Impacts of Graded Doses of Pyridoxine on the Biomarkers, Aspartate Aminotransferase, Lactate Dehydrogenase and Total Antioxidant Capacity in Doxorubicin-Induced Cardiotoxicity in Female Rats

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Abstract

Doxorubicin (DOX), one of the anthracycline family; most widely used antineoplastic drugs and highly effective in treating cancer patients. The intended drug exerted 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 but their use associated with cardiotoxicity adverse effect. Pyridoxine (vitamin B6) is one of the water soluble B vitamins; converted into the active form, pyridoxal 5'-phosphate (PLP). Pyridoxine may have a crucial role in antioxidant mechanism. The aim of the current study was to investigate the possible protective effect of graded doses (5, 10, and 15mg/kg) of pyridoxine hydrochloride intraperitoneally (IP) injected for four consecutive days against single dose of (15mg/kg) doxorubicin-induced cardiotoxicity in female rats IP injected at the fourth day only. Fifty-six (56) Wistar albino female rats were utilized weighing 180-200 gm allocated into eight groups, seven rats each; and by utilizing IP injection as route of administration as follows: **Group I:** distilled water (negative control); **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) prior to doxorubicin (15 mg/kg); **Group VII:** Pyridoxine (10 mg/kg) prior to doxorubicin (15 mg/kg); **Group VIII:** Pyridoxine (15 mg/kg) prior to doxorubicin (15 mg/kg). At the 5^{th} day (after 24 hr from the last treatment), blood was withdrawn and heart tissue homogenate obtained for laboratory evaluation. DOX caused significant elevations in serum biomarker enzymes of aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and significant reduction in heart tissue homogenate content of total antioxidants capacity (TAC). Treatment with 5mg/kg pyridoxine for four consecutive days prior to a single dose 15mg/kg doxorubicin resulted in a non-significant differences in serum enzymes level of AST, LDH, and TAC heart tissue homogenate contents. Besides, treatment with 10 or 15mg/kg pyridoxine for four consecutive days prior to a single dose of doxorubicin produced significant increments in TAC heart tissue homogenate level compared to positive control. Moreover, treatment with 15mg/kg pyridoxine for four consecutive days prior to a single dose 15mg/kg doxorubicin resulted in significant reduction in serum enzymes level of AST and LDH. In conclusion, pyridoxine supplementation might be a promising adjunctive agent for improving oxidative stress and biological markers for preventing DOX-induced cardiac complications.

Keywords: Doxorubicin (DOX), Cardiotoxicity, Pyridoxine, Total antioxidant capacity.

تقييم التأثير الوقائي المحتمل لهايدروكلوريد البيريدوكسين على الأنزيمات Aspartate Aminotransferase AST, Lactate Dehydrogenase LDH ومضادات الأكسدة Total Antioxidant Capacity TOAC لتقليل سمية القلب المستحث بواسطة الدوكسوروبيسين في أناث الجرذان

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الخلاصة

الدوكسوروبيسين أحد الأدوية الفعالة من عائلة الأنثراسيكلين، يستخدم في علاج العديد من الأورام السرطانية الخبيثة لدى المرضى المصابين بالسرطان. ان آلية العلاج الكيميائي الرئيسة لعمل الدواء اعلاه تتضمن إقتحامه للتركيب الحلزوني للحامض النووي الذي بدوره

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يسبب تلف الحامض النووي وتثبيط تولد جسيمات الخلية الضرورية لحفظ ديمومة الخلية الحية. تم تقييد أستخدام هذا الدواء بسبب سمية القلب الناتجة من الجرع التراكمية له التي يمكن أن تؤدي لاحقا الى قصورفي القلب. البيريدوكسين (فيتامين ب٦), من مجموعة فيتامين (ب) الذائبة في الماء. يتحول هذا الفيتامين داخل الجسم إلى فوسفات البيريدوكسال الذي يعد الشكل الفعال للفيتامين اعلاه .يمكن ان يمتلك الفيتامين ب٦ تأثير مهم كمضاد للأكسدة.الهدف من الدراسة هو لتقييم التأثير الوقائي المحتمل لجرعات متدرجة من هايدروكلوريد البايريدوكسين ,(٥ ملغم / كيلو غرام،١٠ ملغم / كيلو غرام،١٥ ملغم / كيلو غرام) كل منها استخدمت لمدة ٤ ايام متتالية ضد سمية القلب المستحثة بولسطة الدوكسوروبيسين الذي اعطي في اليوم الرابع كجرعة منفردة في اناث الجرذان. تم أستخدام ستة وخمسون (٥٦) جرذ انثوي مختبري , قسمت الجرذان المختبرية الي ثمانية مجموعات , كل مجموعة تتكون من سبعة جرذان وباستخدام طريقة الحقن داخل الصفاق وكما يلي: **المجموعة الاولى** حقنت ماء مقطر (٥,. مل / غرام) مرة واحدة في اليوم لمدة أربعة أيام متتالية. **المجموعة الثانية** حقنت (٥ ملغم / كيلو غرام) هايدروكلوريد البايريدوكسين مرة واحدة في اليوم لمدة أربعة أيام متتالية. **المجموعة الثالثة** حقنت (١٠ ملغم / كيلو غرام) هايدروكلوريد البايريدوكسين مرة واحدة في اليوم لمدة أربعة أيام متتالية. **المجموعة الرابعة** حقنت (١٥ ملغم / كيلو غرام) هايدروكلوريد البايريدوكسين مرة واحدة في اليوم لمدة أربعة أيام متتالية . **المجموعة الخامسة** حقنت (٥,. مل / غرام) ماء مقطر مرة واحدة في اليوم لمدة أربعة أيام متتاية وفي اليوم الرابع تم حقن الجرذان بجرعة واحدة من الدوكسوروبيسين (١٥ ملغم / كيلو غرام) . المجموعة السادسة حقنت هايدروكلوريد البايريدوكسين (٥ ملغم / كيلو غرام)جرعة واحدة في اليوم لمدة أربعة ايام متتالة اضافة الى جرعة واحدة (١٥ ملغم / كيلو غرام) من الدوكسوروبيسين الذي حقن في اليوم الرابع فقط. المجموعة السابعة حقنت هايدروكلوريد البايريدوكسين (١٠ ملغم / كيلو غرام) جرعة واحدة في اليوم لمدة أربعة ايام متتالة أضافة الى جرعة واحدة (١٥ ملغم / كيلو غرام) من الدوكسوروبيسين في اليوم الرابع فقط. المجموعة الثامنة حقنت هايدروكلوريد البايريدوكسين (١٥ ملغم / كيلو غرام) جرعة واحدة في اليوم لمدة أربعة ايام متتالة أضافة الى جرعة واحدة (١٥ ملغم / كيلو غرام) من الدوكسوروبيسين في اليوم الرابع فقط. في اليوم الخامس (بعد مرور ٢٤ ساعة على الجرعة الأخيرة) تم سحب عينات من الدم ومن نسيج القلب للجرذان لغرض اجراء التحليلات المختبرية. لوحظ وجود ارتفاع معنوي في مستوى الانزيمات (AST) , (LDH) في مصل الدم أضافة الى أنخفاض معنوي في مستوى مضادات الاكسدة (TAC) لدى مجموعة الجرذان المعاملة بالدوكسوروبيسين لوحده بالمقارنة مع المجموعة المعاملة بالماء المقطر فقط. نتائج الدراسة بينت أن أستخدام البيريدوكسين بجرعة (٥ ملغم / كيلو غرام) لمدة ٤ أيام متتالية قبل أستخدام الدوكسوروبيسين ليس له تأثير معنوي على مستوى الأنزيمات (AST), (LDH) أومضادات الأكسدة (TAC) أضافة الى أن استخدام البيريدوكسين بجرعة (١٠ ملغم / كيلو غرام) أو 15mg/kgقبل جرعة الدوكسوروبيسين أدى الى أرتفاع مستوى مضادات الأكسدة (TAC) بشكل معنوي مقارنة مع المجموعة المعاملة بالدوكسوروبيسين لوحده. نتائج الدراسة بينت أيضا أن أستخدام البيريدوكسين بجرعة (١٥ ملغم / كيلو غرام) له تأثير معنوي في تقليل مستوى الأنزيمات (AST),(LDH) بالمقارنة مع المجموعة المعاملة بالدوكسوروبيسين لوحده. وبذلك نستنتج أن أستخدام البيريدوكسين يمكن ان يكون مركب واعد عندما يستخدم بجرعة مناسبة لمنع أو تقليل سمية القلب المستحث بواسطة الدوكسوروبيسين من خلال تأثيره المضاد للأكسدة (antioxidant), تحسين الإجهاد التأكسدي (oxidative stress) و تحسين المعايير البايولوجية.

الكلمات المفتاحية: الدوكسوروبيسين، سمية القلب،البيريدوكسين، مضادات الأكسدة.

Introduction

Doxorubicin (DOX) is one of the most widely used antineoplastic drugs (1). It is highly effective in treating patients with acute lymphoblastic leukemia, Hodgkinis lymphoma, aggressive non-Hodgkinis lymphomas, breast carcinoma, ovarian carcinoma and many solid tumors (2). DOX exerted 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 (3). The successful use of DOX has been hindered by its most important and common "cardiotoxic" adverse effect which remains the major limitation of its use with strong impaction on life quality and survival (4). The cardiotoxicity of DOX is attributed to complex mechanisms that include oxidative stress through ROS production and possibly cellular iron accumulation, intracellular calcium dysregulation, mitochondrial damage, and apoptosis/necrosis (1). This fact allows the researchers to develop strategies to reduce the toxic effects of DOX without interfering with its antitumor properties. Antioxidants, which are capable of protecting the cells from oxidative injury, should be included in the potential antioxidant therapy. Therefore , there is a need for identifying alternative, natural and safer sources of antioxidants (5).Pyridoxine (vitamin B6) is one of the water soluble B vitamins; converted into the active form, pyridoxal 5'-phosphate (PLP), which is physiologically-active coenzyme of vitamin B6, is mainly involved in the metabolism of amino acids, nucleic acids, glycogen, porphyrin, and lipids. In addition, pyridoxine may have a crucial role in antioxidant mechanism (6-11). The exact antioxidant mechanism of such vitamin may confirmed; on one hand, it may react directly with the peroxy radicals and thereby scavenge radicals and inhibit lipid peroxidation (11-15). On the other hand, pyridoxine may indirectly play an antioxidant role by serving as coenzyme in the glutathione antioxidant. Besides, pyridoxal 5'-phosphat (PLP) serves as a coenzyme in the trans-sulfuration pathway of homocysteine to cysteine, which is an important contributor for synthesizing reduced glutathione (GSH). Again, pyridoxine may directly or indirectly play a role in oxidative stress and the antioxidant defense system was confirmed by others (16).

It has been reported that deficiency of pyridoxine (Pr, B6) or its active form pyridoxal phosphate (PLP, B6) may promote oxidative lipid peroxidation and exacerbates the oxidative stress. Moreover, investigators demonstrated that pyridoxine is strongly inhibit xanthine oxidase (XO) activity, an enzyme responsible for the formation of uric acid and hydrogen peroxide [17]. The aim of the present study was to investigate the possible protective effect of three graded doses (5mg/kg, 10mg/kg, and 15mg/kg) of pyridoxine each administered prior to a single dose (15mg/kg) doxorubicin- induced cardiotoxicity in female rats.

Materials and Methods

Animals

The experiment was performed with the utilization of 56 Wistar Albino female rats (the available sex) 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 randomly divided into eight groups of 7 rats each as follows:

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.

Preparation of doxorubicin hydrochloride solution.

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).

Preparation of pyridoxine hydrochloride solution.

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.

24 hours after the end of treatment (i.e. at day 5), animals were euthanized 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 serum aspartate aminotransferase AST and lactate dehydrogenase LDH enzymes level[18]. The heart of each animal utilized in this study was quickly excised, rinsed in chilled phosphate buffer saline (PBS) solution (pH 7.4) at 4ºC 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 1500×g [or 5000 revolution/minute

(rpm)] at 4 °C. The supernatant is utilized for the estimation of tissue homogenate contents of TAOC. Blood and tissue homogenate samples were stored at −20ºC until analysis process[19].

Analysis

Estimation of serum aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) specifically catalyzes the transfer of the amino group of aspartic acid to ketoglutarate yielding glutamate and oxaloacetate, which is then reduced to malate by the enzyme malate dehydrogenase (MDH) with the oxidization of the coenzyme nicotinamide adenine dinucleotide reduced form (NADH) to oxidized nicotinamide adenine dinucleotide (NAD). Aspartate aminotransferase (AST) determination involves the following reactions [20, 21]:

$$\text{L-Aspartate} + \text{Ketoglutarate} \xrightarrow{AST} \text{Oxalacetate} + \text{L-Glutamate}$$

$$\text{Oxalacetate} + \text{NADH} \xrightarrow{MDH} \text{Malate} + NAD^+$$

The absorbance reduction at 340nm, as a consequence of NADH oxidation, was determined photometrically and is direct proportional to the serum AST activity in the sample. Serum activity of AST is expressed as IU/L.

Estimation of serum lactate dehydrogenase (LDH) activity

Pyruvate is reduced by NADH and this reaction is catalyzed by LDH enzyme, according to the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{LDH} \text{L-Lactate} + AD^+$$

NAD+ is related to the serum enzymatic activity of LDH which is measured by spectrophotometer at 340 nm. Serum LDH activity was expressed as IU/L. [22] [23].

Estimation of Total Antioxidant Capacity (TAC) level

The estimation of TAC in heart tissue homogenate is based on the enzyme-linked immuno-sorbent assay (ELISA) with the utilization of ready-made rat kit for this purpose. The microtiter plate provided in the kit has been pre-coated with an antibody specific to TAC (mixed SOD/CAT /GSH-PX /GSH). Standards or samples are added to the appropriate microtiter plate wells then followed by the addition of the second horseradish peroxidase (HRP) conjugated TAC antibody to bind the analyte and incubated for 60 minutes at 37ºC. After the addition of 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution and incubates for 15 minutes at 37ºC, only wells that contain TAC mixture will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulfuric acid (H_2SO_4) solution and the color change is spectro - photometrically measured at a wavelength of 450nm.The concentration of TAC mixture in the samples is determined by comparing the O.D. of the samples to the standard curve. The Concentration of TAC is expressed as IU/mL [24].

Statistical Analysis

Statistical analyses were carried out by using IBM SPSS (statistical package for social sciences) version 23.0 program. 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.

Results

Impact of three doses 5mg/kg, 10mg/kg, and 15mg/kg pyridoxine hydrochloride, single doxorubicin hydrochloride, and each prior to doxorubicin on the activities of serum AST and LDH enzymes in female rats:

Table (1) and figure [(1),(2)] showed that there were non-significant differences (P>0.05) in the serum activity of AST (figure 1) and LDH (figure 2) in groups of rats IP injected with either 5mg/kg (**Group II**) or 10mg/kg (**Group III**) pyridoxine compared to the corresponding activity in negative control. Moreover, significant reduction (P<0.05) in the serum activity of the intended enzymes in group of animals treated with 15mg/kg (**Group IV**) pyridoxine compared with the negative control. Besides, female rats IP injected with (15mg/kg) doxorubicin (**Group V**) showed a significant increase (P<0.05) in the serum activity of the AST and LDH compared to the negative control animals (**Group I**). Moreover, female rats IP injected with (15mg/kg) doxorubicin (**Group V**) showed a significant increase (P<0.05) in the serum activity of the AST and LDH compared to the negative control animals (**Group I**). Furthermore, serum AST and LDH enzyme activities in female rats IP injected with either 5mg/kg pyridoxine for four consecutive days prior to a single dose of 15mg/kg doxorubicin (**Group VI**) or 10mg/kg pyridoxine for four consecutive days prior to a single dose of

15mg/kg doxorubicin (**Group VII**) were non-significantly different (P>0.05) compared with the corresponding serum enzymes activity in positive control animals (**Group V**). In contrast, the serum activity of the intended enzymes in group of rats treated with 15mg/kg pyridoxine for four consecutive days prior to a single dose of 15mg/kg doxorubicin (**Group VIII**) were significantly reduced (P<0.05) compared to the corresponding serum activity of positive control rats (**Group V**). Moreover, table (1) and figure [(1),(2)] showed that there were significant reduction (P<0.05) in serum AST and LDH activity among groups of animals IP injected with increasing doses of (pyridoxine HCl 5mg/kg, 10mg/kg or 15mg/kg for four consecutive days each prior to a single dose of 15mg/kg doxorubicin HCl) when compared with each other using ANOVA and least significant differences (LSD) analysis.

Impact of three doses 5mg/kg, 10mg/kg, and 15mg/kg pyridoxine hydrochloride, single doxorubicin hydrochloride, and each prior to doxorubicin on the TAOC levels in heart tissue homogenate of female rats

Table (2) and figure (3) showed that there were non-significant differences (P>0.05) in TAOC level in heart tissue homogenate of rats IP injected with 5mg/kg pyridoxine (**Group II**) compared to the negative control (**Group I**). Beside, significant elevation (P<0.05) in heart tissue homogenate TAC levels in groups of animals treated with either 10mg/kg (**Group III**) or 15mg/kg (**Group IV**) pyridoxine compared with the negative control. Besides, female rats intraperitoneally (IP) injected with (15mg/kg) doxorubicin (**Group V**) produced significant reduction (P<0.05) in the heart tissue homogenate level of the (TAC) compared to the corresponding levels in negative control animals (**Group I**). Furthermore, table (2) and figure (3) showed that there were non-significant differences (P>0.05) in the levels of TAC in heart tissue homogenate of female rats IP injected with 5mg/kg pyridoxine for four consecutive days prior to a single dose of 15mg/kg doxorubicin (**Group VI**) compared with the corresponding tissue levels in positive control animals (**Group V**). Besides, the heart tissue homogenate TAC levels in group of rats treated with either 10mg/kg pyridoxine for four consecutive days prior to a single dose of 15mg/kg doxorubicin (**Group VII**) or 15mg/kg pyridoxine for four consecutive days prior to a single dose of 15mg/kg doxorubicin (**Group VIII**) were significantly elevated (P<0.05) compared to the corresponding levels in positive control rats (**Group V**). In addition, significant elevation (P<0.05) in heart tissue homogenate TAC levels were observed among groups of animals IP injected with increasing doses of pyridoxine HCl (5mg/kg, 10mg/kg or 15mg/kg for four consecutive days each prior to a single dose of 15mg/kg doxorubicin HCl) when the previously-mentioned groups compared with each other's using ANOVA and LSD analysis, Table (2) and figure (3).

Table (1): Effects of various treatments on serum activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) enzymes in female rats (N=7).

Groups	Treatment	Serum AST levels (IU/L) Mean ± SEM	Serum LDH levels (IU/L) Mean ± SEM
Group I	Negative Control [distilled water (DW)]	169.6 ± 5.3745	892.175 ± 29.547
Group II	Pyridoxine (5 mg/kg)	167.285 ± 8.620	815 ± 37.965
Group III	Pyridoxine (10 mg/kg)	164.832 ± 8.359	800.808 ± 30.625
Group IV	Pyridoxine (15 mg/kg)	148.314 ± 2.343*	660.857 ± 42.094*
Group V	Positive control [doxorubicin (15 mg/kg)]	315.2 ± 28.691^{*A}	1381.266 ± 143.353^{*A}
Group VI	Pyridoxine (5 mg/kg) + doxorubicin (15 mg/kg)	295.571 ± 4.893 ^{Aa}	1309.714 ± 98.333^{Aa}
Group VII	Pyridoxine (10 mg/kg) + doxorubicin (15 mg/kg)	255.428 ± 37.196^{Ab}	1300.07 ± 65.092^{Ab}
Group VIII	Pyridoxine (15 mg/kg) + doxorubicin (15 mg/kg)	199.571 ± 187.8 ^{Bc}	991.385 ± 90.857^{Bc}

- Each value represents mean ± standard error of means (SEM).
- * Significantly different (*P* < 0.05) with respect to the negative control group.
- Values with non-identical capital letters superscripts (A, and B) are significantly different (*P*<0.05) in comparison with the positive control group (Doxorubicin-treated animals) using unpaired Student t-test.
- Values with non-identical small letters superscripts (a, b, and c) are significantly different (*P*<0.05) among (VI, VII and VIII) groups using ANOVA and LSD analyses.
- N number of animals.

Group I: negative control distilled water; **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) prior to doxorubicin (15 mg/kg); **Group VII:** Pyridoxine (10 mg/kg) prior to doxorubicin (15 mg/kg); **Group VIII:** Pyridoxine (15 mg/kg) prior to doxorubicin (15 mg/kg).

Table (2): Effects of various treatments on levels of the heart tissue homogenate TAC in female rats (N=7).

Groups	Treatment	Tissue homogenate TAC levels (IU/ML) Mean ± SEM
Group I	Negative Control [distilled water (DW)]	3.903 ± 0.276
Group II	Pyridoxine (5 mg/kg)	4.037 ± 0.228
Group III	Pyridoxine (10 mg/kg)	$4.784 \pm 0.281^{*}$
Group IV	Pyridoxine (15 mg/kg)	$4.988 \pm 0.174^{*}$
Group V	Positive control [doxorubicin (15 mg/kg)]	$2.168 \pm 0.248^{*A}$
Group VI	Pyridoxine (5 mg/kg) + doxorubicin (15 mg/kg)	2.634 ± 0.284^{Aa}
Group VII	Pyridoxine (10 mg/kg) + doxorubicin (15 mg/kg)	3.708 ± 0.149^{Bb}
Group VIII	Pyridoxine (15 mg/kg) + doxorubicin (15 mg/kg)	3.982 ± 0.365^{Cc}

- Each value represents mean ± standard error of mean (SEM).
- * Significantly different (*p* < 0.05) with respect to the negative control group.
- Values with non-identical capital letters superscripts (A, B, and C) are significantly different (*P*<0.05) in comparison with the positive control group (Doxorubicin-treated animals) using unpaired Student t-test.
- Values with non-identical small letters superscripts (a, b, and c) are significantly different (*P*<0.05) among (VI, VII and VIII) groups using ANOVA and LSD analyses.
- N number of animals.

Group I: negative control distilled water; **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) prior to doxorubicin (15 mg/kg); **Group VII:** Pyridoxine (10 mg/kg) prior to doxorubicin (15 mg/kg); **Group VIII:** Pyridoxine (15 mg/kg) prior to doxorubicin (15 mg/kg).

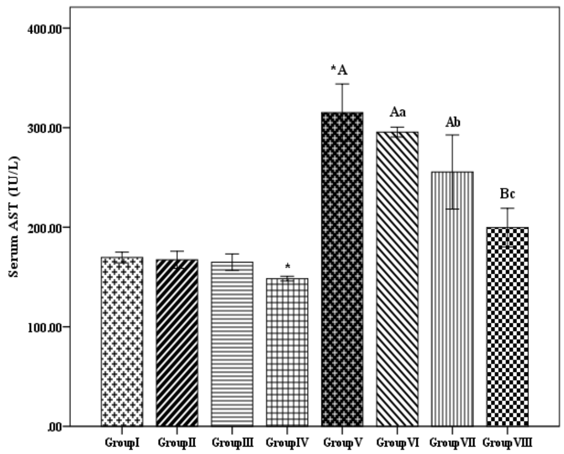

Figure (1): Bar chart showing serum levels of AST in various experimental rats' groups.

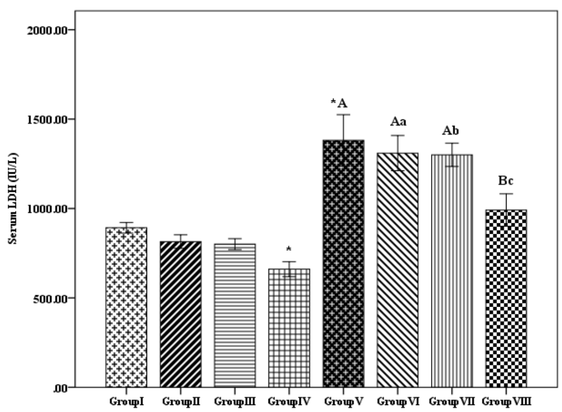

Figure (2): Bar chart showing serum levels of LDH in various experimental rats' groups.

* Significantly different ($p < 0.05$) with respect to the negative control group. Non-identical capital letters (A and B) superscripts are significantly different ($P<0.05$) in comparison with the positive control group (Doxorubicin-treated animals). Non- identical small superscript letter (a, b, and c) are significantly different ($P<0.05$) among (VI, VII and VIII) groups within (a) AST chart, and (b) LDH chart.

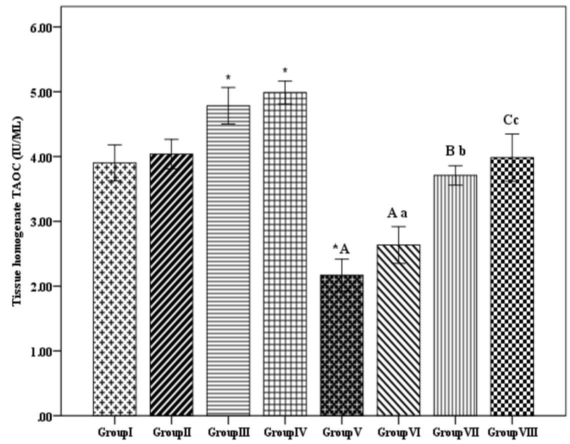

Figure (3): Bar chart showing tissue homogenate enzymes level of total antioxidant capacity (TAOC) in various experimental rats' groups.

* Significantly different ($P<0.05$) with respect to the negative control group. Non-identical capital letters (A, B, and C) superscripts are significantly different (P<0.05) in comparison with the positive control group (Doxorubicin-treated animals). Non-identical small letter superscripts (a, b and c) are significantly different (P<0.05) among (VI, VII and VIII) groups.

Discussion

Cardiac injury is the main limiting factor for the use of DOX as anticancer agent. DOX-induced generation of reactive oxygen species (ROS) seems to be a leading cause of cardiomyopathy [25] [26]. This study investigates the effects of pyridoxine pretreatment, which were used in different doses on DOX-induced acute cardiotoxicity. The data of present study were in agreement with studies performed by others; where, serum AST [27] and LDH [28] enzymes activity were significantly elevated in association with DOX treatment. The increase in serum AST and LDH activity could be attributed to the well-known cardiac toxic effects of DOX, which may lead to the damage of the myocardial cell membrane or it become permeable, that resulted in the leakage of AST and LDH into the blood. This probably accounts for the increase in the activity levels of these marker enzymes in the serum [27]. Pyridoxine at 5mg/kg or 10mg/kg dose each produced non-significant reduction in serum AST and LDH enzymes activity. In contrast, 15mg/kg pyridoxine produced significant decrement in the intended enzymes activity levels; moreover, treatment with pyridoxine 15mg/kg prior to a single dose 15mg/kg DOX restored the activities of enzymes by reducing the marker enzymes (AST and LDH) levels in serum. This may be attributed to the protective role of pyridoxine on the myocardium, reducing the myocardial damage, thereby, restricting the leakage of these enzymes in serum.

It has been reported that TAC include antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px); exist in all oxygen-metabolizing cells to prevent cells from damage exerted by free radicals and provide a repair mechanism for oxidized components [29]. Superoxide dismutase (SOD) dismutases superoxide, the first step generated radical, to hydrogen peroxide and oxygen. Hydrogen peroxide (H_2O_2) is neutralized to H_2O by GSH-Px or CAT. The tissue homogenate enzymatic levels of TAC were significantly reduced ($P<0.05$) in DOX-treated group **(Group V)** this comes in tune with Khan G *et al* (2014) [27] and Al-Harthi SE *et al* (2014) [30]. The current study also showed that pyridoxine 5mg/kg produced non-significant differences in tissue homogenate TAOC level; while pyridoxine at 10mg/kg or 15mg/kg each dose produced a significant increment in TAOC enzyme level in comparison with the negative control group. In addition, data from this study showed that TAC including enzymatic and non-enzymatic cellular antioxidant defense mechanisms in groups of animals treated with either 10mg/kg pyridoxine prior to a single dose of 15mg/kg DOX (**Group VII**) or 15mg/kg pyridoxine prior to a single dose of 15mg/kg DOX (**GroupVIII**) were significantly elevated, this comes in agreements with other study Taş S *et al* (2014)[31], in which, pyridoxine enhanced serum antioxidant paraoxonase and arylesterase enzymes activities; related to pyridoxine ability to reduce oxidative stress [31] ; moreover, pyridoxine may directly or indirectly play a role in oxidative stress and the antioxidant defense system [16]. It was suggested that the intended vitamin may act as a powerful chain-breaking antioxidant in biological systems related to its ability to scavenge peroxyl radicals [14] [32] [33].

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 prior to a single dose of DOX (15mg/kg) has a protective effect and can ameliorate the cardiotoxic effect of DOX that evidenced by a significant reduction in the measured serum biomarkers enzymes rat AST and rat LDH with significant increment in the level of rat TAOC in the heart tissue; therefore, pyridoxine may have a therapeutic value against acute cardiotoxicity induced by doxorubicin in female rats via its antioxidant effects.

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