Biochemical Effect of Ethanolic Extraction of Date Palm on Doxorubicin -Induced Cardiotoxicity in Rats

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Abstract
Doxorubicin (DXR) is a highly effective drug for chemotherapy. However, cardiotoxicity reduces its clinical utility in humans. This study was undertaken to determine the protective effects of administration of Date Palm extract on serum Cardiac Troponins I (CTn1), nitric oxide (NO),creatine kinase(CK-MB), lactate dehydrogenase (LDH) in DXR-induced cardiotoxicity in rats. At the end of the study, animals were sacrificed and and the heart was analyzed histologically.The Dox group showed significantly higher levels of serum NO, LDH, and C K - M B and lower level of C T n1 compared with the control group with inflammation and necrosis in cardiac histopathology. In the DP+MTX DOXgroup, it was detected that DOXtreatment caused a significant increase the levels of serum NO and decresae levels C T n1, LDH, and C K - M B. Collectively, pre-coadministration of DP partially mitigated DOX-induced cardiac injuries via its antioxidant, anti-inflammatory, anti-fibrotic, and anti-apoptotic potential. **Conclusions:** DP exerted a significant protective effect from doxorubicin toxicity.
Keywords: Doxorubicin-induced cardiotoxicity; Nitric oxide.

1. Introduction

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year. Chemotherapy-related cardiotoxicity continues to be one of the limiting factors in the antineoplastic treatment regimens leading to a significant damage of the heart with consequent cardiac failure in treated patients.One of the cytostatic drugs that is often implicated in acute as well as chronic cardiotoxicity is an anthracycline antibiotic (ANT) doxorubicin (DOX) which was isolated in the early 1960s from the pigment-producing bacterium Streptomyces peucetius var. caesius, along with daunorubicin (DAU, also known as daunomycin and rubidomycin) [1-3].The chemotherapeutic use of DOX is limited by their capacity to induce dose-dependent cardiotoxicity action due to reactive oxygen species generation, oxidative stress, topoisomerase-IIb inhibition, and mitochondrial damage leading to cardiac cell death and cardiac dysfunction [4-6].
The proposed cardiotoxic mechanisms of DOX include myocardial injury induced by excessive generation of reactive oxygen species (ROS) via electron exchange with oxygen molecules and redox cycling in the heart, thus resulting in oxidative stress, cellular toxicity ,cardiac inflammation, mitochondrial dysregulation, and apoptosis.The role of reactive oxygen species (ROS) in DOX cardiotoxicity is enhanced by the cardioprotective effect of antioxidants in animals treated with DOX [7-9].Date palm (Phoenix dactylifera) belongs to the Arecaceae botanical family, which contains about 200 genera with around 3,000 species.Dates contain biologically important phytochemical constituents including phenolics (such as vallinic acid and syringic acid) , flavonoids (luteolin, methyl luteolin, quercetin, and epicatechin), and anthocyanins as well as the presence of selenoproteins, coumaric acid, and ferulic acid, dietary fiber, vitamins, and minerals, while their protein contains the majority of essential amino acids[11-13].
The aim of the present study was to investigate the potential ameliorative effect of Date Palm extract on serum CTn1, NO, CK-MB, LDH in rats doxorubicin -induced cardiotoxicity in rats.

2. Materials And Methods

1. Male rats (n = 30) were randomly classified into three groups of ten each.
2. Control group (G1): This group contain 10 adult rats each rat received normal saline daily for thirty days.
3. Group dox (G2): This group contain 10 adult rats treated with DOX 2mg\kg\B.W IP daily for thirty days.
4. Group dox+DP (G3): This group contain 10 adult rats treated with DOX 2mg\kg\B.W IP and Date Palm extract 2mg\kg\B.W oral gavage daily for thirty days.

Ethanolic Extraction of Date Palm

Zahedi dates were purchased from the market in Salah Addin governorate and washed with tap water followed by air dried in shade for one week at room temperature. seeds were separated from the fruits manually and crushed into fine particles and the fruits

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also were grinded, the final grinded fruits and crushed seeds were collected together then were extracted by Soxhelt apparatus by using 99.9% ethanol. The extracted solution was collected and exposed to ambient temperature for one week till it became semi-solid texture and was kept in the fridge until use. Eventual extract diluted with distilled water to be suitable for oral administration [14].

The excised parotid glands were immediately fixed in 10% formalin and processed for paraffin embedding according to standard procedure. Serial sections of five µm thickness of heart were cut and stained with hematoxylin and eosin (H&E). Two examiners unaware of experimental details independently determined the histomorphological changes in the heart using a light microscope [15-18].

In all analyses, p-values < .05 were regarded as statistically significant. Analyses were performed using Stata version 14.

Biochemical study

The Dox group showed significantly higher levels of serum NO, and lower level of C T n1 compared with the control group (49.628±1.340 vs 4.718±0.2281µM: (49.628±1.340 vs 4.718±0.2281: p < 0.0001) and (0.45830±0.0621 vs 0.06483 ±0.00576: pg/mL: p < 0.0001)) respectively. In the DP+MTX DOXgroup, it was detected that DOXtreatment caused a significant increase the levels of serum NO and decresae C T n1 18.160 ±3.17 vs49.628±1.340µM: p < 0.0001) and (0.15170 ±0.01283 vs 0.45830±0.0621: pg/mL) respectively.

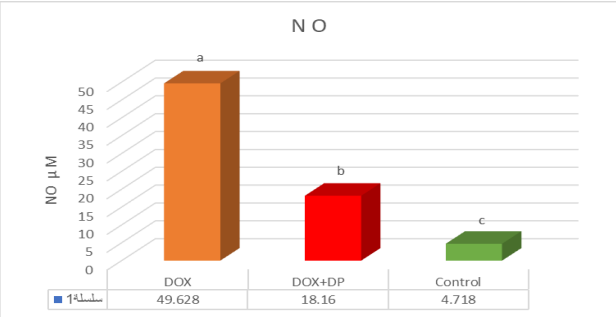

Figure 1. The protective effects of date palm extracts on NO in rats treated with doxorubicin group.

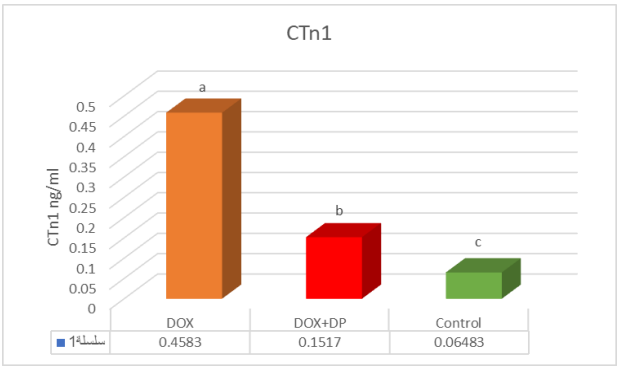

Figure 2. The protective effects of date palm extracts on CTn1 in rats treated with doxorubicin group.

Figure 1 shows highly significant increase in the mean values of serum LDH, and C K - M B in Dox-treated group when compared with control group (160.115±1.775vs 81.467 ± 1.697), and (108.663 ± 2.441 vs160.115±1.775)respectively.Meanwhile, treatment of rats with DP insignificantly increased and restored LDH, and CK- MB activities to their normal levels compared to the DOX alone treated group (152.40±5.94 vs 238.60±16.3) and (108.663 ± 2.441 vs 160.115 ± 1.775) respectively.

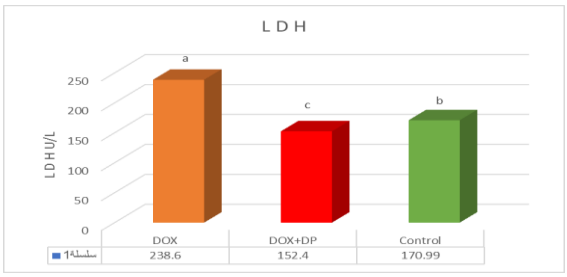

Figure 3. The protective effects of date palm extracts on LDH in rats treated with doxorubicin group.

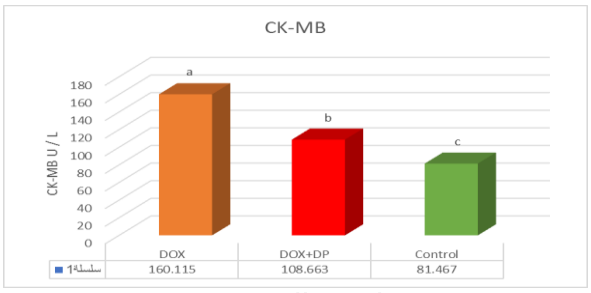

Figure 4. The protective effects of date palm extracts on CK- MB in rats treated with doxorubicin group.

Histological Study

1-control group (G1)

The heart wall was formed by endocardium which facing the cavities of liver and those cavities are filled with blood mass, the myocardium was arranged in the form of longitudinal direction of cardiac muscle fibers and circular direction with presence of fibroblast in the endomysium, also blood vessels of coronary artery were found in between myocardial bundles (fig 1).

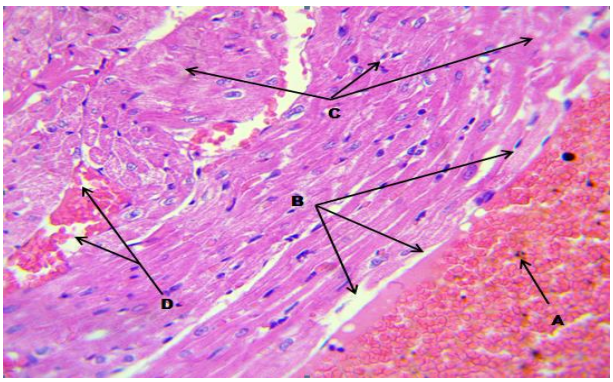

Fig (1) heart wall, blood mass in the cavities of heart (A). endocardium (B) cardiac muscle fibers (C) coronary blood vessels (D) (H & E x40).

2-Doxorubicin qroup (G2)

Myocardium had bundles of cardiac muscle fibers, mostly are containing vacuolated sarcoplasm and cavitation which had great cavities nearby the epicardium, there is myocardial fiber of intact texture (fig 2).

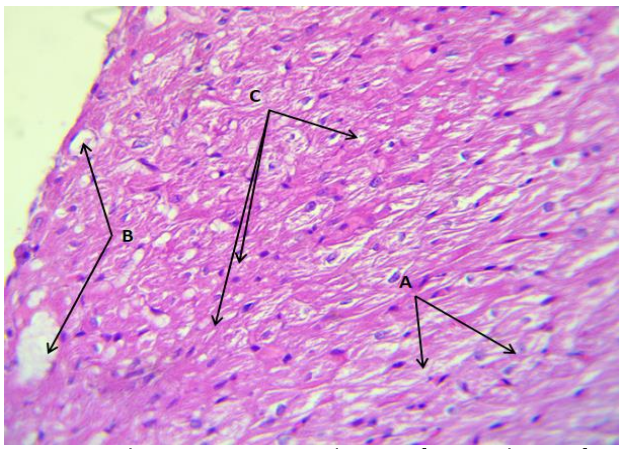

Fig (2). heart tissue, vacuolation of sarcoplasm of cardiac muscle fibers (A), cavitation of muscle bundles (B) intact muscle fibers(C) (H & E x40).

3-Doxorubicine with Date Palm extract group (G3)

Hypertrophy of myocardial fibers were present, surrounded by fibroblasts and macrophages in the endomysium, the sarcoplasm of most cardiac muscle fibers had vacuolation, the cavities of heart found with sever congestion of blood (fig 3).

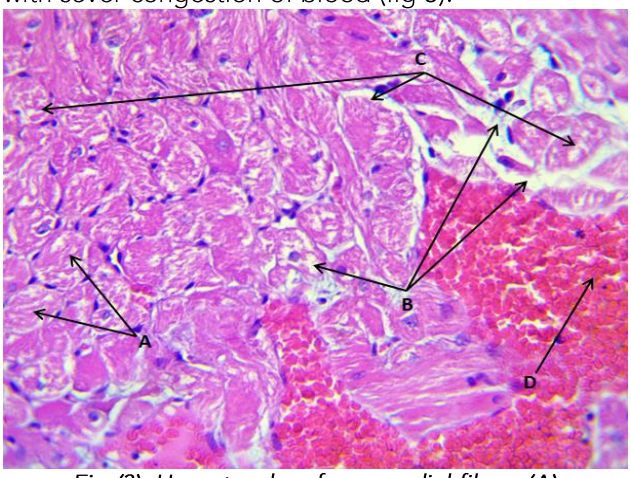

Fig (3). Hypertrophy of myocardial fibers (A) macrophages in endomysium (B) vacuolation of sarcoplasm (C) congestion of blood in heart cavity (D) (H & E x40).

3. Discusion

Endothelial nitric oxide synthase an extremely reactive signaling molecule, responsible for the nitric oxide (NO) production from L-arginine and oxygen. The NO is a remarkable regulator for cellular functions such as vasodilation, platelet aggregation inhibition, neutrophil adhesion, scavenging superoxide (O-2) radicals, and inhibition of xanthine oxidase. The overexpression of inducible nitric oxide synthase (iNOS) caused by inflammation is responsible for the increase in cardiac NO levels caused by DOX. Doxorubicin binds to the eNOS (endothelial NO synthase) reductase enzyme, causing the formation of the Dox semiquinone radical, which reduces free oxygen to superoxide (O2--). This reactive conversion involves an enzymatic one-electron reduction reaction, which has cardiotoxic effects. In the presence of flavoenzymes like NADPH-cytochrome P450 reductase and mitochondrial NADH dehydrogenase, Dox completes the one-electron reduction reaction. There is an imbalance in superoxide free radicals and nitric oxide levels when the drug binds to the eNOS reductase enzyme. Cardiotoxicity is caused by a decrease in nitric oxide levels and an increase in superoxide levels [20, 21].

Troponins are proteins that play an important role in muscle contraction. Troponin C, Troponin T, and Troponin I are three subunits of the troponin complex that work together with tropomyosin to regulate heart muscle contraction and relaxation [. Troponin C, also known as TN-C or TnC, is a calcium-binding protein that is produced in cardiac and skeletal muscle and is encoded by the TNNC1 gene. Troponin I is the inhibitory subunit that prevents myosin from binding to actin. Troponin I comes in three different isoforms: fast and slow skeletal isoforms, as well as a cardiac-specific isoform. Troponin T, the largest subunit (36 kDa), is responsible for cardiac contraction.The N-terminus, also known as the T1 region (interacts with tropomyosin), and the C-terminus, also known as the T2 region, are two functional regions of troponin T.Cardiac Troponins I and T are cardiomyocyte - expressed biomarkers for myocardial injury. Troponin is present in the blood in very small to undetectable amounts in physiological conditions; however, troponin is increased in the blood in pathological conditions such as myocardial injury. [21-24].

Regarding the serum cardiac troponin I (cTn I), this study revealed that, there was a significant elevation of cTn I in the DOX group denoting high cardiomyocyte breakdown which could be due to anemic hypoxia resulting due to alteration in erythropoiesis. The values was returned completely to normal in the combined DOX and DP group.Normal healthy cells contain plenty of LDH and CK enzymes. Both of them are cytosolic enzymes used as a measure or marker of tissue damage [Intesar J. Mohammed,2021]. reflect the integrity of myocardial cell membrane and the degree of myocardial injury [Wang L, Li Y2018,].

Doxorubicin treatment causes significant elevation in plasma LDH when compared with normal control group due to due to cardiac tissue damage, leading to damaged of the the integrity of myocardial cell membrane, with increased permeability, which results in the leakage of intracellular LDH into plasma [25-28].

This increase in serum CK-MB level indicates an injury or damage to cardiac cells by doxorubicin which may be due to the inhibition of nucleic acid and protein synthesis attributed the increase of CK-MB to the excessive production of free radicals and lipid peroxides that might have caused leakage of cytosolic enzymes and to cell membrane damage [29-30].

Pretreatment with 2mg/kg of DP showed marked re in LDH and CK-MB levels compared with

doxorubicin group,This indicates that the pretreatment with DP can prevent the leakage of intracellular LDH and CK-MB into plasma, thus exerting the myocardial protection functions.

Histopathological analysis of cardiac tissue showed that the use of 2 mg/kg DOXcauses the Bundles of myocardial fibers had sarcoplasmic vacuolations and the branches of coronary blood vessels had homolyzed blood in its lumens, atrophy of some cardiac muscle fibers also dected.This alteration may be due to the acumulation of DOX in cardiac muscles' mitochondria because of its high affinity to cardiolipin, a phospholipid which is present in the inner mitochondrial membrane.DOX mediated mitochondrial dysfunction caused by ROS and increased the oxidative stress,activation of enzymatic pathways, and exaggerated immune response.Treatment with DP decreased these necrosis, coagulation and fibrosis which further confirm the cardioprotective effect of DP. [31-34].

4. Conclusions

DP exerted a significant protective effect from doxorubicin toxicity, by increase the serum levels of serum NO and decresae levels C T n1, LDH, and C K - M B.

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