Cardioprotective Effect of Marine Astaxanthin on Doxorubicin-Induced Cardiotoxicity in Normal Rats

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors AMMO, AAAQ, HSAM, HMAK and SEA were involved in sharing in experimental work and writing the manuscript. Author WSR did cytopathological investigation and others read and share in interpretation of data and writing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Doxorubicin (DOX) is an effective antineoplastic drug indicated to treat many cancerous diseases but its clinical usefulness is limited by many side effects. The main and the most serious one is DOX induced cardiotoxicity. Many strategies have been tried to minimize this side effect such as addition of cardioprotective agent to DOX treatment protocols.

Aims: The aim of this work was directed to investigate whether marine astaxanthin (ATX), a xanthophyll carotenoid pigment with potent antioxidant effect, could protect heart against the cardiotoxicity induced by DOX.

Methodology: Forty Male Wister rats were divided into four equal groups and treated for one week as follow: Group I rats were treated with normal saline (2 ml/kg, x7, i.p.) and considered a control group. Group II rats were treated with ATX (40 mg/kg, x7, i.p.). Group III rats were treated with...
1. INTRODUCTION

Doxorubicin was firstly used clinically in cancer therapy in the late 1960s. It is considered as one of the most potent antitumor anthracyclines. DOX could be administered alone or with other chemotherapeutic agents in the treatment protocols of many types of cancers such as leukemias, lymphomas, soft-tissue sarcomas and solid tumors. Unfortunately, its cytotoxic effects are limited by its cardiotoxicity [1,2], the main side effect of DOX, which could lead to congestive heart failure [3]. It has been reported that the cardiomyopathy and congestive heart failure after treatment with DOX is dose-dependent [4,5]. Doxorubicin induces its cardiotoxicity by many mechanisms such as DNA and RNA damages, induction of oxidative stress through liberation of reactive oxygen specious, lipid peroxidation, increase the endoplasmic reticulum-mediated apoptosis, inhibition of autophagy and interference with of calcium homeostasis [2,6]. In addition, DOX metabolism produces superoxide anion and hydroxyl radical which lead to toxic manifestation in the cellular membrane of the normal cells. Also, it has been reported that this toxicity is mediated through cardiac tissues inflammation [7]. Between the importance of DOX in cancer treatment and the increase of the incidence of its induced cardiotoxicity, it has become increasingly important to find pharmacological remedies with protective effects against this serious side effect [7,8]. Variety of approaches have been investigated as the addition of natural compound with chemopreventive or anticancer properties to the DOX treatment protocol [9,10,11]. Astaxanthin is a natural reddish carotenoid pigment belongs to the xanthophylls family. It has a potent antioxidant, anti-inflammatory, anti-lipid peroxidation and cardioprotective effects [12,13,14]. Intraperitoneal administration of ATX leads to faster absorption with higher bioavailability than oral administration in oncorhynchus mykiss [15]. ATX is extensively distributed in all tissues after an oral administration and metabolized by CYP1A following oral ingestion in the rat [16]. The plasma ATX elimination half-life was estimated to be 21 ± 11 hr. after oral dose in human [17]. In our laboratory, we found that ATX potentiated the cytotoxic activity of DOX against the growth of Ehrlich ascites carcinoma cells in vivo (data not shown). Therefore, the present study was undertaken to test whether ATX could protect the heart against DOX-induce cardiotoxicity in normal rats through prevention of oxidative stress.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Doxorubicin hydrochloride was purchased from Ebewe pharma Austria. Astaxanthin was purchased from Haihang Industry Co., Ltd. CI-
MB ELIZA kit (Cat No. E-EL-R1327) was purchased from Elabscience biotechnology Inc. (USA) and CPK kit (Cat No. CF13000120) was obtained from Centronic GmbH, (Germany). Total antioxidan
t capacity kit (Cat No. GT 2513), reduced glutathione kit (Cat. No. GR 2511), glutathione peroxidase kit (Cat No. GP 2524), glutathione -S- trasferase kit (Cat. No. 2519), malondialdehyde kit (Cat No. MD 2529), Catalase kit (Cat. No. CA 2517), Superoxide dismutase kit (Cat. No. SD 2521) were purchased from Biodiagnostic Co. (Dokki, Giza, Egypt).

2.2 Animals and Housing

Male Wistar Albino rats (8-10 weeks of age, 250-300 gm. b.wt.) were provided from the animal house at College of Pharmacy, King Abduaziz University, Jeddah, Saudi Arabia. The rats were acclimatized for 7 days before experiments. A commercial balanced diet and water ad libitum were provided all over the experiments.

2.3 Experimental Design

Forty Male Wister rat were randomly distributed into four equal groups, 10 animals in each group. Rats of group I were injected with normal saline (2 ml/kg, x7, i.p.) and considered as control group. group II animals were treated with ATX (40 mg/kg, x7, i.p.). Rats of group III were treated with normal saline (2 ml/kg, x7, i.p.) and a single dose of DOX (20 mg/kg, i.p.) at day 7. Finally, group IV rats were treated with ATX (40 mg/kg, x7, i.p.) and at day 7 treated with a single dose of DOX (20 mg/kg, i.p.). After 24 and 48 hrs of treatment, rats were anesthetized and prepared gently for collection of blood samples in non-heparinized tubes from each rat by cardiac puncture according to the IACUC recommended standard methods for blood collection. The samples were left to clot for 30 minutes then centrifuged for serum separation which was stored at − 80°C to evaluate different biochemical parameters.

2.4 Evaluation of Cardiotoxicity of DOX Treatment in Presence and Absence of ATX

2.4.1 Evaluation of Serum Creatine Phosphokinase (CPK)

Creatine kinase activity was determined colorimetrically using CPK kit (Centronic GmbH, Germany) according to the method of Szasz et al. [18].

2.4.2 Evaluation of Serum Creatine Kinase MB isoenzyme (CK-MB)

The serum level of CKMB was evaluated in rats' serum using rat creatine kinase MB isoenzyme, CK-MB ELISA Kit, according to the manufacturer protocol.

2.4.3 Measurement of serum antioxidants activities and oxidative stress

The Total serum antioxidant capacity was measured by colorimetric method, according to the method of Koracevic et al. [19]. In the heart tissues homogenate the reduced glutathione was measured according to the method of Beutler et al. [20]. GPx was determined by colorimetric method according to the method of Paglia et al. [21] and Malondialdehyde level was determined by colorimetric method according to the method of Ohkawa et al. [22]. SOD was measured colorimetrically in the cardiac tissues homogenate according to the method of Nishikimi et al. [23].

2.5 Histopathological Examination

After blood collection, rats were sacrificed by gently decapitation, chest opened and hearts were extracted. Heart sample was taken immediately and washed with saline. Part of the left ventricle of the heart was fixed in 10% phosphate buffered formalin and processed for paraffin blocks. Serial histological longitudinal sections of 5-µm thickness were cut, mounted on glass slides and stained with haematoxylin and eosin (H&E) for general structure [24]. Half gm. of the remaining cardiac tissues was homogenized in 5 ml of phosphate buffer saline on ice, using an electric homogenizer (Potters, German).

2.6 Statistical Analysis

Statistical analysis of the data was carried out using computer program package (SPSS, version 21). All data are expressed as mean with their standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to compare differences between experimental groups. It was followed by the least significance difference (LSD) test. However, two-sample t-test and its P-value to analyze the significance of the difference in the samples mean. Differences were considered significant at P < 0.05.
3. RESULTS

3.1 Effect of DOX and/or ATX on Cardiac Enzymes

Table 1 showed the effect of DOX and/or ATX on the serum level of CPK. The level of serum CPK was significantly increased (2.14 and 2.05 fold) after 24 and 48 hrs. of DOX treatment, respectively. On the other hand, addition of ATX to DOX showed a significant decrease in CPK level in compared to DOX treated rats 24 and 48 hrs. of treatment. Table 2 showed the effect of DOX and/or ATX on the serum CK-MB level in rats. There were significant increases in CK-MB level (7.19 and 6.8 fold) in DOX treated rats compared to control after 24 and 48 hrs. treatment, respectively. While, addition of ATX to DOX nearly restored the CK-MB level to the normal at the two time points tested.

3.2 Effect of DOX and/or ATX on the Serum Antioxidant Capacity

Table 3 showed the effect of DOX and/or ATX on TAC level in rats' serum. There was a significant increase in TAC in ATX treated rats (1.26, 1.16 fold) compared to control rats after 24 and 48 hrs. of treatment, respectively. However, there was a significant decrease (1.4 and 1.54 fold) in serum TAC level in DOX treated rats in compare with control after 24 and 48 hrs. of treatment, respectively. Combination DOX and ATX in the treatment protocol showed a significant increase (1.26, 1.11 fold) in serum TAC level compared to control after 24 and 48 hrs. respectively.

Table 1. Effect of DOX and/or ATX on CPK activity in Rats’ serum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>350 ± 6.78</td>
<td>361.53 ± 8.62</td>
</tr>
<tr>
<td>ATX</td>
<td>344.19 ± 9.37</td>
<td>344.19 ± 3.83</td>
</tr>
<tr>
<td>DOX</td>
<td>748.64 ± 11.42a</td>
<td>741.21 ± 6.60 a</td>
</tr>
<tr>
<td>ATX and DOX</td>
<td>411.05 ± 19.21a,b</td>
<td>413.53 ± 17.67a,b</td>
</tr>
</tbody>
</table>

DOX (20 mg/kg, i.p.) was injected in male Wistar rats pretreated either with ATX (40 mg/kg, x7, i.p.) or normal saline (2 ml/kg, x7, i.p.). Data are expressed as mean ± SEM of five male Wistar rats after 24 hrs. and 48 hrs. a Significantly different from control at P-value < 0.05, b Significantly different from corresponding DOX at P-value <0.05, one way ANOVA with LSD post test

Table 2. Effect of DOX and/or ATX on serum CK-MB level

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>106 ± 2.02</td>
<td>106.40 ± 1.96</td>
</tr>
<tr>
<td>ATX</td>
<td>105.80 ± 2.58</td>
<td>108.80 ± 1.28</td>
</tr>
<tr>
<td>DOX</td>
<td>763.80 ± 5.03 a</td>
<td>727 ± 7.41 a</td>
</tr>
<tr>
<td>ATX and DOX</td>
<td>378.60 ± 5.41 a,b</td>
<td>347.60 ± 4.09 a,b</td>
</tr>
</tbody>
</table>

DOX (20 mg/kg, i.p.) was injected in male Wistar rats pretreated either with ATX (40 mg/kg, x7, i.p.) or saline (2 ml/kg, x7, i.p.). Data are expressed as mean ± SEM of five male Wistar rats 24 hrs. and 48 hrs. after treatment. a Significantly different from control at P-value < 0.05, b Significantly different from corresponding DOX at P-value <0.05, one way ANOVA with LSD post test

Table 3. Effect of treatment with DOX and/or ATX on serum total antioxidant capacity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>1.23 ± 0.07</td>
<td>1.31 ± 0.05</td>
</tr>
<tr>
<td>ATX</td>
<td>1.55 ± 0.01 a</td>
<td>1.52 ± 0.01 a</td>
</tr>
<tr>
<td>DOX</td>
<td>0.88 ± 0.01 a</td>
<td>0.85 ± 0.04 a</td>
</tr>
<tr>
<td>ATX and DOX</td>
<td>1.53 ± 0.01 a,b</td>
<td>1.46 ±0.02 a,b</td>
</tr>
</tbody>
</table>

DOX (20 mg/kg, i.p.) was injected in male Wistar rats pretreated either with ATX (40 mg/kg, x7, i.p.) or saline (2 ml/kg, x7, i.p.). Data are expressed as mean ± SEM after 24 and 48 hrs. (n=5). a Significantly different from control at P-value < 0.05, b Significantly different from corresponding DOX at P-value <0.05, one way ANOVA with LSD post test.
Table 4. Effect of DOX and/or ATX on Reduced Glutathione (GSH) Level in Rats' heart homogenate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs.</td>
</tr>
<tr>
<td>Normal saline</td>
<td>118.95 ± 6.10</td>
</tr>
<tr>
<td>ATX</td>
<td>125.39 ± 8.54</td>
</tr>
<tr>
<td>DOX</td>
<td>71.26 ± 0.95 a</td>
</tr>
<tr>
<td>ATX and DOX</td>
<td>104.78 ± 2.71 a, b</td>
</tr>
</tbody>
</table>

DOX was injected (20 mg/kg, i.p.) in male Wistar rats pretreated either with ATX (40 mg/kg, x7, i.p.) or saline (2 ml/kg, x7, i.p.). Data are expressed in mean ± SEM of the experiment in male Wistar rats after 24 hrs. and 48 hrs. (n=5). a Significantly different from control at P-value < 0.05. b Significantly different from corresponding DOX at P-value <0.05, one way ANOVA with LSD post test.

Table 5. Effect of DOX and/or ATX on MDA Level in Rats' heart homogenate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs.</td>
</tr>
<tr>
<td>Normal saline</td>
<td>37.30 ± 1.49</td>
</tr>
<tr>
<td>ATX</td>
<td>33.24 ± 1.79</td>
</tr>
<tr>
<td>DOX</td>
<td>128.36 ± 2.99 a</td>
</tr>
<tr>
<td>ATX and DOX</td>
<td>47.10 ± 1.11 a, b</td>
</tr>
</tbody>
</table>

DOX (20 mg/kg, i.p.) was injected in male Wistar rats pretreated either with ATX(40 mg/kg, x7, i.p.) or saline (2 ml/kg, x7, i.p.). Data are expressed as mean ± SEM of the experiment in male Wistar rats after 24 hrs. and 48 hrs. (n=5). a Significantly different from control at P-value < 0.05. b Significantly different from corresponding DOX at P-value <0.05, one way ANOVA with LSD post test.

Table 6. Effect of DOX and/or ATX on GPx on Level in Rats' heart homogenate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GPx (U/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs.</td>
</tr>
<tr>
<td>Normal saline</td>
<td>6.68 ± 0.37</td>
</tr>
<tr>
<td>ATX</td>
<td>6.41 ± 0.21</td>
</tr>
<tr>
<td>DOX</td>
<td>2.96 ± 0.08 a</td>
</tr>
<tr>
<td>ATX and DOX</td>
<td>4.60 ± 0.08 a, b</td>
</tr>
</tbody>
</table>

DOX (20 mg/kg, i.p.) was injected in male Wistar rats pretreated either with ATX(40 mg/kg, x7, i.p.) or saline (2 ml/kg, x7, i.p.). Data are expressed as mean ± SEM of five male Wistar rats after 24 hrs. and 48 hrs. a Significantly different from control at P-value < 0.05. b Significantly different from corresponding DOX at P-value <0.05, one way ANOVA with LSD post test.

Table 7. Effect of DOX and/or ATX on SOD Activity in Rats' cardiac tissues homogenate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SOD activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs.</td>
</tr>
<tr>
<td>Normal saline</td>
<td>3.57 ± 0.22</td>
</tr>
<tr>
<td>ATX</td>
<td>3.81 ± 0.31</td>
</tr>
<tr>
<td>DOX</td>
<td>2.23 ± 0.02 a</td>
</tr>
<tr>
<td>ATX and DOX</td>
<td>3.35 ± 0.03 a, b</td>
</tr>
</tbody>
</table>

DOX (20 mg/kg, i.p.) was injected in male Wistar rats pretreated either with ATX (40 mg/kg, x7, i.p.) or saline (2 ml/kg, x7, i.p.). Data are expressed as mean ± SEM of five male Wistar rats after 24 hrs. and 48 hrs. a Significantly different from control at P-value < 0.05. b Significantly different from corresponding DOX at P-value <0.05, one way ANOVA with LSD post test.

Table 4 showed the effect of DOX and/or ATX on GSH level in rat's heart homogenate. There were significant decreases (1.7 and 1.8 fold) in GSH level in DOX treated rats compared with control after 24 and 48 hrs. of treatment, respectively. While, in presence of ATX the GSH levels maintained nearly to the normal values (104.6 and 104.78 mg/g tissue) respectively.
3.3 Effect of DOX and/or ATX on Lipid Peroxidation in the Rats Cardiac Tissues

Table 5 showed the effect of DOX and/or ATX on MDA level in the rat's heart homogenate. There were significant increases (3.5 and 3.7 fold) in MDA level in DOX treated rats compared with control after 24 and 48 hrs of treatment, respectively. While, addition of ATX to DOX showed a significant reduction in MDA levels and return it nearly to normal values (47.10 nmol/g tissue and 43.30 nmol/g tissue) after 24 and 48 hrs of treatment, respectively.

Tables 6 showed the effects of DOX on GPx in rat heart homogenate. GPx level in rat heart has been decreased after DOX treatment, while in presence of ATX, GPx levels maintained nearly to normal values (4.60, 5.21 U/mg. tissue) after 24 and 48 hrs of treatment, respectively (Table 6).

3.4 Histopathological Investigation after Treatment with DOX and/or ATX

Fig. 1 showed Light photomicrographs of rat's cardiac tissues from control group treated with normal saline (2 ml/kg) showing normal
branching muscle fiber with central vesicular nuclei. Fibroblasts with flat nuclei are noted in the surrounding endomysium and blood capillaries are present between the cardiac muscle fibers.

Light photomicrograph (Fig. 4) showed the effect of DOX (20 mg/kg) treatment on the myocardium tissues of the rats. DOX treatment showed a marked disruption of normal cardiac architecture, congestion of blood vessels and capillaries, condensed pyknotic peripheral nuclei and multiple areas of fragmented cardiac muscle fibers.

These changes have been attenuated when pretreated with ATX. Treatment with DOX (20 mg/kg) + ATX (40 mg/kg) showed that most of cardiac muscle fibers regained its normal structure but localized areas of myocytolysis and shortening of cardiac muscle fibers are still noted (Figs. 1-5).

![Photomicrograph of myocardium section of a rat after 48 hrs. of treatment with DOX (20mg/kg,i.p.) showing marked disruption of the myocardium architecture. Areas of myocytolysis (★), cardiac muscle fibers with deeply acidophilic sarcoplasm and peripheral pyknotic nuclei (dashed arrows) and perivascular polymorphnuclear cell infiltration are noted (○). (H & E x 400)](image1)

![Photomicrograph of a section of myocardium of a rat after 48 hrs. of treatment with ATX (40mg/kg,x7.i.p.) + single dose of DOX (20 mg/kg i.p.) at day 7 showing most of cardiac muscle fibers regained its normal structure (arrows). But localized areas of myocytolysis (★) and shortening of cardiac muscle fibers are still noted (dashed arrows). (H & E x 400)](image2)

**4. DISCUSSION**

Doxorubicin is one of the effective and widely used antineoplastic drugs indicated for treatment of many kinds of cancers either alone or in combination with other antineoplastic drugs. However, its clinical usefulness is limited by its detrimental adverse effects as cardiotoxicity which may be exaggerated to reach heart failure [25]. Cardiotoxicity is the major and the most serious adverse effect of DOX which limit its clinical usefulness. Many strategies have been tried to minimize this serious side effects by using combination treatment with cardioprotective agent and synthesis of DOX liposomes [7,8,9,10,26,27,28].

Fig. 5. Among the possible potential chemosensitizer is ATX which has cytotoxic activity [29,30,31] and chemoprotective effect against chemotherapy adverse effects [12,29,32]. In our laboratory, it has been proven that ATX sensitized DOX cytotoxic activity against the growth of Ehrlich ascites carcinoma cells in-vivo (data not shown). Therefore, our current study focus on the protective effect of ATX against DOX-induced cardiotoxicity. The mechanisms by which DOX exerts its cardiotoxicity are not clear enough and still under investigation. Although there are several cellular pathways involved in DOX induce cardiotoxicity such as release of vasoactive substances, mitochondrial deteriorations, lipid peroxidation and depletion of the cellular antioxidants such as glutathione. As ROS liberation plays an essential role in the DOX induced cardiotoxicity we and other researchers focused on the potential involvement of ROS in DOX induced cardiotoxicity [2,3,4,6,33].
It is well known that the heart tissues are highly susceptible to oxidative stress due to its inherent decreased detoxifying natural antioxidants [8,11].

In animal's studies, the acute cardiotoxicity induced by DOX was associated with a high level of ROS liberation and lipid peroxidation. Moreover cardiac tissues injuries are associated with elevation of the level of CPK and CK-MB enzymes. It is well known that these enzymes are released from the heart muscle cells when they are injured and their activities in the blood after myocardial injury reflect the extent of damage in its musculature [34,35]. Our results showed that there was a significant reduction in serum total antioxidant capacity, reduced glutathione level, glutathione peroxidase level and superoxide dismutase activity in the cardiac tissues after DOX treatment (Tables 3, 5, 6, 7). In addition, there was a significant increase in the lipid peroxidation in term of malondialdehyde level in the cardiac tissues which was significantly increased to (3.4 and 3.7 fold) 24 and 48 hrs. after DOX treatment, respectively (Table 5).

These results are in a good agreement with others who reported the cardiac toxicity after DOX treatment. Their findings reported the decrease in the serum level of TAC and increase in the level of MDA after DOX administration in the rats' cardiac tissues [11,36,37,38].

Addition of ATX to DOX maintained the serum TAC, SOD, MDA levels and GPx level in rats hearts tissues nearly to the normal values in compare with animal treated with DOX alone.

It has been reported that the treatment with DOX increase CPK level and CK-MB as a sequences of DOX induced cardiotoxicity as a good marker to evaluate the toxic deterioration in cardiac tissues [7,39].

In the present study, there was a significant increase in CPK level after 24 and 48 hrs. of DOX treatment, respectively in compare with control (Table 1).This result was confirmed by a significant increase of the specific cardiac marker CK-MB at the same two time points tested (Table 2). Addition of ATX showed a cardioprotective effect against DOX induced cardiotoxicity. These findings were confirmed by a significant reduction of the total CPK levels in ATX + DOX treated rats compared with rats treated with DOX alone. This cardioprotective effect of ATX were further confirmed by a significant reduction in CK-MB level in ATX + DOX treated rats in compare with animals treated with DOX alone (Table 2).

These results agree with Gross et al.[40], Monroy-Ruiz et al. [41] and Binu et al. [42] who reported that ATX has a cardioprotective effect through scavenging of free radicals involved in deterioration and remodeling of cardiomyocytes and tissues such as superoxide anion and reduction of oxidative stress markers involved in cardiotoxicity from the arachidonic acid and linoleic acid pathways.

In harmony with our results, Nakao et al. [43] reported that ATX protects heart tissues damage through its antioxidant properties. Moreover, Nishigaki et al. [44] stated that ATX minimizes the glycated protein/iron chelate-induced toxicity through suppression of lipid peroxidation and protein oxidation and enhance the activity of antioxidant enzymes in human umbilical vein endothelial cells.

The current study showed that DOX-induced cardiotoxicity is minimized by quenching of ROS and hydrogen peroxide which is one of the proposed molecular mechanisms involved in the DOX induced cardiotoxicity and induction of apoptosis in cardiomyocytes [45].

Our results are in a good agreement with Wang et al. [46] who concluded that quenching of H2O2 or over expression of GPx decreased DOX-induced apoptosis in endothelial cells and cardiomyocytes but not in tumor cells. This may explain that the ATX provides a cardiomyocytes protective effect with potentiation of DOX cytotoxicity in EAC cells.

In contrary to our results, one of the molecular mechanisms of DOX induced cardiotoxicity is induction of apoptosis in endothelial cells and cardiac cells through activation of p53 protein. In our results ATX upregulated the expression of p53 gene in tumor cells (data not shown) as synergistic mechanism to potentiate the DOX cytotoxic effects which may be falsely explained that ATX increases the DOX-induced cardiotoxicity.

This discrepancy could be refuted as reported by Wang et al. [46] who found that DOX caused early activation of p53 in tumor cells that was followed by caspase-3 activation and DNA fragmentation. These findings suggest that the transcriptional activation of p53 in DOX-induced apoptosis in endothelial and cardiac cells may not be as crucial as it is in tumor cells. Therefore,
the cytotoxicity of DOX is potentiated through over expression of p53 gene by ATX in EAC cells but not in cardiomyocytes.

Histopathological studies confirmed the biochemistry results where DOX causes loss of normal organization of cardiac muscle fibers revealing deeply acidophilic sarcoplasm and peripheral pyknotic nuclei. Moreover, numerous areas of muscle fibers shortening are noted. While, rats treated with ATX + DOX have less histopathological deteriorations (Figs. 1-5).

5. CONCLUSION

This research concluded that astaxanthin has the ability to reduce the cardiotoxic effect of DOX through inhibition of oxidative stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the animal studies were approved by the ethical research committee unit at the College of Medicine, King Abdulaziz University (Reference No.112-18).

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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