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Hepatoprotective effect of date palm fruit extract against doxorubicin intoxication in Wistar rats: *In vivo* and *in silico* studies

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ABSTRACT

Objective: To investigate the prophylactic efficacy of date palm fruit extract against doxorubicin-induced hepatotoxicity in Wistar albino rats.

Methods: The rats were equally and randomly assigned to 6 groups: group 1 (untreated control), group 2 and 3 given daily oral administration of prophylactic aqueous extract of date palm fruit at 0.75 and 1.5 mg/kg body weight, respectively, and group 4, 5 and 6 intraperitoneally injected with doxorubicin at 15 mg/kg on day 30. Rats in group 5 and 6 received daily oral administration of aqueous extract of date palm fruit at 0.75 and 1.5 mg/kg body weight, respectively, for 30 d. The phytochemicals identified by GC-MS analysis were analyzed using *in silico* study. Antioxidant enzymes, liver enzymatic, biochemical parameters and histopathological analysis were determined to evaluate hepatoprotective activity of date extract.

Results: Aqueous extract of date palm fruit significantly mitigated doxorubicin-induced changes in activities of liver enzymes, reduced reactive oxygen species levels, and suppressed lipid peroxidation and DNA damage. Moreover, aqueous extract of date palm fruit reduced doxorubicin-induced hepatic lesions. Molecular docking studies showed that most compounds of aqueous extract of date palm fruit identified *via* GC-MS had good interaction with proteins of human pregnane X receptor, oxygenase-1, and CYP2C9.

Conclusions: The aqueous extract of date palm fruit mitigates doxorubicin-mediated DNA damage and hepatotoxicity, and restores normal liver function and may be a promising agent against the deleterious effects of doxorubicin.

KEYWORDS: Date palm; Aqueous extract; Doxorubicin; Hepatotoxicity; Rat; Liver enzymes; Reactive oxygen species; Lipid peroxidation; DNA damage; GC-MS; Molecular docking

1. Introduction

The liver is essential for the metabolism of endogenous substrates and xenobiotics. It carries out basic biochemical processes involved in maintenance of homeostasis. Since the main function of the liver is to eliminate toxins through metabolism, impairment of liver function results in deleterious and pathological changes[1]. Drugs and toxins are among the leading etiopathogenetic

Significance

Date fruits are of high nutritional and therapeutic value and have a diversity of antioxidant, antimicrobial, and anti-proliferative properties. In this study, the biochemical, antioxidant, electrophoretic, histopathological, and *in silico* studies revealed that aqueous extract of date palm fruit can protect against doxorubicin-induced hepatotoxicity.

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mediators associated with the onset of acute hepatic malfunction. Acute hepatic damage due to toxic agents is characterized by hepatocellular membrane injury, oxidative damage, aggravated necrosis of hepatocytes, parenchymal cell infiltration by neutrophils, and enhancement of liver stellate cells, with subsequent progressive inflammation leading to liver damage[2].

Doxorubicin (DOX) is an effective anthracycline glycoside antibiotic with broad-spectrum antitumor activity against different solid tumors. Indeed, studies have demonstrated strong antitumor impact of DOX on cervical, ovarian, uterine, lung, breast, and soft tissue cancers, as well as primary bone sarcomas, Hodgkin's disease, and leukemias[3,4]. Although the mechanism underlying the antitumor effect of DOX is unclear, it is thought that reactive oxygen species might be involved in the process[5]. It is assumed that the most important factor involved in cytotoxic effect of DOX might be oxidative damage to cell membrane lipids and cellular constituents[6]. DOX injection results in generation of hydrogen peroxide, hydroxyl radicals, and superoxide anions. DOX is metabolized to a semiquinone free radical through the action of NADPH-cytochrome P-450, leading to the production of hydroxyl radicals and superoxide anion which trigger lipid peroxidation[7].

Date palm (*Phoenix dactylifera* L.) is a perennial, monocotyledonous and dioecious fruit that belongs to Arecaceae family with 14 species grown in the tropical areas of Southern Asia or Eastern and Northern Africa[8]. Owing to its tremendous nutritional value, excellent yields, and long lifespan, the date palm has been described as the "tree life"[9]. It contains at least 6 vitamins and other functional constituents such as polyphenols, flavonoids, phytomelatonin, and phytoestrogens[10,11]. These compounds exhibit several significant pharmacological properties such as anticancer, antioxidant, anti-inflammatory, antidiabetogenic, antiestrogenic, antiviral, antihypertensive, and anxiolytic potential[12]. The importance of antioxidants is based on their capacity for efficiently scavenging free radicals which cause a variety of diseases[13]. Despite possessing diverse medicinal properties, their pharmacological evidence is largely ambiguous. This study aimed to investigate the possible prophylactic effect of aqueous extract of date palm fruit (DPFAE) against DOX-induced hepatotoxicity in Wistar albino rats using biochemical, histopathological and *in silico* approaches.

2. Materials and methods

2.1. Reagents and drugs

DOX was bought from EBEWE Pharma Ges.m.b.H. Nfg.KG, A-4866 Unterach, Austria. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin were assayed using appropriate kits (EGY-CHEM for Lab Technology, Bader City, Egypt). Hematoxylin and eosin solution and Formaldehyde were purchased from Sigma-Aldrich (St. Louis, USA).

2.2. Preparation of DPFAE

Date palm fruits (DPF) were bought from a date market in Jeddah, KSA, and were identified by a taxonomist in the Faculty of Science, KAU (Specimen voucher number: *Phoenix dactylifera* L. #PD17569). Then, 10 g of the DPF was soaked overnight in 100 mL of distilled water and stirred in a mixer at (25±4)℃. The resultant aqueous extract was filtered and centrifuged at 13 000 *g* at 4℃ for 5 min. The supernatant obtained was preserved at 4℃ until use. The DPFAE was prepared freshly on daily basis just before oral administration to rats *via* intragastric gavage during the experiment as described by Vayalil[14].

2.3. Chromatographic analysis of DPF using gas chromatography-mass spectrophotometry (GC-MS)

Chromatographic analysis (GC-MS) of DPF using bis(trimethylsily1) acetamide silylation reaction was performed as described in a previous study[15]. GC-MS (Agilent Technologies 7890B GC Systems combined with 5977A Mass Selective Detector) was used to determine volatile products. GC-MS analyses were carried out on a column HP-5MS Capillary; 30.0 m × 0.25 mm ID × 0.25 μm film. The carrier gas was helium at a rate of flow of 1.7 mL/min with 1 μL injection. The sample was analyzed with the column held initially for 4 min at 40℃ after injection, then the temperature was increased to 300℃ with a 20℃/min heating ramp, with a 3.0 min hold. Injection was carried out in split-less mode at 300℃. MS scan range was (*m/z*): 50-550 atomic mass units under electron impact ionization (70 eV).

2.4. Experimental design

Male Wistar albino rats weighing 150-200 g (two months old) were purchased from King Fahd Medical Research Center of King Abdulaziz University, KSA. The rats were kept in an animal house at (25±1)℃ in an environment with a 12 h light/12 h dark cycle and were permitted free access to a standard pellet diet and tap water. Before treatment, the rats were acclimatized to the laboratory environment for 7 d.

Sixty healthy male Wistar albino rats (150-200 g) aged 6-8 weeks, were obtained from the King Fahd Medical Research Centre of King Abdulaziz University, KSA. The rats were randomly and equally divided into 6 groups, with 10 rats in each group. Group 1 served as a control group. Group 2 and 3 received daily oral prophylactic dose of DPFAE *via* intragastric gavage [2 mL of DPFAE (0.75 mg/kg bwt) and 4 mL of DPFAE (1.5 mg/kg bwt of DPFAE), respectively] for 30 d. Group 4, 5, and 6 were injected

with a single dose of DOX (15 mg/kg, *i.p.*) at the end of the 30th day of the study. The DOX dose was adopted from a previous study[16]. Rats in group 5 and 6 were given a daily oral protective dose of DPFAE [2 mL of DPFAE (0.75 mg/kg bwt) and 4 mL of DPFAE (1.5 mg/kg bwt), respectively] for 30 d.

2.5. Sample collection

The rats were fasted for 12 h before sacrifice *via* decapitation, followed by sample collection. At 24 h post-DOX injection, the rats were anesthetized with pentobarbitone sodium (60 mg/kg bwt) and blood sample from each rat was withdrawn from the optic vein into a centrifuge tube and kept at 25℃ for 15 min. Serum samples were obtained by centrifuging the blood samples in a cooled centrifuge at 3 000 rpm for 15 min. The serum samples were subjected to assays of ALT, AST, γ-GT, ALP using the optimized standard kinetic method. Total bilirubin, total protein, and albumin were estimated with a direct colorimetric method. Then, the abdomen of each rat was cut open, and the liver was removed and divided into three sections. One section was rinsed immediately in 10% buffered neutral formaldehyde solution and subjected to histological processing for histopathological examination. The second liver section was utilized for DNA extraction, while the third section was used for preparation of liver tissue homogenate.

2.6. Preparation of liver tissue homogenate

The liver tissue homogenate was prepared from freshly-excised liver tissue in line with method described in a previous study[17].

2.7. Oxidative and antioxidative markers

The activities of hepatic superoxide dismutase (SOD) and glutathione peroxidase (GPx) were assayed according to the method of Mubarak *et al*[18]. Glutathione-S-transferase (GST), glutathione reductase (GR), and catalase (CAT) were assayed according to the method of Khan and Sultana[19]. Malonaldehyde (MDA) was estimated in the liver tissue homogenates according to the modified method by Mubarak *et al*[18].

2.8. Extraction of rat liver genomic DNA, and gel electrophoresis

Rat hepatic tissue DNA extraction was performed using QIAGEN, DNeasy, RNeasy, QIAGEN Group. Molecular biology-grade agarose gel (2% agarose gel in 1× TAE buffer) was prepared as indicated by Kumar *et al*[20]. Gel electrophoresis of the DNA extract was conducted at a constant potential difference of 100 V for up to 1 h. The DNA fragments were visualized using UVI Tech. photo-documentation system.

2.9. Pathological study

2.9.1. Tissue preparation procedure

Histopathological evaluation of hepatic tissue sections was performed on all rats (the control and tested groups). Rats that died before the end of the study were excluded. After sacrifice, all excised tissue samples were initially fixed with 10% neutral buffered formalin solution, followed by routine processing, paraffin-embedding, sectioning (4 μm thickness), and staining with hematoxylin and eosin (H&E).

2.9.2. H&E staining procedure

Sections were heated in a 60℃ oven for 1 h before staining to allow for fixation of tissue on the slide. Then, the slides were deparaffinized in xylene (2 changes, each for 10 min). The slides were rehydrated by placing them in decreasing concentrations of alcohol (absolute ethanol for 5 min, 90% ethanol for 5 min, and 70% ethanol for 5 min). Then, the slides were rinsed in distilled water for 2 min. Staining with hematoxylin was done for 2 min, followed by washing in running tap water until the sections turned blue. This was followed by staining with eosin for 1 min, after which the slides were dipped once in 90% ethanol, and then transferred to absolute alcohol (2 changes, each for 2 min). Finally, the sections were cleared in 2 changes of xylene (5 min for each), mounted using Canada balsam, and covered with clean glass slide covers.

2.9.3. Procedure for histopathology evaluation

A pathologist examined each H&E-stained hepatic tissue slide under a light microscope (Olympus CX21FS1) at low and high power magnifications (×200 & ×400). All morphological changes were recorded and compared amongst various groups. Group 2, 3, 4, 5, and 6 were compared with the control group (Group 1), and any observed alterations were reported in descriptive terms and photographed.

2.10. In silico analysis

2.10.1. Preparation of proteins and molecular docking

The crystal structures of three proteins [human pregnane X receptor protein (PDB ID:1ilg), oxygenase-1 (PDB ID:1n3u), and CYP2C9 (PDB ID:4nz2)][21,22] related to liver health were obtained from RCSB PDB database[23]. The crystal structures of the proteins were prepared for docking *via* protonation, removal of water molecules and protein inhibitors, atom fixation, refinement of RMSD gradients, and energy minimization using Molecular Operating Environment suit (MOE, Demo version 2019; Chemical Computing Group Inc; Montreal, QC, Canada). Docking was performed using MOE, with the proteins in a rigid setting, and

ligands in a flexible setting. Active sites were assigned by MOE active site finder in line with previously predicted active sites[21]. Discovery Studio Visualiser 2020[24] was used for visualization of ligand-protein interactions. The structures of bioactive compounds of date fruit analyzed by GC-MS were obtained from NCBI PubChem database and NIST Chemistry WebBook.

2.10.2. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling

ADMET properties of the top 4 compounds of date fruit analyzed by GC-MS were selected for *in silico* prediction using PreADMET profiling, and their absorption rate, plasma clearance, tissue distribution, metabolic effects, and toxicity profiles were estimated as outlined earlier[25].

2.11. Statistical analysis

Quantitative data are expressed as mean±standard deviation (mean±SD). One-way analysis of variance (ANOVA) was used for compsrion amongst groups. All analyses were carried out using SPSS version 21 for Windows (SPSS Inc., Chicago, USA). For all analyses, differences were considered statistically significant at $P<0.05$.

2.12. Ethical statement

Ethical approval for this research was obtained from the Research Ethics Committee of Faculty of Medicine, King Abdulaziz University (Reference No 442-16). The rats received animal care according to the guidelines of the Committee for the Prupose of Control and Supervision of Experiments on Animals, KSA.

3. Results

3.1. GC–MS analysis of DPF

By matching the mass spectra of the bioactive components of DPF with the NIST library, 18 major compound peaks were obtained out of 38 phyto-constituents of DPF date extract, as shown in Figure 1 and Supplementary Table 1. These compounds were glycerol, *L*-threitol (4TMS derivative), *L*-(+)-threose, 2-tris (trimethylsilyl) ether, trimethylsilyloxime, *D*-(+)-arabitol, 2-pentenedioic acid, 2-[(trimethylsilyl)oxy] bis(trimethylsilyl) ester, *D*-(-)-tagatofuranose, pentakis(trimethylsilyl) ether (isomer 1), *D*-pinitol, pentakis (trimethylsilyl) ether, *D*-sorbitol (6TMS derivative), *D*-(+)-galactose, pentakis (trimethylsilyl) ether, pentafluorobenzyloxime (isomer 1), *D*-mannitol (6TMS derivative), *D*-glucopyranose (5TMS derivative), *L*-(+)-tartaric acid (4TMS derivative), palmitic acid (TMS derivative), 3-heptadecen-5-yne, (Z), stearic acid, 9-octadecenoic acid (E-TMS derivative), fumaric acid [di (2-propylphenyl)] ester, and α linoleic acid.

3.2. Serum levels of liver enzymes

Rats treated with DOX on the 30th day exhibited significant elevations in serum activities of ALT, AST, γ-GT, ALP, and total bilirubin, accompanied by significant depletion in serum albumin and total protein, when compared to the control group ($P<0.01$). These results are shown in Table 1. However, there were no significant changes in the serum levels of the liver parameters in rats given DPFAE at 0.75 and 1.5 mg/kg bwt alone, when compared to those of the normal control group ($P>0.05$). Treatment with DPFAE at 0.75 and 1.5 mg/kg bwt significantly decreased serum levels of ALT, AST, γ-GT, ALP, and total bilirubin, and significantly increased serum albumin and total protein levels when compared to the DOX-treated rats ($P<0.01$; Table 1). There were no significant changes in serum levels of these liver parameters between rats treated with DPFAE and the control group.

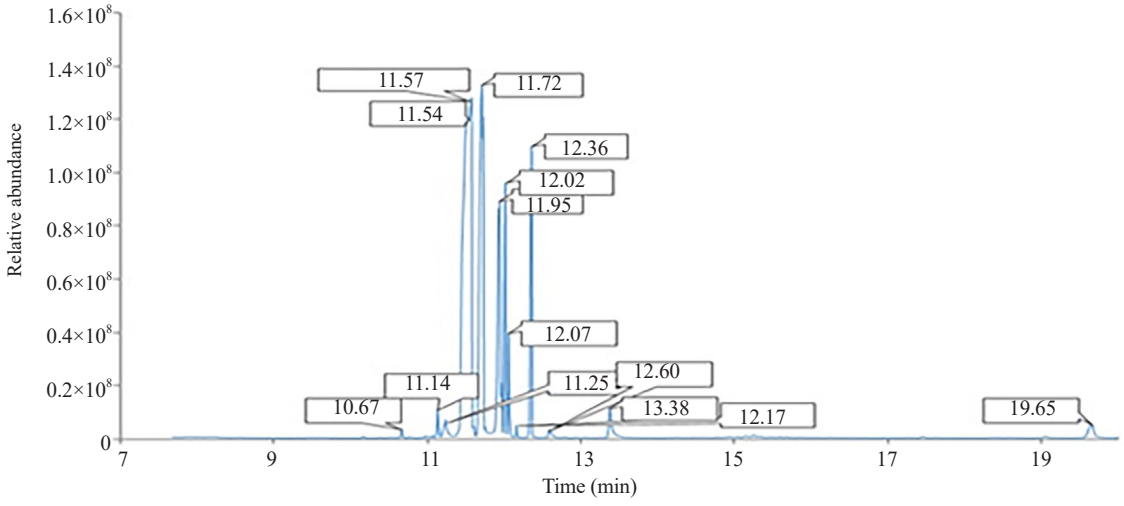

Figure 1. Gas chromatography-mass spectrometric analysis of active ingredients of date palm fruits (DPF).

Table 1. Serum levels of liver enzymes.

Items	Control	DPFAE (0.75 mg/kg bwt)	DPFAE (1.5 mg/kg bwt)	DOX	DOX+DPFAE (0.75 mg/kg bwt)	DOX+DPFAE (1.5 mg/kg bwt)
ALT (U/L)	37.78±4.81	34.64±2.82	36.40±3.14	57.47±7.12*	38.90±4.23$^{\#}$	38.08±2.46$^{\#}$
AST (U/L)	88.54±11.42	86.88±10.69	86.36±8.35	150.43±9.06*	99.24±4.79$^{\#}$	95.10±6.04$^{\#}$
γ-GT (U/L)	10.40±0.71	10.60±0.73	10.22±0.56	15.46±0.84*	11.08±0.48$^{\#}$	10.46±0.93$^{\#}$
ALP (U/L)	104.38±7.23	105.98±6.02	106.96±8.10	196.98±14.20*	109.44±7.48$^{\#}$	106.06±11.52$^{\#}$
TB (μM)	0.78±0.13	0.76±0.11	0.74±0.13	1.51±0.10*	0.85±0.11$^{\#}$	0.80±0.13$^{\#}$
TP (g/dL)	6.27±0.45	6.37±0.36	6.41±0.32	5.05±0.46*	6.19±0.42$^{\#}$	6.27±0.38$^{\#\#}$
ALB (g/dL)	3.80±0.18	3.46±0.20	3.81±0.24	2.92±0.27*	3.66±0.27$^{\#}$	3.78±0.25$^{\#}$

Data are expressed as mean ± SD. Values were statistically tested using ANOVA. $^{*}P$<0.01, compared to the normal control; $^{\#}P$<0.05 and $^{\#\#}P$<0.01, compared to the DOX-treated group. DPFAE: aqueous extract of date palm fruit, DOX: doxorubicin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, γ-GT gamma-glutamyl transpeptidase, ALP: alkaline phosphatase, TB: total bilirubin, TP: total protein, ALB: albumin.

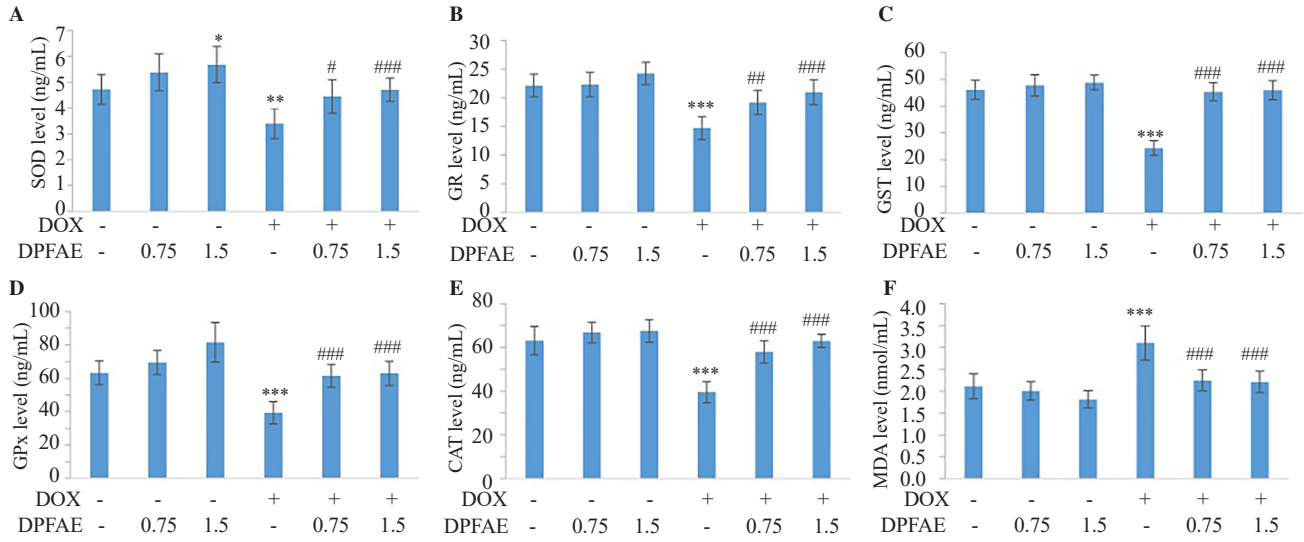

Figure 2. Levels of superoxide dismutase (SOD), glutathione reductase (GR), glutathione-S-transferase (GST), glutathione peroxidase (GPx), catalase (CAT), and malonaldehyde (MDA). All data were expressed as mean ± SD. Values were analyzed by using ANOVA. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 compared to the normal control; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 compared to the DOX group.

3.3. Enzymatic antioxidants and lipid peroxidation markers

Rats treated with DOX on day 30 showed significant decreases in SOD, GR, GST, GPx, and CAT, and significant increases in MDA levels in hepatic tissue homogenate when compared to the control rats (P<0.01; Figure 2). Treatment with DPFAE at 0.75 and 1.5 mg/kg bwt significantly increased serum levels of SOD, GR, GST, GPx, and CAT and significantly reduced MDA levels in the hepatic tissue homogenates when compared to the DOX-treated rats (Figure 2). However, as shown in Figure 2, there were no significant changes between DPFAE-treated rats and the normal control group (P>0.05).

3.4. Electrophoretic evidence of DNA fragmentation

Electrophoretic images of DNA bands are shown in Figure 3. Rats treated with DOX had a markedly higher degree of hepatic DNA damage than the untreated group. Rats treated with DPFAE at 0.75 and 1.5 mg/kg bwt in addition to DOX injection exhibited marked improvement in liver DNA. Interestingly, there was no evidence of DNA fragmentation in rats prophylactically given DPFAE either at 0.75 or 1.5 mg/kg bwt.

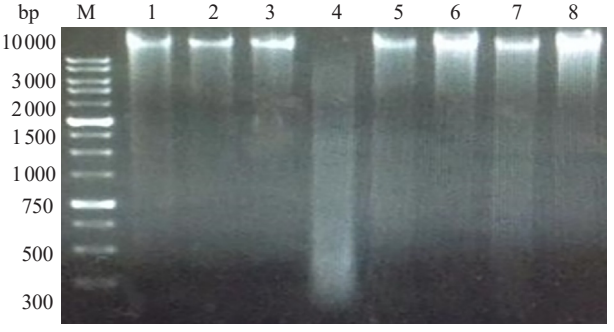

Figure 3. DNA fragmentation. Lane M: a DNA marker with 10000 bp. Lane 1: normal group. Lane 2: DPFAE group (0.75 mg/kg bwt), Lane 3: DPFAE group (1.5 mg/kg bwt). Lanes 4: DOX-treated group. Lanes 5 & 6 DPFAE 0.75 mg/kg bwt + DOX. Lanes 7 & 8 DPFAE 1.5 mg/kg bwt + DOX.

3.5. Histopathological changes

Liver tissue sections from the group 1 rats showed no remarkable lesions and were used as a standard for comparison. Although hepatic sections from the group 2 and group 3 had clear evidence of hypertrophy, there were no other abnormalities in these groups (Figure 4A). In sections from the group 4, there were distinctive features of venous and sinusoidal congestion, with the latter

sometimes spotted at low power as a tigroid pattern depicting hepatocytes with more basophilic cytoplasm and a rather tortuous cord pattern of arrangement (Figure 4B). Various degrees of hepatocyte injury was seen in this group, mostly in the form of reversible degenerative changes manifested as centrilobular ballooning (Figure 4C). Furthermore, foci of centrilobular spotty necrosis were common.

However, in the group 5 and group 6, there was no evidence of severe lesions like those present in the group 4, except for occasional foci of spotty necrosis (in the group 5), while mild congestion and hepatocyte lesions (merely in the form of hypertrophic changes) were the predominant lesions in the group 5 and the only changes in the group 6 (Figures 4D and 4E).

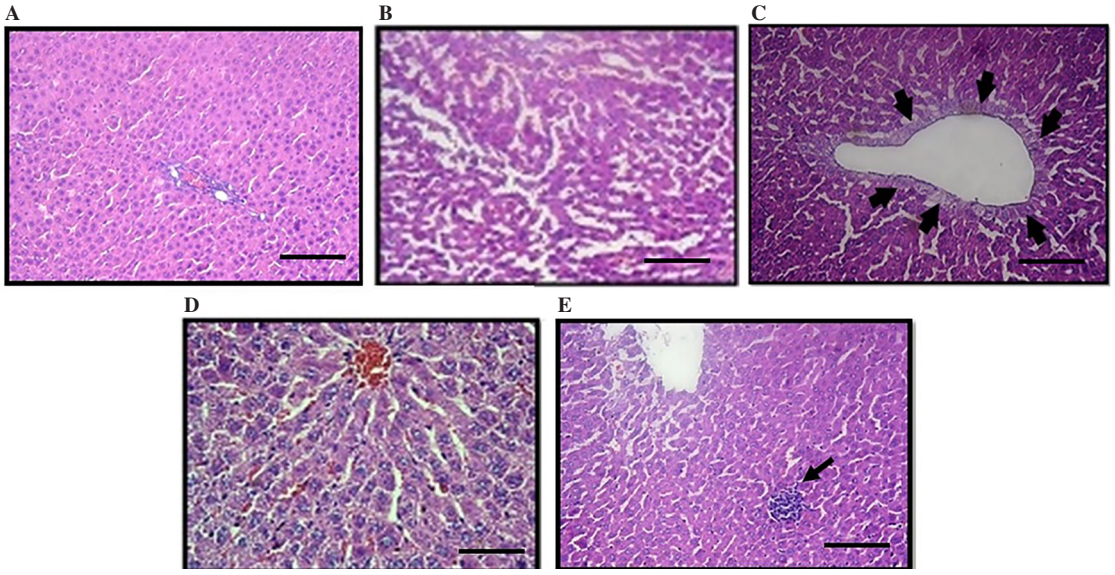

Figure 4. Histological changes in liver tissues in different study groups. The DPFAE prophylactic groups (group 2) showed normal hepatocytic structure with mildly congested portal vein as in A (scale bar = 50 μm, H&E ×200). The DOX-treated group showed marked sinusoidal dilation with the tigroid pattern and hepatocytic degenerative changes as in B (scale bar = 50 μm, H&E ×200) and markedly dilated central vein with a rim of hepatocytes exhibiting ballooning degeneration as in C, note the black arrows (scale bar = 20 μm, H&E ×400). The DPFAE (0.75 mg/kg bwt) protected group showed mildly congested central vein and blood sinusoids with apparently normal hepatocytes as in D (scale bar = 20 μm, H&E ×400) and normal hepatocytes with an area of focal spotty necrosis in group 5 as in E, note the black arrow (scale bar = 50 μm, H&E ×200).

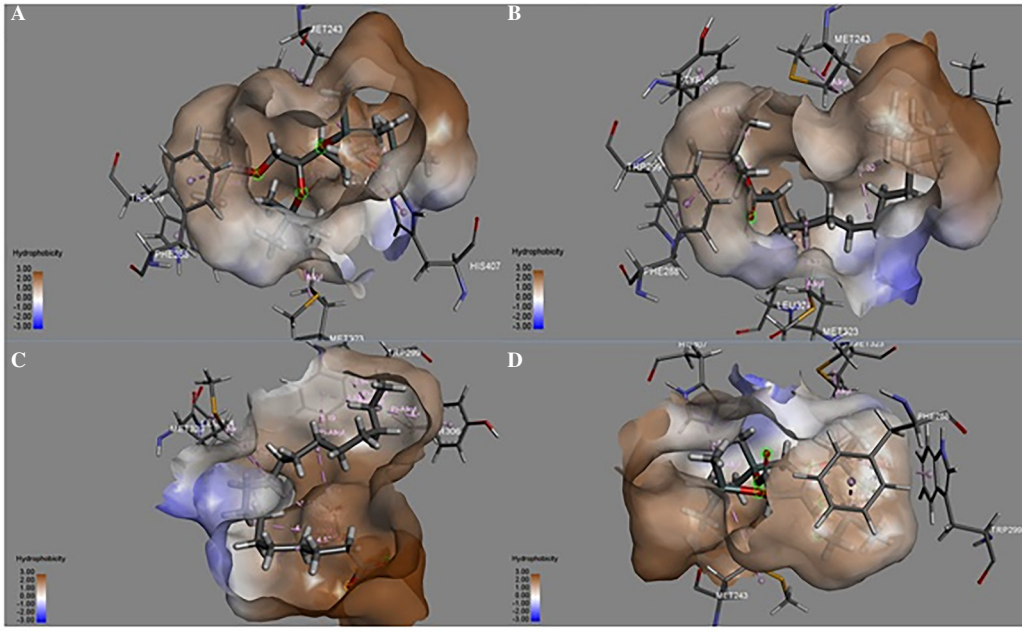

Figure 5. Interaction of human pregnane X receptor protein (1ilg) and selected DPF compounds, the hydrophobic residues of protein active site are shown in brown color. A: *L*-(+)-Threose, tris(trimethylsilyl) ether, trimethylsilyloxime. B: Palmitic acid, TMS derivative. C: Stearic acid. D: *L*-(+)-Tartaric acid, 4TMS derivative.

3.6. Results of in silico analysis

3.6.1. Molecular docking

The bioactive compounds from DPF displayed varying degrees of binding affinity with different selected proteins. Four compounds [*L*-(+)-threose, tris(trimethylsilyl) ether, trimethylsilyloxime, *L*-(+)-tartaric acid (4TMS derivative), palmitic acid (TMS derivative), and stearic acid] produced the highest inhibitory effects on human pregnane X receptor protein (Figure 5), oxygenase-1 (Figure 6), and CYP2C9 (Figure 7), with binding energies higher than −7 kcal/mol as shown in Supplementary Table 2. Most of the other compounds identified through GC-MS showed good interactions with human pregnane X receptor protein (PDB ID:1ilg), as shown in Figure 5.

3.6.2. PreADMET profiling

The pharmacokinetic properties of the ADMET of the best four compounds were predicted using PreADMET profiling. The four compounds showed good (more than 98%) human intestinal absorption, and varying degrees of inhibition of cytochrome P450 (CYPs), as shown in Supplementary Table 3.

4. Discussion

DOX is an effective drug for a wide variety of tumors. A frequent side effect of this drug is hepatotoxicity, which is especially important in the presence of earlier defects in liver functions.

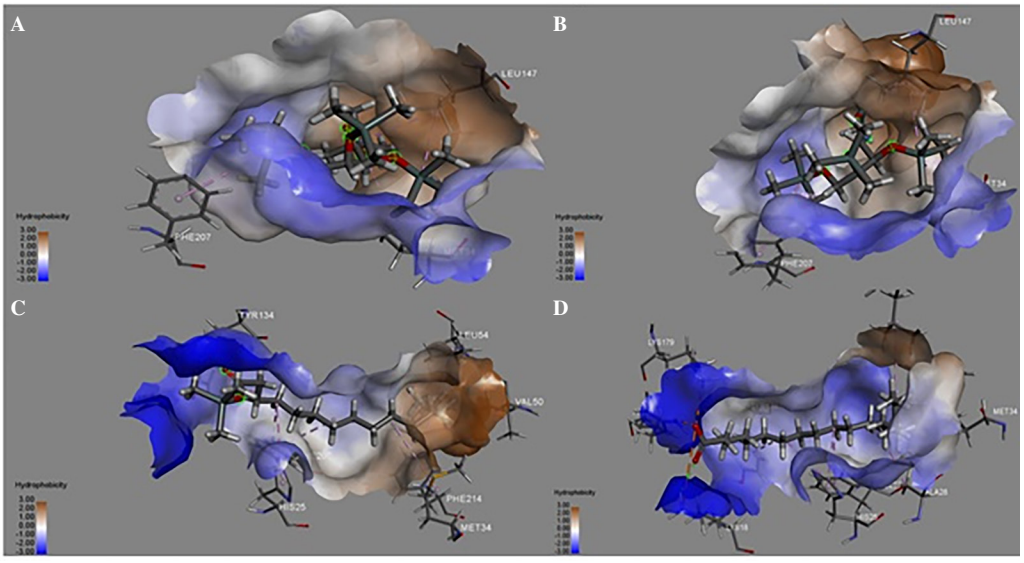

Figure 6. Interaction of oxygenase-1 (1n3u) protein and selected DPF compounds, the hydrophobic residues of protein active site are shown in brown color. A: *L*-(+)-Threose, tris(trimethylsilyl) ether, trimethylsilyloxime. B: Palmitic acid, TMS derivative. C: Stearic acid. D: *L*-(+)-Tartaric acid, 4TMS derivative.

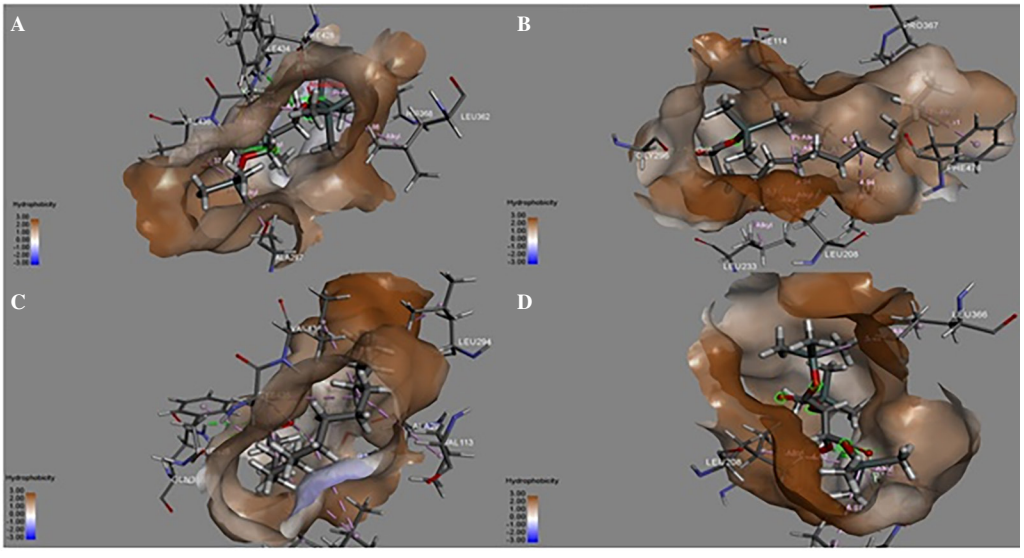

Figure 7. Interaction of CYP2C9 protein (4nz2) and selected DPF compounds, the hydrophobic residues of protein active site are shown in brown color. A: *L*-(+)-Threose, tris(trimethylsilyl) ether, trimethylsilyloxime. B: Palmitic acid, TMS derivative. C: Stearic acid. D: *L*-(+)-Tartaric acid, 4TMS derivative.

Primarily, the study aimed to investigate prophylactic effect of DPFAE against DOX-induced hepatotoxicity based on the biochemical, histopathological, and *in silico* analysis.

The results of GC-MS analysis of date palm revealed the presence of several saturated and unsaturated fatty acids such as stearic acid, palmitic acid, 9-octadecenoic acid, fumaric acid, and α-linolenic acid. The 9-octadecenoic acid, the common name for oleic acid has been classified as an anti-inflammatory agent due to its inhibitory effect on pro-inflammatory signaling and reduction of expressions of cytokines and pro-inflammatory mediators[26]. The presence of α-linolenic acid in date palm mitigates oxidative stress-induced hepatic lesions injury, hepatic steatosis and non-alcoholic liver disease[27,28]. Stearic acid plays a crucial role in protection of cortical neurons against oxidative damage through activation of an endogenous antioxidant system[29].

In addition, the therapeutic potential of date palm is due to its polyphenolic contents[30]. Polyphenols possess a variety of properties: induction of apoptosis, inhibition of oxidation, modulation of CYP enzymes involved in stimulation of pro-carcinogens, upregulation of genes that encode antioxidant enzymes, and potential to alter cellular signaling[31]. The probable mechanism by which date palm reversed DOX-induced hepatotoxicity might be due to its antioxidant properties owing to its polyphenol content[32]. Polyphenols neutralize free radicals *via* hydrogen donation, thereby suppressing their injurious reactions.

The results of the current study showed that DOX increased serum levels of ALT, AST ALP, γ-GT, and total bilirubin, and significantly decreased serum albumin and total protein, which are consistent with a previous study[33]. This reflects lipid peroxidation-mediated damage to hepatocyte membranes and the degree of hepatocellular lesions. Elevation in serum transaminases is the strongest sign of cellular leakage and loss of functional integrity of the hepatocyte membrane[34,35]. The reduction in the serum albumin and total protein could be the result of impaired biological pathways, or it might be attributed to changes in cell membrane permeability in kidney and liver tissues, leading to loss of proteins, particularly albumin through the kidney[36,37].

The precise mechanism underlying DOX-induced hepatotoxicity is not entirely well understood. Most researches support the involvement of a free radical-promoted oxidative stress mechanism. This is based on the chemical structure of DOX which has an affinity to produce peroxynitrite radicals and superoxide anions when the drug is metabolized in the liver[38].

In the present study, DOX-induced hepatotoxicity generated oxidative stress as characterized by decreased SOD, GR, GST, GPx, and CAT and significant increases in MDA in rats, which were mitigated by DPFAE. Thus, depression of oxidative stress could serve as an effective strategy for date palm against DOX-induced hepatotoxicity. SOD utilizes the oxidants as substrates and catalyzes reduction of superoxide radical to H_2O_2. The DOX-induced decrease in SOD activity in the hepatic tissue of rats could be due to the over-generation of free radicals. SOD is a unique self-defense tool against oxidative stress. It constitutes the initial line of defense against the toxic effects of ROS[39]. CAT is considered an indicator of elevated H_2O_2 because it decomposes H_2O_2 which generates hydroxyl radicals, and it is an essential member of the antioxidant defense mechanisms of mammalian cells. CAT converts H_2O_2 to singlet oxygen and water. In the present study, there were significant decreases in the CAT activity in the hepatic tissue of DOX-treated rats. Then serum levels of liver function markers, anti-oxidative markers, and MDA in DOX-induced rats were restored when treated with DPFAE at 0.75 and 1.5 mg/kg bwt. This finding indicates that these two doses of DPFAE were effective in protecting liver tissues from the hazardous effects of DOX.

The significant damage in hepatic DNA from DOX-treated rats could be due to hepatocyte apoptosis. This finding is in agreement with that of Barakat *et al.* who reported marked DNA degradation in DOX-treated rats, relative to the normal control group[40]. The well-established lipophilic character of DOX and its ability to bind to DNA macromolecules are likely to result in high DOX concentrations in hepatic nucleus, thereby resulting in DNA damage[41]. The DOX-induced hepatotoxicity was markedly attenuated *via* treatment with DPFAE.

Histological changes observed in hepatic sections of the DOX-treated groups were mild, but there was less degree of cell injury among the DPFAE-treated rats when compared with the DOX-injected group. The DPFAE-treated groups, whether injected with DOX or not, had hypertrophic changes in hepatocytes. This form of adaptation could be attributed to metabolic enzyme induction that led to increases in endoplasmic reticulum, peroxisomes, and mitochondria[42]. One possible explanation for these changes in hepatocytes is enhanced induction of antioxidant enzymes which was also recorded at the serological level in this study. These adaptive responses enabled hepatocytes in the DPFAE-treated groups to survive under DOX-induced stress and evade cell injury altogether. On the other hand, changes in the group 4 rats injected with DOX without prior exposure to DPFAE resulted in a variety of hepatocyte stress patterns, including a tigroid pattern of hepatocytes. These changes (also referred to as basophilic alteration) could be associated with peliosis which reflects proliferation of a phenotypically and enzymatically-altered group of hepatocytes. Moreover, rats in the group 4 manifested reversible cell injury in the form of centrilobular ballooning. Both tigroid pattern and centrilobular ballooning may likely represent different modes of reversible cell injury in response to oxidative stress induced by DOX. The oxidative stress resulting from lipid peroxidation contributed to cell membrane dysfunction, with subsequent increases in intracellular fluid which led to ballooning degeneration[42].

DPFAE also obviously reduced hepatocytic injury, qualitatively in the form of absence of tigroid hepatocytic alteration and ballooning, and quantitatively in the form of reduction in spotty necrosis. The high level of spotty necrosis observed in the group 4 was only focally encountered in the group 5. This finding indicates the presence of mononuclear inflammatory cells and

acute or ongoing lobular injury that may be originated from DOX intoxication[43].

The demonstrable histopathologic changes reflecting the hepatotoxicity of DOX were all early indications of cell injury which have been reversed towards normalization by DPFAE. It is important to acknowledge that all the changes discussed hitherto were very meticulous observations. Despite their subtleness, it is expected that these findings provide salient insights into the mechanism underlying the hepatotoxic effect of DOX and the opposing protective effect of DPFAE. These observations indicate the need for further electron microscopic studies since electron microscopic examination would more overtly highlight ultrastructure changes that conventional light microscopic examination might not have detected.

The preADMET profiling showed that the four investigated molecules had high intestinal absorption values higher than 98%. The predicted interaction with cytochrome P450 (CYP) protein revealed that all four compounds had inhibitory effects on CYP 2C19, CYP 2C9, and CYP 3A4. These interactions could result in decreases in the ability of these enzymes to metabolize other drugs in the body, leading to accumulation of their metabolites and enhanced pharmacological effects[44]. None of the four molecules inhibited the CYP AD6 protein.

In conclusion, the current study established that date palm (0.75 and 1.5 mg/kg bwt) exerts comparable hepato-protection in a rat model of DOX-induced hepatotoxicity. It could be assumed that this result does not precisely conclude that date palm fruit is an effective hepatoprotective agent against DOX intoxication. To determine the value of date palm as a therapeutic agent, more exploratory and confirmatory studies on chemo-sensitizing potential of date palm as well as investigations of detailed molecular mechanisms are required. Such investigations could be expanded to discover whether date palm offers tissue-specific protection against other types of chemotherapeutic agents.

Conflict of interest statement

The authors declare no conflict of interest.

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Authors' contributions

AMF bought DPF sample, prepared the DPFAE and conducted the experiments; MKA and OASB guided all experiments, interpreted data, performed the statistical analysis and completed the manuscript; the veterinarian HAA supervised the animal care, feeding, DPFAE administration, DOX injection and rats' dissection along with organ extraction; LSS conducted the histopathology and interpreted data. MZ conducted the GC-MS analysis of date palm and participated in writing results and discussion. SBH performed the molecular studies including the DNA extraction and gel electrophoresis. HNA performed the *in silico* studies and participated in writing results and discussion. All authors read and approved the final manuscript.

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