Protective effect of *Zingiber officinale* (ginger) on doxorubicin induced oxidative cardiotoxicity in rats.

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Abstract: *Zingiber officinale Roscoe* (Ginger) is a widespread herbal medicine, it bears an enormous number of pharmacological activities so the present study was undertaken to find out whether oral administration of ethanolic ginger extract (EGE) could exert any protective effect against doxorubicin (DOX) induced cardiotoxicity. DOX was i.p. injected to male albino rats in 4 equal groups; 8 each over a period of 2 weeks (cumulative dose, 15 mg/kg b. wt.). Protection from doxorubicin-oxidative injury was investigated by oral administration of Vitamin E (100 mg/kg) as a standard cardioprotective antioxidant and ethanolic ginger extract (200mg /kg. b. wt) once daily over a period of 6 weeks (4 weeks before and 2 weeks concurrently with doxorubicin). The results revealed that, DOX treatment induced marked ECG alterations & myocardial oxidative damage (represented by reduction in catalase activity and increase in lipid peroxidation & troponin 1 level), decreased survival rate and increased peritoneal fluid. Administration of vit. E and EGE before and concurrently with DOX significantly reduced the oxidative myocardial changes induced by DOX treatment, decreased mortality%, eliminated ascites and improved the ECG tracing. Histopathological observations supported the abovementioned biochemical results. This indicates that ethanolic ginger extract as well as vit.E provide protection from DOX-induced cardiac injury in terms of oxidative stress.
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1. Introduction

Medicinal plants have been used by all civilizations as a source of medicines since ancient times.There has been a growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, due to their natural origin, cost effectiveness and lesser side effects ***(Naik et al., 2003).*** Interest in medicinal plants as a re-emerging health aid in the maintenance of personal health and well-being has been fuelled by rising costs of prescription drugs, and the bioprospecting of new plant-derived drugs ***(Sharma et al., 2010)***.

Active oxygen species and free radicals play an important role in the pathogenesis of several human diseases, such as rheumatoid arthritis, cardiovascular diseases and cancer ***(Hertog et al., 1997)***. The antioxidant defense enzymes have been suggested to play an important role in maintaining the physiological levels of oxygen and hydrogen peroxide and eliminating peroxides generated from inadvertent exposure to xenobiotics and drugs. Any natural compound with antioxidant properties may help in maintaining health when continuously taken as components of dietary foods, spices or drugs ***(Singh, 2000).***

Nowadays, cardiovascular diseases (coronary artery disease, hypertension, heart failure, and stroke) are the leading causes of death in human beings ***(Parabathina et al., 2011)***. Oxidative stress, resulting from an imbalance in the generation of free radicals and antioxidant defense molecules, affects biological macromolecules causing their structural alterations that lead to cell damage and its death ***(Ryter et al., 2007).***This phenomenon is considered to be a major factor in the pathogenesis of a wide variety of disease states, including cardiovascular diseases (heart failure and atherosclerosis) cancer and diabetes ***(Sies, 1985 and Diaz 1997)***.In this regard, reduction of oxidative stress may be a good target for prevention and treatment of these diseases.

Considering the hazards of treatment failure, drug resistance and heavy costs associated with the current cardiac therapy, there is strong motive in the study of natural compounds with free radicals scavenging capacity. Medicinal plants, especially those with traditional use have always been considered as a rich source of antioxidants. *Zingiber officinale* Roscoe is one of these plants.

Among myriad of plants, *Zingiber officinale* Roscoe; commonly known as ginger; belongs to the family Zingeberaceae. and has been used as a spice and natural additives for more than 2000 years ***(Bartley and Jacobs, 2000).***Ginger has been used extensively in folklore medicine to treat a wide range of ailments including stomach pain, diarrhea, nausea, asthma, respiratory disorders ***(Grzanna et al., 2005).***

Ginger contains some chemical constituents as [6]-Gingerol, [6] -Shagol, Methyl [6] – isogingerol and paradol which are responsible for its cardio protective anti-inflammatory, anti-microbial,

antioxidant, anti-proliferative, neuro-protective and hepatoprotective activities ***(Ghosh et al.,2011)***. It also has hypoglycaemic and hypolipidaemic effect ***(Ahmed and Sharma, 1997)***. According to ***Mustafa et al. (1993)***, Z. *officinale* was found to inhibit the activity of cyclooxygenase and lipoxygenase and hence decrease the pain in rheumatism and headaches.

Topic et al. (2002) stated that, *Z. officinale* could inhibit lipid peroxidation by maintaining the levels of antioxidants in the serum of rats treated with malathion. This is in concordance with an earlier study which found that 6- gingerol in *Z. officinale* was a potent scavenger for the peroxyl radical which is the main product of lipid peroxidation ***(Aeschbach et al. 1994).*** Regular intake of ginger in diet can protect against oxidative tissue damage, ***(Nirmala et al., 2007).***

Doxorubicin anthracycline is an anticancer drug used against solid tumors, leukemia, breast cancer, small cell carcinoma of lung and esophageal carcinoma ***(Koti et al., 2009)***. Doxorubicin induced cardiomyopathy may result in progressive heart failure after anti-neoplastic therapy, thus limiting the application of this potent chemotherapeutic agent ***(Osman et al., 2009)***.The mechanisms proposed that doxorubicin bound with ferric iron to stimulate the production of reactive oxygen species (ROS) that leads to impairment of cell functioning and cytolysis and also bound to β- glycoprotein to stimulate the production of caspase ***(Murdoch et al., 2006 and Appia Krishnan et al., 2009)*** and apoptosome that cause DNA damage. DNA damage in proliferative cells activates a pathway that arrest cell division that allow either DNA repair or the induction of cell death by apoptosis ***(L'Ecuyer et al., 2009)***.The vulnerability of the heart to ROS is further intensified by doxorubicin inhibition of ROS neutralizing enzymes ***(Kalivendi et al., 2005)***. Although, cardiotoxicity associated with chronic administration of doxorubicin represents a serious complication of its use, it can also serve as a useful experimental model of cardiomyopathy and congestive heart failure for the evaluation of potential cardioprotective agents ***(Simunek et al., 2004)***.

In the light of aforementioned medicinal properties of *Zingiber officinale*, the present study was undertaken to find out whether oral administration of ethanolic ginger extract could exert any protective effects against doxorubicin (DOX) induced cardiotoxicity on the level of oxidant production.

2. Material & Methods

Plant material:

Ginger rhizomes were purchased from local market in Sharkia, Egypt. For preparation of ethanolic ginger extract(EGE), dried and finely powdered rhizomes of plant (500 g) were macerated with 1250 ml of ethanol 70% for 72 hours at room temperature with interval stirring. The extract was fine-filtered by using gauze and funnel then concentrated on a water bath at 40 °C, then freeze dried. The yielded extract was 5 g semisolid mass which stored at 4°C for further experimental studies.

Agents:

- **Doxorubicin** (ADRIAMYCIN)® each vial contains 20 mg Doxorubicin HCL (Pfizer, Egypt).
- **Vitamin E (**Vitamin E capsules**)** each capsule, 400mg Vitamin E (PHARCO pharmaceuticals, Egypt). Capsules of vitamin E were cut open and poured in a clean container. Vegetable oil was added in a rate of 0.1ml/ rat ***(Zdunczyk et al., 2002)***for dissolving vitamin E to be suitable for administration

Experimental animals:

The study was carried out on 32 adult male albino rats, each weighing 130-170g, obtained from Faculty of Veterinary Medicine, Zagazig University. All rats were kept under observation and acclimatization period of one week to the laboratory environment before starting the experiments. They were kept under hygienic condition in metal cages and fed on barley and milk all over the experimental period and water was provided *ad- libitum*.

Experimental protocol:

After one week of acclimatization, rats were randomly allocated into 4 groups, each of 8 rats as follow:

Group (1): Rats in this group served as **control** and orally received 0.2 ml vegetable oil once daily for 6 weeks.

Group (2): Vegetable oil + DOX (2.5 mg/kg b.wt) i.p. **(DOX)**

Group (3): Vit E (100 mg /kg b.wt) orally (a standard cardioprotective antioxidant (***Khatib et al., 2011)*** + DOX. ***(E+DOX)***

Group (4): 200 mg /kg b.wt of EGE ***(Ansari et al., 2006)*** + DOX. ***(EGE+DOX)***

All rats were treated with vegetable oil or ginger or vit E once daily over a period of 6 weeks (4 weeks before and 2 weeks concurrent with doxorubicin). After 4 weeks from the starting of the experiment, rats of all groups except group (1) were i.p administered DOX at a dose of 2.5 mg/kg b.wt every other day for 2 weeks ***(Hania et al., 2011)***.

Mortality and general condition of rats were observed daily throughout the whole experimental period (6 weeks). Body weights were recorded 2 times per week during the treatment and until the end of experiment.

Electrocardiography (ECG) monitoring:

ECG was recorded 24 hrs after the last dosing of DOX. All rats were fasted overnight but had free

access to water after the last dose of administration. Oscillograph (Bioscience, UK) Instrument was used to record and monitor ECG tracings. Rats from each group were anesthetized with thiopental sodium (40 mg/kg b.wt), needle electrodes were inserted under the skin for the limb lead at position II. For each ECG tracing QT, ST intervals, QRS complex and heart rate were measured.

Determination of serum level of cardiac injury biomarker (Troponin1):

Rats were sacrificed after ECG, blood samples were collected and serum was separated and kept frozen at -20^{o}C until used for estimation of **Troponin 1 (**biomarker of cardiac injury) using chemiluminescence immunoassays according to ***Melanson et al. (2007)***

Oxidant/ antioxidant status of cardiac tissue:

Heart samples (0.5 g each) were homogenized in 5 mL of saline at 4 °C with an electrical homogenizer. Homogenates were then centrifuged at 3000 rpm for 15 min. The resulting supernatant were collected and used for estimation of catalase **(CAT)** activity according the method described by ***Aebi(1984)*** and **malondialdehyde (MDA)** concentrations according the method described by ***Esterbauer et al. (1982).***Tissue homogenates were preserved at -20°C until performing the investigations.

Histopathological examination:

Specimens from the hearts of different groups were kept in 10 % neutral formalin and processed in paraffin wax. Sections of 5 microns thickness were stained with Haematoxyline and Eosin (H & E) and examined microscopically for histopathology ***(Bancroft and Stevens, 1996).***

Statistical analysis:

The obtained data was analyzed by using the statistical package for social science (**SPSS**, **15.0 software, 2008**). Data are presented as mean ± SEM. $P \leq 0.05$ were considered significant.

3. Results

General observation and mortality:

Our results revealed that,37.5% of rats belonged to ***DOX*** treated group were died compared to 12.5% in the ***EGE +DOX*** group,however, no mortality was observed in the other groups (Table 1). Rats in the DOX treated group appeared weak, lethargic with weight loss. those rats also showed scruffy fur with a light yellow tinge, red exudates around the eyes & nose with soft watery feces. Strikingly, rats belonged to that group developed ascites, as determined by a grossly distended abdomen and later confirmed during necropsy. The hallmark gross pathologic changes in DOX treated rats were presence of excessive amounts of pericardial, pleural and peritoneal fluids. These observations were significantly milder in ***E+DOX*** and ***EGE + DOX***. Groups.

Heart weight, body weight and heart weight / body weight ratio:

Administration of DOX to rats significantly *($p<0.05$)* decreased the body weight and heart weight and significantly *($p<0.05$)* increased the ratio of heart weight to body weight compared to the control group. Pre- and concurrent treatment with vit E and ethanolic ginger extract significantly ($p < 0.05$) increased body weight and heart weight and significantly *($p<0.05$)* decreased the ratio of heart weight to body weight compared to DOX treated group (Table 1).

Evaluation of ECG tracing:

ECG tracing showed normal cardiac activity in the control group with a mean heart rate of 210±3.53 beat/min Fig.(1-a). Rats in the DOX treated group showed several ECG changes including bradycardia (141±3.31 beat/min), a significant *($p<0.05$)* prolongation in ST, QT intervals and widening of QRS complex Fig.(1-b).Such ECG abnormalities were obviously improved in the ***E+DOX*** and ***EGE+DOX*** groups as evidenced by normalization of heart rate, ST, QT intervals and QRS complex Fig.(1-c,d) & (Table 2).

Biomarker of cardiac injury:

Troponin I; serum marker indicating myocardial injury; was significantly ($p < 0.05$) elevated in the DOX treated group compared with control. The pre- and concurrent treatment with vit E and ethanolic ginger extract significantly ($p < 0.05$) reduced its level compared with DOX treated group (Table 3).

Oxidant/ antioxidant status of cardiac tissue:

Our results showed that, administration of DOX to rats significantly ($p <0.05$) decreased the cardiac activity of catalase (CAT) and significantly ($p<0.05$) increased the cardiac malondialdehyde (MDA) concentrations compared to the control group. It was observed that, the pre- and concurrent treatment with vit E and ethanolic ginger extract guard against the decreases in the activity of this enzyme and the increases of MDA concentrations (Table 3).

Histopathological examination of the cardiac tissues:

The heart of control group showed normal cardiac myocytes with centrally located nuclei. Rats administered DOX showed typical myocardial toxicity in a form of multifocal areas of coagulative necrosis (Zenker's) and round cells aggregations. The affected cardiac muscles were swollen, more eosinophilic with pyknotic and absence of its nuclei. Sometimes, these myocytes were severely vacuolated (fatty change) and infiltrated with mononuclear cells. Serous pericarditis was focally seen and represented by pale eosinophilic granular material and few lymphocytes infiltration. Congestion of the cardiac

blood vessels, edema and hemorrhages were also detected in the cardiac tissues belonged to such group. Meanwhile, the lesions in ***E+DOX*** group were ameliorated or completely absent except for few swollen, degenerated and rarely necrotic myocytes with no evidence of leukocytes infiltrations. The pre- and concurrent treatment with ethanolic ginger extract was significantly lowered the lesions of DOX that represented by focal Zenker's degeneration and necrosis. Slight edema and extravasated erythrocytes were also seen particularly adjacent the necrosis(Table 4 and Plate I).

Taken together, our results give a strong evidence about the cardioprotective effect of EGE in Dox treated rats represented by the improvement of all the above mentioned parameters. Strikingly, the obtained results of EGE treated group were, to a great extent, similar to those obtained from vit.E treated group.

Table(1): Effect of Vit E and ethanolic ginger extract (EGE) on body weight, heart weight, heart weight to body weight % and mortality % in rats treated with doxorubicin (n=5).

Groups	Body weight (g)	Heart weight (g)	Heart weight/ Body weight%	Mortality %
Control	222.4±3.35^{a}	0.840±0.019^{a}	0.377±0.006^{c}	0
DOX	130±6.51^{c}	0.660±0.040^{b}	0.506±0.009^{a}	37.5
E+DOX	187.6±3.50^{b}	0.776±0.035^{a}	0.414±0.011^{b}	0
EGE+DOX	179.4±5.03^{b}	0.744±0.026^{a}	0.412±0.007^{b}	12.5

Means with the same column carrying different superscripts are significant at ($p < 0.05$).

Table(2): Effects of Vit E and ethanolic ginger extract (EGE)on ECG tracing in rats treated with doxorubicin (n=5).

Groups	QT(sec)	ST(sec)	QRS(sec)	Heart rate (b/min)
Control	0.112±0.01^{b}	0.075±0.005^{b}	0.06±0.006^{c}	210±3.53^{a}
DOX	0.168±0.004^{a}	0.108±0.0049^{a}	0.12±0.006^{a}	141±3.31^{c}
E+DOX	0.132±0.10^{b}	0.084±0.0075^{b}	0.088±0.013^{bc}	184±5.09^{b}
EGE+DOX	0.124±0.11^{b}	0.088±0.0049^{b}	0.092±0.01^{b}	176±5.99^{b}

Means with the same column carrying different superscripts are significant at ($p < 0.05$).

Table(3): Effect of Vit E and ethanolic ginger extract (EGE)on the level of Troponin 1, CAT activity and MDA concentrations in rats treated with doxorubicin (n=5).

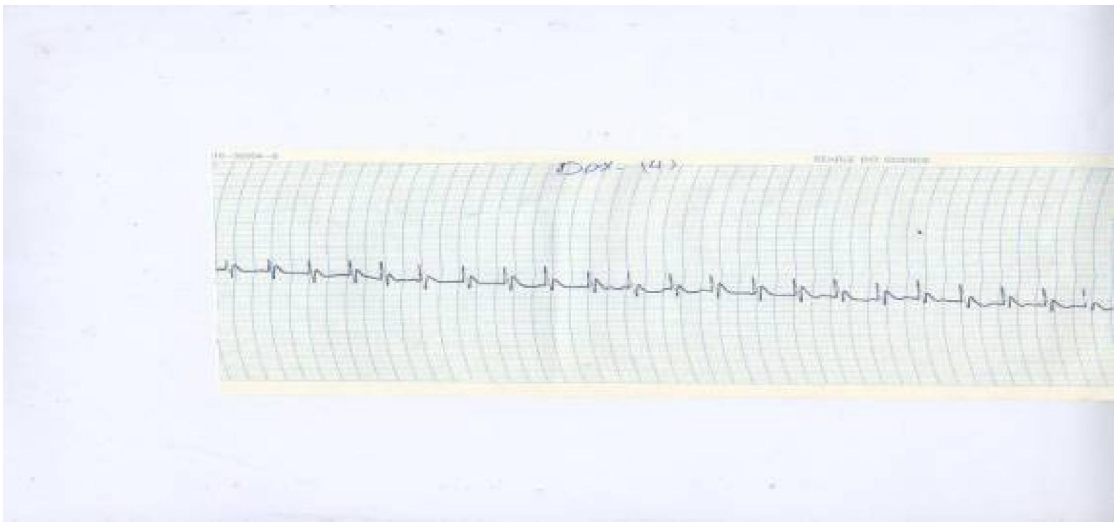
Groups	CAT(U/g.tissue)	MDA(nmol/ g.tissue)	**Troponin I** (ng/ml)
Control	1.7621±0.04^{a}	23.29±2.05^{c}	0.004±0.0024^{d}
DOX	0.743±0.15^{b}	87.62±8.18^{a}	0.122±0.0086^{a}
E+DOX	1.5688±0.017^{a}	43.22±5.22^{b}	0.030±0.0071^{c}
EGE+DOX	1.519±0.03^{a}	39.52±5.02^{c}	0.058±0.0116^{b}

Means with the same column carrying different superscripts are significant at ($p < 0.05$).

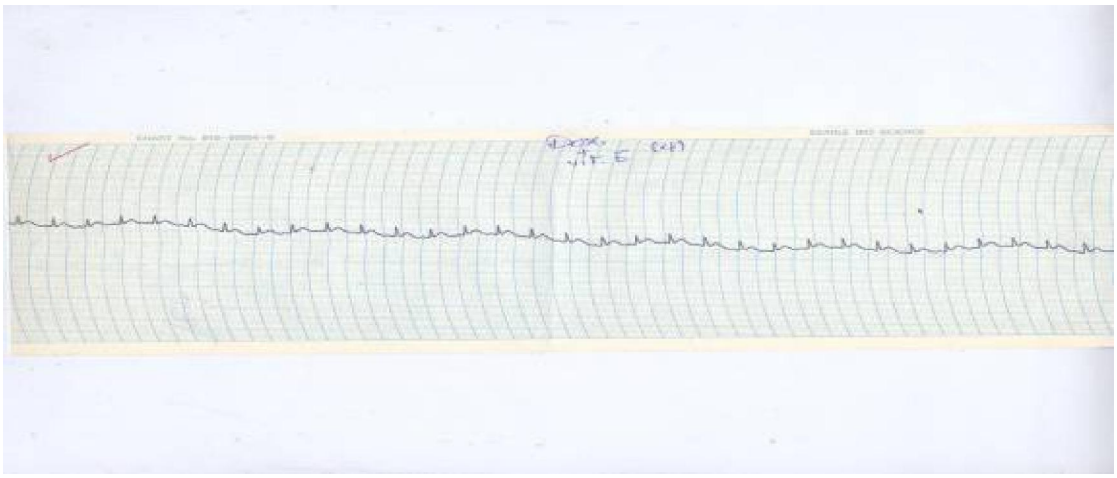
Table (4): Effect of Vit E and ethanolic ginger extract (EGE)on the lesion scores of the heart in rats treated with doxorubicin

Groups	The Lesions in the Hearts				
	Necrosis	Leukocytes aggregations	Degeneration and vacuolations	Edema	Hemorrhage
Control	-	-	-	-	-
DOX	++++	++++	++++	+++	++
E+DOX	±	-	+	+	++
EGE+DOX	++	+	++	+	+

1-a



1-b



1- c

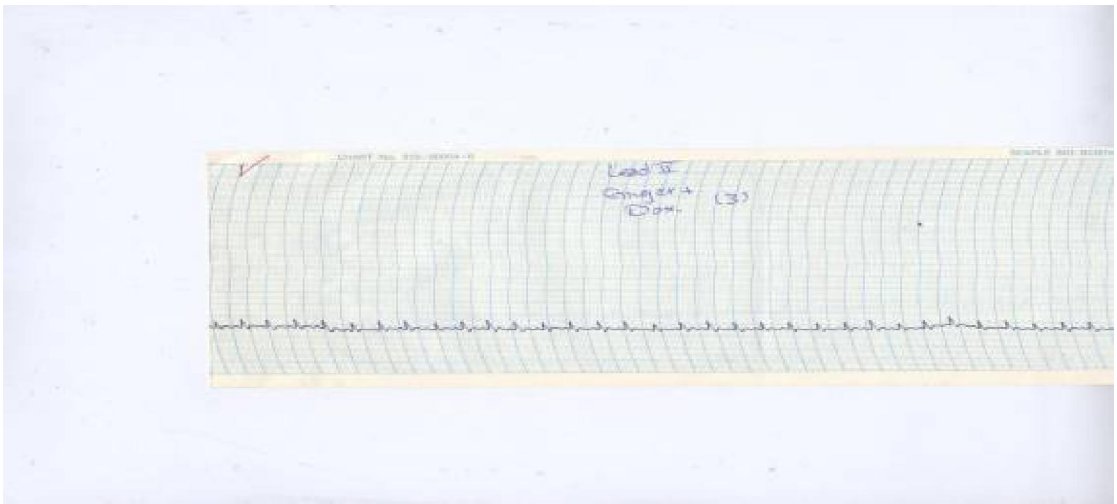


Fig.(1) showing normal ECG tracing in the control rats **(a)** and severe ECG changes in DOX treated rats **(b)** while **(c,d)** illustrating the improving Effects of Vit E and ethanolic ginger extract (EGE) respectively on ECG tracing in rats treated with doxorubicin

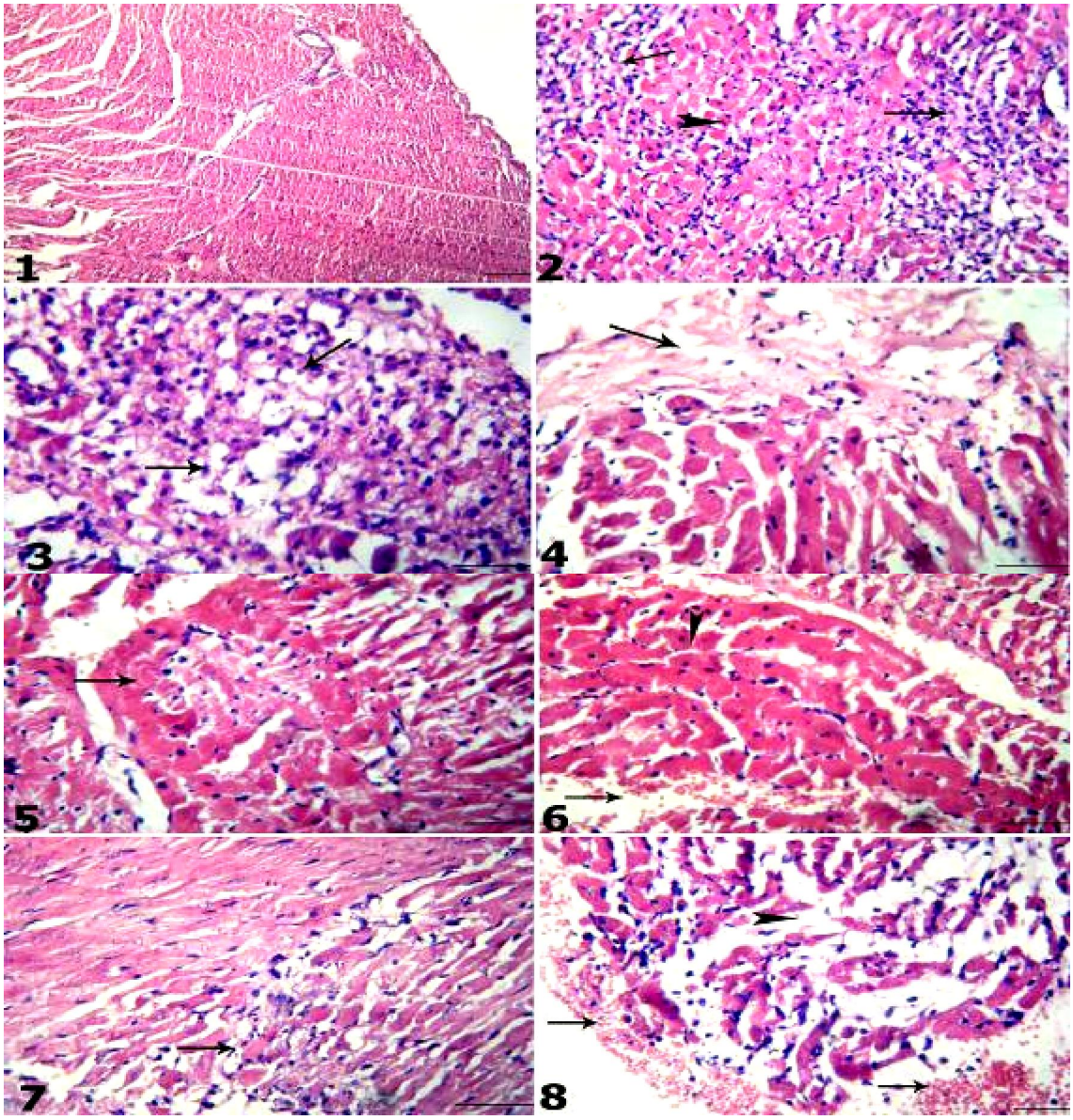


Plate (I): Heart sections from rats exposed to different treatments showing normal cardiac myocytes with centrally located nuclei in the control group **photo (1).** DOX treated group shows extensive Zenker's necrosis (arrowhead) and round cells aggregations (arrows) **photo (2)**, severe vacuolations of myocytes and round cells infiltrations (arrows) **photo (3)** and pale eosinophilic granular material at the pericardium infiltrated with few lymphocytes (arrow) **photo(4)**. DOX + Ginger show focal necrotic area with pyknotic and absent nuclei (arrow) **photo (5)** and hemorrhage (arrow) among the necrotic myocytes (arrowhead) **photo (6)**. DOX + Vit E show few swollen, degenerated and necrotic myocytes (arrow) **photo (7)** and hemorrhage (arrow) and mild edema among normal myocytes (arrowhead) **photo (8)**. **H&E** (Bar = 100 µm).

4. Discussion

For centuries, ginger has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines (***Ghosh et al., 2011).*** It is not only one of the most popular of all the spices but also of the top five antioxidant foods ***(American Journal of Clinical Nutrition, 2006)*** because it contains more than 50 antioxidants ***(Masuda, 2004***).

On the other hand, doxorubicin (DOX) continues to be an effective and widely used broad spectrum

chemotherapeutic agent. However, its clinical use is limited because of its serious dose dependent cardiotoxicity ***(Singal and Iliskovic, 1998)***. Clinical and experimental investigations suggested that increased oxidative stress plays a critical role in subsequent cardiomyopathy and heart failure associated with DOX treatment ***(Mihm et al., 2002).***

The current work was undertaken to find out whether oral administration of ethanolic ginger extract (EGE) could exert any protective effects against DOX induced cardiotoxicity on the level of oxidant production.

In the present study, DOX administration accompanied by a high mortality rate (37.5%) compared to the control group that is consistent with the results obtained by ***El-sayed et al. (2011)*** who found that a single i.p. injection of DOX for rat at a dose of 15 mg /kg b.wt. induced 50% mortality. In a similar way ***Shakya et al. (2011)*** reported that in rats,i.p. administration of Dox in 6 equal injections (each dose containing 2.5 mg/kg body weight) alternatively for 2 weeks to make a total cumulative dose of 15 mg/kg b. w. resulted in 75% mortality. The majority of authors dealing with DOX toxicity consider the resultant cardiomyopathy and the nephropathy are the most important contributors to the mortality observed in the experimental rats after treatment with DOX ***(Herman et al., 2000)***.

Our results revealed that, Live rats belonged to DOX treated group showed excessive fluid accumulation in pericardial, pleural and peritoneal cavities. In keeping with this line, ***Elberry et al. (2010***) illustrated that, a single i.p. administration of DOX (15 mg/kg b.wt) provoked a high mortality, and live rats showed excessive degree of pericardial, pleural and peritoneal effusion. Ascites has been reported to be a characteristic of DOX-induced heart failure ***(Kim et al., 2005)***. The existence of mortality and ascites could be explained on the basis of the development of heart failure.

Accumulation of ascitic fluid as well as significant decreased body weight and heart weight indicating a severe dysfunction in cardiac performance. Our findings are in agreement with those of ***Kelishomi et al.(2008)*** who stated that, administration of DOX (1.25mg/kg i.p.), 4 times per week, to rats for 4 weeks accompanied by 50% mortality rate, significant decline in body mass and severe effusion intensity score. Likewise, ***Momin et al. (2012)*** recorded that, rats treated with Dox at a total cumulative dose of 15 mg/kg i.p for 2 weeks in six divided dosage showed a decrease in body weight, heart weight.

Decrease of body weight caused by DOX administration may be attributed to the direct toxic effects of DOX on intestinal mucosa which associated with apoptosis leading to destruction of the normal mucosal architecture and loss of intestinal stem cells ***(Dekaney et al., 2009)*** and additional indirect action on the gastrointestinal tract could arise from reduced food intake that causes decrease in the secretion of internal hormones resulting in decreased trophic effects to the mucosa and inhibition of protein synthesis ***(Herman et al., 2000)***.

Reduction in heart weight in DOX treated rats indicate loss of myofibrils and myocardial necrosis and cardiomyocyte death***(Weinberg and Singal, 1987)***. This suggestion could be confirmed, in the current study by histopathological observations which revealed myocardial coagulative necrosis, myocytes vacuolization, mononuclear cellular infiltration as well as vascular congestion. Increased heart weight / body weight ratio may be due to severe emaciation of Dox treated rats. The previous changes in body weight, heart weight and heart weight / body weight ratio are attributable, at least in part to the toxic effects of reactive oxygen species (ROS) produced by DOX.

Interestingly, this study give an evidence that the pre- and concurrent treatment with vit E and ethanolic ginger extract ameliorated the development of DOX cardiotoxicity. This was indicated by zero mortality in Vit.E treated group & 12.5% in EGE treated group, the increase in the body weight, and the relative normalization in heart weight as well as heart/body weight ratio in both groups. Their ability to protect against DOX-induced mortality and ascites was considered an early sign of cardioprotection of vit E and ginger which may be attributed to their antioxidant activity.

ECG changes are one of the most reliable parameters for assessing Dox-induced cardiotoxicity ***(Xu et al., 2010).*** In Dox treated rats, significant changes in the ECG, such as prolongation of the QT, ST intervals, widening of the QRS complex and bradycardia have been reported, whereas the QRS-intervals are directly related to cell depolarization, the QT interval is an expression of the late repolarization phase; Dox specifically prolong the later phase by disturbing the ion flux across the myocellular membrane which is related to the morphological injuries caused by Dox. These changes reflected arrhythmias, conduction abnormalities and attenuation of left ventricular function. Similar changes in ECG tracing have been reported by other studies ***(Shah et al., 2009 and Elberry et al., 2010)*** who illustrated that, the ECG changes induced by DOX consisted of prolongation of QT, ST intervals and widening of QRS complex.

In our study, ECG tracings from vit E and ethanolic ginger extract treated groups verified a guardian role for vit E and ginger, represented by

normalization of heart rate, QT, ST, intervals and QRS complex. In a like manner ***Puri et al.(2005) and Shah et al. (2009)*** documented that, vitamin E pretreatment prevented the PR, QT, ST segment and QRS complex changes caused by DOX and there is sufficient evidence proved that, vitamin E protects the rat myocardium from doxorubicin-induced damage.

The production of ROS is considered to be the backbone in DOX-induced cardiotoxicity ***(Takemura and Fujiwara, 2007and Fu et al., 2010)***. ROS can take up electrons from the lipids in cell membranes, resulting in cell damage. This process of oxidation of the fatty membranes is called lipid peroxidation which contributes to oxidant-induced cell death. Malondialdehyde (MDA), a major and stable end product formed of peroxidation, is regarded as a marker of lipid peroxidation ***(Del et al., 2005).*** Furthermore, ROS can also cause mitochondrial structural and functional damage, which may result in cardiomyocyte apoptosis or death ***(Menna et al., 2007)***. Thus, the scavenging of excessive ROS by antioxidants may be effective in preventing oxidative cell death. Cells have evolved different antioxidants to neutralize ROS which can suppress lipid peroxidation, hence these antioxidants are absolutely critical for inhibiting oxidative stress-induced cytotoxicity. Antioxidant enzymes, such as CAT, GPx and SOD, are a class of enzymes capable of inhibiting the oxidation and are major intracellular antioxidant defenses in cells. It has been shown that the over expression of antioxidant enzymes can provide protective effects against the ROS-induced cardiomyocytes damage ***(Liu et al., 2009)***.Current study evidenced that the activity of the cardiac antioxidant enzyme CAT was significantly reduced while that of MDA concentrations were significantly elevated in response to DOX administration compared to the control rats. Such data clearly indicated an overt oxidative stress. These data are in accordance with those reported by ***(Thippeswamy et al., 2011 and Ragavendran et al., 2012)*** who found that, DOX administration in rat evoked a significant decrease in catalase activity and a significant elevation in MDA concentrations**.** The observed decrease in the catalase activity can be explained on the basis of its exhaustion in combating the DOX-induced free radicals which interact with biomembrane resulted in increase of MDA concentrations.

Our findings clearly demonstrated that, Pre- and concurrent treatment of rats with vit E and ethanolic ginger extract significantly guarded against the oxidative stress observed in the DOX group. They mitigated the decrease of catalase activity that explained and confirmed the reduction of MDA concentrations in the cardiac tissues.

Gust as likely, pretreatment with vit E ***(Mohanty et al., 2009)*** and ethanolic ginger extract ***(Ansari et al., 2006)*** enhanced the antioxidant defence, by increase catalase, superoxide dismutase activity and decrease lipid peroxidation against isoproterenol induced oxidative myocardial injury in rats.

In the same context, it is extremely to the purpose to bring up the fact that, vitamin E, the most important lipid-soluble antioxidant, is incorporated into cellular membranes in which it effectively inhibits lipid peroxidation ***(Packer et al., 2001).*** It acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues ***(Traber and Stevens 2011).***

On the other hand, this effect of EGE can be attributed to its high content of polyphenolic compounds (6-gingerol and its derivatives) which have a high antioxidant activity. It has a very good scavenging of 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) and decreased its reducing capacity. The ginger extract is a powerful hydroxyl radicals scavenger and it was shown to inhibit lipid peroxidation ***(Liu et al., 2003)***. The polyphenols in the ginger extract also demonstrated a higher chelatoforming capacity with regard to Fe^{3+}, leading to the prevention of the initiation of hydroxyl radicals which are known inducers of lipid peroxidation ***(Stoilova et al., 2007)***.

On similar ground, ***Sekiwa et al., (2000)*** reported that, glucosides related to gingerdiol from ginger have antioxidative activity. Gingerols pungent principles in ginger, inhibited xanthine oxidase activity responsible for the generation of ROS, such as superoxide anion ***(Chang et al., 1994)***.

Likewise, ***Mansour et al. (2008)*** documented that, 6-gingerol act as a potentially selective cardioprotective agent, against cardiotoxicity induced by doxorubicin by augmentation of endogenous myocardial antioxidants activities.

In fact there is a hypothesis that the cardiac dysfunction associated with DOX is attributable, at least in part, to cardiac cell apoptosis resulted from ROS generation. In supporting to this notion, our results demonstrated that level of troponin 1,which released from damaged myocytes and the most specific highly sensitive powerful biomarker of drug-induced cardiotoxicity ***(Babuin and Jaffe, 2005)*** was extremely elevated in DOX treated group indicating severely damaged heart tissue. Similar observations were previously reported by ***(Ahmed et al., 2005 and Elberry et al., 2010)*** who stated that, i.p. injection of a single dose (15mg/kg B.W.) of doxorubicin resulted in significant increase in serum level of troponin I.

The extent of cardioprotection offered by vit E and ethanolic ginger extract is associated with a significant attenuation of serum levels of troponin I. A

possible explanation is that, vit E and ginger, via their effect against lipid peroxidation, causes stabilization of myocytes membrane and prevents the leakage of troponin1 into serum.

The forementioned biochemical data and ECG abnormalities were further strengthened by histopathological examination of rats' hearts. They showed cardiac injury in the form of multifocal areas of coagulative necrosis (Zenker's) and round cells aggregations. The affected cardiac muscles were swollen, more eosinophilic with pyknotic and absence of its nuclei. Sometimes, these myocytes were severely vacuolated (fatty change) and infiltrated with mononuclear cells. Serous pericarditis was focally seen and represented by pale eosinophilic granular material and few lymphocytes infiltration. Congestion of the cardiac blood vessels, edema and hemorrhages were also detected. The severity of the histological changes was much less in sections from pre and concurrent treated rats with vit E and ethanolic ginger extract. Thus, the observed maintenance of the cardiomyocyte integrity would lead to decreased leakage of cardiac markers.

In conclusion, our data indicated that, in comparable to vit.E, oral administration of ethanolic ginger extract protects against DOX-induced cardiotoxicity in rats as evidenced by improved mortality and ascites, mitigation of ECG abnormalities, restoration of the oxidant/ antioxidant status, reducing the cardiac injury marker (troponin 1) as well as lessening the resultant histopathological changes. These effects can be attributed, at least in part, to its antioxidant activity.

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