

# Myocardial Infarction Markers in Solid Ehrlich Carcinoma Tumor Model Treated With Doxorubicin

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## Abstract

Doxorubicin (DOX) is an anthracyclin antitumor antibiotic closely related to the natural product daunomycin. The most dangerous side effect of DOX is cardiomyopathy leading to congestive heart failure. This study aimed to investigate the cardiotoxicity of DOX by measuring lactate dehydrogenase (LDH), creatine kinase (CK-MB) enzymes, and troponin I level in serum, and measuring malondialdehyde (MDA) level in the heart muscle tissue. Sixty BALB/c male mice were used in this study. Except for mice in the control group, each mouse was implanted subcutaneously with 0.2 ml of the ascites fluid containing  $1 \times 10^6$  Ehrlich carcinoma cells (ECCs) into the right thigh of the hind limb of a mouse. The total period of the experiment was 49 days (7 days before and 42 days after tumor inoculation). Mice were randomly divided into three groups (20 mice per each group) as follows: Group 1: Control group, in which mice received an intraperitoneal (i.p.) injection of 0.2 ml normal saline once weekly on days 0, 7, 14, 21 (for 21 days). Group 2: Solid Ehrlich carcinoma (SEC) control group, in which mice received an intraperitoneal injection of 0.2 ml normal saline once weekly on days 0, 7, 14, 21 (for 21 days) starting one hour after tumor inoculation. Group 3: DOX group, in which mice received DOX (4 mg/kg, i.p.) once weekly on days 0, 7, 14, 21 (for 21 days) starting one hour after tumor inoculation. The results of this study showed that, treatments with an intraperitoneal injection of DOX lead to increase of myocardial infarction markers as serum LDH, CK-MB enzyme activities, and Troponin I level. MDA increases in cardiac tissue homogenate in treated mice with DOX. It can be concluded that DOX treatment of male mice inoculated with SEC Tumor induced a myocardial infarction. Therefore, cancer patients treat with DOX should be checked the serum LDH, CK-MB enzyme activities, and Troponin I level during treatment to avoid myocardial infarction.

**Keywords:** Adriamycin, Doxorubicin, Cardiotoxicity, Myocardial infarction.

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## INTRODUCTION

Cancer is a group of diseases associated with unregulated cell growth and differentiation [1]. The steady increase in global cancer incidence and its associated morbidity and mortality were faced by different strategies of preventions. One of these strategies is chemoprevention by using of synthetic, natural, or biological agents to suppress, reverse, or prevent the initial phases of carcinogenesis or the progression of premalignant cells to invasive disease [2]. Interest in this area of research has markedly increased with an improved understanding of cancer biology and successes in the chemoprevention of prostate, colon and breast cancer. It has become apparent over the last years, that the definition of chemoprevention should include the concept of 'delay', which means that the preventive effect may last for a

definite period. The rate of tumor development is decreased even if the incidence eventually returns to that of the untreated population [3].

Chemoprevention may target a variety of steps in tumor initiation, promotion, and progression [4]. Many agents may have effects throughout the carcinogenic process. Compounds that inhibit cancer initiation are traditionally termed 'blocking agents' acts by preventing the interaction between endogenous free radicals or chemical carcinogens and DNA, leading to a reduction of damage and mutations which contribute cancer initiation, progressive genomic instability and overall neoplastic transformation [5]. Prevention of cancer initiation by downregulating of chronic inflammatory responses and reduce reactive oxygen and nitrogen species production, or by protecting processes include modulation of DNA methyl transferases to

prevent inactivation of tumor suppressor genes. Histone deacetylases inhibition has also been described among a variety of effects of blocking agents on cancer epigenetic mechanisms [6]. Chemopreventive agents may affect at the initiation of cancer by stopping promotion and progression of cancerous cells. The mechanisms contributing to this activity by inhibition of signal transduction pathways and inhibiting the effects of tumor promoters which will lead to cell proliferation. Tamoxifen as anti-estrogens can block this effect in cases of cancers which promoted by estrogens [2]. Recent reports suggest that some chemopreventive agents interfere with cancer cell metabolism and energy homeostasis via effects on pathways such as AMP kinase [7]. The adverse effects of chemotherapeutic agents lead to restriction of chemotherapy. The toxic by-products of some chemotherapeutic drugs are deposited in vital organs such as the heart, liver, and kidneys producing serious organs toxicity [8].

Doxorubicin (DOX), also called adriamycin, is a medication used in cancer chemotherapy. It is derived from bacteria by chemical semisynthesis. It is an anthracyclin antitumor antibiotic closely related to the natural product daunomycin. Like all anthracyclines, it works by intercalating DNA, with the most serious adverse effect being life-threatening cardiotoxicity. It is commonly used in the treatment of a wide range of cancers, including malignancies, soft tissue sarcomas, and solid tumors. It is often used in combination chemotherapy as a component of various chemotherapy regimens [9]. DOX is commonly used to treat Hodgkin's lymphoma, some leukemias, bladder, breast, stomach, lung, ovaries, thyroid cancers, and multiple myeloma [9]. Commonly used doxorubicin-containing regimens are TAC (Taxotere), ABVD (adriamycin, bleomycin, vinblastine, dacarbazine), AC (Adriamycin, cyclophosphamide), FAC (5-fluorouracil, adriamycin, cyclophosphamide) and CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone). Doxorubicin and similar compounds also have antimalarial effects. In 2009, a compound similar in structure to doxorubicin was found to inhibit plasmepsin II (an enzyme unique to the malarial parasite *Plasmodium falciparum*) [10]. Cardiomyopathy is the most dangerous toxic effect of doxorubicin which leading to congestive heart failure. The rate of cardiomyopathy is dependent on its cumulative dose. Another common and fatal complication of DOX is typhlitis, an acute life-threatening infection of the bowel [11]. It also characterized by developing allergic reactions characterized by skin eruptions on the palms of the hand or soles of the feet, swelling, pain, and erythema. DOX-containing regimens can also lead to reactivation of hepatitis B infection and dyspigmentation [12].

The analysis of cardiac biomarkers has become the frontline diagnostic tools for AMI and has

greatly enabled the clinicians in the rapid diagnosis and prompt treatment planning, thereby reducing the mortality rate to a great extent [13]. There are some cardiac markers that can be used in the diagnosis of myocardial infarction among them include aspartate transaminase, alanine transaminase, troponin I, creatine kinase, etc. Creatine kinase (isoenzymes CK-MB) is the enzyme used as a definitive serum marker for the diagnosis or exclusion of acute myocardial infarction [14]. Creatine kinase, lactate dehydrogenase, and their isoenzymes are exhibited better cardiac specificity. Cardiac troponins I and T have replaced the cytosolic enzymes in the role of diagnosing myocardial ischemia and infarction. These markers, known as cardiac risk markers, evolved from diagnostic markers such as troponins or CK-MB, but markers of inflammation also belong to these groups of diagnostic chemicals [15]. The markers that are well suited for the early diagnosis of AMI within the time interval 0-6 hours after symptom onset are myoglobin, H-FABP, and CK-MB isoforms. CK-MB mass measurement is suitable in the 6-24 hours interval; CK-MB based on activity measurement is more sensitive in the 12-24 hours interval, and the other cardiac markers like total CK, cTnT, and cTnI are most reliable after 12 hours from symptom onset. The prolonged diagnostic window of cardiac troponins of several days that is highly sensitive and specific obviates the needs for less specific markers with a long diagnostic window like aspartate aminotransferase (ALT) and CK. Based on the recent recommendations by the ESC/ACC, cTnI and cTnT are the best markers for the confirmation of AMI. CK-MB is the second best marker in the absence of troponins assays [16].

## MATERIALS AND METHODS

### Doxorubicin

Doxorubicin (DOX) is commercially available in powder form for injection and was purchased from Carlo Erba, Turkey. It was dissolved in normal saline and administered by intraperitoneal injection in a dose of 4 mg/kg body weight once weekly for 3 weeks [17].

### Solid Ehrlich Carcinoma Tumor Model

A model of SEC was used, where  $1 \times 10^6$  of the Ehrlich carcinoma cells (ECCs) obtained from the Pharmacology and Experimental Oncology unit of the national cancer institute, Cairo University, Egypt. These were implanted subcutaneously into the right thigh of the hind limb of mice. A palpable solid tumor mass (about 100 mm<sup>3</sup>) was developed within 12 days following implantation of ECCs [18].

### Animals

BALB/c male mice weighing 20–25 gm, obtained from the animal house of the faculty of medicine, Tanta University, Egypt. Animals were kept in individual metabolic cages at 22 °C, 55% relative humidity and 12/12 hours light-dark cycle through the whole period of the study. All the experiments were

conducted according to the Helsinki declaration of animal ethics [19]. The protocol of this study was approved by the ethical committee of the faculty of pharmacy, Helwan University, Egypt.

### Experimental Design

Sixty BALB/c male mice were used in this study. Except for mice in the control group, each mouse was implanted subcutaneously with 0.2 ml of the ascites fluid containing  $1 \times 10^6$  ECCs into the right thigh of the hind limb of a mouse. The day of implantation of ECCs was considered as the zero point (day 0) of the experiment. The total period of the experiment was 49 days (7 days before and 42 days after tumor inoculation). Mice were randomly divided into three equal groups (20 mice per each group) as follows: Group 1: Control group, in which mice received intraperitoneal (i.p.) injection of 0.2 ml normal saline once weekly on days 0, 7, 14, 21 (for 21 days). Group 2: Solid Ehrlich carcinoma (SEC) control group, in which mice received intraperitoneal injection of 0.2 ml normal saline once weekly on days 0, 7, 14, 21 (for 21 days) starting one hour after tumor inoculation [18]. Group 3: Doxorubicin (DOX) group, in which mice received DOX (4 mg/kg, i.p.) once weekly on days 0, 7, 14, 21 (for 21 days) starting one hour after tumor inoculation [17].

On the 40<sup>th</sup> day after tumor inoculation tumor volume was measured. Two days later (42 days after tumor inoculation), the survival rate was determined. Then, the blood samples were collected. Mice were sacrificed and their hearts were excised for further investigation.

Assessment of the time-course effects of different treatments on tumor volume of SEC: Tumor volume was measured using a Vernier caliper starting on the 15<sup>th</sup> day post-implantation and thereafter every 5 days till the last record on the 40<sup>th</sup> day post-implantation prior to scarification of the survived mice. Tumor volume (V) was calculated as  $V \text{ (mm}^3\text{)} = (a^2 \times b) / 2$  Where: (a) represents length of the small diameter (mm), (b) represents length of the large diameter (mm).

Counting of the survival rate: The number of the survived mice in each group was counted on days 7, 14, 21, 28, 35 and 42 after implantation of Ehrlich carcinoma cells then divided by the total number of animals in each group to get the percentage of the survived mice [20].

### Preparation of Blood Serum Samples

On the 42<sup>nd</sup> day after implantation of ECCs, blood was withdrawn from the orbital sinus of the mouse under light ether anesthesia. Serum was separated immediately by centrifugation at 4000 rpm for 10 minutes.

### Preparation of Tissue Homogenates

The hearts of mice were immediately dissected out and freed from the adjacent tissues, washed with cold saline to remove any excess blood, blotted to dry on filter paper and then weighed and were divided into two portions: The first portion was homogenized in cold saline to give 10 % homogenate using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA). The homogenate was centrifuged at 3000 rpm for 10 minutes at 4 °C and the supernatant was prepared for MDA analysis. The second portion was used for histopathological examinations.

Determination of serum lactate dehydrogenase (LDH) activity: using kits supplied by STANBIO, USA according to Buhl and Jackson [21]. Principle: Elevations in serum LDH occur as a result of myocardial disease, liver disease, pernicious and megaloblastic anemias, pulmonary emboli, malignancies, and muscular dystrophy. Analysis of LDH provides a definite diagnosis of myocardial affection. LDH specifically catalyzes the oxidation of lactate to pyruvate with the subsequent reduction of NAD to NADH. The rate at which NADH forms is proportional to LDH activity. The method described determines NADH absorbance increase per minute at 340 nm.

Determination of serum creatine kinase (CK-MB) activity: using kits provided by STANBIO, USA according to Rosalki [22]. Principle: Creatine kinases are dimeric molecules composed of M and B subunits and exist as the iso-enzymes MM, MB, and BB. The subunits M and B are immunologically distinct. CK-MM and CK-MB are distributed primarily in the skeletal muscle and heart muscle, respectively, while CK-BB is present mainly in the brain and in tissues composed of smooth muscle. Following acute myocardial injury, CK-MB activity increases significantly and this elevation is highly specific for laboratory diagnosis of myocardial damage. This method is an optimized UV-test according to the German Society of Clinical Chemistry and International Federation of Clinical Chemistry and Laboratory Medicine [22]. In this procedure, CK activity is measured in the presence of an antibody to CK-M monomer. This antibody to CK-M monomer completely inhibits the activity of CK-MM and half of the activity of CK-MB, while not affecting the B subunit activity of CK-MB and CK-BB. Due to negligible concentrations of CK-BB in the circulation, the remaining activity, multiplied by a factor of 2, represents the activity of the CK-MB isoenzyme.

Determination of serum cardiac troponin I level: using ELISA kits supplied by Sigma Aldrich Co. according to the instructions of the manufacturer. Principle: The troponin I ELISA kit is based on the principle of a solid phase ELISA. The assay system utilizes four unique monoclonal antibodies directed

against certain antigenic determinants on the molecule. Three mice monoclonal anti-troponin I antibodies are used for solid phase immobilization on the microplate wells. The fourth antibody is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the four antibodies, resulting in the troponin I molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 90 minutes' incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A substrate solution (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution changing the color to yellow. The concentration of troponin I is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

**Determination of tumor and cardiac tissue malondialdehyde (MDA) level:** Principle: This method depends on the fact that MDA reacts with TBA producing a thiobarbituric acid reactive substance (allegedly a [TBA] 2 – Malondialdehyde adduct) a pink chromogen. The thiobarbituric acid reactive substances in the tumor and cardiac tissues were measured according to Uchiyama and Mihara method [23].

#### Reagents

- Potassium chloride, prepared as 1.15% solution by dissolving 1.15gm in 100ml distilled water. Orthophosphoric acid  $H_3PO_4$ , prepared as 1% by adding 1 ml of orthophosphoric acid to 99 ml distilled water,
- Thiobarbituric acid (TBA) was prepared as 0.6% aqueous solution by dissolving 0.6gm TBA in 100 ml distilled water, N-butanol.

#### Procedure

0.5 g of tissue with 4.5 ml of KCl 1.15% were homogenized to form 10% homogenate sample, then, 0.5 ml of the homogenate was added to the solution composed of 3 mL of 1% phosphoric acid and 1 mL of 0.6% TBA aqueous solution, Heating the mixture on a boiling water bath for 45 min. After cooling, 4 ml of *n*-butanol, was added then shaken, and the butanol layer was separated by centrifugation at 2000 rpm for 10 minutes. The optical density of the butanol layer was determined at 535 and 520 nm, and the difference of optical density was calculated between the two determinations to be taken as the TBA value. Calculation: The thiobarbituric acid reactive substances were calculated using  $1.56 \times 10^5 \text{ M}^{-1} \text{ Cm}^{-1}$  as molar absorption coefficient and expressed as  $\mu\text{mol/gm}$  wet tissue.

**Histopathological examination:** The SEC and cardiac sections were prepared and stained with hematoxylin and eosin (H&E) (For both tumor and cardiac tissues) and Mallory's trichrome stain and examined under a light microscope.

#### Statistical Analysis

For statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 16.0 was used. Parameters were shown with a mean  $\pm$  Standard error of the mean (SEM). Multiple comparisons were performed using one way analysis of variance (ANOVA) and nonparametric followed by Tukey-Kramer test for post hoc analysis, as appropriate. Unpaired t-test and Mann-Whitney test were used to compare between two different treatment groups. The overall and pairwise comparisons of survival rate were analyzed by applying the Breslow test (generalized Wilcoxon) using the Kaplan–Meier method. Differences between the means of the different groups were considered significant at a level of  $p\text{-value} < 0.05$ .

## RESULTS

The illustrated data in figure (1) showed that administration of DOX (4 mg/kg) to mice resulted in a significant decrease in tumor volume at the 40<sup>th</sup> day post-implantation by 32.5 % compared to the SEC control group and the survival rate which illustrated in table (1) showed that subcutaneous implantation of ECCs resulted in significant decrease in the survival rate at the 42th day post-implantation by 50 % compared to the control group on the other hand the administration of DOX (4 mg/kg) to ECCs-bearing mice resulted in a significant increase in the survival rate by 50 % as compared to the SEC control group, However, this value was still significantly less than the control group.

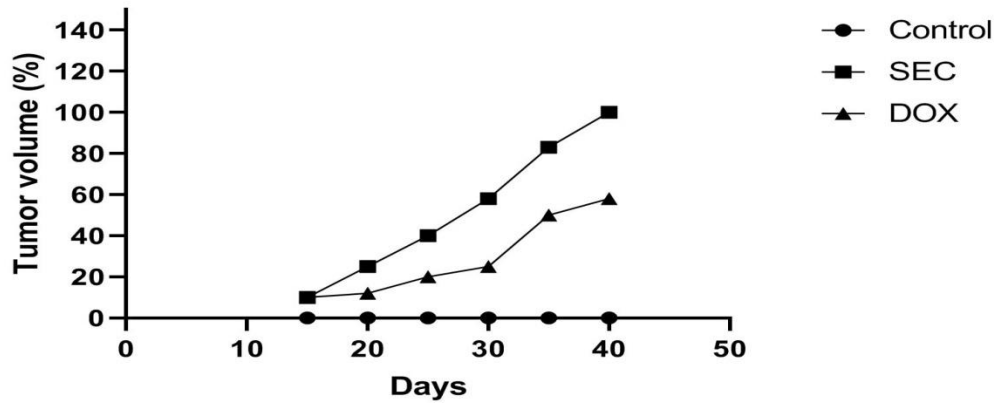
**Table (1): Survival rate as affected by SEC or DOX treatment**

Groups	Survival rate (%)	Survival duration (days; % confidence interval (CI), lower bound to upper bound)
Control	100 %	(42.0 $\pm$ 0.0 days; 95% CI, 42.0–42.0)
SEC	50 % *	(36.4 $\pm$ 2.1 days; 95% CI, 33.11–42.07)
DOX	75% **	(40.6 $\pm$ 0.7 days; 95% CI, 39.69–42.84)

(\*) Significant difference compared to control group ( $p < 0.05$ )

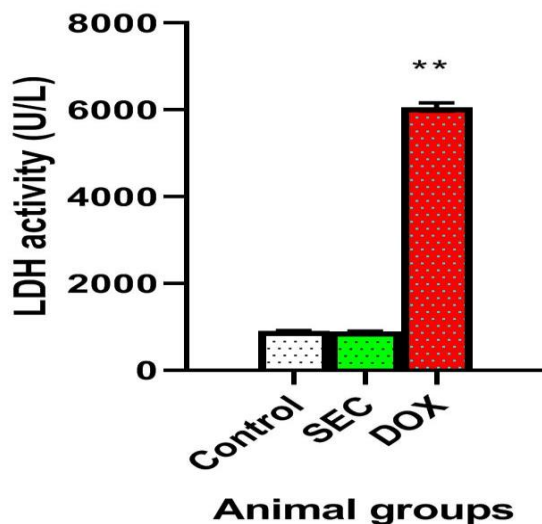
(\*\*) Highly significant difference compared to control group ( $p < 0.01$ )





**Figure (1): Tumor volume as affected by SEC or DOX treatment**

Serum LDH activities which illustrated in figure (2) showed that subcutaneous implantation of ECCs resulted in the non-significant effect on this enzyme activity as compared to the control group, but the administration of DOX (4 mg/kg) to ECCs-bearing mice resulted in a significant increase in serum LDH activity by 5.7 folds as compared to the control group. The other cardiac enzyme CK-MB was showed a significant increase in its activity by 73.2% by administration of DOX as compared to the control group but subcutaneous implantation of ECCs did not show any significant changes in this enzyme activity on comparing with the control group (figure 2).

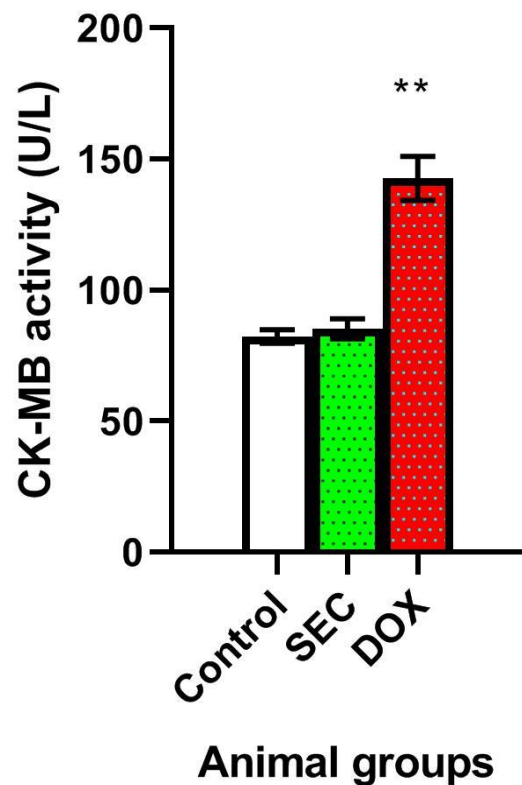


**Figure (2): Serum LDH as affected by SEC or DOX treatment**

(\*) Significant difference compared to control group ( $p < 0.05$ )  
 (\*\*) Highly significant difference compared to control group ( $p < 0.01$ )

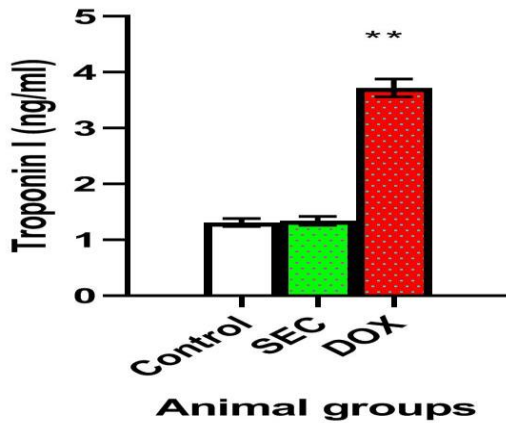
Administration of DOX (4 mg/kg) to ECCs-bearing mice resulted in significant increase in serum cardiac troponin I level by 1.8 folds as compared to the control group, but non-significant effects in SEC group (figure 3). The results in figure (4) showed that subcutaneous implantation of ECCs resulted in non-

significant effect on cardiac tissue MDA level compared to the control group, and administration of DOX (4 mg/kg) to ECCs-bearing mice resulted in significant increase in cardiac tissue MDA level by 56.6% compared to the control group.



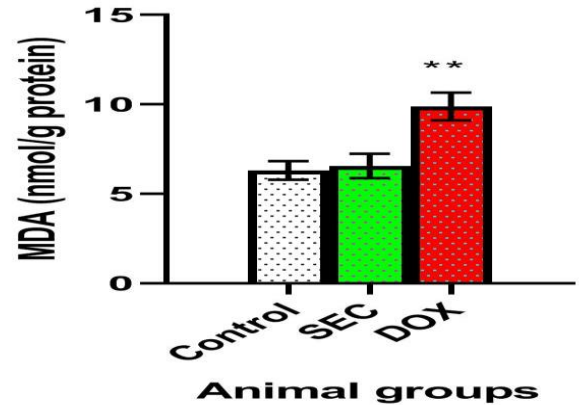
**Figure (3): Serum CK-MB as affected by SEC or DOX treatment**

(\*) Significant difference compared to control group ( $p < 0.05$ )  
 (\*\*) Highly significant difference compared to control group ( $p < 0.01$ )



**Figure (4): Serum troponin I as affected by SEC or DOX treatment**

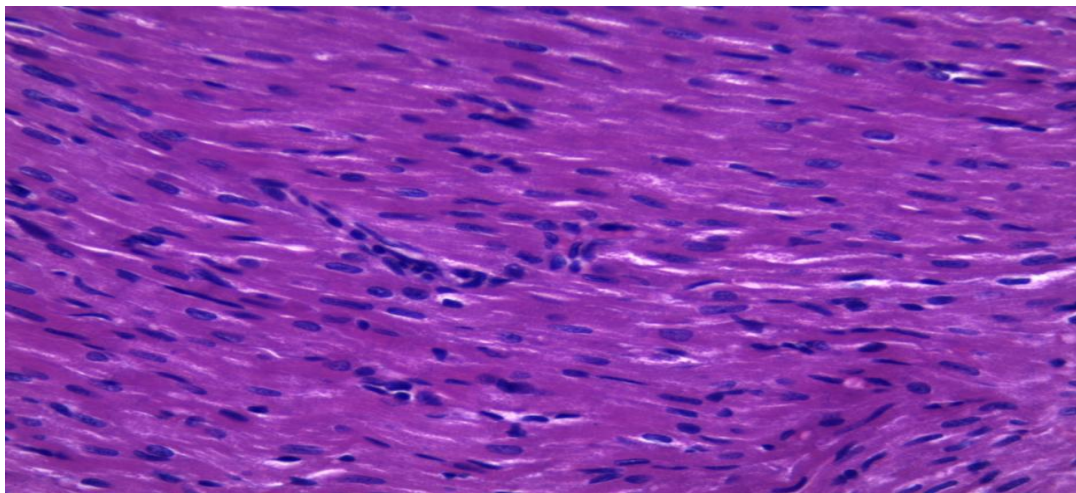
(\*) Significant difference compared to control group ( $p < 0.05$ )  
(\*\*) Highly significant difference compared to control group ( $p < 0.01$ )



**Figure (5): Cardiac tissue MDA level as affected by SEC or DOX treatment**

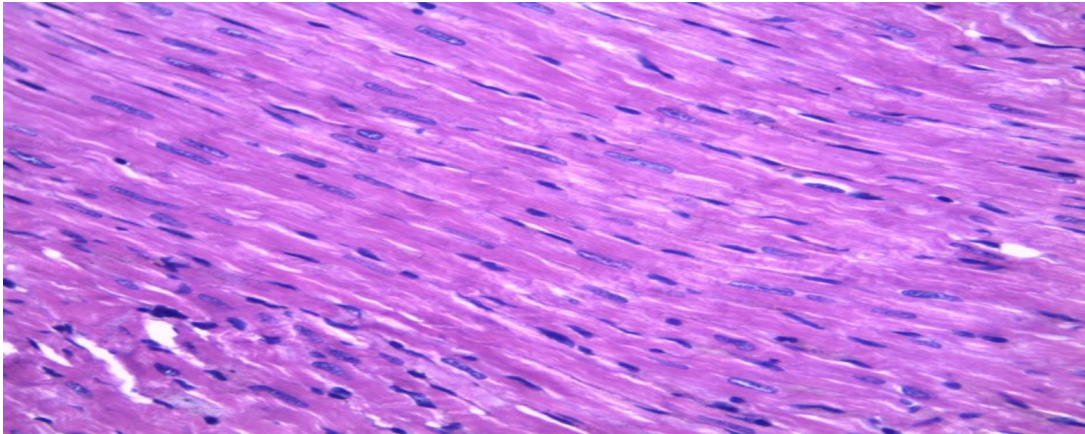
(\*) Significant difference compared to control group ( $p < 0.05$ )  
(\*\*) Highly significant difference compared to control group ( $p < 0.01$ )

The histopathological studies which illustrate in figures (6 – 10) showed that administration of DOX to mice resulted in inflammatory cellular infiltration, disorganization of the heart tissue and interstitial fibrosis of the myocardium. The cellular infiltrate was mostly composed of mature lymphocytes. Connective tissue was clearly increased and fibrosis was evident around myocardial fibers.

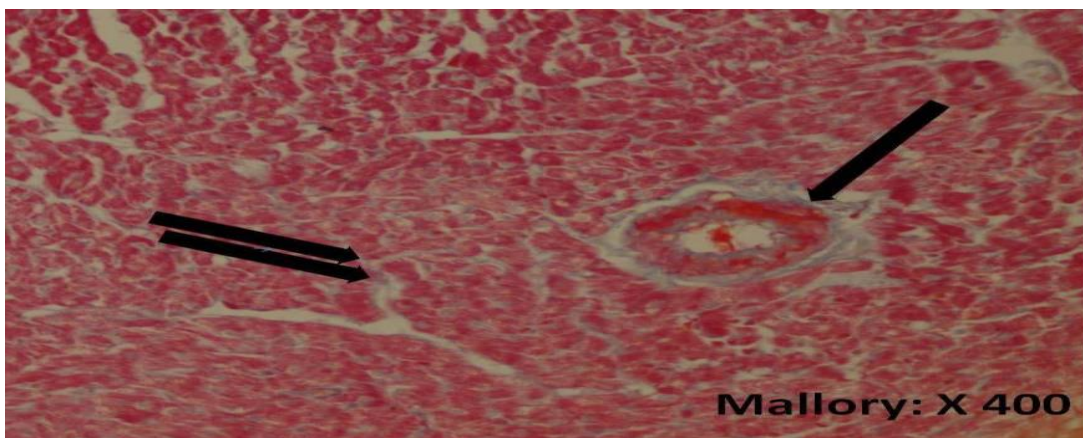


**Fig. (6): A photomicrograph of the cardiac sections from the control group showing normal morphology, consisting of striated muscle fibers (Arrows) and sparse connective tissue (H&E X 400).**

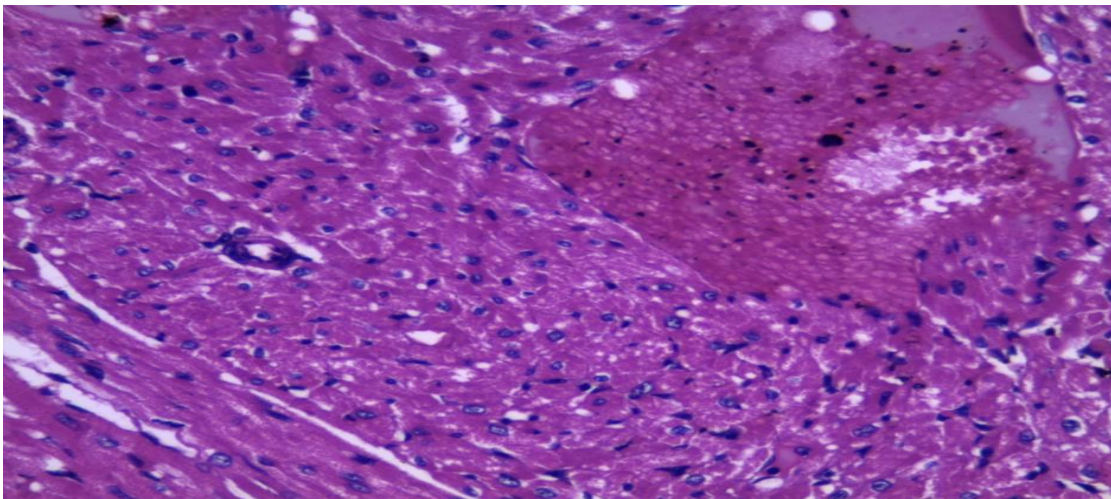




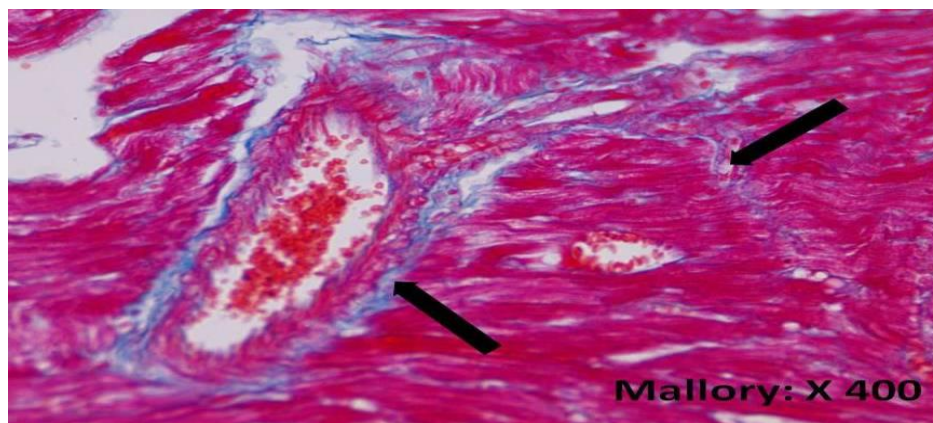
**Fig. (7):** A photomicrograph of the cardiac sections from SEC group showing normal morphology, consisting of striated muscle fibers (Thin arrows), sparse connective tissue with normal blood vessels (Thick arrow) (H&E X 400).



**Fig. (8):** A photomicrograph of the cardiac sections from SEC group showing very minimal fibrous tissue surrounding blood vessels (Arrow) (Mallory×400)



**Fig. (9):** A photomicrograph of the cardiac sections from DOX group showing massive inflammatory cellular infiltration (Thin arrows) and necrosis (Thick arrows) among cardiac muscle cells (H &E ×400).



**Fig. (10): A photomicrograph of the cardiac sections from DOX group showing marked fibrosis among cardiac muscle cells (Arrows) (Mallory×400)**

## DISCUSSION

There are several mechanisms by which DOX is believed to cause cardiomyopathy, including oxidative stress, downregulation of genes for contractile proteins, and p53 mediated apoptosis [24]. Shi *et al.*, [25] attributed DOX-induced cardiotoxicity to abnormal protein processing, hyper-activated innate immune responses, inhibition of neuregulin-1/ErbB signaling, impaired progenitor cell renewal/cardiac repair and decreased vasculogenesis.

The induction of production of free radicals is the best described mechanism through which DOX injures the myocardium. The heart's unique vulnerability to oxidative stress has given this aspect of DOX-induced cardiomyopathy an overwhelming prominence in the literature. DOX is retained in the mitochondrial inner membrane by forming a nearly-irreversible complex with cardiolipin which is required for the functions of the proteins of the electron-transport chain. This leads to disruption of the cardiolipin-protein interface leading to superoxide ( $O_2^-$ ) formation [26]. Other membrane proteins, such as those responsible for the transfer of carnitine, can also be affected by doxorubicin, contributing to the decrease in mitochondrial function. Moreover, it was reported that the binding of DOX to eNOS (nitric oxide synthase) reductase domain results on  $O_2^-$  generation. Also, DOX has a strong affinity for iron, and that the iron complex could cause lipid peroxidation through its interactions with the negatively-charged membranes. This sets up a cycle for free radical generation and the metabolite doxorubicinol is known to interact with thiol groups on proteins, leading to severe damage to the cardiomyocytes [27].

DOX-induced cardiotoxicity is also accompanied by an increase in intracellular calcium levels. Dysregulation of intracellular calcium concentrations is both a result and a cause of ROS-generation. DOX-mediated ROS generation and apoptosis can be inhibited by using a  $Ca^{2+}$  chelator. The ROS can alter normal calcium homeostasis in a variety

of muscle cell types via disruption of normal sarcoplasmic reticulum function. This is accomplished by inhibiting the  $Ca^{2+}$  ATPase pump resulting in impaired  $Ca^{2+}$  handling and/or by directly activating the ryanodine calcium-release channels themselves [28]. DOX was reported to induce the release of calcium from the sarcoplasmic reticulum by increasing the probability that the channel adopts the open state.  $H_2O_2$  generated by DOX can modify key thiol groups on the ryanodine  $Ca^{2+}$ -release channels in the sarcoplasmic reticulum contributing to DOX-induced cardiomyopathy. Also, DOX increases the sensitivity of the mitochondria to intracellular calcium leading to disruption of mitochondrial functions [27].

Both cellular and extracellular factors have an important role in the complex process of myocardial remodeling. DOX was proven to induce significant alterations in the structure and composition of the extracellular matrix contributing to the development of heart failure. It has been shown that DOX enhance the production of matrix metalloproteinases-2 and -9 (MMP-2, MMP-9) in the heart [29]. This is believed to contribute to cardiomyopathy by weakening the collagenous matrix against which the cardiomyocytes work and contributing to pathological remodeling. Both MMP-2 and MMP-9 activities are enhanced by DOX-induced ROS generation. Tissue-inhibitor of metalloproteinase-3 (TIMP, the family which includes MMP-2 and MMP-9) also decreased after DOX administration which is consistent with the apparent increase in MMP-2 and MMP-9 activity in earlier studies [30].

Administration of DOX resulted in cardiotoxicity manifested by a significant increase in serum LDH, CK-MB and troponin I with a significant decrease in cardiac tissue MDA level which associated with significant changes in the histopathological parameters compared to the control and SEC groups. These changes were attributed to the damaging effects of reactive oxygen species generated by the interaction of DOX with iron together with inhibition of DNA



topoisomerase II and stimulation of certain immune and inflammatory responses in the cardiac tissues [25, 27]. Also, DOX was demonstrated to interfere with calcium sequestration by the sarcoplasmic reticulum by altering the calcium pump in the cardiomyocytes. The sodium/potassium pump of the sarcolemma is also affected by DOX, which disrupts the sodium gradient needed for calcium to flow into the sarcolemma of cardiomyocytes. DOX may also interact with the ryanodine receptor, allowing an uncontrolled flow of calcium out of the sarcoplasmic reticulum [31]. Furthermore, DOX may interfere with mitochondrial replication and transcription causing significant imbalances in the electron transport chain and promoting mitochondrial dysfunction [27]. Moreover, alteration of endothelin-1 expression in the cardiomyocytes seen after treatment with DOX provides an additional mechanism of cardiotoxicity. After treatment with DOX, mRNA and plasma levels of endothelin-1 are significantly increased. This increase may potentiate the increased calcium load in the cardiomyocytes, thereby leading to cardiac dysfunction [32].

In the present study, there was a controversy regarding the effect of DOX on oxidative stress in cardiac tissues. In the cardiac tissues, DOX was found to affect cardiac adriamycin-responsive protein (CARP) mRNA expression. CARP is specifically found in the cardiac and muscle tissues and acts as a negative regulator of cardiac-specific gene expression. Overexpression of CARP in cardiomyocytes suppressed cardiac troponin C and atrial natriuretic factor transcription and decreases the production of ROS [33]. CARP is the first example of a cardiac-restricted transcriptional regulatory protein that is sensitive to DOX. DOX was found to exert an inhibitory effect on CARP expression and directly affects the cardiac-specific gene expression of protein-regulating energy and calcium metabolism [33, 34]. Moreover, CARP was proven to enhance the normal pathways of apoptosis in the cardiomyocytes [33].

## CONCLUSION

It can be concluded that Doxorubicin treatment of male mice inoculated with Solid Ehrlich Carcinoma Tumor induced a myocardial infarction. Therefore, cancer patients treat with Doxorubicin should be checked the serum LDH, CK-MB enzyme activities, and Troponin I level during treatment to avoid myocardial infarction.

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