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Full Length Research Paper

Cardioprotective effects of *Curcuma longa L.* extracts against doxorubicin-induced cardiotoxicity in rats

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This study was designed to investigate the possible mechanisms whereby *Curcuma longa* could protect against cardiotoxicity induced by doxorubicin. Administration of doxorubicin (15 mg/kg i.p.) induced cardiomyopathy manifested by significant elevation in serum creatine kinase MB (Ck-MB) and lactate dehydrogenase (LDH) activities. In addition, cardiotoxicity was further confirmed by significant increase in each of serum and cardiac malondialdehyde (MDA) level, serum iron and nitric oxide concentrations, cardiac calcium level, catalase and glucose-6 phosphate dehydrogenase activities as well as by the noticeable reduction in cardiac total antioxidant capacity, vitamin C levels and blood glutathione (GSH) concentration. Oral administration of *Curcuma longa* ethanolic or water extract (200 mg/kg) prior to doxorubicin produced a significant protection which was evidenced by significant reduction in mortality, CK-MB and LDH -activities. Moreover, they significantly increased GSH markedly, decreased cardiac calcium, and cardiac and serum MDA. In addition, both extracts significantly reduced serum nitric oxide, increased cardiac ascorbic acid, and ameliorated the antioxidant enzymes activities. In conclusion, *Curcuma longa* extracts renders resiliency against doxorubicin cardiotoxicity due to their contents of polyphenolic compounds that might serve as novel adjuvant therapy with doxorubicin.

Key words: *Curcume longa* L., doxorubicin, cardiotoxicity, antioxidants.

INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic, is an excellent drug for the treatment of a wide variety of human solid tumors and leukemias. However, its clinical uses are limited by seriously high incidence of cardio-toxicity. An initial acute effect includes hypotension and transient electrocardiographic abnormalities. Meanwhile, the chronic effects may occur several weeks or months after cumulative DOX administration. Cardioxmyophathy is dose dependent which accounts for high mortality (Jensen et al., 2002). However, the mechanisms by which DOX induces cardiac injury and dysfunction are incompletely understood. Some of these include cellular toxicity from metabolites of DOX (Minotti et al., 1995), generation of myocytes. A number of DO X-induced biochemical changes have been identified that can damage cardiac reactive oxygen species (Santos et al., 2007), production of reactive nitrogen species (Pacher et al., 2003); selective inhibition of cardiac muscle gene expression (Ito et al., 1990), disturbance of myocardial adrenergic signaling (Yoshikawa et al., 1994), and induction of cardiac cell apoptosis (Dowd et al., 2001).

DOX causes free radical formation by two major pathways. First, some of flavin-centered, NADPH-depen-dent reductases are capable to produce a non-electron reduction of anthracyclines to anthracycline semiquinone free radicals (Vasquez-Vivar et al., 1997) that induce apoptosis in cardiomyocytes (Martin et al., 2009). Second, anthracycline free radicals may arise via a non-enzymatic mechanism including reactions of anthracyclines and iron (Kotamraju et al., 2002). The heart is particularly vulnerable to the free radicals produced by DOX administration, as it contains less free radical detoxifying substances such as superoxide dismutase, glutathione and catalase than do other

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metabolic organs such as liver or kidney and its highly oxidative metabolism (Olson and Mushlin, 1990). Additionally, DOX has a very high affinity by cardiolipin, a phospholipid that is present in mitochondrial membranes of heart, resulting in the accumulation of DOX inside cardiac cell (Goormaghtigh and Ruysschaert, 1984).

The rhizomes of turmeric (*Curcuma longa* L., Zingiberaceae) play an important role as remedy. Medicinal uses of the rhizomes arise from their contents of volatile oil and curcuminoids (Pothitirat and Gritsanapan, 2006). Curcuminoids are curcumin and two related demethoxy compounds, demethoxycurcumin and bisdemethoxy curcumin. Turmeric has been reported to possess anti-inflammatory, hepatoprotective antiviral activities, and anticancer activity (Radha et al., 2006; Fryer et al., 2009) via its effects on gene expression (Aggarwal et al., 2003). Curcuminoids exhibit free radical scavenging property (Ramsewaket al., 2000). Also, the principle metabolites of curcumin are also bioactive especially tetrahydrocurcumin which inhibits lipid peroxidation in erythrocytes membranes. Turmeric oil is composed of several monoterpene and sesquiterpene compounds such as zingiberene and α-and β-turmerone. It is used as carminative, antifungal and as antiplatelet agent (Lee, 2006).

The cardioprotective effect of the commonly used antioxidant vitamins such as ascorbic acid and vitamin E remains controversial (Qiles et al., 2002). Previous studies have demonstrated that antioxidant compounds have some cytoprotective effect in DOX cardiotoxicity, (Bagchi et al., 2003). It has been reported that *C. longa L* extract is useful in the protection against myocardial injury and preservation of cardiac function (Wattanapitayakul et al., 2005; Kim et al., 2008; Srivastava and Mehta, 2009).

The purpose of the present study was to elucidate whether aqueous and alcoholic extracts of *C. longa L* could contribute to controlling important parameters that have roles in inducing and aggrevating DOX cardiomyopathy. We also used DL-α lipoic acid which has been proven to be an antioxidant and cardioprotective neutraceutical compound (Kumar et al., 2005) for comparison in this experimental model.

MATERIALS AND METHODS

Preparation of plant extracts

Rhizomes of *C. longa* L. were purchased from local herbal shop at Cairo on November 2009 and were identified by Prof. I. El Garf, Faculty of Science, Cairo University .A voucher specimen has been deposited in the herbarium of the National Research Centre, Cairo, Egypt. One hundred gram of the dried powdered plant were extracted with 80% ethanol and then evaporated under reduced pressure. The extracted fraction was completely evaporated in a vacuum oven at a temperature not exceeding 40°C until a constant weight was obtained (6.25 g) from ethanolic extract (*C. longa L* Cl-EtOH). The water extract was obtained by 2 h water reflux, for other 100 g of dried powdered plant followed by evaporation under reduced pressure. Then, the extract was concentrated and lypholized in a freez dryer. The obtained weight from water extract (*C. longa L* Cl-H_2O) was 5.0 g.

Chemicals

Adriamycin, doxorubicin hydrochloride (DOX) was supplied from Pharmacia of Upjohn S.P.A. Research. Thiotacid- DL-α lipoic acid- was purchased from Cid Company. It was used as reference, cardioprotective and antioxidant drug.

Animals

Ninty-six male Sprague-Dawely rats weighing (200 to 250 g) were purchased from Animal House of the National Research Centre. They were kept individually in stainless steel wire bottomed cages at room temperature (25 ± 2°C) under 12 h dark-light cycle. Animals were fed standard pellets diet. The rats had free access to food and tap water. Animal experiments were conducted according to the guidelines of Animal Care and Ethics Committee of the National Research Centre, Egypt.

Experimental design

Animals were randomly assigned to eight groups follows: normal group (SA, n = 6) was given saline. The second group (CL. EtOH, n = 6) was orally administered the alcoholic extract (200 mg/kg/day 0.1 ml). The third group (CL-H_2O, n = 6) was given aqueous extract of *C. longa L* (200 mg/kg/day 0.1 ml). The fourth group (LA, n = 6), was administered lipoic acid (20 $mg.kg^{-1}$/day 0.1 ml), oral gavage (Kumar et al., 2005). The fifth group (DOX, n = 18) was administered adriamycin as a single intraperitoneal injection 15 mg kg^{-1}, 0.1 ml in saline on the seventh day from the beginning of the experiment (Ahmed et al., 2005). Administration of a single dose of doxorubicin to rats has been validated extensively in the study of DOX-induced cardiac dysfunction *in vivo* (VenKatesan, 1998; Dowd et al., 2001). The sixth group (Dox + CL-EtOH, n = 18) that was treated with doxorubicin and alcoholic *C. longa L* extract. The seventh group (DOX + CL-H_2O, n = 18) which was given doxorubicin and aqueous *C. longa L* extract. Finally, the eighth group (DOX + LA, n = 18) was given doxorubicin and lipoic acid. *C. longa L* extracts and lipoic acid were administered seven days before and two days after doxorubicin (Venkatesan, 1998). A high number of animals was used in the DOX treated groups (n = 18) because of the elevated mortality (¤ 50%) in DOX groups that was observed in the pilot study. This mortality rate is consistent with other reports (Ferreria et al., 2007 and Kelishomi et al., 2008). The experiment lasted after nine days after which rats weighed, blood samples were collected under light ether anaesthesia from retro-orbital vein after rats being fasted for 12 h. One part of blood was collected on heparin for determination of hemoglobin which was considered as antioxidant through transitional iron compart-mentalization mechanism (Heffner and Repine, 1989) according to the method described by Reuge (1968) and for the estimation of non protein sulfhydryls, mainly GSH which was measured spectro-photometrically after reacting with dithionitrobenzoic acid to give reduced chromogen, by the procedure of Moron et al. (1979). The other part of blood was left to clot and serum was separated by centrifugation at 3000 r.p.m. for 10 min at 4°C where the clear serum was obtained. Markers for myocardium injury, serum creatine kinase MB and lactate dehydrogenase activities were measured by immunoinhibition assay (Szasz et al., 1974) using Randox Laboratories kit and by kinetic procedure according to Young (1995) using kit provided from Biosystems S.A., respectively. Measurement of endogenous serum nitrite concentration as an

Table 1. Effect of *C. longa* L extracts and lipoic acid on DOX induced changes in heart weight, heart weight to body weight percentage and mortality rate percentage.

Parameters / Groups n=6	Heart weight (g)	Heart weight/body weight percentage	Mortality percentage
Normal	0.70±0.026^{ab}	0.382±0.018^{b}	0
C. longa L EtOH (Cl-EtOH)	0.73 ±0.021^{a}	0.344±0.002^{bc}	0
C. longa L H_2O (Cl-H_2O)	0.63±0.021^{bc}	0.329±0.017^{bc}	0
Lipoic acid (LA)	0.63±0.042^{bc}	0.315±0.015^{c}	0
Doxorubicin (DOX)	0.60±0.036^{c}	0.257±0.002^{d}	50
DOX + (Cl-EtOH)	0.70±0.01^{ab}	0.36±0.005^{ab}	0
DOX + (Cl-H_2O)	0.70±0.037^{ab}	0.338±0.012^{bc}	0
DOX + LA	0.60±0.037^{c}	0.272±0.009^{d}	33.3

Data are expressed as mean ± S.E. Mean values at the same column sharing the same superscript letters are not significantly different (P ≤ 0.05).

indicator of nitric oxide level was applied. It depended on the addition of Griess reagents which converted nitrite into deep purple azo compound which was measured colorimetrically Montgomery and Dymock (1961). Also serum activity of glucose-6 phosphate dehydrogenase, a potent antioxidant enzyme was evaluated by monitoring the rate of NADPH formation at 340 nm using comercially available Biodiagnostic Kit (Langdon, 1960). Finally, iron concentration in serum was evaluated colorimetrically following a procedure described by Williams et al. (1977).

Hearts were dissected washed with saline weighed and immediately homogenized in 0.01 mol/L Tris-HCl ice-cold buffer (pH 7.4) to give 10% homogenate (W/V). The homogenate was centrifuged undercooling at 3200 rpm for 20 min. The supernatant (10%) was used for the determination of malondialdhyde as a marker for cardiac lipid peroxidation value by measuring the presence of thiobarbituric acid reactive substance (TBAR) using chemical method described by Ruiz-Larrea et al. (1994), results were expressed as nmol/g tissue. The supernatant was further diluted with tris-HCl buffer to reach 5% dilution to determine cardiac total antioxidant capacity which was assayed using commercial Biodiagnostic kit according to Koracevic et al. (2001), Catalase activity in the heart was measured spectrophotometrically after H_2O_2 reduction using a method described by Aebi (1984), cardiac ascorbic acid level was estimated chemically using folin phenol reagent (Jagota and Dani, 1982), calcium concentration was determined colorimetrically according to Gindler King (1972) and cardiac protein content was evaluated chemically via procedure of Lowry et al. (1951).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, version 7.5) was used in data analysis. Data were expressed as mean ± S.E. One way analysis of variance (ANOVA) was used to compare between groups. When significant, it was followed by Duncan's multiple range test to clarify the significance between the individual groups. P values less than 0.05 were considered significant.

RESULTS

Doxorubicin treatment induced 50% mortality, this percentage was significantly abolished in rats treated with turmeric extracts, meanwhile it was markedly diminished to 33.3% in those administered lipoic acid. Both the heart weight and the ratio of heart weight/body weight percentage were significantly lower in the DOX than in control group. Co-administration of extracts with DOX produced significant increase in both heart weight and its ratio. On the other hand, LA administration with DOX showed non significant change on the heart weight but its ratio was noticeably higher than in the DOX group. We can notice that CL-EtOH effect was the superior, then Cl-H_2O while that of lipoic was the least (Table 1).

Changes in serum creatine kinase MB and lactate dehydrogenase activities in various experimental groups are represented in (Figure 1 and 2). DOX significantly elevated both activities to be about 2 fold as compared to control group. This revealed an extreme cardiac damage. Treatment with *C. longa L* extracts and lipoic acid markedly restored enzymes activities to normal levels.

The data in (Table 2) represent the effect of *C. longa L* extracts and lipoic acid on DOX-induced biochemical changes that triggered cardiomyopathy. The results demonstrated that DOX treatment extremely increased serum and cardiac malondialdhyde level as a marker for oxidative damage to lipid and consequently for lipid peroxidation to be 2.74 ± 0.323 and 1.57 ± 0.047 respectively, versus 1.26± 0.284 and 1.21 ± 0.097 for the control group. *C. longa L* ethanolic extract, water extract and lipoic acid treatments significantly reduced cardiac lipid peroxides (1.31 ± 0.097, 1.26 ± 0.058 and 1.36 ± 0.037 respectively) as compared to DOX values. Moreover they reduced serum peroxides (1.49 ± 0.017, 2.06 ± 0.346 and 1.29 ± 0.117) as compared to DOX group. Both serum iron and cardiac calcium were significantly increased by DOX (98.5 ±10.3 and 105 ± 7.46, respectively) as compared to those of control (57.5 ± 5.92 and 48.1 ± 2.74, respectively). These findings strongly suggest that DOX increased redox active iron and oxidative stress *in vivo*. *C. longa L* water extract and lipoic acid co-administration with DOX showed nearly similar significant reduction for serum iron level (51.9 ±3.43 and 58.8 ± 0.69) and for cardiac calcium concentration (52.2 ± 2.05 and 52.9 ± 5.16), respectively

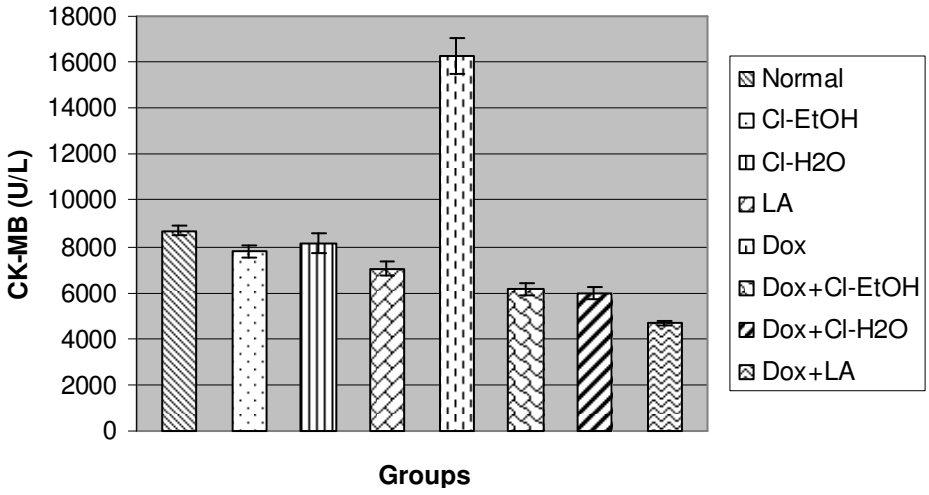

Figure 1. Effect of *C. longa L* extracts and lipoic acid on DOX induced change in serum CK-MB activity.

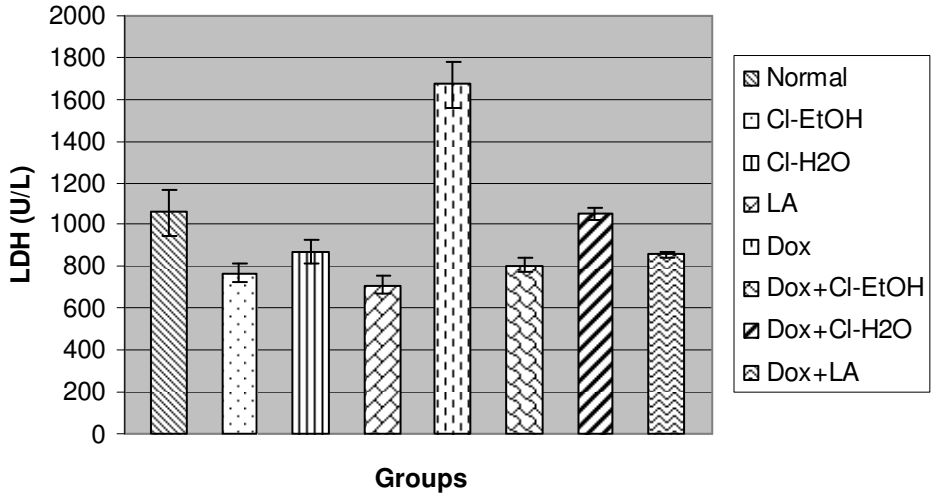

Figure 2. Effect of *Curcuma longa* extracts and lipoic acid on DOX induced change in serum LDH activity.

versus those of DOX. While ethanolic extract showed the least effects although significant, it decreased iron level (79.4 ± 2.78) and calcium level (94.8 ± 3.38) as compared to DOX. Nitric oxide level was noticeably elevated by DOX (29.8 ± 2.69) vs. (25.5 ± 1.39) for control. All the three treatments reduced the increase of nitric oxide by DOX, but the reduction was significant for *C. longa L* EtOH (25.1 ± 0.42) and lipoic (24.7 ± 0.38) and not significant for *C. longa L* H_2O (26.9 ± 0.10). The status of enzymatic, non enzymatic and total antioxidants is shown in (Table 3). There was a marked drop in the concentrations of non enzymatic molecules blood GSH and cardiac ascorbate (0.539 ± 0.013 and 0.62 ± 0.043, respectively), meanwhile, hemoglobin level was slightly increased by DOX (13.2 ± 0.033) as compared to those of the control (0.599 ± 0.027, 1.33 ± 0.032 and 12.8 ± 0.896, respectively). On the other hand, there was a noticeable adaptive increase in the activities of cardiac catalase, a peroxide scavenging enzyme, (21 ± 0.394) and serum glucose 6 phosphate dehydrogenase, a NADPH producer, cardiac total antioxidant capacity was slightly decreased by DOX (30.7 ± 1.52) vs. (32.8 ± 0.538) for control. Co-treatment of *C. longa L* extracts and lipoic acid with DOX remarkably increased ascorbic acid levels (0.95 ± 0.041, 1.35 ± 0.053 and 1.35 ± 0.076, respectively) vs. DOX values. Lipoic acid co-administration with DOX significantly increased glutathione level (0.765 ± 0.042), but both extracts failed to change it. Cardiac catalase activity was markedly normalized by *C. longa L* extracts and lipoic administration with DOX (18.4± 0.829, 16.6 ± 0.401 and 19.6 ± 0.060 respectively) as compared to DOX group. Although, serum G6PD was

Table 2. Effect of *C. longa L* extracts and lipoic acid on DOX-induced biochemical changes that triggered cardiomyopathy in various tested groups.

Parameters / Groups n=6	Serum malondialdhyde (nmol/ml)	Serum iron (µg/dl)	Serum nitric oxide (nmol/L)	Cardiac calcium (mg/gm tissue)	Cardiac malondialdhyde (mol/mg protein)
Normal	1.26±0.248^{c}	57.5±5.93^{cd}	25.5±1.39^{b}	48.1±2.74^{c}	1.21±0.097^{bc}
C. longa L EtOH (Cl-EtOH)	1.20±0.229^{c}	68.9±1.39^{bc}	27.3±0.499^{ab}	72.7±0.68^{b}	1.22±0.039^{bc}
C. longa L H_2O (Cl-H_2O)	1.09±0.096^{c}	61.2±6.52^{cd}	27.2±0.403^{ab}	47.9±3.97^{c}	1.13±0.05^{c}
Lipoic acid (LA)	1.14±0.157^{c}	66.4±3.74^{bcd}	25.5±0.425^{b}	53.6±4.87^{c}	1.25±0.063^{bc}
Doxorubicin (DOX)	2.47±0.323^{a}	98.5±10.3^{a}	29.8±2.69^{a}	106±7.46^{a}	1.57±0.047^{a}
DOX + (Cl-EtOH)	1.49±0.017^{bc}	79.4±2.78^{b}	25.1±0.416^{b}	94.8±3.38^{a}	1.31±0.097^{bc}
DOX + (Cl-H_2O)	2.06±0.346^{ab}	51.9±3.43^{d}	26.9±0.098^{ab}	52.2±2.05^{c}	1.26±0.058^{bc}
DOX + LA	1.29±0.117^{c}	58.8±0.69^{cd}	24.7±0.377^{b}	52.9±5.16^{c}	1.36±0.037^{b}

Data are expressed as mean ± S.E. Mean values at the same column sharing the same superscript letters are not significantly different (P≤0.05).

Table 3. Effect of *C. longa L* extracts and lipoic acid on DOX-induced changes in various antioxidant biomarkers.

Parameters / Groups n=6	Blood glutathione (mmol/L)	Blood hemoglobin (gm/L)	Cardiac catalase (U/mg protein)	Cardiac total antioxidant (mmol/gm tissue)	Cardiac vitamin C (µg /mg protein)	Serum glucose-6-phosphate dehydrogenase (mg/dl)
Normal	0.599±0.027^{b}	12.8±0.896^{b}	18.8±0.220^{abc}	32.8±0.538^{ab}	1.33±0.032^{a}	2.3±0.34^{d}
C. longa L EtOH (Cl-EtOH)	0.536±0.024^{bc}	12.7±0.345^{b}	16.9±0.963^{c}	34.3±0.238^{a}	1.04±0.011^{bc}	3.80±0.352^{c}
C. longa L H_2O (Cl-H_2O)	0.507±0.008^{c}	12.9±0.643^{ab}	16.9±1.095^{c}	33.1±0.505^{a}	1.12±0.027^{b}	4.28±0.343^{bc}
Lipoic acid (LA)	0.576±0.013^{bc}	12.6±0.458^{b}	21.0±0.394^{a}	30.7±1.52^{b}	0.62±0.043^{d}	2.77±0.255^{d}
Doxorubicin (DOX)	0.539±0.013^{bc}	13.2±0.033^{ab}	21.0±0.394^{a}	30.7±1.52^{b}	0.62±0.043^{d}	6.88±0.128^{a}
DOX + (Cl-EtOH)	0.542±0.027^{bc}	12.7±0.361^{b}	18.4±0.829^{bc}	34.1±0.202^{a}	0.95±0.041^{c}	5.03±0.380^{b}
DOX + (Cl-H_2O)	0.543±0.014^{bc}	11.5±0.377^{b}	16.6±0.401^{c}	33.9±0.100^{a}	1.35±0.053^{a}	4.50±0.475^{bc}
DOX + LA	0.765±0.042^{a}	14.6±0.68^{a}	19.6±0.660^{ab}	33.9±0.262^{a}	1.35±0.076^{a}	4.40±0.413^{bc}

Data are expressed as mean ± S.E. Mean values at the same column sharing the same superscript letters are not significantly different (P≤0.05).

significantly amelio-rated by the three treatments, it did not reach near to normal (5.03 ± 0.380, 4.5 ± 0.475 and 4.40 ± 0.414, respectively). However, total antioxidant capacity was extremely elevated (34.1 ± 0.202, 33.9 ± 0.100 and 33.9 ± 0.262, respectively) by co-administration of extracts and lipoic acid with DOX and their effects were equivalent.

DISCUSSION AND CONCLUSION

The anthracycline antibiotic DOX is one of the most effective chemotherapeutic agents against a wide variety of cancers. However, its use is seriously limited by the development of cardio-toxicity that resulted from either acute or chronic drug toxic effects. Among followed strategies to attenuate DOX toxicity are dose optimization, synthesis and use of analogues or combined therapy with antioxidants. The most promising results come from the combination of the drug delivery together with an antioxidant in order to reduce oxidative stress without interference with its antitumor properties (Quiles et al., 2002). Currently, the antioxidant dexrazoxane is the

approved drug indicated for clinical use in conjunction with DOX to alleviate cardiotoxicity. Despite the usefulness of the antioxidant dexrazoxane, its use is often limited. Medicinal plants have recently become a focus of interest because they may play key roles in treating a majority of heart disease with minimal or no side effects. Therefore, our study was designed to examine the cardioprotective actions of *C. longa L* extracts against DOX induced cardiotoxicity.

Our results have shown that intraperitoneal administration of DOX produced signs of cardiomyopathy as it was manifested by excessive fluid accumulation that found in pleural, pericardial, and peritoneal cavities together with ventral edema and enlargement of liver and kidneys (Herman et al., 1988). Compared to control animals, DOX treated animals had increased mortality, 50% of the animals died before termination of the experiment, accumulation of ascites as well as significant decreased heart weight and ratio of heart weight to body weight indicating a severe dysfunction in cardiac performance. Our findings are in agreement with those of Bertinchant et al. (2003) and Kelishomi et al. (2008). These are the results of direct toxic effects on intestinal mucosa and additional indirect action on the gastrointestinal tract arising from reduced food intake (Herman et al., 2000) causing a decrease in secretion of internal hormones and resulting in decreased trophic effects to the mucosa. While the cardiac dysfunction associated with DOX is attributable, at least in part, to cardiac cell apoptosis resulted from reactive oxygen species (ROS) produced by DOX, Konorev et al. (2002) have reported that agents which scavenge ROS protect against DOX induced cardiac apoptosis. However, the majority of authors dealing with this problem considered that cardiomyopathy and nephropathy make the most important contribution to the mortality (Herman et al., 2000). Confirmed with this hypothesis, we found that creatine kinase isoenzyme and lactate dehydrogenase activities, the most specific highly sensitive markers for myocardial cell injury (Yee et al., 2003) were extremely elevated in DOX group indicating severely damaged heart tissue by DOX. Our results are consistent with those of Abd El-Gawad and El-Sawalhi (2004), Abd-Allah et al. (2002), and Venkatesan (1998). The mechanism for the release of these markers seems to be from oxidative damage of DOX to cardiac tissue and the subsequent release of its contents into circulation. Increased formation of free radicals especially the superoxide anion could contribute to the inflammatory cascade in the vessel wall, and might cause arterial endothelial dysfunction. Also, other causes for leakage of cardiac enzymes may be ventricular remodeling, ongoing myocyte degeneration, the presence of coronary artery disease and reduced coronary reserve (Potluri et al., 2004). Normalization of CK-MB and LDH elevated levels and increasing percentage of survivors by *C. longa L* extracts confirms the cardio-protective effects of curcumin (Mohamed et al., 2009; Srivastava and Mehta, 2009; Wattanapitayakul et al., 2005). Curcumin, the main bioactive compound in these plant extracts, increases the cardiac glutathione content, suggesting that it may augment the action of these naturally occurring sulphhydryl groups to maintain membrane integrity with concomitant decrease of enzymes leakage from the cardiolcytes, protection of cardiac tissue from damage, and improvement survival of rats (Venkatesan, 1998). DL alpha lipoic acid is known to be a highly protective agent against chemotherapeutic drugs-induced cardiotoxicity (Mythili et al., 2007). This finding could be confirmed, in the current study, by the significant reduction in mortality, serum CK-MB and LDH activities in LA pretreated group as compared with DOX injected group. Lipids, proteins, and DNA are important targets of ROS and their oxidative products have a variety of biological effects. Lipids are possibly important vehicle for inflicting oxidative damage on cells, resulting in the formation of lipid peroxides that trigger a cascade reaction involving other lipid molecules. The current study showed an increase in cardiac serum TBARS levels, where malondialdhyde is the end product of lipid peroxidation (Nowak et al., 1995) in DOX treated group as compared to control group. Evidence has been provided for DOX induced lipid peroxidation (Hamza et al., 2008; Wang et al., 2007) as a reasonable result for oxidative stress (Singal et al., 2000). Two different ways of free radical formation by DOX have been described, the first implicates the formation of a semiquinone free radical which yields superoxide radicals (Singal et al., 2000) and the second way produces Fe^{2+}-DOX complex (DeBeer et al., 2001) that can reduce oxygen to hydrogen peroxide and other active species. Free radicals are known to attack the highly unsaturated fatty acids of the cell membrane to induce lipid peroxidation and consequently damage membranes (Schinella et al., 2002). *C. longa L* extracts co-administration with DOX significantly decreased lipid peroxidation which was reflected in the decrease levels of TBARS that was demonstrated in previous work (Mohamed et al., 2009). Curcumin, that is responsible for *Curcuma* actions, inhibits lipid peroxidation by scavenging free radicals and thus blocking the lipid chain reaction similar to α-tocopherol (Venkatesan, 1998).

Sreejayan et al. (1997) claimed that the presence of phenolic groups in the structure of curcumin is fundamental to explain its ability to eliminate oxygen free radicals from the medium and that methoxy groups increase this activity. Besides, the phenolic moiety of the curcumin structure can donate hydrogen atoms to deleterious oxy radicals and form the less reactive phenoxy radicals in the process (Arora et al., 1998). Co-administration of LA, a potent free radical scavenger (Kagan et al., 1992) caused a significant fall in the lipid peroxide concentration. Balachandar et al. (2003) demonstrated that lipoic acid protects against DOX-induced lipid peroxidation.

Nitric oxide is an important chemical mediator that can

act as a free radical and can also be converted to highly reactive peroxynitrite anion. NO synthase (NOS) enzyme produces NO from the catalytic conversion of L-arginine to L-citrulline in the presence of oxygen and NADPH (Palmer et al., 1988). Three NOS isoforms exist neural (n NOS), inducible (iNOS or NOS_2), and endothelial (eNOS or NOS_3). DOX binds to all three NOS isoforms (Garner et al., 1999). Our data showed that DOX treatment had significantly increased serum NO that is consistent with Ahmed et al. (2005) and Wang et al. (2007). DOX is thought to increase NO by induction of NOS_2 expression. Where, NOS_2 inhibition or deficiency has been reported to protect against DOX-induced cardiac dysfunction (Pacher et al., 2003). *C. longa L* extracts co-administration with D OX produced significant reduction in NO level which may be attributable to the bioactive substance curcumin, which scavenges free radicals and inhibits nitric oxide synthase activity (Ruby et al., 1995). LA treatment with DOX significantly reduced serum NO level by its ability to quench free radicals (Kumar et al., 2005).

Several pieces of evidence indicate that DOX cardiotoxicity is mainly caused by its interaction with iron. The current results demonstrated that DOX treatment increased serum iron level dramatically. This elevation was previously reported by Othman et al. (2008) and Simunek et al. (2009). Our result was supported by the finding that iron chelation with dexrazoxane that could reduce DOX induced cardiomyopathy (Martin et al., 2009). Following a short exposure to redox-active agent such as DOX, labile iron pools and ROS production rose dramatically in a parallel manner. The release of intracellular iron and the possible involvement of superoxide anions in iron release from ferritin are critical (Deepa and Varalakshmi, 2003). It was reported that superoxide anion formed by redox cycling DOX, mobilizes ferritin iron and promotes oxidative damage (Reif, 1992). It is of value to mention that anthracyclines also cause marked accumulation of oxidative stress products and inactivate the iron regulatory proteins 1 and 2 in cardiomyocytes (Minotti et al., 2001).

Co-administration of turmeric extracts or lipoic acid prior and DOX with significantly ameliorated the increased level of serum iron. A body of accumulated evidence suggests that curcumin and lipoic acid are potent free radical scavengers consequently reduce iron mobilization from ferritin (Reif, 1992). Another possible mechanism by which curcumin reduced iron level may be attributed to its iron chelating activity, since many polyphenolic compounds such as catchin posses this activity (Quiles et al., 2002).

Calcium ion is an important mediator of cell injury. Cytosolic free calcium is maintained at extremely low concentrations and most of it is sequestered in mitochondria and endoplasmic reticulum.

Our data confirms that cardiac calcium level was increased by DOX treatment which is in agreement with that of Kim et al. (2006) and Park et al. (2008). Moreover, increasing proofs indicated that Ca^{2+} overload in myocardial cells could be correlated to DOX induced cardiotoxicity (Li et al., 2002; Huang et al., 2003). As increased calcium activates a number of enzymes with potential deleterious cellular effects. Also, increased intracellular Ca^{2+} levels result in increased mitochondrial permeability and induction of apoptosis. DOX depolymerizes membrane phospholipids, enhances the membrane permeability and increases Ca^{2+} influx, it also inhibits the activity of Na^+ K^+ ATPase, reduces the Na^+-K^+ exchange, and enhances the Na^+-Ca^{2+} exchange (Huang et al., 2003), it decreases the amount of Ca^{2+} released from sarcoplasmic reticulum (Maeda et al., 2001) and of cardiac mitochondria (Zhou et al., 2001). Besides, some studies have shown that application of DOX is accompanied by a significant decrease of mRNA levels for all SR Ca^{2+} transport proteins, including Ca^{2+} ATPase and Ca^{2+} uptake capacity. It was established that H_2O_2 and probably other reactive oxygen intermediates mediated the effects of DOX on gene expression, while it was prevented by antioxidant (Arai et al., 2000).

Pretreatment with turmeric extracts seven days before and two days after DOX administration modulated the increased Ca^{2+} level probably via their membrane stabilizing effects (Venkatesan, 1998). Lipoic acid effect on Ca level was similar to that of turmeric extracts. An explanation of such effect may be attributed to LA ability to quench free radicals that protects membrane polyunsaturated fatty acids from oxidation concomitantly, stabilizes membrane and reduces Ca^{2+} influx.

An important property of natural curcuminoids is to inhibit the negative effects of DOX without interfering with its efficacy as antitumor agent. Where it has been shown in several studies that curcumin induced oncostatic, antiproliferative effects and apoptosis in many cancer cell lines (Wu et al., 2002; Angelini et al., 2008) via inhibiting resistance mediated by p-glycoprotein (Fryer et al., 2009). In Wattanapitayakul et al. (2005) the effects of *C. longa L* extracts on DOX-induced oncotic cell death demonstrated in Hela cells provided their potential use in selectively protecting cardiac cells during DOX treatment. Also, an additive oncostatic action of DOX and *C. longa L* extracts may be present (Notarbartolo et al., 2005). Thus, turmeric might have dual actions, control DOX mediated cardiotoxicity and decreasing oncogenesis.

Normally, superoxide is converted to hydrogen peroxide H_2O_2 that is detoxified by catalase and glutathione peroxidase to water and oxygen. In order to act, glutathione peroxidase needs reduced glutathione. GSH is regenerated from GSSG by the action of the enzyme glutathione reductase, which depends on the availability of NADPH which is generated by G6PD. H_2O_2 and other oxygen radicals can cause oxidation of critical SH groups in proteins and possibly peroxidation of lipids in the cell membranes causing lysis of these membranes.

It is widely accepted that DOX induced oxidative stress by its ability to disturb the above mentioned antioxidant

defense mechanisms of the tissues (El-Missiry et al., 2001). The decrease in the levels of blood GSH, cardiac ascorbic acid and total antioxidant capacity and the increase in cardiac catalase and serum G6PD activities are in agreement with those of Child et al. (2002) and Othman et al. (2008). The increased enzymes activities observed in our rats may be attributed to antioxidant gene over-expression that occurred in the heart in response to DOX treatment (Yilmaz et al., 2006). The induction of these enzymes is an adaptive response induced in cells exposed to oxidative stress. Inspite of the induction of defensive antioxidant enzymes catalase and G6PD, their protective abilities seem to be swamped by enhanced ROS. It is likely that the heart was attempting to detoxify the ROS but this effort was insufficient and the defense system was overwhelmed resulting in over use of GSH and vitamin C.

Prior administration of either turmeric extracts or lipoic acid modulated the levels of all antioxidants whether enzymatic such as catalase and G6PD, or non enzymatic such as GSH and ascorbic acid. Also cardiac total antioxidant capacity was elevated by both treatments. Venkatesan (1998) proved that DOX treated rats that received curcumin displayed augmentation of endogenous antioxidants by elevating their body contents or by preventing their depletion by stress. Meanwhile, Balachandar et al. (2003) demonstrated that lipoic acid ameliorated DOX antioxidant deleterious changes.

Curcumin is a hydrophobic molecule that passes easily through biological barriers into the cytosol of the cell (Quiles et al., 2002) effectively reduces the damage resulting from DOX-induced oxidative toxicity. Also, this lipophilic property permits high level of curcumin as antioxidant since DOX hardly affected the lipophilic antioxidants (Dziegiel et al., 2003).

From the data of the current study, it can be concluded that co-administration of turmeric extracts with DOX protect against acute DOX cardiotoxicity via ameliorating cardiac enzymes, modulating the pathways that trigger cardiac apoptosis such as decreased levels of GSH, increased calcium and over production of oxidant radicals (Childs et al., 2002) and finally, normalizing the antioxidant enzymes. Therefore, we could demonstrate that turmeric supplementation could be used in combination with DOX to protect against cardiomyopathy without attenuating the clinical efficacy of DOX, avoiding the need to take other medications, and improving the patients quality of life.

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