Biochemical and molecular mechanisms associated with the effect of *Phoenix dactylifera* seeds on cyclophosphamide-induced hepato-renal toxicities in mice

Sabry Ali El-Naggar
Tanta University
Mohamed A. Basyouny
Tanta University
Mohammed Alqarni
Taif University
Ahmed S. Haidyrah
King Abdulaziz City for Science and Technology (KACST)
Samer Ezat Amin
Tanta University
Mona Elwan
Tanta University
Ibrahim Omar Barnawi
Taibah University
Karim Samy El-Said (kareem.ali@science.tanta.edu.eg)
Tanta University

Research Article

Keywords: Phytochemicals, Phoenix dactylifera, Cyclophosphamide, Toxicity, Biochemical

Posted Date: September 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-923363/v1

Abstract

Cyclophosphamide (CTX) causes severe side effects. *Phoenix dactylifera* L. showed biomedical values. This study aims to address the biochemical and molecular mechanisms of *Phoenix dactylifera* seeds extract (PDSE) effects on CTX-induced hepato-renal toxicities in mice. Forty male albino mice were divided into four groups: Gp 1 was served as a negative control, Gp2 was injected intraperitoneally (i.p) with PDSE (200 mg/kg) for 30 consecutive days. Gp3 was injected with CTX single dose (200 mg/kg) and Gp4 was injected with CTX then injected with PDSE. Hematological, biochemical, histopathological alterations, and gene expression for pro-inflammatory cytokine were assessed. GC-MS analysis showed the highest percentages of the peak areas were by Ethyl iso-allocholate, 1-Heptatriacotanol, and 9-12-15-Octadecatrienoic acid 2-3-dihydroxypropyl ester. Treatment with PDSE post-CTX-injection ameliorated the hematological, biochemical, and histological alterations by up-regulating the antioxidant biomarkers and downregulating tumor growth factor beta-1 (TGFβ-1), nuclear factor Kappa-beta (NFκ-β), cyclooxygenase-1 (COX-1) genes.

Introduction

Cancer remains one of the foremost common issues around the world. Efforts to find new treatments in recent decades were massive[1]. Although significant progress has been made to control and treat cancer and its progression, the concomitant advance in creating modern cancer drugs is still much slower. Different settings for cancer treatments included surgery, chemotherapy, radiotherapy, and immunotherapy are applying. Till now, chemotherapy is considered the first line of treatment, however, low treatment efficacy is common due to drug resistance and side effects on normal healthy tissues, therefore a significant challenge to find a treatment with less toxicity is an ultimate need[2].

Cyclophosphamide (CTX) is an anticancer agent that is broadly used for the treatment of several types of cancer[3]. For years, CTX is used alone or in a combination with other chemotherapies to treat lymphomas, leukemia, myeloma, lung cancer, and metastatic breast cancer[4]. Not only CTX kills the tumor cells, but also affect the dividing hematopoietic cells and this led to neutropenia and lymphopenia that decreased the efficacy of the immune system[5]. Previous studies have shown that a therapeutic dose of CTX cause liver and kidney toxicities[3,6]. For instance, a previous study showed that a single injection with 4 mg of CTX/mouse caused severe lymphopenia after 3 days post-treatment and decreased the absolute number of white blood cells in the peripheral blood[5].

Some natural antioxidant agents are being used to alleviate oxidative stress-induced post-chemotherapy treatment. In line with this, earlier studies reported the beneficial roles of natural antioxidants on CTX induced toxicity[7,8]. Lowering the side effects by natural products during the treatment of cancer patients may permit to safely administer of a higher and possibly more effective dose of chemotherapy[7]. For instance, *Nigella sativa* oil or thymoquinone administration can lower CTX-induced toxicity via antioxidant mechanisms[9]. Furthermore, *Persea americana* phytoconstituents, propolis, and *Spirulina*

platensis extract can be used for lowering the side effect of CTX[6,10]. Also, it has been reported that different extracts of *Vitex doniana* leaves showed myelo-protective activity in CTX-induced myelotoxicity[11].

The date palms (*Phoenix dactylifera L.*) have been an important crop in Egypt and Middle Eastern countries[12]. Date pit powders are marketed and are used as a non-caffeinated coffee with coffee-related flavor[13]. The date seed contains several biologically active ingredients including flavonoids, tannins, saponins, phenol, and sterols[14]. Preclinical studies have shown that the *P. dactylifera* exhibits antioxidant, anti-microbial, anti-inflammatory, gastroprotective, hepato-protective, nephron-protective, anticancer, and immune-stimulant activities[15,16].

Most of the anti-neoplastic agents are known to cause myelosuppression and neutropenia[5]. To diminish the negative impacts of CTX and its responsive metabolites, extending body's antioxidant protections with common and secure cancer prevention agents is essential. For this reason, there is a require for proficient substances that ensure tissues from the side impacts of CTX-induced harmfulness. This study aimed to address the efficacy of PDSE on hepato-renal alterations induced by CTX in albino mice.

Results

GC-MS analysis of PDSE. Data showed the retention time and the relative abundance of different bioactive phytoconstituents. The peak areas (%) of 11-Dodecen-2-one, 2-methyl-10-undecenal, 1-(cyclopropyl-nitro-methyl)-cyclopentanol, 8, 11, 14-Eicosatrienoic acid, (Z, Z, Z). and Octadecanoic acid, 9, 10-epoxy-18 (trimethylsiloxy), methyl ester was 1.03, 1.14, 2.07, 2.48, and 2.53%, respectively. The peak area of Dodeca-1, 6-dien-12-ol, 6, 10 dimethyl, 12-Methyl-E, E-2, 13-octadecadien-1-ol and 13-Tetradece-11-yn-1-ol were 3.10, 3.39 and 3.91%, respectively. The peak area (%) of Ethyl iso-allocholate, 1-Heptatriacotanol, and 9, 12, 15-Octadecatrienoic acid, 2, 3-dihydroxypropyl ester, (Z, Z, Z) were 5.80, 21.07 and 53.49, respectively (Table 1).

PDSE treatment post-CTX-injection protect against body weight loss. The percentage of change in the total body weight of CTX-treated mice (Gp3) was significantly decreased when compared with the control group (Gp1). Treatment with PDSE alone did not show significant alteration in the body weight gain as compared to Gp1. Treatment with PDSE post-CTX injection (Gp6) protected against the loss of body weights when compared to CTX-injected group (Gp3) (Table 2).

Table 1
Biochemical compounds analyzed by GC-MS of the hydro-alcohol seeds extract of P. dactylifera

No.	RT (min.)	Name	M. F.	M. Wt	Peak area %
1	24.61	11-Dodecen-2-one	$C_{12}H_{22}O$	182	1.03
2	25.51	2-methyl-10-undecenal	$C_{12}H_{22}O$	182	1.14
3	26.83	Dodeca-1,6-dien-12-ol, 6,10 dimethyl	$C_{14}H_{26}O$	210	3.10
4	27.46	13-Tetradece-11-yn-1-ol	$C_{14}H_{24}O$	208	3.91
5	31.04	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	3.39
6	31.41	1-(cyclopropyl-nitro-methyl) cyclopentanol	$C_9H_{15}NO_3$	185	2.07
7	31.97	8,11,14-Eicosatrienoic acid, (Z,Z,Z)	$C_{20}H_{34}O_2$	306	2.48
8	32.61	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	5.80
9	33.27	Octadecanoic acid, 9,10-epoxy-18 (trimethylsiloxy), methyl ester	$C_{22}H_{44}O_4Si$	400	2.53
10	33.87	1-Heptatriacotanol	$C_{37}H_{76}O$	536	21.07
11	34.22	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	$C_{21}H_{36}O_4$	352	53.49

Table 2
Initial body weights, final body weights, % change in body weight of groups under the study.

Groups	I. B. wt. (g.)	F. B. wt. (g.)	% Change in B. wt.
Control	21.48 ± 1.1	31.3 ± 3.6	45.85 ± 4.7
Control/PDSE	21.23 ± 1.2 n.s.	30.97 ± 7.4 n.s.	45.75 ± 4.9 n.s.
CTX	21.3 ± 1.1 n.s.	27.8 ± 6.0 n.s.	30.26 ± 3.5 *
CTX/PDSE	21.5 ± 1.2 n.s.	29.5 ± 3.1 n.s.	37.20 ± 4.1 n.s.

The values represented mean ± SD; I.B.W: Initial body weight; F.B.W: Final body weight. *P* value < 0.05 was statistically significant. **PDSE**: Phoenix dactylifera seeds extract, **CTX**: Cyclophosphamide.

Treatment with PDSE post-CTX-injections ameliorated hematological alterations.

The results showed that as compared to the control group (Gp1), the total number of red blood cells (RBCs), hemoglobin (Hb) concentration and hematocrit (%) did not show significant alterations in all groups (Table 3). Compared to Gp1, treatment with PDSE (Gp2) and CTX-injected group (Gp3) showed

significant increase in the total platelets count. A decrease in the WBCs count was found in the group of mice post a single injection of CTX (Gp3) when compared to Gp1. Treatment with PDSE after CTX injections (Gp4) ameliorated the toxic effect of CTX on WBCs and restores their number close to the normal values (Table 3). Mice were treated with CTX (Gp3) showed a decrease in the total number of lymphocytes and monocytes when compared to the control group (Gp1). Treatment with PDSE post-CTX-injection ameliorated the toxic effect of CTX on these cells and return the numbers close to the normal values.

Table 3
Hematological parameters in different groups of mice treated with PDSE, CTX, or with PDSE post CTX-injection.

Groups	Hb (g/dL)	RBCs ($x10^6$ / µL)	Hct (%)	WBCs ($x10^3$ / µL)	Plat. ($x10^3$ /µL)
Control	11.82 ± 0.3	8.82 ± 0.4	39.3 ± 3.3	8.96 ± 1.5	882.8 ± 31.3
Control/PDSE	11.0 ± 0.5 n.s.	8.7 1 ± 0.1 n.s.	41.9 ± 2.5 n.s.	8.19 ± 1.8 n.s.	1589.3 ± 78.2 ***
CTX	9.80 ± 0.9 n.s.	9.03 ± 0.6 n.s.	38.28 ± 3.6 n.s.	6.31 ± 2.0 *	1026.5 ± 78.1 *
CTX/PDSE	9.98 ± 0.9 n.s.	8.15 ± 0.9 n.s.	35.70 ± 3.7 n.s.	7.93 ± 2.2 n.s.	747.8 ± 179.4 n.s.

The values represented mean ± SD; *P* value < 0.05 was statistically significant; n.s means non-significant. **PDSE**: Phoenix dactylifera seeds extract, **CTX**: Cyclophosphamide.

Treatment with PDSE improved hepato-renal biochemical dysfunctions induced by CTX

Treatment with PDSE alone did not result in significant changes in the level of AST and ALT when compared to their control. Furthermore, the result showed that there was an increase in the liver transaminases AST and ALT in CTX-injected groups (Gp3), when compared to the control group. Treatment with PDSE post-CTX injections ameliorated the toxic effect of CTX on the liver function as evident by decreased AST and ALT (Fig. 1). Treatment with PDSE alone did not show any significant changes in the levels of urea and creatinine when compared to their control, however, CTX injection led to an increase in. Treatment with PDSE post-CTX injections showed a decrease in urea and creatinine levels when compared to the groups of mice injected with CTX (Fig. 2).

PDSE treatments ameliorated the oxidative stress induced by CTX-injections. Treatment with PDSE (Gp2) alone did not result in any significant changes in SOD, CAT, and MDA levels when compared to the control group (Gp1). CTX-injected groups (Gp3) showed a significant decrease in the hepatic SOD and CAT while significant increase in the level of MDA was observed when compared to Gp1. Treatment with PDSE post-CTX injections mitigated the oxidative stress via enhancing the antioxidant status of liver tissues as evident by the increase of SOD and CAT along with decrease in the level of MDA (Fig. 3).

PDSE treatment improve histopathological alterations in liver and kidney tissues. Examination of liver sections of the control group (Gp1) and the PDSE administered mice (Gp2) showed that hepatocytes radiating from the central vein. The hepatocytes had homogenous granular cytoplasm with centrally located nucleus with prominent envelopes and normally distributed chromatin. Liver strands were alternating with narrow blood sinusoids lined by endothelial cells and distinct phagocytic Kupffer cells (Fig. 4a and b). Liver sections of mice after a single dose of CTX-injection (Gp3) showed noticeable disorganized liver architecture, congested blood vessels with fatty exudate, and many obvious mononuclear infiltrations around damaged cells, also widening of blood sinusoids (Fig. 4c). While the CTX/PDSE-treated mice (Gp4) exhibited improvement of hepatic architecture, hepatic cords radiating from the central vein, regular narrow blood sinusoids network, some hepatocytes show hyper-eosinophilia and activated Kupffer's cells (Fig. 4d).

Normal histological structure of control mice, renal parenchyma was appeared as the cortex region, normal glomeruli with normal bowman's space, and normal renal tubules (Fig. 5a). The PDSE-treated mice (Gp2) exhibited the cortex that contains glomeruli with normal Bowman's space and normal appearance of mostly renal tubules (Fig. 5b). The histopathological changes in the kidneys of the CTX-treated mice (Gp3) appeared in the form of destructed, shrunken, and congested glomeruli with irregular bowman's space, most of the renal tubules were damaged, lost their characteristic appearance with intratubular hemorrhage (Fig. 5c). Mice injected with CTX/PDSE (Gp4) showed normal glomeruli and renal tubules, but few numbers of disorganized tubules were observed (Fig. 5d).

Molecular analysis for TGF-β1, NFk-β, and COX-1 genes. Pro-inflammatory cytokines, *TGFβ-1*, *NFk-β*, and *COX-1* genes expression in liver and kidney tissues were assessed by real-time PCR. Concentration of RNA were determined by Nanodrop, and the results showed that the isolated RNA is pure and with considerable concentrations ranged from 1420 to 2340 ng/µl. The obtained results revealed a significant increase in the expression of the pro-inflammatory genes (TGF-β1, NFk-β, and COX-1) in the liver and kidney tissues of CTX-injected mice. Treatment with PDSE post-CTX injection led to significant decrease in the mRNA expression levels of these genes when compared to their controls (Table 4).

Table 4
Fold changes of the mRNA expression of *TGFβ-1*, *NFκ-β, COX-1* and *COX-2* genes in liver and kidney tissues of the different groups.

Tissue/Genes		Groups			
		Gp1 (Control)	**GP2** (PDSE)	**GP3** (CTX)	**GP4** (CTX/PDSE)
Liver	TGFβ-1	1.00 ± 0.06	1.53 ± 0.19*	7.11 ± 0.36***	4.13 ± 0.27***
	NF*κ*-β	1.00 ± 0.06	1.97 ± 0.13***	11.72 ± 0.48***	7.71 ± 0.34***
	COX-1	1.00 ± 0.06	1.43 ± 0.17*	6.82 ± 0.39***	4.23 ± 0.19***
Kidney	TGFβ-1	1.00 ± 0.06	1.23 ± 0.12*	3.51 ± 0.39***	2.11 ± 0.18***
	NF*κ*-β	1.00 ± 0.06	1.53 ± 0.15***	7.63 ± 0.58***	4.27 ± 0.31***
	COX-1	1.00 ± 0.06	1.29 ± 0.13*	4.45 ± 0.42***	3.17 ± 0.37***

The values represented mean ± SD; *P* value < 0.05 was statistically significant; n.s means non-significant.

Discussion

Chemotherapeutic drugs are utilized for the treatment of diverse malignancies, but their therapeutic use is limited due to their adverse side effects such as hepatotoxicity and immune suppression[17,18]. The current study was performed to evaluate the phytochemical constituents of PDSE by using GC-MS analysis. Our results revealed that by GC-MS analysis, PDSE contains several bioactive compounds, which could have potential biomedical applications[19–22]. The results showed that there is a significant decrease in the total body weights of CTX-injected mice. This might be due to CTX side effects on the different body tissues. PDSE treatment after CTX injection protected weights lose[23].

The present study reported a significant decrease in WBCs count CTX injection. These findings could be due to the direct effect of CTX on bone marrow (BM) which led to myelosuppression. Our finding agreed with the previous study showed the analysis of kinetics cellular recovery after CTX treatment; a single dose of CTX treatment (4 mg/mouse) decreased leukocytes number[5,24]. Due to chemotherapy treatment, the dividing hematopoietic cells are influenced and decrease immunity (5). Our findings were agreed with the previous study that showed that natural products and date palms showed considerable potential immunostimulant against CTX-induced immunosuppression in mice[25,26].

Treatment with PDSE post-CTX injection decreases serum ALT and AST activities. This finding agreed with a previous study showed the role of medicinal plants in improvement of hepatotoxicity induced by CTX[8,23]. CTX-induced elevations in the levels of urea and creatinine investigated in our study are inconsistent with the previous studies[27]. Accordingly, treatment with PDSE ameliorated CTX-induced

renal toxicity, as indicated by a steep decrease in urea and creatinine levels, possibly by maintaining the renal cellular membrane integrity. This was consistent with a previous study reported the impact of plant-based formulations to control and modulate anticancer drug-induced nephrotoxicity[23,28]. CTX-induced hepatotoxicity is associated with oxidative stress[29]. In our study, the activities of the antioxidant enzymes, SOD, and CAT in the liver tissues were significantly reduced by CTX treatment. It has been reported that natural antioxidants protect against CTX hepatotoxicity[30]. PDSE treatment increase the activities of SOD and CAT, these confirm the finding of the study reported the enhancement of antioxidant enzyme activities upon administration of PDSE[31]. Treatment with PDSE also protected against lipid peroxidation, which could be attributed to their free radical scavenging activity. These findings were consistent with the previous study indicated the role of medicinal herbs in amelioration of lipids peroxidation[32].

CTX is metabolized by liver cytochrome P450, which likely causes sinusoidal obstacle disorder, coming about in a harmful impact on hepatic cells, in this way actuating rot, hindrance of hepatic veins[33]. It has been reported that in renal tissue, cell death of the proximal tubule epithelium could be induced by CTX[34]. The results reported that the adverse effects of CTX in the hepatic and renal tissue are significantly reversed in post-treated PDSE group. PDSE showed a significant amelioration in the proximal tubular damage[35]. Data also demonstrated significant downregulation in the proinflammatory cytokines (TGFβ-1, NFκ-β, COX-1, and COX-2 genes) upon treatment with PDSE. These findings agreed with previous study reported date fruit extract can prevent liver fibrosis by suppressing genotoxicity and nuclear factor-κB inflammatory pathway[36]. Studies supported our data reported that natural products have anti-inflammatory effects in several inflammatory diseases due to its antioxidant/anti-inflammatory activities[37,38].

Conclusion

Treatment with PDSE either post-CTX-injection significantly ameliorated the hematological, biochemical, and histological alterations induced by CTX toxicity on the liver and kidney organs as shown by improving their functions and upregulation of the antioxidant biomarkers.

Materials And Methods

Chemicals. Cyclophosphamide (CTX) was purchased from Sigma-Aldrich (St Quentin Fallavier, France). Vials were diluted by phosphate buffer saline (PBS) and the concentration was adjusted to 200 mg/kg body weight (b. wt). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) kits were purchased from Bio-diagnostic Company, Egypt.

Collection of plant seeds and extracts preparation. Date (*Phoenix dactylifera* L) was purchased from a market in Tanta city, Egypt. Then, identified by a taxonomist at the Botany Department, Faculty of

Science, Tanta University. Seeds were dried in shade and crushed. Fifty grams of seeds powder was mixed with 500 mL 70% (V/V) ethanol. The extract was filtered and dried under air condition.

Gas chromatography and mass spectrum (GC-MS) profiling. Phytochemical's composition in PDSE were assessed by using Trace GC 1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with TG-5MS column (30 m × 0.25 mm × 0.25 μm film thickness). The injector and MS transfer line temperatures were kept at 270, 260°C, respectively. Diluted samples (1μl) were injected using Auto-sampler (AS1300) coupled with GC in the split mode. The ion source temperature was set at 200°C. The structure determination was done by comparison of mass spectra patterns to WILEY 09 and NIST 11 mass spectral database.

Mice and experimental design. Forty male Swiss albino mice (22 ± 2 g) allowed acclimating for 1 week in the animal house conditions of the Faculty of Science, Tanta University. The institutional animal care committee at the Zoology Department, Faculty of Science, Tanta University-Egypt, approved the experimental design (IACUC-SCI-TU-0089). Temperature and humidity were about 22 ± 1 $^{\circ}$C and 55 ± 5% respectively, light-dark (day/night) cycle was achieved. Mice were given drinking tap water and normal experimental pelleted animal food *ad libitium*. Mice were divided into four groups (n = 10) as the following: negative control mice were injected intraperitoneally (i.p) with 200 μl saline (Gp1), Gp2 was injected i.p with PDSE (200 mg/kg) daily for 30 consecutive days. Gp3 were injected i.p with a single dose of CTX (200 mg/kg) at day 0 and Gp4 were injected with CTX as in Gp3, then injected with PDSE as in Gp2.

Determination of body weight changes. All mice were weighed at the beginning of the experiment as initial body weight (I.B.W) and at the end as final body weight (F.B.W); the difference between the F.B.W and I.B.W was calculated.

Hematological, biochemical, and histopathological studies. Groups of mice were sacrificed after the end of the experiment. Gross examinations were performed macroscopically on all mice during the sacrifice. Blood samples were collected in either heparinized or non-heparinized glass tubes. Whole blood was separated for complete blood count, while serum was used for biochemical analyses by using their kits. Sections from each liver and kidneys of all mice were collected and stored at -80°C for the determination of the oxidant/antioxidant biomarkers and gene expression analysis. Other livers and kidneys sections of all mice were collected and fixed in 10% buffered formalin for histopathological investigation.

Gene expression analysis. Real-time PCR with SYBR Green was used to measure mRNAs expression of tumor growth factor beta-1 (TGF-β1), nuclear factor Kappa-beta (NFκ-β), cyclooxygenase-1, and 2 (COX-1 and 2) genes in the liver tissues with β-actin as an internal reference. The isolated cDNA was amplified using Maxima SYBR Green/ROX qPCR Master Mix following the manufacturer protocol (Thermo scientific, USA, # K0221) and gene-specific primers shown in Table 5. The web-based tool (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) was used to design these primers based on

published sequences. Primer sequence similarity to other known sequences was checked with BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Table 5
Forward and reverse primers sequence for real time PCR.

Gene	Forward primer (′5 —— ′3)	Reverse primer (′5 —— ′3)
TGFb-1	AAGAAGTCACCCGCGTGCTA	TGTGTGATGTCTTTGGTTTTGTCA
NFk-β	CCTAGCTTTCTCTGAACTGCAAA	GGGTCAGAGGCCAATAGAGA
Cox-1	CCCAGAGTCATGAGTCGAAGGAG	CAGGCGCATGAGTACTTCTCGG
Cox-2	GATTGACAGCCCACCAACTT	CGGGATGAACTCTCTCCTCA
β-actin	ACCCACACTGTGCCCATCTA	CGTCACACTTCATGATG

Statistical analysis. Data are expressed as means ± S.D of three replicates. For all statistical tests P-values < 0.05 was statistically significant. Statistical analysis was performed using students' t-test. Data and statistical analysis were performed using Excel 2016 (Microsoft Corporation, USA), and Minitab version 18).

Institutional Review Board statement. The study was carried out in compliance with the ARRIVE guidelines. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Ethical committee at the Faculty of Science, Tanta University, with ethical approval license (IACUC-SCI-TU-0089).

Declarations

Data availability. The data presented in this study are available on request from the corresponding author.

Conflict of interest. All authors declared that there was no conflict of interest.

Acknowledgement. Authors sincere appreciate to Taif University Researchers Supporting Project number (TURSP-2020/309), Taif University, Taif, Saudi Arabia.

References

1. Henderson, B. E. *et al.* Hormonal carcinogenesis.*Carcinogenesis.*21,427–433(2000).
2. Liu, C. C. *et al.* Caffeic acid phenethyl ester as an adjuvant therapy for advanced prostate cancer.*Med. Hypothesis.*80,617–619(2013).
3. Torimura, T. *et al.* Metronomic chemotherapy: possible clinical application in advanced hepatocellular carcinoma.*Trans. Oncol.*6,511–519(2013).

4. Lori, J. *et al.* Doxorubicin and cyclophosphamide for the treatment of canine lymphoma: a randomized, placebo- controlled study.*Vet. Comp. Oncol.*8,188–195(2010).
5. Salem, M. *et al.* Kinetics of rebounding of lymphoid and myeloid cells in mouse peripheral blood, spleen, and bone marrow after treatment with cyclophosphamide.*Cell. Immunol.*276,67–74(2012).
6. El-Naggar, S. A. *et al.* Pretreatment with the Micro-alga, *Spirulina platensis* ameliorates cyclophosphamide-induced hematological, liver and kidney toxicities in male mice.*Ain Shams J. Foren. Med. Clin. Toxicol.*30,1–7(2018).
7. Patra, K. *et al.* Amelioration of cyclophosphamide induced myelosuppression and oxidative stress by cinnamic acid.*Chemico-Biol. Interac.*195,231–239(2012).
8. El-Naggar, S. A. *et al.* Efficacy of *Rosmarinus officinalis* leaves extract against cyclophosphamide-induced hepatotoxicity.*Pharmac. Biol.*54,2007–2016(2016).
9. Kamarzaman, S. *et al.* The prophylactic effect of *Nigella sativa* against cyclophosphamide in the ovarian follicles of matured adult mice: a preliminary study.*Animal. Plant. Sci.*24,81–88(2014).
10. Paul, R. *et al.* Avocado fruit (*Persea americana* Mill) exhibits chemo-protective potentiality against cyclophosphamide induced genotoxicity in human lymphocyte culture.*Exp. Ther. Oncol.*9,221–230(2011).
11. Ufelle, S. A. *et al.* Hematological effects of leaf extract of *Moringa oleifera* Lam in normal and myelo-suppressed Wistar rats.*Afr. J. Biomed. Res.*21,87–90(2011).
12. El-Juhany, L. I. Degradation of date palm trees and date production in arab countries causes and potential rehabilitation.*Aust. J. Basic. Appl. Sci.*4,3998–4010(2010).
13. Baliga, M. S. *et al.* (2011). A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). *Food. Res. Int.* 44, 1812–1822 (2011).
14. Adeosun, A. M. *et al.* Phytochemical, minerals, and free radical scavenging profiles of *Phoenix dactilyfera* L. seed extract.*Taibah. Univ. Med. Sci.*11,1–6(2016).
15. Rahmani, A. H. *et al.* Therapeutic effects of date fruits (*Phoenix dactylifera*) in the prevention of diseases via modulation of anti-inflammatory, antioxidant, and antitumor activity.*Int. J. Clin. Exp. Med.*7,483–491(2014).
16. Taleb, H. *et al.* The antibacterial activity of date syrup polyphenols against S. aureus and E. coli.*Front. Microbiol.*7,198(2016).
17. Benzer, F. *et al.* Curcumin ameliorates doxorubicin-induced cardiotoxicity by abrogation of inflammation, apoptosis, oxidative DNA damage, and protein oxidation in rats.*Biochem. Mol. Toxicol.*32,e22030(2018).
18. Bhattacharjee, P. *et al.* Monitoring HIV prevention programme outcomes among key populations in kenya: findings from a national survey.*PLoS. ONE.*10,e0137007(2015).
19. Lewis, K. *et al.* Prospects for plant-derived antibacterials.*Nat. Biotechnol.*24,1504–1507(2006).
20. Tungmunnithum, D. *et al.* Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview.*Medicin.*5,93–109(2018).

21. Kumar, P. P. *et al.* Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo.*Afr. J. Biochem.*4,191–195(2010).
22. Srivastava, R. *et al.* GC-MS analysis of phytocomponents in petroleum ether fraction of Wrightia tinctoria seed.*Pharmacog.*7,249–253(2015).
23. Qi, Q. *et al.* Protective effect of bergenin against cyclophosphamide-induced immunosuppression by immunomodulatory effect and antioxidation in Balb/c mice.*Molec.*23,2668(2018).
24. Szumilas, P. *et al.* Effect of stem cell mobilization with cyclophosphamide plus granulocyte colony-stimulating factor on morphology of haematopoietic organs in mice.*Cell. Prolif.*38,47–61(2005).
25. Gnanasekaran, S. et al. Immunostimulant and chemoprotective effect of vivartana, a polyherbal formulation against cyclophosphamide induced toxicity in Swiss albino mice. Exp. Ther. Oncol.11,51–61(2015).
26. Nwaneri-Chidozie, V. O. *et al.* Effect of Phoenix dactylifera fruit wine produced by Saccharomyces cerevisae on the haematological and some biochemical parameters in albino rats.*Saudi. J. Life. Sci.* 2,116–121(2017).
27. Djousse, L. *et al.* Total serum bilirubin and risk of cardiovascular disease in the Framingham offspring study. *Americ. J. Cardiol*, **87**, 1196–1200 (2001).
28. Heidari-Soreshjani, S. *et al.* Phytotherapy of nephrotoxicity-induced by cancer drugs: an updated review.*Nephropathol.*6,254–263(2017).
29. Tripathi, D. N. *et al.* Astaxanthin intervention ameliorates cyclophosphamide-induced oxidative stress, DNA damage and early hepatocarcinogenesis in rat: role of Nrf2, p53, p38 and phase-II enzymes.*Mutat. Res.*696,69–80(2010).
30. Kumar, K. B. *et al.* Chemoprotective activity of an extract of *Phyllanthus amarus* against cyclophosphamide induced toxicity in mice.*Phytomed.*12,494–500(2005).
31. Al-Qurainy, F. *et al.* Biochemical and genetical responses of *Phoenix dactylifera* L. to cadmium stress.*BioMed. Res. Int*. 2017,1–9(2017).
32. Zarei, M. & Shivanandappa, T. Amelioration of cyclophosphamide-induced hepatotoxicity by the root extract of *Decalepis hamiltonii* in mice.*Food. Chem. Toxicol.*57,179–184(2013).
33. Basu, S. *et al.* Hepatoprotective activity of *Litchi chinensis* leaf against paracetamol-induced liver damage in rats.*Middl. East. J. Sci. Res.*20,292–296(2014).
34. Asiri, Y. A. Probucol attenuates cyclophosphamide-induced oxidative apoptosis, p53 and Bax signal expression in rat cardiac tissues. *Oxid. Med. Cell. Longev*, **3**, 308–316 (2010).
35. Al-Qarawi, A. A. *et al.* Protective effect of extracts from Dates (*Phoenix dactylifera L.*) on carbon tetrachloride–induced hepatotoxicity in rats.*Int. J. Appl. Res. Vet. Med.*2,176–180(2004).
36. Attia, D. *et al.* Liver stiffness measurement using acoustic radiation force impulse elastography in overweight and obese patients.*Aliment. Pharmacol. Ther.*44,366–379(2016).
37. Bai, T. *et al.* Thymoquinone attenuates liver fibrosis via PI3K and TLR4 signaling pathways in activated hepatic stellate cells.*Int. Immunopharmacol.*15,275–281(2013).

38. Majdalawieh, A. F. & Fayyad, M. W. Immunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: A comprehensive review.*Int. Immunopharmacol.*28,295–304(2015).

Figures

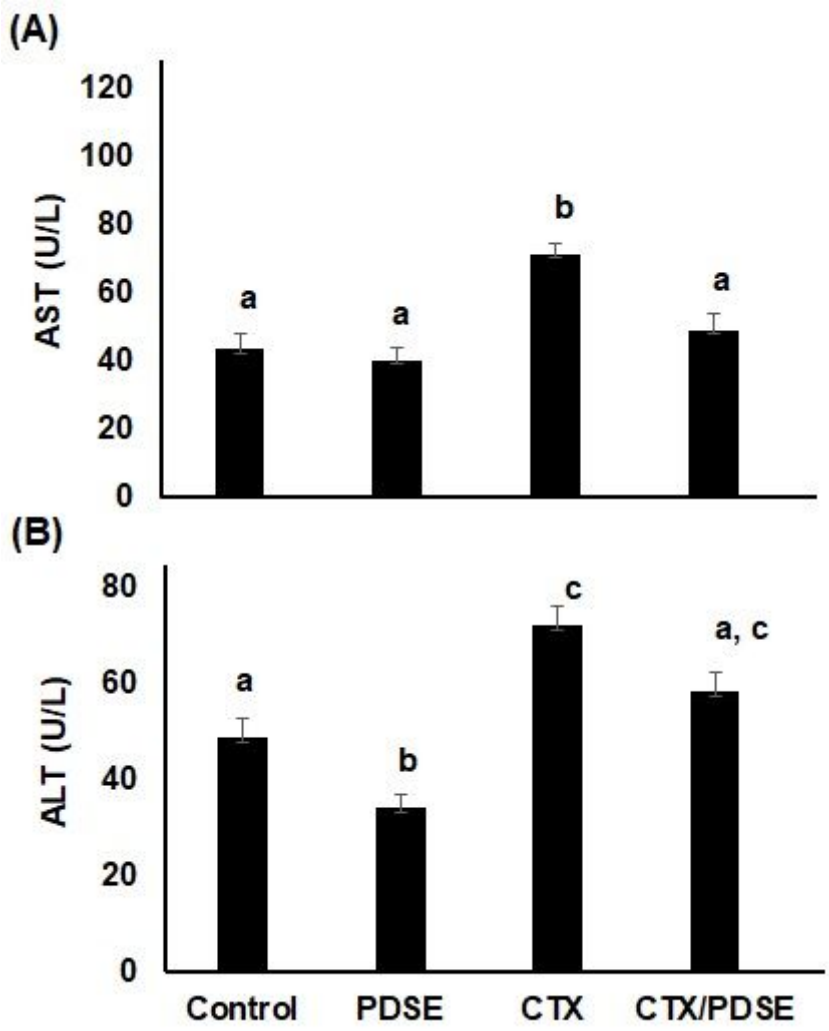

Figure 1

(A and B). Serum levels of aspartate transaminases (AST) (A) and alanine transaminases (ALT) (B) in the different groups of mice.

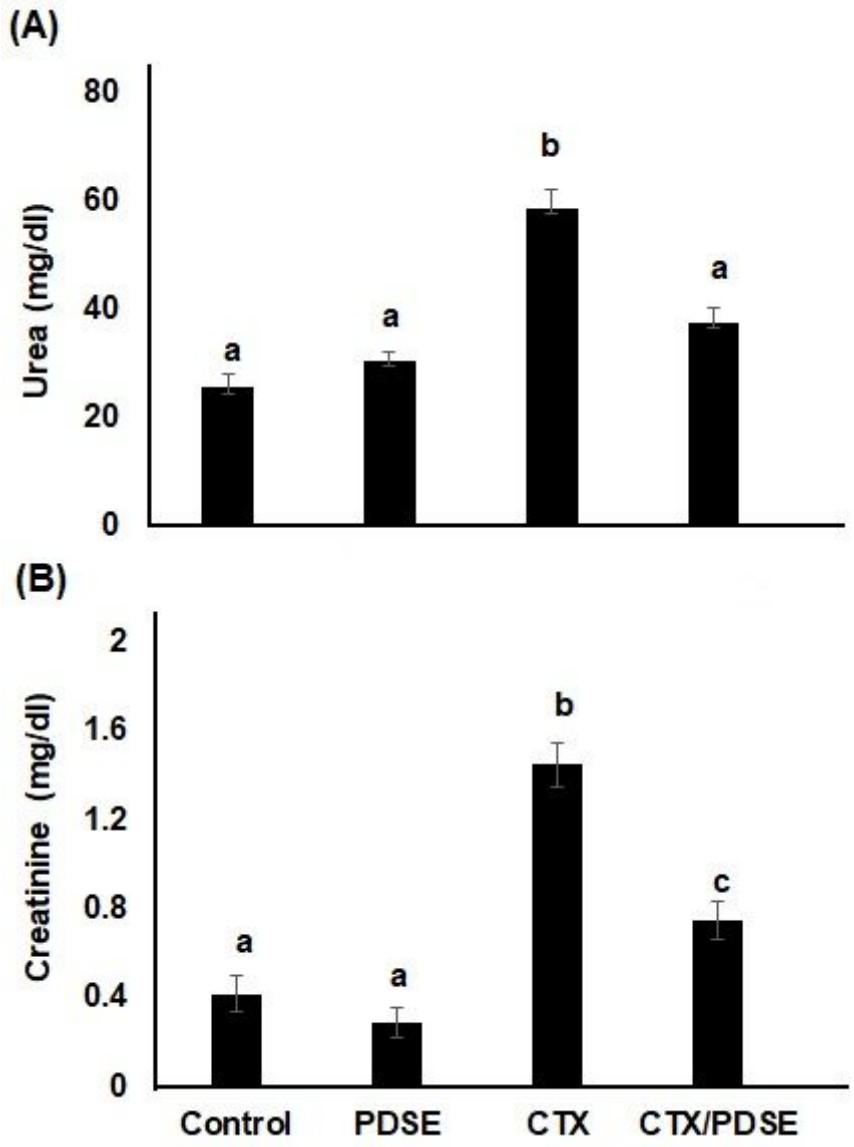

Figure 2

(A and B). Serum levels of urea (A) and creatinine (B) in the different groups of mice.

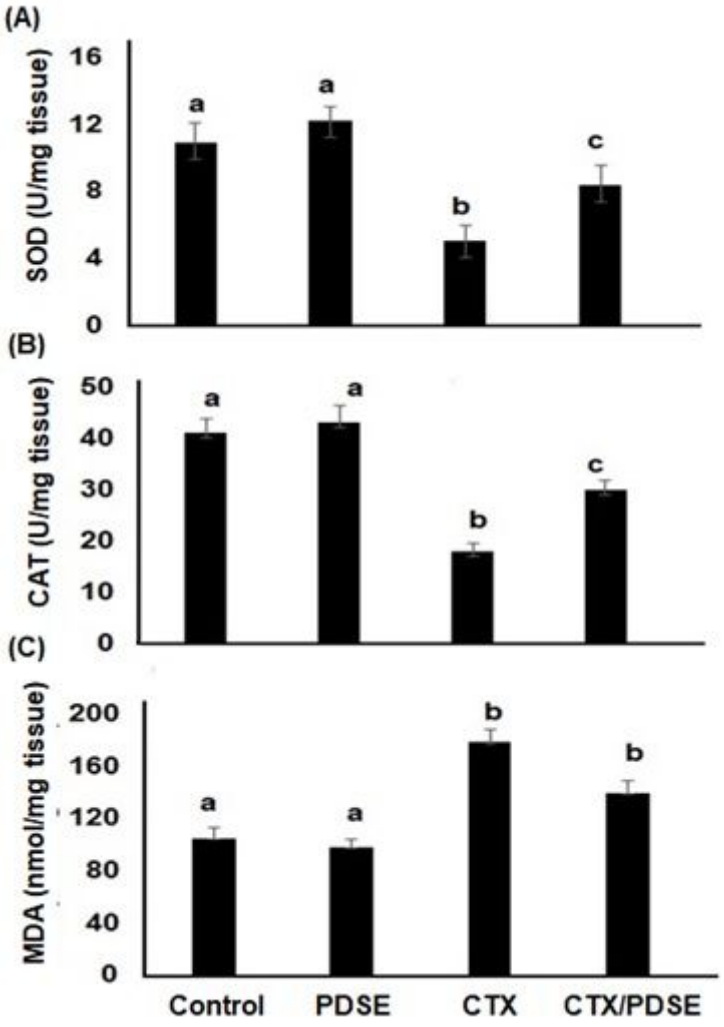

Figure 3

(A-C). Hepatic levels of superoxide dismutase (SOD) (A), catalase (CAT), (B) and malondialdehyde (MDA) (C) in the different groups of mice.

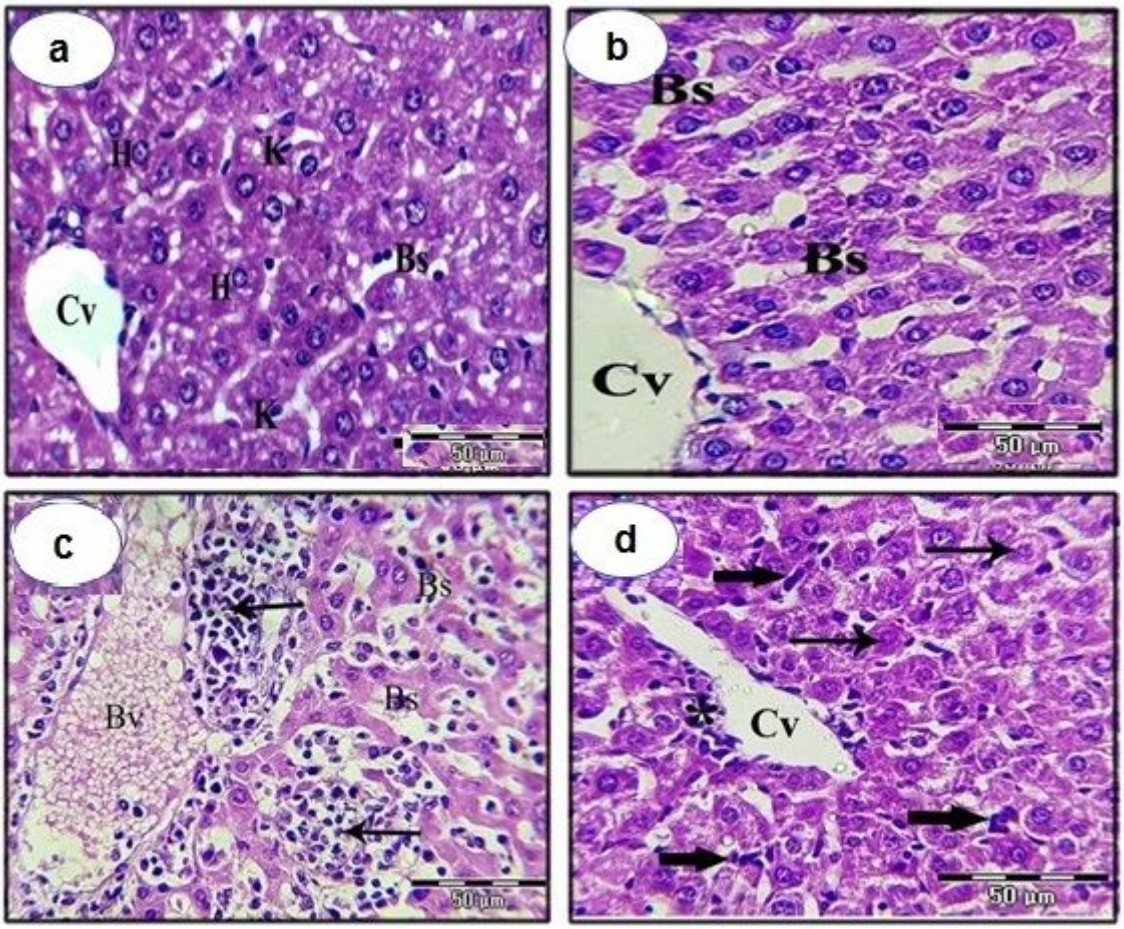

Figure 4

(a-d). Photomicrograph of liver sections (High magnification) stained with H&E from the different groups under the study. (a) Control group showing normal hepatic lobule, central vein (Cv), and radiating polygonal hepatocytes (H), blood sinusoids (Bs) lined by endothelial cells, and distinct phagocytic Kupffer cells (K). (b) PDSE treated mice showing the normal hepatic structure, slightly widening blood sinusoids (Bs) with normal Kupffer cells. (c) CTX-injected group (Gp3) showing noticeable disorganized liver architecture, congested blood vessels (Bv) with fatty exudate and obvious mononuclear infiltration (arrows) around damaged cells, widening of blood sinusoids (Bs) were seen. (d) CTX/PDSE-treated mice exhibits improvement of the hepatic architecture, dilated central vein (Cv), cellular infiltration (*), few hepatocytes show hyper-eosinophilia (arrows) and few activated Kupffer cells (thick arrows).

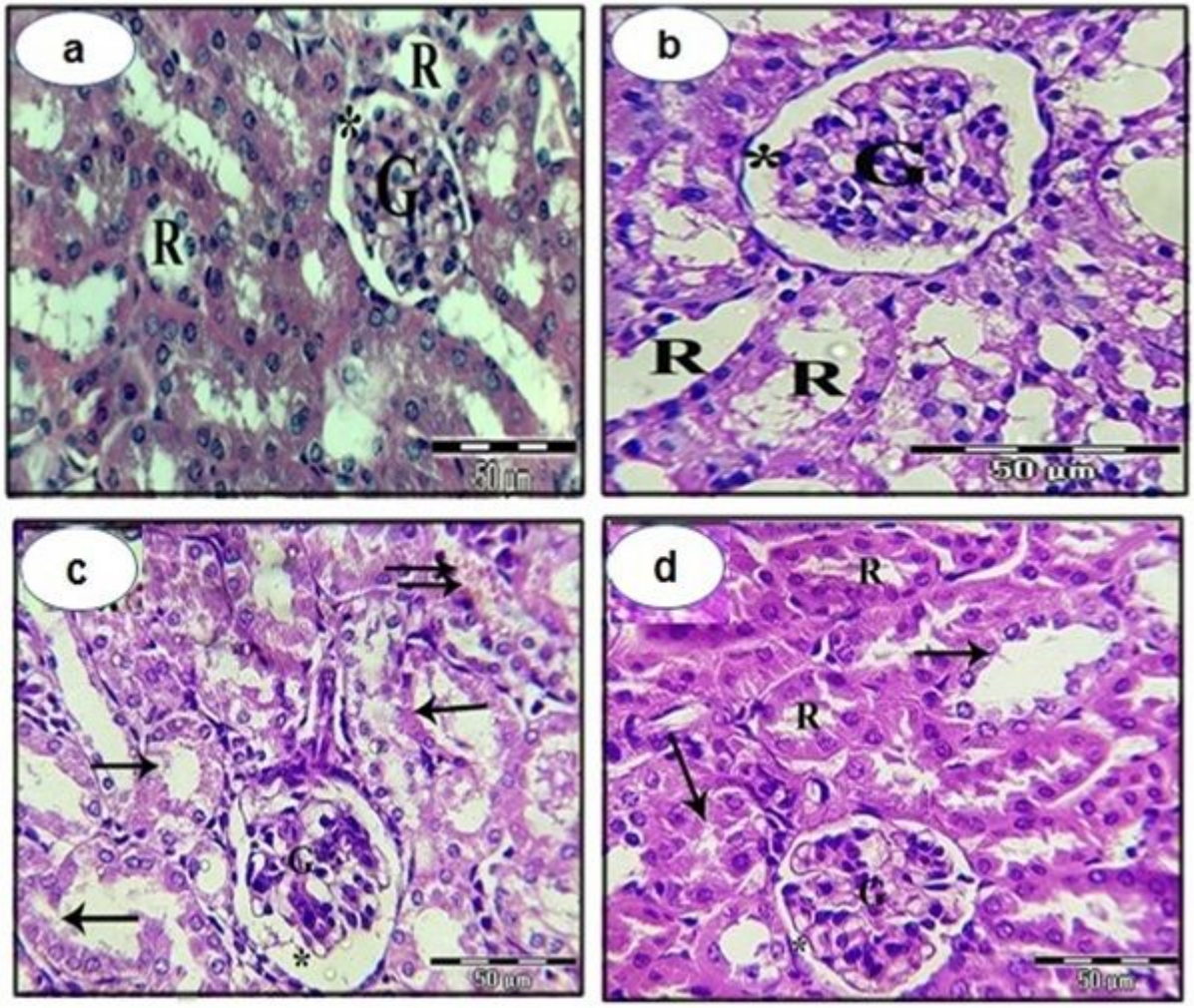

Figure 5

(a-d). Photomicrograph of kidney sections of mice (High magnification) of different groups stained with H&E. (a) Renal cortex of control mice showed normal architecture of renal glomeruli (G) and renal tubules (R). (b) PDSE treated mice exhibit the cortex that contains glomeruli (G) with normal Bowman's space (*) and normal appearance of mostly renal tubules (R) but few tubules have degenerated. (c) The kidney sections of mice after a single dose of CTX injection showing atrophy of the glomeruli (G), irregular Bowman's space (*), destruction of most renal tubules, almost nuclei of the lining epithelia are degenerated (arrows) and intratubular hemorrhage (double arrow). (d) CTX/PDSE-treated mice exhibits normal structure of the kidney tissue with normal glomeruli (G), normal renal tubules (R), but few numbers of disorganized tubules (arrows) were seen.