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Olive Leaf Extract and α-Tocopherol Combination Therapy Attenuates Doxorubicin Induced Cardiotoxicity in Rats

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Abstract

Background: Cardiotoxicity is a serious side effect of Doxorubicin (DOX), which is a very effective drug used in the management of several malignancies. Oxidative stress is thought to play the major role in DOX toxicity. Several antioxidants such as vitamin E and anti-inflammatory substances like Olive Leaf Extract (OLE) used to attenuate DOX toxicity.

Aim: This experimental study tests the hypothesis that, co-administration of both Vit-E and OLE will have a synergistic and/or additive effects, and that their concomitant use is better than using either alone in alleviating DOX Induced Cardiac Toxicity (DICT) in rats.

Material and Methods: For the first 2 weeks, 4 groups of Wistar rats (n=8 in each) received intraperitoneal (i.p) DOX (total 20mg/kg) injections (DOX groups). Three of them also received either daily OLE: 500mg/kg BW or daily a-tocopherol 100mg/kg BW, or both for a period of 4 weeks. A control group of rats that received only i.p. saline was also included. All groups were tested for parameters of myocardial contractility and left ventricular function (LVDP, LVEDP and dp/dt max), Aortic Pressure (AoP), markers of myocardial tissue injury (cTnI, LDH, AST and ANP), and oxidative stress state (MDA, GSH, GPx and SOD).

Results: Combined OLE and Vit-E therapy with DOX improved Myocardial Contractility (MC), Left Ventricular Functions (LVF) and reduces Aortic Pressure (AoP), it also protected from DOX induced cardiac tissue injury and attenuated the accompanying oxidative stress state better than either alone.

Conclusion: The results of the present study confirm the beneficial effects of combined OLE and Vit-E therapy in ameliorating DICT in rats.

Key Words: *Olive oil – Vitamin E – Adriamycin – Heart toxicity – Oxidative stress.*

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Introduction

DOXORUBICIN (DOX) is a very effective and potent antineoplastic drug [1], which is used effectively in the management of several solid and hematological malignancies. Unfortunately, DOX has many acute and cumulative dose-related side effects such as hepatotoxicity, nephrotoxicity, and importantly cardiotoxicity [2], in addition to its adverse effects on the bone marrow and testis. Cardiotoxicity ranges from transient electrocardiographic changes to life-threatening cardiomyopathy, myocarditis, and heart failure that can be

ABBREVIATIONS:

ANP	: Atrial Natriuretic Peptide.
AoP	: Aortic Pressure.
AST	: Aspartate Transaminase.
cTnI	: Cardiac Troponin I.
DICT	: Doxorubicin-Induced Cardiotoxicity.
DOX	: Doxorubicin.
dP/dt max	: Maximum rate of left ventricular pressure rise in early systole.
DTNB	: 5,5`-Dithiobis-2-nitrobenzoic acid.
GPx	: Glutathione Peroxidase.
GSH	: Reduced Glutathione.
GSSG	: Oxidized Glutathione.
LDH	: Lactate Dehydrogenase.
LVEDP	: Left Ventricular End-Diastolic Pressure.
LVDP	: Left Ventricular Developed Pressure.
LVF	: Left Ventricular Functions.
MC	: Myocardial Contractility.
MTI	: Myocardial Tissue Injury.
NADPH	: Nicotinamide Adenine Dinucleotide Phosphate-oxidase.
NO	: Nitric Oxide.
OLE	: Olive Leaf Extract.
OSS	: Oxidative Stress State.
MDA	: Malondialdehyde.
ROS	: Reactive Oxygen Species.
SOD	: Superoxide Dismutase.
Vit-E	: Vitamin E.

fatal. These side effects frequently restrict its use [3].

The mechanisms underlying the pathogenesis of Doxorubicin-Induced Cardiotoxicity (DICT) are not clearly understood. Many factors have been proposed by previous studies including the production of Reactive Oxygen Species (ROS) which produce an Oxidative Stress State (OS S), calcium overloading, mitochondrial dysfunction, alteration in signaling of beta-adrenergic receptors, matrix metalloproteinase enzyme activation, and cytokine release due to activation of innate immune system [4-8]. However, oxidative stress was reported by most of these studies to play the major role in DOX toxicity [9,10]. This effect can be neutralized by antioxidants such as α-tocopherol (Vit-E), and consequently attenuate or prevent the harmful effects of DOX [11,12]. Also, Olive Leaf Extract (OLE) contains Oleuropein and other phenolics such as hydroxytyrosol [13], which showed beneficial anti-inflammatory properties, removal of ROS, protective effects against oxidative injury of myocardium, and suppression of 12– and 5 – lipoxygenase enzymes [14,15].

Several available DOX analogues failed to show a potent antineoplastic efficacy as compared to DOX. So, we should do more work to develop new strategies to attenuate the toxic effects of DOX without interfering with its antineoplastic properties [4]. Therefore, several studies have demonstrated the protective effects of Vit-E, either alone or combined to other antioxidants [11,16,17], against DICT. Also, few ones have examined the effects of Olive Leave Extract (OLE) as a natural anti-inflammatory and antioxidant agent, in the treatment/prevention of DOX toxicity [18-20]. To our knowledge, no previous study has addressed the concomitant use of both OLE and Vit-E in the prevention of DICT.

Therefore, the present study aimed to test the hypothesis that co-administration of Vit-E and OLE will have a synergistic and/or additive, and their concomitant use is better than using either alone in alleviating DICT in rats receiving DOX. The degree of cardiotoxicity was examined both physiologically by assessing the myocardial contractility/left ventricular functions (MC/LVF) using the langendorff perfusion technique and biochemically by determining cardiac injury markers and oxidative stress parameters.

Material and Methods

The study was conducted in Aljouf University, KSA starting from September 2017.

Chemicals:

- Adricin (Doxorubicin 50mg/25ml vial) was purchased from EIMC United Pharmaceutical, Egypt.
- Vitamin-E (α-tocopherol) 400mg capsules were purchased from PHARCO Pharmaceutical, Egypt.
- Olive Leaf Extract (OLE) preparation: OLE was obtained from Olive Research Center, Aljouf, north KSA. Fresh olive leaves were collected from the Olive orchid, exposed to air-drying then were grinded into fine particulate powder to pass a screen of 2mm diameter pores and then stored in a dark and dray place. The extract was prepared by Soxhlet extraction process using 150 gram of olive leaf powder that was soaked in 75% ethanol (1/10, w/v) as a solvent for 24 hours, then the extract was centrifuged at 5000rpm for 10 minutes to evaporate and remove ethanol. Finally, the extract was filtered using Whatman filter papers, and the filtrate was weighed (approximately equaled 60gm) and divided into two vials [21]. During administration period, OLE was kept refrigerated at 4ºC.

Experimental animals:

Forty male Wistar albino rats, aging 12-16 weeks and weighing 200±20gm were used. Rats were acclimatized in the animal house of Aljouf University for ten days prior to the experiment. Animals were housed in cages at room temperature (23-25ºC), about 65% relative humidity and alternate day/night periods of 12hr. Food (pelleted rat chow) and drinking water were available ad libitum. Any animal showed a sign of disease or stress during the acclimatization period was eliminated.

Ethical considerations:

The study protocol and the use of animals for research purposes were approved by the "Permanent Local Committee for Research Bioethics" of Aljouf University (approval NO: 16-16-8/39).

Experimental procedure:

The forty rats were randomly divided into 5 groups, 8 in each of the following groups:

- *Group I (Control group, Saline):* Rats received 8 intraperitoneal (i.p) saline injections over the first two weeks then continued for the next two weeks without any injections.
- *Group II (DOX group):* Rats received equal eight i.p DOX injections (each 2.5mg/kg) over the first two weeks only {the total DOX dose was 20mg/kg BW which is well proved to produce cardiotoxic effects [18]}.

- *Group III (DOX + OLE group):* Rats received the same eight i.p DOX injections plus daily OLE 500mg/kg BW by nasogastric tube over the first two weeks then daily OLE dose was continued alone for the next two weeks.
- *Group IV (DOX + Vit-E group):* Rats received the same eight i.p DOX injections plus daily a-tocopherol 100mg/kg BW by nasogastric tube over the first two weeks then daily ct-tocopherol dose was continued alone for the next two weeks.
- *Group V (DOX + OLE + Vit-E group):* Rats received the same eight i.p DOX injections plus daily OLE and ct-tocopherol by nasogastric tube by the same doses of previous groups over the first two weeks then daily OLE and ct-tocopherol doses were continued for the next two weeks.

Animal scarification:

In all groups, rats were sacrificed by the end of four weeks (the experimental period). The animals were starved for 12 hours prior to blood collection and anesthetized using sodium pentobarbital (50mg/kg; i.p.) and heparin (200IU/kg) was injected just before scarification to prevent blood coagulation, then blood was collected through retro-orbital puncture. Blood samples were collected into dry tubes without anticoagulant allowing clotting then centrifuged and serum was collected and stored at –70ºC until analysis.

Langendorff preparation and hemodynamic parameters:

After collecting blood for biochemical analysis, a transabdominal incision and cut in diaphragm were performed to expose the heart which was rapidly removed with severing of the blood vessels and immediately immersed into cold Krebs Henseleit (KH) buffer (4ºC) solution (mmol): Glucose 11.1, $CaCl_2$ 2, $MgSO_4$ 1.3, NaCl 119, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and KCl 4.7 at pH 7.4 to protect the heart from ischemic injury.

Then aorta was cannulated to perfuse the heart in a retrograde manner via the aorta at a constant rate. KH solution buffer was bubbled with 95% O_2 + 5% CO_2 at 37ºC. The backwards pressure shut the aortic valve, forcing the solution into the coronary vessels, which were normally supply the heart with blood. This allows nutrients and oxygen to feed the cardiac muscle to continue beating for hours after removal from the animal and various parameters can be measured.

The perfusate was pushed into the heart with volume regulated flow (12.5ml/min). Changes in the coronary vessels resistance will result in fluctuations in aortic pressure that can be monitored with a pressure transducer which was attached to a side arm of aortic cannula.

A water-filled balloon was introduced into left ventricle and connected to a pressure transducer to record the Left Ventricular Developed Pressure (LVDP).

One electrode (5V amplitude of 3ms duration) was placed on right atrium and another one on steel cannula to pace the heart (300 beats/minute) to keep the standard cardiac contractile response to the experimentally used drugs and excluding the effects of changes in heart rate and/or arrhythmia periods.

The following parameters were continuously displayed and recorded using a PowerLab data acquisition system:

- Left Ventricular Developed Pressure (LVDP).
- Left Ventricular End-Diastolic Pressure (LVEDP).
- Maximum rate of left ventricular pressure rise in early systole (dP/dt max).
- Aortic Pressure (AoP).

This in vitro isolated organ technique allowed the study of contractile force, coronary resistance and other hemodynamic parameters of the heart under known physiological conditions without the neural and hormonal complications of in vivo, whole animal experiments **[22]**. By the end of perfusion protocol, the hearts were immediately frozen at 70ºC until used for biochemical analysis within 7 days of termination of perfusion protocol.

Estimation of reduced glutathione content in cardiac tissue:

Glutathione (GSH) was measured in heart homogenate, this method was described by **[23]**. Its principle depends on reduction of 5,5`-Dithiobis-2-nitrobenzoic acid (DTNB) by the sulfhydryl group of GSH, the formed product was measured calorimetrically at 412nm.

Glutathione peroxidase activity in cardiac tissue:

Glutathione Peroxidase (GPx) provides a mechanism to detoxify peroxides in living cells. GPx Cellular Activity Assay Kit supplied by (SIGMA-ALDORISH, St. Louis, USA-Catalog Number CGP1) was used, the principle of this test depends on the ability of GPx to oxidize GSH to GSSG, a reaction coupled with another one that consumes NAPH to regenerate reduced glutathione. Colorimetric assessment of decrease in NADPH was done at wave length at 340nm.

Determination of cardiac tissue's superoxide dismutase activity:

Superoxide Dismutase (SOD) activity was determined in heart homogenate according to Marklund [24] method. Principle depends on the inhibition of pyrogallol auto-oxidation by SOD. The inhibition is directly proportional to the activity of SOD in the tested sample. Changes in the absorbance were recorded calorimetrically at wave length 420nm.

Malondialdehyde (MDA) in cardiac tissue:

Cardiac level of Malondialdehyde (MDA) was estimated according to Kei [25] technique. In brief, it was assessed by adding homogenates to Buege-Aust reagent and heated for 15min at 100ºC, then centrifuged, and MDA in determined colorimetrically at 535mm. The standard used in this assay was 1, 1, 3, 3 tetraethoxy propane.

Serum Aspartate Aminotransferase (AST):

Aspartate Aminotransferase (AST) Activity Assay Kit (SIGMA-ALDORISH, St. Louis, USA-Catalog Number MAK055). In this kit, a ketoglutarate accepts an amino group from aspartate to result in the generation of glutamate, resulting in the production of a colorimetric product measured at wave length 450nm.

Serum Atrial Natriuretic Peptide (ANP):

Atrial Natriuretic Peptide EIA Kit supplied by (SIGMA-ALDORISH, St. Louis, USA-Catalog Number RAB00385), and the test was performed according manufacturer instructions.

Serum Lactate Dehydrogenase (LDH):

Lactate Dehydrogenase Activity Assay Kit supplied by (SIGMA-ALDORISH, St. Louis, USA-Catalog Number MAK066). In this kit, LDH reduces NAD to NADH, which is specifically detected by colorimetric (450nm) assay.

Serum Cardiac Troponin I (cTpnI):

Cardiac troponin I is an important diagnostic marker of cardiac injury [26]. It was measured using Troponin I ELISA kit according to instruction of the kit supplied by (SIGMA-ALDORISH, St. Louis, USA-Catalog Number SE120134).

Statistical methods:

Data were coded and entered using the Statistical Package for Social Sciences (SPSS) Version 25 (SPSS Inc., Chicago, IL, USA). Data was summarized using mean and standard deviation for quantitative variables. Comparisons between groups were done using one way-analysis of variance (ANOVA) with multiple comparisons with Post Hoc tests [27]. Correlations between quantitative variables were done using Pearson correlation coefficient [28]. p-values less than 0.05 were considered as statistically significant.

Results

Combined OLE and Vit-E therapy with DOX improves cardiac contractility, Left Ventricular Function (LVF) and reduces Aortic Pressure (AoP) better than either alone.

To assess the effect of DOX injection on cardiac contractility and left ventricular function, we measured LVDP, LVEDP and dP/dt max in different groups. DOX group showed impaired LVF (LVDP, LVEDP and dP/dt max) as compared to Control group, LVF was significantly improved in both DOX + OLE and DOX + Vit-E groups but the maximal improvement in LV function was obtained in DOX + OLE + Vit-E group as shown in Fig. (1A-C). Also, Aortic Pressure (AoP) was measured which was maximally elevated in DOX group as compared to control group, both OLE and Vit-E significantly decrease AoP in DOX + OLE and DOX + Vit-E respectively, but combined OLE and Vit-E therapy with DOX succeeded to reduce the AoP maximally without a significant difference between AoP values in DOX + OLE + Vit-E and control groups as shown in Fig. (1D).

Heart was mostly protected from DOX induced cardiac tissue injury due to concomitant OLE and Vit-E therapy in DOX + OLE + Vit-E group.

To evaluate the degree of cardiac injury following DOX administration we have measured some important cardiac biomarkers; Cardiac Troponin I (cTnI), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST) and Atrial Natriuretic Peptide (ANP). Serum cTnI, LDH and AST levels were significantly elevated in DOX group as compared to control group. Both OLE and Vit-E attenuate the marked rise in cTnI, LDH and AST in DOX + OLE and DOX + Vit-E groups respectively as compared to DOX group, but combined OLE and Vit-E therapy in DOX + OLE + Vit-E group synergistically reduced the levels of cardiac damage biomarkers more than either of them did alone as shown in (Table 1).

Regarding ANP, although a tendency to decrease was noticed with the use of either OLE, or Vit-E, and with the use of a combination of both as compared to the DOX group. However, such decrease was no statistically significant (Table 1).

Combined OLE and Vit-E therapy with DOX attenuates the accompanying oxidative stress state better than either alone.

DOX induced oxidative stress state was also investigated in cardiac tissues by evaluating the levels of Malondialdehyde (MDA), Glutathione (GSH), and antioxidant enzymes i.e. Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD).

MDA level in cardiac tissues as an indicator of tissue lipid peroxidation was significantly increased in DOX group as compared to control group, while the MDA levels were decreased in OLE + DOX and OLE + Vit-E groups as compared to DOX group, but the culminating reduction in MDA level as compared to DOX group was shown in DOX + OLE + Vit-E group approaching its normal baseline in control group (Table 2).

Regarding GSH content as well as GPx and SOD activities in cardiac tissues, both of OLE and Vit-E in DOX-OLE and DOX-Vit-E respectively attenuate the accompanying DOX induced oxidative stress state with better effect in Vit-E, while combined OLE and Vit-E therapy in DOX + OLE + Vit-E group maintained GSH content and nearly recovered cardiac GPx and SOD activities as compared to control group better than either of them alone as shown in (Table 2).

The myocardial contractility represented by Left Ventricular Developed Pressure (LVDP) was significantly negative correlated with the severity of DOX induced cardiac tissue injury represented by serum levels of cTnI (r=–0.873, p<0.005) as shown Fig. (2).

The degree of oxidative stress state represented by MDA levels was significantly positive correlated with the severity of DOX induced cardiac tissue injury represented by serum levels of cTnI (r= 0.793, p<0.005)as shown in Fig. (3).

The degree of oxidative stress state represented by MDA levels was significantly negative correlated with the myocardial contractility represented by LVDP (r=–0.769, p<0.005) as shown in Fig. (4).

Aortic Pressure (AoP) as an indicator of the resistance in coronary vessels was significantly positive correlated with cardiac tissue markers as cTnI and it was having a significant negative correlation with antioxidants as Superoxide Dismutase (SOD) as shown in Figs. (5,6) (r=–0.888, p<0.005) and (r=–0.893, p<0.005) respectively.

Table (1): Effect of doxorubicin, Olive leaf extract and Vitamin E on rat serum levels of Cardiac Troponin I (cTnI), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST) and Atrial Natriuretic Peptide (ANP).

	Control group	DOX group	DOX + OLE group	DOX + Vit E group	DOX + OLE + Vit E group
cTnI (ng/ml)	1.9±0.4	9.5±1.5*	6.8±1.5*#	4.5±0.9*#$	3.4±0.9*#$@
LDH (U/L)	175.0±12.8	259.5±12.1 *	235.8±11.5*#	230.4±20.3 *#	180.1±18.3#$@
AST (U/L)	56.3±13.2	140.6±42.4*	119.6±36.3 *#	116.9±34.1 *#	92.3±15.8*#$@
ANP (pg/ml)	26.6±2.9	31.1±3.5	29.7±4.3	28.2±3.7	28.5±2.6

Values are presented as mean ± SD.
* : p<0.05 in comparison to control group.
# : p<0.05 in comparison to DOX group.
$: p<0.05 in comparison to DOX + OLE group.
@ : p<0.05 in comparison DOX + Vit-E group.

Table (2): Effect of doxorubicin, Olive leaf extract and Vitamin E on rat cardiac tissue levels of Antioxidant Glutathione (GSH), Glutathione Peroxidase (GPx), Antioxidant enzymes Superoxide Dismutase (SOD) and Malondialdehyde (MDA).

	Control group	DOX group	DOX + OLE group	DOX + Vit E group	DOX + OLE + Vit E group
MDA (mmol/g) tissue	23.6±2.8	43.0±6.4*	32.6±3.5*#	28.5±3.4#	21.4±3.1#$@
GSH (mmol/g) tissue	6.4±0.9	4.5±0.9*	5.3±0.76*#	5.6±0.92 *#	6.1±0.54#$
Gpx (U/g) tissue	41.8±5.5	28.2±4.0*	33.1±3.7*#	35.6±2.9 *#	40.0±3.9#$@
SOD (U/g) tissue	201.8±14.7	99.6±12.5*	115 .0±13 .2*#	160.6±15.4 *#$	180.9±16.6*#$@

Values are presented as mean ± SD.
* : p<0.05 compared to control group.
# : p<0.05 compared to DOX group.
$: p<0.05 compared to DOX + OLE group.
@: p<0.05 compared to DOX + Vit-E group.

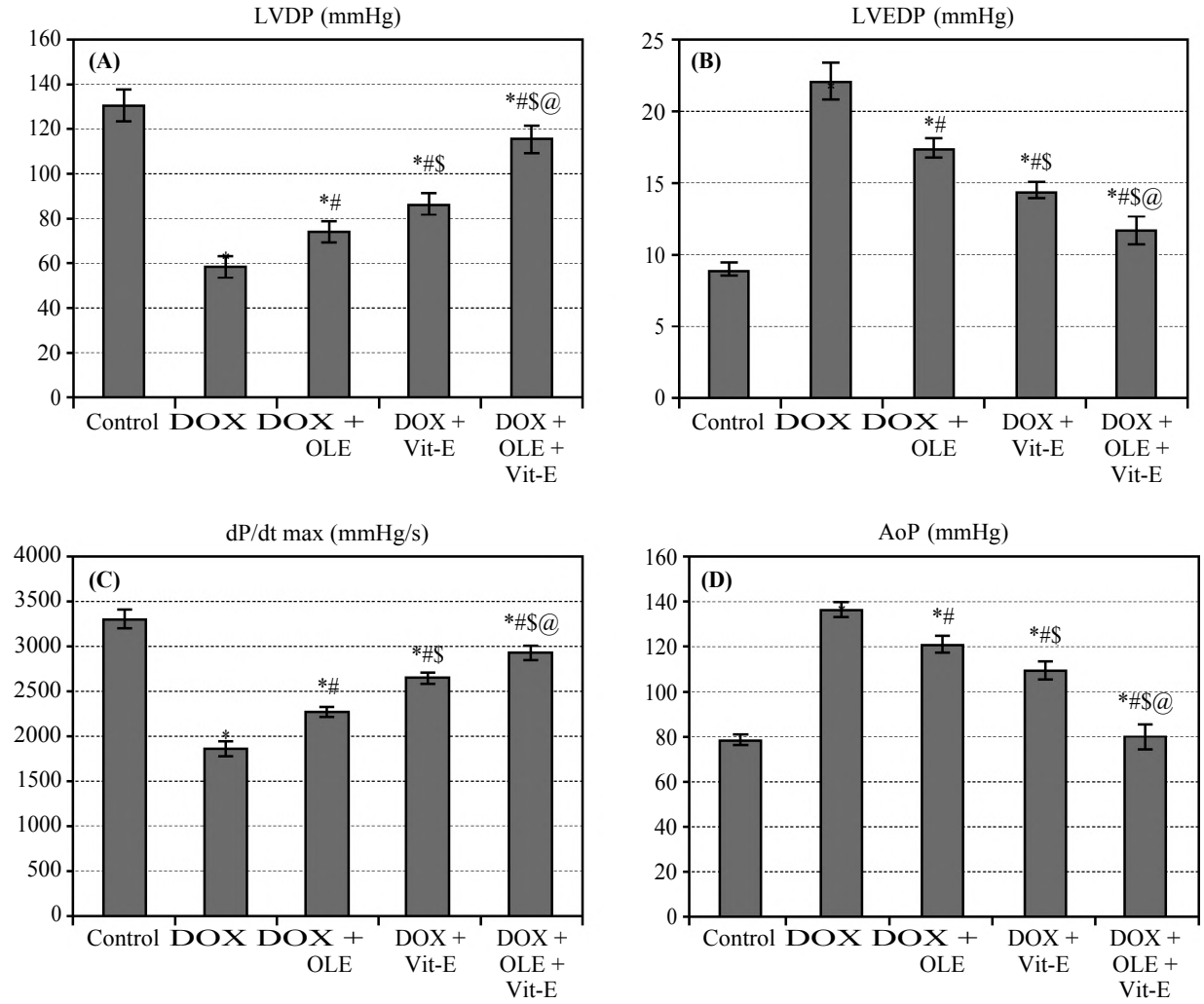

Fig. (1A-D): Comparison between the five studied groups (X-axis) regarding LVDP (A), LVEDP (B), dP/dt max (C) and AoP (D).

Values are presented as mean ± SD.
* : $p<0.05$ as compared to control group.
\# : $p<0.05$ as compared to DOX group.
$: $p<0.05$ as compared to DOX + OLE group.
@ : $p<0.05$ as compared to DOX + Vit-E group.

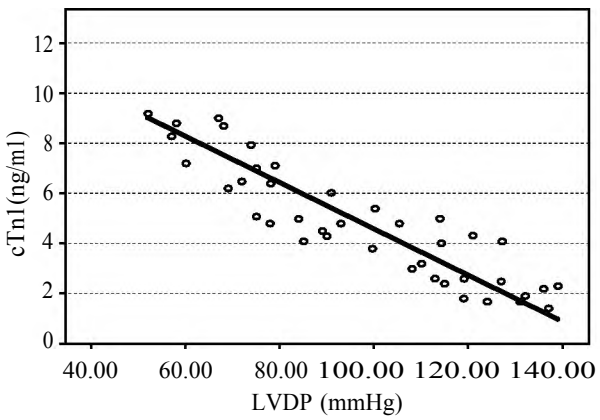

Fig. (2): Correlation between Left Ventricular Developed Pressure (LVDP) and cardiac troponin I (cTnI) and (r=–0.873, $p<0.005$).

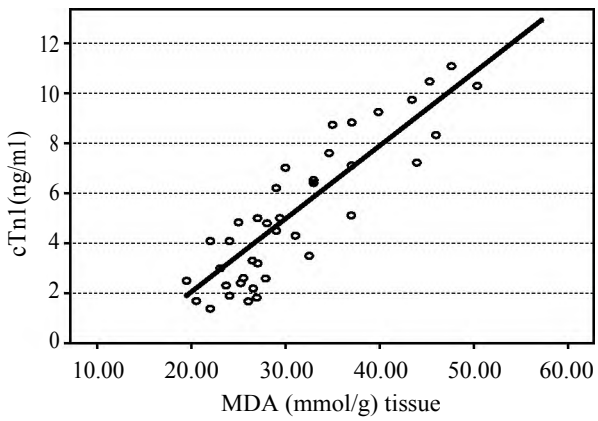

Fig. (3): Correlation between the levels of Malondialdehyde (MDA) and cardiac troponin I (cTnI) (r=0.793, $p<0.005$).

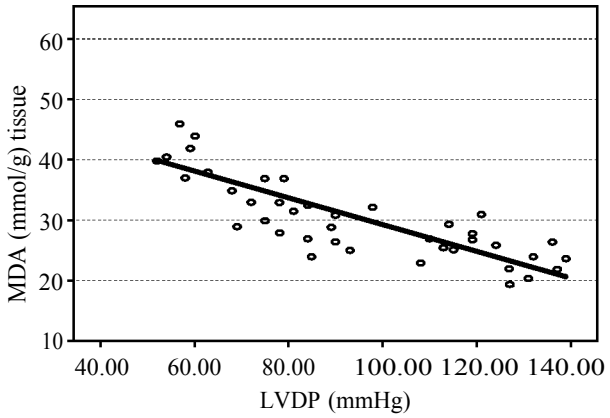

Fig. (4): Correlation between Left Ventricular Developed Pressure (LVEDP) and tissue Malondialdehyde (MDA) (r=–0.769, p<0.005).

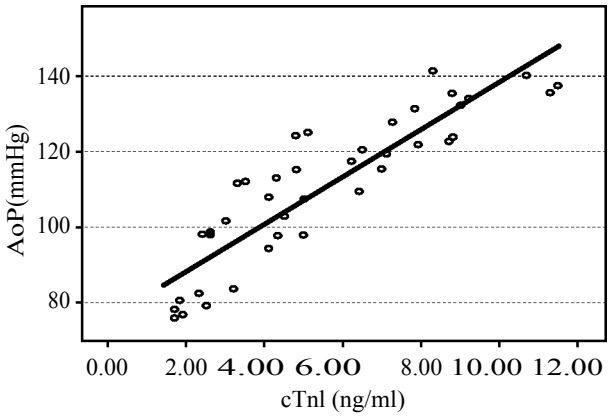

Fig. (5): Correlation between Aortic Pressure (AoP) and serum cTnI levels (r=0.888, p<0.005).

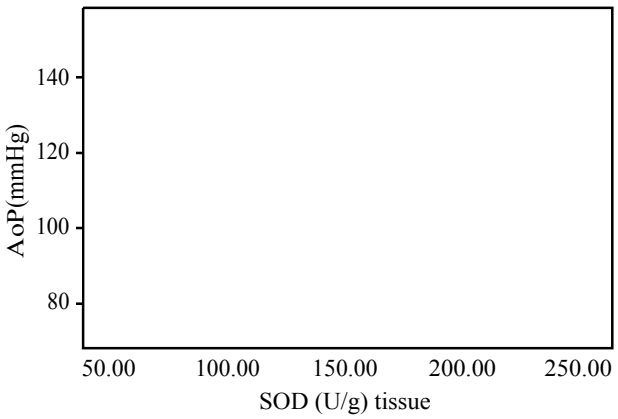

Fig. (6): Correlation between Aortic Pressure (AoP) and tissue; Superoxide Dismutase (SOD) levels (r=–0.893, p<0.005).

Discussion

Although several studies have demonstrated the protective effects of Vit-E, only few studies have examined the effects of OLE [18-20] against Doxorubicin-Induced Cardiotoxicity (DICT). To our knowledge, no previous study has addressed the effects of the concomitant use of OLE and Vit-E on Myocardial Contractility/Left Ventricular Function (MC/LVF), myocardial tissue injury (MTI), and Oxidative Stress State (OSS) in the DICT rat model. This gives the present study its originality as it shows, for the first time, that when compared to controls, the concomitant administration of OLE and Vit-E have not only improved the MC/LVF and attenuates the MTI associated with DOX administration, but also reduced the accompanying OSS as well. Indeed, the beneficial effects of the dual-regimen (OLE + Vit-E) were significantly higher than those observed with the use of either alone. This represents a kind of synergism between both agents, and will potentially improve their therapeutic potentials.

Despite the well-known potent and broad-spectrum antineoplastic activity of DOX against many types of solid and hematological malignancies [29,30], its wide use is limited by the confirmed, acute and cumulative dose-related, multi-organ toxic effects mainly hepatotoxicity [31]. Nephrotoxicity [2,32], myelotoxicity [33], and the serious deterioration of cardiotoxicities including; arrhythmia, cardiomyopathy, and MTI leading to reduced MC and LVF [34].

The mechanisms underlying the DICT have not been fully elucidated until now [35] despite dozens of studies that confirms a multifactorial base of this toxicity. None the less, the generation of ROS with its well known damaging effects on the cell components is thought to play the major role, and it was studied extensively [7-10,36-38], in addition to mitochondrial dysfunction [39,40], and increased cellular necrosis and apoptosis [41,42] together with an increased production of pro-inflammatory cytokines and responses [43,44]. This was confirmed by many interventional studies that showed improved DOX toxicity when oxidative stress and inflammations were reduced [45-47].

Better understanding of the mechanisms underlying DICT will not only help in preventing it, but also maximize benefiting from its high potency as well, putting in mind the failure of alternative strategies such as dose modification, use the DOX analogues, and combination therapy [4]. The findings of the present study add to the pool of evidence that confirms the role of oxidative stress in the pathogenesis of DICT.

In one hand, many studies showed the beneficial, but incomplete, effects of Vit-E in alleviating DICT when used alone or or as analogue in combination with other antioxidants [11,48]. Nowadays,

we are currently at the era of using both as a combination or a pro-drug utilizing nanotechnology in delivering targeted therapy of many cancers [49-51]. In addition to its anti-oxidant effects and role in ameliorating DICT, Vit-E was found to enhance the chemotherapeutic/antineoplastic effect of DOX [52] even against drug resistant cancers [53]. On the other hand, only few studies have investigated the role of OLE in reducing DICT [18-20], but none has assessed the use of both Vit-E and OLE concomitantly in this indication. The hypothesis behind the need of combination is based on the multifactorial basis of DICT, the partial response to the use of either agent alone, and the need to target many of the proposed mechanisms by the multiple constituents of OLE together with Vit-E.

The findings of the present study show that VC/LVF are improved in both DOX + OLE and DOX + Vit-E groups by different proportions, but the maximal improvement was shown in DOX + OLE + Vit-E group. To exclude the possible involvement of other systemic factors to the observed responses, all contractile parameters (LVDP, LVEDP and dP/dt max) and Aortic Pressure (AoP) levels were tested in isolated hearts using Langendorff perfusion technique in all the studied groups.

Several mechanisms may explain the aggravated synergistic and/or additive protective effects of combined DOX + Vit-E + OLE therapy. First, in addition to its anti-inflammatory effects [54], OLE improves contractility via its ability to restore the normal arrangements of cardiomyocyte contractile proteins (myosin, troponin I and dystrophin), which become disturbed with DOX administration [18]. Also, OLE restores the expression of the main transcription factor that regulates cardiac development, GATA-4, in the myocardial cells after being depleted by DOX [18]. Second, Oleuropein, the principal component of OLE, expressed a wide range of beneficial pharmacological cardiovascular effects in vitro studies including; stimulation of NO generation by rat macrophages [55], anti-inflammatory properties [5], anti-oxidant effect against myocardial ischemia/reperfusion injury [14], suppression of eicosanoid generation and platelet aggregation, and suppression of 12-and 5-lipoxygenase enzymes [54]. Third, many in vivo experiments elucidated that OLE has anti-inflammatory, anti-atherogenic, antihypertensive, and hypoglycemic effects, in addition to reducing blood pressure in nitro-L-arginine-induced hypertensive rats, lowering blood cholesterol and other lipids levels [56-58]. In addition, OLE lowered the blood levels of lipids, glucose, creatinine, and liver enzymes in streptozotocin-induced diabetic rats [59]. Furthermore, Oleuropein reduced the size of infarction, serum lipid, and oxidative stress markers in rabbits fed high cholesterol diet [60]. Fourth: A dual interaction between Vit-E and Olive oil has been demonstrated by two earlier studies where Vit-E supplementation increased thein vivo stability and the antioxidant capacity of refined Olive oil in the rat model of DOX toxicity [61], and Olive oil supplemented with Vitamin E increased the mitochondrial coenzyme Q levels and reduced the OSS in livers of the same rat model [62]. Fifth: Some studies have demonstrated the presence of Vit-E as a one of the component of Olive oil [63-64] making the suggestion of beneficial role of higher Vit-E concentrations in addition to the actions of the other components.

In the present study, a marked increase in the cardiac biomarkers (cTnI, LDH, and AST) indicating a serious MTI is demonstrated in the DOX group compared to controls. However, LDH and AST not much increased in DOX + OLE and DOX + Vit-E groups as compared with DOX group and these results are similar, but the marked reduction in their plasma levels have been shown in DOX + OLE + Vit E group. Because of the high sensitivity and specificity of cTnI, absence of histopathological assessment in the present study can't be considered as a limitation. This is supported by the similarity of our results to the work of others [16-18]. Also, our results showed supportive and parallel changes over the studied groups in the form of improved VC/LVF with reduced MTI markers and reduced oxidative stress, which were more prominent in the DOX + Vit-E + OLE group. Another potential limitation of this work is that we did not measure pro-inflammatory cytokines in the studied groups. However, many recent studies that looked at oxidative stress and pro-inflammatory cytokines showed parallel changes in both [45-47], which indicates that changes in one (e.g., oxidative stress) as the case in this study can reflect the other (pro-inflammatory cytokine assay), making simultaneous measurement of both only adds more costs, and therefore unnecessary.

On the other hand, ANP is also an important biomarker of LVF in patients receiving DOX. ANP is synthesized and secreted mainly from atria in response to volume overload, so its mechanism of secretion is secondary to impaired LVF and heart failure [65]. In present study, there was no significant difference in the plasma levels of ANP between different groups, which is different to previous studies [66]. This result may be due to the fact, Brain Natriuretic Peptide (BNP), which is synthesized in ventricles is an earlier predictor of heart

failure than ANP, so it is a very useful, sensitive and specific biomarker of impaired LVF [65], and needs to be addressed in future studies.

The previous changes in MC/LVF parameters together with the changes in the levels of biomarkers of MTI are associated with increased lipid peroxidation and diminished antioxidant enzymatic activity in heart tissue, as DOX induces an OSS by increasing the generation of ROS, enhancing inflammatory reactions, and apoptotic cascades in cardiac tissue [67]. In present study, the minimal increase in MDA level and minimal decrease in GSH, Gpx and SOD enzymatic activity have been shown in the DOX + OLE + Vit-E group as compared with control group while maximal lipid peroxidation and disturbance in the activity of antioxidant enzymes appeared in DOX group. Our current results are in accordance with other authors who found antioxidant activities for both OLE and Vit-E in separate studies and this study showed that, OLE and Vit-E has a strong synergetic antioxidant effects.

Dexrazoxane isthe exclusive FDA-approved drug used clinically to ameliorate DICT. It acts via reducing myocardial mitochondria injury [68,69], chelating iron intracellularly leading to blockade of iron-assisted ROS production [70], and suppressing topoisomerase II enzyme, which has recently been involved in the pathogenesis of DICT [71]. However, the clinical application of dexrazoxane is restricted owing to its interference with the antineoplastic effects of DOX [72], and due to its potential role in stimulating secondary malignancies, which led to its withdrawal from the European market [73]. Consequently, new cardioprotective approaches, techniques and/or drugs are needed.

The combination used in the present study (Vit-E + OLE) may have a role in this respect, but needs to be studied extensively in humans. Indeed, the results of this study can't be extrapolated to humans receiving DOX, and human studies in this area are scanty [74,75]. Therefore, well-designed and well-executed randomized clinical trials involving this combination in cancer patients receiving DOX-containing regimens are warranted.

The ideal drug needs to be effective in protecting from DICT without any negative interference onits antineoplastic potency, and should not lead to any significant side effects nor induce secondary malignancies. Indeed, the combination used in the present study was effective, safe, and together with the shown evidence of Vit-E enhancement of antineoplastic effects of DOX [52], deserves future clinical studies.

In conclusion, the results of the present study confirm the beneficial effects of both OLE and Vit-E in ameliorating DICT in rats, especially when given together. These effects manifests as improved MC/LVF, reduced MTI and decreased OSS and ROS. These synergistic and/or additive effects of OLE and Vit-E are probably related to their multifunctional nature, especially oxygen radical scavenging actions. Clinical trials of this combination are needed and awaited.

References

1- YUAN A., WU J.H., SONG C.C., TANG X.L., QIAO Q., ZHAO L.L., GONG G.M. and HU Y.Q.: A novel self-assembly albumin nanocarrier for reducing doxorubicin-mediated cardiotoxicity. J. Pharm. Sci., 102: 1626-35, 2013.

2- GHARANEI M., HUSSAIN A., JANNEH O. and MADDOCK H.L.: Doxorubicin induced myocardial injury is exacerbated following ischaemic stress via opening of the mitochondrial permeability transition pore. Toxicol Appl. Pharm., 268: 149-56, 2013.

3- WALLACE K.B.: Doxorubicin-induced cardiac mitochondrionopathy. Pharm. Toxicol., 93: 105-15, 2003.

4- YANG J.Q., MAITY B., HUANG J., GAO Z., STEWART A., WEISS R.M., ANDERSON M.E. and FISHER R.A.: G-protein inactivator RGS6 mediates myocardial cell apoptosis and cardiomyopathy caused by doxorubicin. Cancer Res., 73: 1662-7, 2013.

5- LIU C.K., ISMAIL S., BRENNAN O., HASTINGS C. and DUFFY G.P.: Encapsulation of cardiac stem cells in superoxide dismutase-loaded alginate prevents doxorubicin-mediated toxicity. J. Tissue Eng. Regen. Med., 7: 302-11, 2013.

6- HEGER Z., CERNEI N. and KUDR J.: A novel insight into the cardiotoxicity of antineoplastic drug doxorubicin. Int. J. Mol. Sci., 14: 21629-46, 2013.

7- PATEL N., JOSEPH C. and CORCORAN G.B.: Silymarin modulates doxorubicin-induced oxidative stress, Bcl-xL and p53 expression while preventing apoptotic and necrotic cell death in the liver. Toxicol Appl. Pharmacol., 245: 143-52, 2010.

8- ESPINOSA C., LÓPEZ-JIMÉNEZ J.Á. and CABRERA L.: Protective effect of white tea extract against acute oxidative injury caused by adriamycin in different tissues. Food Chem., 134: 1780-5, 2012.

9- EL-MOSELHY M.A. and EL-SHEIKH A.A.K.: Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity. Biomed. Pharmacother., 68: 101-10, 2014.

10- ABO-SALEM O.M.: The protective effect of aminoguanidine on doxorubicin-induced nephropathy inrats. J. Biochem. Mol. Toxicol., 26: 1-9, 2012.

11- HADI N., YOUSIF N.G., AL-AMRAN F.G., HUNTEI N.K., MOHAMMAD B.I. and ALI S.J.: Vitamin E and telmisartan attenuates doxorubicin induced cardiac injury in rat through down regulation of inflammatory response: B.M.C. Cardiovasc. Disord., 12: 63, 2012.

12- HU X.X., FU L., LI Y., LIN Z.B., LIU X., WANG J.F., CHEN Y.X., WANG Z.P., ZHANG X., OU Z.J. and OU J.S.: The Cardioprotective Effect of Vitamin E (Alpha-Tocopherol) Is Strongly Related to Age and Gender in Mice. PLoS One, 10 (9): e0137405, 2015.

13- GONZALEZ-CORREA J.A., MUNOZ-MARIN J., ARREBOLA M.M., GUERRERO A., NARBONA F., LOPEZ-VILLODRES J.A. and De LA CRUZ J.P.: Dietary virgin olive oil reduces oxidative stress and cellular damage in rat brain slices subjected to hypoxia-reoxygenation. Lipids, 42: 921-9, 2007.

14- MANNA C., MIGLIARDI V., GOLINO P., SCOGNAMIGLIO A., GALLETTI P., CHIARIELLO M. and ZAPPIA V.: Oleuropein prevents oxidative myocardial injury induced by ischemia and reperfusion. J. Nutr. Biochem., 15: 461-6, 2004.

15- MILES E.A., ZOUBOULI P. and CALDER P.C.: Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human whole blood cultures. Nutrition, 21: 389-94, 2005.

16- AROZAL W., F.D. SUYATNA F.D., JUNIANTITO V., ROSDIANA D. S., AMURGAM S., AULIA R., MONAYO E.R. and SISWANDI R.: The effect of Mangiferin (Mangifera indica L) in Doxorubicin-induced Cardiotoxicity in rats. Drug. Res., 65: 574-80, 2015.

17- SNEZANA K. BJELOGRLIC, JELENA RADIC, VIKTOR JOVIC and SINISA RADULOVIC: Activity of d,1-a-Toccopherol (Vitamin E) against Cardiotoxicity Induced by Doxorubicin and Doxorubicin with Cyclophosphamide in Mice. Basic & Clinical Pharmacology & Toxicology, 97: 311-9, 2005.

18- KUMRAL A., GIRIŞ M., SOLUK-TEKKE_IN M., OLGAÇ V., DOGRU-ABBASOGLU S., TÜRKOGLU Ü. and UYSAL M.: Effect of olive leaf extract treatment on doxorubicin-induced cardiac, hepatic and renal toxicity in rats. Pathophysiology, 22 (2): 117-23, 2015.

19- ANDREADOU I., PAPAEFTHIMIOU M., ZIRA A., CONSTANTINOU M., SIGALA F., SKALTSOUNIS A.L., TSANTILI-KAKOULIDOU A., ILIODROMITIS E.K., KREMASTINOS D.T. and MIKROS E.: Metabonomic identification of novel biomarkers in doxorubicin cardiotoxicity and protective effect of the natural antioxidant oleuropein. N.M.R. Biomed., 22 (6): 585-92, 2009.

20- ANDREADOU I., SIGALA F., ILIODROMITIS E.K., PAPAEFTHIMIOU M., SIGALAS C., ALIGIANNIS N., SAVVARI P., GORGOULIS V., PAPALABROS E. and KREMASTINOS D.T.: Acute doxorubicin cardiotoxicity is successfully treated with the phytochemical oleuropein through suppression of oxidative and nitrosative stress. J. Mol. Cell Cardiol., 42 (3): 549-458, 2007.

21- TAVAFI M., AHMAD H. and TOOLABI P.: Inhibitory Effect of Olive Leaf Extract on Gentamicin-induced Nephrotoxicity in Rats. Iran J. Kid. Dis., 6 (1): 25-32, 2012.

22- BOPASSA J.C., EGHBALI M., TORO L. and STEFANI E.: A novel estrogen receptor GPER inhibits mitochondria permeability transition pore opening and protects the heart against ischemia-reperfusion injury. Am. J. Physiol. Heart Circ. Physiol., 298: H16-H23, 2010.

23- SEDLAK J. and LINDSAY R.H.: Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem., 25 (1): 192-205, 1968.

24- MARKLUND S.L.: Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal BALB/c mice. Mutat. Res., 148 (1-2): 129-34, 1985.

25- KEI S.: Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta, 90 (1): p. 37-43, 1978.

26- ADAMS J.E. 3rd, BODOR G.S., DÁVILA-ROMÁN V.G., DELMEZ J.A., APPLE F.S., LADENSON J.H. and JAFFE A.S.: Cardiac troponin I. A marker with high specificity for cardiac injury. Circulation, 88 (1): 101-6, 1993.

27- CHAN Y.H.: Biostatistics102: Quantitative Data - Parametric & Non-parametric Tests. Singapore Med. J., 44 (8): 391-6, 2003a.

28- CHAN Y.H.: Biostatistics 104: Correlational Analysis. Singapore Med. J., 44 (12): 614-9, 2003b.

29- FAROLFI A., MELEGARI E., AQUILINA M., SCARPI E., IBRAHIM T., MALTONI R., SARTI S., CECCONETTO L., PIETRI E., FERRARIO C., FEDELI A., FAEDI M., NANNI O., FRASSINETI G.L., AMADORI D. and ROCCA A.: Trastuzumab-induced cardiotoxicity in early breast cancer patients: A retrospective study of possible risk and protective factors. Heart, 99: 634-9, 2013.

30- DAS J., GHOSH J., MANNA P. and SIL P.C.: Taurine protects rat testes against doxorubicin-induced oxidative stress as well as p53, Fas and caspase 12-mediated apoptosis. Amino Acids, 42: 1839-55, 2012.

31- DAMODAR G., SMITHA T., GOPINATH S., VIJAYAKUMAR S. and RAO Y.: An evaluation of hepatotoxicity in breast cancer patients receiving injection Doxorubicin. Ann. Med. Health Sci. Res., 4 (1): 74-9, 2014.

32- INJAC R., BOSKOVIC M., PERSE M., KOPRIVEC-FURLAN E., CERAR A., DJORDJEVIC A. and STRUKELJ B.: Acute doxorubicin nephrotoxicity in rats with malignant neoplasm can be successfully treated with fullerenol C60(OH)24 via suppression of oxidative stress. Pharmacol. Rep., 60 (5): 742-9, 2008.

33- PEREIRA NETO G.B., ANDRADE J.N.B., SOUSA M.G. and CAMACHO A.A.: Holter electrocardiography in dogs showing doxorubicin-induced dilated cardiomyopathy. Arq. Bras. Med. Vet. Zootec., 58: 1037-42, 2006.

34- TORTI F.M., BRISTOW M.M., LUM B.L., CARTER S.K., HOWES A.E., ASTON D.A., BROWN B.W. Jr., HANNIGAN J.F. Jr., MEYERS F.J., MITCHELL E.P. and BILLINGHAM M.E.: Cardiotoxicity of epirubicin and doxorubicin: Assessment by endomyocardial biopsy. Cancer Res., 46 (7): 3722-7, 1986.

35- MINOTTI G., MENNA P., SALVATORELLI E., CAIRO G. and GIANNI L.: Anthracyclines: Molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol. Rev., 56: 185-229, 2004.

36- DUDKA J., GIEROBA R., KORGA A., BURDAN F., MATYSIAK W., JODLOWSKA-JEDRYCH B., MANDZIUK S., KOROBOWICZ E. and MURIAS M.: Different effects of resveratrol ondose-related Doxorubicin-induced heart and liver toxicity. Evid. Based Complement. Alternat. Med., 606183, 2012.

37- BOGHDADY N.A.: Antioxidant and antiapoptotic effects of proanthocyanidin andginkgo biloba extract against doxorubicin-induced cardiac injury in rats. Cell Biochem. Funct., 31 (4): 344-51, 2013.

38- SALEEM M.T., CHETTY M.C. and KAVIMANI S.: Antioxidants and tumor necrosis factoralpha-inhibiting activity of sesame oil against doxorubicin-induced cardiotoxicity. Ther. Adv. Cardiovasc. Dis., 8 (1): 4-11, 2014.

39- FERREIRA A.L., MATSUBARA L.S. and MATSUBARA B.B.: Anthracycline-induced cardiotoxicity. Cardiovasc. Hematol. Agents. Med. Chem., 6: 278-81, 2008.

40- KY B., VEJPONGSA P., YEH E.T. FORCE T. and MOSLEHI J.J.: Emerging paradigms in cardiomyopathies associated with cancer therapies. Circulation Res., 113: 754-64, 2013.

41- WANG Y., LEI T., YUAN J., WU Y., SHEN X., GAO J., FENG W. and LU Z.: GCN2 deficiencyameliorates doxorubicin-induced cardiotoxicity by decreasing cardiomyocyteapoptosis and myocardial oxidative stress. Redox. Biol., 17: 25-34, 2018.

42- GU J., FAN Y.Q., ZHANG H.L., PAN J.A., YU J.Y., ZHANG J.F. and WANG C.Q.: Resveratrolsuppresses doxorubicin-induced cardiotoxicity by disrupting E2F1 mediatedautophagy inhibition and apoptosis promotion. Biochem. Pharmacol., 150: 202-13, 2018.

43- ALUISE C.D., SULTANA R., TANGPONG J., VORE M., St. CLAIR D., MOSCOW J.A. and BUTTERFIELD D.A.: Chemo brain (chemo fog) as a potential side effect of doxorubicinadministration: Role of cytokine-induced, oxidative/nitrosative stress incognitive dysfunction. Adv. Exp. Med. Biol., 678: 147-56, 2010.

44- TIEN C.C., PENG Y.C., YANG F.L., SUBEQ Y.M. and LEE R.P.: Slow infusion rate of doxorubicininduces higher pro-inflammatory cytokine production. Regul. Toxicol. Pharmacol., 81: 69-76, 2016.

45- SANGOMLA S., SAIFI M.A., KHURANA A. and GODUGU C.: Nanoceria ameliorates doxorubicininduced cardiotoxicity: Possible mitigation via reduction of oxidative stress andinflammation. J. Trace. Elem. Med. Biol., 47: 53-62, 2018.

46- HAJRA S., PATRA A.R., BASU A. and BHATTACHARYA S.: Prevention of doxorubicin (DOX)-induced genotoxicity and cardiotoxicity: Effect of plant derived smallmolecule indole-3-carbinol (I3C) on oxidative stress and inflammation. Biomed. Pharmacother., 101: 228-43, 2018.

47- SHAKER R.A., ABBOUD S.H., ASSAD H.C. and HADI N.: Enoxaparin attenuates doxorubicininduced cardiotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. B.M.C. Pharmacol. Toxicol., 19 (1): 3, 2018.

48- SAILO B.L., BANIK K., PADMAVATHI G., JAVADI M., BORDOLOI D. and KUNNUMAKKARA A.B.: Tocotrienols: The promising analogues of vitamin E for cancer therapeutics. Pharmacol. Res., 130: 259-72, 2018.

49- MET IN E., MUTLU P. and GUNDUZ U.: Co-delivery of Doxorubicin and D-a-Tocopherol polyethylene glycol 1000 succinate by Magnetic Nanoparticles. Anticancer Agents. Med. Chem., Doi: 10.2174/ 18715206186661 80313154724. [Epub ahead of print], 2018.

50- OLIVEIRA M.S., MUSSI S.V., GOMES D.A., YOSHIDA M.I., FREZARD F., CARREGAL V.M. and FERREIRA L.A.M.: a-Tocopherol succinate improves encapsulation and anticancer activity of doxorubicin loaded in solid lipid nanoparticles. Colloids. Surf. B. Biointerfaces, 140: 246-53, 2016.

51- LU J., ZHAO W., LIU H., MARQUEZ R., HUANG Y., ZHANG Y., LI J., XIE W., VENKATARAMANAN R., XU L. and LI S.: An improved D-a-tocopherol-based nanocarrier for targeted delivery of doxorubicin with reversal of multidrug resistance. J. Control Release, 196: 272-86, 2014.

52- DANHIER F., KOUHÉ T.T., DUHEM N., UCAKAR B., STAUB A., DRAOUI N., FERON O. and PRÉAT V.: Vitamin E-based micelles enhance the anticancer activity of doxorubicin. Int. J. Pharm., 476 (1-2): 9-15, 2014.

53- OLIVEIRA M.S., ARYASOMAYAJULA B., PATTNI B., MUSSI S.V., FERREIRA L.A.M. and TORCHILIN V.P.: Solid lipid nanoparticles co-loaded with doxorubicin and a-tocopherol succinate are effective against drug-resistant cancer cells in monolayer and 3-D spheroid cancer cell models. Int. J. Pharm., 512 (1): 292-300, 2016.

54- QABAHA K., AL-RIMAWI F., QASEM A. and NASER S.A.: Oleuropein Is Responsible for theMajor Anti-Inflammatory Effects of Olive Leaf Extract. J. Med. Food, 21 (3): 302-5, 2018.

55- VISIOLI F., POLI A. and GALL C.: Antioxidant and other biological activities of phenols from olives and olive oil. Med. Res. Rev., 22: 65-75, 2002.

56- KHAYYAL M.T., EL-GHAZALY M.A., ABDALLAH D.M., NASSAR N.N., OKPANYI S.N. and KREUTER M.H.: Blood pressure lowering effect of an olive leaf extract (Olea europaea) in L-NAME induced hypertension in rats. Arzneimittelforschung, 52: 797-802, 2002.

57- FKI I., BOUAZIZ M., SAHNOUN Z. and SAYADI S.: Hypocholesterolemic effects of phenolic-rich extracts of Chemlali olive cultivar in rats fed a cholesterolrich diet. Bioorg. Med. Chem., 13: 5362-70, 2005.

58- JEMAI H., BOUAZIZ M., FKI I., EL-FEKI A. and SAYADI S.: Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. Chem. Biol. Interact., 176: 88-98, 2008.

59- EIDI A., EIDI M. and DARZI R.: Antidiabetic effect of Olea europaea L. in normal and diabetic rats. Phytother. Res., 23: 347-50, 2009.

60- AL-AZZAWIE H.F. and ALHAMDANI M.S.: Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci., 78: 1371-7, 2006.

61- QUILES J.L., RAMÍREZ-TORTOSA M.C., IBÁÑEZ S., ALFONSO GONZÁLEZ J., DUTHIE G.G., HUERTAS J.R. and MATAIX J.: Vitamin E supplementation increases the stability and the in vivo antioxidant capacity of refined olive oil. Free Radic. Res., 31 Suppl: S 129-S135, 1999.

62- QUILES J.L., RAMIREZ-TORTOSA M.C., HUERTAS J.R., IBAÑEZ S., GOMEZ J.A., BATTINO M. and MATAIX J.: Olive oil supplemented with Vitamin E affects mitochondrial coenzyme Q levels in liver of rats after an oxidative stress induced by adriamycin. Biofactors, 9 (2-4): 331-6, 1999.

63- OKOGERI O. and TASIOULA-MARGARI M.: Changes occurring in phenolic compounds andalpha-tocopherol of virgin olive oil during storage. J. Agric. Food Chem., 50 (5): 1077-80, 2002.

64- GIMENO E., CALERO E., CASTELLOTE A.I., LAMUELA-RAVENTÓS R.M., De La TORRE M.C. and LÓPEZ-SABATER M.C.: Simultaneous determination of alpha-tocopherol andbeta-carotene in olive oil by reversed-phase high-performance liquidchromatography. J. Chromatogr., 81 (1-2): 255-9, 2000.

65- KOH E., NAKAMURA T. and TAKAHASHI H.: Troponin-T and Brain Natriuretic Peptide as Predictors for Adriamycin-Induced Cardiomyopathy in Rats. Circ. J., 68: 163-7, 2004.

66- NOUSIAINEN T., VANNINEN E., JANTUNEN E., PUUSTINEN J., REMES J., RANTALA A., VUOLTEENAHO O. and HARTIKAINEN J.: Natriuretic peptide during the development of doxorubicin-induced left ventricular diastolic dysfunction. J. Intern. Med., 251: 228-34, 2002.

67- KUMRAL A., GIRIŞ M., SOLUK-TEKKEŞIN M., OLGAÇ V., DOGRU-ABBASOGLU S., TÜRKOGLU Ü. and UYSAL M.: Beneficial effects of carnosine and carnosine plus Vitamin E treatments on doxorubicin-induced oxidative stress and cardiac, hepatic, and renal toxicity in rats. Hum. Exp. Toxicol., 35 (6): 635-43, 2016.

68- HIDEG K. and KÁLAI T.: Novel antioxidants in anthracycline cardiotoxicity. Cardiovasc. Toxicol., 7: 160-4, 2007.

69- MOUQUET F., ROUSSEAU D., DOMERGUE-DUPONT V., GRYNBERG A. and LIAO R.: Effects of trimetazidine, a partial inhibitor of fatty acid oxidation, on ventricular function and survival after myocardial infarction and reperfusion in the rat. Fundam. Clin. Pharmacol., 24: 469-76, 2010.

70- ICHIKAWA Y., GHANEFAR M., BAYEVA M., WU R., KHECHADURI A., NAGA PRASAD S.V., MUTHARASAN R.K., NAIK T.J. and ARDEHALI H.: Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. J. Clin. Invest., 124: 617-30, 2014.

71- ZHANG S., LIU X., BAWA-KHALFE T., LU L.S., LYU Y.L., LIU L.F. and YEH E.T.: Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nature Med., 18: 1639-42, 2012.

72- SWAIN S.M. and VICI P.: The current and future role of dexrazoxane as a cardioprotectant in anthracycline treatment: Expert panel review. J. Cancer Res. Clin. Oncol., 130 (1): 1-7, 2004.

73- TEBBI C.K., LONDON W.B., FRIEDMAN D., VILLALUNA D., De ALARCON P.A., CONSTINE L.S., MENDENHALL N.P., SPOSTO R., CHAUVENET A. and SCHWARTZ C.L.: Dexrazoxane-associated risk for acute myeloid leukemia/myelodysplastic syndrome and other secondary malignancies in pediatric Hodgkin's disease. J. Clin. Oncol., 25: 493-500, 2007.

74- SUHAIL N., BILAL N., KHAN H.Y., HASAN S., SHARMA S., KHAN F., MANSOOR T. and BANU N.: Effect of vitamins C and E on antioxidant status of breast-cancer patients undergoing chemotherapy. J. Clin. Pharm. Ther., 37 (1): 22-6, 2012.

75- SUNG L., TOMLINSON G.A., GREENBERG M.L., KOREN G., JUDD P., OTA S. and FELDMAN B.M.: Serial controlled N-of-1 trials of topical Vitamin E as prophylaxis for chemotherapy-induced oral mucositis in paediatric patients. Eur. J. Cancer, 43 (8): 1269-75, 2007.

العلاج المشترك من مستخلص أوراق الزيتون مع ألفا توكوفيرول (فيتامين E) يضعف التأثيرات السامة على عضلة القلب الناتجة عن إستخدام عقار الدوكسوروبيسين فى الجرذان

تعد السمية القلبية أحد الآثار الجانبية الخطيرة لعقار الدوكسوروبيسين، وهو دواء فعال يستخدم فى علاج العديد من الأورام الخبيثة. ويعتقد أن الإجهاد التأكسدى يلعب الدور الرئيسى فى سمية عقار الدوكسوروبيسين. العديد من مضادات الأكسدة مثل فيتامين E والمواد المضادة للإلتهابات مثل مستخلص أوراق الزيتون قد تستخدم لتخفيف سمية عقار الدوكسوروبيسين.

الهدف: تختبر هذه الدراسة التجريبية فرضية أنه سيكون للإعطاء المشترك لكل من فيتامين E ومستخلص أوراق الزيتون تأثيرات تآزرية، وإن إستخدامها المتزامن أفضل من إستخدام أحدهما فى التخفيف من سمية القلب الناتجة عن عقار الدوكسوروبيسين فى الجرذان.

طرق البحث: فى الإسبوعين الأولين، تلقت ٤ مجموعات من فئران التجارب (العدد=٨ فى كل مجموعة) الحقن داخل الصفاق من عقار الدوكسوروبيسين (إجمالى ٢٠مغ/كغ) حقنة. ثلاثة منهم تلقوا إما يوميا مستخلص أوراق الزيتون ٥٠٠مغ/كغ من وزن الجسم أو ألفا توكوفيرول (فيتامين E) ١٠٠ملغم/كغم من وزن الجسم، أو كلاهما لمدة ٤ أسابيع. المجموعة الضابطة تلقت فقط الحقن داخل الصفاق من عقار الدوكسوروبيسين.

تم إختبار جميع المجموعات لمعاملات إنقباض عضلة القلب ووظيفة البطين الأيسر، الضغط الأبهرى، علامات إصابة أنسجة عضلة القلب، وحالة الإجهاد التأكسدى.

النتائج: العلاج المشترك يوميا لمستخلص أوراق الزيتون مع ألفا توكوفيرول (فيتا مين E) مع عقار الدوكسوروبيسين يحسن إنقباض عضلة القلب، وظائف البطين الأيسر ويقلل من الضغط الأبهرى، كما أنه يحمى من الإصابة بنسيج القلب الذى يسببه عقار الدوكسوروبيسين ويضعف حالة الإجهاد المؤكسد المصاحب أفضل من أحد العلاجين بمفرده.

الخلاصة: تؤكد نتائج الدراسة الحالية على الآثار المفيدة للعلاج المشترك لمستخلص أوراق الزيتون مع ألفا توكوفيرول (فيتامين E) فى تحسين أعراض السمية القلبية الناتجة عن إستخدام عقار الدوكسوروبيستين فى الجرذان.