Modulating Effect of Carvedilol on Doxorubicin-Induced Cardiomyopathy and Hepatic Damage

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Abstract: Background: Doxorubicin is an anthracyclin antibiotic that is considered as one of the most effective antitumor agents. The clinical use of doxorubicin soon proved to be hampered by such serious problems as hepatotoxicity and most notably cardiomyopathy. Objectives: The current study aims at evaluating the efficiency of carvedilol as an adjuvant therapy with doxorubicin to protect against doxorubicin - induced cardiomyopathy and hepatic damage. Materials and Methods: Animals were divided into normal group and doxorubicin -treated group injecting doxorubicin as a dose of 2.5 mg/kg/twice weekly/ 3 weeks. Doxorubicin - treated animals were divided into two groups, one kept without further treatment (doxorubicin group) and second group, (doxorubicin + carvedilol), received carvedilol 1mg/kg/ 7 times over a period of 4 weeks including a dose before doxorubicin 1st dose. 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, 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 hepatic tissues of all groups were examined. Results and Conclusions: Our results revealed that doxorubicin caused oxidative stress which plays a major role in doxorubicin -induced cardiomyopathy and hepatic damage. Co-administration of carvedilol in concomitant with doxorubicin caused protection against doxorubicin–induced cardiomyopathy; however, it augmented doxorubicin -induced hepatic damage. Histopathological examination of cardiac and hepatic tissues supported the previous biochemical results. [Journal of American Science. 2010;6(12):20-32]. (ISSN: 1545-1003).

Keywords: Doxorubicin, carvedilol, cardiomyopathy, hepatic damage.

1. Introduction

Almost all clinically used antitumor drugs exhibit toxic side effects affecting heart function. Because of cardiotoxicity during anticancer chemotherapy, effective doses of cytostatics have to be limited, which may worsen antitumor efficacy. Doxorubicin (Dox) is an anthracyclin antibiotic that is considered as one of the most effective antitumor agents. Dox is an essential component of treatment of breast cancer (1), soft tissue sarcomas (2) and many other cancers (3). The immense value of Dox in treating a variety of malignant conditions is unquestioned. However, the clinical use of Dox soon proved to be hampered by such serious problems as the development of resistance in tumor cells (4) and toxicity in healthy tissues, in the form of central nervous system toxicity (5), nephrotoxicity (6) and most notably in the form of cardiomyopathy and congestive heart failure (7). These adverse effects of the drug can preclude its use in some patients and limit the duration of its use in many others (8).

Carvedilol is non cardioselective β-blocker which lacks intrinsic sympathomimetic activity. In addition, it has blocking effects at vascular α1-receptors, antioxidant, and calcium antagonist properties (9). The antioxidant activity of carvedilol was examined in a variety of *in vitro* and *in vivo* assay systems, including physicochemical, biochemical and cellular models. The data indicate that carvedilol prevents electron adduct formation in both aqueous or lipid environments containing either superoxide- or hydroxyl-radical generating systems (10). Furthermore, carvedilol and several of its metabolites are as effective in inhibiting lipid peroxidation in brain and heart membranes (11). The cardioprotective effect of carvedilol has been shown in a variety of *in vitro* and *in vivo* models. The efficacy of carvedilol has been observed with anthracyclin cardiomyopathy and ischemia/ reperfusion (12). One most likely mechanism of cardioprotection by carvedilol is the antioxidant effect (13-15).

The current study aims at evaluating the efficiency of carvedilol as a protective agent against cardiomyopathy and hepatic damage induced by Dox.

2. Materials and Methods:

A- Animals:

We used a total of 32 male albino rats of the Wister strain, weighing 170-200 g, that 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 ± 1^0 C, humidity of 60% ± 10%, and a 12 hr 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 Faculty of Pharmacy, Cairo University.

B- Drugs and chemicals:

Doxorubicin HCL was obtained from Pharmacia & Upjohn, Milan, Italy. Carvedilol was obtained from Cadila Pharmaceuticals Limited, India. Other chemicals in the experiments were of analytical pure grade and supplied by British Drug House (BDH, UK) 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 (22 rats). Dox was dissolved in saline and injected i.p. as total cumulative dose of 15 mg/kg, divided into 6 equal doses, each of 2.5 mg/kg. They were injected twice weekly/ 3 weeks (16). The Dox-treated animals were divided into two groups, one was kept without further treatment termed Dox-group(12 rats), and a second group (10 rats), termed Dox + carvedilol group, received carvedilol as an i.p dose of 1mg/kg / 7 times over a period of 4 weeks including a dose before the 1st Dox dose (17).

D- Serum and Tissue sampling:

Twenty four hours following the last Dox injection, rates were sacrificed by decapitation. A 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 (18). Serum LDH activity was determined using a kit provided also by STANBIO, USA (19). Serum ALT activity was determined, using kit provided by Quimica Clinica Aplicada, Spain (20).

For determination of the biochemical parameters and histopathological changes, hearts and livers were removed by dissection, washed by ice-cold saline and blotted between filter papers.

Histopathological study:

Samples were taken from hearts and livers of rats in different groups and fixed in 10% formol saline for 24 hours. Washing was done in tap water, then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene, embedded in paraffin at 56^O C in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains (21) for histopathological examinations using light microscope.

Biochemical parameters:

The remainder of heart tissue of each rat was weighed and homogenized in ice-cold saline for 1 minute, using an ice-cold Potter Elvejhem glass homogenizer, forming 10% w/v homogenate.

Estimation of cardiac glutathione (GSH) content

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 the determination of GSH (22), depending on the reaction between GSH and 5, 5'- dithio-bis, 2-nitrobenzoic acid to yield a stable yellow colour which can be measured colourimetrically.

Estimation of cardiac malondialdehyde (MDA) level:

Another portion of homogenate was mixed with ice-cold 2.3% KCL (1:1), centrifuged at 3000 r.p.m/15 minutes. The level of MDA was determined in the supernatant depending on measuring the coloured complex formed between thiobarbituric acid reagent and MDA in acidic medium (23).

Preparation of cytosolic fraction for the estimation of glutathione peroxidase (GPx), glutathione-S–transferase (GST) and superoxide dismutase (SOD) activities:

Part of the homogenate was mixed with equal volume of ice-cold Tris- EDTA buffer (pH=7.6), centrifuged at 39.000 r.p.m/4ºC/ 20 minutes. The resulted supernatant was used for the determination of GST; GPx and SOD activities. Determination of GST activity (24, 25) depends on the ability of GST to catalyze the formation of GSH- adduct with 1-chloro-2,4- dinitrobenzene(CDNB). This adduct was measured by noting the net increase in absorbance at 340 nm. Determination of GPx activity (26) is based on following the rate of oxidation of GSH by H_2O_2 in the presence of GPx. Oxidized GSH was determined by following up the decrease in absorbance of the reaction medium at 340 nm, as NADPH was converted to NADP. SOD activity was determined (27) 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 the absorbance at 420 nm. The

presence of SOD in the reaction medium inhibits pyrogallol autoxidation by scavenging the formed superoxide anion. Protein content of the supernatant was determined (28) using bovine serum albumin as standard.

Estimation of nitric oxide (NO) (NO2- / NO3-):

An aliquot of the homogenate was centrifuged at 17.000 r.p.m/ 4ºC/ 20 minutes. The resulted supernatant was used for the determination of NO. Determination of NO radical itself is difficult because of its radical nature and very short half-life. Therefore, determination of the stable oxidation products of NO radical, nitrite ($N0_2^-$) and nitrate ($N0_3^-$) concentrations is used as a measure for the production of NO radical. NO content was determined (29, 30) depending on the colourimetric detection of nitrite with Griess reagent after the enzymatic reduction of nitrate to nitrite using nitrate reductase enzyme. 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.

Statistical analysis

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. Results are presented as the mean ± standard error of the mean (SEM), with the number of observations (n) given in parentheses. All data obtained were submitted to a computerized statistical treatment using SPSS statistical package, version 17. Tables were represented by Microsoft Excel computer program.

3. Results:

Effect of doxorubicin (Dox), separately or in combination with carvedilol, on serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities in rats:

Results of table (1) 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. Carvedilol co-administration caused normalization of LDH as well as significant decrease in CPK serum levels reaching 119.4% of the control value.

Effect of doxorubicin (Dox), separately or in combination with carvedilol, on cardiac malondialdehyde (MDA) and nitric oxide (NO) levels in rats:

Table (2) illustrated that, Dox caused significant increase in MDA and NO levels amounting to 183.36% and 177.7%, respectively, of the control values. Carvedilol co-administration caused normalization of both MDA and NO levels.

Effect of doxorubicin (Dox), separately or in combination with carvedilol, on cardiac glutathione (GSH) content, glutathione peroxidase (GPx), glutathione-S-tranferase (GST) and superoxide dismutase (SOD) activities in rats:

As shown in table (3), Dox administration caused a significant decrease in cardiac GSH level reaching to 64% of the normal value. Co-administration of carvedilol significantly raised GSH content to about 92% compared to the control value. Results of the same table showed significant increase in cardiac activities of GPx, GST and SOD in the Dox-treated rats amounting to 410%, 184% and 225%, respectively, compared to the normal values. Co-administration of carvedilol produced normalization of GST and significant decrease in GPx and SOD activities, accounting to157%, 151% respectively, compared to the control values.

Effect of doxorubicin (Dox), separately or in combination with carvedilol, on serum alanine aminotransferase (ALT) activity in rats:

Results of table (4) revealed that Dox administration caused significant elevation in the serum ALT level to reach 118% of the normal value. Co-administration of carvedilol caused significant elevation in the same enzyme level compared to both Dox-treated and normal group values, reaching 128.8% of the normal control level.

Histopathological examination of the cardiac tissues:

Histopathological examination of the control cardiac section showed normal structure of the myocardium (Figure 1). Sections obtained from rats administrated Dox showed hyalinization the myocardial bundles associated with either inflammatory cells infiltration only or inflammatory cells and oedema in focal manner in between the bundles (Figures 2, 3). With respect to cardiac sections obtained from rats administrated combined therapy of Dox+ carvedilol, no histopathological alterations, compared to normal section, were observed (Figure 4).

Histopathological examination of hepatic tissues:

Examination of liver sections of the different groups illustrated that liver tissue of the normal group showed hepatic lobules with normal architecture (Figure 5). 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 6, 7). In rats administrated Dox + carvedilol, livers were most affected, congestion was observed in both the central and portal veins associated with diffuse kupffer cells proliferation in between the hepatocytes. Moreover, the portal area showed inflammatory cells infiltration (Figures 8, 9).

Table (1): Effect of doxorubicin, separately or in combination with carvedilol, on serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities in rats:

Group	LDH(U/L)	CPK(U/L)
Control	581.17±13 (9)	616.5±20.8 (9)
Doxorubicin	1060.2±29.4 [a, c] (10)	1131.9±32 [a, c] (10)
Doxorubicin + Carvedilol	598.5±23.6 [b] (9)	736.3±41.2 [a, b] (9)

Values are given as means ± SEM (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+ carvedilol group at P<0.05

Table (2): Effect of doxorubicin, separately or in combination with carvedilol, on cardiac malondialdehyde (MDA) and nitric oxide (NO) levels in rats:

Group	MDA nmole /gm tissue	NO nmole / gm tissue
Control	57.6±1.9 (9)	175±11 (9)
Doxorubicin	105.8±4.6 [a, c] (10)	311±18 [a, c] (10)
Doxorubicin + Carvedilol	59.1±3.5 [b] (9)	184±8 [b] (9)

Values are given as means ± SEM (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 + carvedilol group at P<0.05

Table (3): Effect of doxorubicin, separately or in combination with carvedilol, on cardiac glutathione (GSH) content, glutathione peroxidase (GPx), glutathione-S-tranferase (GST) and superoxide dismutase (SOD) activities in rats:

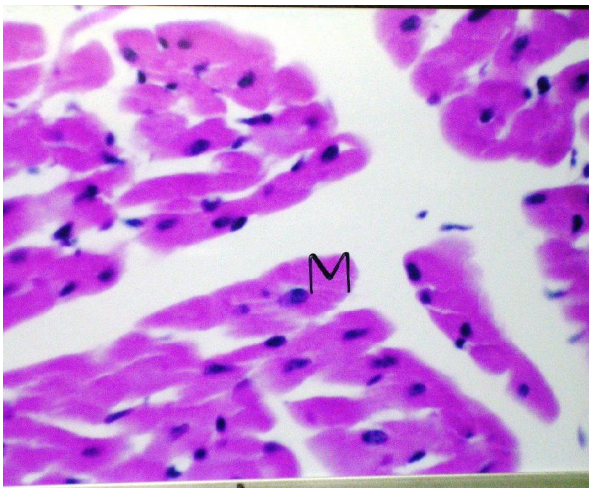
Group	GSH µg/gm tissue	GPx nmoles /min /mg protein	GST nmoles /min/mg protein	SOD U/mg protein
Control	64.3±1.5 (9)	33.6±1.9 (9)	29.6±1.0 (9)	21.3±1.4 (8)
Doxorubicin	41.2±1.1a,c (9)	137.8±7.1a,c (10)	54.7±2.9a,c (9)	48.1±2.2a,c (10)
Doxorubicin +Carvedilol	59.7±2.0a,b (9)	53.0±2.7a,b (8)	36.6±1.59 b (9)	32.3±1.8a,b (9)

Values are given as means ± SEM (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 + carvedilol group at P<0.05

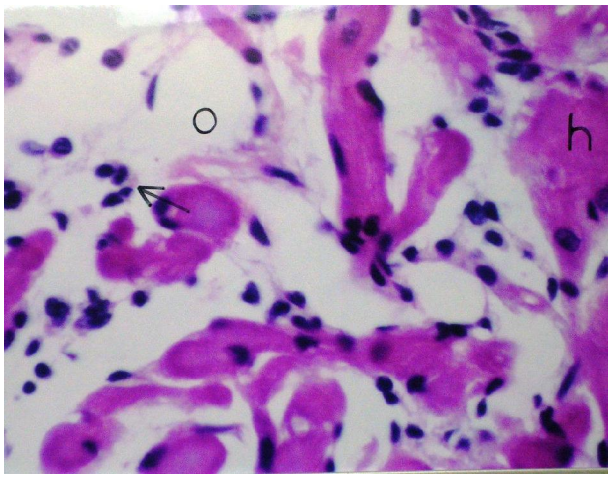
Table (4): Effect of doxorubicin, separately or in combination with carvedilol, on serum alanine aminotransferase (ALT) activity in rats:

Group	ALT (U/L)
Control	52.4±1.8 (9)
Doxorubicin	60.2±1.5[a,c] (10)
Doxorubicin + Carvedilol	69.6±2.6[a,b] (9)

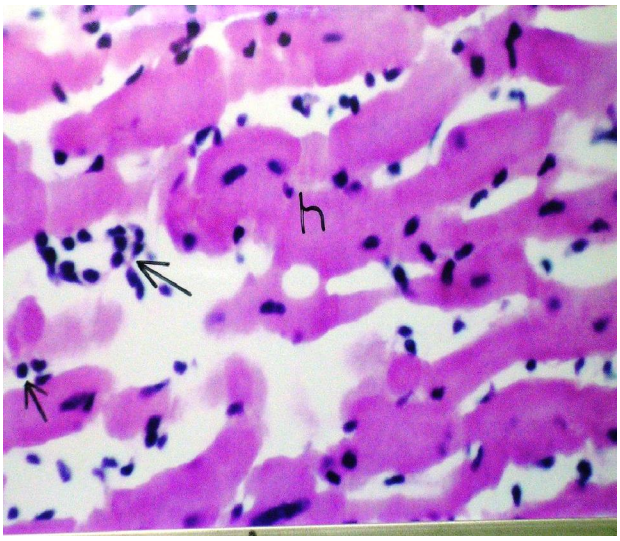
Values are given as means ± SEM (No. of observations are given in parentheses)
a: Significant difference from control group at P<0.05
b: Significant difference from doxorubicin group at P<0.05
c: Significant difference from doxorubicin + carvedilol group at P<0.05



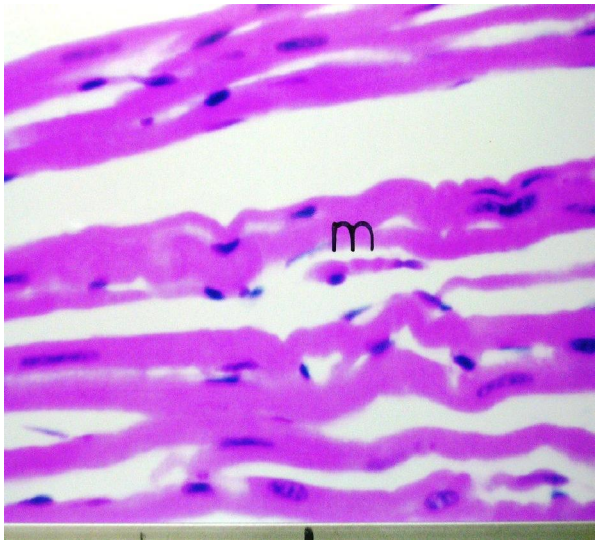
Figure(1): A photomicrograph of cardiac muscle fibers of control group showing normal histological structure of myocardium(M) (H&E x160)



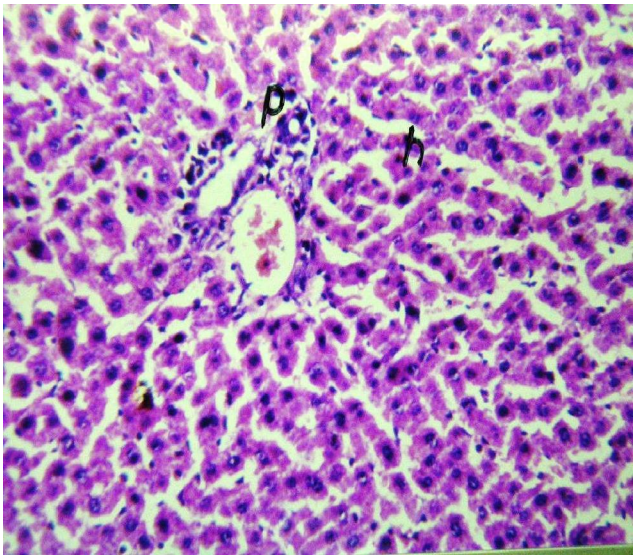
Figure(3): A photomicrograph of cardiac muscle fibers of Dxo group showing oedema(o) with inflammatory cells infiltration (arrow) in focal manner between the myocardial bundles (h). (H&E x160)



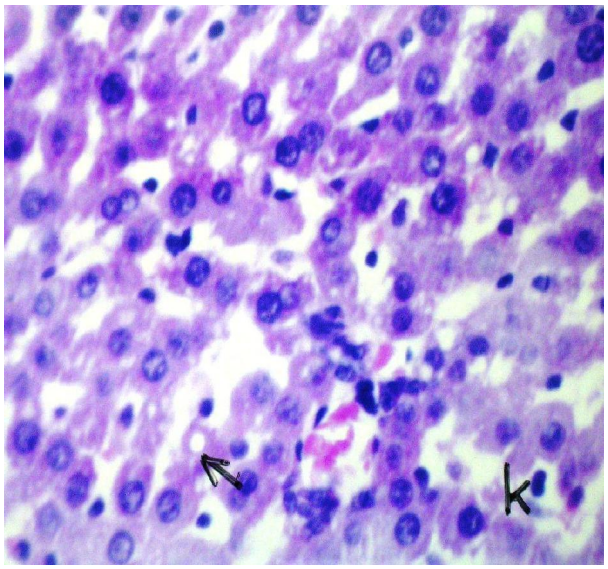
Figure(2): A photomicrograph of cardiac muscle fibers of Dox group showing the inflammatory cells infiltration (arrow) inbetween the hyalinized myocardial bundles(h) (H&E x160)



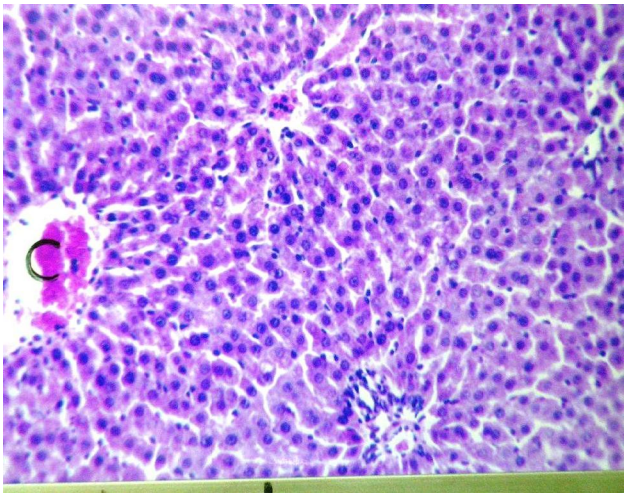
Figure(4): A photomicrograph of cardiac muscle fibers of Dox+ carvedilol group showing intact histological structure of myocardium (m) (H&E x160)



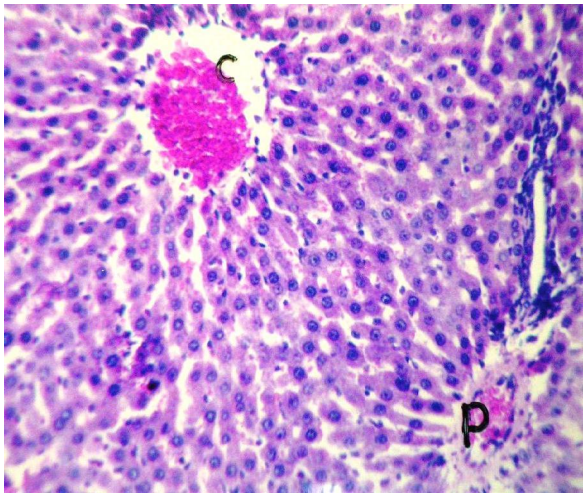
Figure(5): Photomicrograph of liver of normal group showed hepatic lobules (h) and portal vein (p) with normal architecture (H&E x64)



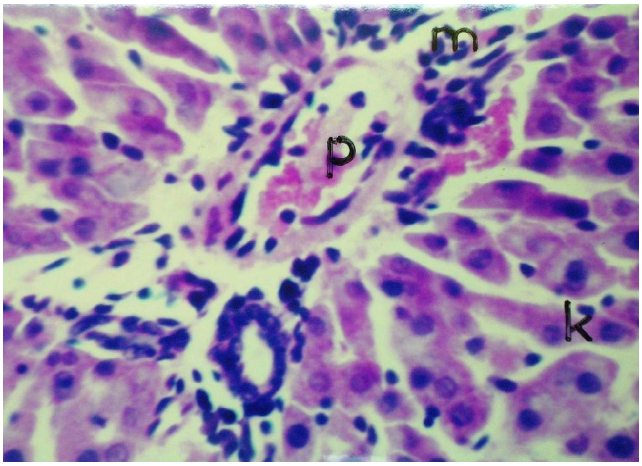
Figure(7): Photomicrograph of liver of Dox group showing diffuse kuffer cells proliferation (k) inbetween the fatty degenrated hepatocytes (arrow) (H&E x160)



Figure(6): Photomicrograph of liver of Dox group showing congestion in central vien(c) (H&E x64)



Figure(8): Photomicrograph of liver of Dox + carvedilol group showing congestion in the central (c) and portal(p) viens. (H&E x64)



Figure(9): Photomicrograph of liver of Dox + carvedilol group showing inflammatory cells infiltration in the portal area (m) around the portal vein (p) with diffuse kuffer cells proliferation (k) inbetween the hepatocyte. (H&E x160)

4. Discussion:

Doxorubicin (Dox) is a potent anticancer drug that is used in treating both hematological and solid tumors. However, severe cardiomyopathy and heart failure have been observed in Dox-treated cancer patients, which limit the clinical dosage of Dox in cancer treatments (31). Oxidative stress is generally held as the mediating mechanism in the multiple biological processes leading to Dox cardiomyopathy, e.g. redox mediated superoxide radical production (32), tissue-specific mitochondrial DNA damage (33) and disturbances of calcium (34) or iron (35). Results of the present study revealed that 15 mg/kg total cumulative dose of Dox induced cardiomyopathy and hepatotoxicity manifested biochemically as significant increase in serum levels of LDH; CPK and ALT. In addition, 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.

Results of table (1) revealed that Dox caused significant increase in the serum levels of LDH and CPK, considered as important markers of cardiac injury. Many previous studies have demonstrated similar elevations in cardiac enzymes activities in rats following Dox administration (36, 37). Leakage of cardiac enzymes directly correlates to ultrastructure damage of heart tissues examined by electron microscope. Dox-induced cardiomyopathy is mainly attributed to increase oxidant production in heart. Dox may undergo a one-electron reduction through a metabolic activation by NADPH-cytochrome P-450 reductase. This reduction leads to the formation of the free radical semiquinone, which in turn can produce a variety of active ROS/RNS, including H2O2, •OH and ONOO (38). These species can attack the cardiomyocyte membrane, damage several macromolecular cellular components, cause protein and lipid peroxidation and consequently lead to cardiomyocyte apoptosis or death (39). This effect would compromise the cellular integrity and potentially account for the leakage of heart enzymes, LDH and CPK, through the membranes and increase their serum activity.

Regarding the effect of Dox administration on cardiac oxidative status, Dox caused significant increase in MDA level (table 2), which is in agreement with previous study (40) who used similar drug regimen. This elevation might be attributed to Dox mediated oxidative stress. The first targets of Dox-mediated free radical damage are various cellular membranes, which are rich in lipids prone to peroxidation. This radical damage results in production of many relatively stable and highly toxic aldehydes, such as MDA. These aldehydes can easily diffuse within the cell, or even cross the plasma membrane, and attack macromolecular targets far from where they were generated and thus act as "second cytotoxic messengers" (41). Table (2) revealed also a marked increase in cardiac NO level in those rats received Dox. This finding is in harmony with previous study (42), which used a model of Dox-induced cardiomyopathy 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 nitric oxide synthase (NOS) expression and, hence, NO release in heart (43). Previous studies suggested that stimulation of endothelial cells with calcium-mobilizing agents activates and dissociates the membrane-bound eNOS (44). Because Dox-induced toxicity is mediated by intracellular H_2O_2 as well as calcium influx, Dox treatment causes an increase in eNOS transcription and protein activity in aortic endothelial cells and thus NO synthesis.

Dox administration, as shown in table (3), caused a significant decrease in cardiac GSH content, which is quiet compatible with previous studies (40, 45). 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 (46). The observed decrease of cardiac GSH content may also be attributed to the enhanced activities of GSH metabolizing enzymes, as shown in the present study. One is GPx which reduces H_2O_2 and various peroxides using GSH as reducing agent, the other is GST which consumes GSH in the conjugation of Dox toxic metabolites (47).

Table (3) showed, also, significant increase in cardiac activity of SOD in the Dox-treated rats, which is consistent with some studies (40, 48). 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 (38), which in turn stimulate SOD as an adaptive response to counteract oxidative stress (49). Also, it was previously showed that ROS can upregulate and induce the synthesis of SOD protein (50). The observed increase in SOD activity might lead to overproduction of hydroperoxides. In consequence, cardiac GPx activity might be stimulated in response to the accumulated peroxides which can subsequently lead to the formation of highly toxic •OH radical through Fenton reaction catalyzed by iron (51). This assumption was supported by our results which showed a significant enhancement in cardiac GPx activity in the Dox-treated group. Another assumed explanation for such increased GPx activity is that multiple doses of Dox alter the activities of antioxidant enzymes in the heart so as to protect against Dox cardiotoxicity (42, 49). Additionally, GPx have been reported to be over expressed in Dox-treated cells, especially those tumor resistant ones (52). This can be considered as intracellular detoxification process for free radical end products (53). In our study, we assumed that this detoxification mechanism can occur also in cardiac myocytes, exposed to Dox administration, to prevent the accumulation of peroxides and the propagation of peroxidation. Table (3), additionally, revealed significant increase in cardiac GST activity in rats treated with Dox, which is in agreement with previously reported results (54). The increase in cardiac GST activity might be related to the fact that GSTs are family of dimeric proteins that posses a multitude of functions including the enzymatic conjugation of GSH to electrophilic xenobiotics (55). 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 (56). Indeed, it has been known that Dox is metabolized, via alkedoreductases, 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 (57). In brief, GST has showed elevation after Dox injection to detoxify Dox and its metabolites and to attenuate the elevated oxidative stress (58). Moreover, Dox-treated cancer cell lines often show elevated GST expression and activity, which is in consistence with our result. Such increased activity of GST enzyme almost certainly leads to increased resistance to Dox treatment, and hence, creation of one of the most problems of Dox therapy (59).

Our results showed also an elevation in serum ALT upon Dox administration (table 4). This result agrees with previous study (60). Leakage of hepatic ALT into the serum is due to Dox-induced oxidative damage to hepatocytes (61). Dox-induced hepatotoxicity may be less severe than its cardiotoxicity. This 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 (62).

The previously mentioned biochemical results produced by Dox administration are supported by the obtained cardiac histopathological results which showed that in the cardiac sections of the Dox-treated rats, serious morphological changes were observed in the myocardium. Also, the examination of liver sections of the same group illustrated that congestion was observed in the central vein in addition to kupffer cells proliferation in diffuse manner between the fatty degenerated hepatocytes.

From the previously mentioned discussion, it has been well established that oxidative stress plays a major role in Dox-induced cardiomyopathy and hepatotoxicity. Thus, the importance of antioxidant co-administration as an adjuvant therapy with Dox in preventing these damages has been emphasized.

Carvedilol is an 1-blocking agent and a potent antioxidant, with a 10-fold greater activity than vitamin E. Some carvedilol metabolites found in human plasma also exhibit antioxidative activity approximately 50- to 100-fold greater than carvedilol. These unique properties of carvedilol may be important in preventing progressive deterioration of left ventricular dysfunction and chronic heart failure (63). Results of the present study revealed that co-administration of carvedilol with Dox could attenuate the cardiotoxicity manifested biochemically by normalization of LDH as well as significant decrease in CPK serum levels. Also, such combined therapy caused normalization of cardiac MDA and NO levels, as well as, significantly raised GSH content. Moreover, co-administration of carvedilol produced normalization of GST, as well as, significant

decreased in cardiac activities of GPx and SOD. Histopathological examination of heart sections supported the alleviating effect of carvedilol on Dox-induced cardiotoxicity. On the contrary, carvedilol caused significant elevation in the serum ALT level compared to both normal and Dox-treated groups. Histology of liver sections showed that liver in this group was most damaged compared to other groups.

Our results showed that, carvedilol effectively prevented cardiomyocyte damage caused by Dox, evidenced biochemically by its ability to reduce the leakage of cardiac enzymes LDH and CPK into the serum. This finding is in harmony with previous results (64). This observed result is attributed to the antioxidant capacity of carvedilol and its ability to protect cellular membranes against oxidative damage preserving their integrity. The protective effect of carvedilol is strongly supported by the present ultra structural results, as carvedilol completely relieved cardiac histopathological damage induced by Dox. This observation is in harmony with results reported previously who related the cardioprotective effect of carvedilol to its positive impact on cardiac mitochondrial function, since, carvedilol prevented the inhibitory effect of Dox on mitochondrial respiration in heart, and also prevented the decrease in mitochondrial Ca^{2+} loading capacity and the inhibition of the respiratory complexes of heart mitochondria caused by Dox. Thus, co-administration of carvedilol decreased the extent of cellular vacuolization and cell death in cardiac myocytes (65, 66).

Our results revealed also that MDA was greatly reduced by the administration of carvedilol concomitantly with Dox, reaching to the normal level. This result is quiet consistent with many previous studies (67, 68). Earlier report (69) showed that carvedilol reduced plasma lipid peroxidation in patients with heart failure. Another possible mechanism is the direct antioxidative property of carvedilol which is attributed to the carbazole moiety of the drug (70). Carvedilol inhibited Fe^{2+}-initiated lipid peroxidation via scavenging free radicals (71) or by sequestering ferric ion (72). Carvedilol can also scavenge lipid radicals directly, thus breaking the chain reaction in membranes (73). On the other hand, as shown in our study, carvedilol caused significant reduction in cardiac NO level, reaching to normal value. This finding is in harmony with previous report (74) demonstrated the effect of carvedilol as a NO quenching agent. Carvedilol protected nitrosylation of intracellular molecule by exogenous NO and reduced intracellular concentration of NO produced by NO donors. Moreover, carvedilol is able to inhibit ROS generation by leukocytes (75) and decrease phagocyte degranulation and the amount of free myeloperoxidase (76), which at the sites of inflammation may function as a catalytic sink for NO (77).

As shown in our results, carvedilol produced remarkable inhibition of the observed GSH depletion in rats treated with Dox, which is in harmony with previous results (78, 67) that used carvedilol dose similar to that used in the current study. This result can be explained depending on the fact that carvedilol is potent antioxidant, since it reduced the production of free radicals in heart and thus the consumption of endogenous antioxidants especially GSH (46). Moreover, a reduced energy breakdown offered by carvedilol is expected to maintain cellular viability, thus avoiding membrane damage, alteration of ionic homeostasis and the occurrence of oxidative stress. Therefore, the positive effects on glutathione and –SH group metabolism which were found after treatment with carvedilol are most likely the consequence of a generalized cardiac protection due to the β-blocking-mediated energy saving (64).

Our study revealed also that carvedilol caused significant decrease in SOD and GPx activities in heart when administered concomitantly with Dox. This effect is, to some extent, similar to previous observation (67). Carvedilol protects a variety of cultured cells from oxygen radical- induced damage when subjected to either artificial oxygen-radical generating systems, such as Fe2+/vitamin C, or endogenous oxygen radical generating systems, such as xanthine-xanthine oxidase which involves the release of superoxide ions. This effect of carvedilol may result from its ability both to scavenge superoxide ions and to inhibit the production of superoxide radicals, the latter being inferred from the observation that carvedilol inhibits superoxide release from phorbol ester-activated neutrophils (79). The reduction in superoxide radical production may in turn relief the adaptive increase in SOD activity preceding Dox administration, as shown in our results. This decrease in cardiac SOD activity might be considered as a direct mechanism for the concomitant decrease in cardiac GPx activity observed in the present study, since no more H2O2 to be declared by GPx.

Regarding the effect of carvedilol on Dox-induced elevation in cardiac GST, the present study showed that carvedilol caused significant decrease in such enzymatic activity, reaching to the normal level. This finding might be related to previous observations that carvedilol could reverse cellular drug resistance by inhibiting MRP1 drug efflux system (80, 81). GST catalyzes the conjugation of Dox and its metabolites to GSH (47). Glutathione conjugates of Dox show high affinities toward the MRP1 pump, a drug efflux protein mediates efflux of GSH conjugates (82). The inhibitory effect of carvedilol on MRP1 can, in turn, reduce the efflux of GSH conjugates of Dox and thus

down regulates the production of the conjugating enzyme, GST.

Our results revealed that in the group administered a combined therapy of Dox+carvedilol, significant elevation in serum ALT level, with respect to both control and Dox- administrated group levels, was shown. Histopathological examination of liver sections of this group supported the biochemical result in that liver tissues of rats belonging to this group were shown to be most damaged compared to other groups, given that, congestion was observed in both the central and portal veins associated with diffuse kupffer cells proliferation in between the hepatocytes and the portal area showed inflammatory cells infiltration.

5. Conclusion:

Oxidative stress plays a major role in Dox-induced cardiomyopathy and hepatic damage. Carvedilol could effectively attenuate the cardiomyocyte damage caused by Dox as evidenced by the biochemical measurements and histopathological examination of cardiac tissue. Unfourtiontly, carvedilol augmented Dox-induced hepatic damage as evidenced by the present biochemical result and the histopathological examination of the hepatic tissue.

Competing Interests:

The authors declare that they have no competing interests.

Authors' Contributions :

SSI: participated in designing the point of research and the plan of work; followed up all the practical experiments; reviewed the statistical analysis; participated in analysis and interpretation of data; drafted the manuscript; the corresponding author for article publication; read and approved the version to be published.

MMB: participated in designing the point of research and the plan of work, read and approved the version to be published.

HSH: carried out the experimental works and performed the statistical analysis.

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