Review

Doxorubicin Cardiotoxicity in Acute Lymphoblastic Leukemia: Possible Protective Role of Grape Seed Extract Proanthocyanidins

Abstract

Aim: To evaluate early DOX cardiotoxicity in asymptomatic leukemic patients and to explore whether GSE proanthocyanidins would prevent the DOX-induced cardiotoxicity.
Study design: Comparative study.

Place and Duration of study: Mansoura University Hospital, between January 2011 and May 2013 forty two newly diagnosed ALL patients were enrolled in this study. Their ages ranged from 9 to14 years. They were divided into two groups; group I was treated with Doxorubicin while Group II was treated with Doxorubicin plus GSE all over the study period. All patients underwent clinical, echocardiographic and laboratory evaluations at the end of induction (phaseI) and at the end of CNS intensification (phaseII). Serum malondialdehyde (MDA) level, high sensitive cardiac troponin T (hscTnT), N-terminal pro brain natriuretic peptide (NT-proBNP) , creatine kinase(CK) and CK -MB isoenzyme activity were determined

Results: There was significant reduction in mean values of ejection fraction(EF),fractional shortening(FS) and Vitamin C while there was highly significant increase in mean values of hscTnT, NT- ProBNP and significant increase in mean value of CK and MDA at the end of phase II in both groups. There was also significant negative correlation between NT-proBNP and EF at the end of phase I &II. Coadminstration of DOX and GSE (group II) significantly improved echocardiographic findings (EF and FS) as well as vitamin C level. It also significantly reduced the DOX cardiotoxicity as revealed by the reduction of biochemical cardiac markers elevation (hscTnT , NT-pro BNP and CK activity) and oxidative injury marker (MDA).

Conclusion: The current study suggested that cardiotoxicity markers could be valuable beside echocardiographic evaluation in the early detection of DOX-induced subclinical cardiotoxicity and GSE has a potential application as a cardioprotective agent against DOX induced cardiotoxicity.

Keywords*: Doxorubicin, Cardiotoxicity markers, GSE.*

1. Introduction

Cardiotoxicity is a well-known and potentially serious complication of anticancer therapy that can significantly impair patient's quality of life and also substantially increase health care costs. Anthracyclines represent the greatest risk for development of

cardiotoxicity[1,2] especially in children[3]. The first anthracycline (ANT) antibiotics, Daunorubicin and doxorubicin have been isolated early in the 1960s and are still widely used for cancer chemotherapy [4].

The acute form of doxorubicin-induced cardiotoxicity can manifest as early as a few minutes after doxorubicin treatment in the form of acute hypotension, transient rhythm disturbances[5] or depression of left ventricular function. Meanwhile chronic ANT cardiotoxicity is characterized by irreversible progressive left ventricular dysfunction and congestive heart failure (HF). It can be divided into 2 distinct types. First, early onset chronic progressive cardiotoxicity usually presents within a year of treatment with a peak incidence 1-3 months after chemotherapy[6] ; Second, late-onset progressive cardiotoxicity that occurs years or even decades after chemotherapy has been completed, usually in survivors of childhood cancers[6,7].

Echocardiograms are the most frequently used modality in the screening for cardiac disease during or after chemotherapy. They provide means to evaluate subclinical cardiotoxicity which was defined as abnormal left ventricular systolic function, including the shortening fraction (FS), ejection fraction (EF), the velocity of fiber shortening corrected for heart rate or the stress velocity index[8]. However, early screening is limited by the subtle nature of the initial injury and the insensitivity of the available methods[5,9].

Biochemical markers of cardiac injury, especially cardiac troponins and natriuretic peptides, have been investigated in the assessment of cancer therapy-induced cardiotoxicity[10,11]. Cardiac troponins — cardiac troponin T (cTnT), cardiac troponin I (cTnI) — and myocardial isoenzyme of creatine kinase (CK-MB) are cardiospecific markers that show structural injury of cardiomyocytes from various causes, including cardiotoxic effect of anticancer therapy [12].

Many studies reported that N-terminal fragment of the brain natriuretic peptide (NT-proBNP) concentrations increased with the severity of ventricular dysfunction and heart failure[10,13]. It was also considered as a promising marker for both exclusion and detection of early ventricular dysfunction after potentially cardiotoxic anticancer therapy[13,14].

Grape seed extract (GSE) is a natural extract from the seeds of Vitis vinifera. It contains the most beneficial groups of plant flavonoids, proanthocyanidins oligomers [15].The catechol structure of proanthocyanidins enables them to scavenge free oxygen radicals [16]. In addition, it has been demonstrated to modulate the activity of antioxidant enzymes system to limit free radical production[17]. Also due to wide range of biological activity including anti-inflammatory and anticancer, GSE is considered a popular dietary supplement [18]. This study aims to evaluate early onset DOX cardiotoxicity by both echocardiographic and biomarkers of myocardial injury in asymptomatic ALL children treated with DOX chemotherapy and to investigate the possible cardio protective effects of GSE.

2. Materials and Methods

2.1 Study subjects

Between January 2011 and May 2013, forty two newly diagnosed ALL children were recruited for this study. Their age ranged from 9 to 14 years (mean 10.75 ±1.45 yrs).The following exclusion criteria were applied: known cardiovascular risk factors history of coronary artery disease, haemodynamically significant valvular heart disease, left ventricular ejection fraction <55%, clinical evidence of kidney or liver diseases and children with BMI ≥ 95 percentile for age & sex. They were divided into two groups; group I was DOX treated while Group II was DOX plus proanthocyanidin GSE extract treated (150mg/day)[19] all over the study period. They were subjected to thorough history, clinical, radiological and laboratory assessment.

A written informed consent was obtained from the parents or guardians of participants**.** The study was approved by the research ethics committee of Mansoura University.

2.2 Drugs

Doxorubicin hydrochloride was provided in the form of Adriamycin vials (25mg/m^2) according to BFM protocol . Grape seed proanthocyanidin extract was administered in the form of Gervital capsules (GSE; 150mg). It was provided by Arab Company for Pharm. and Medicinal plants (Mepaco, Egypt) and stored at 4°C until used.

2.3 Sampling

Fasting venous blood samples were obtained from patients, the day after the end of four doses of DOX chemotherapy (at the end of induction -phase I) then after 3 months (at the end of CNS intensification -phase II). Blood was collected into plain vacutainer tubes; sera were separated and frozen at -70°C until time of analysis.

2.4 Biochemical markers of cardiotoxicity

Serum concentrations of hscTnT (high sensitive cardiac troponin T) and NT-proBNP were determined by electro-chemiluminescence immunoassay, sandwich technique using 4^{th} generation Troponin T high sensitive STAT and proBNP II kits, Elecsys 2010; Roche Diagnostics. The lower limits of detection were 0.01 ng/ml and 5pg/ml respectively [20, 21]. The 99th percentile value for a normal reference population was 13.5 ng/L measured with a CV <10% [20] and the 95^{th} percentile for normal NT-proBNP levels in children from 6 to 14 yrs was 157 pg/ml [22] respectively. Serum CK and CK-MB isoenzyme activity were determined using CK NAK liquiUV kit and immunoinhibition by monoclonal antibody to CK-M subunit respectively, Human, Germany[23].

2.5 Assessment of oxidative status

Serum ascorbic acid (vitamin C) was measured by colorimetric method according to Jacob (1990)[24]. It was oxidized by copper to form dehydroascorbic acid, which reacts with acidic 2, 4 dinitrophenyle hydrazine to form a red bishydrazone, which is measured at 520 nm. Serum MDA level was estimated according to Asakawa and Matsushita (1980)[25] by the thiobarbituric acid (TBA) method. This method is based on the principle that TBA reacting with lipid peroxide, hydroperoxide, and oxygen-labile double bond to form the color adducts with maximal absorbance at 530 nm. The samples were heated with TBA under acidic conditions and the pink color formed was read at 530nm.

2.6 Echocardiography

Each patient underwent a detailed standard echo Doppler cardiographic study. Echocardiography was performed approximately one month and 3 months after the completion of chemotherapy. Assessment was done by an experienced cardiologist who was unaware of the participants' condition. Echocardiograms were obtained using a SONOS 5500 (Hewlett Packard, Andover, Mass, U.S.A.), and images were obtained using 8MHz phased array transducer[26].Two dimensional M-mode measurements, obtained from a parasternal short-axis view according to the guidelines for M-mode echocardiography of the American Society of Echocardiography[27].

LV dysfunction was defined as EF < 55% and FS ≤ 29% [28, 29]. Quantification of echocardiographic parameters was based on the recommendations of the American Society of Echocardiography´s Guidelines and Standards Committee and the Chamber Quantification Writing Group [30].

2.7 Statistical analyses

Data were analyzed with SPSS version 16 for Windows. The normality data was first tested with one-sample Kolmogorov-Smirnov test. Continuous variables are presented as mean ±

SD (standard deviation) and the cardiac biomarkers hscTnT & NTproBNP as median and range . Comparisons between continuous or categorical variables were performed using the Student t-test, Mann–Whitney. *P* value < 0.05 was considered as significant while *P* value≤0.001 as highly significant. Pearson´s correlation coefficient and Spearman correlation coefficient were used to measure strength of linear correlation between two parametric and non parametric variables respectively. Percent of change was calculated as mean or median of the parameter at the end of phase I ─ mean or median of the parameter at the end of phase II / mean or median of the parameter at the end of phase I × 100

3 Results

3.1 Study population

Forty two patients; thirty males (71.4%) and twelve females (28.6%) were participated in this study. Their age ranged from 9 to 14 yrs (mean; 10.75 ±1.45 yrs). The mean age of group I is 11.10± 38 while for group II is 10.46± 42 yrs.

3.2 Echocardiographic outcomes: There is significant reduction in mean values of EF and FS in both groups at phase II, with FS remained above the lower limit of normal (Table 1). The percent of decline is greater in group I than group II (29.4 & 14.8 Vs 17.6 & 8 % respectively) (Figure 1).

3.3 Biochemical markers of cardiotoxicity outcomes: There is highly significant increase in median values of hscTnT, NT- ProBNP and significant increase in mean value of CK at the end of phase II in both groups, noting that median values of hscTnT& NT-proBNP are exceeding the upper limit of normal populations at the end of phase II (Table 2) and percent of increase between the two phases of these markers is greater in group I than group II(133.3, 129.7, 59 Vs 25, 17.7, 11.4 % respectively) (Table 2& Figure 2). There are significant negative correlations between cardiotoxicity markers (CK, hscTnT & NT- ProBNP) and EF at phase I. Meanwhile, significant negative correlations are observed between each of CK, CK-MB& NT- ProBNP and EF, while highly significant negative correlation are observed between CK-MB and FS at the end of phase II (Table 3).

3.4 Oxidative status outcomes: There are no significant difference between both groups in each of mean values of vitamin C and MDA at the end of phase I. However there is significant higher mean value of serum vitamin C in group II Vs group I at the end of phase II and the percent of decrease between the two phases is greater in group I than group II (11.4 Vs 0.86 %) (Table 4 and Figure 3). Meanwhile there is significant lower mean value of MDA in group II Vs group I at the end of phase II, and the percent of increase (38.5 Vs 12.9 %) is greater in group I than group II (Figure 3).

Table (1): Echocardiographic parameters among studied groups.

Echo parameters after chemotherapy	Group I n:20	Group II n:22	*P* value
	Mean ± SD		
EF(%) at end of phase I	59.5± 4.6	56.6±4.1	0.04*
EF(%) at end of phase II	42 ± 5	46.6±5.3	0.006 *
FS(%) at end of phase I	38.4 ±3.7	33.6 ±2.4	≤0.001**
FS(%) at end of phase II	32.7 ±4	30.9±1	0.05*

EF: ejection fraction**.** **FS:** shortening fraction.

Table (2): Biochemical markers of cardiotoxicity among studied groups.

Cardiac markers	Group I n:20	Group II n:22	*P* value
	Mean± SD		
CK(U/L) at end of phase I	122.6 ± 55.2	134.09± 37	0.40
CK(U/L) at end of phase II	195 ±32.4	149.3 ±70.8	0.01*
CK-MB (U/L) at end of phase I	17.2 ± 6.2	17.2± 8	0.99
CK-MB (U/L) at end of phase II	26.6 ±8.3	22.17±8.46	0.09
	Group I n:20	**Group II n:22**	***P* value**
	Median(range)		
hscTnT(pg/ml) at end of phase I	13.5(4-20)	16(3-45)	0.30
hscTnT(pg/ml) at end of phase II	31.5(22-120)	20(5-60)	≤0.001**
NT-proBNP(pg/ml) at end of phase I	111(20-270)	99(19.4-310)	0.60
NT- proBNP(pg/ml) at end of phase II	255(130-773)	116(42-779)	≤0.001**

Table (3): Correlations between echocardiographic parameters and cardiotoxicity biochemical markers.

Cardiotoxicity biochemical markers		EF	FS
(n:42)		**r▪(*P* value)**	
CK	Phase I	-0.4**(.003)	0.189(0.2)
	Phase II	-0.3*(0.02)	0.18*(0.2)
CK-MB	Phase I	-0.29(0.06)	-0.127(0.4)
	Phase II	-0.3*(0.02)	-0.5** (.001)
hscTnT	Phase I	-0.349*(0.02)	0.03(0.8)
	Phase II	0.07(0.6)	0.08(0.6)
NT-Pro BNP	Phase I	-0.4* (0.002)	-0.2(0.1)
	Phase II	-0.3*(0.04)	-0.01(0.9)

▪ Correlation coefficient

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed)

Table (4): Levels of serum Vitamin C and MDA levels in the studied groups.

Oxidative status	Group1 No=20 Mean±SD	Group2 No=22 Mean±SD	*P* value
Vitamin C(mg/dl) at end of phase I	1.14±0.2	1.15 ±0.17	0 .80
Vitamin C(mg/dl) at end of phase II	1.01 ± 0.17	1.14 ± 0.23	0.05*
MDA(mg/dl)at end of phase I	20.5 ±3.7	20.8 ±.5.7	0.80
MDA(mg/dl) at end of phase II	28.4 ±3.2	23.5 ±.7.25	0 .01*

Figure (1): Percent of decline between phase I and phase II in both EF and FS among the studied groups.

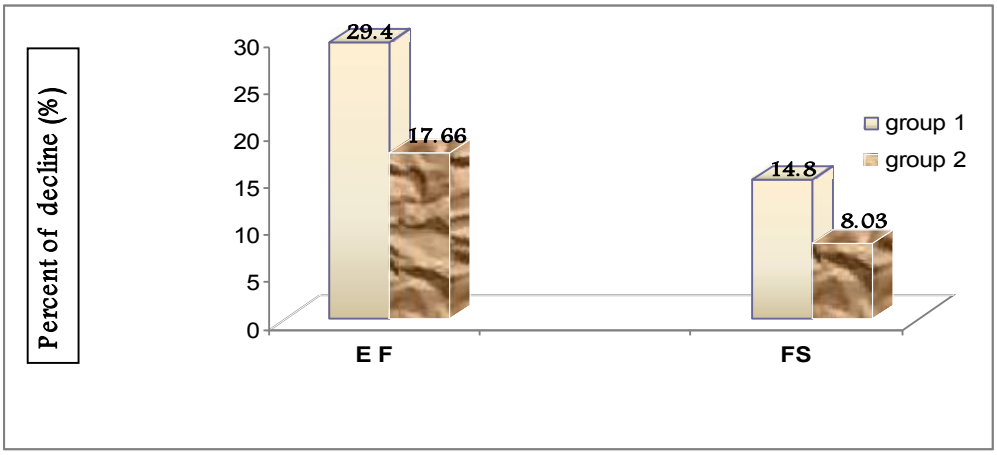


Figure (2): Percent of increase (%) between phase I and phase II in biochemical markers of cardiotoxicity among the studied groups.

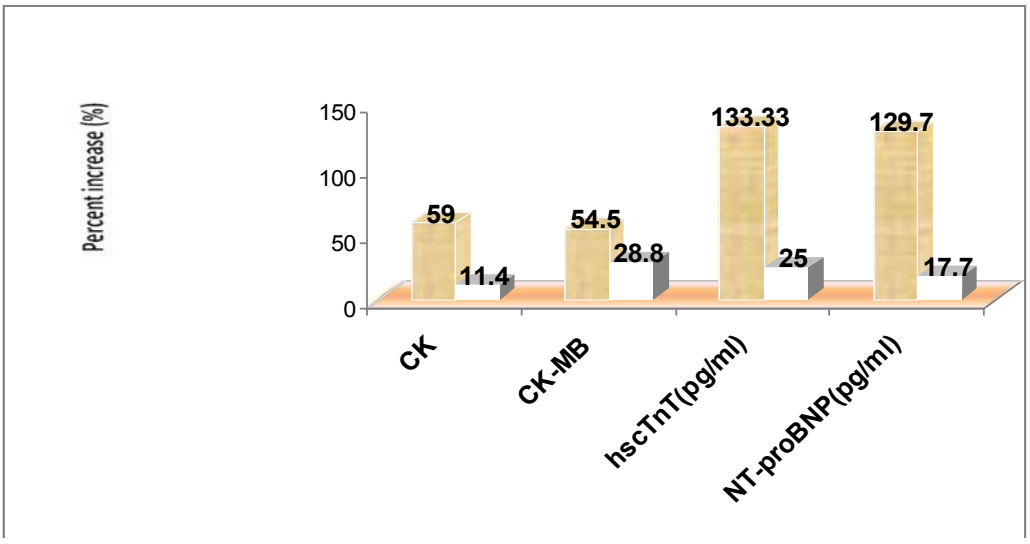
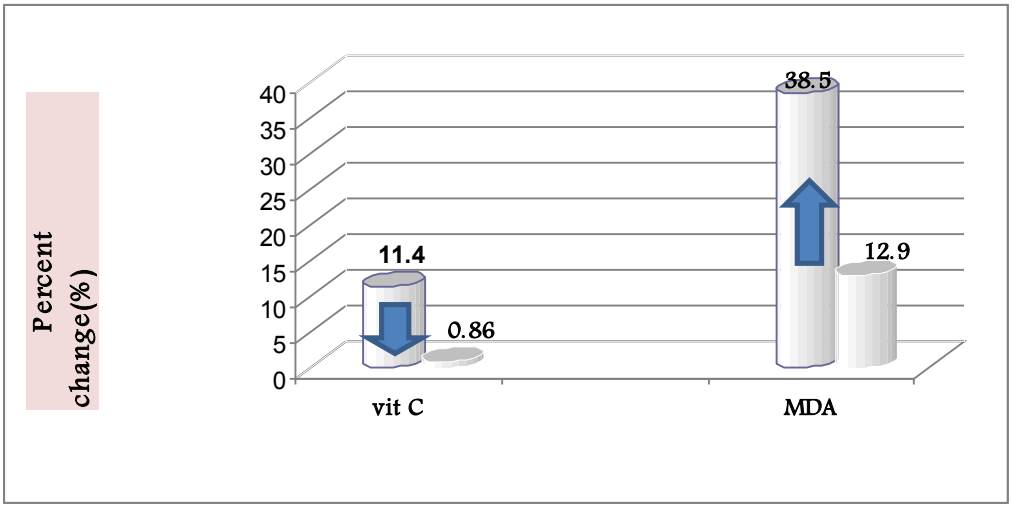


Figure (3): Percent of change (%) between phase I and phase II in Vitamin C and MDA among the studied groups.



4. Discussion

It was postulated previously that LVEF might not be an accurate predictor of congestive heart failure [31], and not very sensitive for early diagnosis of preclinical heart disease[5]. However the ECHO findings of the current study showed significant reduction in both EF and FS at the end of phase II which is more evident in group I, with percent of decline of EF in group I (29 %) exceeding that suggested previously (20%) for defining cardiac event [31]. Also the percent of decline of FS in group I (14.8%) was exceeding the published guidelines for monitoring of ANT treatment in pediatric population [32].

Swain et al., [31] assumed evaluation of serum cardiac troponin T levels as a promising test for ANT-induced cardiotoxicity in pediatric patients and suggested that, the magnitude of elevation of serum cTnT levels as a predictor of left ventricular dilatation. The current study revealed increased mean and median serum levels of CK, hscTnT and NT-ProBNP in both groups at end of phase II but were significantly higher in group I. This coincides with previous findings of Urbanova et al.[33] who decided that these biomarkers are considered more sensitive markers of subclinical cardiotoxicity than conventional electrocardiographic and echocardiographic methods; Roziakova et al.,[13] who concluded that higher levels of NTproBNP detected in childhood leukemia survivors after low ANT cumulative doses might reflect an initial stage of ANT cardiotoxicity before the development of echocardiographic abnormalities; and others [13,14,34].

Not only elevation in cardiac biomarkers but persistent elevations that may reflect the presence of an underlying reduced functional myocardial reserve or reduced cardiac tolerance to cardiac stressors Roziakova et al.,[13]. Therefore there was previous attempt to precisely define a rising pattern of hscTnT as high as 84% from the baseline indicative of acute cardiac injury to be distinguished from minor to modest changes in serial results indicative of biological and analytical variation, and for the long term, a >3-fold increase in troponin will be required [20] . The current study revealed percent of increase of 133.3 for hscTnT between the two phases in group I Vs 25 % in group II.

Our results showed significant negative correlation between cardiotoxicity biomarkers and echocardiographic findings in both phases specially CK, hscTnT and NT-proBNP in phase I and CK, CK-MB and NT-proBNP in phase II. Which was in contrary to results of Mavinkurve-Groothuis et al.[35] who did not find any significant relation between elevated biomarkers NT-proBNP, and cTnT and echocardiographic parameters in children with ANT-induced cardiotoxicity . Cil et al. [36] also concluded that the association between higher NT-proBNP levels and reduced left ventricular EF in asymptomatic breast cancer patients after DOX administration could be an early indication of subclinical acute ANT cardiotoxicity, also Sherief et al. [37] deduced that NT-pro-BNP could be used as a sensitive cardiac biomarker in monitoring of ANT-induced cardiotoxicity; and these support our finding of the significant negative correlation between NT-proBNP and EF in both phases.

Noteworthy, co-administration of GSE with DOX in group II attenuated the decreased ECHO parameters (EF and FS) , and significantly reduced the increased mean values of biochemical markers of cardiotoxicity especially CK, hscTnT and NT-proBNP than in group I who took DOX only. This confirms the beneficial role of GSE in protection against Dox-induced cardiotoxicity elicited previously in animal models [16, 38]. Other previous experimental studies suggested that grape seed proanthocyanidins have a potent protective effect on myocardial ischaemia-reperfusion injury in cardiomyocytes by scavenging the reactive oxygen species generated during ischaemia/reperfusion [39], also, attenuated isoproterenol-induced myocardial damage in rats by resisting free radical attacks and preventing oxidative reactions [40].A human trial also demonstrated that grape seed extract supplementation improved plasma antioxidant capacity in high cholesterol subjects [41].

Regarding the oxidative status of the patients, Vitamin C has decreased in both groups at the end of phase II but it was lower in group I. While MDA level has increased in both groups at the end of phase II but was higher in group I. These results confirmed that DOX treatment increased the MDA level (a marker of lipid peroxidation and an indicator of oxidative injury) [16,42] , which agree with the opinion that free oxygen radicals damage play an important role in DOX-induced cardiotoxicity, secondary to the relatively low expression of antioxidant enzymes, such as catalase and superoxide dismutase in the heart [3,43] .

The great antioxidant capability of proanthocyanidins could be attributed to its specific catechol structure, that enables them to combine with free oxygen radicals and to chelate metals, such as copper and iron, involved in reactive oxygen species generation [16] via stimulation of various forms of cytochrome P450 [44]. In addition to antioxidant properties, proanthocyanidin has been demonstrated to modulate the activity of antioxidant enzymes such as cyclooxygenase and lipoxygenase to limit free radical production [45]. Also, proanthocyanidins have been hypothesized to improve the celluar redox status by modulating the glutathione synthesis pathway against oxidative stress [17]. Moreover, Catechins, the monomeric units of proanthocyanidins, have been reported to possess protective effects to vascular endothelial cells through the inhibition of endothelial NADPH oxidase activity [16].

The absence of any difference in mean values of vitamin C and MDA between the two groups at the end of phase I while significant difference were observed at the end of phase II could point to the optimum required duration of proanthocyanidins to act as an efficient phytochemical antioxidant , it was observed previously that 8 weeks of dietary treatment with a grape seed extract, a proanthocyanidin-rich complex, significantly improved the ferric-reducing antioxidant power in plasma as compared with the control group [46] .To the best of our knowledge the current study is the first study that evaluate the cardioprotective role of proanthocyanidine in ALL children with early onset DOX- induced cardiotoxicity, however further evaluations on a large scale were recommended.

In conclusion, our study showed that the CK, hscTnT and NT-ProBNP would be useful sensitive, inexpensive and readily available biomarkers added to echocardiographic findings for identification of early onset DOX-induced cardiotoxicity and this could be of particular benefit to ensure close monitoring of patients and to start early preventive or therapeutic management of LV dysfunction. Moreover, GSE elicits a typical protective effect on DOX-induced cardiotoxicity as evidenced by decreased MDA levels and improving the oxidative status of the patients as revealed by increased vitamin C level. Decrement of cardiotoxicity biochemical markers also confirmed the cardioprotective role of GSE.

Competing Interests

We declared that we have no competing interests

Authors' Contributions

This work was carried out in collaboration between all authors. SAE designed the study & wrote the protocol with RE & drafted the manuscript. RE diagnosed & managed the enrolled patients. AM & RME were responsible for laboratory analysis. REM wrote &revised the final manuscript. Authors have analyzed the data, managed the literature searches, read & approved the final manuscript.

Consent

A written informed consent was obtained from the parents or guardians of participants.

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