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Role of radish root extract in modulating doxorubicin- induced cardiotoxicity in male rats

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Abstract: This study aimed to evaluate the role of radish root extract (RRE) in modulating doxorubicin (DOX)- induced cardiotoxicity in male rats. DOX was injected intraperitoneally in 8 equal doses (each containing 2.5mg/kg BW) twice weekly for 4 weeks, whereas RRE (30mg/kg BW) was given orally, 6 days/week for the same duration. DOX-treated rats elicited marked increase in serum levels of cardiac troponin-I and enzymes; creatine kinase-MB, lactate dehydrogenase and aspartate aminotransferase, with significant decrease in the body weight (BW), heart weight (HW) and heart /body weight ratio. Results also showed increased cardiac xanthine oxidase , malondialdehyde, protein carbonyl and nitric oxide , with decreased antioxidants; reduced glutathione , superoxide dismutase and catalase, as well as total antioxidant capacity. Increased serum inflammatory markers (tumor necrosis factor α and C-reactive protein), and adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) were also recorded. Besides, a decrease in RBCs count, Hb content, Ht%, MCV, MCH and platelets count with increased leucocytes count were observed. Supplementation of RRE tended to protect against DOX-induced cardiotoxicity, as evidenced via normalizing heart weight, cardiac biomarkers, oxidative stress and inflammatory response. Thus, recommendation for regarding consumption of RRE during DOX treatment should be made.

keywords: Radish root extract, doxorubicin, oxidative stress, inflammation, heart weight, cardiac biomarkers

1.Introduction

Doxorubicin (DOX) is one of the most common antineoplastic drugs [1]**.** It is widely used for treating several forms of cancers including, solid tumors, leukemia, lymphoma, and breast cancer [2]. However, its clinical application is largely hindered by severe cardiotoxic effect that can progress into cardiomyopathy [3] and congestive heart failure [4].

Pathogenesis of DOX cadiotoxicity is multifactorial and is directly related to its metabolic activation [5]. Excessive free radical production and mitochondrial dysfunction have suggested as a primary causative mechanism in DOX-induced cardiotoxicity [6]. In this regard, it has indicated that successful prevention or treatment of DOX-mediated cardiotoxicity depends largely on antioxidant potential of different agents and their ability to react with high free radicals [7]. Even though, there is no specific curative therapy are available.

Interestingly, several dietary plants must show potent antioxidant activities. Radish (*Raphanus sativus*) is one of the most studied vegetable crops worldwide [8]. Radish has wide pharmaceutical importance, mostly attributed to its antioxidant activities [8]. Different parts of radish including, leaves, roots and seeds can be applied in folk medicine as laxative, digestive aid and appetizer [9].Meanwhile, radish is used as a remedy for urinary infections, stomach diseases and ulcers [10]. The use of radish has also suggested to exert effective hepatoprotective effect [11] and to show hypoglycemic and hypolipidemic properties which may help to prevent diabetes [12]. In addition, radish extracts have shown to poses an inhibitory effect on lipid peroxidation and to show potent anti-inflammatory activity [13]. Apart from this, radish may also offer

considerable benefits as an anticancer agent against different tumors [14].

Based on the wide health benefits of radish and the paucity of reports about its cardioprotective properties, the present study was carried out to explore if the use of radish root extract could protect against cardiotoxicity induced by DOX in male rats

2. Materials and methods

Animals

A total of thirty adult male Wistar albino rats (200±10 g) were obtained from the animal house of Biological Products and Vaccines, Cairo, Egypt. They were housed in stainless steel cages with normal rodent diet and water *ad libitum*, under controlled temperature (25 ± 2^0C) 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.

Doxorubicin dosing

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

Plant material and preparation of extract

Radish (*Raphanus sativus*) was obtained from herbal market at Mansoura city, Egypt. It was authenticated by an expert herbalist at Botany department, Faculty of Science, Mansoura University. The roots were separated, cleaned, air dried and grinded to get fine powder. 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].

Phytochemical screening of radish root extract

Radish root extract (RRE) was screened for the main phytochemical constituents, using standard colorimetric methods. Total polyphenols were determined using Folin-Ciocalteu reagent as described by [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 [19] and expressed as catechin equivalent (mg CE/g DWE), while total tannins were measured based on vanillin hydrochloride method as described by [20] and expressed as tannic acid equivalent (mg TA/g DWE). Meanwhile, total alkaloids were quantitatively determined using ammonium hydroxide for precipitation according to described method by [21]. The percentage level of alkaloids was determined using the formula: Alkaloid (mg %) =Final weight of sample/ Initial weight of sample x100.

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 [22]. The assay is based on 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.

Animal grouping and treatment

The animals 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) [17] 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, while RRE was given orally, 6 days/week for the same period. Animals were weighed at the start and end of the study to obtain the body weight change.

Samples collection and processing

At the end of the experimental period, overnight fasted rats were sacrificed under light ether anesthesia and blood samples were collected, allowed to clot and sera were separated for biochemical analysis. Other portions were received on EDTA as anticoagulant for hematological determination

. . The heart was then excised, washed in ice cold saline, blotted dry, weighed and homogenized for further analysis. Heart weight (HW) to body weight (BW) ratio was calculated according to the formula: HW/BW% = HW (g) /BW (g) x100 [23].

Biochemical assessments

Serum cardiac troponin-I (cTn-I) was measured using ELISA kits supplied by Cusabio Co. USA, while enzymes, 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 kits 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, respectively. Cardiac malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and total antioxidant capacity (TAC) 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) was assessed using ELISA kits obtained from Kamiya Biomedical Co. (Seattle, WA, USA). Red blood cells (RBCs) , total white blood cells (WBCs) and platelets (PLts) counts were measured by hemocytometer Neubauer slide [24]. Hemoglobin (Hb) content was measured colorimetrically by Randox kits according to [25], while HCT% was measured according to [26]. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were calculated as mentioned by [27] .

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 and Discussion

Phytochemical screening

The identified phytoconstituents of RRE are presented in Table 1. Obtained data revealed high concentration of total phenolic content in the range of 55.68 GA/g DWE, whereas the concentration of total flavonoids and total tannins were 8.59 CA/g DWE and 9.35TA/g DWE, respectively. Meanwhile, the percentage level of alkaloids seemed to be in the range of 12.80 mg%.

DPPH scavenging activity

The antioxidant activity of RRE was evaluated based on the ability to scavenge DPPH free radical. Results showed that the plant extract inhibits DPPH with IC50 value of 1.2 mg/ml (Table 1).Notably, the antioxidant effect is proportional to disappearance of DPPH in test samples, thus the lowered IC50 value as shown in this study indicates the potential of plant extract to scavenge free radicals that may be related to the high content of total polyphenols

Table1. Phytochemical analysis of radish root extract

Phytochemical	Content
Polyphenols (mg	55.68
Flavonoids (mg	8.59
Tannins (mg TA/g	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); antioxidant activity of radish root extract against DPPH radical expressed in IC50 value

Cardiac biochemical markers

Assessment of cardiac toxicity markers in DOX- treated rats showed elevation in serum level of cTn-I and 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 biomarkers was recorded. No significant changes were noticed in normal animals received RRE compared to control animals (Table2)

Table2: Cardiac toxicity markers in in the studied groups

	CON	SAL	REE	DOX	DOX+REE
cTn-I(ng/ml)	0.027±0.002	0.028±0.002	0.031±0.001	0.064±0.003[a]	0.052±0.001[ab]
CK-MB(U/l)	54.18±3.73	53.97±2.05	54.71±2.79	221.90±6.128[a]	144.00±15.15[ab]
LDH(U/l)	270.80±16.13	267.80±12.51	263.10±8.98	844.20±45.07[a]	395.40±18.66[ab]
AST(U/l)	89.53±4.66	90.34±6.47	89.68±4.11	243.80±15.36[a]	173.30±5.72[ab]

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: cardiac 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 $P< 0.05$

Body weight, heart weight and heart/body weight ratio

DOX treated rats elicited significant reduction in body weight, heart weight and heart/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 (Table3)

Table3: Body weight, heart weight and heart/body weight ratio

	CON	SAL	REE	DOX	DOX+REE
BW(g)	243.2±2.24	238.1±2.77	245.4±2.51	175.7±3.00[a]	234.5±3.00[ab]
HW(g)	0.71±0.041	0.69±0.044	0.74±0.033	0.57±0.029[a]	0.71±0.044[b]
HW/BW%	0.32±0.01	0.31±0.01	0.31±0.01	0.29±0.002[a]	0.31±0.01[b]

Data are expressed as the average of six animals ± SE in each group. CON: control; SAL: saline; RRE: radish root extract; DOX: doxorubicin; BW:body weight; HW: heart weight. a: significant compared with control group at $P< 0.05$; b: significant compared with DOX group at $P< 0.05$

Cardiac oxidative stress and antioxidant markers

Results showed significant reduction in cardiac SOD, CAT, GSH and TAC, accompanied

by notable elevation of XOD, MDA and PC in DOX- treated rats compared to the control animals. These alternations were found to be ameliorated when rats were administered DOX with REE comparing with animals received DOX alone. Indeed, no significant alterations were observed upon administration of REE to normal rats (**Table4).**

Table4. Cardiac oxidative stress and antioxidant markers in the studied groups

	CON	SAL	REE	DOX	DOX+REE
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]
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]

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

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 value of these parameters compared to DOX group. However, no marked changes were detected in normal rats received REE alone (Table5).

Table5 . Serum inflammatory markers and adhesion molecules in the studied groups

	CON	SAL	REE	DOX	DOX+REE
TNF- α(Pg/ml)	5.75±0.08	5.73±0.09	5.86±0.20	30.250.34[a]	25.73±0.34[ab]
CRP(mg/l)	0.81±0.04	0.85±0.06	0.72±0.07	2.65±0.16[a]	1.84±0.10[ab]
VCAM-1(Pg/ml)	6.48±0.21	6.60±0.20	6.07±0.27	11.13±0.21[a]	9.20±0.26[ab]
ICAM-1(Pg/ml)	34.58±1.37	38.25±1.79	37.69±0.94	56.50±1.61[a]	46.02±0.37[ab]

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 $P< 0.05$

Hematological parameters (RBCs, Hb, Hct%, MCV, MCH, WBCs, Plts)

As shown in Table 6, DOX treated rats exhibited significant decrease in RBCs count, Hb content, HCt%, MCV, MCH and PLt count with marked increase in WBCs count when compared with control. Nevertheless, marked improvement was observed in all these parameters when DOX group was supplemented with REE, however, no significant alterations were noticed in normal rats that received REE only

Table6: Hematological parameters (RBCs, Hb, Hct%, MCV, MCH, WBCs, Plts) 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]
WBCs(10^3/µL)	7.85±1.080	6.68±0.71	7.47±0.32	10.83±0.70[a]	8.85±0.45[b]
Plts(10^3/µL)	371.00±9.18	365.80±34.19	378.80±28.08	325.80±9.59[a]	365.20±2.66[b]

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; WBCs: white blood cells; Plts: platelets. a: significant compared with control group at $P< 0.05$; b: significant compared with DOX group at $P< 0.05$

Discussion

DOX is one among the most effective and widely used anticancer drugs. However, cardiotoxicity comprises its clinical effectiveness [28]. In this study, DOX- induced cardiotoxicity is manifested by significant elevations in serum levels of cardiac enzymes (CK-MB, LDH, AST) and cTn-I which are highly sensitive and specific biomarkers of

myocardial injury [5]. These observations are in agreement with previous data showing leakage of cardiac enzymes and cTn-I into circulation upon myocytes membrane damage by DOX [5].

Co- administration of RRE with DOX treatment was effective in reducing serum levels of cardiac enzymes and cTn-I near to control values, which goes in line with the plant use in preventing cardiotoxicity in various experimental models [29]. Radish extract might exhibit cardioprotective activities mostly due to presence of wide variety of bioactive compounds. Results from the present study realized presence of phenolic compounds, mainly galic acid among major radish constituents, which may aid in preventing myocardial damage through its membrane stabilizing properties, thereby limiting cardiotoxicity accomplished by DOX [30].

Beyond this, the present study exhibited notable bodyweight loss with concomitant decreases in heart weight and ratio of heart weight to body weight upon DOX treatment, as in agreement with findings of [31]. Loss of body weight seems to be driven by direct toxic effect of the drug on intestinal mucosa with reduced food utilization [32], whereas decreased heart weight may be the result of local damage of heart tissue [33]. Sever histological alterations characterized by degeneration, separation and disorganization of cardiac muscle fibers with inflammatory cell infiltration and congested blood vessels were also observed in DOX-treated rats.

Supplementation of radish extract to DOX group displayed potential ability for preventing body weight loss, decreased heart weight and histopathological changes compared to the DOX intoxicated animals, suggesting diminished tissue damage and preserved structural integrity based on the higher content of galic acid in this plant extract [34].

Currently, it was accepted that oxidative stress and increased production of reactive oxygen species (ROS) are closely involved in DOX-induced cardiotoxicity [35]. Elevated ROS can cause damage to cellular macromolecules including lipids and proteins, allowing generation of toxic MDA and PC products that trigger tissue oxidative damage [36].

Xanthine oxidase (XOD) is a key enzyme involved in generation of ROS. Increased activity of XOD is implicated in several pathogenic conditions associated with overproduction of ROS [37]. This agrees with findings of [38] where exposure of rats to DOX causes elevation in cardiac XOD activity with progressive increase in free radicals production.

Cellular antioxidants are extremely important in preventing free radicals and combating its toxicity [39]. In this respect, the present data exhibited elevated oxidative stress indices (XOD, MDA, PC), with diminished antioxidants (GSH, SOD, CAT) and TAC in cardiac tissue of DOX treated rats. Indeed, administration of radish extract as a major source of antioxidants succeeded in reducing oxidative stress and enhancing cardiac antioxidant capacity in DOX-treated rats.

As given by the present study, the antioxidant activity of radish extract is dependent mainly on its flavonoid contents particularly catechins. This result coincides with previous data showing the ability of catechins to scavenge ROS by donating a hydrogen atom or by single electron transfer [40]. It also can prevent injury caused by free radical via activating antioxidant enzymes or chelating metal ions such as iron, thereby results in decreased oxidative injury [40].Thus, consumption of radish root extract could be useful in preventing cardiac oxidative damage evoked by DOX.

Accumulating evidence suggested inflammation as a major factor participating directly in development of myocardial injury by DOX [41]. One explanation is that oxidative stress evoked by DOX induces expression of toll-like receptors (TLRs) required for transcription of NF-*k*B and production of several inflammatory mediators such as tumor necrosis factor-α (TNF-α) [5],[42]. TNF-α is a proinflammatory cytokine produced by immune cells (macrophages, T cells and dendritic cells). Besides, TNF-α is also synthesized by cardiomyocytes in various disease conditions [43]. Expression of TNF-α appears to be crucial in the inflammatory reactions occurring during development of heart failure [44]. In humans, increased levels of circulating TNF-α, together with the soluble TNF-α receptors 1 and 2 have

been established as biomarkers for heart failure [45]. Even though, studies suggested that TNF-α adversely affects cardiac myocytes via increased expression of inducible nitric oxide (iNOS) and production of NO [46]. NO is a free radical being important for normal cardiac function. Higher levels of NO is cytotoxic, allowing direct interaction with O_2^- to produce peroxynitrite ($ONOO^-$),which is a powerful oxidant playing a key role in tissue destruction [47]. It is hence logical to establish an association between inflammatory response and oxidative stress [4].

In this view, the present data showing increased levels of TNF-α and NO, suggesting prevalence of proinflammatory/ pro-oxidant environment owing to DOX treatment which is thought to disrupt myocardial structure and function. Countering these effects by administration of radish root extract in this study confirmed the plant bioactive properties probably due to its higher content of tannic acid [10].Tannic acid is a naturally occurring polyphenol has an ability to reduce inflammatory markers such as, TNF-α [17]. Besides, it was found to suppress iNOS [48], which may inhibit NO production and alleviate cardiotoxic effects induced by DOX.

C-reactive protein (CRP) is an acute phase protein synthesized in liver in response to inflammatory processes [49]. CRP plays a crucial role in the expression of adhesion molecules in endothelial cells, the progression of atherosclerotic lesion, survival of endothelial progenitor cell and expression of tissue factor, the key initiator for thrombosis [50].

CRP has been described as an independent risk for occurrence and progression of cardiovascular disease via triggering a variety of inflammatory events [51]. Importantly, elevated CRP serves to promote migration of circulating leucocytes into site of inflammation, allowing their adhesion to vascular walls via adhesion molecules, leading to injury of vascular endothelial cells [52].

In this view, prior studies indicated that DOX exposure leads to significant increase in expression of the two adhesion molecules, VCAM-1 and ICAM-1 by endothelial cells, which have a crucial role in progression of vascular injury and myocardial damage [53]. In the present study, DOX treatment showed similar increase in serum levels of adhesion molecules with concomitant elevation in CRP and total leukocytes, which in all have been alleviated by radish extract supplementation, suggestion its potential for combating enhanced inflammatory response. In this respect, several studies strongly indicated the contribution of polyphenols in protecting against inflammation responses caused through different pathological conditions [54]. Results of the present study revealed presence of high concentration of total polyphenols in radish root extract which are beneficial in improving cellular inflammatory pathways and their molecular targets, including endothelial structure and function [55].

Other than, treatment with polyphenols rich diets was effective in reducing the inflammatory mediators such as CRP [56], thereby limiting leukocytes infiltration and progress of cardiovascular injury .Taken together, it seems possible to consider radish root extract as a proper remedy for DOX-induced cardiotoxicity via its prominent anti-inflammatory properties.

Besides toxic effects on cardiomyocytes, DOX has also proved to cause bone marrow suppression [57]. Bone marrow plays an important role in blood cells production, which is essential for sustenance of life [58]. DOX like other chemotherapeutic drugs can damage bone marrow hematopoietic cells through increased generation of ROS [59]. This effect is widely vitrified by significant reduction in almost all types of blood cells in the peripheral circulation [59]. In the present study, DOX treatment was accompanied by marked reduction in RBCs count, Hb content, HCt%, MCV and MCH levels which may be a result of reduced erythropoiesis and impaired iron metabolism [60]. Reduction of RBCs count in patients undergoing DOX treatment predisposes to development of anemia [61] which may be a leading cause for progression of cardiac toxicity.

Marked reduction in platelets count was also demonstrated following DOX treatment, confirming development of thrombocytopenia [57] which may lead to bleeding or even death under sever conditions [62]. Similar observations were reported in the present study,

where DOX treatment showed significant decrease in platelets count, however a marked elevation in number of WBCs was demonstrated. A finding which is in harmony with earlier data showing rapid mobilization of some WBCs from bone marrow reserves as a compensatory mechanism to loss of WBCs by treatment of the chemotherapy medication, cyclophosphamide [62]. In the present study, administration of radish root extract helped to prevent different hematological changes induced by DOX treatment through increasing RBCs count, related indices and platelets count, with decreased WBCs near to normal values. These effects are confirmed by previous data illustrating the ability of radish root extract to protect against dimethoate deleterious effects on blood cellular components [61]. This effect may be explained based on the abundant presence of polyphenolic compounds, particularly catchine in radish extract. Supplementation of green tea rich in catchine was effective in alleviating age associated hematological complications in rats, which may be related to its strong antioxidant effects on hematopoietic cells [63].So, radish extract can be recommended as proper natural product for combating DOX negative impact on hematopoietic process.

In conclusion, the present data demonstrated protective efficacy of radish root extract against DOX-induced myocardial injury, mostly through modulating oxidative stress and inflammatory response. The efficacy of the plant extract can also confirmed via inhibiting various hematological changes, suggesting that radish extract may be recommended as a complementary supplement for cardiac protection during DOX treatment based on its wide variety of active phytochemicals. However, further studies are required to validate clinical utility of this extract4.

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