VITAMIN E POTENTIALLY AMELIORATES ADRIAMYCIN-INDUCED CARDIOTOXICITY

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Abstract:

Background: It is well known that antioxidants protect agents against adriamycin (Doxorubicin)-induced cardiotoxicity. However, the optimal agent or its optimal dose is still controversial.

Aim of the work: to examine the possible ameliorative effects of vitamin E (in different doses) for adriamycin-induced cardiotoxic effects

Methods: Fifty male rats were divided into 5 equal groups. Control rats received normal saline. Group (2): Rats received adriamycin (2 mg/kg) intraperitoneally every other day for 15 days. Group (3): rats received adriamycin as in group 2 and 100 mg Vitamin E/kg BW/day orally for 15 days. Groups (4) and (5): as in group 3, but vitamin E dose increased to 200 mg and 300 mg, respectively. CK, LDH, CK-MB, CAT, SOD, MDA, and GSH levels were measured. Finally, cardiac muscle histopathology was performed and documented.

Results: Biochemical indicators increased, SOD, CAT, and GSH decreased, and MDA increased with adriamycin usage. In a dose-dependent way, vitamin E improved symptoms (the higher dose was associated with better improvement). Biochemical signs did not return to normal even with the greatest vitamin E intake. CK was strongly and negatively linked with SOD, CAT, and GSH but proportionally correlated with MDA. CK-MB correlated with LDH. Histopathological testing verifies these findings.

Conclusion: Oxidative stress plays a significant role in adriamycin cardiotoxicity. Vitamin E in different doses ameliorated the cardiotoxic effects in a dose-dependent manner. Thus, concomitant administration of vitamin E could protect the heart and extend adriamycin's use as an anticancer agent.

Keywords: Doxorubicin; Cardiac Toxicity; Oxidative Stress; Biomarkers

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INTRODUCTION

Adriamycin (doxorubicin) is an effective chemotherapeutic agent used to treat a wide range of cancers (e.g., sarcomas, breast cancer, Hodgins's, non-Hodgkin's lymphomas, and leukemias). It is isolated from a fungus known as S. Peucetius (1). However, apart from the usual side effects of chemotherapeutic anticancer drugs, adriamycin clinical use is limited by its potential cardiotoxicity reported among 25.0% of patients (2).

Adriamycin cardiotoxic effects are reported in two phases, the first is the acute phase, which occurs 48-72 hours after the first drug administration and usually affects 11.0% of patients, and it is reversible by proper treatment. It manifests in the form of ventricular or atrial arrhythmias, QT interval prolongation, T-wave abnormalities, and atypical ST segments (3).

The phase is chronic toxicity, which is reported with repeated drug use for a long time (several weeks or months). Cardiotoxic manifestations were reported among 60.0% of patients, including tachycardia, hypotension, or ventricular failure (4).

It may be advanced to cardiomyopathy, congestive heart failure, and systolic dysfunction (2).

Different pathophysiological mechanisms were proposed to explain adriamycin-induced cardiac toxicity, including oxidative stress, topoisomerase II inhibition, reduction of DNA binding or alkylation, cardiomyocyte-specific gene expression, direct damage to the cell membrane, dysfunction of adrenoreceptors and dysregulation of calcium (5).

Calcium dysregulation and oxygen radicals, often known as reactive oxygen species (ROS), are the two crucial elements that cause apoptosis by activating caspases (a group of cysteine proteases) (6). Also, these factors stimulate inflammatory cell infiltration and production of proinflammatory cytokines leading to inflammation of cardiac tissue and cardiomyocyte death. All these effects reduce the systolic performance of the left ventricle with atrophy of its walls (7, 8).

Vitamin E (α-tocopherol) is one of the fat-soluble vitamins. It is an essential nutrient characterized by its antioxidative and anti-inflammatory properties (9). In addition, it is the major lipid-soluble antioxidant with potential anti-toxic effects (10). These effects could be attributed to its biological actions, which include reducing ROS toxic effects, effective removal of free radicals, modification of the oxidative stress damaging effects (11), and cell membrane protection from harmful effects of lipid peroxidation (12).

Thus, it is proposed that vitamin E could possibly protect cardiac muscle from Adriamycin-induced cardiotoxic effects.

The Aim of the work: The current study was designed to examine the possible ameliorative effects of vitamin E (in different doses) for adriamycin-induced cardiotoxic effects

MATERIAL AND METHODS

Fifty male albino rats were used in the current experiment, with an average weight of 180 g. The Faculty of Veterinary Medicine at Cairo University in Egypt provided all of the animals, and they were all housed in ventilated rooms for the duration of the experiment. The temperature was adjusted at 25.0±2.0 with 12 hours of light/dark cycles, with free access to food and water (ad libitum).

They were divided into equal 5 groups (every 10 rats): The Control group (1): rats were given saline, 150 mg LC/kg body weight (BW)/day, as described by Tousson, et al. (13). Group (2): rats received adriamycin (2 mg/kg) every other day for 15 days by intraperitoneal injection. Group (3): rats received adriamycin (2 mg/kg) every other day, as in group 2, and oral 100 mg Vitamin E/kg BW/day once daily for 15 days. Group (4): rats received adriamycin (2 mg/kg) every other day, as in group 2, and oral 200 mg Vitamin E/kg BW/day once daily for 15 days. Group (5): rats received adriamycin (2 mg/kg) every other day, as in group 2, and oral 300 mg Vitamin E/kg BW/day once daily for 15 days.

Ethical aspects:

The Damietta Faculty of Medicine, Al-Azhar University, Dameitta, Egypt's local research and ethics council, examined and approved the study protocol with registration number (IRB 00012367-22-09-002). It was completed per the international animal use and care principles.

Blood sampling for biochemical analysis: At the end of the study, rats were anesthetized by ether, and the heart was directly punctured to obtain blood samples in capillary tubes. The samples were left for coagulation and then centrifuged at 3000rpm for 15 minutes to obtain the serum for biochemical analysis. Samples were kept at -20°C

till the time of biochemical analysis. Heart injury indicators were measured in the serum samples. These indicators included lactate dehydrogenase (LDH), creatine kinase (CK), and CK-MB levels, as described previously by Neumeier, et al. (14), Buhl and Jackson (15), Szasz, et al. (16)

A sampling of the heart tissues to evaluate oxidative stress: Following the collection of blood samples, the hearts of the rats were removed and cleaned with a 0.9% solution of ordinary saline. Then, using an electrical homogenizer surrounded by ice, 1 gm of the heart tissue was collected and homogenized with cold phosphate buffer (10 ml) of pH 7.4. Then, Nethylmaleimide was added to inhibit GSH from oxidizing, and centrifugation was performed at 5000 rpm for 30 minutes, as described by Aboubakr, et al. (17). The supernatant was used for the determination of catalase (CAT), superoxide dismutase (SOD) activities, MDA, and GSH concentrations as described by Nishikimi, et al. (18), Aebi (19), Habig, et al. (20), Uchiyama and Mihara (21).

Histopathological examination

A slice of the preserved heart was fixed for 24 hours in a 10% buffered neutral formalin solution and embedded in paraffin. After fixation, 6 µm thick segments were prepared and stained by hematoxylin and eosin (HE) and investigated by a senior histopathologist under the light microscope, as described by Bancroft and Gamble (22).

Chemicals

Adriamycin was acquired from EIMC United Pharmaceuticals Company in a clear vial containing 10 mg of Adriamycin hydrochloride dissolved in 0.9% normal saline. The Egyptian pharmaceutical firm Pharco provided the vitamin E.

Statistical analysis of data:

The collected data were fed to the statistical package of social sciences, version 22 (IBM®SPSS® Inc., USA). Numerical values were presented by the arithmetic mean, and standard deviation, and groups were compared by the One Way Analysis of Variance (ANOVA) test. Furthermore, the cardiotoxic indicators were correlated with the oxidative stress indicators by calculating Pearson's correlation coefficient. P value < 0.05 was regarded as significant.

RESULTS

In the current work, treatment of rats with adriamycin (2 mg/kg) every other day for 15 days by intraperitoneal injection was associated with a significant rise of biochemical markers of cardiac toxicity (CK, LDH, and CK-MB), and a significant reduction of SOD, CAT, GSH with the significant increase of MDA. The concomitant use of vitamin E was associated with significant improvement of both indicators of cardiac toxicity and oxidative stress in a dose-dependent manner (the higher dose was associated with better improvement). However, values did not return to the normal level (i.e., the concurrent use of vitamin E (100 to 300 mg) was associated with amelioration of cardiotoxic effects of adriamycin in a dose-dependent manner; the higher tested dose (300 mg) was associated with the best effect) (Figure 1, 2).

Figure (3) shows the bivariate correlation between biochemical markers of cardiac toxicity and oxidative stress indicators. It revealed that CK was significantly and inversely correlated with SOD, CAT, and GSH but proportionately correlated with MDA. In addition, LDH and CK-MB showed a similar correlation.

Histopathological results: In the heart tissues of the control group, there was regular cell distribution and normal cardiomyocyte architecture. No vacations of the cytoplasm or myocardial degeneration were observed (Figures 4 and 5). Nevertheless, in the adriamycin-treatment group, there was an increase in cellular degeneration, with the presence of cytoplasmic vaculations and marked cellular infiltration (Figure 6). In concurrent treatment with vitamin E, the cardiomyocytes preserved the normal cellular architecture, normal cell distribution, and no cytoplasmic vaculations. But with mild cellular infiltration (Figure 7).

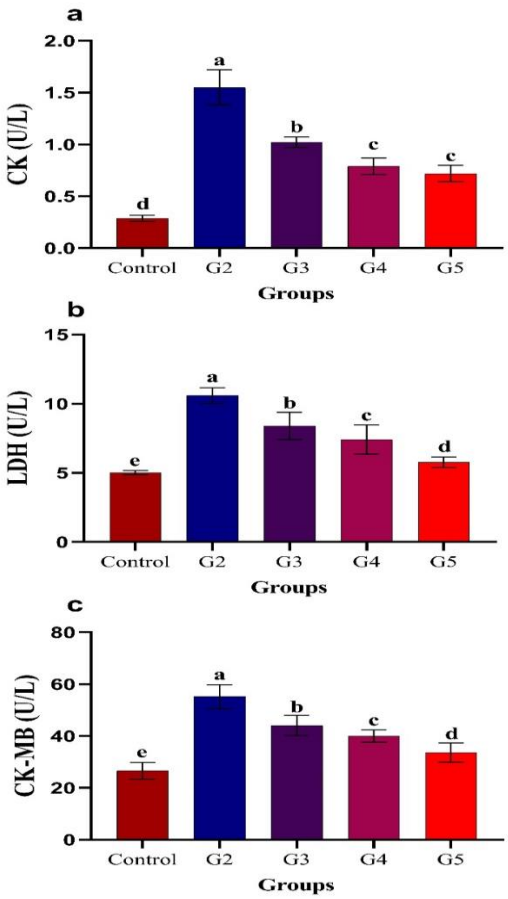


Figure 1. Effect of individual treatment with doxorubicin on the levels of Biochemical markers of cardiac toxicity. G2= Adriamycin, G3= Adriamycin plus vitamin E (100mg/kg/day); G4 = Adriamycin plus vitamin E (200mg/kg/day); G5 = Adriamycin plus vitamin E (300mg/kg/day); LDH: lactate dehydrogenase; CK= creatine kinase; CK-MB= Creatine kinase-MB. Mean values in each column followed by a different lower-case-letter (a, b, c, d) are significantly different by Tuky test at $p \leq 0.05$.

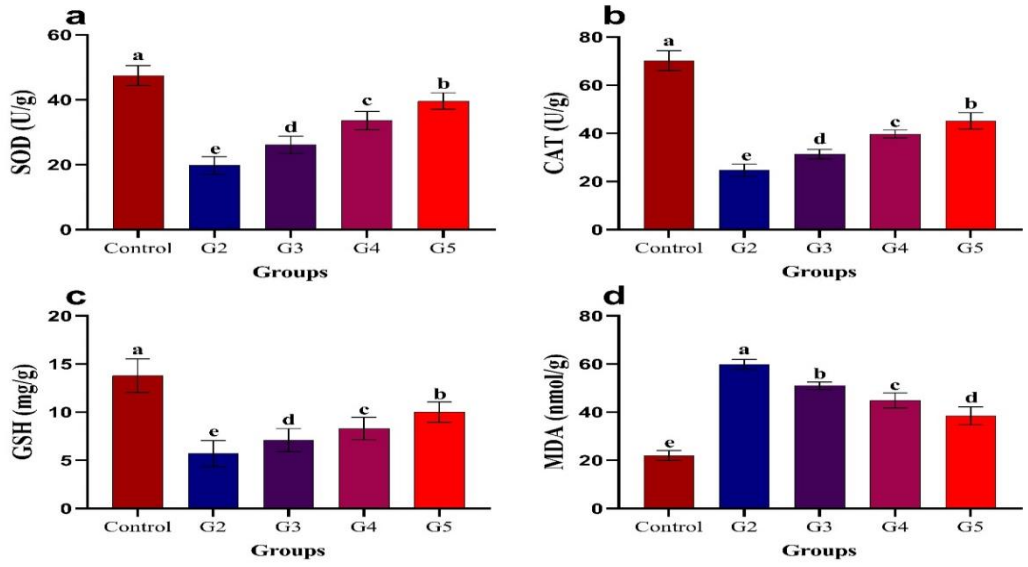


Figure 2. Effect of individual treatment with doxorubicin on the levels of indicators of Oxidative stress. G2= Adriamycin, G3= Adriamycin plus vitamin E (100mg/kg/day); G4 = Adriamycin plus vitamin E (200mg/kg/day); G5 = Adriamycin plus vitamin E (300mg/kg/day) Mean values in each column followed by a different lower-case-letter (a, b, c, d) are significantly different by Tuky test at $p \leq 0.05$.

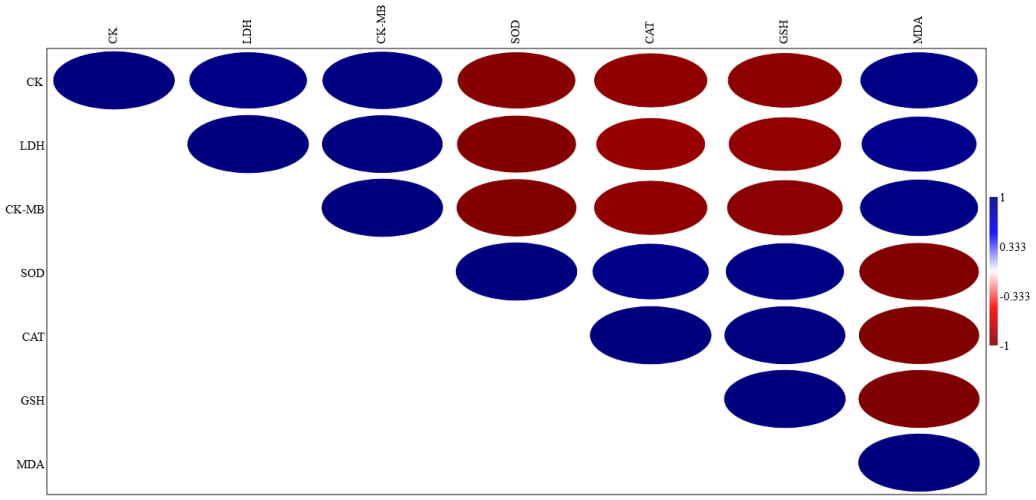
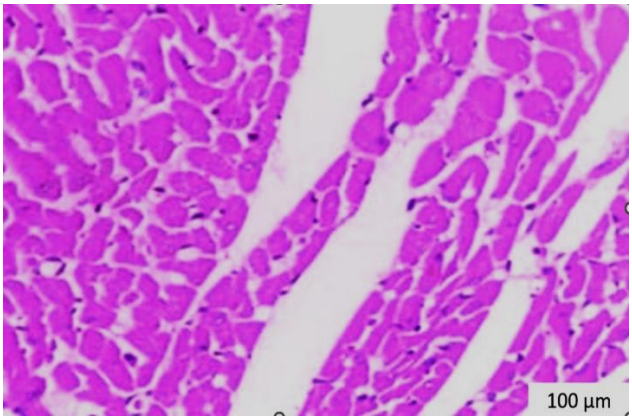
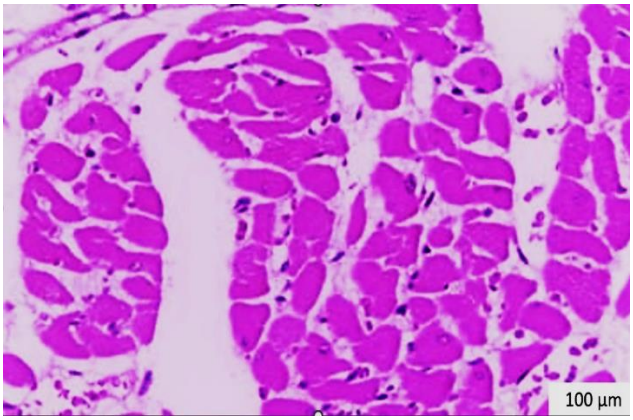


Figure 3. Pearsons correlation between biochemical markers of cardiac toxicity and indicators of oxidative stress



Figures 4. Photomicrograph of H & E stained section showed normal architechiture of cardiac muscle



Figures 5. Photomicrograph of H & E stained section showed normal architechiture of cardiac muscle

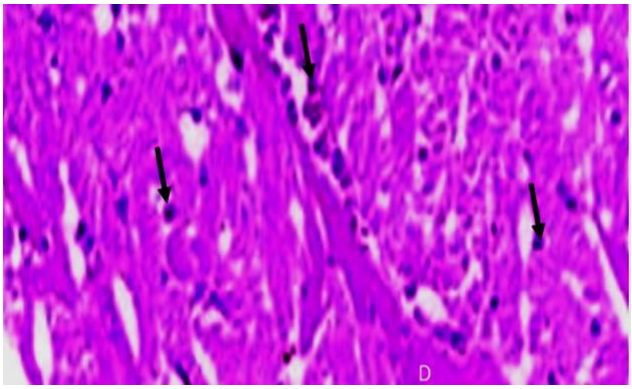


Figure 6. Photomicrograph of H & E stained section showed cellular degeneration (D) and marked cellular infiltrations (arrows).

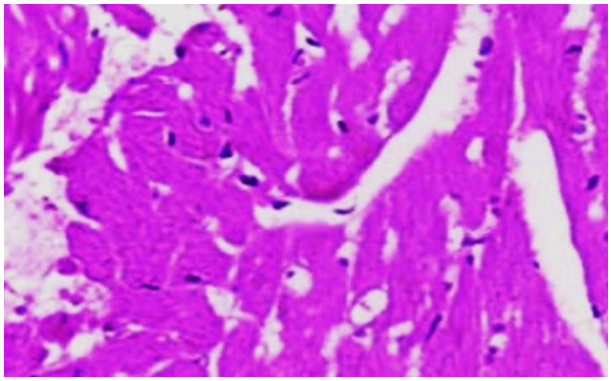


Figure 7. Photmicrogrph of group 5 (Adriamycin plus 300 mg of vitamin E, showed a marked reduction of cellular infiltration and absent cellular degeneration.

DISCUSSION

The results of the current work indicated that adriamycin was associated with a significant increase in biochemical indicators of cardiac muscle injury (e.g., LDH, CK, CK-MB). These harmful effects occur by different mechanisms.

Oxidative stress was proposed as one of these mechanisms. It was tested and confirmed in the current work by the powerful correlation between indicators of cardiac toxicity and oxidative stress markers. The concurrent use of vitamin E was

associated with significant amelioration of the cardiotoxic effects, and it is ascribed to its antioxidant actions in a dose-dependent manner (the higher dose was the best; 300 mg in the current work).

In accordance with the current work, According to Oda and Derbalah (23), the degradation or narcotization of the cell membranes of cardiac myocytes causes the activities of elevated serum enzymes indicative of heart damage to rise. Abou Elazab, et al. (24) achieved the same results. Additionally, according to Abdel-Samia, et al. (25), adriamycin negatively affected the myocardium's structure, caused severe myocardial damage, and caused several degenerative changes, including substantial vacuolization, myofibril loss, and myocytolysis. Furthermore, the adriamycin group serum levels of cardiotoxic indicators were significantly increased. They added these effects were alleviated by the concomitant treatment of Vitamin E (100 mg); however, it did not reach the normal level. Thus, in the current work, we tried to use higher doses of vitamin E, suggesting that higher doses will lead to better effects or even complete prevention of adriamycin cardiotoxic effects.

In accordance with the current findings, Shamlan (26) reported that intraperitoneal injection of DOX at a dose of 2.5 mg/kg, given six times in a row over a period of two weeks, significantly increased the MDA level and advanced oxidation protein products while concurrently lowering the total oxidant capacity and catalases in the heart muscle. This toxic effect is due to its intermediate metabolite, semiquinone (an intermediate free radical), with the formation of free oxygen radicals. At the same time, the heart's antioxidant capacity is limited compared to other organs like the liver, with the net result of cardiac injury (27, 28).

Another study found that Wistar rats' heart tissues had higher MDA levels and lowered GSH, CAT, and SOD activity levels after receiving 2 mg/kg of DOX intraperitoneally every 48 hours for 12 days (29).

As CK-MB and LDH are the main myocardial enzymes released in cases of myocardial injury and increased with the progress of injury (30), they are the best indicators of myocardial damage and toxicity. The usage of DOX in the control group significantly boosted the present study's findings. This increase was ameliorated by the use of vitamin E in different doses. The same outcomes were recorded by Eisvand, et al. (28), Azizi, et al. (30), Momin, et al. (31) also reported that the intraperitoneal injection of DOX in a dose of 3mg/kg for 6 injections in a period of 2 weeks was associated with a significant elevation of LDH serum levels in rats. This was attributed to DOX's reduction of oxygen and/or glucose supply to cardiac muscle, with subsequent damage of myocyte membrane and leakage of cellular enzymes in the bloodstream (31).

Histopathological evidence of cardiac muscle damage was obtained in the current study and the previous literature. The changes include loss of the normal architecture of the cardiomyocytes, cytoplasmic vaculations, cellular infiltrations, and increased deposition of collagen fibers (32).

Another mechanism to explain the cardiotoxic effects of DOX was the increased cellular programmed death (apoptosis). However, it is the native mechanism of its anticancer effects. Interestingly, its anticancer apoptotic effects are due to the increased production of reactive oxygen species. Unfortunately, due to the cardiac muscle's limited antioxidant capacity, DOX damaged the normal cardiac muscles (6).

Hadi, et al. (33) found a substantial rise in CK-MB activity after adriamycin administration in a clinical experiment. This shows that doxorubicin has caused harm or damage to cardiac muscle cells. The suppression of protein and nucleic acid production could bring this on. According to Sridharan and Shyamaladevi (34), the considerable rise in CK-MB was caused by excessive free radical and lipid peroxide formation, and enzyme leakage due to cell member damage CK-MB was greatly decreased after antioxidant therapy.

CONCLUSION

In adriamycin cardiotoxicity, oxidative stress damage could play a significant role in cardiotoxicity. Vitamin E, by its antioxidant action, in different doses, ameliorated the cardiotoxic effects in a dose-dependent manner. The best ameliorative effect was observed with the dose of 300 mg. Thus, concomitant administration of vitamin E could protect the heart from the cardiotoxic effects of adriamycin and thus extend its use as an anticancer agent. However, future studies must explore other molecular mechanisms involved in adriamycin cardiotoxic effects and search for other protective agents.

Author contributions: All authors have sufficiently contributed to the Study and agree with the results and conclusions.

Funding: No funding source is reported for this study.

Ethical statement: The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Al-Azhar University Damietta, Egypt, with reference No IRB 00012367-22-09-002/2022.

Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and Conclusions are available upon request from the corresponding author.

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