

Effects of Treatment with Doxorubicin on Proinflammatory and Prooncogenic Mediators in Solid Ehrlich Carcinoma (SEC) Tumor Model

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Abstract

Background: Doxorubicin is used for treatment of solid tumors blood cancers, however, its cardiotoxicity, cardiomyopathy, and congestive heart failure, are the major limitation of its use. Doxorubicin treatment induces inflammation in various cancer cell lines. Exposure urothelial cells to doxorubicin (DOX) show an increase in IL-1 β and prostaglandin E2, similarly, studies have shown that high IL-8, TNF- α and NF κ B. This study aimed to investigate the effects of doxorubicin treatment of inflammatory and prooncogen mediators in heart muscle and tumor tissue. **Methods:** 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 1×10^6 Ehrlich carcinoma cells (ECCs) into the thigh of the hind limb. Mice were divided into three groups (20 mice per group) as follow: 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), 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, 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. **Results:** administration of DOX to ECCs-bearing mice resulted in a significant increase in cardiac tissue IL-6 level by 4 folds compared to the control group and subcutaneous implantation of ECCs resulted in a significant increase IL-6 in tumor tissue. Administration of DOX to ECCs-bearing mice resulted in a significant decrease in tumor tissue IL-6 and TNF- α levels. SEC group showed a significant increase in tumor tissue SphK1 activity compared to the control group and administration of DOX ECCs-bearing mice resulted in the non-significant effect on the tumor tissue SphK1 activity. **Conclusion:** Treatment with DOX, leads to increase in inflammatory mediators as, IL-6 and TNF- α , in cardiac muscle tissue amelioration of these parameters in tumor tissue. SphK1 enzyme activity was increased by tumor induction.

Keywords: Doxorubicin, inflammatory mediators, Prooncogenic mediators, interleukins, Sphingosine kinase-1.

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INTRODUCTION

Resistance to drugs and failure of treatment remains a major problem in cancer therapy. Cancer resistance were classified on the basis of response to chemotherapy into two broad categories: primary and acquired [1]. Primary resistance precedes initial chemotherapy and acquired resistance involved in genetic changes after clinical intervention until tumor cells develop resistance phenotypes. Acquired resistance, in one of its forms is mediated by the interaction of tumor cells with their microenvironment. The tumor cells circumvent the apoptotic effects of chemotherapy by cell adhesion-mediated resistance, in which tumor cell integrins adhere to fibroblast or the extracellular matrix; and soluble factor-mediated

resistance, which induces the stroma to produce growth factors, chemokines, and cytokines [2, 3]. The theme of chemotherapeutic effects that, it induces signaling events that eliminate and control tumor cells and stimulates signals that could minimize their clinical efficacy and promote metastatic development. Advances in our understanding of the molecular mechanisms that elucidate cancer progression and the etiology of drug resistance have identified various crucial targets. Unsurprisingly, some of these targets promote inflammation events and seem to play a role in angiogenesis, tumor proliferation, and metastasis [4].

The inflammatory changes play an important role, in particular tumor necrosis factor alpha (TNF- α) and nuclear factor kappa B (NF κ B) signaling pathways.

The study of Ohta *et al.*, [5] assessed the effects of cisplatin treatment on ovarian cancer cells on nuclear factor kappa B activation and concluded that cisplatin enhanced its phosphorylation significantly, mediated by the PI3/Akt signaling cascade. This finding is consistent with the coexpression of NFκB transcription factors p65 and p50 in ovarian cancer patients who received a chemotherapy regimen that included cisplatin [6].

NFκB signaling is a converging point for controlling downstream signaling cascades that include, interleukin 1 (IL-1), IL-6, IL-8, TNF-α and transcription of other inflammatory genes [7]. The important cytokine in angiogenesis regulation, cell proliferation, and invasion is IL-6. Receptor system of IL-6 involves STAT-3- and extracellular signal regulated kinase (ERK)-mediated pathways. STAT-3 plays multiple roles in cell proliferation and survival and through activation of *bcl-2*, *c-myc*, and *cyclin-D* and persistent activation of STAT-3 is involved in tumorigenesis in a variety of leukemias [8, 9]. Activation of extracellular signal regulated kinase (ERK) induces cell proliferation by phosphorylation of transcription factors such as ELK1 and c-FOS. IL-8 has been implicated in cancer progression, particularly in mediating angiogenesis in various cancer types including pancreatic, colon, non-small-cell, lung carcinoma, and melanoma [10, 11].

Cisplatin treatment leads to increment of TNF-α, and a variety of pharmacological inhibitors attenuate cisplatin nephrotoxicity mediated by TNF-α. Treatment of mouse kidneys with salicylate, leads to attenuation of cisplatin-induced increase in TNF-α mRNA and also reducing TNF-α levels in serum. Rutin treatment on Wistar rats has a beneficial effect on cisplatin's deteriorative effects through inhibition of TNF-α and NFκB pathway-mediated inflammation. Likewise, administration of luteolin in kidneys of mice significantly reduced TNF-α and NFκB, as well as COX-2 expression [12].

Doxorubicin is used for treatment of solid tumors blood cancers, however, its cardiotoxicity, cardiomyopathy, and congestive heart failure, are the major limitation of its use [13, 14]. The studies of Sauter *et al.*, [15] and Kang *et al.*, [16] stated that doxorubicin treatment induces inflammation in various cancer cell lines. Exposure urothelial cells to doxorubicin show an increase in IL-1β and prostaglandin E2, similarly, studies have shown that high IL-8, TNF-α and NFκB monocyte chemotactic protein-1 and G-CSF84-expressing mice have better outcomes from doxorubicin-induced cardiac damage and mortality and if they are pretreated with an IL-1 receptor antagonist. Activation of NFκB and inflammatory cytokines had been approved by doxorubicin effect on adipose tissue, thereby increasing serum total cholesterol, triglyceride, and low-density lipoprotein levels. This can be explained by doxorubicin induced downregulation of

peroxisome proliferator activated receptor gamma, which is present in white adipose tissue. The sequence of events can be summarized as a reduction in circulating free fatty acid clearance, macrophage recruitment, and activation of NFκB and inflammatory cytokines [17].

Functional studies indicated that IL-6 can increase the invasion and migration of Renal cell carcinoma and trigger the epithelial-mesenchymal transition and the clinical data also revealed that serum IL-6 was undetectable in healthy people while presented in metastatic Renal cell carcinoma patients. Higher serum levels of IL-6 were correlated with worse prognosis outcomes of renal cell carcinoma [18]. This study aimed to investigate the effects of doxorubicin treatment on proinflammatory and prooncogenic mediators in heart muscle and tumor tissue.

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 [19].

Solid Ehrlich Carcinoma (SEC) Tumor Model

A model of SEC was used, where 1×10^6 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 mm³) was developed within 12 days following implantation of ECCs [20].

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 Helsinki declaration of animal ethics [21]. 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 1×10^6 ECCs into the right thigh of the hind limb of 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 [20].

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 [19]. On the 40th day after tumor inoculation tumor volume was measured. Two days later (42 days after tumor inoculation), the survival rate was determined. Mice were sacrificed and heart and tumor 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 15th day post-implantation and thereafter every 5 days till the last record on the 40th day post-implantation prior to scarification of the survived mice. Tumor volume (V) was calculated as $V (\text{mm}^3) = (a^2 \times b) / 2$ 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 [22].

Preparation of Tissue Homogenates

The heart and tumor tissue 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. Each of heart and tumor was divided into two portions, the first portion 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 further biochemical analysis.

The second portion: was used for histopathological and immunohistochemical examinations.

Determination of tumor tissue sphingosine kinase-1 (SphK1) activity

Using ELISA kits supplied by MyBioSource, Inc., USA, according to the manufacture's protocol.

Principle

Sphingosine kinase 1 (SphK1) catalyzes the phosphorylation of sphingosine to sphingosine-1-phosphate. SphK1 has been implicated in proliferation, survival, migration and regulation of calcium homeostasis, development and regulation of the cardiovascular and nervous systems, inflammation, immunity, and cancer growth. SphK1 activity assay is prepared by the addition of sphingosine kinase, sphingosine (substrate) in reaction buffer and initiated by the addition of ATP. The reaction is then stopped by adding the ATP detector after the chosen reaction time. The luminescent signal is inversely correlated with the kinase activity.

Determination of tumor and cardiac tissue tumor necrosis factor-alpha (TNF-α)

Using mouse TNF-α ELISA kits supplied by RayBiotech, Inc. according to the instructions of the manufacturer.

Principle

TNF-α ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of TNF-α in serum, plasma and tissue homogenate supernatants. This assay employs an antibody specific for mouse TNF-α coated on a 96-well plate. Standards and samples are pipette into the wells and TNF-α present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Mouse TNF-α antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TNF-α bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Determination of tumor and cardiac tissue interleukin-6 (IL-6) level

Using mouse IL-6 ELISA kits supplied by Sigma Chemical Co. according to the instructions of the manufacturer.

Principle

Mouse IL-6 ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of IL-6 in biological samples, such as serum, plasma, cell culture supernatants, urine, and/or cell and tissue lysates. This assay employs a specific capture antibody coated on a 96 well plate. Standards and samples are pipetted into the wells and IL-6 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and a biotinylated detection antibody specific for IL-6 is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to

the amount of IL-6 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Histopathological and Immunohistochemical 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 (For cardiac tissues) and examined under light microscope.

Statistical Analysis

For statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 16.0 was used. Parameters were shown with mean ± Standard error of 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 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-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 40th 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 a 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 ± 0.0 days; 95% CI, 42.0–42.0)
SEC	50 % *	(36.4 ± 2.1 days; 95% CI, 33.11–42.07)
DOX	75 % *#	(40.6 ± 0.7 days; 95% CI, 39.69–42.84)

* Significantly different from the control group (P < 0.05)
 # Significantly different from SEC control group (P < 0.05)

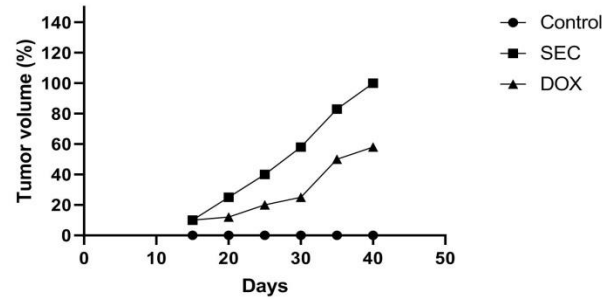


Figure (1): Tumor volume as affected by SEC or DOX treatment

The illustrated results in figure (2) showed that subcutaneous implantation of ECCs resulted in the non-significant effect on cardiac tissue IL-6 level compared to the control group. On the other hand, administration of DOX (4 mg/kg) to ECCs-bearing mice resulted in a significant increase in cardiac tissue IL-6 level by 4 folds compared to the control group. The data in figure (3) showed that, subcutaneous implantation of ECCs resulted in a significant increase in tumor tissue IL-6 level by 5.8 folds compared to the control group and administration of DOX (4 mg/kg) to ECCs-bearing mice resulted in a significant decrease in tumor tissue IL-6 level by 42.2% as compared to SEC control group. However, this value was still significantly higher than the control group.

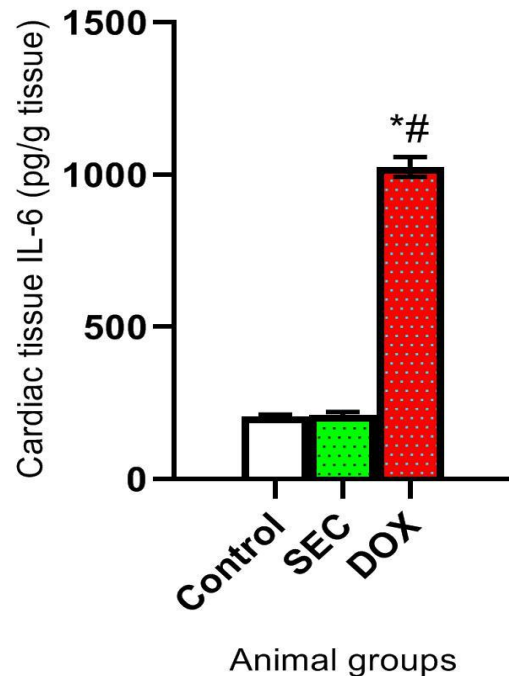


Figure (2) Cardiac tissue IL-6 as affected by SEC or DOX treatment

* Significantly different from the control group (P < 0.05)
 # Significantly different from SEC control group (P < 0.05)

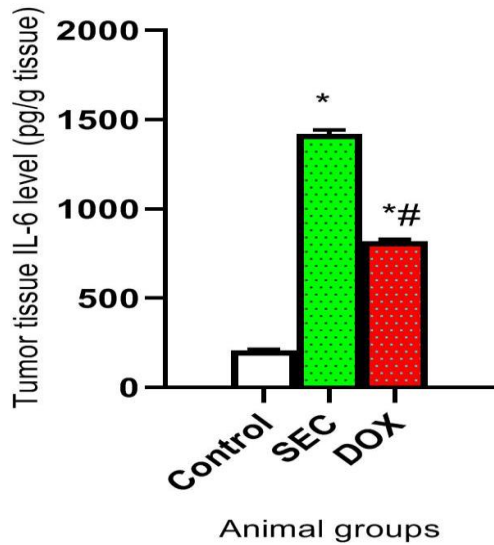


Figure (3) Tumor tissue IL-6 as affected by SEC or DOX treatment

* Significantly different from the control group ($P < 0.05$)
 # Significantly different from SEC control group ($P < 0.05$)

Figure (4) showed that subcutaneous implantation of ECCs resulted in the non-significant effect on cardiac tissue TNF- α level compared to the control group and administration of DOX (4 mg/kg) to ECCs-bearing mice resulted in a significant increase in cardiac tissue TNF- α level by 2.7 folds as compared to the control group. Subcutaneous implantation of ECCs resulted in a significant increase in tumor tissue TNF- α level by 5.1 folds compared to the control group. Administration of DOX (4 mg/kg) to ECCs-bearing mice resulted in a significant decrease in tumor tissue TNF- α level by 38.4% as compared to SEC control group. However, this value was still significantly higher than the control group (Figure 5).

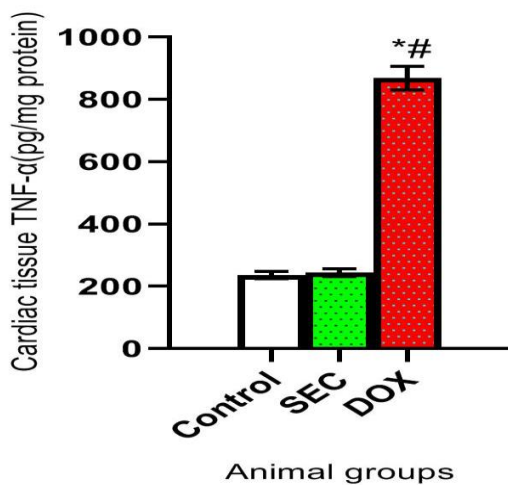


Figure (4) Cardiac tissue TNF- α as affected by SEC or DOX treatment

* Significantly different from the control group ($P < 0.05$)
 # Significantly different from SEC control group ($P < 0.05$)

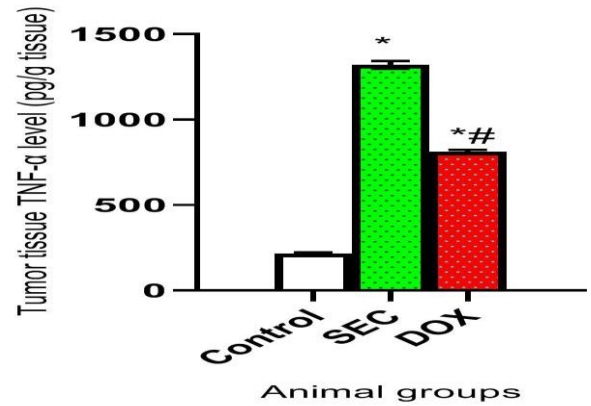


Figure (5) Tumor tissue TNF- α as affected by SEC or DOX treatment

* Significantly different from the control group ($P < 0.05$)
 # Significantly different from SEC control group ($P < 0.05$)

Subcutaneous implantation of ECCs resulted in a significant increase in tumor tissue SphK1 activity by 80.9 % compared to the control group and administration of DOX (4 mg/kg) to ECCs-bearing mice resulted in the non-significant effect on the tumor tissue SphK1 activity compared to SEC control group. However, this value was still significantly higher than the control group (Figure 6).

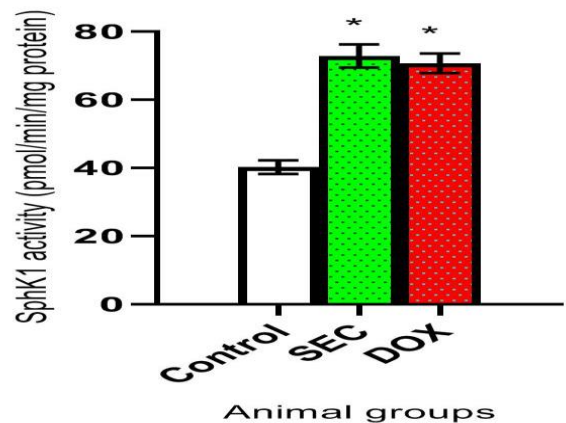


Figure (6) SphK1 as affected by SEC or DOX treatment

* Significantly different from the control group ($P < 0.05$)

The histopathological studies on cardiac and tumor tissues indicated 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 (Figures 7-11). Subcutaneous implantation of ECC into the right thigh of the hind limb of mice resulted in the development of Ehrlich solid tumor (At day 42 post-implantation) showing sheets of small, higher chromatophilic tumor cells of a variable shape representing cell proliferation regions surrounding areas of necrosis and differentiated cells.

Intraperitoneal administration of doxorubicin to mice resulted in the improvement of the histopathological

picture manifested as sheets of malignant cells with focal necrosis (Figures 12,13).

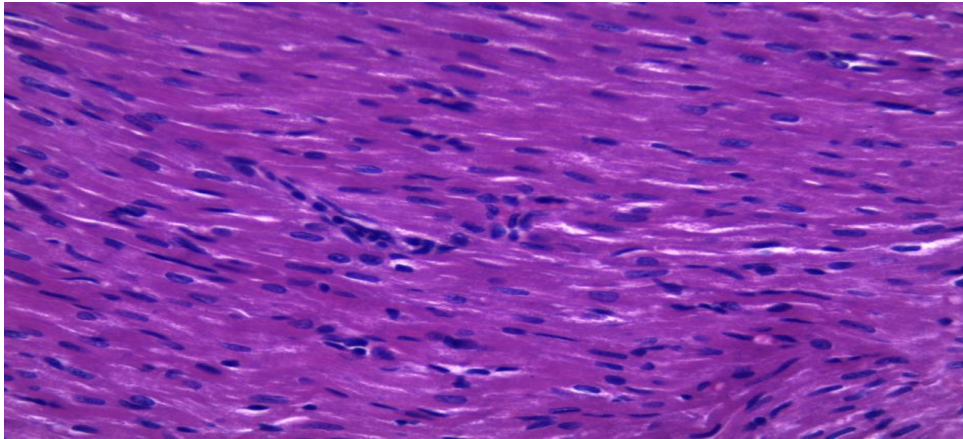


Fig. (7): A photomicrograph of the cardiac sections from the control group showing normal morphology, consisting of striated muscle fibers and sparse connective tissue (H&E X 400).

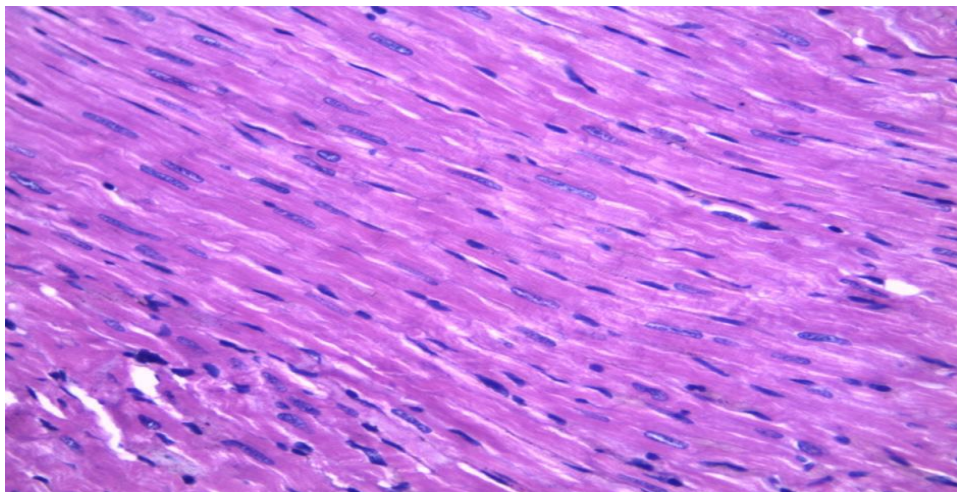


Fig. (8): A photomicrograph of the cardiac sections from SEC group showing normal morphology, consisting of striated muscle fibers, sparse connective tissue with normal blood vessels (Thick arrow) (H&E X 400).

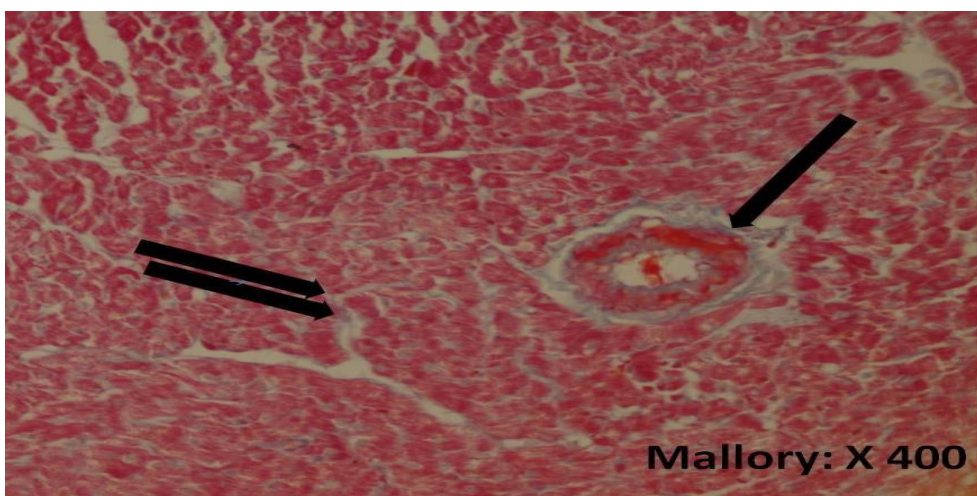


Fig. (9): A photomicrograph of the cardiac sections from SEC group showing very minimal fibrous tissue surrounding blood vessels (Arrow) (Mallory X 400)

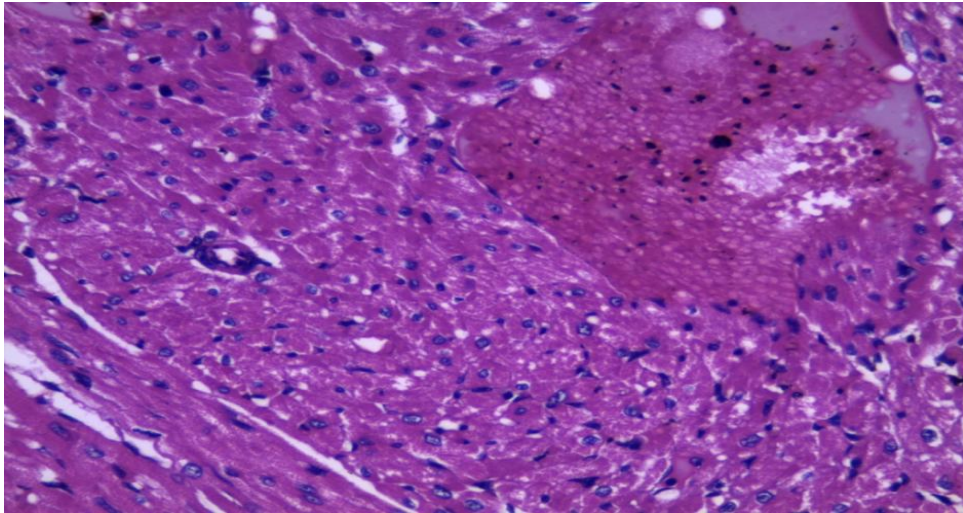


Fig. (10): A photomicrograph of the cardiac sections from DOX group showing massive inflammatory cellular infiltration and necrosis among cardiac muscle cells (H &E ×400)

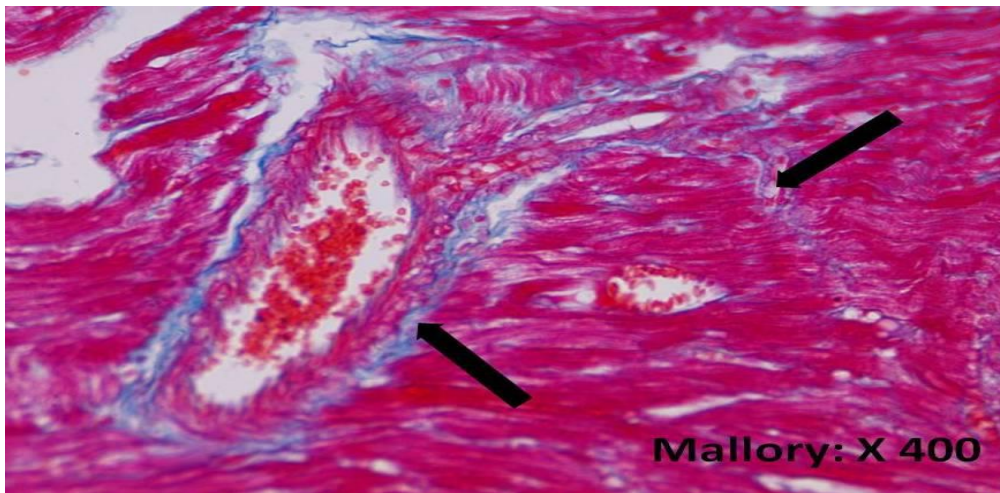


Fig. (11): A photomicrograph of the cardiac sections from DOX group showing marked fibrosis among cardiac muscle cells (Arrows) (Mallory×400)

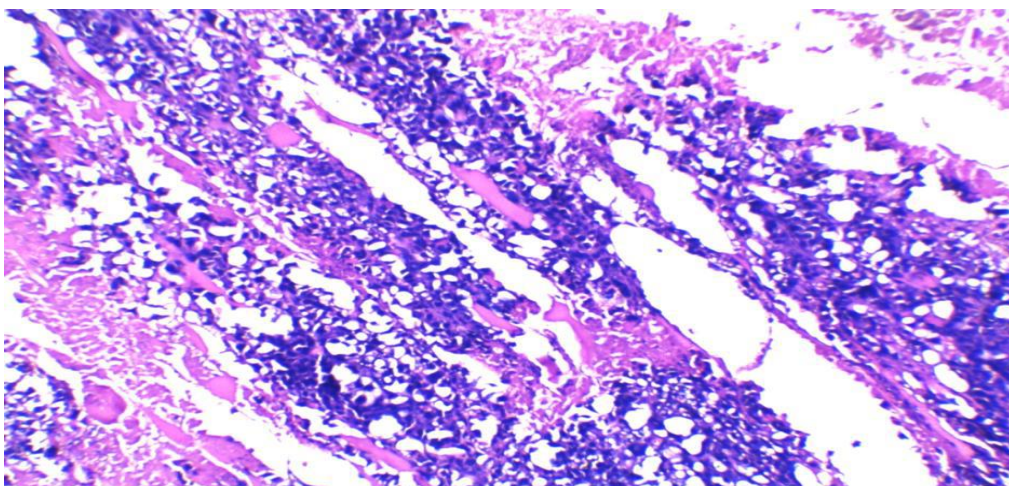


Fig. (12): A photomicrograph of Ehrlich solid tumor showing sheets of small, higher chromatophilic tumor cells of variable shape representing cell proliferation regions (Thin arrows) surrounding areas of necrosis (H&E X 250).

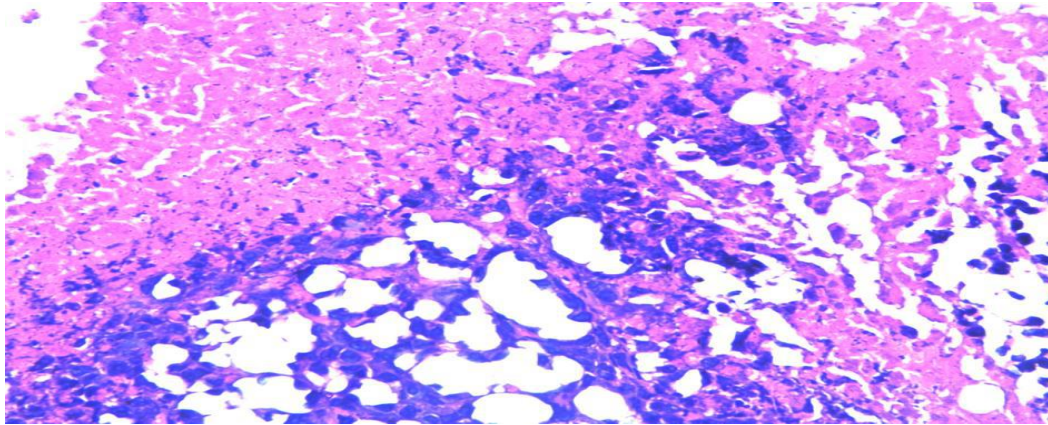


Fig. (13): A photomicrograph of SEC sections from mice that received doxorubicin showing sheets of malignant cells with focal necrosis (H&E X 250).

DISCUSSION

In the present study, implantation of ECCs resulted in a significant increase in tumor tissue TNF- α and IL-6 compared to the control group which was in the same line with other studies which reported that proinflammatory cytokines such as TNF- α and IL-6 have an important role in cancer development [23]. TNF- α and IL-6 are perhaps the best characterized protumorigenic cytokines that were initially suspected to be involved in cancer due to their ability to activate the oncogenic transcription factors such as nuclear factor kappa B (NF- κ B), activator protein 1 (AP-1) and signal transducers and activators of transcription 3 (STAT3) in epithelial cells [24].

Sphingosine kinase-1 (SphK1) is an enzyme responsible for phosphorylation of sphingosine to produce sphingosine-1-phosphate (S1P) which is a key regulator of cell growth, differentiation and migration [25, 26]. SphK1 was reported to increase the production of TNF- α and IL-6 through activation of STAT3 and NF- κ B which may participate in the pathogenesis of cancer. Moreover, S1P is a mediator of calcium influx during neutrophil activation by IL-6 leading to the production of superoxide toxic radicals that lead to further destruction of various body tissues [27]. Many evidences demonstrate that SphK1 is over-expressed in many tumor types and that inhibitors of SphK1 may sensitize tumors to chemotherapy [28]. The results of the present study showed that subcutaneous implantation of ECCs resulted in a significant increase in tissue SphK1 activity which in turn increased the production of TNF- α and IL-6 by tumor tissues compared to the control group. Overexpression of SphK1 was found to enhance the expression of Bcl-2 and suppress the expression of Bim in endothelial cells [29].

Administration of DOX resulted in cardiotoxicity manifested by a significant increase in TNF- α and IL-6 associated with a significant changes in the histopathological and immuno-histochemical 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 [30, 31]. 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 [32]. Furthermore, DOX may interfere with mitochondrial replication and transcription causing significant imbalances in the electron transport chain and promoting mitochondrial dysfunction [30]. 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 [33].

Sphingolipids play a critical role in the cell biological functions. Among them, ceramide and sphingosine induce apoptosis and inhibit cell proliferation while sphingosine 1-phosphate (S1P) inhibits apoptosis and promotes cell survival and proliferation. The balance between ceramide/sphingosine and S1P forms the so-called "sphingolipid-rheostat", which decides the cell fate [34]. Sphingosine kinases are a group of enzymes that catalyze the phosphorylation of sphingosine to S1P [35]. There are two isoforms of sphingosine kinases known as SphK1 and SphK2 [26]. SphK1 was proven to increase the production of the proinflammatory cytokines such as TNF- α and IL-6 through activation of STAT3 and NF- κ B which may participate in proliferation and growth of cancer cells [36]. Moreover, S1P which is produced by the action of SphK1 was

proven to inhibit apoptosis, possibly through its effect on the Phosphatidylinositol 3-Kinase/Akt pathway [37].

From the above mentioned data, inhibition of SphK1 activity might represent an effective targeted therapy for cancer. Nagahashi *et al.*, [38] reported that a specific inhibitor of SphK1 can suppress tumor-induced angiogenesis and lymphangiogenesis and can induce apoptosis. Paugh *et al.*, [39] suggested that a selective sphingosine kinase 1 inhibitor may integrate multiple molecular therapeutic targets in human leukemia. The most prominent mechanisms of this effect were enhanced apoptosis and cleavage of Bcl-2 with alteration of the Akt pathway. Also, Xu *et al.*, [40] reported that concurrent targeting of Akt and SphK1 may inhibit the growth of tumor cells, possibly through the affection of ceramide production, and subsequent cell death and apoptosis.

CONCLUSION

This study concluded that, treatment with DOX, leads to increasing in inflammatory mediators as, IL-6 and TNF- α , in cardiac muscle tissue. On the other hand, DOX ameliorate these parameters in tumor tissue. Prooncogenic mediator, SphK1 enzyme activity was increased by tumor induction and DOX treatment.

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