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Therapeutic Potential of Radish Root Extract Against Doxorubicin-induced Cardiotoxicity in Male Rats Via Alleviating Disrupted Redox Homeostasis, Inflammation and Coagulation Activity

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Abstract

Cardiotoxicity is a major limiting factor for clinical use of doxorubicin (DOX) in chemotherapy. This study aimed to evaluate effectiveness of radish root extract (30mg/kg BW) to attenuate doxorubicin (DOX) - induced cardiotoxicity and to explore involved mechanisms. Phytochemical analysis of tested plant and assessment of antioxidant capacity against DPPH free radical were also performed. Treatment of male rats with DOX (2.5mg/kg BW, i.p.) twice weekly for 4 weeks elicited myocardial toxicity characterized by increased serum cTn-I, CK-MB, LDH and AST, decreased heart weight to body weight ratio, and myocardial degeneration. Other findings emphasized increased xanthine oxidase, lipid peroxidation, protein carbonyl, and nitric oxide, with diminished endogenous antioxidants in heart tissue, indicating provoked oxidative stress. Elevated serum inflammatory markers (TNF-α, CRP), adhesion molecules (VCAM-1, ICAM-1), fibrinogen and platelet activating factor, accompanied by marked thrombocytopenia were also validated. Results of radish analysis revealed strong scavenging activity against DPPH and presence of different polyphenols; flavonoids, tannins and alkaloids as a major phytoconstituents. Supplementation of radish extract has shown to be effective against toxic manifestations of DOX, most likely due to its radical scavenging and antioxidant activities. Therefore, radish extract can be suggested as a promising cardioprotective remedy for patients on DOX- based chemotherapy.

Keywords: Doxorubicin; cardiotoxicity; radish root extract; oxidative stress; adhesion molecules; platelet activating factor.

1. Introduction

Doxorubicin (DOX) is an anthracycline chemotherapeutic drug widely used for treating several forms of malignancies [1]. Regardless of DOX effectiveness, its clinical application is largely impeded by severe cardiotoxic effect that can progress into irreversible cardiomyopathy and congestive heart failure [2]. Several mechanisms seemed to be responsible for DOX cardiotoxicity; among which oxidative stress has emerged as a major factor. DOX may cause oxidative stress through its enzymatic reduction into unstable semiquinone radical which in turn undergoes redox cycling in the presence of molecular oxygen (O_2) to form reactive oxygen species (ROS) including superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) [3]. Other pathways may involve direct interaction of DOX with iron to form iron-DOX complex, thereby increases iron accumulation and generation of ROS [4]. Heart is a more susceptible organ towards DOX generating radicals due to lowered antioxidant defenses and higher oxidative metabolism of cardiomyocytes [5]. An imbalance between antioxidant capacity and production of ROS causes damage to membrane lipids and different cellular components with resulting myofibrillar degeneration and cardiotoxicity [6]. Nevertheless, it has also suggested that raised ROS plays a direct role in triggering inflammatory response [7], thrombus formation and coagulation pathways [8], which are together believed to be key mediators in progression of DOX cardiotoxiciy.

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Lately, the use of natural plant antioxidants has gained much attention for their high ability to prevent various cardiovascular diseases [9]. Radish (*Raphanus sativus* L.) is an edible root vegetable belongs to the family Cruciferae [10]. Radish has widely described to contain high concentration of antioxidant constituents which are useful for human health [11]. Roots and leaves of radish have been used as a traditional medicine for treatment of stomach disorders, ulcers, inflammation and urinary infections [9]. Administration of radish extracts can aid in preventing harmful ailments, such as liver injury, diabetes [9], hyperlipidemia and atherosclerosis [12]. Radish has also shown to confer excellent treatment for asthma and other respiratory complains [13]. Besides, radish has considered as an effective antibacterial, antiviral and antitumor agent [14].

In view of the wide-ranging health benefits of radish and lack of reports regarding its cardioprotective activities, current investigation was done to determine efficacy of radish root extract for combating DOX-associated cardiotoxicity in adult male rats and to clarify the possible underlying mechanisms.

2. Materials and methods

2.1. Animals

Adult male Wistar albino rats (200±10 g) were obtained from the animal unit of Helwan Farm-VACSERA, Cairo, Egypt. They were housed in stainless steel cages with normal rodent diet and water *ad libitum*, under controlled temperature (23±2^{o}C) and 12-h light/dark cycle. Animals were acclimatized to housing conditions for one week. All experiments were carried out according to guidelines of National Research Council [15] and with approval of the local experimental animal's ethics committee at Mansoura University.

2.2. Doxorubicin dosing

Dose protocol based on previous experimental model of DOX-induced cardiotoxicity was applied, where DOX was given at dose of 2.5mg/kg BW dissolved in 2.5ml saline [16].

2.3. Plant material and preparation of extract

Radish (*Raphanus sativus* L.) was obtained from herbal market at Mansoura city, Egypt. It was authenticated by an expert herbalist at Botany department, Faculty of Science, Mansoura University. Roots were separated, cleaned, and air dried. Powdered roots (500g) were extracted using 70% ethanol for 18 h in a Soxhlet apparatus. Radish root extract was filtered and concentrated under reduced pressure at 50°C until dryness. The yield of dry extract was 29.60 g (5.92%) that was suspended in distilled water for oral administration [17].

2.4 Phytochemical analysis of radish root extract

Radish root extract (RRE) was analyzed for the main phytochemical constituents, using standard colorimetric methods. Total polyphenols were determined using Folin-Ciocalteu reagent as described by Lin and Tang [18]. Gallic acid was used as a standard and the total polyphenols were expressed as mg gallic acid equivalent per gram dry weight extract (mg GA/g DWE). Total flavonoid content was measured using aluminum chloride method as described by Zhishen *et al.* [19] and expressed as catechin equivalent (mg CE/g DWE), while total tannins were measured based on vanillin hydrochloride method as described by Sadasivam and Manickam [20] and expressed as tannic acid equivalent (mg TA/g DWE). Meanwhile, total alkaloids were quantitatively determined using ammonium hydroxide for alkaloids precipitation according to previous method by Harborne [21]. The percentage level of alkaloids was determined using the formula: Alkaloid (mg%) = Final weight of sample/ Initial weight of sample x100.

2.5 Radical scavenging activity of radish root extract

The scavenging activity (antioxidant activity) of RRE was determined against 2, 2-diphenyl-1-picrylhydrazil (DPPH) free radical as described by Pratap *et al.* [22]. The assay is based on the capacity of the plant extract to scavenge DPPH. Thus, the antioxidant effect is proportional to disappearance of DPPH purple color in test samples following its scavenging. The absorbance was measured spectrophotometrically at 517 nm and the percentage of inhibition of DPPH (I%) was calculated using the equation: I% = A blank- A sample/A blank x100; where the results were expressed as IC50 value, corresponding to the inhibiting concentration of 50% of the free radical.

2.6 Animal grouping and treatment

A total of thirty rats with no significant differences of initial body weight were randomly assigned to five groups of six rats each. The first served as untreated control group. The second group received saline (2.5ml/kg BW) as a vehicle. In the third and fourth groups, rats received RRE (30mg/kg BW) and DOX (2.5mg/kg BW) respectively. Rats of the fifth group received both DOX (2.5mg/kg BW) plus RRE (30mg/kg BW). DOX or saline was intraperitoneally injected twice weekly for 4 weeks [16], while RRE was given orally, 6 days/week for the same period

[17]. The body weight of rats in each group was recorded at the end of the study and the differences in the body weights were calculated to evaluate the body weight changes.

2.7 Samples collection and processing

At the end of the experimental period, overnight fasted rats were sacrificed under light ether anesthesia. Blood samples were collected and divided into two separated portions. The first was collected in EDTA containing tube as an anticoagulant for hematological assessments using auto-hematology analyzer (Genrui KT-6400), while the second portion was collected without anticoagulant and centrifuged to separate serum for biochemical analysis. The heart was then excised, washed in ice cold saline, blotted dry and weighed. One half was dissected free, weighed, and homogenized for further analysis, while the other was fixed in 10% buffered formalin for histological examination.

2.8 Heart weight to body weight ratio

Heart weight (HW) to body weight (BW) ratio was calculated according to the formula:

HW/BW% = HW (g) /BW (g) x100 [23].

2.9 Biochemical assessments

Serum cardiac troponin-I (cTn-I) was measured using ELISA kit supplied by Cusabio Co., USA, while enzyme activities of creatine kinase-MB (CK-MB), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were assessed using kits from Horiba ABX Co., France. Cardiac xanthine oxidase (XOD) was measured using ELISA kit supplied by Cusabio Co., USA, while protein carbonyl (PC) and nitric oxide (NO) were measured using kits from Cayman Chemical Co., USA and Bioassay Co., USA. Cardiac superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), total antioxidant capacity (TAC) and malondialdehyde (MDA), as indicator of lipid peroxidation were determined using kits from Bio-diagnostic Co., Giza, Egypt. Serum C-reactive protein (CRP) was measured using kit from Sigma Diagnostic Ltd, Hungary, whereas tumor necrosis factor-α (TNF-α) and intercellular adhesion molecule-1(ICAM-1) were measured using ELISA kits from Biovisio Co., USA. Serum vascular cell adhesion molecule-1(VCAM-1) and platelet activating factor (PAF) were assessed using ELISA kits obtained from Kamiya Biomedical Co. (Seattle, WA, USA), while fibrinogen was measured using ELISA kit from Immunology Consultants Laboratory, Inc., USA.

2.10 Histopathological examination

Fixed heart specimens were dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax. Sections of 4 to 5 µm thickness were prepared and stained with hematoxylin and eosin (H & E) for microscopic examination. Cardiac histopathological changes were scored as severe (+++), moderate (++), mild (+) and nill (-) based on the method modified by Alpsoy *et al*. [24])

2.11 Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.04 (GraphPad Software Inc., San Diego, CA, USA). One way analysis of variance (ANOVA) was adopted to evaluate significance between the individual groups at $P < 0.05$ and the results were expressed as means ± SE (n=6).

3. Results

3.1 Phytochemical screening

The identified phytoconstituents of RRE are presented in Table 1. Total polyphenol content was 55.68 mg GA/g DWE, whereas total flavonoid and total tannin content were 8.59 mg CA/g DWE and 9.35mg TA/g DWE, respectively. Meanwhile, the percentage level of alkaloids was 12.80 mg%.

Table1. Phytochemical screening and DPPH scavenging activity of radish root extract.

Phytochemical constituents	Inference
Polyphenols (mg GA/g DWE)	55.68
Flavonoids (mg CA/g DWE)	8.59
Tannins (mg TA/g DWE)	9.35
Alkaloids (mg %)	12.80
DPPH (IC50 mg/ml)	1.25

Data represent polyphenols content expressed in mg equivalent gallic acid (mg GA/g DWE); flavonoids content expressed in mg equivalent catechin (mg CA/g DWE); tannins content expressed in mg equivalent tannin (mg TA/g DWE); scavenging activity of radish root extract against DPPH radical expressed in IC50 value.

3.2 DPPH scavenging activity

The antioxidant activity of RRE was evaluated based on the ability to scavenge DPPH free radicals. Results showed that the plant extract inhibits DPPH with IC50 value of 1.2 mg/ml (Table 1). Of importance is that lowered IC50 value of DPPH as shown in the test samples, may indicate the potential of used extract to scavenge free radicals that may be related to the high antioxidant activity of the polyphenols of this plant extract.

3.3 Cardiac toxicity markers

Assessment of cardiac toxicity markers in DOX-treated rats showed elevation in serum level of cTn-I

and enzyme activities of CK-MB, LDH and AST compared to control group. Indeed, these changes appeared to be improved following oral consumption of RRE, where significant reduction in cardiac markers was recorded. No significant changes were noticed in normal animals received RRE compared to control animals (Fig.1).

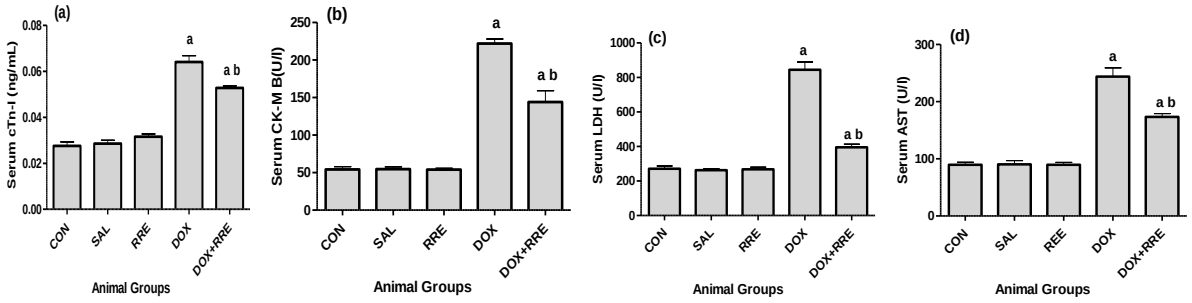

Fig1. Cardiac toxicity markers; cTn-I(a), CK-MB(b), LDH(c), AST(d) in the studied groups. Data are expressed as the average of six animals ± SE in each group. CON: control; SAL: saline; RRE: radish root extract; DOX: doxorubicin; cTn-I: troponin-I; CK-MB: creatine kinase-MB; LDH: lactic dehydrogenase; AST: aspartate aminotransferase. a: significant compared with control group at $P< 0.05$; b: significant compared with DOX group at $P< 0.05$.

3.4 Body weight, heart weight and heart weight/body weight ratio

DOX treated rats elicited significant reduction in the body weight, heart weight and heart weight to body weight ratio as compared to control group. Indeed, a reverse pattern was exhibited when DOX group was supplemented by RRE, showing significant elevation in the body and heart weights compared to DOX group. However, no significant weight changes were observed in rats received RRE alone compared to healthy control group (Fig.2).

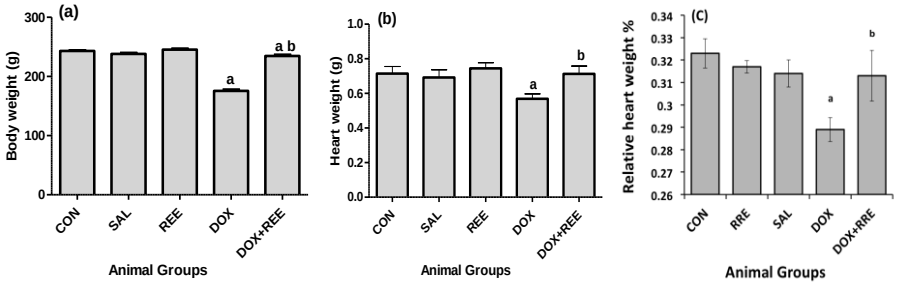

Fig.2.Body weight (a), heart weight (b) and heart/body weight ratio(c) in the studied groups. Data are expressed as the average of six animals ± SE in each group. CON: control; SAL: saline; RRE: radish root extract; DOX: doxorubicin. a: significant compared with control group at $P< 0.05$; b: significant compared with DOX group at $P< 0.05$.

3.5 Cardiac oxidative stress and antioxidant markers

Results showed marked elevation in levels of XOD, lipid peroxidation and protein carbonyl, accompanied by significantly reduced SOD, CAT, GSH and TAC in cardiac tissue of DOX- treated rats compared to the control animals. These alternations were found to be ameliorated when rats were administered DOX plus RRE comparing with animals received DOX alone. Indeed, no significant alterations were observed upon administration of RRE to normal rats (Table 2).

Table2. Cardiac oxidative stress and antioxidant markers in the studied groups

	CON	SAL	RRE	DOX	DOX+RRE
XOD(ng/g)	0.72 ±0.05	0.67 ±0.06	0.80 ±0.04	2.77±0.15[a]	2.04 ±0.03[ab]
MDA(nmol/g)	29.06±0.37	29.47±0.73	28.96±0.48	56.31±1.08[a]	42.32±1.02[ab]

PC (µmol/g)	0.95 ±0.03	0.91±0.07	0.98 ±0.05	3.63±0.08[a]	2.94 ±0.04[ab]
NO(µmol/g)	11.86±0.59	10.70 ±0.63	10.59 ±0.48	22.59±1.13[a]	16.40±0.43[ab]
SOD (U/g)	55.18±3.58	50.33±2.86	54.95±6.19	15.34±3.11[a]	24.37±0.99[ab]
CAT(U/g)	26.15±1.76	27.19±1.47	27.43±1.79	12.37±0.26[a]	18.40±1.36[ab]
GSH (mg/g)	17.26±0.38	16.69±0.32	16.86 ±0.61	10.19±0.28[a]	12.82±0.32[ab]
TAC (mM/g)	3.50 ±0.07	3.56 ±0.04	3.67 ±0.08	1.43±0.14[a]	2.60 ±0.06[ab]

Data are expressed as the average of six animals ± SE in the studied groups. CON: control; SAL: saline; RRE: radish root extract; DOX: doxorubicin; XOD: xanthine oxidase; MDA: malondialdehyde; PC: protein carbonyl; NO: nitric oxide; SOD: superoxide dismutase; CAT: catalase; GSH: glutathione; TAC: total antioxidant capacity. a: significant compared with control group at $P<0.05$; b: significant compared with DOX group at $P< 0.05$.

3.6 Serum inflammatory markers and adhesion molecules.

Serum inflammatory markers (TNF-α, CRP) and adhesion molecules (VCAM-1, ICAM-1) were found to be significantly increased in DOX-treated rats as compared to control group. Administration of RRE to DOX- treated rats attained significant reduction in values of these parameters compared to DOX group. However, no marked changes were detected in the normal rats received REE alone (Fig.3).

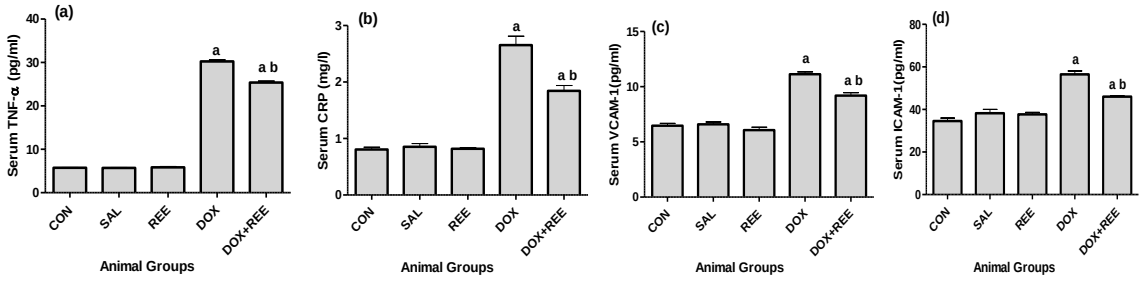

Fig.3. Serum inflammatory markers; TNF-α (a), CRP (b) and adhesion molecules; VCAM-1(c), ICAM-1 (d) in the studied groups. Data are expressed as the average of six animals ± SE in each group. CON: control; SAL: saline; RRE: radish root extract; DOX: doxorubicin; TNFα: tumor necrosis factor α; CRP: C-reactive protein; VCAM-1: vascular cell adhesion molecules-1; ICAM-1: intercellular adhesion molecule-1.a: significant compared with control group at $P< 0.05$; b: significant compared with DOX group at $P< 0.05$.

3.7 Platelets count, serum fibrinogen and PAF

Significant decline in platelets count with marked elevation in serum fibrinogen level and PAF activity were demonstrated in DOX treated rats as compared to control group. Co-administration of RRE substantially prevented observed alternations in the coagulation factors, whereas no significant changes were noticed when RRE was given to normal untreated rats (Fig.4).

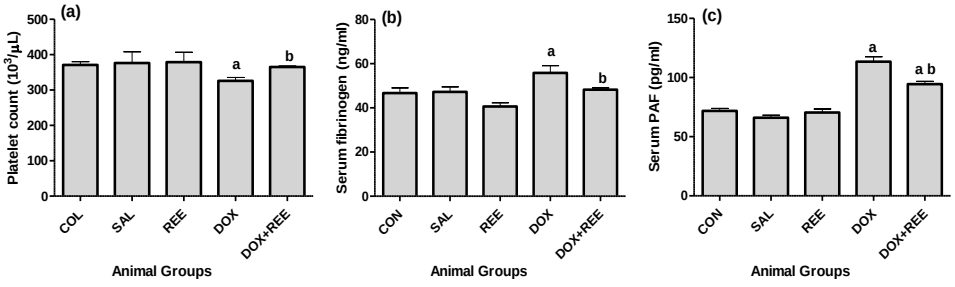

Fig.4. Platelet count (a), serum fibrinogen (b) and PAF(c) in the studied groups. Data are expressed as the average of six animals ± SE in each group. CON: control; SAL: saline; RRE: radish root extract; DOX: doxorubicin; PAF: platelet activating factor. a: significant compared with control group at $P < 0.05$; b: significant compared with DOX group at $P< 0.05$.

3.8 RBCs count, Hb content and other related indices

DOX treated rats exhibited significant decrease in RBCs count, Hb content, HCt%, MCV and MCH when compared with control group. Nevertheless, marked improvement was observed in all these parameters when DOX group was supplemented with RRE, however, no significant alterations were noticed in the normal rats received RRE (Table 3).

Table3. RBCs count, Hb content and other related indices in the studied groups

	CON	SAL	REE	DOX	DOX+REE
RBCs(10^6/µL)	6.55±0.50	6.08±0.42	7.40±0.79	3.50±0.74[a]	5.93±0.66[b]
Hb(g/dL)	13.10±0.30	12.73±0.37	13.17±0.88	7.22±0.65[a]	10.57±0.46[ab]
HCT%	43.23±1.00	42.02±1.23	43.45±2.91	23.82±2.16[a]	34.87±1.53[ab]
MCV(fL/cell)	72.10±4.41	67.06±4.46	67.72±6.20	55.45±4.14[a]	65.47±1.41[b]
MCH(Pg/cell)	23.01±0.95	22.56±2.18	21.86±1.16	17.07±1.29[a]	20.23±0.29[ab]

Data are expressed as the average of six animals ± SE in the studied groups. CON: control; SAL: saline; RRE: radish root extract; DOX: doxorubicin; RBCs: red blood cells; Hb: hemoglobin, HCt%: hematocrit%; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin. a: significant compared with control group at $P < 0.05$; b: significant compared with DOX group at $P < 0.05$.

3.9 Histological observations and cardiac lesions scoring

Heart tissue from control, saline and RRE groups showed normal structure and arrangement of muscle fibers. Rats in DOX- treated group showed marked histopathological alternations characterized by degeneration, separation, and disorganization of muscle fibers, along with congestion of blood vessels scored as severe (+++) and inflammatory cell infiltration scored as moderate (++). Administration of RRE tended to lessen DOX-induced myocardial damage, with mild (+) degenerative changes, separation, and congestion of blood vessels still present (Plate 1&Table 4).

Table 4. Cardiac lesion scores in the studied groups

	CON	SAL	RRE	DOX	DOX+RRE
Degeneration of muscle fibers	(-)	(-)	(-)	(+++)	(+)
Separation of muscle fibers	(-)	(-)	(-)	(+++)	(+)
Disorganization of muscle fibers	(-)	(-)	(-)	(+++)	(-)
Inflammatory cell infiltration	(-)	(-)	(-)	(++)	(-)
Congested blood vessels	(-)	(-)	(-)	(+++)	(+)

Cardiac lesions scoring represented as: (-) nil, (+) mild, (++) moderate and (+++) severe.

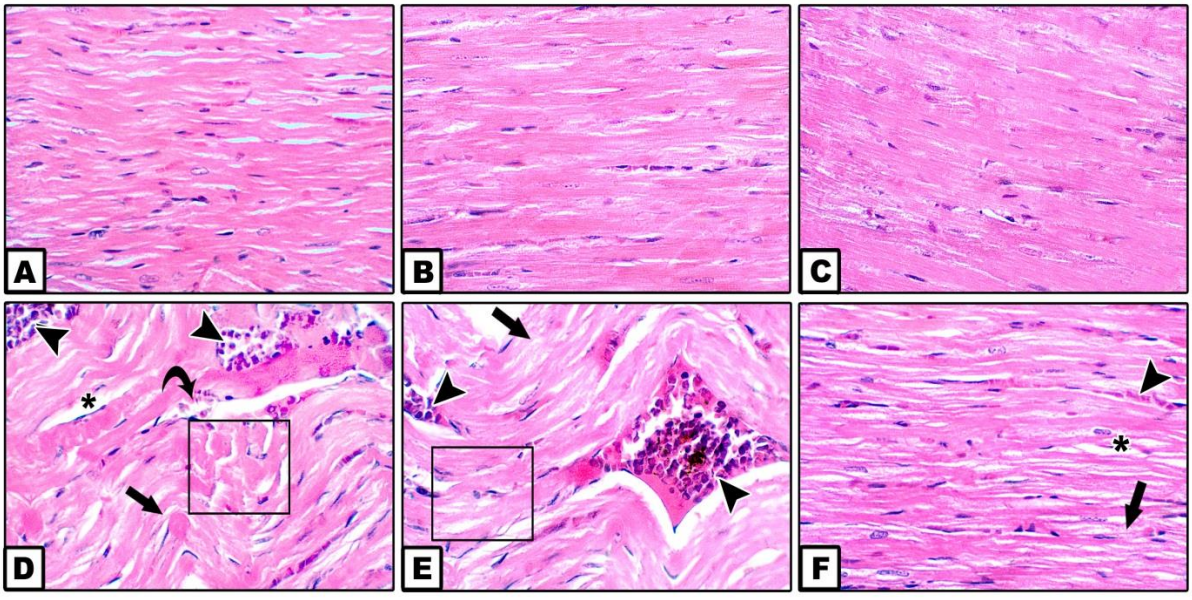

Plate1. Photomicrograph of heart sections in control (A), saline (B) and REE (C) groups showing normal appearance and well organization of cardiac muscle fibers. DOX-treated rats (D, E) showed degeneration (arrow), separation (black asterisk) and

disorganization (square) of muscle fibers, inflammatory cell infiltration (curved arrow) and congested blood vessels (arrowhead). However, administration of DOX+REE (F) exhibited near normal cardiac muscle structure with slight degenerative changes (arrow), separation (black asterisk) and congestion of blood vessels (arrowhead) (H&E, x400).

4. Discussion

Cardiac toxicity is a common complication of DOX treatment [25]. Incidence of cardiac toxicity is often associated by significant elevation in serum levels of cTn-I, CK-MB, LDH and AST which are sensitive biomarkers of myocardial injury [3]. cTn-I is considered as a specific myocardium protein, whereas CK-MB, AST and LDH are not specific and almost existed in other tissues [26]. Regardless, both biomarkers showed marked serum elevations in the current study following DOX treatment, suggesting alteration of cell membrane permeability and leakage of these proteins into circulation [27]. Interestingly, intake of RRE with DOX was effective in reducing levels of serum cTn-I and the marker enzymes near to control values, indicating cardioprotective effect of this plant most likely due to its various bioactive constituents. Results from the present study revealed that RRE possesses high concentrations of polyphenols mainly galic acid, which is important for maintaining normal membrane structural integrity, thus limiting leakage of cardiac marker proteins [28].

Reduced heart weight and heart weight to body weight ratio was also noticed in the current study. Similar results were demonstrated by Zare *et al* [29] who contributed heart weight reduction to loss of myofibrils driven by DOX- induced cardiotoxicity. This notion was further confirmed by the present histopathological findings characterized by myofibrillar degeneration and disorganization with appearance of inflammatory cell infiltration and congested blood vessels. Indeed, combined administration of RRE with DOX successfully reduced these pathogenic changes, providing further insights into cardioprotective activity of used plant extract.

Recent attention has been given to the role of reactive oxygen species (ROS) and oxidative stress in the pathogenesis of DOX-related cardiotoxicity [30]. Antioxidants are enzymatic and non-enzymatic molecules which dramatically delay or prevent oxidative damage by ROS [31]. Xanthine oxidase (XOD) is an enzyme that delivers electrons directly to molecular oxygen (O_2), thereby promoting ROS production and redox imbalance [32]. Increased activity of cardiac XOD, coupled to failure of antioxidant defences may ultimately result in cascade of oxidative reactions including lipid peroxidation and protein carbonyl production, which are reliable indices for progression of myocardial damage [33]. In the present study, DOX treatment elicited significant elevation in levels of XOD, lipid peroxidation and protein carbonyl, paralleled by marked reduction in cardiac antioxidant markers; GSH, SOD, CAT and TAC, which in all were significantly attenuated following intake of RRE. This effect could be contributed to presence of flavonoids particularly catechin that ascribed to scavenge ROS and chelate metal ions, such as iron [34]. Besides, the plant extract has shown to reduce DPPH free radical which serves as indicator for its antioxidant activity. Consumption of RRE can therefore be useful in preventing DOX cardiotoxicity via its potential to reduce deleterious effects of oxidative stress [35].

Beyond this, it has been established that inflammation is largely involved in DOX- induced cardiotoxicity [36]. Increased ROS by DOX may cause activation of nuclear factor kappa-B (NFkB) pathway and production of proinflammatory cytokines, including TNF-α in the myocardium [37]. Aberrant TNF-α production has been shown to play a pivotal role in the development of chronic heart failure in human via triggering various inflammatory reactions [38]. Increased levels of TNF-α have also demonstrated to promote expression of inducible nitric oxide synthase (iNOS), leading to enhanced NO generation. Higher levels of NO are cytotoxic, allowing direct interaction with $O_2^{\bullet-}$ to produce peroxynitrite ($ONOO^-$) which is a powerful oxidant playing a key role in myocardial destruction [39]. In this study, DOX treatment showed markedly increased TNF-α and NO levels that were brought down by administration of RRE, suggesting its potential for combating triggered inflammatory response. These results are supported by previous data showing anti-inflammatory activity of the fresh radish juice in experimental animal models of acute and chronic inflammation [40]. Herein, it is very likely that presence of tannic acid in radish extract may contribute to the plant anti-inflammatory action through down regulating TNF-α [41] and iNOS [42] production, thereby targeting generation of NO and progression of cardiac toxicity.

CRP is an acute phase protein produced mainly in response to inflammatory reactions [43]. Elevated CRP has been ascribed as independent risk factor for occurrence and development of various cardiovascular diseases owing to its ability to promote migration of circulating leucocytes into site of tissue inflammation, allowing their adhesion to vascular walls with resulting injury of vascular endothelial cells [44]. In this view, prior studies reported that DOX exposure leads to marked increase in expression of the two adhesion molecules; VCAM-1 and ICAM-1 by endothelial cells which have a

crucial role in triggering inflammation and vascular injury [45]. In the current study, DOX treatment showed significant increase in serum levels of CRP, VCAM-1 and ICAM-1 which together have been alleviated by ingestion of RRE. This effect is likely attributable to presence of alkaloids in RRE as clearly observed herein. In agreement, Souza *et al* [46] implicated alkaloids ability to protect against inflammatory response caused through different pathological conditions. Moreover, available flavonoids in RRE may act more specifically through modulating inflammatory cells infiltration [47], thereby limiting progression of endothelial dysfunction and cardiovascular injury. Hence, it can suggest that protective effect of RRE is ultimately related to its anti-inflammatory properties which may affectedly target DOX cardiotoxicity.

Despite these issues, there is accumulating evidence that increased coagulation activity is closely related to DOX-induced cardiotoxicity. The exact mechanism is not entirely known; however, platelets are considered to play a central role [48]. Effect of DOX on platelets was extensively studied; where it has shown to directly induce platelets toxicity with consequent thrombocytopenia [49] as notably seen in the current study. Even though, DOX has also suggested to cause translocation of phospholipid phosphatidylserine (PS) from the inner side to the surface of platelets. Generated ROS by DOX serve to mediate PS translocation through enhancing scramblase and suppressing flippase activities leading to platelets pro-activation [48]. This promotes binding of platelets to fibrinogen via GPIIb/IIIa receptors, leading to platelets aggregation and increased thrombotic events [50]. Of importance is that platelet-fibrinogen binding is strengthened by high fibrinogen levels even in the presence of thrombocytopenia [51]. Hence, the current data showing raised serum fibrinogen in animals exposed to DOX could be indicative for enhanced coagulation activity. In this respect, PAF has been described as a potent mediator of platelets aggregation. More details were explained by Palur Ramakrishnan *et al*. [52] who suggested stimulated platelets activity by PAF as a vascular risk factor in patients with coronary artery disease. Thus, PAF inhibition might convey therapeutic benefits in certain cardiovascular disorders [53]. In relation, the present data exhibited elevated serum PAF activity in DOX-treated rats which seemed to be successfully attenuated following intake of RRE, suggesting reduced platelets aggregation. This effect may account for abundant presence of flavonoids in used plant extract, which have shown to possess anti-platelet activities, particularly through blocking GPIIb/IIIa receptors and platelets binding to fibrinogen [54]. Flavonoids have also known as inhibitors for PAF pathway [55]. Hence, it is conceivable that RRE could inhibit DOX cardiotoxicity through its anticoagulant properties.

Beyond its coagulant effect, DOX has frequently shown to induce diversity of hematologic alterations which might be indicative of myelosuppression. This effect is widely vitrified by significant abnormalities in almost all types of blood cells with particular emphasis on the reduction in circulating RBCs [56]. As already mentioned, the present study showed significantly decreased RBCs count, Hb content, HCt%, MCV and MCH levels in DOX treated animals compared to control group. This could be contributed to impaired erythropoiesis and defective iron metabolism [57]. Nevertheless, it has recognized that increased generation of ROS by DOX can promote oxidative hemolysis of RBCs and decreased survival of oxidized RBCs in circulation [56]. Currently, there is increasing evidence that disruption of RBCs production in patients undergoing DOX treatment predisposes to prevalence of anemia [58] which may be a leading cause for reduced oxygen carrying capacity and hypoxic state both in heart and endothelial cells [59]. Therefore, it is tempting to speculate that damage of hematopoietic cells by DOX could play a role in myocardial injury and subsequent cardiotoxicity. In the current study, administration of RRE helped to alleviate hematologic abnormalities induced by DOX treatment through increasing RBCs count and related indices near to normal values. This effect is confirmed by previous data illustrating the ability of RRE to protect against dimethoate deleterious effects on blood cellular components [60], which may be explained based on its natural polyphenols with high antioxidant potential [60]. So, radish extract can be recommended as a beneficial agent for combating DOX negative impact on hematopoietic system.

In conclusion, RRE effectively attenuated DOX-induced cardiotoxicity via suppressing oxidative stress and enhancing cellular defenses against inflammatory response, coagulation activity and cardiac structural alterations. Therefore, radish extract could be considered as a potential cardioprotective agent for patients on DOX-based chemotherapy.

5. Conflicts of interest

The authors declare that they have no conflict of interest.

6. Acknowledgments

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7. References

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