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Phoenix dactylifera seeds extract alleviates doxorubicin-induced cardiotoxicity in male rats

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ABSTRACT

Background: *Phonix dactylifera* have been bio-applied for management of diseases. Exploring new applications for date seed by-products can benefit the date producing countries. Doxorubicin (DOX) causes several adverse effects including cardiotoxicity **Aim:** This study aims to investigate the impact of *P. dactylifera* seeds extract (PDSE) on cardiotoxicity. **Methods:** Thirty two male rats were equally divided into: Gp1 was the control; Gp2 was i.p. injected with PDSE (300 mg/kg), Gp3 was i.p injected with DOX (4 mg/kg). Gp4 was injected with DOX as in Gp3, and then administered with PDSE as in Gp2. Biochemical and molecular investigations were evaluated. **Results:** Treatment with PDSE led to improvement in the cardiotoxicity induced by DOX in male rats that evidenced by significant improvement in the biochemical parameters including cardiac functions, oxidative stress biomarkers, and gene expression of TGF-β/Smad-7 genes in the heart tissues. **Conclusion:** *Phonix dactylifera* seeds extract showed potent ameliorative impact against the cardiotoxicity adverse effect by improving cardiac function biomarkers, oxidative stress, and involved TGF-β/Smad-7 pathway.

1. Introduction:

Chemotherapeutic drugs are widely used for cancer treatment. In spite of their efficacy, the adverse effects on the vital organs are associated **(1)**. Finding new avenues to decrease the adverse effects of chemotherapy is necessary **(2)**. Chemotherapy causes several side effects due to promotion of oxidative stress, which consequently led to cardiotoxicity **(3)**. Doxorubicin (DOX) is an antineoplastic agent for different malignancies. However, its uses are restricted due to cardiotoxicities **(4)**. It has been reported that DOX engender mitochondria-dependent apoptotic pathway in cardiomyocytes **(5)**.

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Phoenix dactylifera demonstrated several biomedical applications; it has potential to be used in pharmaceutical industries **(6)**. Date seeds are usually discarded after consuming date fruits, which are generated in large amount as waste products, these by-products could represent sources for phytomedicine **(7)**. Previous studies have reported that the *P. dactylifera* exhibits immune-stimulant, antioxidant, antidiabetic, and anticancer activities **(8)**. Furthermore, it has been reported that *Phoenix dactylifera* seeds extract (PDSE) relive hepatic toxicity mediated by carbon tetrachloride via inhibiting oxidative stress **(9)**. This study investigated the impact of PDSE on cardiotoxicity in rats.

2. Material and Methods:

Chemicals:

Doxorubicin (DOX) was purchased from Al-Hekma Company, Egypt.

Preparation of *P. dactylifera* seeds extract:

P. dactylifera seeds were identified, authenticated, and complied with relevant institutional and national guidelines. Seeds were dried, mashed into powder, then 50g was mixed with 70% ethanol and the *P. dactylifera* seeds extract (PDSE) was obtained **(10)**.

Experimental design:

Thirty two male rats (120 ± 20 g) were treated according to guidelines for experimental animal's uses in research which was approved by Ethical committee at the Faculty of Science, Tanta University, with ethical approval license (IACUC-SCI-TU-0238). Rats were divided into: Gp1 was negative control, Gp2 was administered with modified dose of PDSE (300 mg/kg) i.p. daily or a month **(10),** which is 1/10 of the LD_{50}, Gp3 was injected with DOX (4 mg/kg) i.p once a week for a month **(11)**. Gp4 was injected with DOX as in Gp3, and then administered with PDSE as in Gp2. Sera samples were separated for biochemical analyses. Furthermore cardiac tissues were isolated for determination of the oxidative stress biomarkers and gene expression investigations. All groups were weighted and the percentages of body weight change were determined.

Determination of biochemical parameters:

Serum creatinine kinase-MB (CK-MB), Troponin I**,** Lactate dehyrogenase (LDH)**,** and aspartate aminotransferase (AST) were determined according to the manufacturers' protocols **(12-15)**. Furthermore, cardiac catalase (CAT), superoxide dismutase (SOD) activities, reduced glutathione (GSH), and malondialdehyde (MDA) levels were assessed **(16-19)**.

Molecular analysis:

Real-time PCR was used to assess TGF-β and Smad-7 genes expression in the heart tissues following the manufacturer protocol (Thermo Scientific, Waltham, MA, USA, # K0221). The β-actin primers were TGCCTGACGGTCAGGTCA (forward) and CAGGAAGGAAGGCTGGAAG (reverse). TGF-β primers were TGCCTGACGGTCAGGTCA and CAGGAAGGAAGGCTGGAG, respectively. Smad-7 primers were CCCCATCACCTTAGTCGACTCT (forward) and GACAGTCTGCAGTTGGTTTGAGA (reverse). The isolated cDNA were amplified using Maxima SYBR Green/ROX qPCR Master Mix following the manufacturer protocol and gene specific primers. The web-based tool was used to design these primers. The sequences of the targeted primers were checked with BLAST. The quantities critical threshold (Ct) of target gene was normalized with

quantities (Ct) of housekeeping gene beta actin by used the $2^{-\Delta\Delta Ct}$ method **(20)**.

3. Results:

3.1. Effect of PDSE on the % of body weight changes in DOX-intoxicated rats:

The results reported that DOX-injected rats showed a significant decrease ($p \leq 0.05$) in the % of B.W. change that represented 19.24 % when compared to the negative control group (34.81 %). Rats that injected with DOX and treated with PDSE demonstrated significant increase ($p \leq 0.05$) in the % B.W. change (27.73 %) when compared to rats treated with DOX alone (Table 1).

3.2. Effect of PDSE treatment on serum creatine kinase and troponin-I:

By determining the cardiac function test including creatine kinase MB (CK-MB) and troponin I, results showed significant increase in the CK-MB activity and troponin I in the DOX-intoxicated group that represented 822.45 U/L and 1.15 ng/ml, respectively, when compared to the control group that represented 178.43 U/L and 0.086 ng/ml, respectively. Treatment of DOX-injected rats with PDSE led to significant decrease in the CK-MB activity and troponin I levels by 51.94% and 33.51%, respectively, when compared to the DOX-intoxicated group (Figure 1).

3.3. Effect of PDSE treatment on serum LDH and AST activities:

Group injected with DOX showed significant increase in the sera LDH and AST activities. Treatment with PDSE after DOX injection in rats led to significant decrease in the activities of LDH and AST by 59.07%, and 64.65%, respectively (Figure 2).

3.4. Effect of PDSE treatment on antioxidants/oxidants hemostasis

Rats that injected with DOX showed significant decrease in SOD, CAT, and GSH levels accompanied with significant increase in the MDA levels. Rats that injected with DOX and treated with PDSE showed significant improvement in oxidative stress biomarkers by increasing SOD, CAT, and GSH levels up to 22.8 ± 2.3 U/g, 7.6 ± 0.4 U/g, and 3.3 ± 0.2µmol/g, respectively when compared to DOX-intoxicated rats (Table 2).

3.5. Treatment with PDSE inhibit TGF-β/Smad-7 pathway:

The results show that by using β-actin housekeeping gene, there were significant up-regulation ($p < 0.05$) in TGF-β genes and significant down-regulated in Smad-7 expression level the heart tissues of DOX-injected group. However, treatment of DOX-injected rats with PDSE led to significant down-regulation ($p < 0.05$) in the mRNA expression levels of cardiac TGF-β genes and significant up-regulated in cardiac Smad-7 expression level (Figure 3).

4. Discussion

Tumor therapy by using anticancer therapeutic agents resulted in destruction of various biochemical and physiological homoeostasis. The antitumor DOX showed wide applications for treating several malignancies, however, its uses are limited by cardiotoxicities development; therefore, several researchers have been interested in the preventive impacts of natural agents against DOX-induced cardio-toxicity **(21)**. *Phoenix dactylifera* are rich in minerals, vitamins, phytochemicals, and dietary fibers, it is effective candidate to be anticancer, antidiabetes, anti-inflammatory, and for relive of cardiovascular diseases **(22, 23)**. The impacts of PDSE against cardiotoxicity by DOX in rats were evaluated.

Treatment with PDSE increased the % of b.wt change in DOX-intoxicated rats. Due to increased food intake and PDSE-enhanced intestinal mucosa, as

previously documented, groups treated with PDE saw improvements in body weight as compared to inebriated groups **(23)**. Furthermore, the current study was extended to evaluate biochemical cardiac parameters including CK-MB, troponin I, LDH, and AST levels. Our findings revealed the significant increase of these biochemical parameters in the sera of DOX-intoxicated group. Treatment with PDSE led to significant improvement in cardiac functions that evidenced by decrease in the previous parameters. These findings could be due to due to the excessive ROS generation that agreed with several studies demonstrated the ameliorating effects of natural agents against DOX cardiotoxicity **(24-26)**.

The data showed that group of rat that injected with DOX showed significant decrease of SOD, CAT, and GSH levels along with significant increase in the MDA levels. Rats that injected with DOX and treated with PDSE showed significant improvement in oxidative stress biomarkers by increasing SOD and CAT activities, decreasing MDA level. Several studies reported the negative impacts of DOX on the antioxidant status and the beneficial role of natural agents including in experimental animals **(26-28).** TGF-β1 gene downregulation leads to suppression of myocardial fibrosis and apoptosis **(29)**. The results also reported significant up-regulation in the expression levels of TGF-β genes accompanied with significant down-regulated in Smad-7 expression level the heart tissues of DOX-injected rats. PDSE administration led to significant down-regulation of cardiac TGF-β genes, significant up-regulated in cardiac Smad-7 expression level. These findings indicated that the ameliorative effect of PDSE on the cardiotoxicity involved the TGF-β1/Smad pathway, which in line with previous report of who demonstrated that desferrioxamine mitigates DOX-induced cardiotoxicity in rat by inhibiting TFG-β/Smad pathway **(30)**.

5. Conclusion

In the present study, treatment with PDSE showed potential ameliorative effect versus cardiotoxicity mediated by DOX in rats by improving cardiac function biomarkers, antioxidants/oxidants status in cardiac tissues.

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Table (1): Initial, final body weight, and % of B.W. changes

Groups	I.B.W. (g)	F.B.W. (g)	% B.W. change
NC	135 ± 3.26	182 ± 2.96	34.81[a]
PDSE	130 ± 2.96	180 ± 3.69	38.46[a]
DOX	139 ± 3.29	166 ± 3.46	19.42[b]
DOX/PDSE	137 ± 3.43	175 ± 3.24	27.73[a,c]

All data were represented as mean ± S.D. **I.B.W.:** Initial body weight, **F.B.W.:** Final body weight, **B.W.:** Body weight, **NC:** Negative control; **PDSE:** *Phonix dactylifera* seeds extract; **DOX:** Doxorubicin. Groups don't share a letter are significantly different ($p \leq 0.05$).

Table (2): Cardiac antioxidants/oxidants parameters

Groups	SOD (U/ g)	CAT (U/ g)	GSH (µmol/g)	MDA (nmol/g)
NC	34.6 ± 3.7[a]	11.5 ± 0.9[a]	5.2 ± 0.3[a]	48.4 ± 3.2[a]
PDSE	37.9 ± 3.8[a]	12.7 ± 1.4[a]	5.9 ± 0.4[a]	43.7 ± 2.9[a]
DOX	8.7 ± 0.8[b]	2.3 ± 0.3[b]	1.2± 0.1[b]	186.8 ± 6.7[b]
DOX/PDSE	22.8 ± 2.3[c]	7.6 ± 0.4[c]	3.3 ± 0.2[c]	97.7 ± 6.9[c]

All data were represented as mean ± S.D. **SOD:** Superoxide dismutase; **CAT:** Catalase. **GSH:** Reduced glutathione; **MDA:** Malondialdehyde; **NC:** Negative control; **PDSE:** *Phonix dactylifera* seeds extract; **DOX:** Doxorubicin.Groups don't share a letter are significantly different ($p \leq 0.05$).

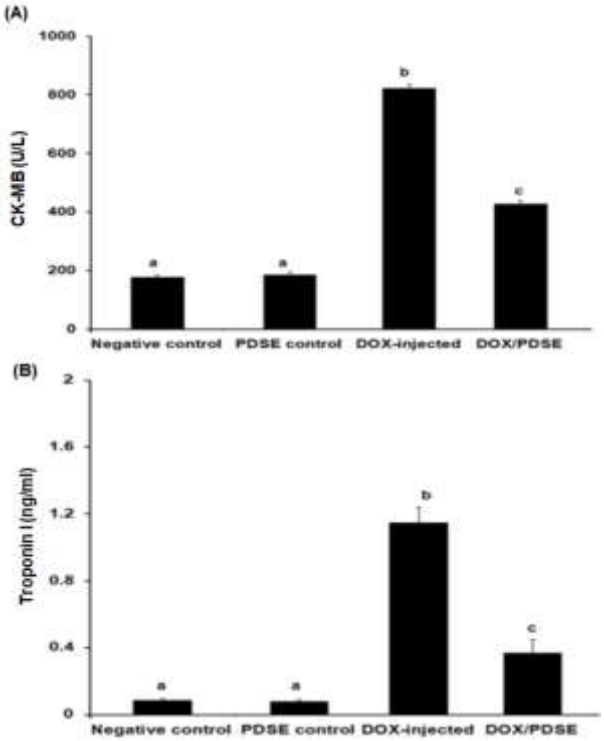

Fig. (1): Creatine kinase MB activity **(A),** and troponin-I level **(B)** in the different groups. All data were represented as mean ± S.D. Groups don't share a letter are significantly different ($p \leq 0.05$).

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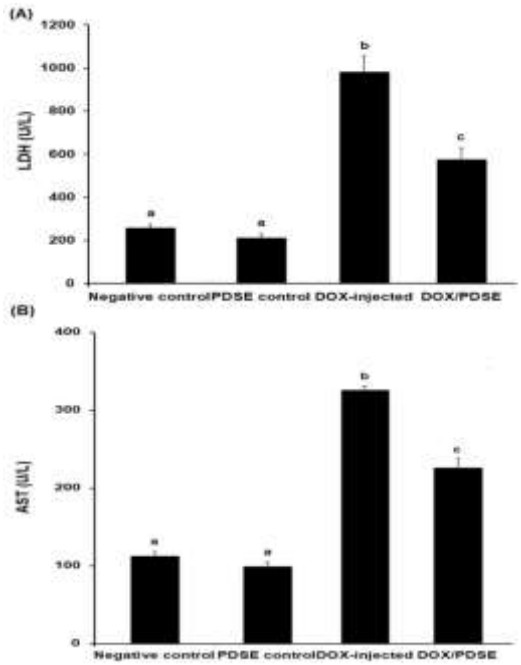

Fig. (2): Lactate dehydrogenase **(A)**, and aspartate transaminase **(B)** activities. All data were represented as mean ± S.D. Groups don't share a letter are significantly different ($p \leq 0.05$).

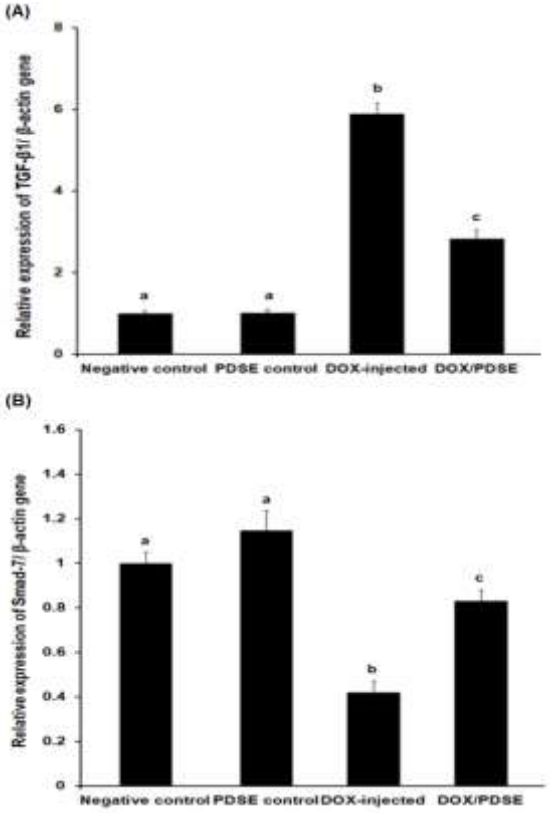

Fig. (3): Gene expression analysis of TGF-β1 **(A)**, and Smad-7 **(B)** genes by RT-PCR in the different groups under the study. The values represented as means ± S.D.; Means that do not share a letter are significantly different. $P < 0.05$ was considered to be statistically significant.