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Research Article

Cardioprotective Effect of Grape Seed Extract on Chronic Doxorubicin-Induced Cardiac Toxicity in Wistar Rats

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Abstract

Purpose: The aim of the present study was to determine the ability of grape seed extract (GSE) as a powerful antioxidant in preventing adverse effect of doxorubicin (DOX) on heart function.

Methods: Male rats were divided into three groups: control, DOX (2 mg/kg/48h, for 12 days) and GSE (100 mg/kg/24h, for 16 days) plus DOX. Left ventricular (LV) function and hemodynamic parameters were assessed using echocardiography, electrocardiography and a Millar pressure catheter. Histopathological analysis and *in vitro* antitumor activity were also evaluated.

Results: DOX induced heart damage in rats through decreasing the left ventricular systolic and diastolic pressures, rate of rise/decrease of LV pressure, ejection fraction, fractional shortening and contractility index as demonstrated by echocardiography, electrocardiography and hemodynamic parameters relative to control group. Our data demonstrated that GSE treatment markedly attenuated DOX-induced toxicity, structural changes in myocardium and improved ventricular function. Additionally, GSE did not intervene with the antitumor effect of DOX.

Conclusion: Collectively, the results suggest that GSE is potentially protective against DOX-induced toxicity in rat heart and maybe increase therapeutic index of DOX in human cancer treatment.

Introduction

Doxorubicin (DOX), an anthracycline antibiotic, is well-known as one of the most widely-used chemotherapeutic drugs which has been shown to be highly effective in the treatment of a broad spectrum of human cancers.[1] Despite its broad therapeutic effectiveness, clinical studies have reported that the major limiting factor of DOX chemotherapy is its significant cardiotoxic effects, which often results in irreversible degenerative cardiomyopathy and heart failure.[1,2] Although the exact mechanism by which DOX results in cardiotoxicity is not clearly understood, but most studies support the hypothesis that DOX induces oxidative stress through enhanced reactive oxygen species (ROS) production.[1-3] Considering that the heart is vulnerable to free radicals due to its less developed antioxidant defense mechanisms,[4] cellular injury can strongly be related to DOX-induced oxidative stress. Given that free radicals play a pivotal role in DOX-induced damage to the myocardium, antioxidants could protect the heart against DOX-toxicity.

One of the phytochemicals extensively investigated in recent years is grape seed extract (GSE). This extract, an excellent source of natural antioxidants, is used in the pharmaceutical, cosmetic and food industries.[5,6] Effects of GSE on improvement of liver function,[7] reducing infarct size and cardiac arrhythmias,[8] lipid profile[9] and lipid peroxidation[10] in patients with type II diabetes have been reported previously. Some studies have unequivocally demonstrated that GSE has substantial potential for scavenging free radicals in both *in vitro* and *in vivo* experimental models.[5,6,11] An excellent example comes from recent investigations where grape seed proanthocyanidins extract has been shown to be a superior scavenger against superoxide anion and hydroxyl radicals in comparison with vitamins C, E and β-carotene.[12]

In this context, a large number of preclinical and clinical studies have shown a broad spectrum of pharmacological and therapeutic benefits of GSE against oxidative stress,

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degenerative disease like cardiovascular dysfunctions and various types of cancers.[5,6,11-14]

Given that the protective effects of GSE on oxidative stress, cardiovascular diseases and neoplasm is dependent on its free radical scavenging capability and its antioxidant impacts and since the DOX-induced cardiotoxicity is mainly mediated through free radical production, natural antioxidants like GSE may offer an effective and safe means to counteract some of the problems and bolstering the antioxidant defense systems against cardiovascular diseases via neutralizing harmful free radicals. Therefore, the aim of the present study was to determine the ability of GSE to reduce the DOX-induced cardiotoxicity in a rat model.

Materials and Methods

Materials

The following materials were used in the experiments: DOX hydrochloride (Exir Nano Sina Company, Iran), Ketamine hydrochloride and Xylazxine (Alfasan, Netherlands), heparin (Hospira, USA), human breast adenocarcinoma MCF7 cell line (Pasteur Institute of Iran,), MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), RPMI 1640, DPPH (1, 1-diphenyl-2-picrylhydrazyl; Sigma; Germany), fetal calf serum, DMSO (Dimethyl sulfoxide), penicillin, streptomycin, L-glutamine and sodium pyruvate (Gibco, USA).

Animals and ethics

Adult male Wistar rats (180–220 g, aged 8–10 weeks) were obtained from Pasteur institute of Iran. Animals were housed in a room with a 12:12-h light/dark cycle and had access to rodent chow and tap water ad libitum. All experiments were performed according to the protocols approved by the Committee on the Ethics of Animal Experiments of the Tabriz University of Medical Sciences. All efforts were made to minimize animal suffering.

Preparation of Grape Seed Extract

The GSE used in this study was prepared as described previously.[7,8] Briefly, grape seeds (Vitis vinifera) were washed with water and crushed, the crude extract was partitioned between H_2O and n-hexane for separating lipoid compounds, then GSE was prepared by using ethanol 95% and water (water/ethanol, 30/70) as solvents with mechanical agitation for 2 to 3 h, this process was repeated twice. Then the organic solvent was evaporated and dried extract residue was kept at 4 °C for treatments.

Drug Treatment and Experimental Groups

All experiments were conducted in a quiet room during the light period (between 8:00 a.m. and 1:00 p.m.). A summary of the experimental design is shown in Figure 1; eighteen rats were divided into three experimental groups (six animals in each group). Drug solutions were freshly prepared before administration. Group 1 received saline only intraperitoneally (IP) and served as control (Ctrl), group 2 received DOX (2mg/kg/48h, IP for 12 days; DOX was dissolved in normal saline) and group 3 received GSE (100 mg/kg/day, IP for 16 days; GSE was administered in normal saline) and from day 4 received DOX (2 mg/kg/48h, IP for 12 days). The dose of GSE was chosen based on previous reports[15-18] and our pilot study.

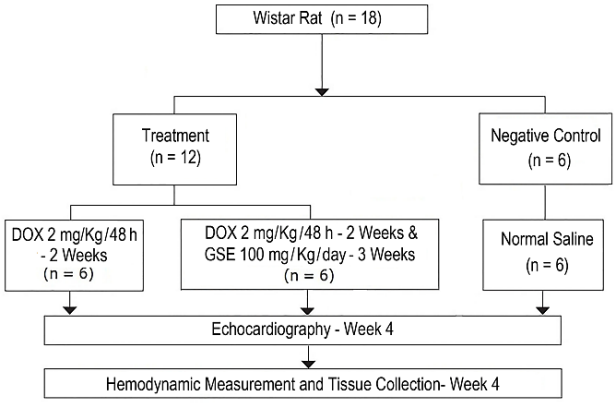

Figure 1. Experimental design, more details of the design are provided in section "Drug Treatment and Experimental Groups"

Echocardiography

Rats were sedated with ketamine (10-20 mg/kg IP) and transthoracic echocardiography was performed with a digital color Doppler ultrasound system (iVis 60 Expert Vet CHISON Medical Imaging, China) as described previously.[19] Briefly, animals were positioned in a chest closed supine form. The transducer was placed gently in the left parasternal position. The left ventricular end diastolic dimension (LVEDD) and left ventricular end systolic dimension (LVESD) were measured using M-mode tracing. The percentage of change in LV cavity dimension; fractional shortening (FS) and ejection fraction (EF) were measured as follows:[20]

$$\text{Fractional shortening (\%)} = [(\text{LVEDD} - \text{LVESD})/\text{LVEDD}] \times 100;$$

$$\text{Ejection fraction (\%)} = [(\text{LVEDD}^3 - \text{LVESD}^3)/\text{LVEDD}^3)] \times 100.$$

Electrocardiography

Forty-eight hours after last DOX administration, rats were anesthetized with a combination of xylazine (10 mg/kg, IP) and ketamine (100 mg/kg, IP) and kept warm with a heating lamp. Electrocardiograms (ECG) were recorded using three stainless steel needle electrodes inserted subcutaneously into the left forepaw and hind paws of the rats.[19] They were connected to a bio-amplifier (Bio Amp ML136; ADInstruments; Australia) to record and analyze ECG data using Lab Chart7 software (ADInstruments; Australia).

Hemodynamic study

Animals were anesthetized with ketamine (100 mg/kg, IP) and xylazine (10 mg/kg, IP). In order to prevent blood coagulation, rats received a subcutaneous injection of heparin (2000 U/kg). After 10 min of ECG recording, the neck of the rat was opened longitudinally and the right

carotid artery was exposed and released, ligated distally and stay sutures were placed proximal to the carotid artery. A small opening was then made in the artery with mini-scissors and a 2F micromanometer-tipped pressure transducer catheter (SPR-407; Millar Instruments) was inserted to the artery for evaluation of arterial blood pressure (BP). A catheter was inserted gently into the LV to record data for hemodynamic analysis using Lab Chart 7 software (ADInstruments). The heart rate (HR), LV pressure at the ends of both systole and diastole (LVESP, LVEDP), maximum rate of rise of left ventricular pressure (max dP/dt), minimum rate of rise of left ventricular pressure (min dP/dt), end-diastolic pressure (EDP) and contractility index, a major determinant of cardiac output and an important factor in cardiac compensation, were calculated. The R-R interval, which is the interval from the peak of one QRS complex to the peak of the next and the QT interval on the surface electrocardiogram, an indirect measure of time between ventricular depolarization and repolarization, was also measured.

Body weight and heart/body weight ratio

We monitored body weight development at the beginning and end of the study in all groups. Heart weight (HW)/body weight (BW) ratio was calculated.[21]

Histopathological analysis

At the end of study, the animals were euthanized and the hearts were excised, weighted, then washed with normal saline and finally fixed in 10% neutral buffered formalin, as previously described.[21] After fixation, the tissues were processed using the standard histological method, embedded in paraffin and tissue sections were cut and stained with hematoxylin and eosin. The histopathologic slides were examined by a veterinary pathologist and compared under a light microscope. The hematoxylin-eosin (H&E) stained sections were used for the following purposes: 1) morphological analysis of the myocardium, 2) inflammation and tissue damage assessment. Inflammation and tissue damage were determined by counting the number of mononuclear inflammatory cells (Lymphocytes and Macrophages) in H&E stained sections by randomly counting 100 microscopic fields over a total area 1.5 mm^2 at 400 × magnifications.[22]

In vitro antitumor activity

In order to determine the effect of GSE on DOX-inhibited growth and proliferation of the malignant cell line MCF-7 (human breast cancer cells), cell viability was evaluated by MTT assay according to the manufacturer's instructions. Briefly, the cells were distributed (5000 cells/well) in 96-well plates and maintained in RPMI-1640 medium supplemented with 10% fetal-calf serum and antibiotics (Penicillin G 50,000 units/l. Streptomycin 38,850 units/l and Nystatin 9078 units/l), in an incubator at 37˚C with a humidified atmosphere of 10% CO_2 and the cells were grown for 24 h. The cells were then exposed to a series of concentrations of free DOX (0.1, 0.5, 1, 5 and 10 µg/ml) and/or GSE (250 and 500 µg/ml) and incubated for 24 h (the drugs were dissolved in 100 µl of DMSO and then diluted with RPMI 1640). At the end of incubation time, MTT (20 µl with the concentration of 5 mg/ml) was added to each well and the plates incubated for further 6 h. Then, the culture medium was removed, 200 µl of DMSO was added to each well and the plates were shaken for 10 min. Finally, the optical density was measured at 550 nm using a microplate reader (AD 340; Beckmann Coulter). All the experiments were performed in triplicate.[19,21]

Statistics

All data were analyzed using SPSS software (Version 13.0). Student's t-test or one-way analysis of variance (ANOVA) followed by a Tukey's HSD post hoc test were used to analyze the statistical significance of the differences between groups, as needed. All data are presented as the mean ± standard error of the mean (SEM) of at least 6 rats in each group. A *p*-value less than 0.05 was considered statistically significant.

Results

Echocardiographic analysis

To evaluate the influence of the GSE and DOX on LV remodeling and function, a series of echocardiography studies were conducted. As shown in Figure 2 and Table 1, statistical analysis revealed that DOX treatment significantly decreased the FS ($p<0.01$) and EF ($p<0.01$), as compared with the Ctrl group. In addition, data analysis indicated that GSE treatment significantly increased the FS ($p<0.01$) and EF ($p<0.01$) in comparison with DOX group. Increase of FS and EF in GSE group reached to normal values as in Ctrl group with no significant differences. Moreover, there was no significant change in LVDD and LVSD.

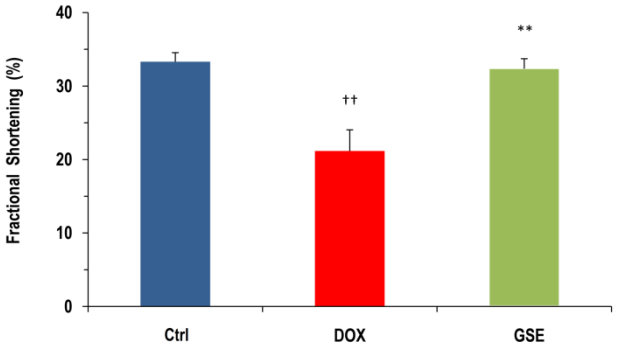

Figure 2. The effects of DOX (doxorubicin, 12 mg/kg) alone or in combination with GSE (grape seed extract, 100 mg/kg) on left ventricular fractional shortening in rats. Values are expressed as mean ± SEM. (n=6). ††: $p<0.01$ *vs.* Ctrl group; **: $p<0.01$ *vs.* DOX group.

Electrocardiographic recordings

Table 2 summarizes the significant changes in ECG recordings. ECG features in the Ctrl was normal. The data analysis revealed that DOX administration significantly changed the HR, RRI and QA parameters

(p<0.05) in comparison with Ctrl group. Moreover, statistical analysis demonstrated that GSE administration significantly improved the ECG parameters including HR and RRI, as compared to the DOX group (p<0.05).

Table 1. Echocardiographic analyses of left ventricular fractional shortening and ejection fraction in rat heart

Parameter	Group		
	Ctrl	DOX	GSE
LVDD (mm)	6.60±0.05	6.30±0.12	6.53±0.08
LVSD (mm)	4.40±0.07	4.97±0.217	4.41±0.07
FS (%)	33.31±1.24	21.16±2.88††	32.34±1.38**
EF (%)	70.19±1.71	50.03±5.10††	68.83±1.80**

Changes of left ventricular fractional shortening and ejection fraction in study groups, Ctrl=control, DOX=doxorubicin (12 mg/kg); GSE=grape seed extract (100 mg/kg), the values are expressed as mean ± SEM (n=6). ††: p<0.01 *vs.* Ctrl group and **: p<0.01 *vs.* DOX group.

Table 2. Electrocardiogram parameters

Parameter	Group		
	Ctrl	DOX	GSE
HR (BPM)	221.9±10.9	186.5±11.1†	233.9±9.100*
RRI (S)	0.274±.014	0.328±0.020†	0.270±0.010*
QA (µV)	1.171±3.18	14.07±5.760†	0.074±0.004
QTI (S)	0.072±0.005	0.076±0.004	0.074±0.004

Ctrl=control, DOX=doxorubicin, GSE=grape seed extract + DOX, RRI=RR interval, HR=heart rate, S=second, BPM=beats per minute. QA: Q amplitude, QTI: QT interval, the values are expressed as mean ± SEM (n=6). †: p<0.05 *vs.* Ctrl group,*: p< 0.05 *vs.* DOX group.

Blood pressure measuring

Table 3 shows that DOX treatment consistently and significantly decreased the systolic pressure (p<0.001), diastolic pressure (p<0.05) and mean pressure (p<0.01) in comparison with the Ctrl group. However, there was no significant change following GSE treatment, as compared to the DOX group.

Table 3. Arterial and left ventricular function parameters in study groups

Parameter		Group		
		Ctrl	DOX	GSE
Artery	Systolic pressure	88.06±1.85	71.74±1.84†††	81.08±1.85
	Diastolic pressure	68.25±2.28	53.32±4.66†	60.01±7.92
	Mean pressure (mmHg)	78.27±1.51	62.38±3.39††	70.46±7.42
Left Ventricle	Max Pressure (mmHg)	86.92±1.98	23.17±2.24†††	47.30±7.04**
	Min Pressure (mmHg)	1.03±0.86	5.01±0.95††	-2.44±1.25***
	Systolic Duration (s)	0.13±0.01	0.04±0.01†††	0.1±0.01***

Ctrl=control; DOX=doxorubicin (12 mg/kg); GSE=grape seed extract (100 mg/kg); the values are expressed as mean ± SEM (n=6). †: p<0.05, ††: p<0.01 and †††: p<0.001 *vs.* Ctrl group. *: p<0.05, **: p<0.01 and ***: p<0.001 vs. DOX group.

Left ventricular function analysis

DOX treatment markedly decreased the max pressure, (p<0.001, Table 3), contractility index (p<0.05, Figure 3) and the max dP/dt (p<0.05, Figure 4) and increased the EDP (p<0.001, Figure 5), min pressure (p<0.01, Table 3) and the min dP/dt (p<0.05, Figure 4) relative to the Ctrl group. In addition, GSE exposure significantly elevated the max pressure (Table 3), contractility index (Figure 3), min dP/dt (p<0.01, Figure 4), the min pressure (Table 3) and max dP/dt (p<0.001, Figure 4), while reduced the EDP (p<0.05, Figure 5), as compared to the DOX group.

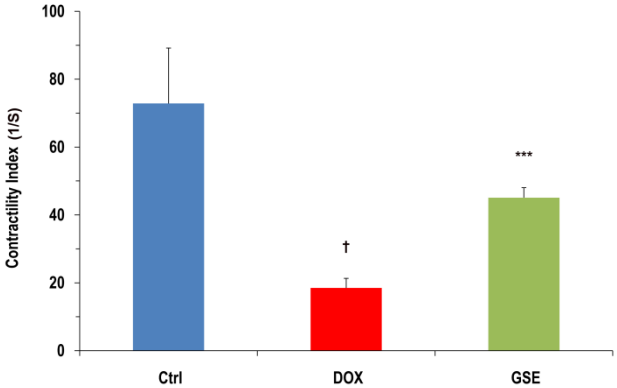

Figure 3. The effects of DOX (doxorubicin, 12 mg/kg) alone or in combination with GSE (grape seed extract, 100 mg/kg) on contractility index (1/S). Values are expressed as mean ± SEM. (n=6), †: p<0.05 *vs.* Ctrl group; ***: p<0.001 *vs.* DOX group.

Body weight development and heart/body weight ratio

As indicated in Table 4, the data analysis revealed that DOX treatment significantly resulted in decreased BW (p<0.001), HW (p<0.001) and HW/BW ratio (p<0.001) in comparison with the Ctrl group. Moreover, the results indicated that GSE treatment significantly increased BW (p<0.001), HW (p<0.001) and HW/BW ratio (p<0.01) relative to the DOX-treated group.

Histopathological results of heart tissue

The histopathological changes in the rats' myocardium of all study groups are shown in Figure 6. The Ctrl group exhibited normal morphological findings. There were significant changes in DOX group including:

cytoplasmic vacuolization, interstitial edema, hyaline degeneration and Zenker's necrosis, as compared to the Ctrl group. Furthermore, DOX appeared to have significant adverse effects on rat cardiac tissue, i.e. focal to extensive hemorrhages, accumulation of acute inflammatory cells, injured vascular structures, necrotic changes in the nuclei of cardiomyocytes and mild cardiac fibrosis. In GSE group the myocardial damage was dramatically attenuated, as compared to the DOX group. There was also little evidence of pathological changes in the cardiomyocytes following GSE treatment. Therefore, it could be speculate that GSE leads to cell preservation and decreased necrosis, cytoplasmic vacuolization and maintained a normal morphology and structure for the cardiac muscle. The numbers of mononuclear inflammatory cells in study groups are illustrated in Figure 7. The numbers of inflammatory cells including lymphocytes and macrophages in DOX group were significantly higher than Ctrl group ($p<0.001$) and GSE treatment significantly decreased the number of these cells in comparison with the DOX group ($p<0.001$).

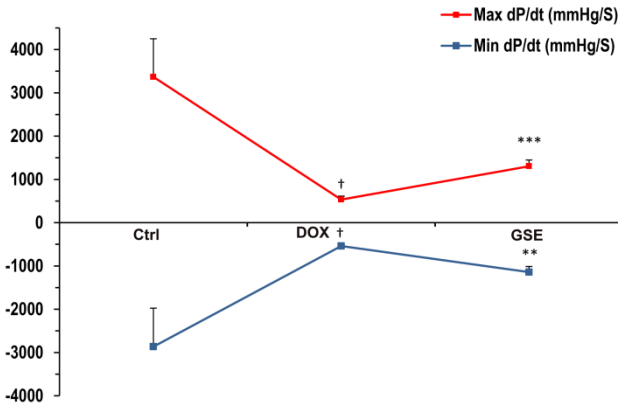

Figure 4. The effects of DOX (doxorubicin, 12 mg/kg) alone or in combination with GSE (grape seed extract, 100 mg/kg) on max dP/dt (mmHg/s) and min dP/dt (mmHg/s) alterations in rats. Values are expressed as mean ± SEM. (n=6). †: $p<0.05$ *vs.* Ctrl group; **: $p<0.01$ and ***: $p<0.001$ *vs.* DOX group.

Table 4. Body weight and heart/weight ratio in study groups.

Group	IBW (gr)	FBW (gr)	HW (gr)	HW / BW
Ctrl	201.33±1.02	221.16±1.7000	0.90±0.0200	0.004±0.000100
DOX	210.00±0.89	181.17±1.61†††	0.54±0.02†††	0.003±0.0001†††
GSE	208.33±1.68	227.66±2.04***	0.78±0.02***	0.003±0.00001**

IBW=initial body weight; FBW=final body weight; HW=heart weight; BW=body weight.
Ctrl=control; DOX = doxorubicin (12 mg/kg); GSE = grape seed extract (100 mg/kg), the values are expressed as mean ± SEM (n=6). †††: $p<0.001$ *vs.* Ctrl group, **: $p<0.01$ and ***: $p<0.001$ *vs.* DOX group.

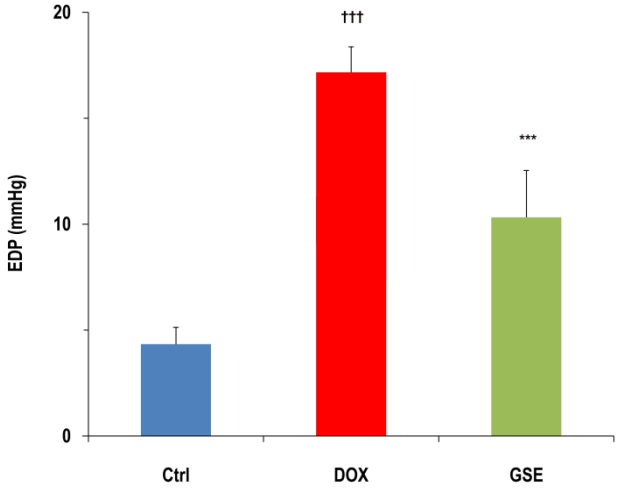

Figure 5. The effects of DOX (doxorubicin, 12 mg/kg) alone or in combination with GSE (grape seed extract, 100 mg/kg) on EDP (%). Values are expressed as mean ± SEM. (n=6). †††: $p<0.001$ *vs.* Ctrl group and ***: $p<0.001$ *vs.* DOX group.

Cytotoxicity assays

To evaluate the antitumor activities of GSE alone or in combination with DOX, MCF-7 cell line was used as tumor cells in MTT assay. As illustrated in Figure 8, the data analyses revealed that DOX produced cell toxicity dose-dependently. The results indicated that GSE alone, at dose 500 µg/ml, resulted in cell toxicity. However, co-administration of GSE at this dose with DOX did not affect the cytotoxicity. Therefore, these findings demonstrate that GSE does not change the DOX-induced cell toxicity, *in vitro*, $p>0.05$.

Discussion

DOX is widely used for the control and management of variety of human cancers, whereas, its consumption is limited by side effects. Cardiomyopathy is the most important toxic outcome in patients receiving DOX.[23,24] In the current study, it has been demonstrated that GSE has protective effect on DOX-induced cardiotoxicity in rat heart. The animal model used in this study was described previously[21] and characterized by injuries similar to what reported by others.[17,18,25-27] Alterations in physiological parameters are well known as one of the toxic effects of DOX, which is characterized by reduced body and heart weights.[28-30] Our findings here confirmed the literature reports that DOX treatment leads to decreased both body and heart weights in animals[31] and that GSE treatment increased body and heart weights, as compared to DOX group.

Our data supported previous findings in which DOX administration significantly resulted in increased left ventricular dysfunction and decreased the FS and EF in the echocardiographic assessment.[21,32] Treatment with GSE significantly reversed the effects of DOX on left

ventricular function, EF and FS, as compared to DOX group. In addition, in line with previous findings,[21,33-37] we found that DOX exposure resulted in reduced aortic, systolic, diastolic and mean pressure as well as decreased max pressure, min pressure, EDP, max dP/dt, min dP/dt and contractility. These adverse effects were reversed by GSE treatment.

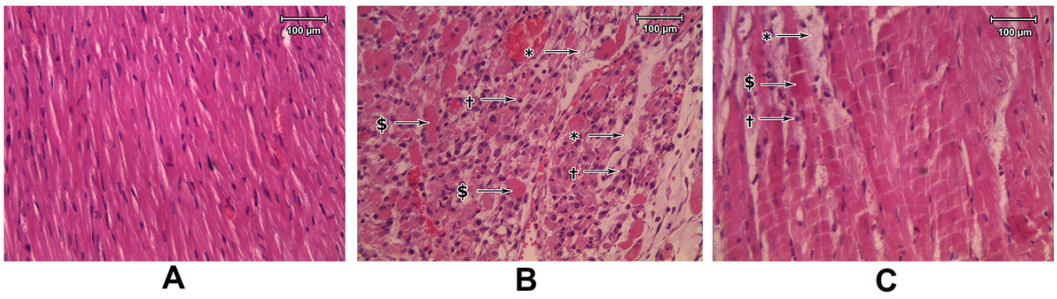

Figure 6. Effect of grape seed extract (GSE) on doxorubicin (DOX)-induced histopathological alterations in cardiac tissues (H&E, 400×). A: control group shows normal histological pattern. B: DOX group shows hyaline degeneration and Zenker's necrosis ($), infiltration of acute inflammatory cells (†) and inter cardiomyocytes edema (*): The lesions indicate severe pathological changes in the myocardium. C: GSE group shows hyaline degeneration and Zenker's necrosis ($), infiltration of acute inflammatory cells (†) and inter cardiomyocytes edema (*): These changes indicate slight histopathological injury in the cardiac tissue of GSE group.

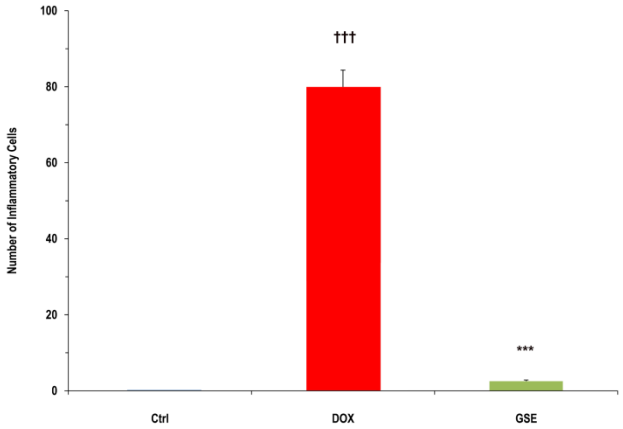

Figure 7. The numbers of mononuclear inflammatory cells (Lymphocytes and Macrophages) in study groups. DOX = doxorubicin (12 mg/kg) alone or in combination with GSE = grape seed extract (100 mg/kg). Values are expressed as mean ± SEM (n=6), †††: p<0.001 *vs.* Ctrl group and ***: p<0.001 *vs.* DOX group.

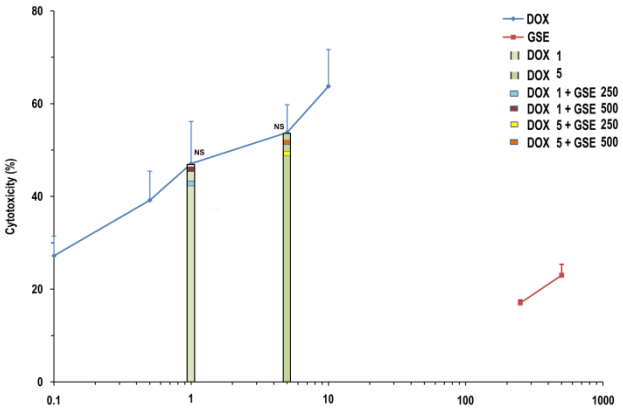

Figure 8. Cytotoxicity of DOX (doxorubicin; 1 and 5 µg/ml) alone or in combination with GSE (grape seed extract; 250 and 500 µg/ml) against MCF-7 cells. The results are mean values ± SEM of three independent experiments performed in triplicate. NS: There was no a significant difference between DOX alone or in combination with GSE, p>0.05.

A recent study provided interesting evidence indicating that GSE treatment has cardioprotective effect in high-fat diet-induced cardiac dysfunction and DOX-induced cardiotoxicity in animals. Treatment with GSE highly improved heart rate and pressure and this protection was associated with iron and calcium accumulation and ROS generation in the myocardium,[18,38] hence, they recommended GSE as an option for the prevention of DOX-induced cardiotoxicity. Further confirmation comes from another study showing that GSE treatment in combination with DOX in mice significantly protected the heart tissue by improving its antioxidant activity; leading to this conclusion that GSE acts as a potent antioxidant to prevent heart damage.[27,39]

In the line with this evidence, Karthikeyan et al have confirmed the efficacy of GSPE as a cardioprotective agent in alleviating isoproterenol-induced myocardial injury in rats.[40] They demonstrated that GSPE administration at doses of 100 and 150 mg/kg positively alters the levels of glutathione, ascorbic acid, a-tocopherol, ceruloplasmin, mitochondrial cytochrome, phospholipids and adenosine triphosphate and also restores normal mitochondrial function. This experimental evidence indicates that GSPE may serve as a potential therapeutic tool in promoting cardiovascular health.

We investigated ECG alterations because it was found that the severity of changes in ECG is directly related to the known DOX-induced cardiotoxicity in humans and animals.[41,42] Our results clearly indicate that DOX resulted in myocardial injury as indicated by the increase in the RR interval and QA and the decrease in HR of the ECG records. It has been documented that these ECG changes are associated with the prolongation of action potential duration and DOX could strongly affect the recovery phase of the transmembrane action potential, influencing preferentially Ca^{2+} movements across the cellular membrane.[26,43,44] In addition, it has been reported that DOX alters calcium homeostasis in

the myocardium.[18] In fact, several previous studies demonstrated the role of Ca^{2+} disturbances in DOX-induced cardiotoxicity *in vivo*[45] and *in vitro*,[46] they also raise a major discrepancy as shown by lower levels of myocardial calcium following DOX administration compared to plethoric studies where higher levels of calcium after DOX treatment was observed. On the other hand, it was found that GSE affects the levels of calcium in the heart tissue,[18] it is possible that GSE normalized the DOX-induced ECG alterations in a positive way.[25] In the present study, GSE treatment was also able to prevent the development of ECG changes induced by DOX and to confirm that GSE has a cardioprotective effect against DOX-induced cardiac dysfunction.

In this study typical histopathological alterations such as noticeable interstitial edema, focal myocardial fibrosis, perinuclear vacuolation and myocardial necrosis was observed following DOX treatment as reported in different experimental animal models,[21,47-49] including noticeable interstitial edema, focal myocardial fibrosis, perinuclear vacuolation and myocardial necrosis. Treatment with GSE decreased the infiltration of inflammatory cells including lymphocytes and macrophages into the myocardium of rats significantly, in comparison to DOX group (Figure 7). Our data confirmed previous findings suggesting GSE attenuated the detrimental impacts of DOX on morphology and ultrastructure of heart tissues in histopathology studies.[27,31]

Furthermore, the results of the MTT assay indicated that DOX exerts a dose-dependent cytotoxic effect on MCF7 cells and that GSE treatment in combination with DOX had no significant effect on DOX-induced cell toxicity and GSE alone showed cytotoxicity effect on MCF7 cells.[50] In addition, it was reported that procyanidin, an antioxidant flavonoid of the GSE, exhibited antitumor activity on MCF7 cells. In this regard, other studies have demonstrated that procyanidins induce cytotoxicity on several tumor cells such as human adenocarcinoma cells A549,[31] human colorectal cancer HT29, LoVo cells,[51] A-427 human lung cancer cells, CRL-1739 human gastric adenocarcinoma cells and K562 chronic myelogenous leukemic cells.[52]

During the two past decades tremendous effort has been put into uncovering the molecular mechanisms and/or intracellular targets involved in the DOX-induced cardiotoxicity and different hypotheses have been developed to explain this phenomenon,[53-55] but no single one of these was able to fully explain it.[2] Rather, DOX cardiotoxicity appears to be a multifactorial process that results in cardiomyocytes death with typical apoptotic features and heart failure as the terminal downstream event.[53,56,57] It has long been established that DOX anticancer actions are closely associated with DNA intercalation, topoisomerase-II inhibition and apoptosis. The most important cardiotoxicity actions of DOX are related to oxidative stress. It appears that such difference in mechanisms is not fully justified.[2,53] It seems there is some overlapping between the beneficial (anticancer/therapeutic) and detrimental (cardiotoxic) effects of DOX, in fact, they share common effectors such as oxidative stress and both involve apoptosis.[2] On the other side, it has been reported that antioxidants can be protective against DOX-induced cardiotoxicity through their free radical scavenging capability.[58,59] There are antioxidant flavonoids such as procyanidin B4, catechin and gallic acid in GSE which can protect DNA from oxidative damage in a dose-dependent manner.[60] For instance, GSPE treatment has been shown to significantly inhibit DOX-induced cardiotoxicity as indicated by decreased DNA damage and histopathological changes in the cardiac tissue of mice.[61] In addition, recent studies demonstrated the bioavailability of grape seed proanthocyanidins to the target organs exhibiting a superior protection against oxidative DNA damage and oxidative stress relative to vitamin C, E and β-carotene.[12] In support of our findings, it has been reported that various chemical compounds such as carvedilol,[62] rosmarinic acid,[63] dexrazoxane[64,65] as well as herbal agents including GSE,[18,39] saffron extract[66] and garlic extract[67] found to potentially be protective against DOX-induced cardiotoxicity. Based on reported studies GSE produces protective effect by several mechanisms including antioxidant effect,[68,69] decreasing the number of apoptotic cells,[70] prevention of DNA fragmentation,[15,71] regulation of the expression levels of the pro-apoptotic protein Bax-alpha,[72] increasing anti-apoptotic protein Bcl-2[73] and inhibition of apoptotic signaling pathways.[74,75] This study is a comprehensive descriptive study but did not aim to investigate the protection mechanism(s) of GSE on DOX-induced cardiomyopathy. Further mechanistically approach studies on GSE-induced cardioprotection and examining different doses of GSE will enrich the study.

Conclusion

In conclusion, hemodynamic, ECG, echocardiographic, histopathologic and MTT results in this study confirm the protective effects of GSE on DOX-induced cardiotoxicity, probably through antioxidant and anti-inflammatory mechanisms. Taken together, our results support the notion to introduce GSE as a potential drug candidate for co-administration with DOX in human chemotherapy in order to increase DOX therapeutic index. Further investigations by clinical trials to examine GSE cardioprotective effect in DOX users are necessary to confirm this claim.

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Ethical Issues
Not applicable.

Conflict of Interest
The authors report no declaration of interest.

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