabetic and kidney function indices

Serum fasting glucose level, uric acid, creatinine and urea were estimated using an automatic analyzer (ci16200, Abbott, USA). Insulin was estimated by rat insulin ELISA kit (BioVendor company, Laboratorni medicina a.s. Karasek 1767/1, 621 00 Brno Czech Republic)

Determination of inflammatory and angiogenic biomarkers

Tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF) were determined in kidney tissue, using commercially available ELISA kit in accordance to the guidance provided by the manufacturer (R&D Systems, USA). Serum C–reactive protein (CRP) was measured utilizing latex-enhanced immunonephelometry method (Siemens Dade Behring, Germany).

Histopathological examination

Small segments of kidney were treated with 10 % buffered formalin for fixation and then dehydrated and implanted in a paraffin wax. The kidney specimens were cut into sections (3–4-μm), stained with hematoxylin-eosin and examined by a light microscope.

STATISTICAL ANALYSIS

The results of the current study were represented as the mean ± SD. Statistical analysis was carried out utilizing one-way analysis of variance (ANOVA) followed by Bonferroni as a post-ANOVA test. The changes among the values were statistically significant at $p \leq 0.05$.

RESULTS

Effect of plant extracts on hyperglycemia markers

Hyperglycemic rats showed a marked raise in serum glucose and a drop in insulin content versus control animals (Table 1). Ingestion of CFE and /or PDE, markedly ameliorated the glucose and the insulin levels versus diabetic untreated rats ($P \leq 0.001$).

Effect of plant extracts on kidney hypertrophy

Diabetic rats had decreased body weights and increased relative kidney weights (kidney hypertrophy) compared with normal rats (Table 2). Administration of CFE and /or PDE appreciably restored the animal body weights and reduced the renal weight in the diabetic treated rat group**.** Treatment with the combination of CFE and PDE was the effective one in ameliorating the rat body weights and reducing the kidney hypertrophy.

Effect of plant extracts on inflammatory markers

As shown in Figures 1 and 2 respectively, the diabetic animals displayed marked increase in the levels of serum CRP and renal TNF-α as compared with the control rats, this deterioration was reversed after the plant extracts administration.

Effect of plant extracts on renal angiogenesis

Figure 3 shows a significant increase in the angiogenic factor (VEGF) in the renal of diabetic rats with relation to the control ones ($P \leq 0.001$). Intake of the plant extracts lonely or in a combination, markedly reversed the elevation in this marker.

Impact of plant extracts on kidney function

Diabetic rats showed increases in serum levels of kidney function markers (uric acid, creatinine and urea) in comparison with the control rats. CFE and /or PDE treatment, pronouncedly reduced serum kidney function markers (Table 3).

Effect of plant extracts on histopathological changes

The impacts of the two plant extracts lonely or in a combination on renal histomorphological changes in the diabetic rats are presented in Figure 4. Diabetic rats showed significant vacuolar degeneration of tubules (Figure 4b); glomerular degeneration (Figure 4c) and infiltration of

inflammatory immune cells (Figure 4d). While, treatment of diabetic rats with CFE and/or PDE (Figures 4e, f & g respectively), restored the normal structural and morphological architecture of renal glomeruli and tubules with respect to the diabetic rats.

Table 1

Effect of CFE and /or PDE on hyperglycemic indices in serum of diabetic rat groups

Parameters	Control	Diabetes	Diabetes + CFE	Diabetes + PDE	Diabetes + CFE+ PDE
Glucose (mg/dl)	80.57±3.67	285.77±16.75^{a}	130.94±5.5^{b*}	100.67±7.3$^{c*\$}$	85.9±5.9*
Insulin (pg/ml)	360.36±12.55	125.67±7.27^{a}	245.7±10.5 $^{a*\$}$	230.75 ± 6.7$^{a\$}$	289.56± 12.5^{b*}

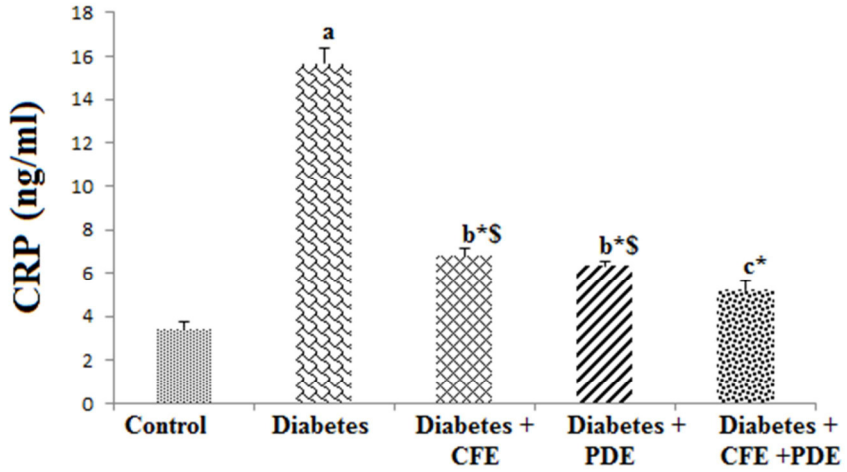
Data are presented as mean ± S.D. (n=10). Significant difference at: $^{a}P \leq 0.001$, $^{b}P \leq 0.01$, $^{c}P \leq 0.05$ compared with the control group; $^{*}P \leq 0.001$ compared with diabetic group; $^{\$}P \leq 0.05$, compared with the combination group (CFE+ PDE).

Table 2

Effect of CFE and /or PDE on renal hypertrophy in diabetic groups.

Parameters	Control	Diabetes	Diabetes + CFE	Diabetes + PDE	Diabetes + CFE+ PDE
Body weight change(g)	+70.43±5.17	-10.40±1.2^{a}	+ 50.90±5.30$^{c*\$}$	+ 60.76±3.70 $^{b*\$}$	+65.80±4.50*
Relative kidney weight (g /100g body weight)	0.2±0.07	0.49±0.05^{a}	0.29±0.06$^{b*\$}$	0.27±0.07$^{b*\$}$	0.22±0.03*

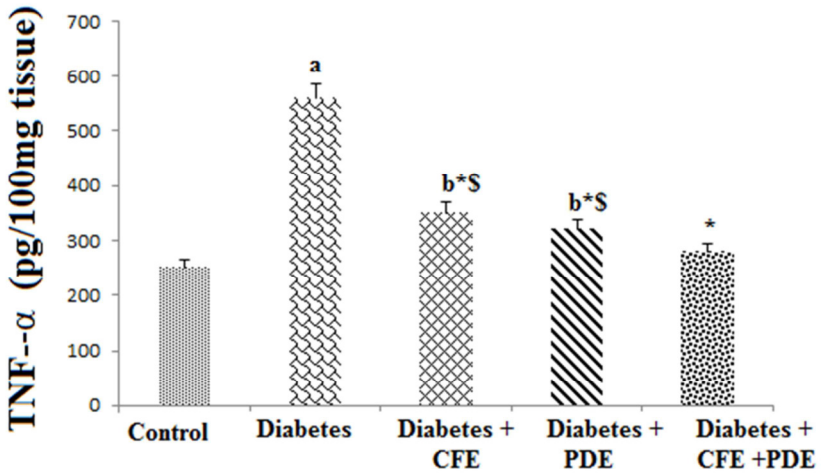
Data are presented as mean ± S.D. (n=10). Significant difference at: $^{a}P \leq 0.001$, $^{b}P \leq 0.01$, $^{c}P \leq 0.05$; $^{\$}P \leq 0.001$ compared with diabetic group; $^{\$}P \leq 0.05$, compared with the combination group (CFE+ PDE).



Values are represented as mean±SD (n=10); $^{a}P \leq 0.001$, $^{b}P \leq 0.01$, $^{c}P \leq 0.05$ compared with the control group, $^{*}P \leq 0.001$ compared with diabetic group, $^{\$}P \leq 0.05$ compared with combination group (CFE+ PDE).

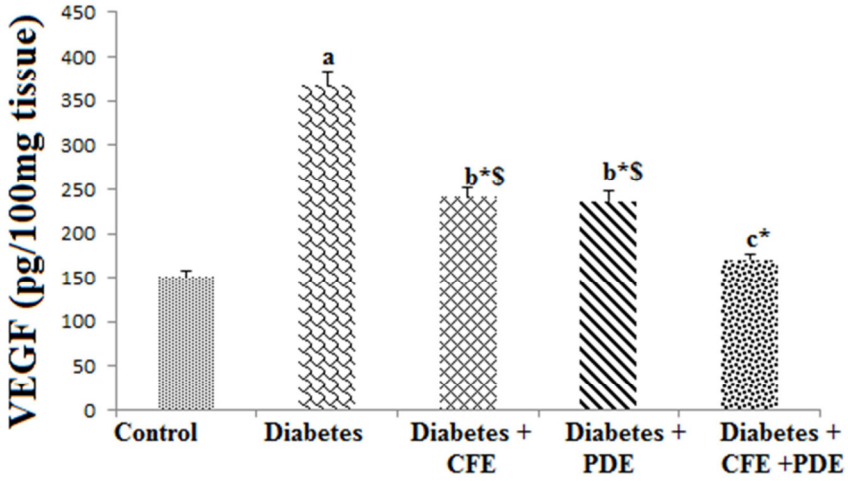
Figure 1

Effect of daily oral administration of CFE and /or PDE on the level of serum CRP in diabetic rats.



Values are represented as mean ±SD (n=10); [a]P ≤ 0.001, [b]P ≤ 0.01 compared with the control group, [*]P ≤ 0.001 compared with diabetic group, [$]P ≤ 0.05 compared with combination group (CFE+ PDE).

Figure 2
Effect of daily oral administration of CFE and /or PDE on the level of renal TNF-α in diabetic rats.



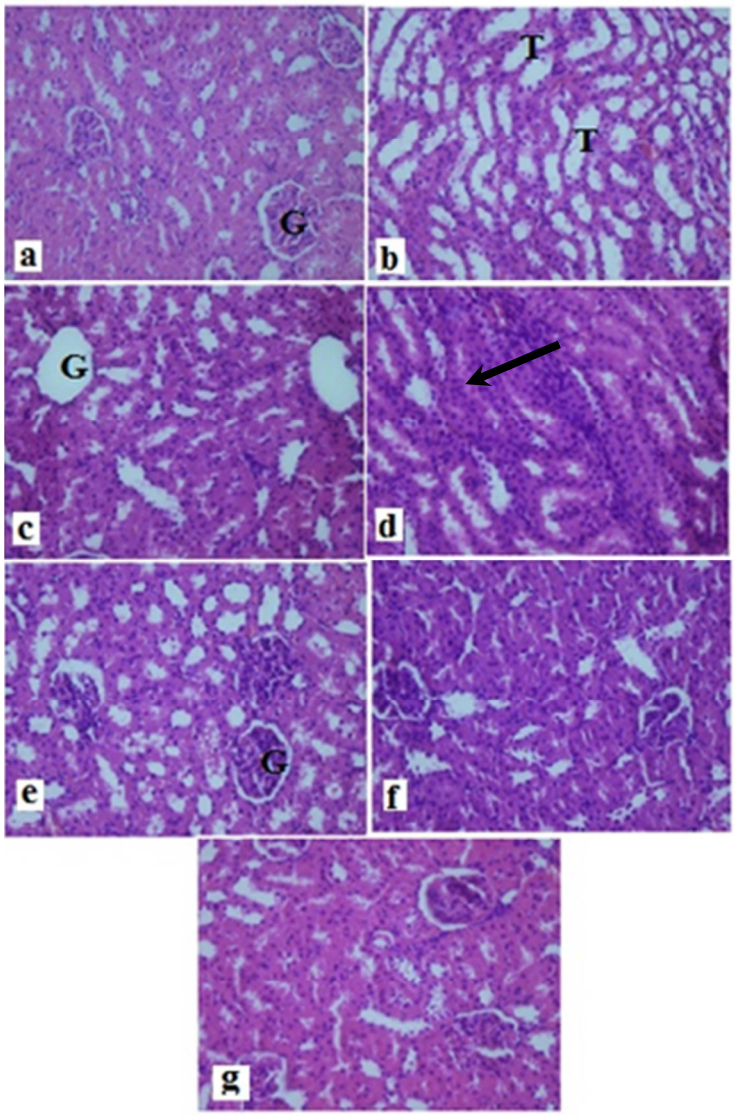
Values are represented as mean ±SD (n=10); [a]P ≤ 0.001, [b]P ≤ 0.01, [c]P ≤ 0.05 compared with the control group, [*]P ≤ 0.001 compared with diabetic group, [$]P ≤ 0.05 compared with combination group (CFE+ PDE).

Figure 3
Effect of daily oral administration of CFE and /or PDE on the level of renal angiogenicVEGF in diabetic rats.

Table 3
Effect of CFE and /or PDE on renal function indices in serum of diabetic rat groups

Parameters	Control	Diabetes	Diabetes + CFE	Diabetes + PDE	Diabetes + CFE+ PDE
Uric acid	2.90±0.18	9.6±0.50 [a]	3.80±0.12 [c*$]	3.52±0.15 [c*$]	2.67±0.23 [*]
Creatinine	0.59±0.08	1.96±0.153 [a]	0.89±0.01 [b*$]	0.9±0.04 [b*$]	0.68±0.04 [*]
Urea	15.50±2.02	48.9±6.2 [a]	22.8±0.90 [c*]	24.50±2.5 [c*]	16.8±0.93 [*]

Data are presented as mean ± SD (n=10); [a]P ≤ 0.001, [b]P ≤ 0.01, [c]P ≤ 0.05 compared with the control group, [*]P ≤ 0.001 compared with diabetic group, [$]P ≤ 0.05 compared with combination group (CFE+ PDE).



(a) Kidney picture of control rat showing normal renal glomeruli and tubules (b, c & d) Kidney pictures of diabetic rats , (b) showing severe vacuolar degradation of tubules; (c) showing glomerular degradation and (d) showing infiltration of inflammatory immune cells (arrow). (e, f & g) Kidney sections of diabetic rat treated with CFE, PDE C. and their combination (CFE+ PDE) respectively, showing more or less normal kidney architecture (H&E X 400).

Figure 4
Kidney histomorphologic pictures of control and diabetic rats.

DISCUSSION

Extensive evidence revealed that DN is the most common reason of renal failing[3]. Drugs of plant origin is now accepted than the synthetic ones for controlling diabetic complexity for their safety and multifunctional roles. This investigation demonstrated the potential protective mechanism(s) of CFE and /or PDE versus renal damage induced by hyperglycemia in rats. To the extent of our information, this is the first work illustrating the protective impact of CFE and /or PDE versus renal damage in diabetic state. Significant elevation in the serum glucose level and a drop in insulin level were observed in STZC induced diabetic rats, indicating development of diabetic state in rats. Treatment with CFE and /or PDE markedly modulated the deviation in these parameters in hyperglycemic rats with respect to diabetic untreated ones. The combination of the two plant extracts was the efficient in regulating these diabetic markers, suggesting that both plant extracts may improve the capability of diabetic rats to utilize the excess blood glucose by promoting the pancreatic beta-cells to produce more insulin. Similarly, the glycemic modulating efficacy of both *C. flexuosus* leaf extract and *P. dactylifera* fruit extract were previously reported [12-13]. In line with previous investigations, the current research illustrated that diabetic rats had decreased body weights and increased relative kidney weights compared with control ones, indicating renal hypertrophy [4,19]. The lowering in the body weights might be an indication of a decrease in the rate of protein synthesis coupled with an excessive breakdown of structural proteins as an alternative

source of energy due to the low availability of carbohydrates[19]. Renal hypertrophy may be due to the expansion of extracellular matrices which is prominent in diabetic nephropathy[20]. Administration of CFE and /or PDE appreciably restored the animal body weights and reduced the renal weight in the diabetic treated rat group**.** Treatment with the combination of CFE and PDE was the effective one in ameliorating the rat body weights and reducing the kidney hypertrophy. The pathogenicity of DN have been related to hyperglycemia induced chronic renal inflammation[7]. The event of renal inflammation can be initiated in DN by overexpression of proinflammatory molecules[7,21]. The present study showed up-regulation of serum CRP and renal TNF-α in diabetic rats with relation to non-diabetic ones**.** Elevation of such mediators in diabetic rats may be a mechanism by which diabetic hyperglycemia accelerates renal inflammatory deterioration. Some authors have reported that over-production of CRP during diabetes is one of the diabetic mechanism that lead to the progression of renal failure[22]. CRP induced by high glucose, significantly can up-regulate other inflammatory molecules, including TNF-α, and IL-1β via an NF-κB-dependent mechanism**[21].** CRP can also promote the production of monocyte chemoattractant protein-1 (MCP-1) and fibrotic growth factors (such as TGF-β1, connective tissue growth factor) which synergistically able to cause renal inflammation and fibrosis[22]. On the other hand, it has reported that production of TNF-α is a toxic to renocytes and can cause their direct injury through inducing apoptosis and necrotic cell death[23]. Beside, reported toxic behavior of TNF-α on renocytes include the induction of transcription proteins, expression of cytokines and cell adhesion proteins, involved in the generation of other inflammatory molecules which collectively have an important action in renal failing in diabetes[24]. Our finding may propose that CRP and TNF α act synergistically as inflammatory cofactors of high glucose to induce kidney failing. Thus, a prophylactic strategy that ameliorates the expression of inflammatory proteins could prevent organ dysfunction. Treatment of diabetic rats with CFE and /or PDE markedly reduced the concentration of TNF-α and CRP versus diabetic untreated animals. The possible mechanism by which the two plant extracts elicit anti-inflammatory effect in diabetic rats might be through inhibiting the production of inflammatory proteins. The suppressing effect of *P. dactylifera* fruit extract on the expression of inflammatory cytokines has been demonstrated in an experimental animal model**[25]**. Also some dietary cucurbits show suppressing impact on the inflammatory proteins, namely interleukin (IL)-1β and TNF-α in sera of lipopolysaccharide (LPS)-inflamed mice[26]. Our work also demonstrated a pronounced increment in the angiogenic factor (VEGF) in the kidney of hyperglycemic animals. Similarly, excessive amount of VEGF was observed in response to hyperglycemia in diabetic rat[27]. VEGF is the primary angiogenic growth factor that promotes diabetic nephropathy in animals and human[27-28]. Overexpression of this factor can provoke inflammatory immune response and promote renal hypoxia, leading to proteinuria[29]. VEGF can cause thickening of tubular basement membrane, and renal interstitial fibrosis by stimulating extracellular matrix deposition, leading to impair in renal performance[30-31].VEGF has been implicated in the suppression of endothelial NO production, which act as anticoagulant and anti-inflammatory factor, beside its role in vascular relaxation, thus causing vascular remodeling and inflammation[32]. The dramatic increase in renal VEGF in diabetic rats was significantly reduced by CFE and/or PDE treatment, indicating their beneficial anti-angiogenic effect. The combination of the two extracts was potential in reducing the angiogenic factor. To the best of found knowledge, this is the first work investigating the beneficial role of CFE against angiogenesis. However, previous study revealed the role of date fruit extract in reducing the expression of VEGF in experimentally induced liver damage[25]. Creatinine, uric acid and urea are the major biomarkers utilized of renal failing. The increment in these indices are potential pathological indicators of DN development[33]. Damaging in the renal tissue was evident with the elevation of these markers in serum of diabetic animals presented in this work. Histopathological examination of kidney picture of hyperglycemic animals showed obvious damages in the renal tissue as noticed by remarkable vacuolar degeneration of tubules, glomerular damages and infiltration of inflammatory immune cells. Treatment with the plant extracts, lonely or in a combination significantly abated the increased in kidney function biomarkers and reduced the aforementioned histo-cytological alterations, suggesting the renoprotective impact of the used extracts against diabetic nephropathy.

CONCLUSION

Our work indicated that CFE and/or PDE exert protective impact versus DN by suppressing the induced inflammatory proteins and angiogenic factor, thus proposing that these plant extracts might have potential curative use for preventing and/or treatment of diabetes induced renal disorder.

AUTHORS CONTRIBUTION STATEMENT

Amna A Saddiq planned the experiments, all authors carried out the experiment. Azza M Mohamed performed the statistical analysis of the results and drafted the article.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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