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Original article

Possible mechanisms for the renoprotective effects of date palm fruits and seeds extracts against renal ischemia/reperfusion injury in rats

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ABSTRACT

Purpose: This work investigates the possible renoprotective effects of date palm fruits and seeds extract against renal ischemia and their underlying mechanisms.
Methods: 108-Sprague Dawle male rats were randomly allocated into 6 equal groups differently receiving aqueous or methanolic fruit and seed extracts. Assay of serum creatinine, BUN and TNF-α, morphological examination of the left kidney, markers of the redox state (MDA, CAT, and GSH), the expression of TNFα and Nrf2 genes at the level of mRNA, the expression of caspase-3 and TGF-β proteins by immunohistochemistry were performed.
Results: 45-min renal I/R caused significant deterioration of kidney functions (increase in serum creatinine and BUN) and morphology ($P < 0.001$) and significant reduction in CAT activity and GSH levels with significant increase in serum TNF-α and MDA concentration and the expression of Nrf2, caspase-3, TNF-α, and TGF-β in kidney tissues. Pre-treatment with either date palm fruit or seed extracts significantly improved kidney functions and morphology ($P \leq 0.001$) with a significant increase in the expression of Nrf2 and CAT activity, and GSH concentration and a reduction in serum TNF-α and expression of caspase-3, TNF-α, and TGF-β ($P < 0.001$).
Conclusions: Administration of date palm extracts exhibited a renoprotective effect against renal I/R injury.This renoprotective action might be due to their antioxidants, anti-apoptotic and anti-inflammatory actions. Moreover, aqueous fruit extracts offered powerful renoprotective effect than aqueous seed extracts, and aqueous fruit and seed extracts were generally more effective than methanolic extracts.

1. Introduction

Acute kidney injury (AKI), a serious health issue in hospitalized patients with a high rate of mortality [1], is due to renal vascular, glomerular and tubular damage [2]. It has been estimated that acute renal failure (ARF) can develop in 5% of hospitalized patients and nearly 10 % of them require kidney transplantation [3]. One of the most common causes of AKI and ARF is the ischemia/reperfusion (I/R) injury [4]. Renal I/R injury is a common sequelae for shock, sepsis, renal transplantation, cardiovascular surgery, partial nephrectomy, clamping of renal arteries during resection of renal tumors [5–7]. In kidney transplantation, renal I/R injury may result in primary renal dysfunction, delayed graft dysfunction, allograft dysfunction and graft rejection [8].

The mechanisms underlying renal I/R injury are complex and include oxidative stress, production of inflammatory cytokines and necrotic and apoptotic cell death [9–12]. The imbalance between local O_2supply and needs leadsto excessive production of reactive oxygen species (ROS), which cause damage to tubular epithelial cells, eventually leading to necrotic and apoptotic cell death [2,13]. Excessive production of ROS during both ischemia and reperfusion results in marked lipid peroxidation, whose products exert mutagenic and

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Table 1
Baseline and endpoint of serum creatinine (mg/dl) and BUN (mg/dl) levels at 24 h, 48 h and 7 d in the 6 experimental groups.

	24 h		48 h		7 days	
	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint
Serum creatinine (mg/dl)						
Sham group	0.52 ± 0.13	0.83 ± 0.19*	0.53 ± 0.12	0.68 ± 0.13*	0.52 ± 0.07	0.58 ± 0.07*
I/R group	0.54 ± 0.08	3.50 ± 0.8*,a	0.61 ± 0.05	2.70 ± 0.67 *,a	0.51 ± 0.10	1.53 ± 0.30*,a
ASE group	0.61 ± 0.08	1.53 ± 0.10*,a,b	0.62 ± 0.05	1.18 ± 0.35*,a,b	0.59 ± 0.10	1.05 ± 0.17*,a,b
AFE group	0.56 ± 0.09	1.78 ± 0.40*,a,b	0.63 ± 0.04	1.15 ± 0.23*,a,b	0.58 ± 0.11	0.90 ± 0.14*,a,b
MSE group	0.51 ± 0.09	1.70 ± 0.19*,a,b	0.62 ± 0.03	1.08 ± 0.17*,a,b	0.54 ± 0.09	0.93 ± 0.09*,a,b
MFE group	0.52 ± 0.07	1.85 ± 0.34*,a,b	0.60 ± 0.04	1.17 ± 0.32*,a,b	0.53 ± 0.11	0.80 ± 0.07*,a,b
Serum BUN (mg/dl)						
Sham group	23.3 ± 4.50	25.0 ± 3.08	19.9 ± 3.50	22.6 ± 3.20*	21.4 ± 3.60	23.6 ± 3.02
I/R group	20.5 ± 3.2	62.0 ± 12.50*,a	19.0 ± 2.70	49.7 ± 6.10*,a	19.70 ± 4.0	41.0 ± 5.30*,a
ASE group	20.73 ± 1.25	38.25 ± 2.50*,a,b	20.0 ± 3.07	33.48 ± 1.76 *,a,b	21.0 ± 2.0	28.34 ± 4.04*,b
AFE group	24.97 ± 3.59	43.25 ± 3.59*,a,b	20.0 ± 2.50	35.32 ± 2.25*,a,b	22.0 ± 3.1	31.16 ± 4.41*,b
MSE group	23.75 ± 2.48	38.75 ± 4.27*,a,b	21.0 ± 2.08	30.0 ± 4.24 *,a,b	22.0 ± 3.10	27.86 ± 3.81*,b
MFE group	22.76 ± 4.43	40.73 ± 2.89*,a,b	20.0 ± 2.20	41.33 ± 2.66 *,a,b	20.0 ± 3.0	33.88 ± 5.37*,b

All data were expressed as mean ± SEM.
BUN: blood urea nitrogen, sham group: right nephrectomy without left renal ischemia, I/R: 45 min left renal ischemia with right nephrectomy, AFE group: aqueous fruit extract, MFE: methanolic fruit extract, ASE: aqueous seed extract, and MSE: methanolic seed extract.
* Significant versus baseline value of the same group (paired T-test).
[a] Significant vs sham group of the same time period.
[b] Significant vs I/R group of the same time period.

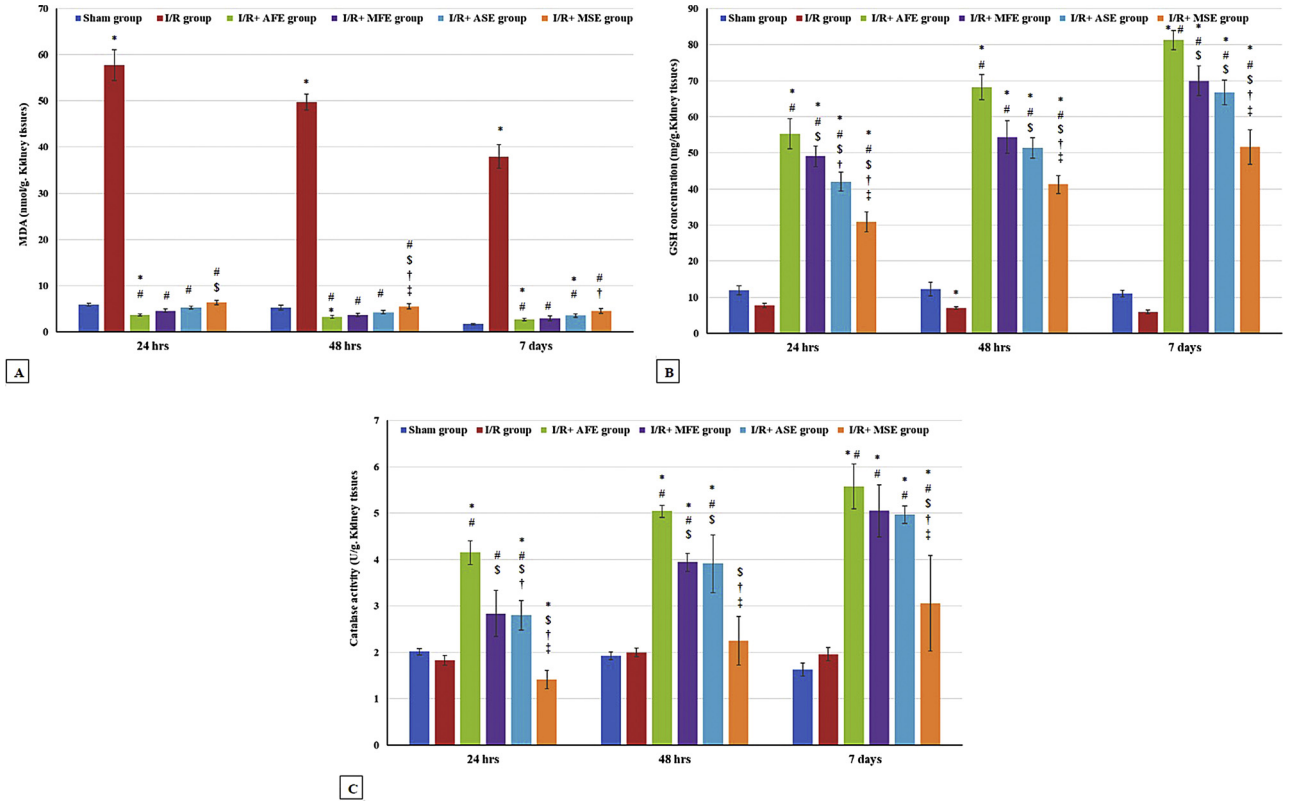

Fig. 1. Oxidative stress markers in kidney tissues A) malondialdehyde (MDA) (nmol/g kidney tissues), B) reduced glutathione (GSH) (mg/g. kidney tissues) and C) catalase enzyme (CAT) (U/g kidney tissues. I/R = ischemia/reperfusion, AFE = aqueous fruit extracts, MFE = methanolic fruit extracts, ASE = aqueous seed extracts, and MSE = methanolic seed extracts, *significant vs sham group, # significant vs I/R group, $ significant vs I/R + AFE group, † significant vs I/R + MFE group and ‡ significant vs I/R + ASE group.

proteotoxic effects, by forming covalent adducts with both DNA and proteins, thus activating necrotic and apoptotic cell death pathways [14,15]. The expression of apoptosis markers, like caspase-3, and the production/expression of oxidative stress markers, such as malondialdehyde (MDA) and nuclear erythroid-related factor 2 (Nrf2), as well as the production of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α) and transforming growth factor-β (TGF-β) were significantly increased as a result of I/R [9–12].

Natural antioxidants have beneficial effects on the prevention of human diseases via their powerful abilities to scavenge free radicals and ROS [16]. Date palm (Phoenix dactylifera *L*) has a powerful antioxidant effect due to its high contents of both, coumaric and ferulic acids,

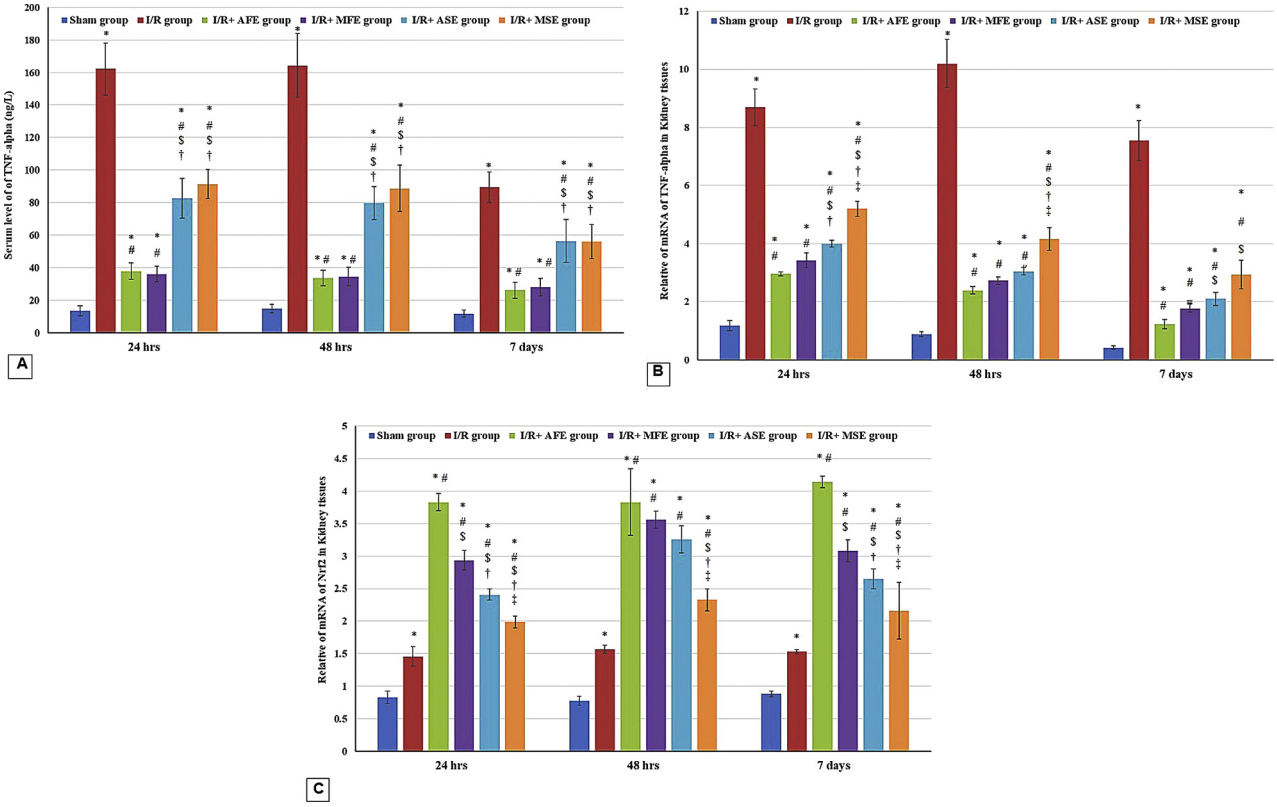

Fig. 2. Effect of palm date extracts on A) the serum level of TNF-α (ng/L), B) relative expression of Nrf2 and C) relative expression of TNF-α in kidney tissues. I/R = ischemia/reperfusion, AFE = aqueous fruit extracts, MFE = methanolic fruit extracts, ASE = aqueous seed extracts, and MSE = methanolic seed extracts, *significant vs sham group, # significant vs I/R group, $ significant vs I/R + AFE group, † significant vs I/R + MFE group and ‡ significant vs I/R + ASE group.

Table 2
Acute tubular necrosis (ATN) score in kidney tissues from different studied groups.

	24 h	48 h	7 days
Sham group	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)
I/R group	3.5 (3.0 – 4.0)*	3.0 (2.0 – 4.0)*	2.5 (1.0 – 4.0)*
I/R + AFE group	1.0 (0.0 – 2.0)*,#	1.0 (0.0 – 1.0)*,#	0.0 (0.0 – 0.0)#
I/R + MFE group	2.0 (1.0 – 3.0)*,#	1.5 (0.0 – 2.0)*,#	1.0 (0.0 – 1.0)#,$
I/R + ASE group	2.0 (1.0 – 3.0)*,#	2.0 (1.0 – 3.0)*,#,$	1.0 (0.0 – 2.0)*,#,$
I/R + MSE group	2.5 (1.0 – 4.0)*,$	2.5 (1.0 – 4.0)*,$	1.5 (0.0 – 3.0)*,#,$
P value (K-W)	< 0.001	0.002	0.008

All data were expressed as median, (minimum-maximum). Kruskal Wallis test. AFE group: aqueous fruit extract, MFE: methanolic fruit extract, ASE: aqueous seed extract, and MSE: methanolic seed extract. P < 0.05 was considered significant.

* Significant vs sham group.
# Significant vs I/R group. AFE group.
$ Significant vs I/R + AFE group.

flavonoids, phenolic compounds, and anthocyanins [17]. Several *in-vitro* studies [18,19], and in-vivo studies [20–22], demonstrated powerful antioxidant properties of date palm fruits and seeds extracts. Also, El-Mousalamy et al., concluded that both, aqueous and methanolic extracts of date palm fruit and seeds, protect the kidneys against diabetic nephropathy in rats via their antioxidant effects [23]. In the current work, we hypothesized that date palm extracts might protect the kidney from ischemic injury via its potent antioxidant activities. Therefore, the current study tried to examine the effects of aqueous and methanolic extracts of date palm fruits and seeds on renal I/R injury and to explore their possible underlying mechanisms in rats.

2. Materials and methods

2.1. Experimental animals

At the Medical and Experimental Research Center (MERC), Mansoura University, Faculty of Medicine,108 male Sprague-Dawley rats, aged 6–8 weeks and weighing 190 ± 10 g, were housed in separate cages at a temperature of 20 – 22 °C and 12-hrs light, 12-hrs dark cycle with free food and water access. Experimental protocols and techniques were performed and approved in accordance with the guidelines of the Mansoura university institutional review board (IRB)(no.:R/19.03.462).

2.2. Preparation of extracts

The extraction procedure of the date palm fruit and seed was performed according to El-Mousalamy et al. [23]. Briefly, 100 g of the fresh blended fruit or seed of date palm were completely extracted with 1 l of either methanol (methanolic extract) or water (aqueous extracts) and the mixture sieved and the remaining fluids (methanol or water) in the extract was evaporated to get the concentrated crude extract. Then, the crude extract was reconstituted in saline and given per gastric gavage in 1 mL saline and given for five weeks before ischemia and one week after.

2.3. Animal model of renal I/R injury and stidy design

The renal I/R rat model of this study consists of left renal ischemia for 45-min followed by right nephrectomy as mentioned previously in Hussein et al., study [10]. The experimental groups include 6 main groups (each group, 18 rats) as follow; 1) Sham group, in which right nephrectomy without left renal ischemia and rats received 1 mL saline

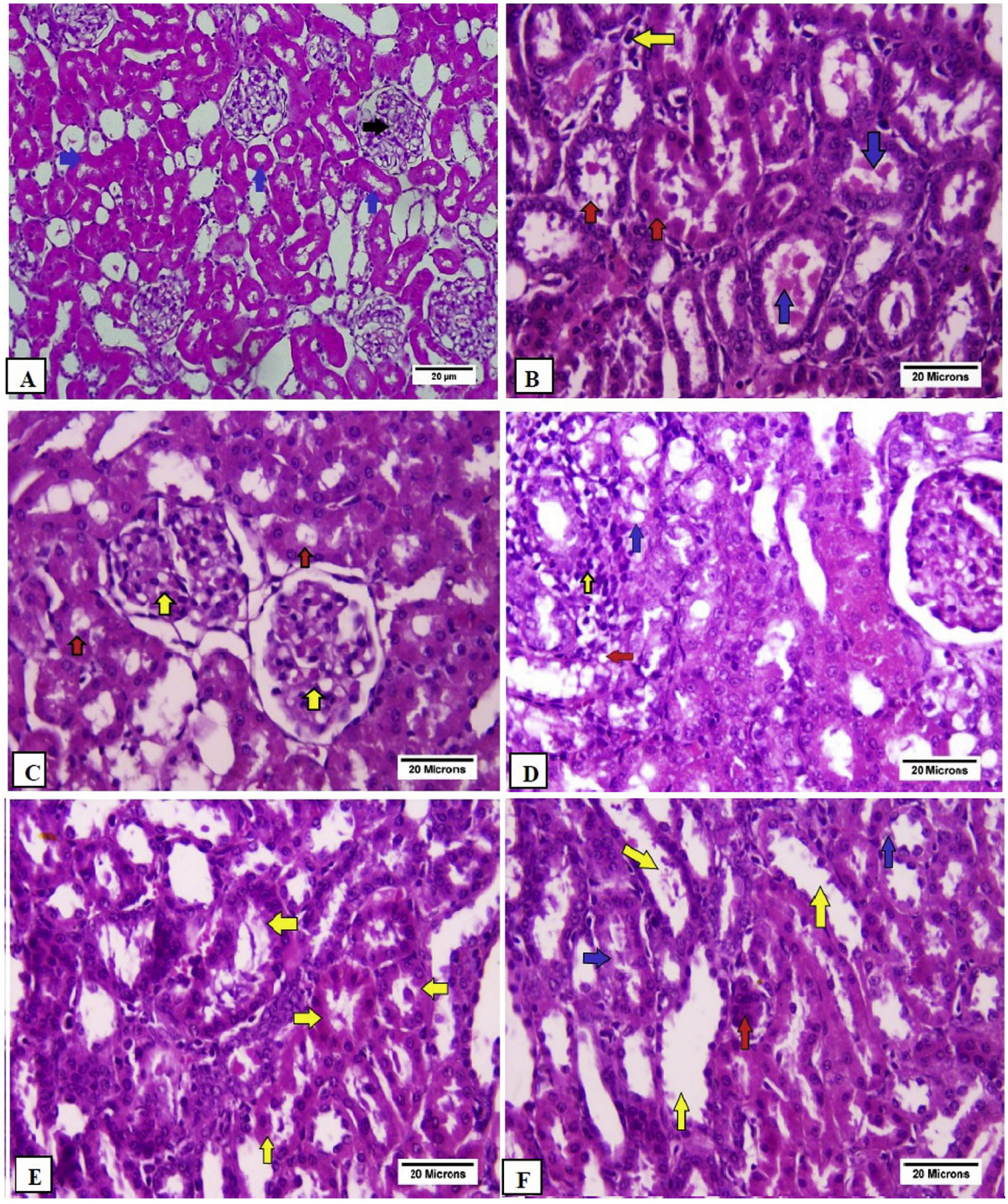

Fig. 3. Kidney specimens stained with H&E showing A) normal proximal tubular epithelium (blue arrows) and glomerular structure (black arrows) (sham group, H&E, 200x), B) proximal tubular dilatation and intratubular casts (blue arrows), necrosis tubular epithelia cells (red arrows) and interstitial inflammatory cell infiltrate (yellow arrows (I/R group, H&E, 400×), C) nearly normal tubular epithelia cells with intact brush borders (red arrows) and intact glomerular structure (yellow arrows) (I/R + AFE, H&E, 400×), D) interstitial inflammatory cell infiltrate (yellow arrows), tubular cell vacuolization (blue arrows) and tubular epithelial cell necrosis and loss of brush borders (arrows red) (I/R + MFE, H&E, 400×), E) tubular cell necrosis of epithelia cells (yellow arrows) (IR + ASE, H&E, 400×) and F) cystic dilatation of the renal tubules (yellow arrows), detached epithelia cells (red arrows) and necrosis of tubular epithelial cells (I/R + MSE, H&E, 400×).

via gastric gavage 2) I/R group, in which 45 min left renal ischemia with right nephrectomy and 1 mL saline via gastric gavage 3) I/R + AFE (aqueous fruit extract) group, as I/R group, but rats received aqueous fruit extract dissolved in 1 mL saline at a dose of 4 mg/kg body weight (b.w.) daily, 4) I/R + MFE (methanolic fruit extract) group, as the previous group but received methanolic fruit extract dissolved in 1 mL saline at a dose of 5 mg/kg b.w. daily, 5) I/R + ASE (aqueous seed extract) group, received aqueous seed extract dissolved in 1 mL saline at a dose of 10 mg/kg b.w. daily, and 6)I/R + MSE (methanolic seed extract) group, received methanolic seed extract dissolved in 1 mL saline at a dose of 5 mg/kg b.w. daily. Then, rats in each group were allocated into 3 subgroups including 24 h, 48 h, and day 7.

2.4. Urine and blood samples collection and kidney harvesting

Animals were placed in metabolic cages at the end of the experiment to collect 24 h urine. At the time of sacrifice, rats were anesthetized using sodium thiopental (120 mg/Kg, intraperitoneal (i.p.)) to

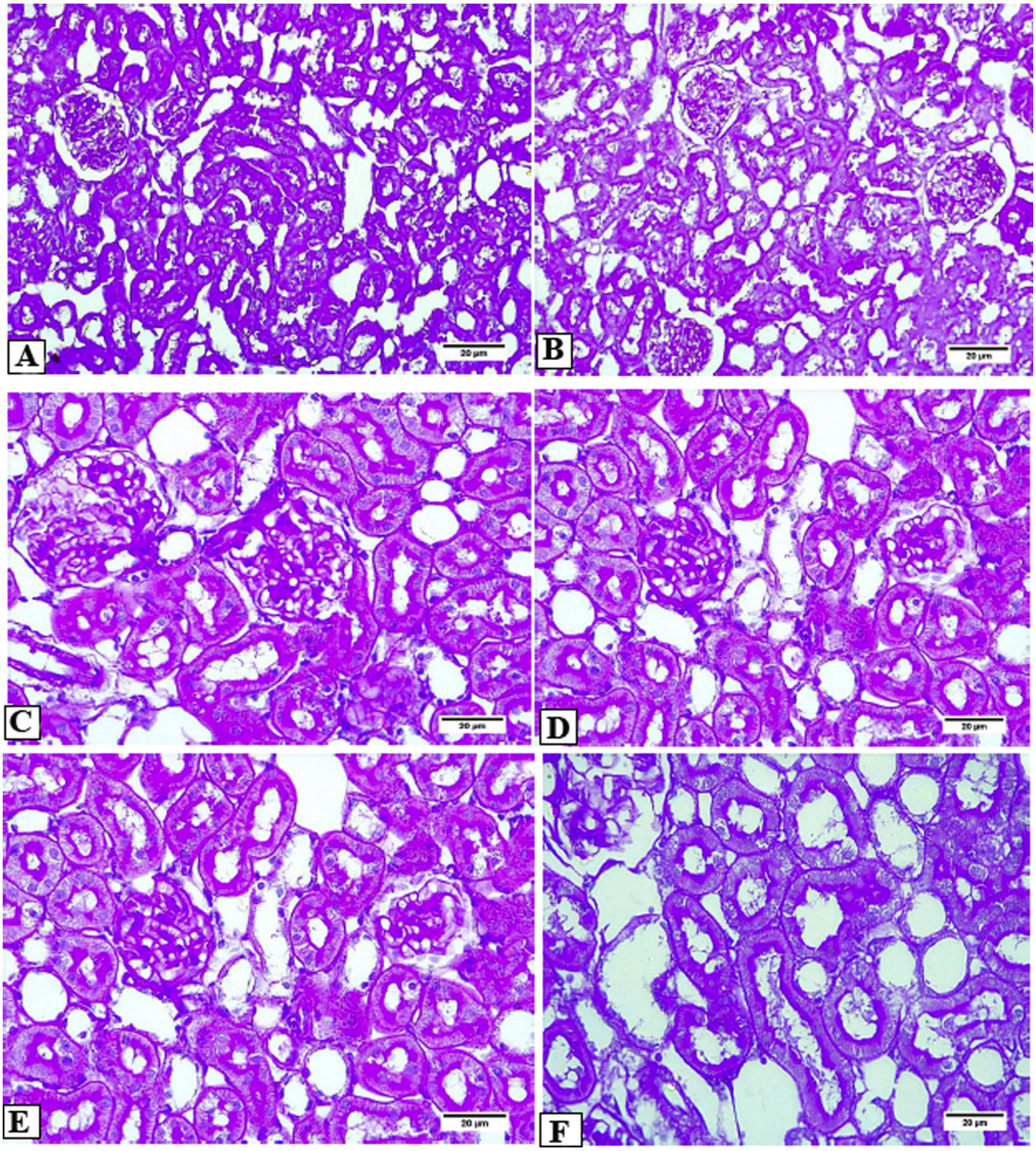

Fig. 4. The periodic acid–Schiff (PAS) stain for the brush border of the tubular epithelial cells. A) intact brush border of renal tubular epithelial cells (red arrows) (Sham group at 48 h, 200×), B) marked loss of tubular epithelia cell borders (black arrows) (I/R group at 48 hs, 400×), C) loss of few brush borders (black arrows) (I/R + AFE group at 48 hs, 400×) D) moderate loss of brush borders (black arrows) (I/R + MFE group at 48 hs, 400×), E) moderate loss of brush borders (black arrows) (I/R + ASE group at 48 hs, 400×) and F) moderate loss of brush borders (black arrows) (I/R + MSE group at 48 hs, 400x).

collect blood samples from the heart and the abdomen by rapidly open the midline laparotomy and harvested the left kidney. Blood samples were left to clot, then centrifuged to collect serum. The collected urine and serum were stored until the time of analysis at −20 °C. The harvested kidney was bisected into 2 halves, one half was stored at −80 °C until the time of the biochemical and molecular analysis, and the second half was stored in neutral formalin (10 %) for histopathological and immunostaining examination.

2.5. Biochemical assay of creatinine, BUN and TNF-α in serum and oxidative stress markers in renal homogenates

Serum creatinine and blood urea nitrogen (BUN) were measured using commercially available kits (Diamond Diagnostics, city, Egypt). Also, the level of tumor necrosis factor-α (TNF-α) in serum was determined by an ELISA kit (Immunotech, France) according to manfarctuer instructions. About 50 mg of the left renal cortical tissues was uniformly broken in cold buffer (50 mM KHPO4, pH 7.5 in 1 mM EDTA) to a homogenate that centrifuged at 4000 rpm for 15 min at 4 °C to get the supernatant. The activity of catalase (CAT) enzyme and the concentrations of malondialdehyde (MDA) and reduced glutathione (GSH) were assessed in the supernatant using a commercially available kits (Bio-Diagnostics, Dokki, Giza, Egypt).

2.6. Quantification of TNF-α and Nrf2 expression

The expression of TNF-α and Nrf2 mRNA in renal homogenates using real-time PCR was assessed as previously described by Hussein

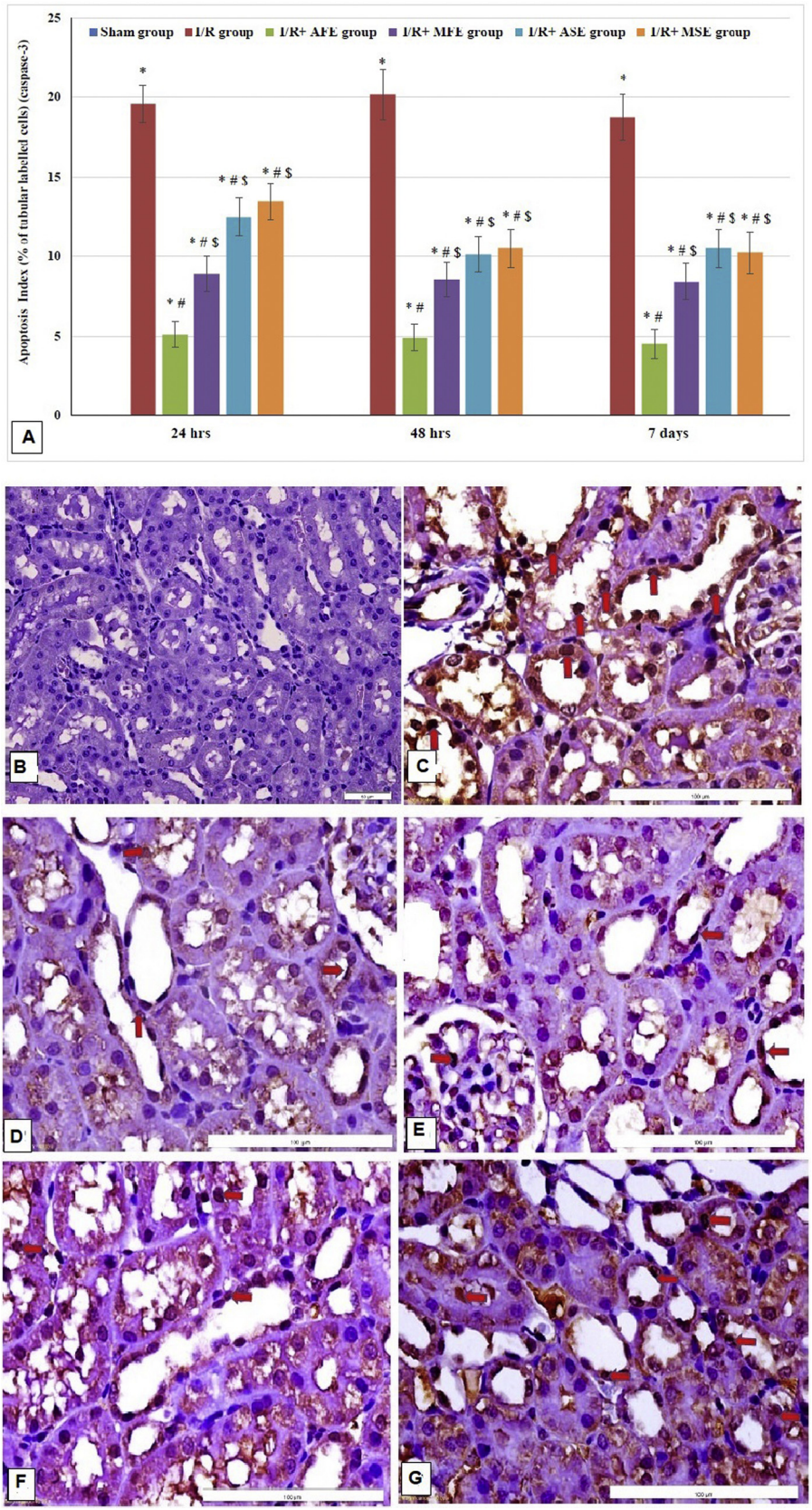

Fig. 5. The effect of palm date fruit and seed extracts on the expression of capase-3 protein in kidney tissues. A = the score of apoptotic labelling index in different studied groups by immunohistochemical examination. Kidney specimen showing B) no expression of caspase-3 in renal tubules (sham group, 200×), C) high nuclear expression of caspase-3 (brown cloured) in proximal and distal tubules (average no. 25 nuclei) (I/R group at 48 h, red arrows, 400×), D) low nuclear expression of caspase-3 (average no. 5 nuclei) in proximal and distal tubules (I/R + AFE group at 48 h, red arrows, 400×), E) moderate nuclear expression of caspase-3 in proximal, distal tubules and glomeruli (average no. 8 nuclei) (I/R + MFE group at 48 h, red arrows) (400×), F) moderate nuclear expression of caspase-3 in proximal and distal tubules (average no. 9 nuclei) (I/R + ASE group at 48 h, red arrows) (400×) and G) moderate nuclear expression of caspase-3 in proximal and distal tubules (average no. 12 nuclei) (I/R + MSE group at 48 h, red arrows) (400×). I/R = ischemia/reperfusion, AFE = aqueous fruit extracts, MFE = methanolic fruit extracts, ASE = aqueous seed extracts, and MSE = methanolic seed extracts, *significant vs sham group, # significant vs I/R group, $ significant vs I/R + AFE group, † significant vs I/R + MFE group and ‡ significant vs I/R + ASE group.

et.al [11], whereas gene expression quantity was calculated using $^{2\Delta}$ct (2-(Ct of target gene-Ct of GAPDH) formula [24].

2.7. Histological examination and immunohistochemical assessment

Four-micron thick kidney sections were processed from paraffin blocks and stained with hematoxylin and eosin to prepare the

Table 3
Scoring of the TGF-beta expression in kidney tissues in different studied groups.

	24 h	48 h	7 days
Sham group	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)
I/R group	2.5 (1.0 – 3.0)*	2.5 (1.0 – 3.0)*	2.0 (1.0 – 2.0)*
I/R + AFE group	0.5 (0.0 – 1.0)*,#	0.5 (0.0 – 1.0)*,#	0.0 (0.0 – 0.0)#
I/R + MFE group	1.5 (1.0 – 2.0)*,#	1.0 (0.0 – 1.0)*,#	1.0 (0.0 – 1.0)#,$
I/R + ASE group	2.0 (1.0 – 3.0)*,#,$	1.5 (0.0 – 3.0)*,#,$	1.0 (0.0 – 2.0)*,#,$
I/R + MSE group	2.0 (1.0 – 3.0)*,$	2.0 (1.0 – 3.0)*,$	1.0 (0.0 – 2.0)*,#,$
P value (K-W)	0.001	0.005	0.02

All data were expressed as median, (minimum-maximum). Kruskal Wallis test. MFE: methanolic fruit extract, ASE: aqueous seed extract, and MSE: methanolic seed extract. P < 0.05 was considered significant.

* Significant vs sham group.

Significant vs I/R group. AFE group.

$ Significant vs I/R + AFE group. AFE group: aqueous fruit extract.

histological slides that were examined using light microscope by an expert pathologist. The cortex and outer medulla were eamined for tubular dilatation, intratubular casts, epithelial cell vacoluization, loss epithelial cell brush borders, tubular cell necrosis, and intertitial hemorrhage and neutrophil cell infiltration [25]. The severity of renal tubular lesions or acute tubular necrosis (ATN) was graded according to the Jablonski et al. [26] scale; 0 = normal; 1 = necrosis of individual cells; 2 = necrosis of cells in adjacent cells of proximal convoluted tubules with survival of surrounding tubules; 3 = necrosis confined to the distal third of the proximal convoluted tubules with a band of necrosis extending across the inner cortex; and 4 = necrosis affecting all three segments (S1,S2 in the cortex and S3 in the outer medulla) of the proximal convoluted tubules.

Slides and immunostaining for immunohistochemical analysis of caspase-3 and TGF-β were prepared as mentioned in the work of Hussein et al., [11,12]. Caspase-3 appeared as cytoplasmic and nuclear brown staining in glomerular and tubular epithelial cells. For each slide, the apoptotic index of caspase-3 was assessed in renal tubular cells (excluding necrotic tubules) by counting the number of positive nuclei in ten randomly, non-overlapping HPF [27]. TGF-β expression appeared as cytoplasmic bronze-colored staining. The expression was quantified according to the percentage of cells showing positive staining under high power field (400x) into 0 = no expression or < 5%, 1 = 5–25 %, 2 = 25–50 %,and 3 = 50–75 % and 4 = > 75 % [28].

2.8. Statistical analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS)(Version 16). One-way ANOVA followed by Tukey's test was used for quantitative data and Kruskal Wallis test was used for qualitative data with a statistical significance considered when *P*-value ≤ 0.05.

3. Results

3.1. Serum creatinine and BUN

Serum creatinine values were comparable in different groups. At the endpoints, serum creatinine was significantly higher in the I/R group than the sham group ($p < 0.05$) at 24 h, 48 h, and 7 days. On the other hand, the treated groups (I/R + AFE,I/R + MFE, I/R + ASE, and I/R + MSE groups) have significantly lower levels of serum creatinine than I/R group ($p < 0.05$). Moreover, serum creatinine was significantly lower in I/R + AFE group than other treated groups (I/R + ASE, I/R + MFE, and I/R + MSE) ($p < 0.05$) at 24 h after I/R injury whereas no significant difference found among the treated groups at both, 48 h and day 7 (Table 1).

The basal values of serum BUN in different groups were of no significant difference. At the endpoints, serum BUN was significantly higher in the I/R group than the sham group ($p < 0.05$) at the three different time intervals. A significant reduction in the level of serum BUN compared to the I/R group ($p < 0.05$)was found in the treated groups; I/R + AFE, I/R + MFE, I/R + ASE, and I/R + MSE. Moreover, fruit extracts showed a significant decrease in serum creatinine compared to the seed extracts ($p < 0.05$) at 48 h after I/R injury (Table 1).

3.2. Markers of oxidative stress

At 24 h, 48 h, and 7 days, the concentration of MDA in kidney tissues was significantly higher in the I/R group than the sham group ($p < 0.05$) and significantly lower in the treated groups (I/R + AFE, I/R + MFE, I/R + ASE, and I/R + MSE) than the I/R group ($p < 0.05$). Moreover, I/R + AFE, I/R + MFE and I/R + ASE showed significant attenuation in MDA concentration than I/R + MSE at 48 h ($p < 0.05$) (Fig. 1A). On the other hand, the concentration of GSH in kidney tissues, was significantly lower in the I/R group than the sham group at 48 h only ($p < 0.05$). Also, compared to I/R group, the concentration of GSH was significantly higher in I/R + AFE, I/R + MFE, I/R + ASE, and I/R + MSE groups at the different time intervals ($p < 0.05$). Moreover, aqueous extracts (seed and fruits) caused significant improvement on the GSH concentration than the methanolic extracts (seed and fruits) ($p < 0.05$) with the fruit extracts being of a significant improvement on the GSH than the seed extracts ($p < 0.05$) (Fig. 1B). Finally, the activity of catalase enzyme showed non-significant difference between the sham and the I/R groups. On the other hand, I/R + AFE, I/R + MFE, and I/R + ASE groups have significantly higher activity for the catalase enzyme than the I/R group at 24 h, 48 h, and day 7 and the I/R MSE group at day 7 only ($p < 0.05$) (Fig. 1C).

3.3. Serum level of TNF-α and the expression of TNF-α and Nrf2 at the level of mRNA

The serum level of TNF-α was significantly higher in the I/R group than the sham group ($p < 0.05$) at 24 h, 48 h and day 7. On other hand, serum TNF-α level was significantly attenuated in all treated groups compared to I/R group at different time intervals ($p < 0.05$). Moreover, the fruits extracts (aqueous and methanolic) lead to a significant decrease in the TNF-α concentration compared to seed extracts (aqueous and methanolic) ($p < 0.05$) (Fig. 2A).

On the other hand, compared to sham group, the expression of the TNF-α mRNA in kidney tissues was significantly higher in the I/R group and in all the treated groups at 24 h, 48 h and day 7 ($p < 0.05$). On the other hand, compared to I/R group, all treated groups (I/R + AFE, I/R + MFE, I/R + ASE, and I/R + MSE) showed significant downregulation in the expression of Nrf2 in kidney tissues at different times ($p < 0.05$). Moreover, the expression of TNF-α was significantly downregulated in fruit eactracts groups (I/R + AFE and I/R + MFE) compared to seed extracts groups (I/R + ASE and I/R + MSE) at different time intervals ($p < 0.05$), and upregulated in I/R + MSE compared to I/R + ASE group at 24 and 48 h ($p < 0.05$) (Fig. 2B).

Regarding the expression of Nrf2 in kidney tissues, its expression was significantly higher in the I/R group compared to the sham group ($p < 0.05$) and in the treated groups compared to the I/R group ($p < 0.05$). Moreover, fruits and seed aqueous extracts caused a significant upregulation of the Nrf2 expression than the methanolic extracts of both ($p < 0.05$) and the fruits extracts caused significant upregulation in the expression of Nrf2 than the seed extracts ($p < 0.05$) (Fig. 2C).

3.4. Histopathological examination

The score of acute tubular necrosis (ATN) in kidney tissues was significantly higher in the I/R group than the sham group ($P < 0.001$)

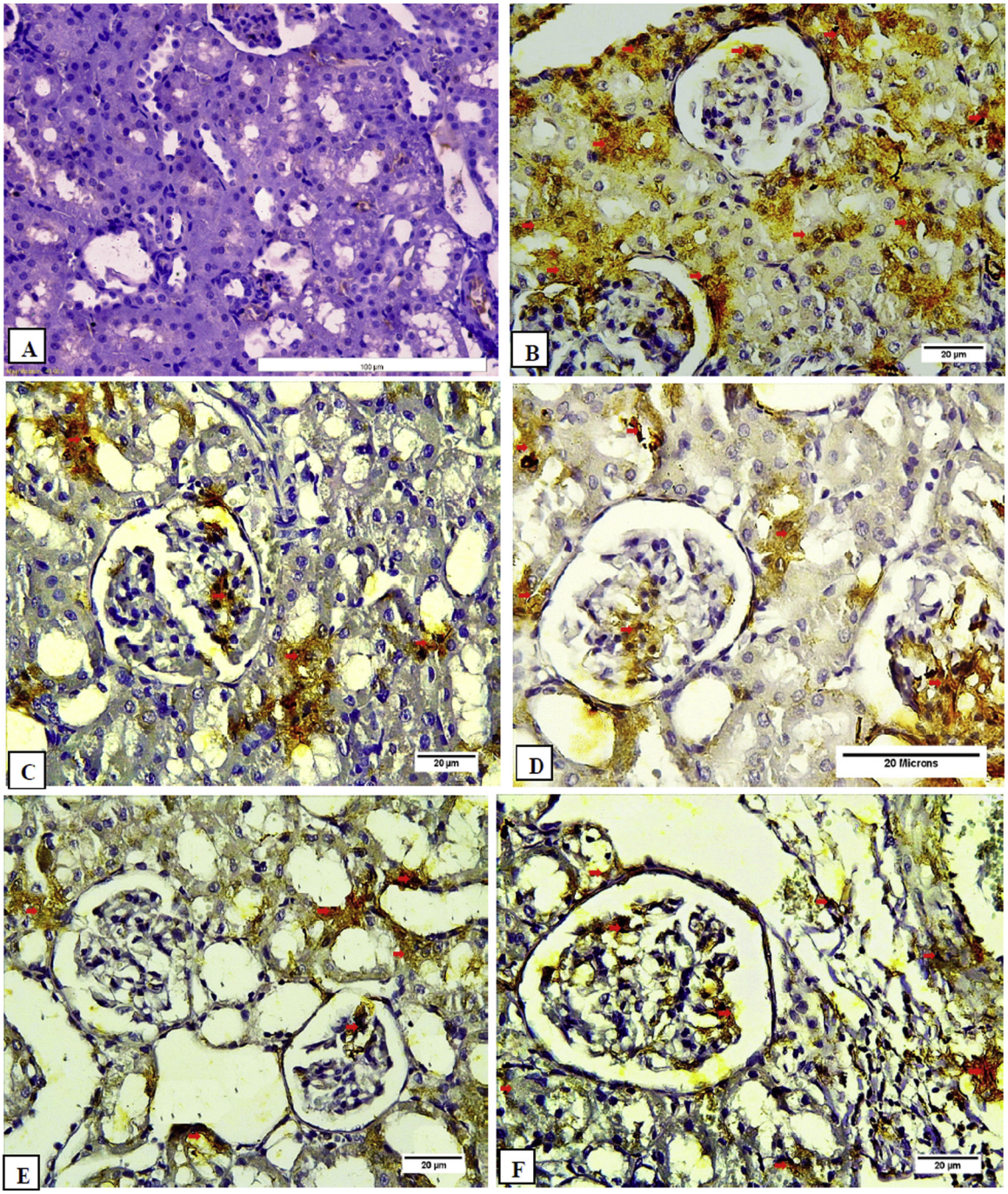

Fig. 6. The expression of the TGF-β in the kidney specimens by immunohistochemistry. Kidney specimen showing A) no expression of TGF-β1 in glomerulus and renal tubules (sham group) (100×), B) strong cytoplasmic expression of TGF-β1 in renal tubules and glomeruli (I/R group, 400×), C) low cytoplasmic expression of TGF-β1 in renal tubules and glomeruli (I/R + AFE group at 48 h, red arrows) (400×), D) moderate cytoplasmic expression of TGF-β1 in renal tubules and glomeruli (I/R + MFE group at 48 h, red arrows) (400×), E) moderate cytoplasmic expression of TGF-β1 in renal tubules and glomeruli (I/R + ASE group at 48 h, red arrows) (400×) and F) moderate cytoplasmic expression of TGF-β1 in renal tubules and glomeruli (I/R + MSE group at 48 h, red arrows) (400×).

and became significantly improved in the I/R + AFE, I/R + MFE, I/R + ASE, and I/R + MSE groups compared to the I/R group ($P < 0.05$) (Table 2). Kidneys harvested from the sham group showed normal preserved glomerular and tubular structures with H&E stain (Fig. 3a) while the I/R group showed a proximal tubular epithelial cell necrosis, intratubular cast formation, and inflammatory cell infiltration with marked loss of brush border (Fig. 3b). Kidney specimens obtained from the treated groups showed minimal tubular necrosis with minimal loss of brush borders (Fig. 3c–f).

Periodic acid–Schiff (PAS) stain was used to examine the brush border of the tubular epithelial cells. Fig. 4a shows an intact brush border of proximal tubular epithelium in the sham group, whereas Fig. 4b shows proximal tubular necrosis with marked loss of brush border in the I/R group. I/R + AFE and I/R + MFE groups show a minimal loss of brush border of proximal tubular epithelial cells (Fig. 4c and d, respectively). On the other hand, I/R + ASE and I/R + MSE groups show a moderate loss of brush border of proximal tubular epithelial cells (Fig. 4e and f, respectively).

3.5. Immunhistochemical examination of caspase-3 and TGF-β

I/R group showed a higher expression of caspase-3 protein than the sham group ($P \leq 0.001$) with reduced expression in the treated groups (I/R + AFE, I/R + MFE, I/R + ASE, and I/R + MSE) compared to the I/R group ($P < 0.05$). Moreover, its expression was significantly lower in the AFE group compared to other treated groups ($P \leq 0.01$) (Fig. 5A). A negative cytoplasmic, and nuclear expression of the caspase-3 protein in the kidney specimens obtained from the sham group (Fig. 5B), while those of the I/R group showed marked cytoplasmic and nuclear expression of caspase-3 in glomeruli and proximal and distal tubules (Fig. 5C). In the treated groups, all were exhibited moderate and minimal cytoplasmic and nuclear expression of caspase-3 protein in proximal tubular cells (Fig. 5D–G).

The expression of TGF-β protein was higher in the I/R group compared to the sham group ($P \leq 0.001$) with an improved expression in the treated groups in comparison to the I/R group ($P < 0.05$). AFE group has lower expression of the TGF-β protein than the other treated groups ($P \leq 0.01$) (Table 3). Kidney specimens obtained from the sham group showed a negative TGF-β expression (Fig. 6A), while the I/R group was of a marked membranous expression of TGF-β protein in glomeruli and proximal and distal tubules (Fig. 6B). A moderate to minimal membranous expression of TGF-β protein in proximal tubular cells were revealed in the treated groups (Fig. 6C–F).

4. Discussion

Notwithstanding, the recent advances in the management of renal I/R injury using surgical, medical, and pharmacological methods, the later remains a major problem that needs further investigation on the possible underlying mechanisms and for the discovery of a new line of therapy [29]. In the current work, a well-known rat model of renal I/R injury was adopted, in which left renal ischemia for 45-min with contralateral right nephrectomy was done. This study showed that renal I/R injury caused a significant rise in serum creatinine and BUN in the I/R group suggesting the impairment of kidney glomerular and tubular functions. Besides, it led to a deterioration of kidney morphology in the form of necrosis of proximal tubular epithelial cells, loss of brush border, cystic dilatation of renal tubules and marked leucocytic infiltration in the kidney tissues consistent with the results of preliminary studies [9–1230]. Moreover, treatment with aqueous and methanolic fruit and seed extracts caused attenuations in the alterations of kidney function tests (creatinine and BUN) and renal morphology associated with I/R, with fruit extracts exerting more pronounced effects than seed extracts, and aqueous extracts of both kinds being generally more effective than the respective methanolic extracts. The differences in the quality and the quantity of the substances in each extract could explain the differences in the degree of renoprotective effects of the extracts. In a previous study, fruit extracts yielded 8 antioxidants substances with a high concentration of p-coumaric acid, caffeic acid, and ferulic acid, whereas seed extracts yielded 5 antioxidant substances with a high concentration of caffeic and gallic acid with the aqueous extracts having higher concentrations of the antioxidant substances than the methanolic extracts [23]. El Arem et al., explained the renoprotective effect of the aqueous extracts of date palm against dichloroacetic acid-induced nephrotoxicity by its potent antioxidant capacities [31]. In addition to the phenolic compounds just mentioned, the antioxidant substances identified in the latter study also included flavonoids like quercetin, luteolin and rutin [32].

In the current study, we investigated several mechanisms involved in the pathogenesis of renal I/R injury such as oxidative stress. Several reports have investigated the role of oxidative stress and ROS in the pathogenesis of renal I/R injury [9,30]. In agreement with these studies, the current study demonstrated an oxidative imbalance in the redox state of the kidney tissues undergoing I/R, as evidenced by the increased levels of MDA, a marker of lipid peroxidation, and the reduced levels of GSH and of the activity of CAT, a detoxifying enzyme. The excessive production of ROS in kidney tissues during the oxidative stress leads to apoptotic and necrotic cell death, therefore, targeting this process will aid in the management of the renal I/R injury. In this study, all date palm extracts caused a reduction in the MDA with an increase in the GSH and CAT activity, suggesting the antioxidant effect of date palm extracts in renal I/R injury, especially for the aqueous fruit extract. Moreover, the role of Nrf2 as a major transcription factor for the antioxidant enzymes such as superoxide dismutase (SOD) and heme oxygenase (HO)-1 in the antioxidant effect of the extracts was investigated. Preliminary studies have demonstrated the possible renoprotective effect of Nrf2 against the oxidative injury during the renal I/R injury [9–11]. Upregulation of the Nrf2 by palm date extracts in renal I/R injury is reported for the first time in this study, which found a mild upregulation of Nrf2 in the I/R group suggesting the upregulation of the endogenous protective mechanisms in the kidney with the ischemic insult. Moreover, the palm date extracts caused a marked increase in the expression of Nrf2 when compared to the I/R group suggesting that the induction of Nrf2 transcription factor might be a possible underlying mechanism for the renoprotective action of palm date extracts.

An inflammatory response during renal I/R injury has been reported in several previous studies [33,9–12], which was associated with the expression of several inflammatory cytokines such as TNF-α and TGF-β [9–12].Gaur and Aggarwal showed that the activation of the TNF-α production was associated with cell death through the induction of the apoptotic pathways [34]. The current work reported a significant increase in the serum level of TNF-α and in the expression of TNF-α gene at the level of mRNA in kidney tissues obtained from I/R group of this study which is consistent with the previous findings [9,11]. Moreover, we found that treatment with palm date fruit and seed extracts leads to an attenuation in the serum level of TNF-α and in the expression of TNF-α, suggesting that the anti-inflammatory effects of these extracts that might be associated with the renoprotective action of the extracts against renal I/R injury.

TGF-β is a cytokine which plays an important role in renal I/R injury as a participant in the inflammatory cascade within minutes after the ischemic damage [11]. Its expression at the levels of mRNA and protein was increased in the damaged and regenerating proximal tubules in less than 12 h up to 14 days after ischemia [35]. The upregulated TGF-β triggers the development of renal glomerulosclerosis and interstitial fibrosis [36,37]. Here, a significant increase in the expression of TGF-β1 at the level of protein by immunohistochemistry assay in kidney tissues of ischemic rats was shown compared to those of the sham-operated rats. In addition, both palm date extracts (fruit and seed) caused reduction in the expression of TGF-β suggesting anti-inflammatory effects of the extracts during renal I/R injury.

Apoptosis, also known as programmed cell death, is one of the renal cell death mechanisms during renal I/R injury, that were investigated in this study.Execution of the process of apoptosis is performed by caspases, a group of cysteine proteases [9]. These caspases act by irreversibly cleaving their substrates after aspartic acid residues. Several caspases have been involved in the apoptotic cell death during renal I/R such as caspase-3 and caspase-9 [9]. This report found an increase in the caspase-3 expression at the protein level in the I/R group which is in line with several related studies [9–1130]. The current study demonstrated immunoreactivity for caspase 3 mainly in cytoplasm and with lesser extent in the nucleus which is in agreement with Orhan and Bolkent, [38]. Moreover, fruit and seed extracts lead to a significant reduction in the caspase-3 expression at different time intervals suggesting their anti-apoptotic effects. Although, we examined several mechanisms in the current study, still some points should be cleared such as the role of inflammatory cells including neutrophils, macrophages and dentridtic cells in renal I/R injury. This is considered as one of the limitations of the current study and futures studies are needed to explore more about the renoprotective action of palm date extracts in

renal I/R injury.

In conclusion, pre-treatment of rats with date palm extracts (fruit and seed) has a renoprotective effect against renal I/R injury that could be due to attenuation of the oxidative stress, inflammatory cytokines (TNF-α and TGF-β), apoptotic cell death (caspase-3), and upregulation of the transcription factor, Nrf2 and the renoprotective effects of fruit extracts being more marked than those of seed extracts. Future clinical studies on the effects date palm extracts would be highly recommended taking into consideration the possible pharmaceutical applications of the extracts.

Author contributions

Mansour A. Alghamdi: shared in the idea, collection of data and analysis and draft writing, Abdelaziz M. Hussein: shared in the idea, the induction of animal model, biochemical analysis, data analysis and paper writing, Laith N. Al-Eitan: shared in the idea, data analysis, and paper writing. Eman Elnashar: shared in histopathological examination, Ahmed Elgendy: sample collections and paper writing, Asim M.Abdalla: data collection and analysis, Seham Ahmed: induction of model and biochemical analysis; Wael A. Khalil: shared in histopathological examiniation and analysis.

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Declaration of Competing Interest

All authors declared that there was no conflict of interest in this work.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2020.110540.

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