Impact of Graded Doses of Pyridoxine on Doxorubicin-induced Cardiotoxicity in Female Rats

Doaa K. Abdul Ridha¹, Nada N. Al-Shawi ², Ahmed Q. AL-Awadi³
The National Center for Drug Control and Research (NCDCR)¹, Ministry of Health/Environment, Baghdad, Iraq.
Department of Pharmacology and Toxicology, College of Pharmacy², Baghdad University, Baghdad-Iraq.
Department of Pathology, College of Veterinary Medicine³, Baghdad University, Baghdad-Iraq.
¹Corresponding author’s E-mail: haider_bahaa@yahoo.com

Received: 25-07-2017; Revised: 28-08-2017; Accepted: 18-09-2017.

ABSTRACT

The aim of this study was to investigate the possible protective effect of three graded doses (5, 10, and 15mg/kg) of pyridoxine hydrochloride intraperitoneally injected against (15mg/kg) doxorubicin-induced cardiotoxicity in female rats. 56 Wister albino female rats were utilized weighing 180-200 gm divided into eight groups, seven rats in each; Group I: negative control [distilled water (DW)]; Group II: Pyridoxine (5mg/kg); Group III: Pyridoxine (10mg/kg); Group IV: Pyridoxine (15mg/kg); Group V: doxorubicin (15 mg/kg); Group VI: Pyridoxine (5 mg/kg) + doxorubicin (15 mg/kg); Group VII: Pyridoxine (10 mg/kg) + doxorubicin (15 mg/kg); Group VIII: Pyridoxine (15 mg/kg) + doxorubicin (15 mg/kg). Histological examination, serum biomarker enzymes of creatine kinase myocardial bound (CK-MB), N-terminal Pro brain natriuretic peptide (NT-ProBNP) and cardiac tissue homogenate content of malondialdehyde (MDA) and caspase-3 (CASP-3) were monitored at the end of study to evaluate DOX cardiotoxicity. DOX caused significant elevations in serum levels of CK-MB, NT-ProBNP, and the heart tissue content of MDA and CASP-3 (P<0.05). Pretreatment with (10 and 15mg/kg) pyridoxine resulted in significant reduction (P<0.05) in serum level of CK-MB, and pyridoxine in 15mg/kg showed a significant reduction (P<0.05) in serum level of NT-ProBNP, heart tissue content of MDA and CASP-3; compared to positive control group. Histopathological studies of the heart showed marked cardiac muscle damage in doxorubicin-treated rats and the damage was alleviated in pyridoxine-treated rats prior to doxorubicin administration (Group VIII). In conclusion, vitamin B6 supplementation might be a promising adjunctive agent for improving oxidative stress and biological markers for preventing DOX induced cardiac complications.

Keywords: Doxorubicin (DOX), cardiotoxicity, oxidative stress, apoptosis, pyridoxine.

INTRODUCTION

Doxorubicin (DOX), one of the most potent anthracycline antineoplastic agents used in the treatment of lymphoid malignancies and solid tumors in both adults and children. The intended drug has been reported to exert its activity mainly by intercalation with DNA and by this means it inducing damage to the DNA and inhibiting the synthesis of macromolecules that are essential to maintain cell life. More widespread use of DOX has been limited by a dose-dependent and cumulative cardiotoxicity that can subsequently lead to heart failure. Cardiotoxicity is defined by the National Cancer Institute as the ‘toxicity that affects the heart’, this definition includes a direct effect of the drug on the heart but also an indirect effect that may occur due to enhancement of hemodynamic flow alterations or due to thrombotic events. The main hypothesis for the underlying mechanism of anthracycline-associated cardiotoxicity (AAC) has been suggested that DOX induces an iron mediated increase in ROS, referred to as the “ROS and iron hypothesis”. According to this hypothesis, in the presence of iron, DOX leads to futile redox cycling, inducing substantial ROS production and cellular damage. Oxidation of the aglycone portion of DOX results in the formation of a semiquinone radical, which can rapidly revert to the parent compound by using O2 as an electron acceptor. This redox cycle leads to the formation of superoxide anion (O₂⁻), which is spontaneously converted to hydrogen peroxide (H₂O₂) or by superoxide dismutase (SOD) enzyme. Subsequently, H₂O2 may be converted to highly toxic hydroxyl (•OH) radicals in the presence of heavy metals, such as iron, through the Fenton reaction. In addition, DOX can interact with iron directly to form a DOX-Fe complex, resulting in iron cycling between Fe (II) and Fe (III) forms and substantial ROS production. The role of iron in DOX-induced cardiotoxicity has been supported by several studies. Systemic iron accumulation increases DOX-induced damage. Other possible mechanisms are the induction of apoptosis, mitochondrial DNA damage, changes in ATP production, down-regulation of mRNA expression for sarcoplasmic reticulum calcium ATPase.

Pyridoxine (vitamin B6) is one of the water soluble B vitamins that assist in the metabolism of proteins, fats, and carbohydrates. Pyridoxine, although not classified as a classical antioxidant compound, has recently been shown to have highly efficient antioxidant effects. It was demonstrated that pyridoxine acts as a highly efficient hydroxyl radical (•OH) quencher with a capacity for scavenging up to eight of such radical type. Pyridoxine deficiency was also suggested to be associated with atherogenesis since it may influence long-chain...
polyunsaturated fatty acids biosynthesis, -increase lipid peroxidation, and -affects antioxidant defense.\(^{11}\)

The aim of the present study was to evaluate the possible protective effect of three graded doses of pyridoxine against doxorubicin-induced cardiotoxicity in female rats.

**MATERIALS AND METHODS**

**Animals**

The experiment was performed with the utilization of 56 Wistar albino female rats weighing 180-200 gm (age: 4 months). Rats were obtained from the Animal House of the College of Pharmacy/University of Baghdad and from the Animal House of the National Center of Drug Control and Research (NCDCR). They were maintained on normal conditions of temperature (25 ±2°C), humidity and under a 12 h light/dark cycle. They were fed standard rodent pellet diet and they have free access to water *ad libitum*. The animals had no manifestation of any illness upon examination. They were left for two weeks without interference for acclimatization. The study was approved by the Graduate Studies and the Ethical Committees of the College of Pharmacy, University of Baghdad.

**Experimental Design**

Rats were divided into eight groups of 7 rats each:

- **Group I:** Healthy female rats intraperitoneally (IP) injected with 0.5ml of distilled water (D.W.) once daily for four consecutive days. This group served as a healthy negative control.
- **Group II:** Healthy female rats IP injected with 5mg/kg pyridoxine hydrochloride once daily for four consecutive days.
- **Group III:** Healthy female rats IP injected with 10mg/kg pyridoxine hydrochloride once daily for four consecutive days.
- **Group IV:** Healthy female rats IP injected with 15mg/kg pyridoxine hydrochloride once daily for four consecutive days.
- **Group V:** Healthy female rats IP injected with 0.5ml D.W. for four consecutive days. At day 4, a single dose of doxorubicin hydrochloride (15mg/kg) was IP injected. This group served as a positive control.
- **Group VI:** Healthy female rats IP injected with 5mg/kg pyridoxine hydrochloride (10mg/ml) once daily for four consecutive days. At day 4, a single dose of doxorubicin hydrochloride (15mg/kg) was IP injected.
- **Group VII:** Healthy female rats IP injected with 10mg/kg pyridoxine hydrochloride (10mg/ml) once daily for four consecutive days. At day 4, a single dose of doxorubicin hydrochloride (15mg/kg) was IP injected.
- **Group VIII:** Healthy female rats IP injected with 15mg/kg pyridoxine hydrochloride (10mg/ml) once daily for four consecutive days. At day 4, a single dose of doxorubicin hydrochloride (15mg/kg) was IP injected.

**Doxorubicin hydrochloride preparation**

Fifty milligrams (50mg) of doxorubicin hydrochloride (ADRIBLASTINA® POWDER/Actavis S.P.A. Pasteur/Italy) powder for injection is dissolved in 10ml D.W to obtain 5mg/ml or (3mg/0.6ml concentration per 200g rat weight).

**Pyridoxine hydrochloride preparation**

Two milliliters (2ml) of pyridoxine HCl (100mg) ampoule (Pyridoxine hydrochloride Injection USP 100mg/2ml/Strides Arcolab Limited/India) diluted to 10ml with D.W to obtain 10mg/ml or 1mg/0.1ml concentration.

**Sample preparation**

After euthanization of animals by anesthetic diethyl ether (May and Baker, England), blood was withdrawn from carotid artery from the neck of each rat utilized in this study, and placed in labeled centrifuging tubes, then allowed to clot for 20 min at room temperature and then centrifuged at 3000 (rpm) for 15 minutes; the supernatant separated and was used for the estimation of creatine kinase (CK-MB) isozyme and N-terminal-probrain natriuretic peptide (NT-ProBNP) levels. The heart of each animal utilized in this study was quickly excised, rinsed in chilled phosphate buffer saline (PBS) solution (pH 7.4) at 4°C to dismount thoroughly the excess blood, then heart tissues blotted with filter paper weighed, and minced to small pieces; where, 1g heart tissue was put in tube containing 10 ml of phosphate buffer saline (PBS) solution prepared at the previously-mentioned pH value, to obtain 10% tissue homogenate. The tube containing the heart tissues was put in a beaker containing ice (ice path) then homogenized with the aid of homogenizer (Success Technic Industries, Malaysia) at set 3 for 1 minute at 4°C. After that, the homogenate was centrifuged by the cooled centrifuge (Hittich Rotanta, England) for 15 minutes at 1500xg [or 5000 revolution/minute (rpm)] at 4°C. The supernatant is utilized for the estimation of malondialdehyde (MDA) and CASP-3. Blood and tissue homogenate samples were stored at −20°C until analysis process.

**Estimation of creatine kinase CK-MB isozyme activity**

The procedure involves measuring creatine kinase (CK) activity in the presence of a monoclonal antibody specific to the CK-M monomer. This antibody (anti CK-M antibody that are present in the reagent of the kit, Biolabo, France) completely inhibits the activity of CK-MM and half of CK-MB while not affecting the B subunit of CK-MB and CK-BB. Only the activity of the non-inhibited B monomer subunit, representing half of the CK-MB activity, is measured. The method assumes that CK-BB activity in the specimen is essentially zero. The CK-MB activity is obtained by multiplying the CK-B activity by two.
The reaction scheme is as follows:

\[
\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{HK (Hexokinase)}} \text{ATP} + \text{Glucose} \xrightarrow{+G-6-PDH} \text{ADP} + \text{G-6-P} \xrightarrow{6-Phosphogluconate + \text{NADPH} + \text{H}^+} \text{Creatine} + \text{ATP}
\]

The increase in the absorbance due to the conversion of NADP\(^+\) in to NADPH, measured at 340nm by the autosampler apparatus (Kenza, Biolabo, France) is proportional to the CK-MB activity in the specimen, serum activity of CK-MB is expressed as IU/L.\(^{12}\)

**Estimation of serum N-terminal-probrain natriuretic peptide (NT-ProBNP) level**

NT-pro BNP considers as a strong biomarkers of outcome in chronic HF; their serum level estimation in rat is performed by utilizing the enzyme-linked immunosorbent assay ELISA (MYBioSource, USA). The microtiter plate provided in the kit has been pre-coated with an antibody specific to NT-ProBNP. The concentration of NT-ProBNP in the samples is measured spectrophotometrically at a wave length of 450 nm by means of a microplate reader by comparing the optical density (O.D) of the samples to the standard curve. Serum level of NT-ProBNP is expressed as ng/mL.\(^{13}\)

**Estimation of Malondialdehyde (MDA) levels**

The oxidative stress in heart homogenates was assessed by measuring lipid peroxidation end product the malondialdehyde (MDA) content. The estimation of the intended content was based on ELISA (MYBioSource, USA) (a quantitative sandwich enzyme immunoassay technique). The content of MDA in the heart tissue homogenate was spectrophotometrically measured at a wave length of 450 nm by means of a microplate reader through comparing the optical density (O.D) of the samples to the standard curve.\(^{14,15}\) The contents of MDA in heart homogenate were expressed as nmol/ml.

**Estimation of caspase-3 (CASP-3) level**

The estimation of caspase-3 level employs the quantitative sandwich enzyme immuno-sorbent assay (ELISA) technique (Elabscience Biotechnology, USA). The OD proportional to the concentration of Casp-3 was measured spectrophotometrically at a wave length of 450 nm ± 2 nm.\(^{15}\)

Quantification of the concentration of Casp-3 in the heart tissue homogenate is achieved by comparing its measured OD to the standard reference curve that is prepared using known standard concentration. The concentration of Casp-3 is expressed as nanogram per milliliter (ng/mL).\(^{16}\)

**Histopathological examination**

Heart tissues were prepared for histological examination according to the method of Junqueira et al (1995).\(^{17}\) At the end of the experiment, hearts were rapidly dissected out and washed immediately with PBS and fixed in 10% buffered formalin. The fixed tissues were embedded in paraffin and serial sections (5μm thick) were cut. Each section was stained with hematoxylin and eosin (H&E). The analyses were microscopically performed.

**Statistical Analysis**

The significance of difference between the mean values was calculated utilizing unpaired Student's t-test. The numeric data were expressed as mean ± standard error of means (SEM). Besides, the statistical significance of the differences among various groups was determined by one-way analysis of variance (ANOVA) and least significant decrease (LSD). The level of significance was set at (P<0.05) for all data presented in the results of this study. Statistical analyses were carried out by SPSS 23.0 program.

**RESULTS AND DISCUSSION**

Impact of three doses 5mg/kg, 10mg/kg, and 15mg/kg pyridoxine hydrochloride, single doxorubicin hydrochloride, and their combination on the levels of the serum CK-MB and NT-ProBNP in female rats

Female rats intraperitoneally (IP) injected with (15mg/kg) doxorubicin (Group V) produced a significant increase (P<0.05) in the serum activity of the CK-MB enzyme and the level of the prohormone NT-ProBNP compared to the negative control animals (Group I); non-significant differences (P>0.05) were determined in the serum activity of the previously-mentioned -enzyme and the prohormone level in groups of rats IP injected with 5mg/kg (Group II), 10mg/kg (Group III), and 15mg/kg (Group IV) of pyridoxine compared to the negative control. Serum CK-MB activity in female rats IP injected with a combination of 5mg/kg pyridoxine plus a single dose of 15mg/kg doxorubicin (Group VI) were non-significantly different (P>0.05) compared with the corresponding isoenzyme serum activity level in positive control animals (Group V); moreover significant reduction (P<0.05) in serum CK-MB activity in groups of rats treated with either 10mg/kg pyridoxine plus a single dose of 15mg/kg doxorubicin (Group VII) or 15mg/kg pyridoxine plus a single dose of 15mg/kg doxorubicin (Group VIII) were observed; compared to the corresponding activity in positive control rats (Group V). Concerning NT-ProBNP, the serum levels in (Group VI) and (Group VII) showed a non-significantly different (P>0.05) compared with the corresponding prohormone level in positive control animals (Group V). Besides, the intended prohormone level in Group VIII-treated rats was significantly decrease (P<0.05) compared to the corresponding level in positive control rats (Group V). Tables 1 and figure 1 Show a non-significant differences (P>0.05) in serum level of CK-MB
with a significant differences ($P<0.05$) in serum level of NT-ProBNP; among groups of animals IP injected with increasing doses of pyridoxine HCl (5mg/kg, 10mg/kg or 15mg/kg each with a single dose of 15mg/kg doxorubicin HCl) when these groups was compared with each other's using ANOVA and least significant decrease (LSD) analysis.

**Table 1:** Serum levels of (a) CK-MB and (b) NT-ProBNP in various experimental female rats' groups (N=7)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Serum CK-MB levels (IU/L) Mean ± SEM</th>
<th>Serum (NT-ProBNP) levels (ng/ml) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Negative Control [distilled water (DW)]</td>
<td>513.124 ± 20.374</td>
<td>0.49 ± 0.071</td>
</tr>
<tr>
<td>Group II</td>
<td>Pyridoxine (5 mg/kg)</td>
<td>503.638 ± 52.226</td>
<td>0.452 ± 0.033</td>
</tr>
<tr>
<td>Group III</td>
<td>Pyridoxine (10 mg/kg)</td>
<td>456.655 ± 37.354</td>
<td>0.420 ± 0.027</td>
</tr>
<tr>
<td>Group IV</td>
<td>Pyridoxine (15 mg/kg)</td>
<td>429.498 ± 61.215</td>
<td>0.411 ± 0.058</td>
</tr>
<tr>
<td>Group V</td>
<td>Positive control [doxorubicin (15 mg/kg)]</td>
<td>794.591 ± 51.929*</td>
<td>0.868 ± 0.125*</td>
</tr>
<tr>
<td>Group VI</td>
<td>Pyridoxine (5 mg/kg) + doxorubicin (15 mg/kg)</td>
<td>644.762 ± 50.153*</td>
<td>0.735 ± 0.048*</td>
</tr>
<tr>
<td>Group VII</td>
<td>Pyridoxine (10 mg/kg) + doxorubicin (15 mg/kg)</td>
<td>575.668 ± 27.677Ba</td>
<td>0.571 ± 0.044Ab</td>
</tr>
<tr>
<td>Group VIII</td>
<td>Pyridoxine (15 mg/kg) + doxorubicin (15 mg/kg)</td>
<td>554.824 ± 31.142*Ca</td>
<td>0.53 ± 0.077Bc</td>
</tr>
</tbody>
</table>

- Each value represents mean ± standard error of means (SEM).
- *= Significantly different ($p < 0.05$) with respect to the negative control group.
- Non-identical superscripts capital letters (A, B, and C) are significantly different ($P<0.05$) in comparison with the positive control group (Doxorubicin-treated animals).
- An identical small superscript letter (a) within the column of CK-MB are non-significantly different ($P>0.05$) among (VI, VII and VIII) groups.
- Non-identical small superscripts letters (a, b, and c) within the column of NT-ProBNP are significantly different ($P<0.05$) among (VI, VII, and VIII) groups.
- N = number of animals.

**Figure 1:** Bar chart showing serum levels of (a) CK-MB and (b) NT-ProBNP in various experimental rats' groups.
- *= Significantly different ($p < 0.05$) with respect to the negative control group, (A, B, and C) non-identical capital letters superscripts; significantly different ($P<0.05$) in comparison with the positive control group (Doxorubicin-treated animals); An identical small superscript letter (a) are non-significantly different ($P>0.05$) among (VI, VII and VIII) groups within (a) CK-MB chart; (a, b, and c) small superscripts letter within (b) NT-ProBNP chart are significantly different ($P<0.05$) among (VI, VII, and VIII) groups.
Impact of three doses 5mg/kg, 10mg/kg, and 15mg/kg pyridoxine hydrochloride, single doxorubicin hydrochloride, and their combination on the levels of the tissue homogenate MDA and CASP-3 in female rats:

Female rats intraperitoneally (IP) injected with (15mg/kg) doxorubicin (Group V) caused a significant increase ($P<0.05$) in the heart tissue homogenate content of the MDA and CASP-3 activity compared to the negative control animals (Group I). Non-significant differences ($P>0.05$) in the tissue homogenate content of MDA and CASP-3 level in group of rats IP injected with 5mg/kg pyridoxine (Group II) compared to the negative control. Beside, significant decrease ($P<0.05$) in the MDA content and CASP-3 enzyme activity in groups of animals treated with either 10mg/kg (Group III) or 15mg/kg (Group IV) pyridoxine alone compared with the negative control. Moreover, tissue homogenate MDA content and CASP-3 activity in female rats IP injected with either combination of 5mg/kg pyridoxine plus a single dose of 15mg/kg doxorubicin (Group VI) or 10mg/kg pyridoxine plus a single dose of 15mg/kg doxorubicin (Group VII) were non-significantly different ($P>0.05$) compared with the corresponding content and enzyme activity in positive control animals (Group V); significant decrease ($P<0.05$) in the intended content and enzyme activity in group of rats treated with 15mg/kg pyridoxine plus a single dose of 15mg/kg doxorubicin (Group VIII) compared to the corresponding activity in positive control rats (Group V) were observed. Table 2 and figure 2. Additionally; non-significant differences ($P>0.05$) in MDA content and CASP-3 enzyme activity in heart tissue homogenate among groups of animals IP injected with increasing doses of pyridoxine HCl (5mg/kg, 10mg/kg or 15mg/kg each with a single dose of 15mg/kg doxorubicin HCl) were observed when these group was compared with each other’s using ANOVA and least (LSD) analysis as shown in table 2 and figure 2.

Table 2: Tissue homogenate contents of (a) MDA and (b) CASP-3 enzyme activity in various experimental female rats' groups (N=7).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Tissue homogenate MDA levels (nmol/ml) Mean ± SEM</th>
<th>Tissue homogenate CASP-3 levels (ng/ml) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Negative Control [distilled water (DW)]</td>
<td>2.158 ± 0.109</td>
<td>0.666 ± 0.068</td>
</tr>
<tr>
<td>Group II</td>
<td>Pyridoxine (5 mg/kg)</td>
<td>2.042 ± 0.189</td>
<td>0.611 ± 0.081</td>
</tr>
<tr>
<td>Group III</td>
<td>Pyridoxine (10 mg/kg)</td>
<td>1.769 ± 0.135</td>
<td>0.483 ± 0.027</td>
</tr>
<tr>
<td>Group IV</td>
<td>Pyridoxine (15 mg/kg)</td>
<td>1.712 ± 0.166</td>
<td>0.462 ± 0.035</td>
</tr>
<tr>
<td>Group V</td>
<td>Positive control [doxorubicin (15 mg/kg)]</td>
<td>3.250 ± 0.285</td>
<td>1.644 ± 0.190</td>
</tr>
<tr>
<td>Group VI</td>
<td>Pyridoxine (5 mg/kg) + doxorubicin (15 mg/kg)</td>
<td>2.921 ± 0.015</td>
<td>1.535 ± 0.176</td>
</tr>
<tr>
<td>Group VII</td>
<td>Pyridoxine (10 mg/kg) + doxorubicin (15 mg/kg)</td>
<td>2.711 ± 0.131</td>
<td>1.503 ± 0.074</td>
</tr>
<tr>
<td>Group VIII</td>
<td>Pyridoxine (15 mg/kg) + doxorubicin (15 mg/kg)</td>
<td>2.351 ± 0.293</td>
<td>1.11 ± 0.143</td>
</tr>
</tbody>
</table>

- Each value represents mean ± standard error of mean (SEM).
- * = Significantly different ($P < 0.05$) with respect to the negative control group.
- Non-identical capital letters superscripts (A and B) are significantly different ($P<0.05$) in comparison with the positive control group (Doxorubicin-treated animals).
- An identical superscript small letter (a) within MDA and Casp-3 column is non-significantly different ($P>0.05$) among (VI, VII and VIII) groups.
- N = number of animals.

Histopathological changes:

Histopathological observation of the H&E stained transverse sections through the cardiac muscles, showed normal tissues in section (Fig. 3-A). The histopathological changes in hearts of female rats IP injected with (15mg/kg) doxorubicin were manifested and characterized by necrosis of cardiac myocytes, severe infiltration of inflammatory cells mainly neutrophils and severe vacuolation in the cytoplasm of the cardiac muscles (myocytolysis) (Figures 3-B); histological examination of the heart section of rats IP injected with (5 and 10mg/kg) pyridoxine for 4 consecutive days showed normal heart architecture (Figures 3-C,3-E); heart of rat IP injected with 15mg/kg pyridoxine showed normal cardiac muscle and a minor congestion of blood vessel (figure 3-G); Histological examination of the heart sections of rats injected IP with 5mg/kg pyridoxine for 4 consecutive days plus a single dose 15mg/kg doxorubicin showed congestion of blood
vessels and infiltration of inflammatory cells in the interstitial tissue (figure 3-D); Histological examination of the heart section of rats injected IP with 10mg/kg pyridoxine for 4 consecutive days plus a single dose 15mg/kg doxorubicin showed few neutrophils in blood vessel and infiltration of inflammatory cells mainly mononuclear cells and loss of differential stain of the cardiac muscles (figure 3-F); Histological examination of the heart section of rats IP injected with 15mg/kg pyridoxine for 4 consecutive days plus a single dose 15mg/kg doxorubicin showed normal heart architectures (figure 3-H).

Figure 2: Bar chart showing the tissue homogenate contents of (a) MDA and (b) CASP-3 enzyme activity in various experimental rats' groups.

* = Significantly different ($p < 0.05$) with respect to the negative control group, (A, B, and C) non-identical capital letters superscripts; significantly different ($P<0.05$) in comparison with the positive control group (Doxorubicin-treated animals); (a) small letters superscripts within (a) and (b) charts of MDA and Casp-3 respectively, are non-significantly different ($P>0.05$) among (VI, VII and VIII) groups.

Figure 3: Hematoxylin and eosin-stained sections of rat heart, which were examined under high power of light microscope: (A) Represents negative control rats received D.W. intraperitoneally, showing normal myocardial fibers, no vacuolation, necrosis or inflammation ($\times40$); (B) Represents (15mg/kg) DOX alone treated rats, showed abnormal heart section, necrosis of cardiac myocytes ($\times40$) (black arrow); (C) represents (5mg/kg) pyridoxine treated rats, showed normal...
heart architecture (×20); (D) Represents (5mg/kg) pyridoxine + (15mg/kg) DOX treated rats, showed congestion of blood vessels (×20) (red arrow); (E) Represents (10mg/kg) pyridoxine treated rats, showed normal heart architecture (×20); (F) Represents (10mg/kg) pyridoxine + (15mg/kg) DOX treated rats, showed few neutrophils in a blood vessels (×20) (blue arrow); (G) Represents (15mg/kg) pyridoxine treated rats, showed normal cardiac muscle and a minor congestion of blood vessel (×20) (yellow arrow); (H) Represents (15mg/kg) pyridoxine + (15mg/kg) DOX treated rats, showed normal heart architectures (×20).

Anthracyclines including doxorubicin induce cardiotoxicity by a cumulative and dose-dependent manner leading to myocardial damage that ranging from mild disturbance of cardiac biomarkers to irreversible cardiomyopathy. Since doxorubicin is necessary in cancer treatment, protection against its cardiotoxicity represents an important challenge to prevent detrimental effects on the heart functions while maintaining the same anticancer efficacy, from this point of view many trials have taken place in an attempt to reduce doxorubicin induced cardiotoxicity. As previously mentioned DOX cardiotoxicity attributed to complex mechanisms that include oxidative stress and free radical generation, membrane lipid peroxidation, mitochondrial damage and iron-dependent oxidative damage to macromolecules, intracellular calcium dysregulation, and apoptosis/necrosis. Acute DOX-induced cardiotoxicity alters the organization of the cardiomyocytes and induces apoptosis, which is a potentially modifiable and preventable form of myocardial tissue loss.

The results of present study confirmed the results performed by others; where, a single dose of DOX (15 mg/kg IP) induces acute cardiotoxicity in rats. The results showed that DOX induce myocardial injury that represented by a significant increase (P<0.05) in -serum CK-MB enzyme activity, -prohormone NT-Pro BNP, -cardiac tissue contents of MDA, and -CASP-3 enzyme activity compared to the negative control group (tables 1 and 2, figures 1 and 2), this comes in tune with many previous reports that demonstrated significant elevation of serum CK-MB, NT-Pro BNP, tissue homogenate MDA, and CASP-3.

The mechanism of cardiotoxicity induced by a doxorubicin may be attributed to increased oxidative stress, possibly through induction of NADPH oxidase, the major enzyme responsible for the formation of ROS in the cardiovascular system. CK-MB isozyme distributed primarily in the heart muscle and its elevation represents an indicator of myocardial dysfunction. NT-proBNP, a promising candidate marker for the exclusion and detection of ventricular dysfunction after potentially cardiotoxic anticancer therapy, detection was able to support an early diagnosis of “chemotherapy related cardiac dysfunction” CRCD in the absence of LVEF decrease and is more sensitive than echocardiography for early cardiac changes. NT-proBNP is a sensitive test and has a moderate relationship with the left ventricular (LV) systolic and diastolic function, making it a useful cardiac marker for the monitoring of early anthracycline cardiotoxicity; the NT-proBNP level inversely correlated with ejection fraction, LV dysfunction associated with an abnormally high NT-proBNP level.

Malondialdehyde (MDA), an end product of lipid peroxidation, one of the best indicators of oxidative stress. The elevation in MDA content can be attributed to the effects of free radicals generated as a result of DOX treatment, on NADH dependent microsomal lipid peroxidation and thus initiates a lipid radical chain reaction causing oxidative damage to cell membrane.

Additionally, DOX treatment induced significantly an increase in cleavage of CASP-3, one marker of apoptosis in the heart tissue. Caspase-3 is a cytosolic protein that exists normally as an inactive precursor with a higher molecular weight (about 32 kDa). Cleaved proteolytically into low molecular weights (11, 17, and 20 kDa) when a cell undergoes apoptosis.

The results of the current study demonstrated that there were significant reduction of serum CK-MB in (10 and 15 mg/kg) pyridoxine pretreated groups (Group VII) and (Group VIII), besides serum NT-ProBNP level and tissue homogenate MDA and CASP-3 contents were significantly reduced in 15mg/kg pyridoxine pretreated groups (Group VIII). This comes in tunes with the Sibel Tas, et al. the reduction in the previously-mentioned parameters might be attributed to protective antioxidant effects of pyridoxine. It was suggested that such vitamin may act as a powerful chain-breaking antioxidant in biological systems related to its ability to scavenge peroxyl radicals.

The significant decrease in CASP-3 cleavage observed after pyridoxine pretreatments (figure 2 b). This may illustrate the protective role of pyridoxine on the myocardium and the possible restoration of the myocardial cell membrane damage and reduce their permeability, thereby restricting the leakage of these enzymes into the blood stream.

CONCLUSIONS

According to the results obtained from this study, it could be concluded that each of the pyridoxine doses (10 and 15mg/kg) administered once daily for 4 consecutive days to female rats has a protective effect on rats heart when administered with a single (15mg/kg) doxorubicin induced acute cardiomyopathy; via its antioxidant, and anti-apoptosis effects.

Acknowledgements: This article was abstracted from M.Sc. thesis submitted to the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad/Iraq. The authors are thankful to the Department of Pharmacology and...
Toxicology at The College of Pharmacy, Baghdad University, Baghdad-Iraq, and The National Center for Drug Control and Research (NCDCR), Ministry of Health/Environment, Baghdad, Iraq for their continuous encouragement and support.

REFERENCES
echocardiographic outcomes, J Clin Oncol, 30 (10), 2012, 1042–1049.


Source of Support: Nil, Conflict of Interest: None.