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

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Authors' contributions

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

Original Research Article

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ABSTRACT

Aim: To evaluate early doxorubicin (DOX) cardiotoxicity in asymptomatic leukemic patients and to explore whether Grape seed extract (GSE) proanthocyanidins would prevent the DOX-induced cardiotoxicity.
Study Design: Prospective randomized double blind study.
Place and Duration of Study: This study was conducted in Mansoura University Hospital, between January 2011 and May 2013. Forty two newly diagnosed acute lymphoblastic leukemia (ALL) patients were enrolled, their ages ranged from 9 to14 years. They were divided into two groups; group I received Doxorubicin-containing chemotherapy while Group II was treated with Doxorubicin-containing chemotherapy plus GSE all over the study period. All patients underwent clinical, echocardiographic and laboratory evaluations at the end of induction (phase I) and at the end of CNS intensification (phase II). Serum malondialdehyde (MDA) level, high sensitive cardiac

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troponin T (hscTnT), N-terminal pro brain natriuretic peptide (NT-proBNP), creatine kinase (CK) and CK -MB isoenzyme activity were determined

Results: There were significant reduction in mean values of ejection fraction (EF), fractional shortening (FS) and Vitamin C, while there were highly significant increase in mean values of hscTnT, NT- ProBNP and significant increase in mean values of CK and MDA at the end of phase II in both groups. There were also significant negative correlations between each of CK and 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 decrement in the elevated values of biochemical cardiac markers (hscTnT, NT-pro BNP and CK activity) and oxidative injury marker (MDA).

Conclusion: Biochemical cardiac markers have the potential to be used, besides echocardiographic measurements, in the early detection of DOX-induced subclinical cardiotoxicity. GSE is promising as a cardioprotective agent against DOX induced cardiotoxicity in children with ALL.

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 (DOX) have been isolated early in the 1960s and are still widely used for cancer chemotherapy [4].

The acute form of DOX-induced cardiotoxicity can manifest as early as a few minutes after DOX 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 present in two distinct subtypes, the first is the early onset progressive subtype, occurring within a year of treatment with a peak incidence 1-3 months after chemotherapy [6]. The second is the late-onset progressive cardiotoxicity, occurring years or even decades after chemotherapy has been completed, usually in survivors of childhood cancers [6,7].

Doxorubicin-induced cardiotoxicity is suggested to be through lipid peroxidation and the generation of free radicals by anthracycline-iron complexes, which induce apoptosis and cardiac myocytes damage as the heart is particularly poorly protected against oxidative stress [8,9]. The resulting cardiomyocyte apoptosis induced by reactive oxygen species, is distinct from apoptosis induced by DOX in tumor cells [10].

Several maneuvers with various advantages and disadvantages were used to detect the cardiac effect of DOX. Echocardiograms are the most frequently used modality in the screening for cardiac disease during or after chemotherapy. They provide means to evaluate subclinical cardiotoxicity, defined as abnormal left ventricular systolic function [11]. The adopted diagnostic approach depended mainly on the estimation of left ventricle ejection fraction (LVEF) or left ventricle fractional shortening (LVFS) using conventional echocardiography. Although this approach showed low sensitivity toward early prediction

[5,9], it is still used for monitoring left ventricular function in both clinical practice and clinical trials [12].

Biochemical markers of cardiac injury, especially cardiac troponins and natriuretic peptides, have been investigated in the assessment of cardiotoxicity induced by anti-cancer therapy [8,13]. 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 [14].

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 [8,15]. It was also considered as a promising marker for both exclusion and detection of early ventricular dysfunction after potentially cardiotoxic anticancer therapy [15,16].

Several approaches -mostly undertaken in adults- have been studied in order to reduce cardiotoxicity induced by anticancer therapy. These attempts have focused on three main approaches. The first is by decreasing myocardial concentrations of ANTs and their metabolites by dose limitation and schedule modification. The second is by developing less cardiotoxic ANT analogues and formulations and the third is by the administration of cardioprotective agents during and after chemotherapy to attenuate the effects of ANTs on the heart without altering the anti-tumor activity of the drug [17,12]. Currently, the most effective cardioprotectant for use in children is dexrazoxane. Although the results of the dexrazoxane studies in children are promising, evidence to make a recommendation for its' use to prevent ANT cardiotoxicity in children is limited [18].

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 [19]. The catechol structure of proanthocyanidins enables them to scavenge free oxygen radicals [20]. In addition, it has been demonstrated to modulate the activity of antioxidant enzymes system to limit free radical production [21]. Based on wide range of biological activities including anti-inflammatory and anticancer effects, GSE is considered a popular dietary supplement [22]. This study was aimed to evaluate early onset DOX cardiotoxicity by both echocardiographic and biomarkers of myocardial injury in asymptomatic ALL children treated with DOX-containing chemotherapy and to investigate the possible cardio-protective effects of GSE.

2. MATERIALS AND METHODS

2.1 Study Design

Prospective double blinded randomized clinical trial was carried out. The evaluator assessing outcome (cardiologist & pediatrician) and study subjects were blinded about the randomization of treatment assignments. Randomly assigned treatment to consecutively numbered patients through opaque envelops. An expert in research methods was recruited from Public Health department in Mansoura faculty of medicine to implement the randomization process.

2.2 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±.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 and sex. They were divided into two groups. Group I received chemotherapy containing cardiotoxic DOX and Group II received chemotherapy containing cardiotoxic DOX plus GSE proanthocyanidine (150mg/day) [23] 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.

2.3 Drugs

Doxorubicin hydrochloride was provided as Adriamycin vials (25mg/m^2 per dose for 4 doses weekly), in combination with other chemotheraputics according to modified BFM protocol (prednisone, vincristine, L- asparaginase, cyclophosphamide, 6- mercaptopurine, arabinosyl cytosine and age dependent dose of methotrxate). 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.

2.4 Sampling

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

2.5 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 [24,25]. The 99th percentile value of hscTnT for a normal reference population was 13.5ng/L, with a CV <10% [24] and the 95^{th} percentile for normal NT-proBNP levels in children from 6 to 14 yrs was 157pg/ml [26]. Serum CK and CK-MB isoenzyme activities were determined using CK NAK liquiUV kit and immunoinhibition by monoclonal antibody to CK-M subunit, Human, Germany [27].

2.6 Assessment of Oxidative Status

Serum ascorbic acid (vitamin C) was measured by colorimetric method [28]. It was oxidized by copper to form dehydroascorbic acid, reacting with acidic 2, 4 dinitrophenyle hydrazine to form a red bishydrazone which is measured at 520 nm. Serum MDA level was estimated by the thiobarbituric acid (TBA) method [29]. This method is based on reaction of TBA with lipid

peroxide, hydroperoxide and oxygen-labile double bond to form the color adducts with maximal absorbance at 530 nm.

2.7 Echocardiography

Each patient underwent a detailed standard echocardiographic Doppler examination. It 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. M-mode measurements, obtained from a parasternal short-axis view according to the guidelines for M-mode echocardiography of the American Society of Echocardiography [30].

LV dysfunction was defined as EF < 55% and FS ≤ 29% [31,32]. 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 [33].

2.8 Statistical Analyses

Data were analyzed using SPSS version 16 for Windows. The normality data were first tested with one-sample Kolmogorov-Smirnov test. Continuous variables are presented as mean ± SD (standard deviation) and the cardiac biomarkers hscTnT and NTproBNP as median and range. Comparisons between continuous variables were performed using the Student t-test (parametric) and Mann–Whitney (non parametric). *P* value < 0.05 was considered statistically 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%) participated in this study. Their ages 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 remaining above the lower limit of normal (Table 1). Although percent decline between the two phases is greater in group I than group II (29.4 & 14.8 Vs 17.6 and 8% respectively), their *P* values do not reach statistical significance (*P*=0.5, 0.8) (Fig. 1).

Table 1. Echocardiographic parameters among the 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*

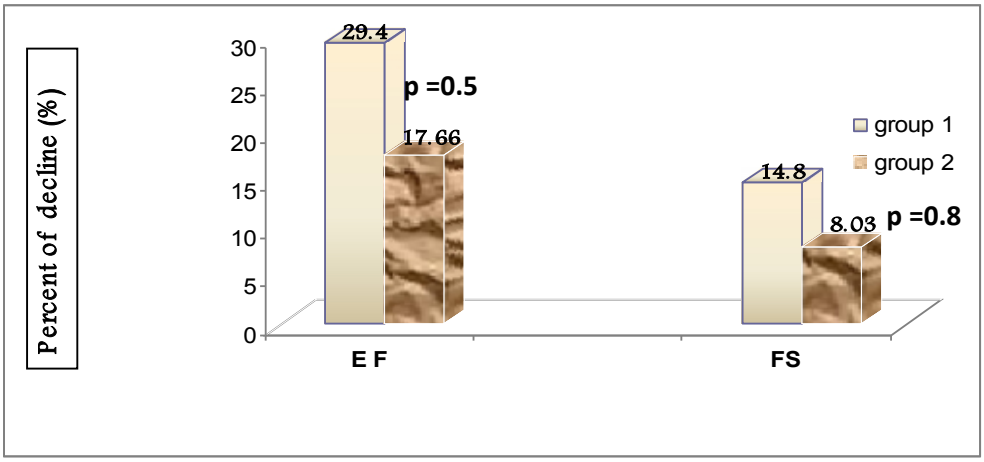

Fig. 1. Percent decline between phase I and phase II of EF and FS in the studied groups

3.3 Biochemical Markers of Cardiotoxicity Outcomes

There is a 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 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 and Fig. 2). There are significant negative correlations between cardiotoxicity markers (CK, hscTnT and NT- ProBNP) and EF at phase I. Meanwhile, there are significant negative correlations between each of CK, CK-MB and NT- ProBNP and EF, also a highly significant negative correlation is 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 mean values of vitamin C and MDA at the end of phase I. However there are significantly higher mean value of serum vitamin C and significantly lower mean value of MDA in group II Vs group I at the end of phase II. Although percent change between the two phases of vitamin C (decrease) and MDA (increase) is greater in group I than group II (11.4 Vs 0.86 % & 38.5 Vs 12.9 % respectively) their *P* values do not reach statistical significance (*P*=0.4, 0.1) (Table 4 and Fig. 3).

Table 2. Biochemical markers of cardiotoxicity among the 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
	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 biochemical markers of cardiotoxicity

Biochemical markers of cardiotoxicity (N:42)		EF	FS
		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)

*** Significant at the 0.01 level* * *Significant at the 0.05 level*

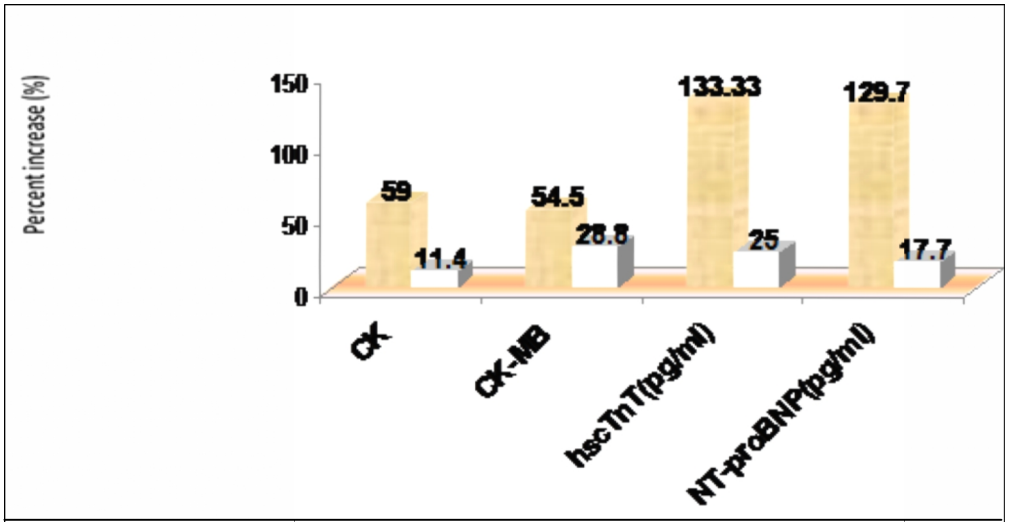

Fig. 2. Percent increase between phase I and phase II of biochemical markers of cardiotoxicity in the studied groups

Table 4. Levels of serum Vitamin C and MDA in the studied groups

Oxidative status	Group1 N=20 Mean±SD	Group 2 N=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*

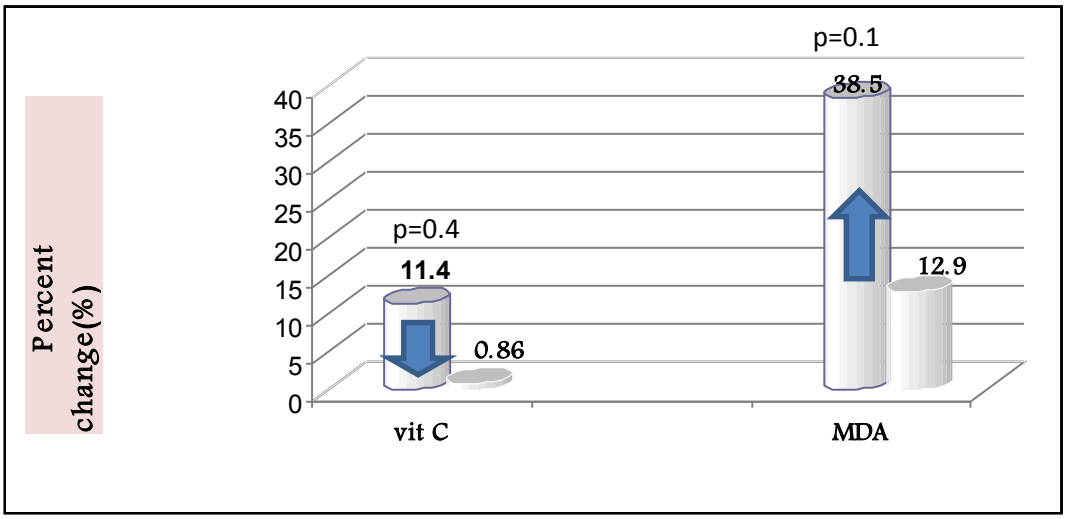

Fig. 3. Percent change between phase I and phase II of Vitamin C and MDA in the studied groups

4. DISCUSSION

It was postulated previously that LVEF might not be an accurate predictor of congestive heart failure [34] and is 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 a percent decline of EF in group I of 29 %, which exceeded what was suggested previously (20%) for defining cardiac event [34]. Also the percent decline of FS in group I (14.8%) was exceeding the published guidelines (≥ 10%) for monitoring of ANT treatment in pediatric population [35].

Swain et al. [34] assumed evaluation of serum cardiac troponin T levels as a promising test for ANT-induced cardiotoxicity in pediatric `patients and suggested the magnitude of elevation of serum cTnT levels as a predictor of left ventricular dilatation. The current study revealed an increase in mean and median serum levels of CK, hscTnT and NT-ProBNP in both groups at the end of phase II but were significantly higher in group I. This coincides with previous findings of Urbanova et al. [36] who considered these biomarkers as more sensitive markers of subclinical cardiotoxicity than conventional electrocardiographic and echocardiographic methods. Roziakova et al. [15] reported 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.

Persistent elevation of cardiac biomarkers are essential, because they reflect the presence of an underlying reduced functional myocardial reserve or reduced cardiac tolerance to cardiac stressors [15]. Therefore there was previous attempt to precisely define a rising pattern of hscTnT as high as 84% from the baseline to be an indicator of acute cardiac injury, while for the long term change (0-8 weeks), a 3-fold increase in troponin will be required [24]. The current study revealed a percent 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. This was contradictory to the results of Mavinkurve-Groothuis et al. [37] 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. [38] 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 indicator of subclinical acute ANT cardiotoxicity. This lends support to the current data showing significant negative correlation between NT-proBNP and EF in both phases.

Noteworthy, co-administration of GSE with DOX in group II attenuated the decrement of ECHO parameters (EF and FS) and reduced the increment of mean values of biochemical markers of cardiotoxicity especially CK, hscTnT and NT-proBNP more than group I who administered DOX only. This confirms the beneficial role of GSE in protection against Dox-induced cardiotoxicity elicited previously in animal models [20,39]. Previous in vitro 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 [40]. It also, attenuated isoproterenol-induced myocardial damage in rats. This action was achieved through resisting free radical attacks and preventing oxidative reactions [41]. Antioxidant activities of grape phenolic compounds have been also investigated in vivo and demonstrated that GSE supplementation improved plasma antioxidant capacity in high cholesterol subjects [42]. It reduced oxidative stress and improved reduced glutathione/oxidized glutathione in obese type 2 diabetic subjects [43]. On the other hand, another study showed that chronic dietary supplementation of grape juice exhibits a neutral antioxidative effect in humans [44]. This inconsistency may be related to the low absorption of grape phenolics since the absorption rate of polyphenol antioxidants is generally less than 1% [45].

Regarding the oxidative status of the patients, Vitamin C was decreased and MDA was increased in both groups at the end of phase II but group I showed the lowest vitamin C and the highest MDA values. These results confirmed that DOX treatment increased the MDA level (a marker of lipid peroxidation and an indicator of oxidative injury) [20]. This confirms the opinion that free oxygen radicals damage plays 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 [3,8,9,46].

The marked antioxidant capability of proanthocyanidins could be attributed to the specific catechol structure that enables them to combine with free oxygen radicals and chelate metals, such as copper and iron, involved in reactive oxygen species generation [20]. This action was achieved through stimulation of various forms of cytochrome P450 [47]. 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; they also have been hypothesized to improve the celluar redox status by modulating the glutathione synthesis pathway against oxidative stress [21]. Moreover, Catechins, the monomeric units of proanthocyanidins have been reported to possess protective effects on vascular endothelial cells through inhibition of endothelial NADPH oxidase activity [20].

The absence of any difference in mean values of vitamin C and MDA between the two groups at the end of phase I and the presence of significant difference 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 GSE, significantly improved the ferric-reducing antioxidant power in plasma when compared to the control group [48] .To the best of our knowledge the current study is the first to evaluate the cardioprotective role of proanthocyanidine in ALL children with early onset DOX- induced cardiotoxicity based on both echocardiographic and biochemical markers of cardioyoxicity, however some limitations are encountered. The current study concentrated on the left side and did not evaluate the right side of the heart as well as for the pulmonary pressure. Another limitation of the study is that assessment of the left ventricular systolic function was carried out by relatively older routine modality. The study also did not evaluate left ventricular diastolic indices and left atrial volume index. Further evaluation on a large scale is recommended.

5. CONCLUSION

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. 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 promising protective effect on DOX-induced cardiotoxicity as evidenced by increased vitamin C, decreased MDA levels and decrement of cardiotoxicity biochemical markers.

CONSENT

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

ETHICAL APPROVAL

The study was approved by the research ethics committee of Mansoura University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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