Biological and Biomedical Journal

Journal homepage: http://www.ESBBS.org

Phoenix dactylifera seeds extract ameliorates the hepato-renal toxicities that induced by cyclophosphamide in male mice

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ARTICLE INFO

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Keywords:
Phytochemicals, *Phoenix dactylifera*, Cyclophosphamide, Hepato-renal, Toxicity.

P-ISSN: 2974-4334
O-ISSN: 2974-4342
DOI: 10.5455/BBJ.145174

ABSTRACT

Cyclophosphamide (CTX) causes severe side effects on certain vital organs. *Phoenix dactylifera* seeds extract (PDSE) showed promising biomedical applications. This study aims to evaluate the phytochemical composition PDSE and its impact on CTX-induced hepato-renal toxicities in mice. The phytochemical compounds of PDSE were determined with gas-chromatography mass spectrometry (GC-MS). Forty male CD-1 albino mice were divided into four groups (n=10) as the following: Group 1 (Gp1) was served as a negative control. Gp2 was injected with PDSE (100 mg/kg) interperitoneally (i.p) daily for 30 days. Gp3 had injected with CTX (200 mg/kg) once, at day 0. Gp4 had injected with CTX/PDSE as in Gp3 and Gp2. Hematological, some biochemical parameters (Liver enzymes, kidney functions and antioxidant/oxidant biomarkers), histopathological alterations and gene expression for some pro-inflammatory cytokines in the liver and kidney tissues were assessed. GC-MS analysis showed that the 1-Heptatriacotanol and 9, 12, 15-Octadecatrienoic acid, 2, 3-dihydroxypropyl ester showed the highest peak areas (21.07 and 53.49%, respectively). The results showed that the treatment with PDSE for 30 days significantly ameliorated the hematological, biochemical, and histological alterations post CTX injection as evidenced by improving the liver/kidney functions, increasing the antioxidant enzymes, and down-regulating the tumor growth factor beta-1 (TGFβ-1), nuclear factor Kappa-beta (NFκ-β), cyclooxygenase-1 and 2 (COX-1 and 2) genes in both of the liver and kidney tissues. Furthermore, treatment with PDSE decreased the histopathological changes in the liver and kidney tissues that were induced by CTX-toxicity. Collectively, treatment with PDSE after CTX-injection showed potent ameliorating effect on both liver and kidney.

1. Introduction

Cancer remains one of the most common health problems worldwide. Efforts to find new safe and effect treatments in recent decades were conducted (Henderson et al., 2014). Till now, different settings to treat cancer included surgery, conventional chemotherapy, radiotherapy, and immunotherapy are used. The conventional chemotherapy known as the first line of treatment; however, treatment failures are common due to drug resistance and due to its adverse effects on normal healthy tissue. Therefore, a significant challenge to find a treatment with less toxicity is an ultimate need (Liu et al., 2013). Cyclophosphamide (CTX) is an anticancer agent that is used for several types of cancer treatment (Torimura et al., 2013; El-Naggar et al., 2015). CTX is used alone or in combination with other chemotherapies for cancer treatment (Satyanarayana et al., 2021). CTX not only kills the tumor cells, but also affects the dividing hematopoietic cells which in turn led to lymphopenia (Salem et al., 2012). The therapeutic dose of CTX could cause liver and kidney toxicities (Torimura et al., 2013; El-Naggar et al., 2015; El-Naggar 2018).

Some antioxidant agents are being used to alleviate oxidative stress induced by chemotherapy. In line with this, a previous study reported the beneficial roles of natural agents on CTX induced toxicity (El-Naggar et al., 2016). 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 (Singh et al., 2018). For instance, *Persea*

americana phytoconstituents, propolis and *Spirulina platensis* extract can be used for lowering the side effect of CTX (Paul et al., 2011; El-Naggar et al., 2015; El-Naggar et al., 2018). In addition, it has also been demonstrated that various crude and fractions of *Vitex doniana* leaves extracts have myelo-protective activity in CTX-induced myelo-toxicity (Ufelle et al., 2011). The date palms (*Phoenix dactylifera L.*) have been an important crop in Egypt and Middle Eastern countries (El-Juhany 2010). Date pit powders are marketed and are used as a non-caffeinated coffee with coffee-related flavor (Baliga et al., 2011). The date seed contains alkaloids, flavonoids, tannins, saponins, phenol, and sterols (Adeosun et al., 2016). Preclinical studies have shown that the *P. dactylifera* exhibits free radical scavenging, antioxidant, anti-mutagenic, anti-microbial, anti-inflammatory, gastro-protective, hepato-protective, nephron-protective, anticancer, and immune stimulant activities (Rahmani et al., 2014; Taleb et al., 2016).

Most of the anti-neoplastic agents are known to cause myelo-suppression and neutropenia (Salem et al., 2012). To reduce the adverse effects of CTX and its reactive metabolites, it is essential to increase the body's antioxidant defenses with natural and safe antioxidants. For this reason, there is a need for efficient substances that protect the healthy tissues from the side effects of CTX-induced toxicity. Therefore, this study aimed to address the ameliorative role of the PDSE on hepato-renal hematological, biochemical, and histopathological alterations that induced by CTX in albino mice.

2. Materials and Methods

2.1. 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 amino transferase (AST), alanine amino transferase (ALT), urea, creatinine, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) kits were purchased from Bio-diagnostic Company, Egypt.

2.2. Collection of plant seeds and extract preparation

Date (*Phoenix dactylifera* L), was purchased from local market in Tanta city, Egypt. The plant materials were identified and authenticated by taxonomists at Botany Department, Faculty of Science, Tanta University. Seeds were collected and dried in shade then crushed in a mortar. Fifty grams (50 g) of seeds powder were mixed vigorously with 500 mL 70% (V/V) ethanol. The hydro-alcoholic extract was filtered, and the solvent was dried under air condition, then the extract was weighed and suspended in 0.9 % sterile saline for further processing.

2.3. Gas chromatography and mass spectrum (GC-MS) profiling

Phytochemicals and secondary metabolites composition in PDSE were assessed by using Trace GC 1310-ISQ mass spectrometer "GC-MS" (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The column oven temperature was initially held at 50 ˚C and then increased by 70 ˚C/min to 230 ˚C hold for 2 min increased to the final temperature 300 ˚C by 30˚C/min hold for 2 min. The injector and MS transfer line temperatures were kept at 270, 260 ˚C, respectively. Helium was used as a carrier gas with constant flow rate of 1ml/min. The solvent delay was 3 min and diluted samples of 1µl were injected automatically using Auto-sampler (AS1300) coupled with GC in the split mode. EL mass spectra were collected at 70 eV ionization voltages over the range of m/z 45-600 in full scan 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.

2.4. 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, before being divided into groups. Animals were used for two different experimental designs. The institutional animal care committee at Zoology Department, Faculty of Science, Tanta University-Egypt, approved the experimental design (IACUC-SCI-TU-0089). Target values for temperature and relative humidity were about 22 ± 1 °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 had injected intraperitoneally (i.p) with 200 µl saline (Gp1), Gp2 had injected i.p with PDSE (100 mg/kg) daily for 30 consecutive days. Gp3 had injected i.p with a single dose of CTX (200 mg/kg) at day 0 and Gp4 had injected with CTX as in Gp3 and injected with PDSE as in Gp2.

2.5. Determination of body weight changes

All mice were weighted 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 and considered as the percentage of the total body weigh changes.

2.6. Hematological, biochemical, and histopathological studies

According to the experimental plan, groups of mice were sacrificed after the end of the experiment, under ethyl ether anesthesia. Gross examinations were performed macroscopically on all mice during 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 determination of the oxidant/antioxidant biomarkers and gene expression analysis. Other liver and kidneys sections of all mice were collected and fixed in 10% buffered formalin for histopathological investigation.

2.7. 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 of the different groups, 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 1. The web-based tool (http://www-genome.wi.mit.edu/cgibin/primer/primer3_www.cgi) was used to design these primers based on published rat sequences.

To ensure primer sequence is unique for the template sequence; we checked similarity to other known sequences with BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Table 1. Forward and reverse primers sequence for real time PCR.

Gene	Forward primer (/5 ------ /3)	Reverse primer (/5 ------ /3)
TGFb-1	AAGAAGTCACCCGCGTGCTA	TGTGTGATGTCTTTGGTTTTGTCA
NF*k*-β	CCTAGCTTTCTCTGAACTGCAAA	GGGTCAGAGGCCAATAGAGA
Cox-1	CCCAGAGTCATGAGTCGAAGGAG	CAGGCGCATGAGTACTTCTCGG
Cox-2	GATTGACAGCCCACCAACTT	CGGGATGAACTCTCTCCTCA
β-actin	ACCCACACTGTGCCCATCTA	CGTCACACTTCATGATG

3. Results

3.1. GC-MS analysis of PDSE

GC-MS chromatogram of PDSE showed the retention time (RT) 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 2).

3.2. PDSE treatment post CTX-injection protect against body weight loss

The final body weight of CTX-treated mice (Gp3) was significantly decreased ($p < 0.05$) when compared with control group (Gp1). Treatment with PDSE alone did not show significant alteration in the body weight gain as compared to Gp1 ($p > 0.05$). Treatment with PDSE post CTX injections protected against the loss of body weights when compared to CTX-injected groups (Gp3) (Table 3).

Table 2. Biochemical compounds analyzed by GC-MS of the hydro alcoholic 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

RT: Retention time; M.F: Molecular formula; M. Wt: Molecular weigh

Table 3. Initial body weights, final body weights, % body weigh change of different groups under the study.

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

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

3.3. PDSE treatment post CTX injections ameliorated hematological changes

The results showed that as compared to the control group (Gp1), the total number of red blood cells (RBCs), hemoglobin (Hb) concentration and hematocrit (%) value did not show significant alterations ($p > 0.05$) in all groups (Table 4). Compared to Gp1, PDSE (Gp2) treatment showed an increase in the total platelets count ($p < 0.05$). A dramatic increase in the total white blood cells (W.B.Cs) count was found in the group of mice post 30 days of a single injection of CTX (Gp3) when compared to the WBCs count in the control group (Gp1) ($p < 0.05$). 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 4). Group of mice which treated with CTX at day 0 (Gp3) showed a significant increase in the total number of lymphocytes, monocytes and neutrophils when

compared to Gp1. Treatment with PDSE post CTX-injection ameliorated the toxic effect of CTX on these cells and returned the numbers close to the normal values (Table 5).

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

Treatment with PDSE alone did not show any significant changes in AST and ALT activities when compared to their control ($p > 0.05$).

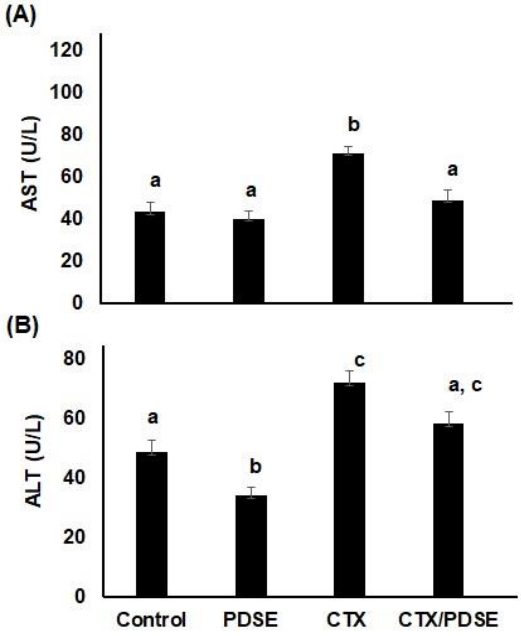

Fig. 1 (A and B). Serum levels of aspartate transaminases (AST) **(A)** and alanine transaminases (ALT) **(B)** in the different groups of mice.

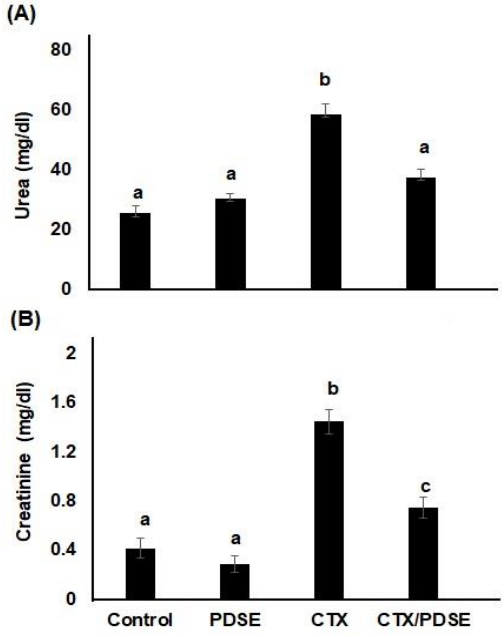

Fig. 2 (A and B). Serum levels of urea **(A)** and creatinine **(B)** in the different groups of mice.

The result showed that there was an increase in the liver transaminases AST and ALT activities in CTX injected groups (Gp3), when compared to their control group ($p < 0.05$). Treatment with PDSE post CTX injections ameliorated the toxic effect of CTX on the liver function as evident by decreasing the activities of AST and ALT (Fig. 1). Treatment with PDSE alone did not show any significant alterations in the level of urea and creatinine levels when compared to their control ($p > 0.05$). CTX injection led to an increase in the level of urea and creatinine when compared to their levels in the control group ($p > 0.05$). Treatment with PDSE post CTX injections significantly decrease in the levels of urea and creatinine ($p < 0.05$) when compared to the groups of mice that injected with CTX alone (Fig. 2).

3.5. PDSE treatments mitigated the oxidative stress induced by CTX-injections

Treatment with PDSE (Gp2) alone did not result in any significant changes in the activities of SOD, CAT and MDA levels when compared to control group (Gp1). CTX injected groups (Gp3) showed decrease in the hepatic antioxidant activities of SOD and CAT and increased the level of MDA when compared to Gp1 ($p < 0.05$). 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 and decrease the level of MDA (Fig. 3).

3.6. PDSE treatment improve histopathological alterations in liver and kidney tissues

Examination of liver sections of 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 envelops and normal distributed chromatin. The liver strands were alternating with narrow blood sinusoids lined by endothelial cells and distinct phagocytic Kupffer cells (Fig. 4a and b). The liver sections of mice (Gp3) after 30 days of single dose of CTX injection 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 central vein, regular narrow blood sinusoids network, some hepatocytes show hyper eosinophilia and activated Kupffer's cells (Fig. 4d).

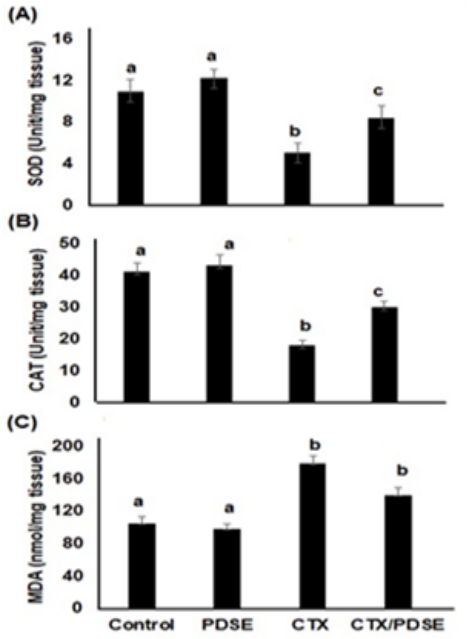

Fig. 3 (A-C). Hepatic levels of superoxide dismutase (SOD) **(A)**, catalase (CAT) **(B)** and malondialdehyde (MDA) **(C)** in the different groups of mice.

The concentration and purity of the extracted RNA were determined by Nanodrop, and the results revealed that the isolated RNA is pure and with considerable higher concentrations ranged from 1420 to 2340 ng/µl. The obtained results revealed significant ($p < 0.001$) increase in the expression of the pro-inflammatory genes (*TGF-β1, NFk-β, COX-1 and COX-2*) in the liver and kidney tissues of CTX-injected mice. Treatment with PDSE post CTX injection led to significant ($p < 0.001$) decrease in the mRNA expression levels of these genes when compared to their controls (Table 6).

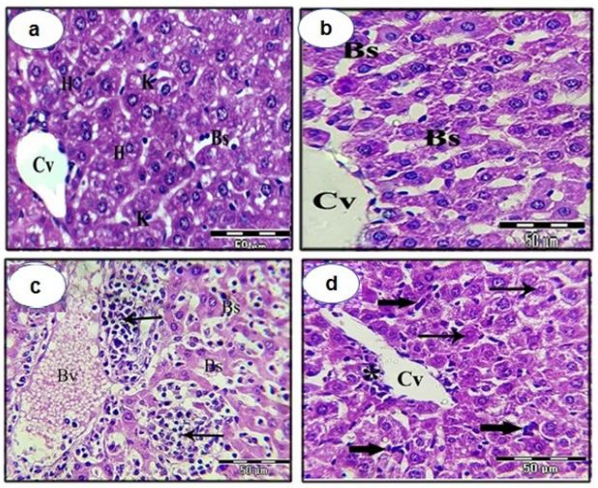

Fig. 4 (a-d). Photomicrograph of liver sections (High magnification) stained with H&E from the different groups under the study. **(a)** Control group showing normal hepatic lobule, central vein (Cv) and radiating polygonal hepatocytes (H), blood sinusoids (Bs) lined by endothelial cells and distinct phagocytic Kupffer cells (K). **(b)** PDSE treated mice showing normal hepatic structure, slightly widening blood sinusoids (Bs) with normal Kupffer cells. **(c)** CTX-injected group (D-0) 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 (D-0)/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).

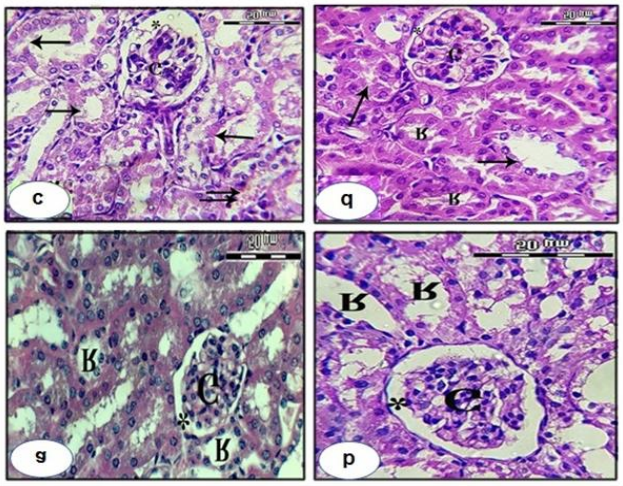

Fig. 5 (a-d). Photomicrograph of kidney sections of mice (High magnification) of different groups stained with H&E. **(a)** Renal cortex of control mice showed normal architecture of renal glomeruli (G) and renal tubules (R). **(b)** PDSE treated mice exhibits the cortex that contains glomeruli (G) with normal Bowman's space (*) and normal appearance of mostly renal tubules (R) but few tubules were degenerated. **(c)** The kidney sections of mice after 33 days of 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 intertubular hemorrhage (double arrow). **(d)** CTX (D-0)/PDSE treated mice exhibits normal like structure of the kidney tissue with normal glomeruli (G), normal renal tubules (R), but few numbers of disorganized tubules (arrows) were seen.

4. Discussion

The conventional chemotherapeutic drugs are used for the treatment of different malignancies, but their therapeutic use is limited due to their adverse side effects on the vital organs (Benzer et al., 2018). Side effects such as hepatotoxicity, renal toxicity, and bone marrow suppression were associated with CTX treatment (Bhattacharjee et al., 2015). The current study was conducted to evaluate the phytochemical constituents of PDSE by using GC-MS analysis and is to evaluate its ameliorative role on the hepatorenal toxicities induced by CTX injection. The results showed that PDSE contains several bioactive compounds including octasiloxane, linoleate, palmitate and pregnane. Such these compounds have the nature of phenolic, flavonoids and fatty acids which could have potential effects as antibacterial, antioxidant and anticancer agents (Tungmunnithum et al., 2018). GC-MS profiling showed that there are some bioactive compounds such as 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z) ethyl palmitate and n-Hexadecanoic had antioxidant, and anti-inflammatory activities (Kumar et al., 2010). Previous studies reported that 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-2,3-dihydroxypropyl ester (14.35 %) had analgesic, antipyretic, anticonvulsant, antiseptic effects (Srivastava et al., 2015).

In this study, a significant decrease in the total body weight of mice that injected with CTX has been found. The decrease might be due to CTX adverse effect on the vital body organs (El-Naggar et al., 2017). Treatment with PDSE after CTX injection may protect mice from losing weight. This finding was in accordance with the previous study that showed that treatment with natural products protects experimental animals to lose weight upon CTX injection (Qi et al., 2018). Ater 30 days of CTX, the number of WBCs was significantly increase than those of the normal control group. This observation is consistent with the capability of CTX to induce mobilization of hematopoietic stem cells from BM to circulation (Szumilas et al., 2005). PDSE may act as an immunostimulant and enhance the immune system which agrees with a previous study reported by Sforcin (2007). These results were also consistent with Nwaneri-Chidozie et al., (2017) that reported some hematological changes upon treatment with date palms in experimental animals.

CTX injection led to an elevation of serum ALT and AST in mice. PDSE treatment post CTX injection improves the activity of these enzymes. This finding agreed with previous study that showed the role of medicinal plants in protection and treatment of hepatotoxicity induced by CTX in experimental animals (El-Naggar et al., 2015). CTX-induced elevations in the levels of urea and creatinine. 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 previous study that reported the impact of plant-based formulations to control and modulate anticancer drug-induced nephrotoxicity indices (El-Naggar et al., 2015; Heidari-Soreshjani et al., 2017). Cellular antioxidants defenses involving the enzymes, SOD and CAT detoxify the free radicals and the consequent oxidative stress. CTX-induced hepatotoxicity is associated with oxidative stress caused by the reduction in the antioxidant enzymes (Raj-asekaran et al., 2002). Other studies have shown that CTX treatment is associated with induction of oxidative stress by the generation of free radicals (Tripathi and Jena 2010). In this study, the activities of the antioxidant enzymes, SOD and CAT in the liver tissues were significantly reduced by CTX treatment indicating pronounced oxidative stress. SOD was drastically reduced in the liver of CTX-injected mice, which would lead to an increase in the levels of H_2O_2 and lowered activities of CAT further contributes to the compromised cellular antioxidant defense. Previous report suggest that natural antioxidants protect against CTX hepatotoxicity (Kumar and Kuttan 2005). Treatment with PDSE post CTX injection increase the activities of SOD and CAT that indicated the improvement of antioxidant status of mice livers, these observations confirm the finding of studies, which reported the enhancement of antioxidant enzyme activities upon administration of PDSE to experimental animals *(*Ragab et al., 2013; Al-Qurainy et al., 2017). Treatment with PDSE after CTX injection protected against the lipid peroxidation, which could be attributed to the free radical scavenging activity of the extracts as well as suppressing oxidative stress. These findings were consistent with the previous study indicated the role of medicinal herbs in ameliorating the lipids peroxidation induced by CTX administration in experimental animals (Zarei and Shivanandappa 2013).

Earlier studies have reported that the therapeutic dose of CTX could cause liver and kidney toxicity (El-Naggar et al., 2015, 2018). CTX is extensively metabolized by the liver cytochrome P450 system, which probably causes sinusoidal obstruction syndrome, resulting in a direct toxic effect on hepatic sinusoidal cells, thus inducing necrosis, obstruction, and obliteration of hepatic veins (Basu et al., 2014). Schwerdt et al., (2006) have revealed that in the renal tissue, cell death in the proximal

tubule epithelium could be induced by the acrolein and chloro-acetaldehyde derived from CTX. Asiri (2010) in another study showed that the tubular epithelium damage caused by the direct adverse effect of acrolein is the first cellular mechanism for CTX toxicity and the increased production of ROS through intracellular phosphoramide mustard. Pre-treatment with *Spirulina platensis* (1000 mg/kg/body weight) ameliorated the hepatic and renal dysfunctions and decreases the hepatic and renal histological changes which were induced by CTX (El-Naggar et al., 2016). The results reported that the adverse effects of CTX in the hepatic and renal tissue are significantly reversed as evidenced by the histopathological examination of the hepatic and renal tissue in post-treated groups with PDSE. Al-Qarawi et al., (2004) reported the importance of *P. dactylifera extracts* in rat models showed significantly reduced the increase in plasma creatinine and urea concentrations and ameliorated the proximal tubular damage. Data also demonstrated significant downregulation in the proinflammatory cytokines (TGFβ-1, NFκ-β, COX-1 and 2 genes) upon treatment with PDSE. These findings agreed with Attia et al., (2016) who reported that date fruit extract can prevent liver fibrosis by suppressing genotoxicity and nuclear factor-κB inflammatory pathway and by promoting collagen degradation. Several studies supported our data, which reported that natural products have anti-inflammatory effects in several inflammatory diseases due to its antioxidant/anti-inflammatory activities as it significantly decreased TNF-*α*, NFκ-β and COX-2 levels, which involved several signaling pathways including NFκ-β, p38 MAPK and ERK1/2 pathways (Bai et al., 2013; Majdalawieh and Fayyad, 2015).

5. Conclusion

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

Conflict of interest

All authors declared that there was no conflict of interest.

Acknowledgements

The author thanks, greatly honors, and expresses deep gratitude to the Zoology Department, Faculty of Science, Tanta University, Egypt, for providing their laboratory to carry out this study.

Author contributions

SR conceptualized the study, performed the experiments, analysis date and wrote the draft. The author read and approved the final manuscript.

Funding

No funding was received from any person or organization for this study.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate All experimental procedures were conducted in accordance with the ethical standards and were approved by the Institutional Animal Care and Use Committee (IACUC) at National Organization for Drug Control and Research (NODCAR) (approval no. NODCAR/III/41/2019).

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