Research Article

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Ameliorating oxidative stress and inflammation by Hesperidin and vitamin E in doxorubicin induced cardiomyopathy

Doxorubicin ile İndüklenmiş Kardiyomiyopatide Hesperidin ve E Vitamini ile Oksidatif Stres ve İnflamasyonun İyileştirilmesi

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Abstract

Background: Doxorubicin (DOX) is a common chemotherapeutic drug. However, it causes cardiomyopathy which reduces its clinical use in human cancer therapy.
Objective: The purpose of our study was to assess the cardioprotective effect of hesperidin (HSP) and vitamin E (VIT.E) against DOX-induced cardiomyopathy.
Material and methods: Seventy rats were allocated into seven groups: control, HSP (50 mg/kg, orally), VIT.E (100 mg/kg orally), DOX [4 mg/kg, intraperitoneally (i.p.)], DOX + HSP, DOX + VIT.E and DOX + HSP + VIT.E.
Results: Our findings showed that serum lactate dehydrogenase (LDH), creatine kinase (CK), myeloperoxidase (MPO), cardiac catalase and caspase activities as well as cardiac malondialdehyde (MDA) and serum nitric oxide (NO) concentrations were reduced DOX + HSP or DOX + VIT.E or DOX + VIT.E + HSP groups compared to DOX group. Whereas, cardiac reduced glutathione (GSH) level, serum arylesterase, and paraoxonase activities were higher in rats injected with DOX and administrated with HSP and VIT.E than that of rats injected with DOX only. Cardiac histopathology of DOX group showed some changes that were improved during administration with HSP and VIT.E.
Conclusion: HSP and VIT.E possess a protective effect against DOX-induced cardiomyopathy via inhibiting oxidative stress, inflammation, and apoptosis.

Keywords: Cardiomyopathy; Doxorubicin; Hesperidin; Vitamin E; Oxidative stress.

Öz

Giriş: Doksorubisin (DOX) yaygın bir kemoterapötik ilaçtır. Bununla birlikte, kardiyomiyopatiye neden olduğu için bu durum ilaçın insan kanser tedavisinde klinik kullanımını azaltır.
Amaç: Çalışmamızın amacı, DOX ile indüklenen kardiyomiyopatiye karşı hesperidin (HSP) ve vitamin E'nin (VIT.E) kardiyoprotektif etkisini değerlendirmektir.
Gereç ve Yöntemler: Yetmiş sıçan yedi gruba ayrıldı: kontrol, HSP (50 mg/kg, oral), VIT.E (100 mg/kg oral), DOX [4 mg/kg, intraperitoneal (ip)], DOX + HSP, DOX + VIT.E ve DOX + HSP + VIT.E.
Bulgular: Bulgularımız, DOX + HSP veya DOX + VIT.E veya DOX + VIT.E + HSP grupları DOX grubu ile karşılaştırıldığında serum laktat dehidrojenaz (LDH), kreatin kinaz (CK), miyeloperoksidaz (MPO), kardiyak katalaz ve kaspaz aktivitelerinin yanı sıra kardiyak malondialdehid (MDA) ve serum nitrik oksit (NO) konsantrasyonlarının azaldığını gösterdi. Oysa kardiyak redükte glutatyon (GSH) düzeyi, serum arilesteraz ve paraoksonaz aktiviteleri DOX ile HSP ve VIT.E enjekte edilen sıçanlarda sadece DOX enjekte edilen sıçanlara göre daha yüksekti. DOX grubunun kardiyak histopatolojisi HSP ve VIT.E ile uygulama sırasında düzelen bazı değişiklikler gösterdi.
Sonuç: HSP ve VIT.E, oksidatif stres, inflamasyon ve apoptozu inhibe ederek DOX ile indüklenen kardiyomiyopatiye karşı koruyucu bir etkiye sahiptir.

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Anahtar Kelimeler: Doxorubicin; Kardiyomiyopati; Hesperidin; Vitamin E; Oksidatif Stres.

Introduction

Doxorubicin (DOX) is a widely used chemotherapeutic drug for the treatment of several hematogenous and solid human malignancies [1]. However, irreversible myocardial damage, resulting in cardiomyopathy and subsequent congestive heart failure reduces its clinical use in human cancer therapy [2]. Also, 41% of cancer patients who received DOX therapy are affected by various cardiac problems and liver function abnormalities [3]. DOX-induced cardiomyopathy has been suggested to involve free radical generation, inhibition of nucleic acid and protein synthesis, myofibrillar degeneration, and mitochondrial abnormalities [4, 5]. Moreover, DOX administration could decrease the endogenous antioxidants that scavenge the free radicals so supplementation of exogenous antioxidants may improve antioxidant defense system [6].

Hesperidin (HSP) a flavanone glycoside found in citrus fruits (i.e. lemon and sweet orange) has wide pharmacological and biological effects. It could act as an antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial agent, [7] as well as its effects on the vascular system [8].

The fat-soluble Vitamin E (VIT.E) is one of the body's main free radical scavengers that protect cell membranes from lipid peroxidation. Also, it maintains the health of red blood cells enhances blood circulation and supports healthy muscle and nerve function. Moreover, VIT.E has antioxidant effects in other cell organelles [9]. However, no data regarding the effectiveness of VIT.E in combination with HSP in a doxorubicin-induced cardiomyopathy model have been reported.

In the current study, we explored the cardioprotective effect of VIT.E alone or with HSP in DOX-induced cardiomyopathy in rats by assessment of their effects on oxidative stress and inflammatory as well as apoptotic parameters.

Materials and methods

Drugs and chemicals

DOX hydrochloride was purchased from Pfizer (Bentley, Australia). HSP, and VIT.Ecapsules, as well as all other chemicals, were purchased from Sigma (St. Louis, MO, USA).

Experimental design

Seventy adult males of *Sprague Dawley* rats, weighing 130–150 g, were purchased from the Organization for Biological Products and Vaccines, Helwan, Cairo, Egypt. Rats were maintained under standard animal house conditions under 20–22°C. Rat experimentation was consistent with the guidelines of Ethics by the Guide for the Use and Care of Laboratory Animals in accordance with animal care committee of the Faculty of Science, Tanta University, and Tanta, Egypt.

Rats were randomly allocated into seven groups each with 10 rats: (1) in control group, rats received normal rat food; (2) in HSP group, rats were intra-gastric administrated with 50 mg hesperidin/kg body weight three times per week for 3 weeks, [10]; (3) in VIT.E rats were intra-gastric administrated with 100 mg VIT.E/kg body weight, [11] two times per week for 3 weeks; (4) DOX group was intra-peritoneal injected with 4 mg doxorubicin/kg body weight three times per week for 2 weeks, [12]; (5) DOX + HSP group was intra-gastric administrated with hesperidin and intra-peritoneal injected with doxorubicin; (6) in DOX + VIT.E group, VIT.E was intra-gastric administrated and DOX was intra-peritoneal injected; (7) in DOX + HSP + VIT.E, HSP and VIT.E were intra-gastric administrated and DOX was intra-peritoneal injected. The administration of HSP or/and VIT.E started 1 week before DOX injection and continued for the next 2 weeks with DOX injection.

At the end of the experiment, rats were sacrificed by puncture under diethyl ether anesthesia. Blood was collected, allowed to clot at room temperature and centrifuged at 3000 rpm for 10 min to separate serum. Serum samples were kept at –20°C for various biochemical analyses.

In addition, hearts from different rats were removed, washed in ice-cold saline (0.9%) and divided into two parts; one of them was fixed in 10% formalin for histopathological analysis the other part was homogenized (10% w/v) in 0.05 M phosphate buffer pH 7.4 then centrifuged at 3000 rpm for 15 min. The supernatant was used for determination of the biochemical parameters.

Biochemical analyses

Estimation of serum LDH and CK activities and lipid profile biomarkers

LDH and CK activities were assayed by commercial kit supplied by Salucea (Germany). Serum triglycerides (TG),

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HDL-cholesterol and total cholesterol (TC) levels were assayed by commercial kit supplied by Biodiagnostics (Cairo, Egypt). Serum LDL-cholesterol was calculated using the following formula: LDL = TC-HDL – TG/5.

Determination of serum nitric oxide (NO) level and myeloperoxidase (MPO) activity

Serum nitric oxide was evaluated by measuring nitrite (NO_2) and nitrate (NO_3) metabolites [13]. The concentration of oxidative end products is estimated by reduction of NO_3 to NO_2 using cadmium. Briefly, Samples were treated with cadmium after deproteinization with 30% zinc sulfate. Then Griess reagent reacts with NO_2 producing pink color that measured at 540 nm against reagent blank. The NO_2 standard curve was constructed with a set of serial concentrations ranging from 5–100 μM.

Serum MPO oxidized hydrogen peroxide in the presence of *o*-diasnsidine as substrate to produce a brown color which is spectrophotometrically measured at 405 nm [14].

Determination of protein content in heart tissue

The heart tissue protein level was estimated with the Folin-Lowry method using bovine serum albumin (BSA) as standard [15]. BSA different concentrations (1–10 μg/mL) were used to plot a standard curve.

Measurement of heart lipid peroxidation, heart reduced glutathione (GSH) level and catalase activity

Lipid peroxide in heart tissue was estimated colorimetrically as malondialdehyde (MDA). The method determines MDA, the end product of lipid peroxidation, which can react with thiobarbituric acid to yield a pink colored complex exhibiting a maximum absorption at 532 nm [16].

Heart GSH level was determined by Ellman method [17]. In brief, 5,5′-dithiobis-2-nitrobenzoic acid (DTNP) was reduced with GSH to produce a yellow color, which is spectrophotometrically measured at 412 nm. The GSH standard curve (0.25–5 mM) was plotted to determine GSH concentration.

Heart tissue catalase activity was estimated according to Luck [18] by determining of the rate for H_2O_2 decomposition at 240 nm.

Determination of serum arylesterase (AE) and paraoxonase (PON) activities

Serum AE was measured using *p*-nitrophenyl acetateas a substrate as described previously [19]. Briefly, 50 mM phosphate buffer, pH 7.5, 5 μL serum and 0.5 μmol *p*-nitrophenyl acetate (acetone 0.3%) were mixed in a total volume of 1 mL, incubated at 37°C for 2 min and the change in absorbance at 450 nm was recorded. AE activity was calculated as nmol of *p*-nitrophenol produced per minute under assay conditions using extension coefficient for *p*-nitrophenol ($\varepsilon = 17.000\ M^{-1}\ cm^{-1}$).

PON was assayed using paraoxon as substrate according to Gi et al. [20]. Briefly, calcium chloride 1 mM, 0.1 M glycine – NaOH buffer pH 10, sodium chloride 0.3 M, 50 μL of serum and paraoxon 2 mM were mixed in a total volume of 1 mL. Then, the mixture was incubated at 37°C for 5 min and the absorbance change at 450 nm was recorded. PON activity was calculated as nmol of *p*-nitrophenol produced per minute using *p*-nitrophenol extension coefficient.

Determination caspase 3 activity in heart tissue

Caspase 3 activity was determined by Biovision (USA) commercial kit. The method is carried out by spectrophotometric detection of *p*-nitroaniline chromophore (pNA) produced by caspase 3 action on labeled substrate DEVD-pNA. The absorbance was measured at 405 nm [21].

Histopathological analysis

The histopathological analysis of heart was performed out according to Scheuer and Chalk [22] using Harris hematoxyline and eosin staining technique. Briefly, the heart tissue samples were fixed in 10% formalin, prepared as 4–6 nm thick sections, stained with eosin and examined by a pathologist.

Statistical analysis

Data are represented as the mean ± SE. Data analysis was performed by GraphPad 6.0 software (San Diego,

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CA, USA) using one-way ANOVA followed by Tukey test and p-values less than 0.05 was considered statistically significant.

Results

Effect of HSP and VIT.E on cardiac parameters

Serum LDH and CK of DOX-treated rats were significantly elevated from control group. On the opposite side, serum LDH and CK activities in rats of DOX + HSP or DOX + HSP + VIT.E were significantly declined from DOX group (Figure 1).

Effect of HSP and VIT.E on lipid profile

Serum TG and TC levels showed a significant increase in DOX and DOX + VIT.E groups than control rats. Moreover, serum LDL level showed significant elevation in DOX, DOX + HSP, DOX + VIT.E and DOX + HSP + VIT.E groups than control group. Conversely, there was a significant decrease in TC, TG and LDL levels in DOX + HSP, DOX + VIT.E and DOX + HSP + VIT.E groups than DOX group. Also, a serum HDL of DOX and DOX + VIT.E group was significantly lower than that of control group. In contrast, there was a significantly more serum HDL of DOX + HSP, DOX + VIT.E and DOX + HSP + VIT.E groups than serum HDL of DOX group (Figure 2).

Effect of HSP and VIT.E on cardiac antioxidant parameters and apoptotic marker (caspase 3)

Heart MDA was significantly higher in DOX group than heart MDA of control group. While, in DOX + HSP or DOX + HSP + VIT.E groups, heart MDA was significantly decreased than that of DOX group. Serum NO level was significantly more in DOX and DOX + VIT.E groups than serum NO of control group. Conversely, there was a significant decrease in NO level in DOX + HSP, DOX + VIT.E and DOX + HSP + VIT.E groups as compared to DOX group. Serum MPO activity was significantly higher in DOX and DOX + HSP groups than control group serum MPO. Whereas, a significant more MPO activity was detected in DOX + HSP + VIT.E group than MPO activity of DOX + HSP and DOX + VIT.E groups as compared to DOX group. GSH level in heart tissue was significantly lower in DOX group than control GSH level. Furthermore, GSH level showed a significant increase in DOX + HSP, DOX + VIT.E and DOX + HSP + VIT.E groups as compared to DOX group (Figure 3).

Cardiac catalase activity was significantly increased in DOX and DOX + VIT.E groups as compared to control group. In contrast, a significant decrease was recorded in catalase activity of DOX + HSP and DOX + HSP + VIT.E groups with a slight decrease in DOX + VIT.E group as compared to DOX group (Figure 4C). In addition, Serum AE and PON activities were significantly reduced in DOX group as compared to control group. Serum AE was significantly elevated in DOX + HSP, DOX + VIT.E and DOX + HSP + VIT.E groups than DOX group (Figure 3C). Moreover, serum PON activity was significantly increased in DOX + VIT.E and DOX + HSP + VIT.E groups with a slight

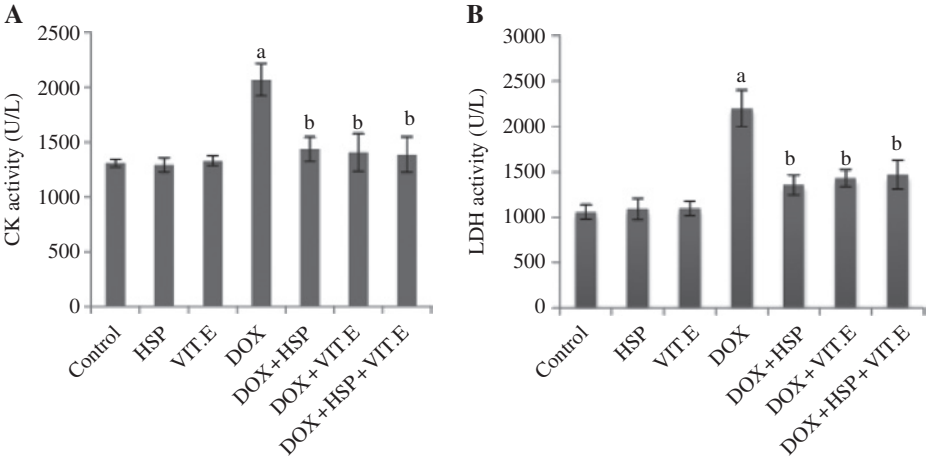


Figure 1: Serum CK (A) and LDH (B) activities were induced by DOX cardiomyopathy in rats and treated with HSP and VIT.E.
DOX: doxorubicin (4 mg/kg, i.p.); HSP: hesperidin (50 mg/kg, orally); VIT.E: vitamin E (100 mg/kg orally). Data are represented as mean ± SE, n = 10. (A) CK. (B) LDH. "a: $p < 0.05$" was significant versus control, "b: $p < 0.05$" was significant versus DOX group.

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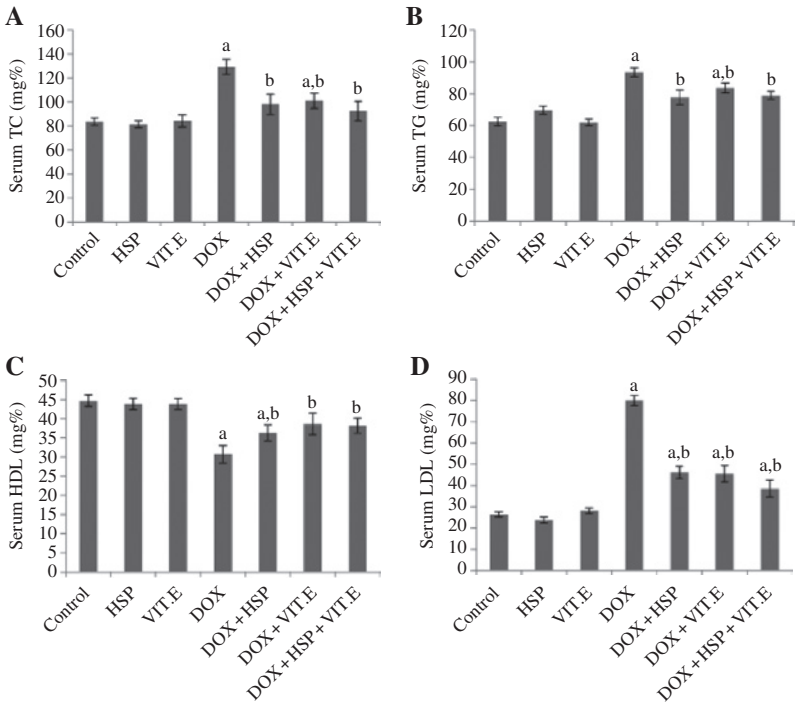


Figure 2: Serum lipid profile in rats in DOX induced rats cardiomyopathy and treated with HSP and VIT.E.
DOX: doxorubicin (4 mg/kg, i.p.); HSP: hesperidin (50 mg/kg, orally); VIT.E: vitamin E (100 mg/kg orally). Data are represented as mean ± SE, n = 10. (A) TC. (B) TG. (C) HDL. (D) LDL. "a: $p < 0.05$" recorded significance versus control, "b: $p < 0.05$" showed significance versus DOX group.

increase in DOX + HSP group as compared to DOX group (Figure 3D). Furthermore, cardiac caspase-3 activity in DOX group showed a significant elevation than control group. Conversely, there was a significant decrease in caspase-3 activity in DOX + HSP, DOX + VIT.E and DOX + HSP + VIT.E groups than caspase 3 activity of DOX group (Figure 4D).

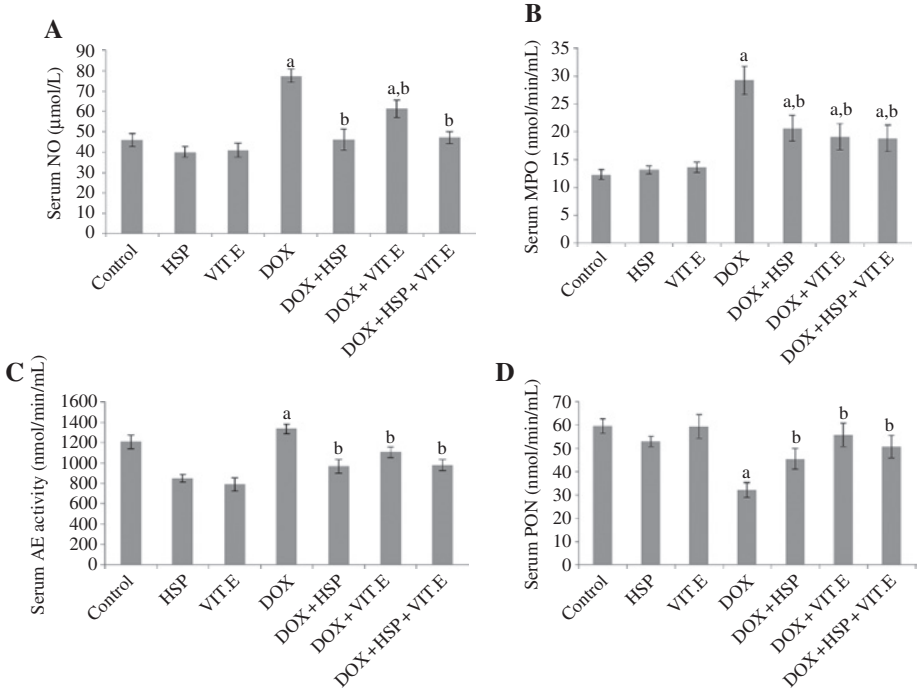


Figure 3: Serum NO (A), MPO (B), AE (C) and PON (D) activities in DOX induced rats cardiomyopathy and treated with HSP and/or VIT.E.
DOX: doxorubicin (4 mg/kg, i.p.); HSP: hesperidin (50 mg/kg, orally); VIT.E: vitamin E (100 mg/kg orally). Data are represented as mean ± SE. "a: $p < 0.05$" showed significance versus control. "b: $p < 0.05$" showed significance versus DOX group.

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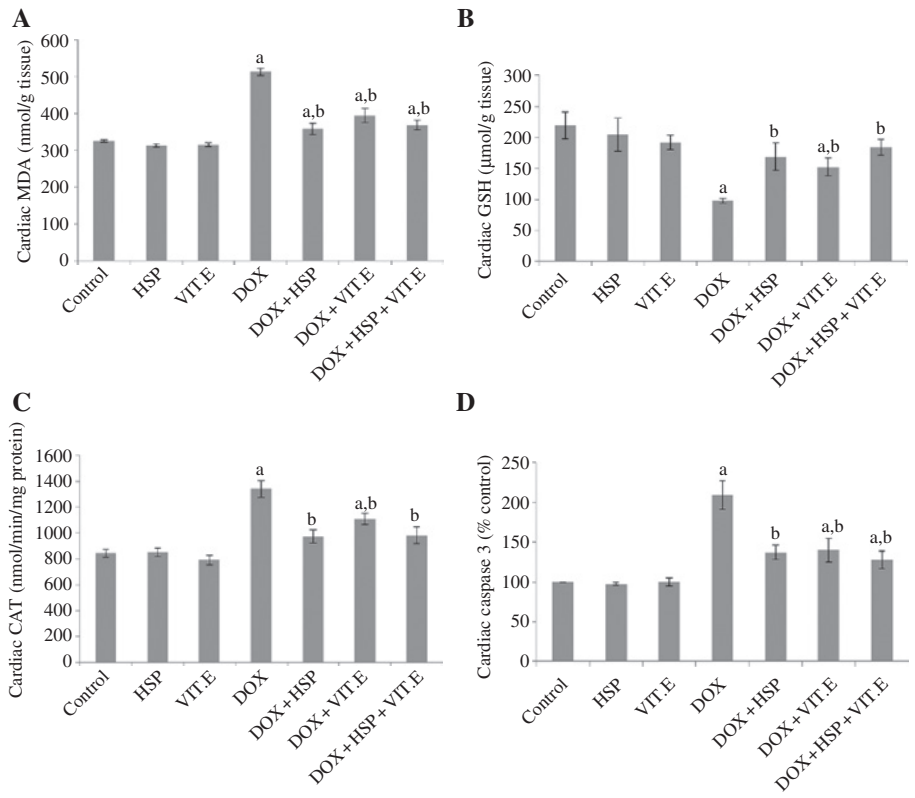


Figure 4: Cardiac MDA (A), GSH (B) levels, CAT (C) and caspase 3 (D) activities in DOX induced rats cardiomyopathy and treated with HSP and/or VIT.E.

DOX: doxorubicin (4 mg/kg, i.p.); HSP: hesperidin (50 mg/kg, orally); VIT.E: vitamin E (100 mg/kg orally). Data are expressed as mean ± SE. "a: $p < 0.05$" showed significance versus control. "b: $p < 0.05$" showed significance versus DOX group.

Heart histopathological analysis

Heart histopathological examination of control untreated group showed a normal structure of myocardial muscle cells in the myocardial bundles (Figure 5A and B). The histology of heart in rats administrated with HSP or VIT.E (Figure 5C–F, respectively) showed no alteration compared to control group.

DOX group showed extensive loss of myofibril, inflammatory cells infiltration, vacuolization of cytoplasm, and apoptosis of cardiomyocytes compared to control group (Figure 6).

In contrast to DOX injected rats, DOX + HSP group showed a well-preserved myocardium with low myofibril loss (Figure 7A and B). In addition, the histology of heart from DOX + VIT.E group showed fewer cytoplasmic vacuolization, decreased loss of myofibrils and lower infiltration in inflammatory cells when compared to DOX group (Figure 7C and D). Also, in heart tissue of DOX + HSP + VIT.E group we observed a lesser loss of myofibrils and a lower cytoplasmic vacuolization but no inflammation as compared to DOX group (Figure 7E and F).

Discussion

DOX-induced cardiomyopathy is mainly due stimulation of cardiac oxidative stress, which lead to depletion of endogenous antioxidants and free radical accumulation in the myocardium [23]. Therefore, the great interest has been focused on the use of natural antioxidants which scavenge free radicals can protect the heart from reactive oxygen species (ROS) induced by cancer chemotherapy [24, 25].

In this study, DOX injection led to severe cardiac injury increasing serum activities of LDH and CK in DOX-injected rats than that of control rats. These results confirmed the previous studies [26, 27]. The mechanism for the elevation of these markers seems is from the oxidative damaging effect of DOX to cardiac tissue and the subsequent excessive release of these markers into circulation [28].

On the other hand, oral administration of HSP and VIT.E either in single or in combination to DOX-injected rats could decrease the elevated levels LDH and CK (Figure 1) which might be due to their antioxidant and ROS scavenging properties. These results are similar with that of Adel-Raheem and Abdel-Ghany [28] who reported that

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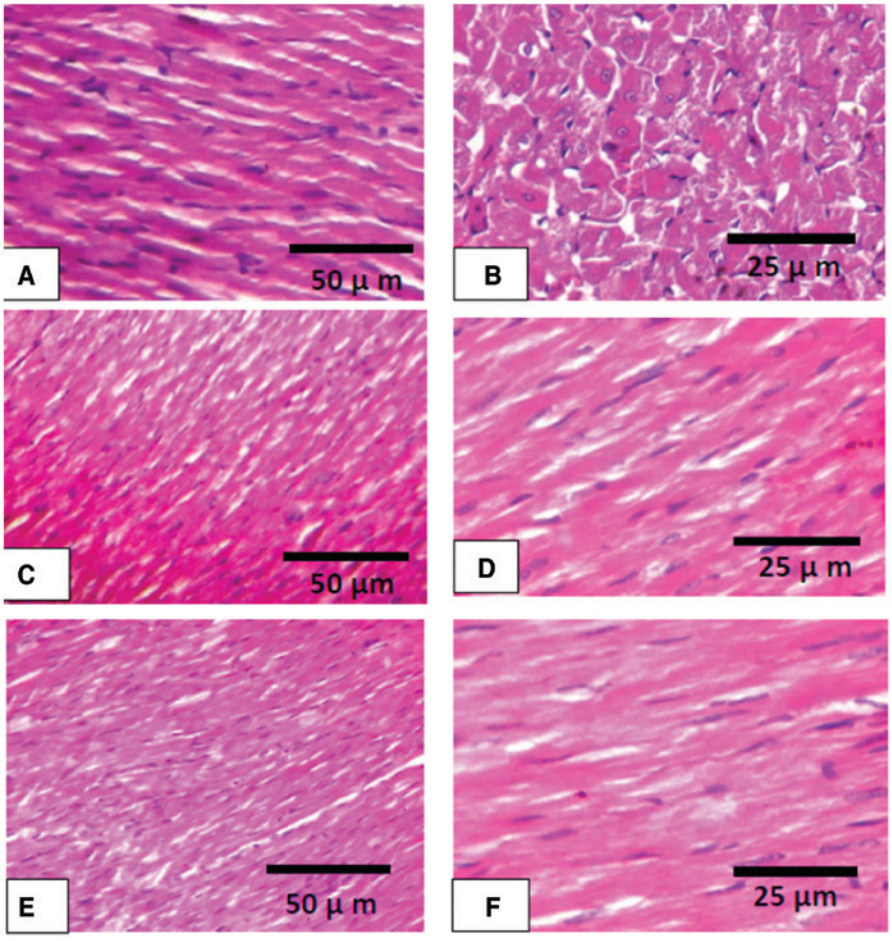


Figure 5: Histopathology of heart sections in control group (A, B), HSP group (C, D) and VIT.E group (E, F).
The photomicrographs of hearts from control untreated group (A and B), HSP group (C and D) and VIT.E group (E and F) showing normal structure of cardiomyocytes in muscle bundles.

prior administration of HSP to DOX injected rats significantly decreased the elevated activities of both enzymes. Also, Hadi et al. [11] showed that pretreatment of VIT.E to DOX group significantly decreased such elevation.

Lipids play a potent role in cardiovascular diseases. DOX was showed to interfere with the metabolism and biosynthesis of lipids [26]. In this study, serum triglycerides and total cholesterol were elevated in rats injected with DOX and in rats injected with DOX and administrated with VIT.E as compared to control rats. Also, serum LDL was elevated in rats injected with DOX and in rats injected with DOX and administrated with HSP and VIT.E either in single or in combination than serum LDL of control rats. Moreover, serum HDL was reduced DOX and DOX + HSP groups than serum HDL of control rats. Such elevation in lipid profile is matched with those of Chennuru and Saleem [26] who reported that DOX treatment significantly increased the serum levels of TG, TC, and LDL while decreased HDL level.

Doxorubicin generates ROS and the dissociation of the eNOS into monomers. Doxorubicin also causes the release of cytochrome C oxidase by entering mitochondria and prolongs the opening time of calcium channels in the sarcoplasmic reticulum, which activates calcineurin leading to apoptosis. Also, Oxidative stress activates Heat Shock Factor 1 and produces more Heat Shock Protein 25, which increases the pro-apoptotic proteins [23]. Furthermore, oxidative stress-induced inflammation and apoptosis in diabetic cardiomyopathy and this could be modulated by a natural product called Myricitrin [29].

Oxidative stress acts an important role in DOX-induced cardiotoxicity by inducing lipid peroxidation which negatively affects heart compared to other body organs due to overt oxidative metabolism and few antioxidant defenses in this organ [24].

In the current study, lipid peroxidation expressed as MDA was elevated in hearts DOX group than that of control rats; this supports an oxidative mechanism of

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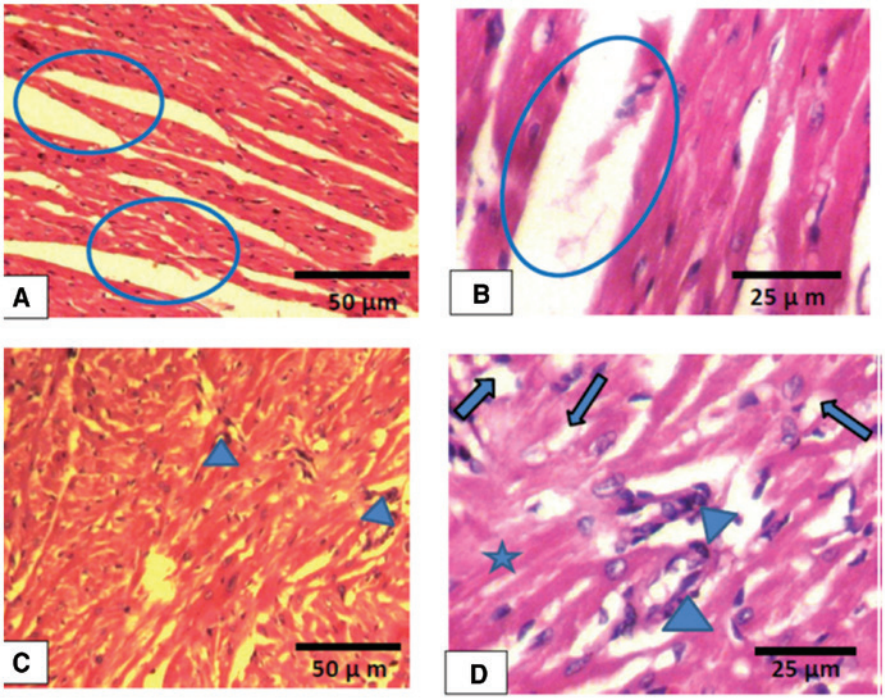


Figure 6: Histopathology of heart sections in DOX group.
The photomicrograph of heart of rat injected with DOX. Showing extensive loss of myocardial fibers (circle) (A and B), vacuolization of cytoplasm (arrow) (D), inflammatory cells infiltration (arrow head) (C and D), and apoptosis (star) (D).

DOX cardiotoxicity. This result was consistent with that of Trivedi et al. [25] and Abdel-Raheem et al. [30] who reported increased cardiac MDA in rats after DOX treatment.

Oral administration of HSP or VIT.E either in single or in combination to the DOX injected rats decreased the elevated MDA levels suggesting that the cardioprotective effects of HSP and VIT.E is because of their antioxidant action. These results are matched with that of Abdel-Raheem and Abdel-Ghany [28] who reported that HSP decreased oxidative stress markers including MDA in DOX-treated rats and Hadi et al. [11] who showed that treatment with VIT.E was significantly reduced DOX-induced MDA increase.

NO is mediating many physiological functions, such as local inflammation and tissue destruction [31]. Inducible nitric oxide synthase (iNOS) activity is elevated by various stimuli, including inflammatory cytokines and DOX. In case of DOX, excessive NO and anion superoxide were produced and interacted together forming peroxynitrite ($ONOO^-$) which causes fibrosis, hypertrophy, and dilation of heart tissue [31].

In our current study, an elevation in serum NO (expressed as NO_2/NO_3) was detected in DOX-injected rats and DOX + VIT.E group than serum NO level of the control group. This result was contradicted with that of Abdel-Raheem et al. [30] who found that DOX decreased serum NO_2 and NO_3 levels in studying the protective effects of Nicorandil, against DOX-induced rats cardiotoxicity.

MPO has mainly located in neutrophils primary granules and its main role is killing microorganisms; however, its excess oxidant production under certain conditions leads to tissue damage. MPO was recorded as a marker of cardiotoxicity in breast cancer patients treated with anthracyclins and Herceptin [32]. Our results showed that serum MPO activity was higher in DOX-injected rats, and DOX + HSP group than that in control rats, which indicate induction of an inflammatory response, accumulation of neutrophils and lipid peroxidation contributed to the cardiac injury. Administration of HSP and VIT.E to DOX-injected rats significantly decreased serum MPO activity than that of DOX group showing the anti-inflammatory effect of HSP and VIT.E (Figure 3B).

Our study also showed that cardiac GSH was lower in DOX group than control group. This depletion which clearly indicates an overt oxidative stress induced by DOX as previously reported by Swamy et al. [33].

Administration of HSP or/and VIT.E to the DOX-injected rats significantly increased GSH level compared to rats injected with DOX only. This confirms the antioxidant properties of HSP and VIT.E. In agreement with our results Abdel-Raheem and Abdel-Ghany [28] showed that administration of HSP to rats before DOX maintained the

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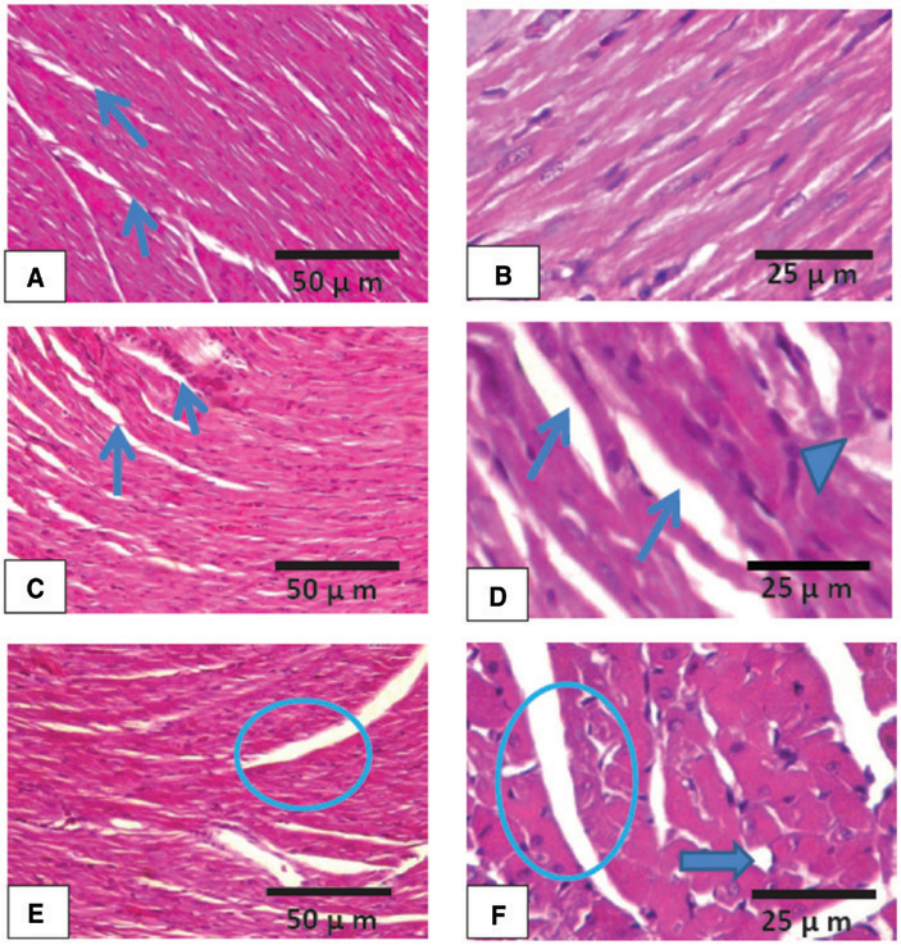


Figure 7: Histopathology of heart sections in DOX + HSP group (A, B), DOX + VIT.E group (C, D) and DOX + HSP + VIT.E group (E, F). The photomicrograph of heart of rat injected with DOX and administrated with HSP showing well preserved myocardium with low myofibril loss (arrow) (A and B). Heart photomicrograph of rat injected with DOX and administrated with VIT.E showing myofibril loss (arrow) and inflammation (arrow head) (C and D). Photomicrograph of heart of rat injected with DOX and administrated with HSP and VIT.E showing myofibril loss (circle) and vacuolization of cytoplasm (arrow head) (E and F).

GSH content at a higher level compared to that of DOX-treated rats.

Additionally, cardiac CAT activity was highly increased in DOX-injected rats and in rats administrated with VIT.E and injected with DOX. This result was in the same line of El-sayed et al. [34] who showed that DOX treatment significantly released the activity of cardiac catalase to be about two fold than the control group. Treatment with HSP or/and VIT.E to DOX-injected rats significantly decreased CAT activity than that of DOX + VIT.E group when both compared to that of DOX group.

PON1 is synthesized in the liver and associated with HDL in serum. Also, it plays an essential role as an antioxidant to prevent the oxidation of LDL which is involved in atherosclerosis development. PON1 exhibits a substrate-dependent activity polymorphism; It hydrolyzes many toxic metabolites including paraoxon or diethyl *p*-nitrophenyl phosphate, other organophosphorus compounds, lactones, and non-phosphorous arylesters [35].

As well as its protective role on vascular disease, PON1 is used as a biomarker of diseases involving in oxidative stress, since PON1 protects against oxidation, inflammation, [36] and a marker for liver diseases [37].

In our study, activities of serum arylesterase and paraoxonase were significantly reduced in DOX injected rats as compared to control rats (Figure 3C and D). This decrease is due to excessive oxidative stress caused by DOX. Also, DOX injection causes liver function abnormalities which may decrease in arylesterase and paraoxonase activities [3].

Oral administration of VIT.E alone or with HSP to DOX injected rats significantly increased paraoxonase and arylesterase activities than their activities in DOX group. Administration of HSP alone to DOX-injected rats caused a

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significant elevation in paraoxonase activity with a slight reduction in arylesterase activity than DOX group.

In the previous study of Chen et al. [38], it has been indicated that oxidative stress induced by DOX causes cardiomyocytes apoptotic death through caspase 3 activation [39]. In our study, caspase 3 activity was highly elevated in DOX injected rats than its activity in control rats.

Administration of HSP or/and VIT.E to the DOX group significantly decreased caspase 3 activity. These results are matched with study of Trivedi et al. [25] who showed that hesperidin (a derivative of HSP) treatment with DOX suppressed caspase 3 in rat cardiomyocytes.

In our histological observations DOX injection produced severe toxicity in cardiac tissues such as myocardial degeneration including myofibrillar loss, inflammatory cell infiltration, cytoplasmic vacuole formation and apoptosis of cardiomyocytes. These histopathological changes are in agreement with another investigator [26] who reported that DOX-treated rats have severe myocardial necrosis with muscles subendocardial loss. Also, in a study of Donia et al. [40] hesperidin ameliorated the cytotoxic effect of doxorubicin while enhancing its anti-tumor effect.

Administration of HSP to DOX-injected rats almost preserved the normal myocardium architecture. The hearts in DOX + VIT.E and DOX + HSP + VIT.E groups showed a significant improvement in architecture and less myofibril loss as compared to DOX-injected rats. These results are in accordance with that of Abel-Raheem and Abel-Ghany [28] who reported that HSP-DOX treated rats showed normal myocardial structure in comparison DOX-treated rats.

In conclusion, our study demonstrated that DOX injection caused cardiac injury in rats through elevation of serum LDH and CK activities and myofibril loss observed in histopathological examination. The toxicity of DOX is due to oxidative stress as evidenced by the elevation of cardiac MDA, serum NO levels as well as cardiac CAT and serum MPO activities with the depletion of cardiac GSH level, arylesterase, and paraoxonase activities. In addition, ROS released from DOX metabolism caused apoptotic tissue damage as manifested by elevation of cardiac caspase 3 activity. Administration of HSP and VIT.E with DOX ameliorate oxidative stress and antioxidant parameters and subsequently apoptotic tissue damage and cardiac injury. Thus, adjuvant administration of HSP and VIT.E with DOX could be a new promising solution in the treatment of fatal cardiac complications of DOX.

Conflict of interests statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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