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Full Length Research Paper

The possible protective effect of *Zingiber officinale* extract on cyclophosphamide-induced cardiotoxicity in adult male albino rats

Nashwa Mohamad M. Shalaby[1], Abeer Ramzy H. Mahmoud[1*], Nagah El-Sayed M. Ali[2], Nahla E. Ibrahem[3] and Noura Hussein Abdel H. Mekawy[3]

[1]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Zagazig University, Egypt.
[2]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Banha University, Egypt.
[3]Department of Histology, Faculty of Medicine, Zagazig University, Egypt.

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Cyclophosphamide, a cytotoxic alkylating agent, is an extensively used antineoplastic agent. Cardio-toxicity is considered as one of the limiting side effects of its use, which is attributed to oxidative stress. *Zingiber officinale* is powerful antioxidants against free radicals and oxidative attacks. The aim of this study is to investigate the possible protective effects of *Z. officinale* against cyclophosphamide induced cardio-toxicity in adult male albino rats. We used 30 adult male albino rats, divided into five groups; Group Ia (-ve control), Group Ib (+ve control), Group II (*Z. officinale* treated group; 200 mg/kg/day orally), Group III (cyclophosphamide treated group; single dose of 150 mg/kg I.P.), and Group IV (cyclophosphamide and *Z. officinale* treated group; rats received *Z. officinale* as before followed by single dose of cyclophosphamide (150 mg/kg)). At the end of experiment, we studied biochemical parameters: oxidative markers (MDA, GSH and Catalase), and Troponin i. The heart tissue was examined by light and electron microscope to evaluate histo-pathological changes and immune-histochemical technique for localization of inducible nitric oxide synthase (iNOS) in the cytoplasm of cardiomyocytes. The result showed increase in troponin I and disturbance of oxidative markers in cyclophosphamide treated group compared to control groups. Whereas these results had significantly improved in cyclophosphamide and *Z. officinale* treated group. Light and electron microscopic examination revealed disruption in the heart tissue histo-architecture in cyclophosphamide group with strong positive cytoplasmic iNOS immunoreaction in numerous cardiomyocytes by histochemical examination unlike that in cyclophosphamide and *Z. officinale* treated group which returned near normal. In conclusion, cyclophosphamide has a cardiotoxic effect which can be prevented by *Z. officinale* supplementation. Further studies about cyclophosphamide toxic effect on the heart and about *Z. officinale* role in protection are recommended.

Key words: Cyclophosphamide, *Zingiber officinale*, cardiotoxicty.

INTRODUCTION

Cyclophosphamide is an alkylating agent having antineoplastic activity and immunosuppressive properties in many conditions like organ transplantation and systemic lupus erythematosus (Nagler et al., 2013).

Cardiotoxicity associated with high-dose cyclophosphamide has been described as a complication of several therapeutic regimens (Kamezaki et al., 2005) and the incidence of fatal cardiomyopathy varies from 2.0 to 17.0%, depending on the different regimens and patient populations (Taniguchi, 2005). It has been suggested that in order to benefit from the cyclophosphamide at higher doses, a protective agent was needed that would eliminate the toxic side effects of cyclophosphamide. Plasma antioxidant concentration has shown a decrease of patients who had a high dose chemotheraphy (Sabuncuoglu and Ozgunes, 2011).

Numerous studies had shown that cyclophosphamide exposure enhances intracellular reactive oxygen species (ROS) production, suggesting that biochemical and physiological side effects may result from its oxidative stress (Manda and Bhatia, 2003). *Zingiber officinale* (ginger), family Zingiberaceae is obtained from the underground stems or rhizomes of Zingiber. It is consumed as a fresh paste, dried powder, slices preserved in syrup, or candy or for flavoring tea. The underground stem or rhizome of this plant has been used as a medicine in Asian, Indian and Arabic herbal traditions since ancient times (Altman and Marcussen, 2001). It has been used in herbal medicine practice for the treatment of arthritis, rheumatologic conditions and muscular discomfort. Also, it has also been suggested for the treatment of various other conditions, including atherosclerosis, migraine headaches, rheumatoid arthritis, high cholesterol, ulcers, depression, and impotence. In addition to these medicinal uses, ginger continues to be valued around the world as an important cooking spice and is believed to help against common cold, flu-like symptoms, and even painful menstrual periods (Grant and Lutz, 2000). Its root contain polyphenol compounds (6-gingerol and shogaol), which have a high antioxidant activity (Stoilova et al., 2007). We aimed to investigate the possible beneficial protective effect of *Z. officinale* extract on cyclophosphamide induced cardiotoxicity in adult male albino rats.

MATERIALS AND METHODS

Animals

In this study 30 male Sprague-Dawley albino adult rats, weighing 200–220 g, were used. Animals were fed *ad libitum* and housed in pairs in steel cages, having a temperature-controlled environment (22 ± 2°C) with 12 h light/dark cycles. Animal housing and handling were ethically considered. Painless procedures were conducted.

Chemicals

Cyclophosphamide was purchased from SIGMA-Aldrich (St. Louis, MO), HCY and CEPM were purchased from Santa Cruz Biotechnology (Dallas, TX).

***Zingiber officinale*:** Fresh ginger (*Z. officinale* Roscoe, Zingiberacae) rhizomes were purchased from local commercial sources. The botanical authentication and extraction were done at the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University.

Extraction of *Z. officinale* ethyl acetate extract

Fresh ginger rhizomes (400 gm) were divided, macerated and saturated with cold methanol (2 L) .The extract was filtered and subjected to drying. The residues were re-extracted for 3 times. The dried extract was dissolved in least amount of methanol (50 mL) and distilled water H2O was added, then fractionation was done by ethyl acetate using separating funnel at room temperature. *Z. officinale* ethyl acetate extract was evaporated to dryness under reduced pressure at 40°C using rotatory evaporator (Hei- VAP Value Digital, Germany) (Lakshmi and Sudhakar, 2010).

Experimental protocol

The experimental animals were randomly divided into five equal groups each of 6 rats.

(i) Group Ia served as -ve control group and received regular diet and water for 10 days.
(ii) Group Ib (+ve control; gum acacia treated group): Each rat received a daily 0.5 ml gum acacia 1% aqueous solution (used for preparation of oral suspension of *Z. officinale* for 10 days by oral gavage.
(iii) Group II (*Z. officinale* treated group) (6 rats) Rats received a daily 200 mg/kg/day of *Z. officinale* for 10 days by oral gavage (El-Sharaky et al., 2009).
(iv) Group III (cyclophosphamide treated group) (6 rats) rats were injected intra-peritoneal with single dose of cyclophosphamide (150 mg/kg) (Gado et al., 2013).
(v) Group IV (cyclophsphamide and *Z. officinale* treated group) (6 rats) rats received *Z. officinale* as before followed by single dose of cyclophosphamide (150 mg/kg). Forty eight hour after cyclophosphamide treatment, under light ether anesthesia, venous samples from the retro-orbital plexus were obtained by capillary glass tubes as described by Schemere (1967). Rats were sacrificed and heart of each rat was dissected and grossly inspected to assess any gross abnormalities; then they were washed with cold normal saline and used for oxidative markers measurement, light and electron microscopic examination and immunohistochemical staining.

Biochemical parameters

Oxidative markers

(1) Determination of MDA (nmol/ g tissue) : MDA was assayed

*Corresponding author. E-mail: lovelylomy_2009@yahoo.com

colorimetrically according to the method proposed by Ohkawa et al. (1979).
(2) Determination of GSH (mmol/g tissue): GSH was assayed colorimetrically according to the method proposed by Beutler et al. (1963).
(3) Determination of catalase activity (U/g): Catalase activity was assayed colorimetrically according to the method proposed by Aebi (1983).

Troponin i: Was measured according to the method proposed by Collinson et al. (2001).

Light microscope examination

The heart was fixed in 10% formalin solution. After fixation, tissues were embedded in paraffin blocks and processed for 5 um thickness sections. These sections were stained by Hematoxylin and Eosin stains (Bancroft and Layton, 2013) and then examined by light microscope.

Immunohistochemical technique

It was used for localization of inducible nitric oxide synthase (iNOS) in the cytoplasm of cardiomyocytes. For the immunohistochemical staining of iNOS, paraffin sections were cleaned in xylene, hydrated, and then placed in phosphate buffered saline (PBS; pH 7.6). Sections were treated with 3% hydrogen peroxide and then washed with PBS. Sections were incubated, first with 1% pre-immune rabbit serum to decrease non-specific staining then with a monoclonal antibody against iNOS (1:200 dilutions; (clone 1A4, code No. 0034851). Detection of the antibody was performed using a biotin–streptavidin detection system. The positive results for iNOS immunoreactions were indicated by brown cytoplasmic staining (Kiernan, 2008; Bancroft et. al., 2013).

Electron microscope examination

Minute specimens were rinsed in 0.1M phosphate buffer saline (PBS) pH 7.2 to remove blood from the surface. heart tissues greater than 2 cm long were minced into smaller pieces of approximately 3 × 3 mm and were fixed in 3% glutaraldehyde, buffered with phosphate buffer for 3 h. It was rinsed twice with phosphate buffer for 10 min per rinse. The tissues were then fixed in 2% aqueous osmium tetraoxide for 2 h and rinsed in 3 changes of distilled water for 10 min. Each dehydration was accomplished by immersion in a graded series of ethanol solutions of 25, 50, 75, 95 and 100%. Infiltration with propyleneoxide and embedding with increasing concentrations of propylene oxide was followed by dehydration . Thin sections (600 nm) were obtained by use of ultra-microtome and were placed on a copper 200 – mesh grid. They were stained with uranyl acetate and lead citrate and examined with GEOL-TEM1010 electron microscopic (Goodhew et al., 2003).

Histo-morphometrical analysis

The image analyzer computer system Leica Qwin 500 (Leica Ltd, Cambridge, UK) at the Image Analyzing Unit of the Pathology Department, Faculty of Dentistry, Cairo University (Egypt) was used to evaluate diameter of cadiac myocytes in H&E stained slides and optical denisty for iNOS. It was measured using the interactive measure menu. Ten readings from five non-overlapping sections from each rat of all groups were examined.

Statistical analysis

The obtained data from biochemical (troponin i and oxidative stress markers (MDA & GSH & catalase activity) and morphometrical (diameter of cardiac myocytes and optical density of iNOS) analysis were expressed as mean ± SD (standard deviation) and subjected to one-way analysis of variance (ANOVA) and post hoc test using Statistical Package for the Social Sciences (SPSS) version 22.0. ANOVA was used for comparison between different groups (more than two groups), with p value less than 0.05 (the level of significance). Least significant difference (LSD) was used to find the statistical difference between the groups when ANOVA was statistically significant (P value <0.05) (SPSS Inc., 2013).

RESULTS

Biochemical and statistical results

The biochemical findings among negative control, gum acacia and *Z. officinale* treated groups were statistically non-significant as regard all studied parameters (Table 1). While there were significant differences in the form of increase in troponin i and disturbance of oxidative markers in heart tissues in cyclophosphamide treated group when compared with the 1st three groups (-ve control, +ve control and *Z. officinale* treated group) (Table 1).

On supplementation of *Z. officinale* in (group IV), there was significant improvement of those parameters when compared with those in cyclophosphamide group (Group III).

Morphometrical and statistical results

Statistical analysis of the diameter of cardiac myocytes among negative control, gum acacia and *Z. officinale* treated groups, was not statistically significant. While, there was statistically significant decrease in diameter of cardiac myocytes of cyclophosphamide treated group (III) when compared with the previous groups (Table 2). A highly significant increase in the diameter of cardiac myocytes of cyclophosphamide and *Z. officinale* group (IV) was detected in comparison to that of cyclophosphamide treated group (III).

Statistical analysis of optical density of iNOS among negative, gum acacia and *Z. officinale* treated groups was not statistically significant as regard the studied parameters, while there was statistically significant increase of optical density of iNOS in cyclophophamide treated group (III) and a highly significant decrease in optical density of iNOS of cyclophophamide and *Z. officinale* group (IV) in comparison with cyclophophamide treated group (III) (Table 3).

Table 1. Statistical comparison among -ve control (Ia), +ve control (Ib), *Z. officinale* treated (II), cyclophophamide treated (III) and cyclophophamide and *Z. officinale* treated (IV) groups as regard mean values of troponin i, oxidative stress markers using ANOVA with post hoc test.

Variable / Group	Group Ia	Group Ib	Group II	Group III	Group IV	F	P
MDA(nmol/g tissue)	50.35±4.36^{aa,b}	49.35±4.33^{aa,b}	48.95±3.09^{aa,b}	89.16±17.28^{B}	60.15±18.33^{a}	12.96	<0.001
GSH(mmol/g tissue)	159.45±50.75^{aa,b}	160.05±50.65^{aa,b}	163.55±50.55^{aa,b}	315.66±55.45^{B}	181.35±55.65^{a}	9.85	<0.001
Catalase activity (U/g tissue)	0.91±0.28^{aa}	0.90±0.27^{Aa}	0.89±0.28^{Aa}	0.32±0.10^{Bb}	0.85±0.12^{aa}	7.67	<0.001
Troponini	5.18±0.85^{aa}	5.20±0.86^{aa}	5.19±0.75^{Aa}	2.44±0.72^{B}	3.99±0.75^{a}	14.21	<0.001

All values are expressed as Mean± SD, N: Number of rats in each group=6 rats, SD: standard deviation, P: Probability P: <0.01 highly significant, a:Significant versus cyclophophamide treated group (P<0.05), aa: highly significant versus cyclophophamide treated group(P<0.01), b: Significant versus cyclophophamide *Z. officinale* treated group (P<0.05), bb: highly Significant versus cyclophophamide *Z. officinale* treated group (P<0.01).

Table 2. Statistical comparison among negative control (Ia), gum acacia (Ib), *Z. officinale* treated (II), cyclophosphamide treated (III) and cyclophosphamide and *Z. officinale* treated (IV) groups as regard mean values of cardiac myocyte diameter using ANOVA and post hoc test.

Variable / Group	Group Ia	Group Ib	Group II	Group III	Group IV	F	P
Cardiac myocyte diameter	14.88±2.35^{aa}	14.55±2.46^{aa}	14.63±2.5^{5aa}	10.52±1.28^{bb}	14.04±0.36^{aa}	4.99	<0.01

All values are expressed as Mean± SD, N: Number of rats in each group=6 rats,SD: standard deviation, P:Probability P: <0.01 highly significant, a:Significant versus cyclophophamide treated group(P<0.05).aa: highly significant versus cyclophophamide treated group (P<0.01), b: Significant versus cyclophophamide *Z. officinale* treated group (P<0.05), bb: highly Significant versus cyclophophamide *Z. officinale* treated group (P<0.01).

Table 3. Statistical comparison among negative control, gum acacia, *Z. officinale* treated, cyclophophamide treated and cyclophophamide and *Z. officinale* treated groups as regard mean values of optical density of iNOS using ANOVA and post hoc test.

Variable / Group	Group Ia	Group Ib	Group II	Group III	Group IV	F	P
Denisty	50.05±1.35^{aa}	51.2±1.31^{aa}	49.06±1.39^{aa}	69.52±0.28^{bb}	52.04±1.36^{aa}	295.7	<0.0.1

All values are expressed as Mean± SD, N: Number of rats in each group=6 rats, SD: standard deviation, P:Probability P: <0.01 highly significant, a:Significant versus cyclophophamide treated group (P<0.05), aa: highly significant versus cyclophophamide treated group(P<0.01), b: Significant versus cyclophophamide *Z. officinale* treated group (P<0.05), bb: highly Significant versus cyclophophamide *Z. officinale* treated group (P<0.01).

Histopathological result

Light microscopic examination

H&E staining: Histological examination of H&E stained sections of control rats of left ventricular myocardium showed muscle fibers in different directions with acidophilic sarcoplasm, central pale oval nuclei. Delicate connective tissues separating cardiac myocytes were seen (Figures 1 and 2). While cyclophosphamide treated rats histopathogical examination revealed myocardial cell damage, necrosis and massive interstitial hemorrhage (Figure 3). Upon supplementation of *Z. officinale* there was partial prevention of the

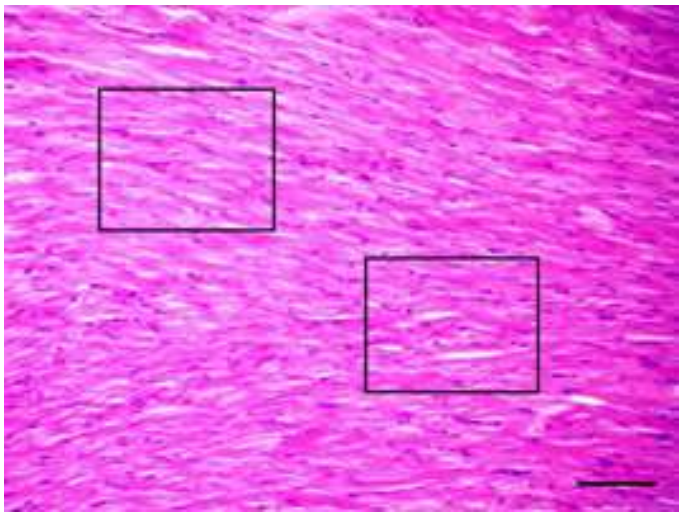

Figure 1. A photomicrograph of a section from the left ventricular myocardium of a control rat showing branching and anastomosing cardiac myocytes (rectangle) (H & E X 200).

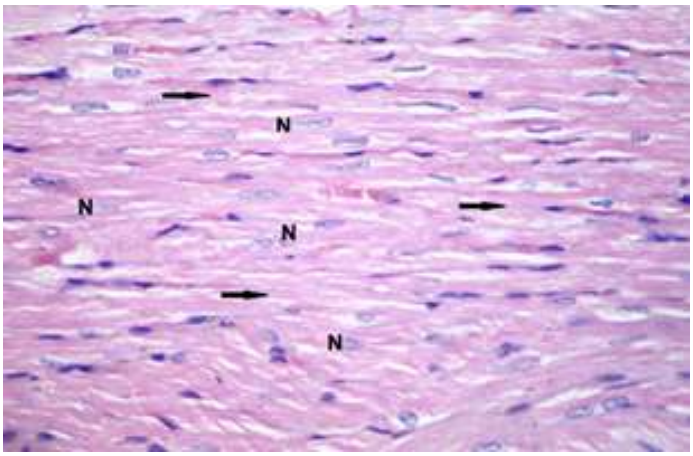

Figure 2. A photomicrograph of a section from the left ventricular myocardium of a control rat group showing muscle fibers in different directions and contained acidophilic sacroplasm (Arrow) and central pale oval nuclei (N) and delicate connective tissue separating cardiac myocytes were seen. (H & E X 400).

above pathological changes (Figure 4).

Immunohistochemical results: Immunohistochemical-stained sections of the control rats of left ventricular myocardium revealed weak positive cytoplasmic iNOS immunoreaction in cardiac myocytes (Figure 5). While, cyclophosphamide-treated rats showed strong positive cytoplasmic iNOS immunoreaction in numerous cardiac myocytes (Figure 6). On the other hand, immunohistochemical-stained sections of cyclophosphamide and *Z. officinale* supplemented rats revealed moderate cytoplasmic iNOS immunoreaction in most of the muscle cells (Figure 7).

Transmission electron microscopic examination

An electron micrograph of a section from left ventricular myocardium of a control group showed cardiac myocytes

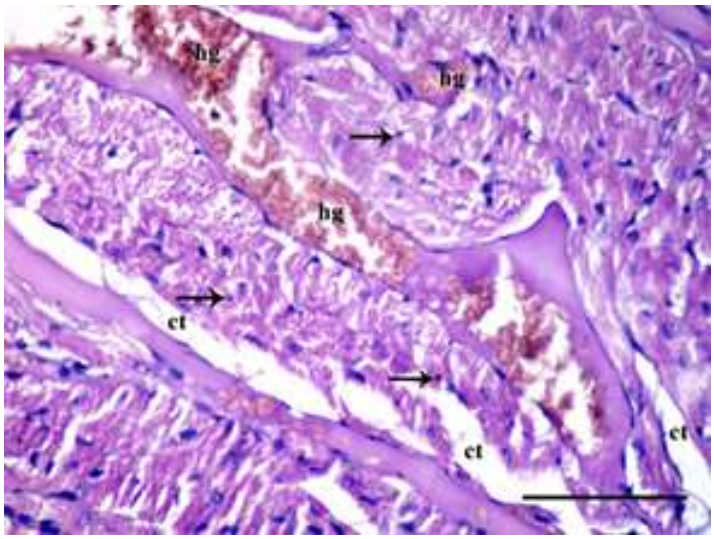

Figure 3. A photomicrograph of a section from the left ventricular myocardium of a cyclophosphamide treated rat showing areas of hemorrhage (hg). Their nuclei were darkly stained (Arrow) .Cardiac muscle fibers are separated by wide connective tissue spaces (ct((Hx & E X 400).

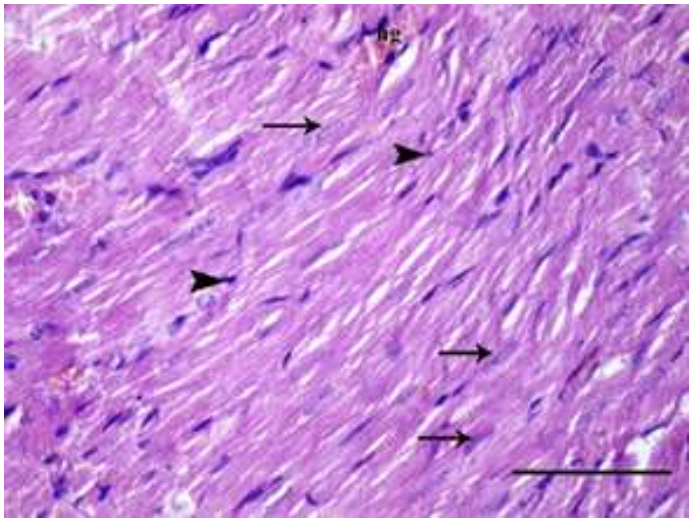

Figure 4. A photomicrograph of a section from the left ventricular myocardium of a cyclophosphamide and *Z. officinale* treated rat showing that some of muscle fibers are normal and contained acidophilic sacroplasm and central pale oval nuclei (Arrow). Some muscle fibers revealing necrosis and their nuclei were darkly stained (Arrow head). Hemorrhage in heart interstitium (hg) is also observed (H & E X 400).

with euchromatic nucleus with dispersed chromatin. Myofibrils revealed alternating dark and light bands and dark prominent Z-lines. Numerous mitochondria were seen between the myofibrils. Adjacent cells were connected by intercalated discs which appeared as dark irregular lines (Figure 8). While, an electron micrograph of a section from left venricular myocardium of cyclophosphamide treated group showed cardiac

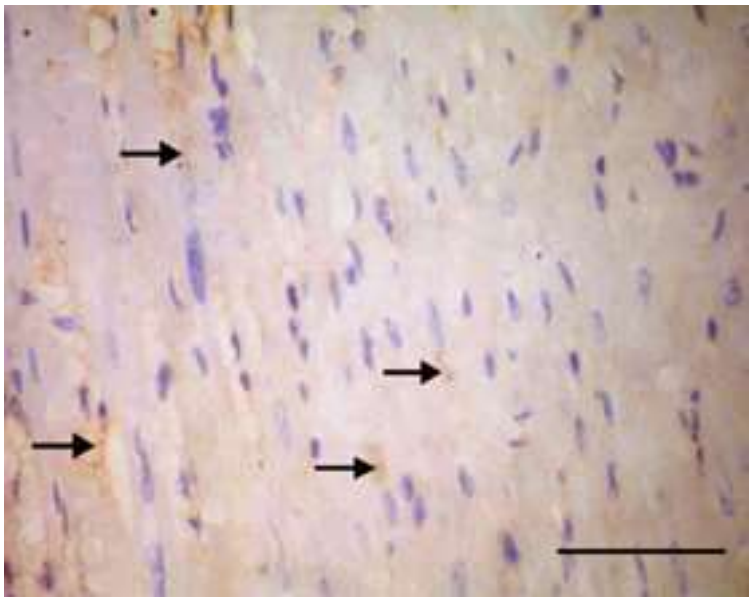

Figure 5. A photomicrograph of a section from left ventricular myocardium of a control rats showing weak positive cytoplasmic iNOS immunoreaction in the cardiac myocytes (Arrow) (Immunoperoxidase for iNOS, X 400).

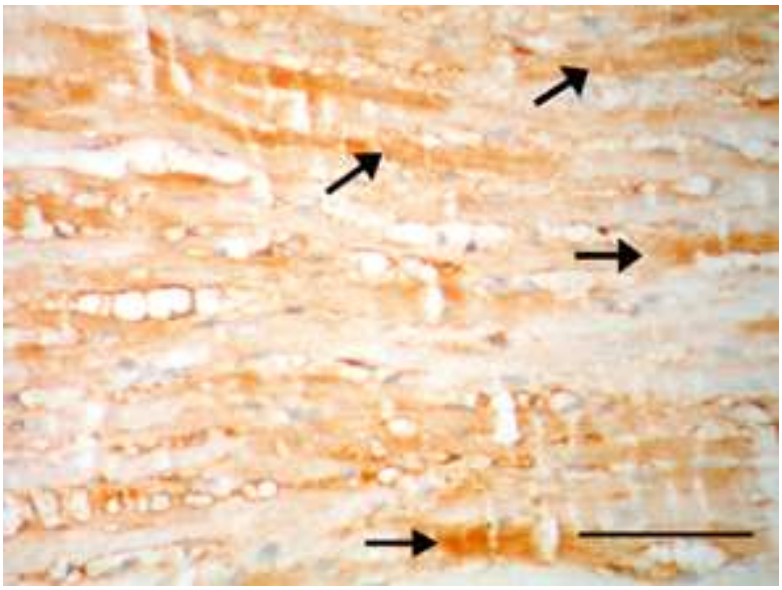

Figure 6. A photomicrograph of a section from the left ventricular myocardium of showing strong positive cytoplasmic iNOS immunoreaction in numerous cardiac myocytes (Arrow).(Immunoperoxidase for iNOS, X 400).

myocyte with some vacuolations. Nucleus with dispersed and peripheral chromatin condensation was observed (Figure 9). Upon supplementation of *Z. officinale* there was a partial prevention of these pathological changes (Figure 10).

DISCUSSION

Cyclophosphamide is a nitrogen mustard alkylating agent with potent antineoplatstic, immunosuppressive, and immunomodulatory effects (Baba et al., 2012).

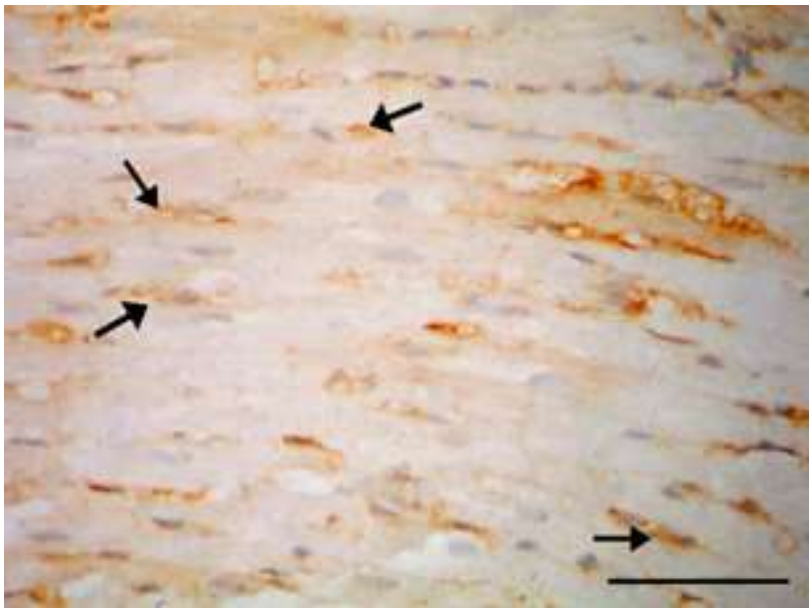

Figure 7. A photomicrograph of a section in the left ventricular myocardium of cyclophosphamide and *Z. officinale* treated rat showing moderate cytoplasmic iNOS immunoreaction reaction in most of the muscle cells (Arrow) (Immunoperoxidase for iNOS, X 400).

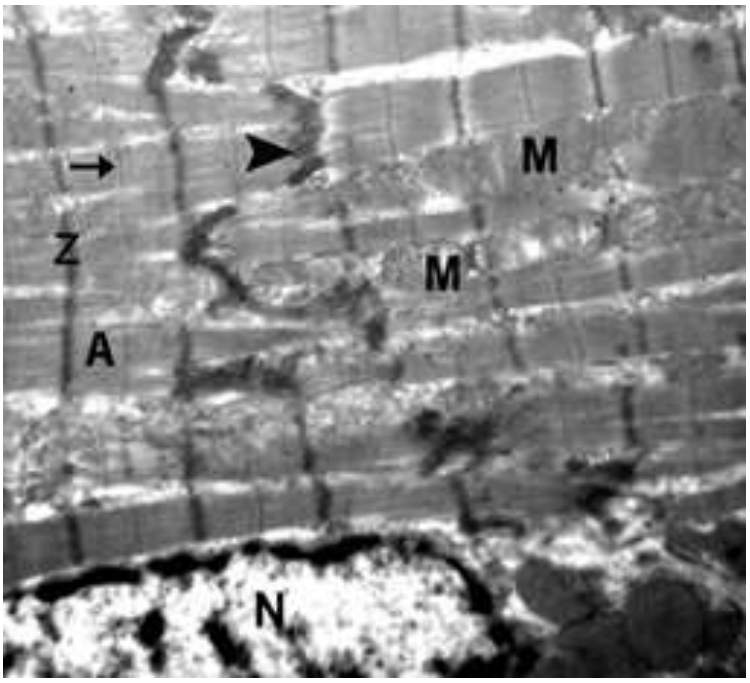

Figure 8. An electron micrograph of a section from left ventricular myocardium of a control group showing cardiac myocytes with euchromatic nucleus (N) with dispersed chromatin. Myofibrils with alternating dark (A) and light bands (arrow) and dark prominent Z-lines (Z) are seen. Numerous mitochondria (M) appear between the myofibrils. Adjcent cells are connected by intercalated discs (arrow head) which appear as dark irregular lines (TEM X 1000).

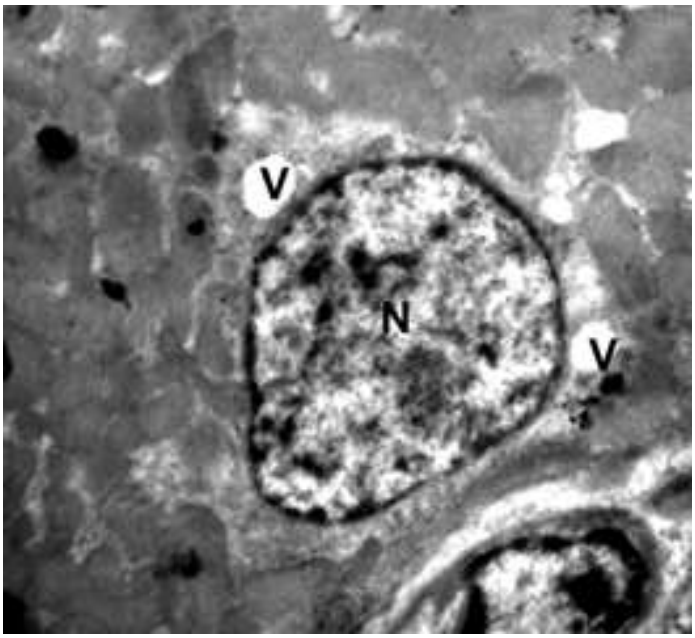

Figure 9. An electron micrograph of a section from left ventricular myocardium of cyclophosphamide treated group showing cardiac myocyte with some vacuolation (V). Nucleus (N) with dispersed and peripheral chromatin condensation is observed (TEM X 1000).

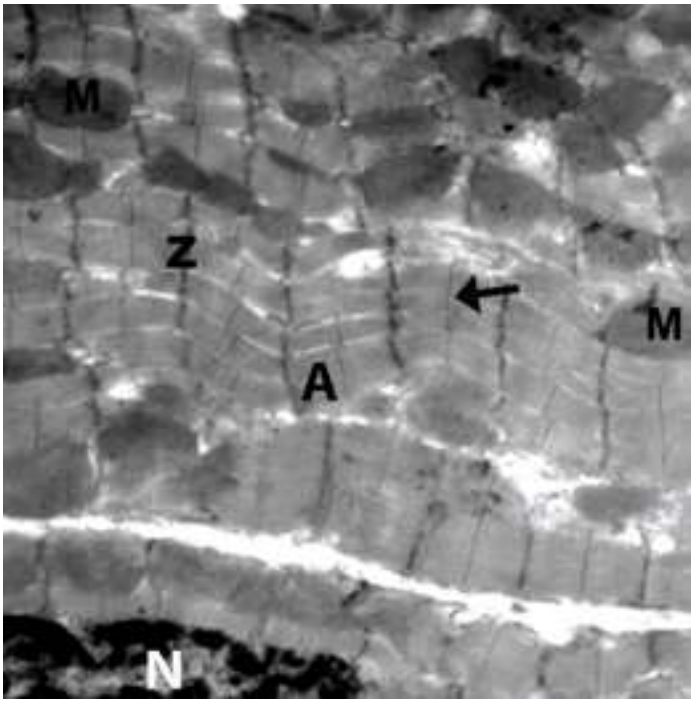

Figure 10. An electron micrograph of a section from left ventricular myocardium of cyclophosphamide and *Z. officinale* treated group showing cardiac myocytes with heterochromatic nucleus (N). Dark prominent Z-lines (Z), alternating dark (A) and light bands (arrow) can be seen. Numerous mitochondria (M) are also observed between the myofibrils (TEM X 10000).

Cyclophosphamide is proved to be a cardiotoxic as it induced hemorrhagic myocarditis showing a typical clinical course invariably leading to mortality. Also, toxic endothelial damage by cyclophosphamide causes extravasation of toxic metabolites and results in myocyte damage, interstitial hemorrhage, and edema. The incidence of fulminant congestive heart failure is reported to be 5–19% (Kamezaki et al., 2005; Morandi et al., 2005). Cyclophosphamide cardiotoxic effect is related more to the magnitude of the dose administered during a single cycle rather than the cumulative dose over a period of time; cardiac failure associated with cyclophosphamide can be seen several days following administration (Dow et al., 1993).

Ginger (*Z. officinale* Rosco), a member of the family Zingiberaceae, well-known as a spice has been used for over 2000 years (Hasan et al., 2012). It contains biological active compounds as terpenes and oleoresin (Rahmani et al., 2014). Its broad spectrum of biological activities includes antioxidant, antimicrobial, antitumor or anti-diabetic effects (Masuda et al., 2004). It causes the suppression of both cyclooxygenase and lipoxygenase metabolites and arachidonic acid (Dugasani, 2010). The present study proved cardiotoxic effect of cyclophosphamide presented by significant increase in troponin I with disturbance of oxidative markers in heart tissue which was reversed significantly by supplementation of *Z. officinale* due to its antioxidant action. There was a significant decrease in the mean cardiac myocyte diameter in cyclophosphamide treated group as compared with all other groups. Some investigators have reported results similar to those of the current study (Iqbal et al., 2008).

According to Pai and Nahata (2000), high dose cyclophosphamide is associated with acute cardiotoxicity which is possibly related to an increase in free oxygen radicals. This increase would be mediated by elevated intracellular levels of the actual cytotoxic metabolite phosphoramide mustard. Cyclophosphamide administration has been proved to increase lipid peroxidation and deplete the antioxidant molecules as glutathione (GSH), catalase, and superoxide dismutase (Dorr and Lagel, 1994). Many studies have suggested that the use of antioxidants in combination with chemotherapy may prolong the survival of patients compared with expected outcome without antioxidant supplements (Manda and Bhatia, 2003; Sudharsan et al., 2006). These results are in line with Gado et al. (2013) who reported the possible cytoprotective effect of antioxidant against cyclophosphamide induced cardiotoxicity. Cetik et al. (2015) reported a dose-dependence on the cyclophosphamide -induced cardiotoxicity with disturbance of oxidative stress marker prevented by intake of carvacrol as antioxidant.

According to Chakraborty et al. (2017) combination of curcumin with piperine exhibited profound cardio-protection effect against cyclophosphamide induced cardiotoxicity. Among the main active phytochemicals in *Z. officinale* gingerols, gingerdiol, shogaols, zingerone, and zingibrene are claimed to have antioxidant activity (Khaki et al., 2009). Some studies showed that *Z. officinale* treatment provided antioxidant effects by raising tissue concentrations of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These antioxidants are important protection against oxidative stress due to their ability to detoxify free radicals, such as reactive oxygen species (ROS) (Harliansyah et al., 2005). Another study done by Morakinyo et al. (2010) supported the antioxidant property of ethanolic extract of *Z. officinale*. The present study revealed histopathological changes by both light and electron microscope in cyclophosphamide -treated rats which partially prevented upon supplemation of *Z. officinale*. These results are in line with Cetik et al. (2015) who reported histological findings as hemorrhagical lesions on myocardium and disruption of myocardial fibers prevented by intake of carvacrol as antioxidant.

Viswanatha-Swamy et al. (2013) proved massive change in the myocardium showing a varying degree of vacuolar changes in the cardiac muscle fibers mainly in the form of degeneration of myocardial tissue, vacuolization of the cardiomyocytes, infiltration of inflammatory cells and myofibrillar loss with cyclophosphamide treated group.

According to Omole et al. (2018) cyclophosphamide treated group shows distorted and wavy myocardial fibers with focal fatty change in some area of myocardial fibers. Loss of cellular constituents of the myocardial cells, myofibrillar loss and hypertrophic myocardial fiber with inflammation. improvement in histoarchitecture were noticed with use of Kolaviron as cardioprotective antioxidant. The result of immunohistochemical-stained sections of the control rats of the heart revealed weak positive cytoplasmic iNOS immunoreaction in cardiomyocytes. While, cyclophosphamide -treated rats showed strong positive cytoplasmic iNOS immunoreaction in numerous cardiomyocytes. On the other hand, immunohistochemical-stained sections of *Z. officinale* supplemented rats revealed moderate cytoplasmic iNOS immunoreaction in most of the muscle cells.

Inducible nitric oxide synthase is an enzyme induced by inflammatory cytokines or endotoxins, cellular disturbance and hypoxia as a defense mechanism. Cyclophosphamide -induced myocardial dysfunction led to hypoxia, with subsequent increased iNOS expression. This induction may be mediated by hypoxia inducible factor- 1α gene expression (HIF-1α) which is a transcriptional factor for iNOS. Several studies supported that hypoxia-induced up-regulation of iNOS expression in several cell types, including cardiomyocytes (Simone et al., 2010; Tekin et al., 2010).

Fitzpatrick et al. (2005) stated that the up-regulation of iNOS expression is accompanied by increased nitric oxide (NO) production. The role of NO in cardiac function is complex, dependent on the level of NO produced.

Loren et al. (2009) mentioned that increased NO has been associated with relaxation of cardiac cells by an increase in cyclic GMP levels and likely plays an important role in modulating myocardial contractile function, ion channel activation, regulation of intracellular calcium, phosphorylation of contractile proteins.

Conclusion

According to the results of this study, administration of cyclophosphamide in adult male albino rats induced cardiotoxicity at the histopathological and biochemical levels which can be alleviated by supplementation of *Z. officinale* extract.

RECOMMENDATIONS

Human trials should be carried out, to establish the potential protective effects of *Z. officinale* in human intoxications. Future experiments are required to evaluate the possible protective molecular mechanisms of *Z. officinale* against cyclophosphamide -induced cardiotoxicity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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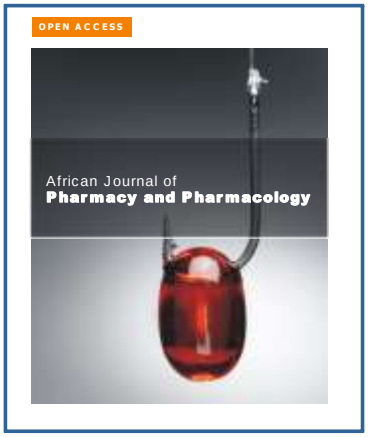

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