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ORIGINAL ARTICLE

Allicin ameliorates doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis

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Abstract

Purpose Doxorubicin (DOX) is a highly active antineoplastic agent; however, its clinical use is limited due to associated cardiotoxicity. This study was performed to evaluate the beneficial effects of allicin, a dietary garlic active constituent against DOX-induced cardiotoxicity.

Methods Forty male Swiss albino mice were divided into five groups, which received normal saline, oral allicin (20 mg kg^{-1} once daily), intraperitoneal DOX (on the 7, 9 and 11th day of the experiment), or DOX plus once daily allicin at 10 or 20 mg kg^{-1}. Sera were collected for evaluation of cardiac injury markers and proinflammatory cytokines. Additionally, heart tissue spacemen were harvested for determination of oxidative stress markers, as well as for histopathological examination and immunohistochemical analysis.

Results DOX administration induced significant ($p < 0.05$) reductions in cardiac tissue level of reduced glutathione and activities of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase). Moreover, it induced significant ($p < 0.05$) elevations in cardiac tissue concentrations of nitric oxide and malondialdehyde as well as serum levels of cardiac injury biomarkers (lactate dehydrogenase,

creatine kinase, and creatine kinase-MB) and proinflammatory cytokines (interleukin-1β, and tumor necrosis factor-alpha). The histopathological examination showed necrotic and degenerative changes in the cardiac tissue, while immunohistochemical analysis revealed marked myocardial expression of activated caspase-3 and cyclooxygenase-2, following DOX adminstration. Allicin pretreatment significantly improved ($p < 0.05$) all examined parameters, and restored the cardiac architecture.

Conclusion The current study demonstrated that allicin effectively mitigates cardiac oxidative damage, apoptosis and inflammation, induced by acute DOX intoxication. Therefore, allicin could be a promising cytoprotective agent against DOX cardiotoxicity.

Abbreviations
CAT	Catalase
CK	Creatine kinase
CK-MB	Creatine kinase-myocardial B fraction
COX2	Cyclo-oxygenase-2
DOX	Doxorubicin
GPx	Glutathione peroxidase
GSH	Glutathione
LDH	Lactate dehydrogenase
IL-1β	Interleukin-1β
MDA	Malondialdehyde
NO	Nitric oxide
8-OHdG	8-Oxo-2′-deoxyguanosine
SOD	Superoxide dismutase
TNF	Tumor necrosis factor

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Introduction

Doxorubicin (DOX) is a highly active antineoplastic agent, used in the treatment of several malignancies, including breast, ovarian, lung and uterine cancers as well as soft-tissue sarcomas [1]. However, its clinical use is limited due to its cumulative multi-organ toxicities including cardiotoxicity [2–4]. Several hazardous effects, including lipid peroxidation, oxidative stress, DNA/RNA damage, inhibition of autophagy, endoplasmic reticulum-mediated apoptosis and disturbance of calcium homeostasis have been associated with DOX-induced cardiotoxicity [5–7]. The metabolism of DOX through NADPH-cytochrome P-450 enzyme leads to the formation of superoxide anions and hydroxyl radicals, which in turn cause injury to cellular membranes [8]. Moreover, cardiac inflammatory changes were reported due to DOX overdosage [9].

Due to the high efficacy of DOX as a chemotherapeutic drug, research efforts are directed to prevent and treat its associated cardiotoxicity. Phytochemicals are widely available, plant-derived, small molecules with proven antioxidant, anti-inflammatory, and anti-apoptotic activities [10]. The beneficial effects of garlic against cardiovascular disorders, such as stroke, coronary artery disease and hypertension are well known [11, 12]. Among the bioactive substances in garlic, allicin is a highly active organosulphur compound [13].

Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is naturally produced by the action of the enzyme alliinase on the stable precursor S-allyl cysteine-S-oxide (alliin), when garlic cloves are macerated [13]. Recent studies have shown that oral administration of allicin reduces systemic blood pressure and protects against coronary endothelial dysfunction and cardiac hypertrophy in rats [13, 14]. Other animal studies reported that allicin offers multi-organ protection against the toxicity of several drugs and chemicals [15–20]. Based on these findings, allicin may be effective in mitigating the side-effects of DOX. In view of these facts, we performed this study to investigate the beneficial effects of allicin against DOX-induced cardiotoxicity in mice.

Materials and methods

Chemicals

Doxorubicin (ADRIAMYCIN®: a vial contains 2 mg/ml of Doxorubicin HCl) was produced by Pfizer, Egypt), while allicin (25% powder) in a clinical formulation was purchased from AnHui Ruisen Biological Technology Co., Ltd., China (CasNo: 539-86-6). The ELISA kits, used for analyzing cytokines [Tumor necrosis factor (TNF-α) and Interleukin-1β (IL-1β)] and 8-Oxo-2′-deoxyguanosine (8-OhdG: a marker of DNA oxidative damage) were obtained from R&D (Mannheim, Germany) and Cayman Chemical (MI, USA) companies, respectively. The kits for creatine kinase (CK) and creatine kinase-MB (CK-MB) were purchased from Stanbio TM (Texas, USA), while lactate dehydrogenase (LDH) Kits were obtained from Randox Laboratories. All other kits were purchased from Biodiagnostics Co. (Cairo, Egypt). Other chemicals, used in this study, were of analytical grades.

Animals and experimental design

Forty male Swiss albino mice (10 weeks age, 25 ± 2 g) were obtained from the animal breeding unit, Faculty of Veterinary Medicine, Zagazig University. Mice were housed in stainless steel cages in a clean well-ventilated room at an adjusted temperature (24 ± 2 °C) and relative humidity ($50 \pm 5\%$). They were provided rodent diet and water ad libitum. Animal care and housing, as well as the experimental protocol were approved by Animal Care and Ethics Review Committee at the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (The approval no. 201609).

After acclimatization for 1 week, mice were randomly divided into five groups (each of 8). The first group (control) orally received physiological saline. The second group (Allicin$_{20}$) received allicin at a dose of 20 mg kg^{-1} BW for 2 weeks. The third group (DOX) received an oral saline solution for 2 weeks with intraperitoneal DOX injection at a dose of 10 mg kg^{-1} BW at 7, 9, and 11th day [21]. The fourth (DOX-Allicin$_{10}$) and fifth groups (DOX-Allicin$_{20}$) received intraperitoneal DOX injection (same dose of third group) plus allicin at a dose of 10 and 20 mg kg^{-1} BW [17], respectively, for 2 weeks.

Serum collection and tissue preparation

At the fourteenth day of the experiment, blood samples were collected in non-heparinized tubes and left for 30 min at room temperature to clot. The samples were then centrifuged at 3000 rpm for 20 min to obtain clear sera, which were preserved at −20 °C until used for biochemical assays. The mice were later killed by cervical decapitation under isoflurane anesthesia and the cardiac tissues were immediately removed from each mouse and washed in physiological saline. A half gram from each tissue was homogenized in 5 ml of phosphate buffer (pH 7.4) on ice, using an electric homogenizer. Homogenates were then centrifuged at 3000 rpm for 15 min at 4 °C and the resulting supernatants were kept at −20 °C until later use. The remaining cardiac specimens were immediately fixed in 10% neutral buffered formalin for histopathological and immunohistochemistry examination.

Assessment of serum biochemical parameters

The stored sera were used for evaluation of cardiac injury biomarkers. Serum aspartate transferase (AST) and LDH levels were assessed according to the methods of Reitman and Frankel [22] and Babson et al. [23], respectively. The CK activity was determined according to the method of Szasz et al. [24], while serum CK-MB activity was assessed according to the method illustrated by Wurzburg et al. [25]. Serum IL-1β/TNF-α and 8-OHdG concentrations were measured using commercially available ELISA kits from R&D (Mannheim, Germany) and Cayman Chemical Co. (MI, USA), respectively, according to the manufacturer's instructions.

Assessment of oxidative/antioxidant status

Cardiac tissue homogenates were used for assessment of antioxidant enzymes' activity, catalase [(CAT) according to the method of Aebi [26]], superoxide dismutase [(SOD) according to the method illustrated by Nishikimi et al. [27]], and glutathione peroxidase [(GPx) according to Paglia et al. [28]]. Reduced glutathione (GSH), a non-enzymatic antioxidant biomarker, was assessed according to the method of Beutler et al. [29]. Lipid peroxidation was evaluated by measuring the malondialdehyde (MDA) concentration according to the method developed by Mihara et al. [30], while nitric oxide (NO) concentration was determined according to Green et al. [31].

Histopathological and immunohistochemical examination

The formalin-fixed cardiac specimens were dehydrated in an ascending grade of ethyl alcohol, cleared in xylol, embedded in paraffin wax, and cut at 4–6 μm thick sections. They were later subjected to staining with Harri's Hematoxylin & Eosin (H&E) for light microscopic examination. The paraffin sections were then stained using primary monoclonal antibodies against cyclooxygenase-2 (COX2) and caspase-3. The binding of antibodies was visualized by avidin–biotin complex (ABC kit, Vector laboratories) and the immunostaining reaction was labeled with diaminobenzidine (DAB) as a chromogen and counterstained with Mayer's Hematoxylin.

Statistical analysis

All values were expressed as the mean ± standard error of mean (SEM). The data were analyzed, using the one way analysis of variance (ANOVA), followed by post hoc Duncan's test. A p value <0.05 was considered statistically significant.

Results

Serum biochemical analysis

Intraperitoneal administration of DOX alone in mice caused a significant ($p < 0.05$) elevation in serum levels of AST, LDH, CK and CK-MB, compared to control mice. However, mice pretreated with both doses of allicin (10 and 20 mg kg^{-1} BW) for 2 weeks showed a dose-dependent significant ($p < 0.05$) reduction in the levels of these parameters, when compared to DOX-overdosed mice (Table 1).

Moreover, DOX administration resulted in significant increases ($p < 0.05$) in serum levels of IL-1β, TNF-α and 8-OHdG (89.14 ± 2.37 pg/ml; 346.93 ± 14.45 pg/ml and 25.65 ± 0.92 ng/ml, respectively), when compared to control mice (38.65 ± 1.88 pg/ml; 185.05 ± 7.74 pg/ml and 13.83 ± 0.6 ng/ml, respectively). Pretreatment of DOX-intoxicated mice with both doses of allicin at 10 and 20 mg kg^{-1} BW resulted in dose-dependent significant reductions ($p < 0.05$) in serum levels of IL-1β (57.88 ± 2.61 and 39.79 ± 1.28 pg/ml, respectively), TNF-α (238.25 ± 6.47 and 189.54 ± 8.36 pg/ml, respectively) and 8-OHdG level (20.28 ± 1.11 and 13.92 ± 0.75 ng/ml, respectively), when compared to DOX-treated mice (Fig. 1).

Table 1 Serum enzymes' activities and biochemical parameters' concentrations in control and treated groups

	AST (U/L)	LDH (U/L)	CK (U/L)	CK-MB (U/L)
G1	30.83[a] ± 2.77	123.07[a] ± 10.81	210.92[a] ± 9.39	40.70[a] ± 3.204
G2	28.70[a] ± 2.77	116.75[a] ± 10.81	201.83[a] ± 9.39	38.58[a] ± 3.204
G3	173.62[b] ± 8.40	373.30[b] ± 13.70	495.52[b] ± 13.07	118.66[b] ± 5.60
G4	121.94[c] ± 2.61	189.02[c] ± 5.86	303.49[c] ± 7.14	66.46[c] ± 4.05
G5	51.07[d] ± 2.75	135.87[a] ± 10.28	241.71[d] ± 9.48	46.83[a] ± 3.45

Data are expressed as mean ± SE ($n = 8$). Values having different alphabetic superscripts within the same row are significantly different ($p \leq 0.05$)

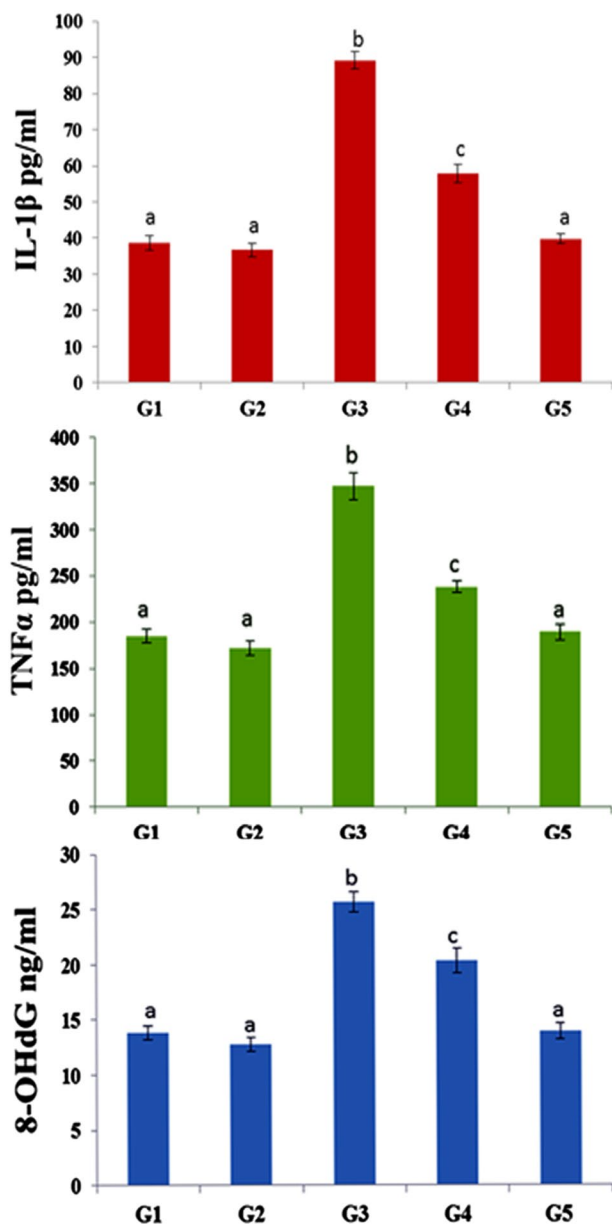*AST* Aspartate transaminase, *LDH* Lactate dehydrogenase, *CK* Creatine kinase, *CK-MB* Creatine kinase-MB

Fig. 1 Serum levels of proinflammatory cytokines and DNA marker in control and different treated groups. IL-1β; interleukin-1beta, TNFα; tumor necrosis factor alpha, 8-OHdG; 8-Oxo-2′-deoxyguanosine. Statistics; One-way ANOVA-test followed by Duncan's Multiple Range Test; $n = 8$ ($P \leq 0.05$), different letters indicate statistical significance ($P \leq 0.05$)

Effect on cardiac lipid peroxidation and antioxidant biomarkers

Doxorubicin-intoxicated mice showed significantly ($p < 0.05$) higher levels of NO and MDA, compared to control mice. These elevations were significantly ($p < 0.05$) ameliorated by treatment with both doses of allicin (10 and 20 mg kg^{-1} BW). Similarly, intraperitoneal DOX

adminstration induced significant reductions in GSH concentration and the activity of cardiac CAT, SOD, and GPx enzymes, compared to untreated mice. However, oral allicin administration at (10 and 20 mg kg^{-1} BW) doses induced a significant ($p < 0.05$) dose-dependent increase in all of these parameters (Table 2).

Histopathological examination results

Examined H&E sections of the control group revealed normal myocardial architecture (a network of branching and anastomosing cardiac myofibers (each cardiomyocyte contains oval, central and euchromatic nuclei), separated by a loose connective tissue of endomysium with flat-nucleated fibroblasts and numerous blood capillaries (Fig. 2a).

Sections of DOX-treated animals showed fragmentation and degeneration of the myocardial tissue, manifested by focal myofibrillar loss, detached areas of cardiomyocytes, dispersed vacuoles, and eosinophilia of some myofibers. The cardiomyocytic nuclei had more than one appearance: either pyknotic and condensed or faint like a ghost. Moreover, sections displayed abundant congestion of blood vessels, extravasation of blood, and a widespread infiltration by fibroblasts and polymorphonuclear cells (Fig. 2b).

The histological sections of DOX-allicin$_{10}$-treated group showed reorganization of cardiac myofibers with less cytoplasmic vacuoles and myofibrillar loss, but congestion of blood vessels was still evident (Fig. 2c). Further attenuation of DOX-induced histological changes was observed in DOX-allicin$_{20}$-treated group with seemingly normal myocardial architecture, well-organized cardiac myofibers and a significant improvement in the myocardial nuclei profile. Congested blood vessels and vacuoles were occasionally observed (Fig. 2d).

Immunohistochemical examination results

The expression of COX2 in the myocardial wall was limited to the cardiomyocyte cytoplasm and fibroblastic nuclei. The control group demonstrated negative immunoreaction (Fig. 3a), while the most intense immunoreactivity was observed in the myocardial sections of DOX-treated mice (Fig. 3b). Treatment with allicin at doses of 10 and 20 mg kg^{-1} reduced COX2 expression in cardiomyocyte cytoplasm, but induced no change in fibroblastic nuclei expression (Fig. 3c, d).

The expression of caspase-3 in the myocardial wall was localized in the cardiomyocyte cytoplasm. The sections from control mice showed no immunoreaction (Fig. 4a), while an intense immunoreactivity was observed in the sections from DOX-overdosed group (Fig. 4b). This immunoreactivity was significantly ameliorated in sections, obtained from

Table 2 Cardiac tissue antioxidants and oxidative markers in control and treated groups

	MDA (nmol/g)	NO (Umol/g)	GSH (mg/g)	GPX (U/g)	SOD (U/g)	CAT (U/g)
G1	$41.97^a \pm 2.85$	$47.39^a \pm 2.25$	$98.87^a \pm 4.19$	$28.96^a \pm 1.32$	$17.22^a \pm 0.95$	$2.68^a \pm 0.06$
G2	$39.92^a \pm 5.31$	$42.96^a \pm 1.67$	$103.30^a \pm 4.19$	$31.39^a \pm 1.32$	$19.15^a \pm 0.95$	$2.81^a \pm 0.06$
G3	$125.95^b \pm 7.07$	$135.30^b \pm 10.75$	$43.80^b \pm 3.26$	$13.92^b \pm 0.67$	$8.35^b \pm 0.59$	$0.74^b \pm 0.07$
G4	$73.09^c \pm 2.70$	$83.85^c \pm 5.22$	$65.33^c \pm 3.14$	$19.82^c \pm 1.83$	$12.93^c \pm 0.43$	$1.85^c \pm 0.09$
G5	$45.48^a \pm 2.80$	$59.18^a \pm 4.72$	$93.82^a \pm 4.75$	$27.46^a \pm 1.59$	$16.07^a \pm 1.69$	$2.52^a \pm 0.11$

Data are expressed as mean $\pm$ SE ($n = 8$). Values having different alphabetic superscripts within the same row are significantly different ($p \leq 0.05$)

MDA Malondialdehyde, *NO* Nitric oxide, *GSH* Glutathione, *GPX* Glutathione peroxidase, *SOD* Superoxide dismutase, *CAT* Catalase
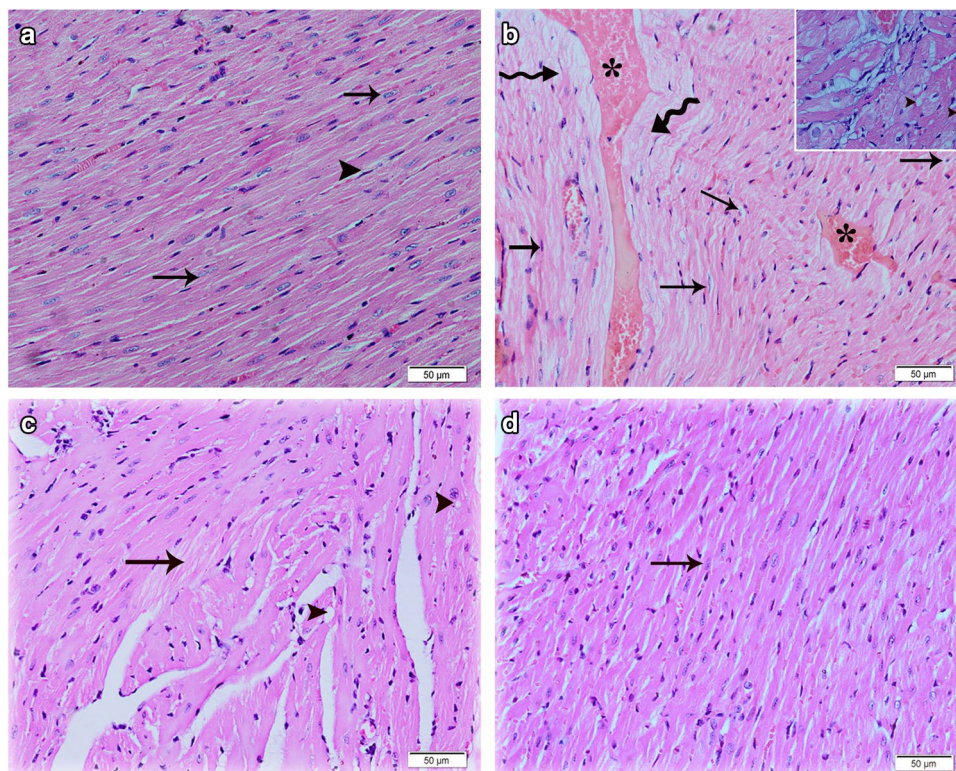

Fig. 2 Photomicrographs of examined groups stained with H&E. **a** Control group: Normal architecture of cardiac wall with branching and anastomosing myofibers bounded with endomysium, cardiomyocytes have central oval, euchromatic nuclei (*arrow*). Note that they are surrounded with numerous blood capillaries and flat nuclei of fibroblasts (*arrowhead*). **b** DOX-treated group: Focal myocytolysis (*curved arrow*) with either pyknotic or fade nuclei (*arrow*). Note the congested blood vessels (*asterisk*). *Inset* showing vacuolar degeneration of cardiomyocytes (*arrowhead*). **c** Allicin treated group (III) at a dose 10 mg/kg: Reorganization of cardiac myofibers with myocytolysis (*arrow*) and small vacuoles (*arrowhead*) of some cardiomyocytes. **d** Allicin treated group (IV) at a dose of 20 mg/kg: well-organized cardiac myofibers and oval and euchromatic cardiomyocytic nuclei (*arrow*). ×20 *scale bar* 50 μm

mice treated with DOX plus 10 or 20 mg kg^{-1} of allicin (Fig. 4c/d).

Discussion

The well-known cardiotoxic effects are primarily attributed to induction of inflammation [9] and overproduction of free radicals at high DOX doses [2, 32]. The heart is considered the main target organ for DOX-induced oxidative stress due to the lower levels of antioxidant enzymes (SOD, CAT, and GSH) in the cardiac muscle, compared to other organs [33, 34]. Moreover, the mitochondria in the cardiac muscle contains cardiolipin, which has a high affinity to DOX, leading to DOX accumulation within the cardiac mitochondria, impairment of the respiratory chain, and

induction of apoptotic death [35]. All these events (oxidative damage, apoptosis, and inflammation) were confirmed in our biochemical, histopathological, and immunohistochemical analyses.

Reduced glutathione (GSH) is a non-enzymatic antioxidant, which scavenges reactive oxygen species (peroxynitrite, hydroxyl radical, lipid peroxyl radical, and H_2O_2) and other free radicals [36]. It acts as a substrate for GPx; therefore, unavailability of GSH results in reduced GPx activity [37]. Other antioxidant enzymes include SOD and CAT, which catalyze the breakdown of superoxide anions and hydrogen peroxide, respectively. Echoing other studies [8, 38, 39], we detected a significant reduction in the activities of cardiac SOD, CAT and GPx following DOX adminstration. Moreover, we observed a significant increase in cardiac tissue levels of NO, MDA, and 8-OHdG in DOX-intoxicated mice. Other studies explained this finding by DOX-induced upregulation of inducible nitric oxide synthase (iNOS), increasing NO production, lipid peroxidation, and DNA damage [40–43].

Interestingly, pretreatment of DOX-overdosed mice with allicin (10 and 20 mg kg^{-1} BW) significantly restored antioxidant enzymes' (GPx, SOD, CAT) activity and GSH concentration and reduced NO, MDA, 8-OHdG levels in the cardiac tissue. Similar effects were described for allicin in the protection against acrylamide [17], cyclophosphamide [20], and gentamicin [18] cytotoxicity. Former studies have suggested that the antioxidant effect of allicin can be direct (by interaction with hydroxylated molecules or transferring its allylic hydrogen to the oxidized peroxyl radicals) or indirect through upregulation of phase II detoxifying enzymes (hemeoxygenase-1, glutathione-S-transferases, NAD(P) H-quinine oxidoreductase, and γ-glutamyl-cysteine synthetase) in a nuclear factor related-2 factor (Nrf2)-dependent pathway [44–46].

Confirming former studies [47, 48], our biochemical analysis showed that DOX induced an acute inflammatory reaction, manifested in elevation of serum levels of TNF-α and IL-1β. Moreover, we detected increased levels of serum cardiac injury markers (LDH, CK and CK-MB) in DOX-overdosed mice. Interestingly, allicin pretreatment lowered serum levels of inflammatory and cardiac injury biomarkers, which further confirms its anti-inflammatory and membrane-stabilizing effects [14, 49]. Liu and colleagues showed that allicin can offer cardioprotection through other mechanisms, e.g., allicin ameliorated cardiac hypertrophy and fibrosis via attenuating reactive oxygen species-dependent Jun N-terminal kinase (JNK1/2) signaling pathway. They also showed that allicin can prevent apoptosis by activating extracellular signal-regulated kinase (ERK) and PI3 K/Akt/GSK3β survival pathways [50].

Our histopathological analysis demonstrated cardiomyopathic changes following DOX adminstration, such as extensive sarcoplasmic vacuolization particularly around the blood vessels, myofibrillar loss, and distortion of normal architecture. Moreover, it demonstrated nuclear

Fig. 3 Photomicrographs showing the expression of COX2 in the myocardium of the examined groups: (**a**) control, (**b**) DOX-treated group showing strong intensity of cardiomyocyte cytoplasm (*asterisk*) and fibroblast nuclei (*arrow*). Reduced COX-2 expression in allicin treated groups at a dose 10 (**c**) and 20 mg/kg B. wt. (**d**) (×20 *scale bar* 50 μm)
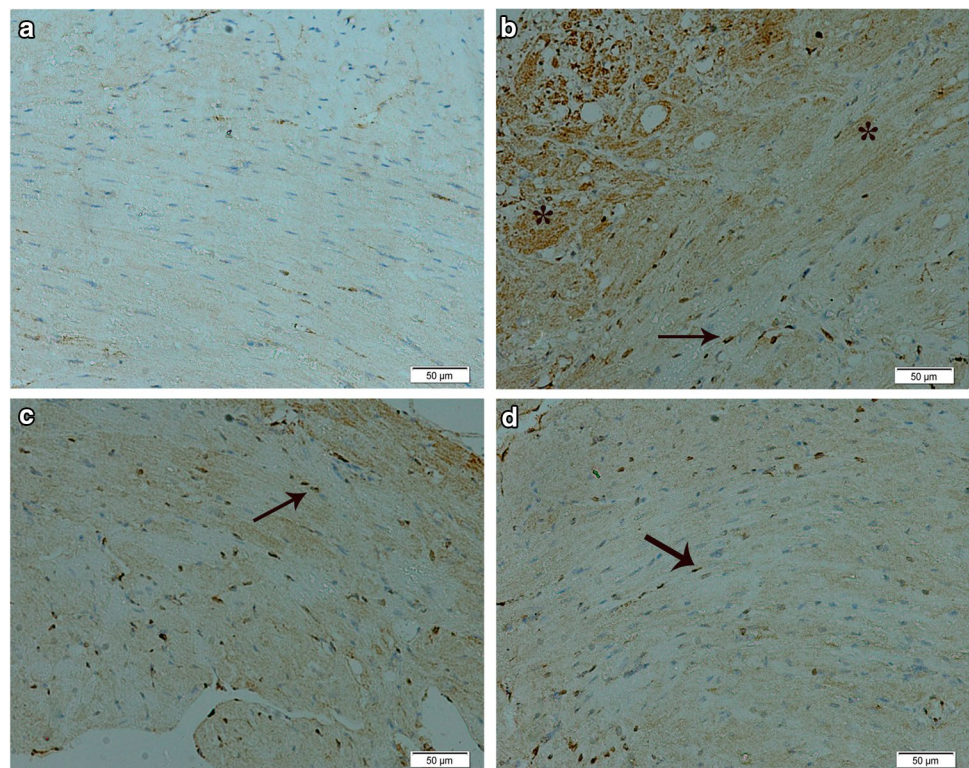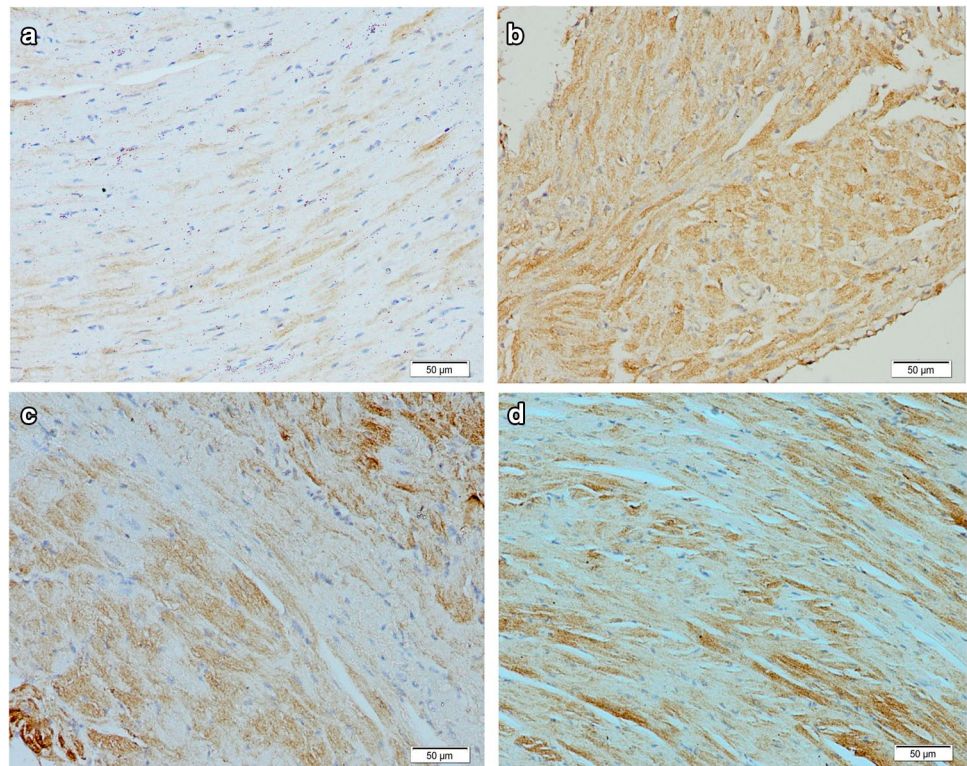
Fig. 4 Photomicrographs showing the expression of caspase-3 in the myocardium of the examined groups: (**a**) control, (**b**) DOX-treated group, allicin treated at a dose of 10 (**c**) and 20 mg/kg B. wt (**d**) (×20 *scale bar* 50 μm)

manifestation of degeneration, such as pyknotic or fading nuclei. These changes have been recorded earlier by several investigators who suggested oxidative stress, lipid peroxidation, and apoptosis as the underlying mechanisms for these observations [51–53]. Other studies have demonstrated that cellular vacuolization reflects dilatation of the smooth endoplasmic reticulum within cardiomyocytes [54, 55], while myofibrillar loss may be due to reduced mRNA expression of cardiac-specific proteins, such as troponin I and myosin light chain 22 [56].

Immunohistochemical analysis showed that DOX-induced apoptosis can be mediated by elevated cellular levels of caspase-3 enzyme. This was previously demonstrated by Davitashvili et al. who showed that DOX causes a marked release of cytochrome C into the cytoplasm, as well as an increased expression of caspase-3 and caspase-9 in cardiomyocytes [57]. Another apoptotic mechanism of DOX that was not investigated in this study is disrupting calcium homeostasis. Doxorubicin increases the opening likelihood of sarcoplasmic reticulum Ca channels and inhibits Na^+–Ca^{2+} exchanger membrane proteins, inducing cellular and mitochondrial Ca overload, which may disrupt cellular metabolism, increase free radicals generation, and induce apoptosis [58].

In the current study, allicin-pretreated groups exhibited an improvement in the histological profile of the myocardium. The cardioprotective effects of allicin were dose-dependent, i.e., cardiac tissues subjected to the higher allicin dose (20 mg kg^{-1}) displayed nearly normal histological architecture. These improvements are probably related to the previously mentioned antioxidant, anti-inflammatory, and antiapoptotic properties of allicin. Based on these findings, future clinical trials should consider allicin as a promising protective agent against DOX cardiotoxicity. Moreover, future animal studies should test the cytoprotective effect of allicin against other chemotherapeutic agents, especially in animal models with malignant tumors.

Conclusion

In experimental mice, DOX-induced cardiac oxidative damage following the exhaustion of cardiac antioxidant defense, as well as apoptotic and inflammatory reactions. Pretreatment with allicin significantly ameliorated the biochemical and histological changes, induced by DOX. Therefore, allicin may be a promising cytoprotective agent against DOX cardiotoxicity in human cancer patients.

Ethical approval This manuscript include animal experiments, and the animal handling, housing and care, as well as the experimental protocol were approved by Animal Care and Ethics Review Committee at the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (The Approval No. 201609). This article does not contain any studies with human participants performed by any of the authors.

References

1. Injac R, Perse M, Obermajer N, Djordjevic-Milic V, Prijatelj M et al (2008) Potential hepatoprotective effects of fullerenol C60(OH)24 in doxorubicin-induced hepatotoxicity in rats with mammary carcinomas. Biomaterials 29:3451–3460

2. Berthiaume JM, Wallace KB (2007) Adriamycin-induced oxidative mitochondrial cardiotoxicity. Cell Biol Toxicol 23:15–25

3. Hao G, Yu Y, Gu B, Xing Y, Xue M (2015) Protective effects of berberine against doxorubicin-induced cardiotoxicity in rats by inhibiting metabolism of doxorubicin. Xenobiotica 45(11):1024–1029

4. Olson RD, Boerth RC, Gerber JG, Nies AS (1981) Mechanism of adriamycin cardiotoxicity: evidence for oxidative stress. Life Sci 29:1393–1401

5. Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ et al (2012) Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J Mol Cell Cardiol 52:1213–1225

6. Li DL, Wang ZV, Ding G, Tan W, Luo X et al (2016) Doxorubicin blocks cardiomyocyte autophagic flux by inhibiting lysosome acidification. Circulation 133:1668–1687

7. Ky B, Vejpongsa P, Yeh ET, Force T, Moslehi JJ (2013) Emerging paradigms in cardiomyopathies associated with cancer therapies. Circ Res 113:754–764

8. Oz E, Ilhan MN (2006) Effects of melatonin in reducing the toxic effects of doxorubicin. Mol Cell Biochem 286:11–15

9. Deepa PR, Varalakshmi P (2005) Biochemical evaluation of the inflammatory changes in cardiac, hepatic and renal tissues of adriamycin-administered rats and the modulatory role of exogenous heparin-derivative treatment. Chem Biol Interact 156:93–100

10. Abushouk AI, Ismail A, Salem AMA, Afifi AM, Abdel-Daim MM (2017) Cardioprotective mechanisms of phytochemicals against doxorubicin-induced cardiotoxicity. Biomed Pharmacother 90:935–946

11. Bordia A, Verma S, Vyas A, Khabya B, Rathore A et al (1977) Effect of essential oil of onion and garlic on experimental atherosclerosis in rabbits. Atherosclerosis 26:379–386

12. Reinhart KM, Coleman CI, Teevan C, Vachhani P, White CM (2008) Effects of garlic on blood pressure in patients with and without systolic hypertension: a meta-analysis. Ann Pharmacother 42:1766–1771

13. Elkayam A, Peleg E, Grossman E, Shabtay Z, Sharabi Y (2013) Effects of allicin on cardiovascular risk factors in spontaneously hypertensive rats. Isr Med Assoc J 15:170–173

14. Sun X, Ku DD (2006) Allicin in garlic protects against coronary endothelial dysfunction and right heart hypertrophy in pulmonary hypertensive rats. Am J Physiol Heart Circ Physiol 291:H2431–H2438

15. Suddek GM (2014) Allicin enhances chemotherapeutic response and ameliorates tamoxifen-induced liver injury in experimental animals. Pharm Biol 52:1009–1014

16. Chan JY, Tsui HT, Chung IY, Chan RY, Kwan YW et al (2014) Allicin protects rat cardiomyoblasts (H9c2 cells) from hydrogen peroxide-induced oxidative injury through inhibiting the generation of intracellular reactive oxygen species. Int J Food Sci Nutr 65:868–873

17. Zhang L, Wang E, Chen F, Yan H, Yuan Y (2013) Potential protective effects of oral administration of allicin on acrylamide-induced toxicity in male mice. Food Funct 4:1229–1236

18. El-Kashef DH, El-Kenawi AE, Suddek GM, Salem HA (2015) Protective effect of allicin against gentamicin-induced nephrotoxicity in rats. Int Immunopharmacol 29:679–686

19. Abdel-Daim MM, Abdelkhalek NK, Hassan AM (2015) Antagonistic activity of dietary allicin against deltamethrin-induced oxidative damage in freshwater Nile tilapia; *Oreochromis niloticus*. Ecotoxicol Environ Saf 111:146–152

20. Ashry NA, Gameil NM, Suddek GM (2013) Modulation of cyclophosphamide-induced early lung injury by allicin. Pharm Biol 51:806–811

21. Pecoraro M, Del Pizzo M, Marzocco S, Sorrentino R, Ciccarelli M et al (2016) Inflammatory mediators in a short-time mouse model of doxorubicin-induced cardiotoxicity. Toxicol Appl Pharmacol 293:44–52

22. Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63

23. Babson SR, Babson AL (1973) An improved amylase assay using dyed amylopectin. Clin Chim Acta 44:193–197

24. Szasz G, Waldenstrom J, Gruber W (1979) Creatine kinase in serum: 6. Inhibition by endogenous polyvalent cations, and effect of chelators on the activity and stability of some assay components. Clin Chem 25:446–452

25. Wurzburg U, Hennrich N, Lang H, Prellwitz W, Neumeier D et al (1976) Determination of creatine kinase-MB in serum using inhibiting antibodies (author's transl). Klin Wochenschr 54:357–360

26. Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

27. Nishikimi M, Appaji N, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46:849–854

28. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70:158–169

29. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888

30. Mihara M, Uchiyama M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278

31. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS et al (1982) Analysis of nitrate, nitrite, and [15 N] nitrate in biological fluids. Anal Biochem 126:131–138

32. Ahmed HH, Mannaa F, Elmegeed GA, Doss SH (2005) Cardioprotective activity of melatonin and its novel synthesized derivatives on doxorubicin-induced cardiotoxicity. Bioorg Med Chem 13:1847–1857

33. De Beer EL, Bottone AE, Voest EE (2001) Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review. Eur J Pharmacol 415:1–11

34. Zhou S, Starkov A, Froberg MK, Leino RL, Wallace KB (2001) Cumulative and irreversible cardiac mitochondrial dysfunction induced by doxorubicin. Cancer Res 61:771–777

35. Ascensao A, Magalhaes J, Soares JM, Ferreira R, Neuparth MJ et al (2005) Moderate endurance training prevents doxorubicin-induced in vivo mitochondriopathy and reduces the development of cardiac apoptosis. Am J Physiol Heart Circ Physiol 289:H722–H731

36. Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition 18:872–879

37. Griffith OW (1999) Biologic and pharmacologic regulation of mammalian glutathione synthesis. Free Radic Biol Med 27:922–935

38. Raskovic A, Stilinovic N, Kolarovic J, Vasovic V, Vukmirovic S et al (2011) The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. Molecules 16:8601–8613

39. Hou XW, Jiang Y, Wang LF, Xu HY, Lin HM et al (2009) Protective role of granulocyte colony-stimulating factor against adriamycin induced cardiac, renal and hepatic toxicities. Toxicol Lett 187:40–44

40. Wang GY, Wang YM, Zhang LN, Li Q, Yue H et al (2007) Effect of resveratrol on heart function of rats with adriamycin-induced heart failure. Zhongguo Zhong Yao Za Zhi 32:1563–1565

41. Aniss HA, Said Ael M, El Sayed IH, Adly C (2014) Amelioration of adriamycin-induced cardiotoxicity by *Salsola kali* aqueous extract is mediated by lowering oxidative stress. Redox Rep 19:170–178

42. Yilmaz S, Atessahin A, Sahna E, Karahan I, Ozer S (2006) Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. Toxicology 218:164–171

43. Palmeira CM, Serrano J, Kuehl DW, Wallace KB (1997) Preferential oxidation of cardiac mitochondrial DNA following acute intoxication with doxorubicin. Biochim Biophys Acta 1321:101–106

44. Rabinkov A, Miron T, Mirelman D, Wilchek M, Glozman S et al (2000) S-Allylmercaptoglutathione: the reaction product of allicin with glutathione possesses SH-modifying and antioxidant properties. Biochim Biophys Acta 1499:144–153

45. Rabinkov A, Miron T, Konstantinovski L, Wilchek M, Mirelman D et al (1998) The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. Biochim Biophys Acta 1379:233–244

46. Prasad K, Laxdal VA, Yu M, Raney BL (1995) Antioxidant activity of allicin, an active principle in garlic. Mol Cell Biochem 148:183–189

47. Wong J, Smith LB, Magun EA, Engstrom T, Kelley-Howard K et al (2013) Small molecule kinase inhibitors block the ZAK-dependent inflammatory effects of doxorubicin. Cancer Biol Ther 14:56–63

48. Tangpong J, Cole MP, Sultana R, Estus S, Vore M et al (2007) Adriamycin-mediated nitration of manganese superoxide dismutase in the central nervous system: insight into the mechanism of chemobrain. J Neurochem 100:191–201

49. Okada Y, Tanaka K, Sato E, Okajima H (2006) Kinetic and mechanistic studies of allicin as an antioxidant. Org Biomol Chem 4:4113–4117

50. Liu C, Cao F, Tang Q-Z, Yan L, Dong Y-G et al (2010) Allicin protects against cardiac hypertrophy and fibrosis via attenuating reactive oxygen species-dependent signaling pathways. J Nutr Biochem 21:1238–1250

51. Chatterjee K, Zhang J, Tao R, Honbo N, Karliner JS (2008) Vincristine attenuates doxorubicin cardiotoxicity. Biochem Biophys Res Commun 373:555–560

52. Shi J, Zhang L, Zhang Y-W, Surma M, Payne RM et al (2012) Downregulation of doxorubicin-induced myocardial apoptosis accompanies postnatal heart maturation. Am J Physiol Heart Circ Physiol 302:H1603–H1613

53. Yalçin E, Oruç E, Çavuşoğlu K, Yapar K (2010) Protective role of grape seed extract against doxorubicin-induced cardiotoxicity and genotoxicity in albino mice. J Med Food 13:917–925

54. Boucek RJ Jr, Miracle A, Anderson M, Engelman R, Atkinson J et al (1999) Persistent effects of doxorubicin on cardiac gene expression. J Mol Cell Cardiol 31:1435–1446

55. Singh G, Singh AT, Abraham A, Bhat B, Mukherjee A et al (2008) Protective effects of *Terminalia arjuna* against doxorubicin-induced cardiotoxicity. J Ethnopharmacol 117:123–129

56. Takemura G, Fujiwara H (2007) Doxorubicin-induced cardiomyopathy: from the cardiotoxic mechanisms to management. Prog Cardiovasc Dis 49:330–352

57. Davitashvili D, Museridze D, Svanidze I, Gegenava L, Sanikidze T (2009) Investigation of oxidative stress-induced alterations in the rat brain cortical cellular culture and their correction with vitamins E and C. Georgian Med News 177:73–77

58. Waring P (2005) Redox active calcium ion channels and cell death. Arch Biochem Biophys 434:33–42