

Role of Selenium in Attenuating Cardiac and Hepatic Damages Induced By the Antitumor Agent, Doxorubicin

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Abstract: Background and Objectives: The clinical use of doxorubicin, one of the most effective antitumor agents, soon proved to be hampered by such serious problems as the development of cardiomyopathy and liver damage. The current study aims at evaluating the role of trace element, selenium, in attenuating cardiac and hepatic damages induced by the antitumor agent, doxorubicin. **Materials and Methods:** Animals were divided into normal control group and doxorubicin -treated group injecting doxorubicin i.p. as 6 equal doses of 2.5 mg/kg, twice weekly/ 3 weeks. The doxorubicin - treated animals were divided into 2 groups, one kept without further treatment (doxorubicin -group), second group, (doxorubicin + selenium) received selenium (Na Selenite) 0.5 mg/kg orally, 3 times/week/4 weeks including one week before the doxorubicin 1st dose. Serum creatine phosphokinase, lactate dehydrogenase, as cardiac damage markers, and alanine aminotransferase, as indicator of hepatic damage, were measured. Malondialdehyde and nitric oxide levels, as cardiac oxidative status indices, cardiac glutathione content, glutathione peroxidase, glutathione-S-transferase and superoxide dismutase activities, as measures for cardiac antioxidant capacity, were also investigated. Histopathological changes in cardiac and liver tissues were examined. The results were analyzed statistically by one-way analysis of variance with subsequent multiple comparisons using Tukey test. **Results:** doxorubicin induced significant increase in serum lactate dehydrogenase; creatine phosphokinase; alanine aminotransferase activities, cardiac nitric oxide, malondialdehyde levels, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase activities, and reduction in glutathione content. Selenium co-administration caused significant decrease in serum lactate dehydrogenase and creatine phosphokinase levels; normalization of serum alanine aminotransferase; significant decrease in cardiac malondialdehyde, nitric oxide levels, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase activities and significant elevation in cardiac glutathione content, compared to doxorubicin -treated group values. Histopathological examination of cardiac and liver tissues supported the previous biochemical results. **Conclusions:** Chronic doxorubicin administration caused cardiomyopathy and hepatic damage. Selenium co-administration produced partial, but significant, protection against cardiomyocyte damage; however, it alleviated hepatic damage-induced by the antitumor agent, doxorubicin.

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Key words: Doxorubicin, cardiomyopathy, liver damage, selenium

Introduction:

Anthracyclines rank among the most effective anticancer drugs ever developed [1]. The first anthracycline was isolated early in the 1960s from the pigment producing *Streptomyces Peucetius*, a species of actinobacteria [2] and was named doxorubicin (Dox). Doxorubicin is an essential component of treatment of breast cancer [3], soft tissue sarcomas [4] and many other cancers [5]. Because Dox has been shown to produce free radicals, it was suggested earlier that free

radical injury might be a mechanism of Dox antitumor activity [6]. There now appears to be general agreement that oxidative stress is unlikely to be a significant contributor to the antitumor activity of Dox [7]. Liver is the main site of Dox metabolism, reduction of side chain carbonyl group by NADPH-Cyto P450 yields a more polar and toxic metabolite, doxorubicinol. Such metabolite accumulates in the heart and contributes significantly to chronic cumulative cardiotoxicity of Dox [8]. The enormous value of Dox in treating a variety of solid and

hematologic malignant conditions is unquestioned. However, as with any other anticancer agent, the clinical use of Dox soon proved to be hampered by such serious problems as the development of resistance in tumor cells [9] or toxicity in healthy tissues, most notably in the form of chronic cardiomyopathy and congestive heart failure[1]. These adverse effects of the drug can preclude its use in some patients and limit the duration of its use in many others [10].

Selenium (Se) plays an important biological role in living organisms, mostly through its incorporation in a family of proteins, selenoproteins. The main biological form of Se is selenocysteine, a cysteine analog that is synthesized from a serine bound to tRNA. The biological roles ascribed to Se include the prevention of cardiovascular disease [11] and cancer [12]. In the heart, Se supplementation caused increase in the cardiomyocyte glutathione peroxidase (GPx) activity, the total antioxidant activity, glutathione (GSH) concentration and catalase activity, leading to decreased generation of reactive oxygen species (ROS)[13]. The present study aims at evaluating the attenuating effect of Se, as an adjuvant therapy, on Dox- induced cardiac and hepatic damages.

Materials and Methods:

A. Animals:

Total numbers of 32 male albino rats of the Wister strain, weighing 170-200 g, were used in the present study. The animals were obtained from the central animal facility at the Faculty of Pharmacy, Cairo University, Cairo, Egypt. All rats were housed in a room with a controlled environment, at a constant temperature of $23 \pm 1^{\circ}\text{C}$, humidity of $60\% \pm 10\%$, and a 12 hrs light/dark cycle. The animals were housed in groups and kept at constant nutritional conditions throughout the experimental period. The experimental protocols were approved by the Ethical Committee of Cairo University.

B- Drugs and chemicals:

Doxorubicin HCL was obtained from Pharmacia & Upjohn, Milan, Italy. Sodium selenite was obtained from Sigma Chemical Company, USA. Other chemicals in the experiments were of analytical pure grade and supplied by British Drug House (BDH, UK), Merck (Germany) and Sigma Chemical Company (USA).

C- Experimental design:

Animals were divided into a normal control group (10 rats), receiving the appropriate volume of saline i.p, and Dox-treated group. Doxorubicin was dissolved in saline and injected i.p., as a total cumulative dose equal to 15 mg/kg, divided into 6 equal doses, 2.5 mg/kg each. They were injected twice weekly/ 3 weeks [14]. Dox-treated animals were divided into two groups, one kept without further treatment (Dox-group), and a second group (Dox + Se) received Se, as sodium selenite, 0.5 mg/kg, orally, 3 times/week/4 weeks including one week before the 1st Dox dose[15].

D- Serum and Tissue sampling:

24 hours following the last Dox injection, rats were sacrificed by decapitation. Blood sample of each animal was collected into a dry centrifuge tube. Serum was separated by centrifugation at 3000 r.p.m. /15 minutes and used to determine creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and alanine aminotransferase (ALT). Serum CPK activity was determined using a kit provided by STANBIO, USA. CPK catalyses the transphosphorylation of ADP to ATP through a series of coupled enzymatic reactions. NADH is provided at a rate directly proportional to CPK activity. The method determines NADH absorbance increase per minute at 340 nm [16]. Serum LDH activity was determined using a kit provided, also, by STANBIO, USA. LDH specifically catalyzes the oxidation of lactate into pyruvate with subsequent reduction of NAD to NADH. Rate of NADH formation is proportional to LDH activity. The method described determines NADH absorbance increase per minute at 340 nm [17]. Serum ALT activity was determined, using a kit provided by Quimica Clinica Aplicada, Spain[18].

Histopathological study:

The hearts and livers were removed by dissection, washed by ice-cold isotonic saline and blotted between two filter papers. Autopsy samples were taken from heart and liver in different groups of rats and fixed in 10% formol saline for 24 hrs. Washing was done, then, serial dilutions of alcohol were used for dehydration. Paraffin bees wax tissue blocks were prepared for sectioning the studied tissues. The obtained sections were stained by hematoxylin and eosin stains [19] for histopathological examinations through the light microscope.

Biochemical parameters:

10% w/v homogenate was prepared in ice-cold deionized water for the remainder of the heart tissues of the different groups.

Measurement of cardiac oxidative status indices: A portion of the homogenate was mixed with ice-cold 2.3% KCL (in ratio of 1:1) and centrifuged at 3000 r.p.m./15 minutes. Thiobarbituric acid (TBA) – reactive substance, malodialdehyde (MDA), content was determined in the supernatant [20], depending on measuring the coloured complex formed between TBA and MDA in acidic medium. Another aliquot of homogenate is centrifuged at 17.000 r.p.m./4°C/20 minutes. The resulted supernatant was used for the determination of nitric oxide (NO), as nitrite (NO_2^-) and nitrate (NO_3^-) concentrations [21], using Griess reagent after the enzymatic reduction of nitrate to nitrite. The Griess reaction involves the reaction of nitrite with sulfanilamide in an acidic solution to yield a diazonium salt, followed by coupling with N-(1-naphthyl) ethylenediamine to yield a colored azo dye that can be measured colourimetrically at 540 nm.

Measurement of some cardiac antioxidant systems: A portion of homogenate was mixed with ice-cold 7.5% sulfosalicylic acid (in a ratio 1:1) and centrifuged at 3000 r.p.m./ 15 minutes. The resulted supernatant was used for determination of GSH [22]. Another part of homogenate was mixed with equal volume of ice-cold Tris- EDTA buffer (pH =7.6), centrifuged at 39.000 r.p.m./ 4° C/ 20 minutes. The supernatant was used for determination of superoxide dismutase SOD; glutathione peroxidase (GPx) and glutathione S-transferase (GST). Determination of GST activity [23, 24] depends on the ability of GST to catalyze the formation of glutathione adduct with 1-chloro,2,4 dinitrobenzene(CDNB). This adduct was measured by noting the net increase in absorbance at 340nm. Determination of GPx [25] depends on measuring the rate of oxidized GSH formation, by following up the decrease in absorbance of the reaction at 340 nm as NADPH was converted to NADP. Superoxide dismutase activity was determined [26], depending on the fact that the spontaneous autoxidation of pyrogallol, at alkaline pH less than 9.5, produces superoxide anion, which in turn enhances further oxidation of pyrogallol with a resultant increase in absorbance at 420 nm. The presence of SOD in the reaction medium retards pyrogallol autoxidation by scavenging the formed superoxide anion.

Statistical analysis:

The results were analyzed statistically by one-way analysis of variance (ANOVA test) with subsequent

multiple comparisons using Tukey test. Differences were considered statistically significant at p less than 0.05. The results were presented as the mean \pm standard error of the mean (SEM). Data obtained were submitted to a computerized statistical treatment using SPSS statistical package, version 17. Graphs were represented by Harvard graphics version 4 computer program.

Results:

Results revealed that Dox caused significant increase in serum levels of LDH and CPK, amounting to 182.4% and 183.6% respectively, as compared to the normal values (Fig. 1). Selenium co-administration caused significant decrease in the activities of LDH and CPK reaching to 139.5% and 153.6%, respectively of the control values. Figure (2) illustrated that, Dox caused a significant increase in cardiac MDA and NO contents, amounting to 183.36% and 177.7%, respectively, compared to the control values. Concomitant administration with Se caused significant decrease in MDA and NO levels reaching to 126% and 120%, compared to the control values.

As shown in figure (3), Dox administration caused a significant decrease in cardiac GSH level reaching to 64% of the normal values. Co-administration of Se significantly elevated GSH content to about 77.9% of the control values. Figure (4) showed significant increases in cardiac activities of GPx and GST in the Dox-treated rats, amounting to 410% and 184% respectively, compared to the normal values. Meanwhile, the co-administration of Se caused significant decrease in the levels of GPx and GST to about 166% and 136% of the normal values. Figure (5) showed significant increases in cardiac activity of SOD in the Dox-treated rats amounting to 225% compared to the normal value. Co-administration of Se caused significant decrease in the level of SOD to about 172% of the normal values. Results of figure (6) revealed that Dox administration caused significant elevation in the serum ALT level to reach 118% of the normal control level. Selenium co-administration caused normalization of the elevated ALT level.

Cardiac histopathological results showed that; in the control sections, the cardiac muscle fibers were grouped in bundles with connective tissue in between. The single muscle fiber had acidophilic cytoplasm and a central nucleus (Figure 7). In the cardiac sections obtained from rats administrated Dox, hyalinization was observed in the myocardial bundles associated with either inflammatory cells infiltration only or inflammatory cells and edema in focal manner in

between the bundles. Edema was also noticed in the subendocardial layer. The subendocardial adipose tissue was infiltrated by inflammatory cells (Figures 8, 9, 10). In the cardiac sections obtained from rats administrated Dox + Se, there was mild hyalinization in the myocardial muscle bundles (Figure 11).

Examination of liver sections of the different groups illustrated that: Liver tissue of the normal group showed hepatic lobules with normal architecture (Figure 12). In case of liver sections of rats administrated Dox, congestion was observed in the central vein, in addition to kupffer cells proliferation in diffuse manner between the fatty degenerated hepatocytes (Figures 13). In case of liver sections of rats administrated Dox + Se, least liver damage was shown, just kupffer cells proliferation was observed in between hepatocytes (Figure 14).

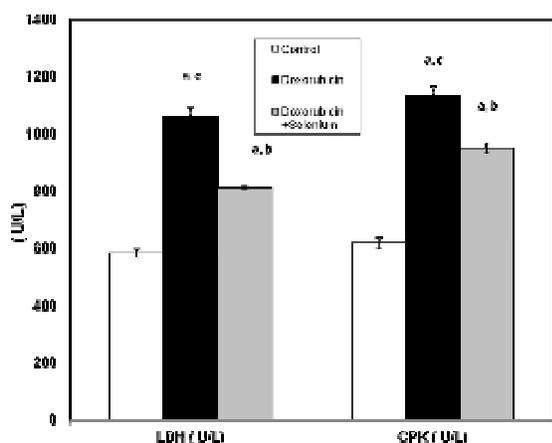


Fig (10): Effect of selenium on doxorubicin-induced alterations in serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities in rats. Values are given as mean \pm SE (No. of observations are given in parentheses). a: Significant difference from control group at P<0.05; b: Significant difference from Doxorubicin group at P<0.05; c: Significant difference from Doxorubicin + Selenium group at P<0.05

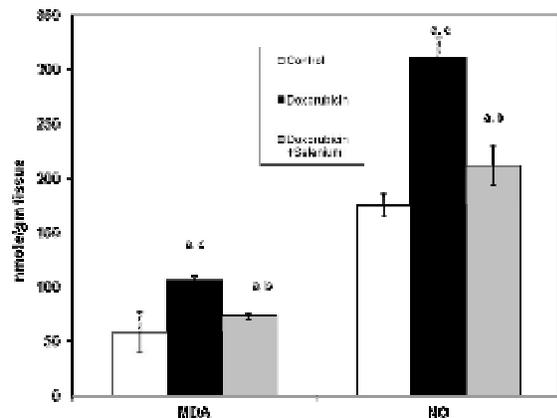


Fig (12): Effect of Selenium on doxorubicin-induced alterations in cardiac malondialdehyde (MDA) and nitric oxide (NO) levels in rats. Values are given as mean \pm SE (No. of observations are given in parentheses). a: Significant difference from control group at P<0.05; b: Significant difference from Doxorubicin group at P<0.05; c: Significant difference from Doxorubicin + Selenium group at P<0.05

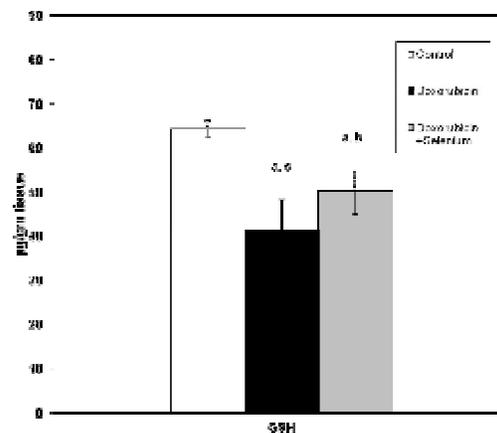


Fig (13): Effect of Selenium on doxorubicin-induced alterations in cardiac glutathione (GSH) content in rats. Values are given as mean \pm SE (No. of observations are given in parentheses). a: Significant difference from control group at P<0.05; b: Significant difference from Doxorubicin group at P<0.05; c: Significant difference from Doxorubicin + Selenium group at P<0.05

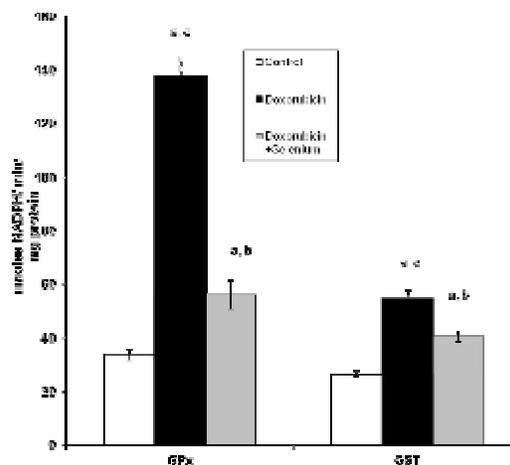


Fig (14): Effect of Selenium on doxorubicin-induced alterations in cardiac glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities in rats. Values are given as mean \pm SE (No. of observations are given in parentheses). a: Significant difference from control group at P<0.05; b: Significant difference from Doxorubicin group at P<0.05; c: Significant difference from Doxorubicin + Selenium group at P<0.05

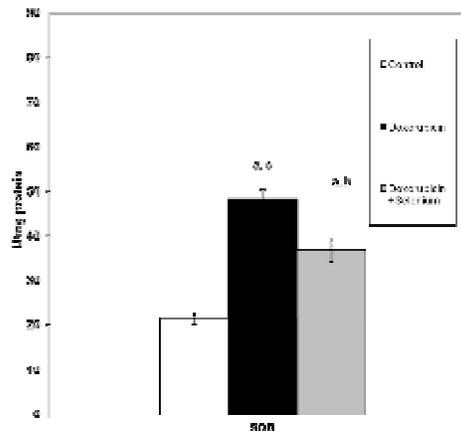
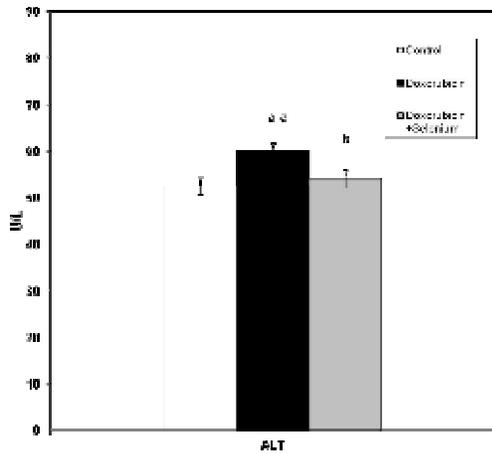
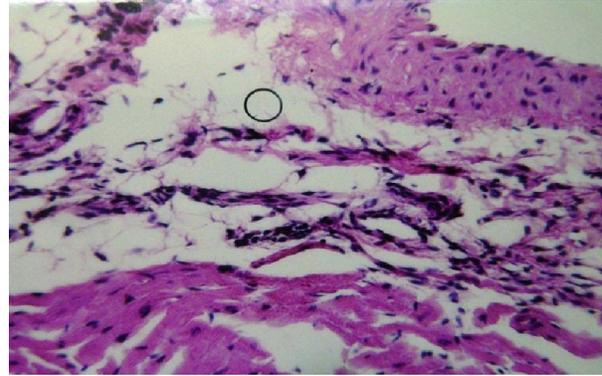


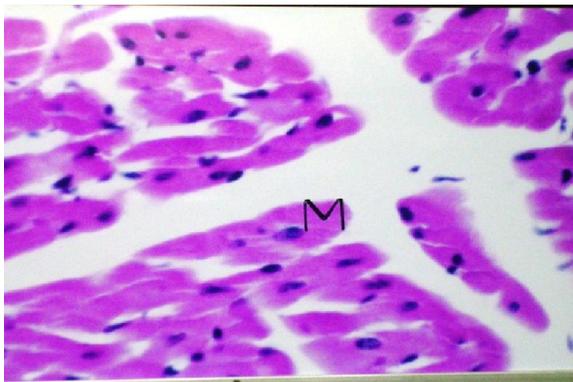
Fig (15): Effect of Selenium on doxorubicin-induced alterations in cardiac superoxide dismutase (SOD) activity in rats. Values are given as mean \pm SE (No. of observations are given in parentheses). a: Significant difference from control group at P<0.05; b: Significant difference from Doxorubicin group at P<0.05; c: Significant difference from Doxorubicin + Selenium group at P<0.05



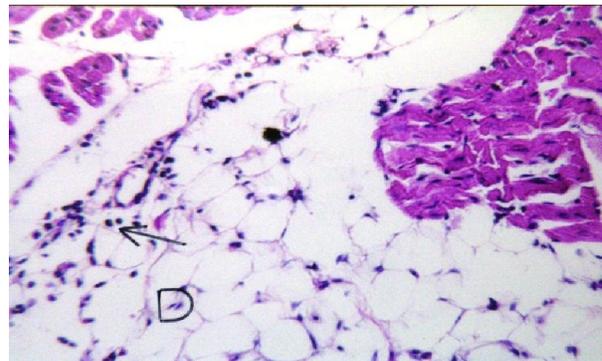
Fig(6):Effect of Selenium on doxorubicin- induced alteration in serum alanine aminotransferase (ALT) activity in rats. Values are given as mean \pm SD. Statistical significance is given in parentheses as follows: (p) between Doxorubicin and Selenium; (q) between Doxorubicin and Doxorubicin + Selenium; (r) between Doxorubicin and Selenium.



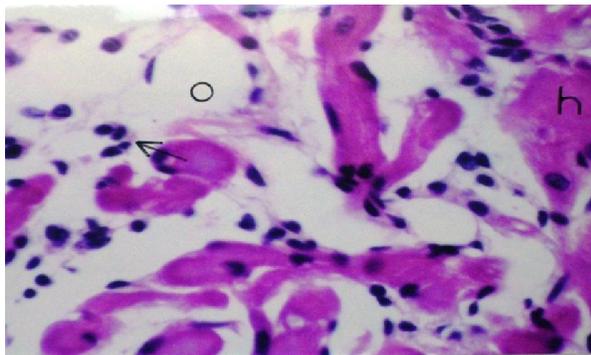
Figure(9): A photomicrograph of cardiac muscle fibers of Dox group showing Subendocardial oedema(o). (H&E 64)



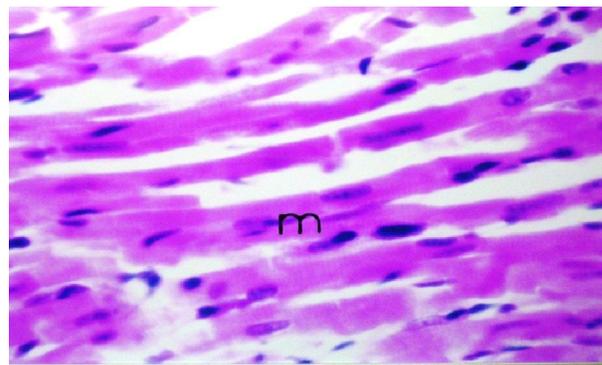
Figure(7): A photomicrograph of cardiac muscle fibers of control group showing normal histological structure of myocardium(M) (H&E 160)



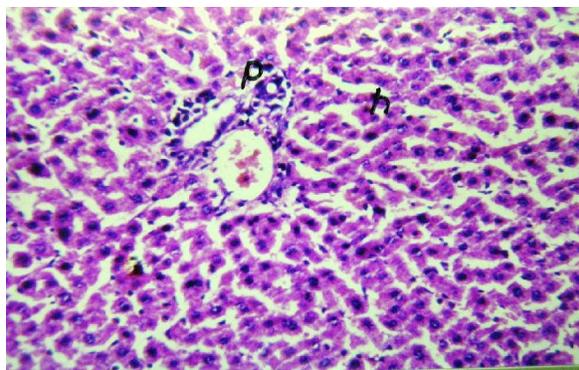
Figure(10): A photomicrograph of cardiac muscle fibers of Dox group showing inflammatory cells infiltration (arrow) in the subendocardial adipose tissue(D) (H&E 64)



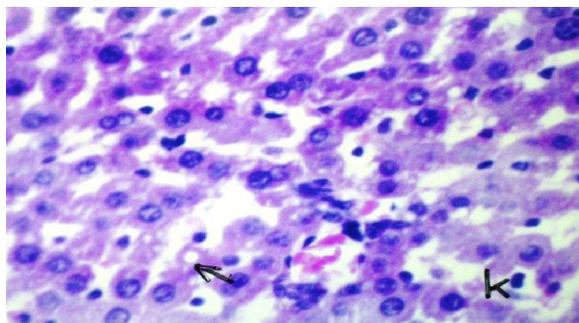
Figure(8): A photomicrograph of cardiac muscle fibers of Dox group showing Oedema(o) with inflammatory cells infiltration (arrow) in focal manner between the myocardial bundles. (H&E 160)



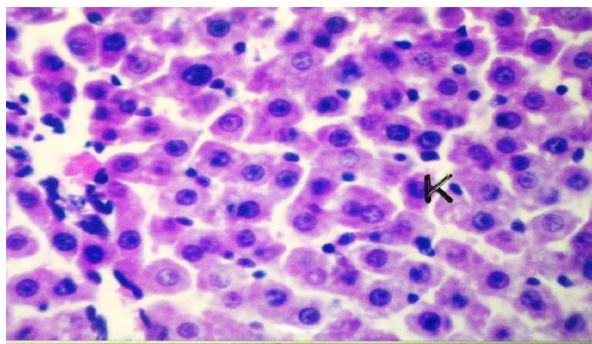
Figure(11): A photomicrograph of cardiac muscle fibers of Dox+Se group showing mild hyalinization in myocardial bundles(m) (H&E 160)



Figure(12): Photomicrograph of liver of normal group showed hepatic lobules (h) and portal vein (p) with normal architecture (H&E 64)



Figure(13): Photomicrograph of liver of Dox group showing diffuse kuffer cells proliferation(k) inbetween the fatty degenerated hepatocytes (arrow) (H&E 160)



Figure(14): Photomicrograph of liver of Dox + Se group showing diffuse kuffer cells proliferation(k) inbetween the fatty hepatocytes (H&E 160)

Discussion:

Doxorubicin-induced cardiomyopathy has long been a serious side effect in treating human cancers, which limits the clinical dosage of Dox [27]. The mechanism of Dox-induced cardiotoxicity is attributed

to the formation of ROS and subsequent changes of membrane fluidity and integrity. Oxidative stress is generally held as the mediating mechanism in the multiple biological processes leading to Dox cardiotoxicity [28]. Nutritional strategies designed to augment cellular defense systems have been identified as a promising approach to combat oxidative stress-associated disease conditions. In this respect, dietary supplementation with Se, potentially adjusting antioxidant enzymatic status, could offer protection in preventing free radical-induced cardiac injury. In the present study, role of trace element, selenium, in attenuating cardiac and hepatic damages induced by antitumor agent, doxorubicin was studied.

Results of the present study revealed that 15 mg/kg total cumulative dose of Dox induced cardiac and hepatic damages, manifested biochemically by significant increase in serum activities of LDH; CPK and ALT. Additionally, Dox caused elevation in cardiac NO, MDA levels, SOD, GPx, GST activities, and reduction in GSH content. Histopathological examination of heart and liver sections of Dox-treated animals supported these biochemical results. Selenium administration, concomitant to Dox therapy, caused significant decrease in the serum activities of LDH and CPK; cardiac MDA, NO levels, GPx, GST and SOD activities and significant elevation in cardiac GSH content, compared to Dox-treated group values, as well as normalization of serum ALT level.

The present results showed significant increase in serum levels of LDH and CPK in Dox-treated group. These enzymes are considered important markers of cardiac injury. Many previous studies have demonstrated similar results in rats following Dox administration [29, 30]. Different types of Dox cardiotoxicity can be recognized [31]: "Acute" cardiotoxicity occurs during Dox administration, however, these effects are never of major concern because these are generally reversible and/or clinically manageable. "Early chronic" cardiotoxicity develops later in the Dox treatment course and characterized by dilated cardiomyopathy, with subsequent development of congestive heart failure [32]. It is now well established that Dox cardiotoxicity may manifest even decades after the completion of anticancer treatment [33]. Co-administration of Se with Dox therapy resulted in decrease in the elevated activities of serum LDH and CPK. This finding is in harmony with this stated by Simoni *et al.*[34], who reported that the elevation in serum LDH activity, as a result of hemoglobin cardiotoxicity, is significantly decreased by Se dietary supplementation. Our biochemical results are supported by the histopathological examination of

the cardiac tissue, since, the marked morphological changes shown in the hearts of Dox- treated animals have been partially preserved by Se administration.

Dox therapy caused significant increase in MDA level. Previous studies reported similar results [35, 36]. This elevation might be attributed to Dox mediated oxidative stress. Heart tissue is rich in mitochondria, which occupy about forty percent of the total intracellular volume of cardiomyocytes [37]. Dox has high affinity for cardiolipin, a negatively charged phospholipid abundant in the mitochondrial inner membrane, leading to mitochondrial accumulation of Dox [38]. Under clinically relevant plasma Dox concentrations, the heart becomes a site of redox reactivity. The quinone functionality of Dox is transformed, in the presence of NADH, into a semiquinone via one-electron reduction by complex I of the electron transport chain [39]. The semiquinone form reacts with O₂ to produce a superoxide radical (O^{•-}), whereby Dox returns to the quinone form. The cycling of Dox between quinone and semiquinone generates large amounts of O₂, which further give rise to a variety of ROS/RNS species [40]. ROS can damage membrane lipids and other cellular components and consequently lead to cardiomyocyte apoptosis or death [41]. Our results showed that lipid peroxidation induced by Dox is significantly decreased in the pretreatment of Se, as manifested by significant reduction in the elevated level of cardiac MDA, which is consistent with previous studies [34, 42]. Previously, it was reported that Se supplementation can protect against free radical damages by increasing myocardial Se content and improving the expression and activity of GPx [43].

The present study revealed a marked increase in cardiac NO level in those Dox-treated rats. This finding is in agreement with the results reported by Saad *et al* [36] who used a model of doxorubicin chronic cardiotoxicity similar to that used in our study. The increase in NO level can be explained on the basis of the ability of Dox to mediate the induction of NOS expression and NO release in heart [44]. Several reports indicate that exposure of endothelial cells to H₂O₂ promotes eNOS expression [45]. Previous studies also suggested that stimulation of endothelial cells with calcium-mobilizing agents could activate eNOS [46]. Because Dox-induced toxicity is mediated by intracellular H₂O₂ as well as the calcium influx, Dox treatment causes an increase in eNOS transcription and protein activity in aortic endothelial cells and thus NO synthesis. On the same line, recent study provides evidence of upregulation of iNOS gene and protein expressions in Dox-induced cardiomyopathy[47]. The

concomitant overproduction of NO and ROS is known to yield highly reactive nitrogen species, peroxynitrite, which may attack and destroy important cellular biomolecules [48]. Selenium, in the present study, caused significant decrease in the elevated cardiac NO level shown in the Dox-treated group, which is in agreement with that reported by Ayaz and Turan [49]. The exact mechanism by which Se influence cardiac NO synthases expression is unknown. Of interest in this context is the report that treatment of nuclear extracts of lipopolysaccharide-activated human T cells with relatively high concentrations of selenite inhibited nuclear factor - B binding and thus decreased NO production [49]. This is because nuclear factor - B is a transcription factor that regulates a number of cellular genes, such as those encoding iNOS [50].

Doxorubicin administration, as shown in our results, caused a significant decrease in cardiac GSH content, which is quiet compatible with previous studies [35]. The overproduction of ROS, caused by Dox administration, can account for this decrease in GSH content, as these species are detoxified by endogenous antioxidants mainly GSH causing their cellular stores to be depleted [51]. The decrease of cardiac GSH content may also be attributed to the enhanced activities of GSH metabolizing enzymes by Dox administration, as shown in the present study. One is GPx which reduces H₂O₂ and various peroxides using GSH as reducing agent. The other is GST which consumes GSH in the conjugation of Dox toxic metabolites [52]. The present study showed that cardiac GSH concentration is higher in (Se+ Dox) -treated rats rather than those administered Dox alone, which is in agreement with recent results [53]. Such effect might be attributed to the antioxidant properties of Se and its ability to reduce Dox- induced oxidative stress. Selenium reduces the consumption of GSH by ROS [53]. Because GSH is one of the essential compounds for maintaining cell integrity [54] and the GSH redox cycle is one of the most important intracellular antioxidant systems, the increase in GSH content could be one of the mechanisms for cardiac protection by Se supplementation [53]. In addition, we assumed that the observed increase in cardiac GSH content might be related to the decreased activities of GSH-utilizing enzymes, GPx and GST, shown in the (Dox + Se) - treated group, leading to preservation of their substrate, GSH.

Our results showed significant increase in cardiac activity of SOD in the Dox-treated rats, which is consistent with some studies [35, 55]. The increase in SOD activity can be explained on the basis that the redox cycling of Dox between quinone and

semiquinone forms generates large amounts of O_2 [40], which in turn stimulate SOD as an adaptive response to counteract oxidative stress. The increased activity of SOD could lead to overproduction of hydroperoxides, in consequence, GPx might be stimulated in response to the accumulated peroxides. This assumption was supported by our results, which showed a significant enhancement in cardiac GPx activity in the Dox-treated group, and also by some authors [36]. On the same line, GPx have been reported to be over expressed in Dox-treated cells, especially those tumor resistant ones [56]. The current study revealed that Se administration caused significant decrease in Dox-induced elevation in SOD cardiac activity, which still higher than that of the normal group value. This result is in harmony with several previous studies. Selenium has been reported to decrease the elevated activity of SOD in heart as a result to cadmium toxicity [57] and hemoglobin mediated cardiotoxicity [34], which used Se dose similar to that used in our study. It is now well established that Se, through its incorporation in selenoproteins, could actively protect against free radicals generation, and hence, ROS- induced damage [58]. As a result of this protective effect, Se consumption could attenuate superoxide radical production, and consequently, decreased the activity of such antioxidant enzyme. Also, our study showed that pretreatment with Se relieved Dox induced hyperactivity of cardiac GPx, which is in harmony with Ayaz and Turan [49]. Glutathione peroxidase is one of the most active antioxidant enzymes in the myocardium [59], and selenium, present in its active site, is essential for its activity. One of the major roles of this essential trace element in the body is to act as cofactor of this key antioxidant enzyme in which it contributes to both catalytic activity and spatial conformation [60]. Therefore, any significant modification of Se status would lead to changes in the activity of GPx and have important consequences on the susceptibility of the tissues to oxidative stress [13, 61]. Selenium has prophylactic action, when it is administered before doxorubicin, it increases myocardial selenium content and improves both expression and activity of GPx. This may account for the increased cardiac GPx activity in (Se+ Dox)-treated group, compared to the normal control value, as shown in our results. Upon pretreatment with Se, myocardial tissues became already protected, and therefore, when exposed to Dox, we assume that there is no need for farther dramatic increase in cardiac GPx activity as an adaptive mechanism. This might be an explanation for the decrease in GPx cardiac activity in (Se+Dox) group value, compared to that result shown in the group treated with Dox alone. Additionally, the obtained biochemical results were supported by each other, since, as we mentioned later, Se supplementation caused

decrease in the SOD activity, which consequently, leads to decreased production of H_2O_2 and hence, decreased activity of GPx enzyme.

Results of the present study revealed significant increase in cardiac GST activity in rats treated with Dox, which is in agreement with many studies [62]. GSTs are family of dimeric proteins that possess a multitude of functions including the enzymatic conjugation of GSH to electrophilic xenobiotics [63]. It has been reported that cellular exposure to xenobiotics and antioxidants leads to coordinated induction of a battery of genes encoding detoxifying enzymes including GST [64]. Indeed, GSTs belong to phase II enzymes that in contrast to phase I, who can participate in both metabolic activation and inactivation, predominately participate in the detoxification of xenobiotics [65]. It has been known that Dox is metabolized via aldehyde reductases yielding C13 hydroxyl derivative, doxorubicinol. This metabolite is actually more polar and toxic than Dox itself. Doxorubicinol accumulates in the heart and contributes significantly to chronic cumulative cardiotoxicity induced by Dox [8]. It has been reported that Dox toxic metabolites are efficiently conjugated with reduced GSH, a reaction that is catalyzed by GST [52]. Moreover, GST, due to its peroxidase activity, can serve to reduce Dox-induced peroxides [52]. In brief, GST has showed elevation after Dox injection to detoxify Dox and its metabolites and to attenuate the elevated oxidative stress [66]. The current study revealed that Se caused significant decrease in Dox-induced elevation in GST cardiac activity, which still higher than that of the normal group value. This result is in agreement with previous studies stated that Se afforded reduction in cadmium- induced elevation in GST cardiac activity [57]. The protective effects of Se are mainly related to its physiological antioxidant properties, and hence, decreased generation of ROS and RNS. Thus, Se supplementation could decrease the activity of GST enzyme responsible for the detoxification of such free radicals.

Our results showed an elevation in serum ALT upon Dox administration which agrees with many previous studies [67, 68] and supported by the present histopathological examinations. Doxorubicin -induced hepatotoxicity might be less severe than its cardiotoxicity, which can be related to the fact that liver mitochondria, unlike cardiac mitochondria, lack the NADH-related pathway of reducing equivalents from the cytosol to the respiratory chain. As a result, liver mitochondria do not generate significant amounts of Dox semiquinones [69]. Selenium co-administration was shown to decrease the elevated serum ALT when

administered with Dox, as reported previously [34]. Selenium supplementation could reduce hepatotoxicity by rendering hepatic tissues less susceptible to lipoperoxidative attack by the drug [70]. Selenium prevents hepatocyte oxidative damage and thus leakage of liver enzymes into serum as in the cases of cadmium hepatotoxicity [71]. This biochemical result is supported by the histopathological examination of liver sections of the different groups which illustrated that, in the liver sections of rats administered Dox, congestion and kupffer cells proliferation were observed, while sections from rats administered Dox+Se showed least liver damage.

In conclusion, Se supplementation produced partial, but significant, protection against Dox-induced cardiomyocyte damage; however, such trace element could alleviate the Dox- induced hepatic damage, as evidenced by the biochemical measurements and histopathological examinations of the cardiac and hepatic tissues.

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