

Role of Alpha Lipoic Acid on Cyclophosphamide Induced Cardiotoxicity in Adult Male Albino Rat: Histological Study

Original
Article

Manar. A. Bashandy and Omyma I. Zedan

Department of Anatomy and Embryology, Faculty of Medicine, Menoufiya University, Egypt

ABSTRACT

Background: Cyclophosphamide is a chemotherapeutic agent used for treatment of leukemia, lymphoma and myeloma. Several studies reported wide range of cyclophosphamide induced cardiotoxicity in cancerous patients. Alpha lipoic acid is a natural antioxidant used widely for prevention and treatment of different conditions.

Aim of the work: This work was conducted to study the role of alpha lipoic acid in cyclophosphamide induced cardiotoxicity basing on histological and ultrastructural studies.

Material and Methods: Forty-four adult male Albino rats were divided into four groups: group I (control), group II (alpha lipoic acid -treated) in a dose (25 mg/kg body weight/day) given orally, group III (cyclophosphamide -treated) in a dose (200 mg/kg body weight) single dose injected intraperitoneally and group IV (alpha lipoic acid plus cyclophosphamid-treated). The experiment was conducted for ten days. Cardiac muscle specimens were subjected to light, electron microscopic and immunohistochemical study.

Results: Microscopic examination of cardiac muscle of cyclophosphamide treated group (III) showed focal disruption and vacuolation of cardiac muscle fibers with mononuclear infiltration between cardiac muscle fibers. Ultrastructurally, destruction of myofibrils, swollen, irregular arranged mitochondria and dilated SER were noted. Some nuclei showed chromatolysis and others were heterochromatic with irregular nuclear membrane. These results were confirmed by significant decrease in Bcl2. The intercellular spaces were wide contained dilated congested blood vessels and collagen. Alpha lipoic acid administration improved cardiac muscle architecture but still not attained the complete normal morphology

Conclusion: Supplementation of alpha lipoic acid in concomitant with cyclophosphamide (CYP) can ameliorate the CYP induced cardiac injury.

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Corresponding Author: Omyma Ibraheem Zedan, MD, Department of Anatomy and Embryology, Faculty of Medicine, Menoufiya University, Egypt, **Tel.:** +20 1002235985, **E-mail:** draomymazidan@hotmail.com

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INTRODUCTION

Cardiac complications resulted from chemotherapy administration represented a major factor in morbidity and mortality of cancer patients^[1]. Cyclophosphamide (CYP) is one of the most popular used chemotherapeutic agents. It is commonly used as therapeutic anti-neoplastic agent for acute and chronic leukemia as well as lymphoma and myeloma^[2]. Because of CYP immunosuppressive property, it is popularly used before organ transplantation^[3].

Comparing to other antineoplastic agents, cardiomyopathy reported to complicate administration of CYP was early and of acute lethal onset of about two to three weeks after administration of 180 – 200 mg^[4]. It is dose dependent cardiotoxicity^[1]. On the other hand, cardiomyopathy induced by other chemotherapeutic agents was revealed to occur after long duration (delayed action) of intake.

CYP cardiotoxicity ranged from acute heart failure to congestive heart failure was reported^[5] after intake of

125 mg/kg as a single dose. The pathogenesis involved for CYP cardiotoxicity was thought to be due to endothelial capillary damage with subsequent escape and leak of erythrocytes and plasma proteins into myocardium^[4]. reported histological findings suggestive of acute pericarditis associated with hemorrhagic myocarditis and fibrin microthrombi precipitated in the capillaries^[6]. It was attributed myocardial wall thickness because of cardiac muscle oedema and hemorrhage leading to restricted left ventricular compliance during diastole and ending by restrictive cardiomyopathy^[1].

CYP is anti-cancerous agent present in inactive form and need cytochrome P-450 system for its metabolic activation. Hydroxylated active metabolite of CYP e.g. phosphoramidate mustard, nitrogen mustard and acrolein were thought to be toxic. Phosphoramidate mustard had possible alkylating activity for DNA while acrolein were thought to impair antioxidant system with subsequent production of reactive oxygen free radicles as hydrogen peroxide and superoxide dismutase. The cardiac inner mitochondrial

membrane can be damaged by reactive oxygen species with resulting reduction in detoxifying capacity induced by oxygen radicals of cardiac mitochondria^[7].

Lipoic acid (LA) is a natural antioxidant organosulfur agent that can be synthesized from octanoic acid and cysteine in mitochondria^[8]. It represented a cofactor for some dehydrogenase enzymes specifically needed during energy metabolism in mitochondria^[9]. It can be supplied exogenously either in food or synthetic food supplements^[10]. Lipoic acid can be uptake in different food types as spinach, broccoli, yeast extract, heart, liver and kidney. It is termed as universal antioxidant as it can perform its action either in membranous or aqueous phase^[11].

Several clinical studies have reported that, LA had beneficial effect in improving certain neurological disorders, liver cirrhosis, diabetes mellitus and myocardial injury induced by ischemia-reperfusion. Other studies had revealed that, LA had protective function against adriamycin induced tissue injury, heavy metal toxicity, lead acetate induced neurotoxicity and kidney stone^[12]. Hence, it was of interest to study the possible ameliorative role of alpha lipoic acid in cyclophosphamide induced cardiotoxicity basing on histological and ultrastructural studies.

MATERIAL AND METHOD

Animals

Forty-four adult male Albino rats were chosen with a weight ranging from (200-250 grams). The rats were selected from breeding animal house present in medical college, Menofya University. They could get free access to standard food and tap water. Good hygienic state and normal temperature were provided to the animal during the study.

Drugs and chemicals

Cyclophosphamide

It was obtained as a vial (98%) was obtained from Baxter Onchology GmbH, Kantstrasse 2 (Halle, Germany). Each vial contained 1 gm/ 10 ml. The vial was dissolved in 25 ml saline. The calculated dose was given to rat.

Alpha lipoic acid

It was used in tablet form (Thiotacid) obtained from EVA Pharma Company for Pharmaceuticals and medical Appliance, Egypt . Each alpha lipoic acid tablet contained 600 mg. Each tablet was dissolved in 100 ml saline. The estimated dose was administered by rat.

Experimental protocol

The experiment was performed following guidelines already set by Menofya University Ethical Committee. The rats were kept into 4 groups with eleven animals in each.

Group I: Control group were administered saline (vehicle) orally for the whole duration of the study (ten days).

Group II: Rats in this group administered alpha lipoic acid (25 mg/kg body weight/day) dissolved in a saline by oral route by use of curved needle to supply the antioxidant immediately into the stomach in a process known as gavage process for ten days^[11].

Group III: Cyclophosphamide was injected intraperitoneal to each rat in this group in a dose of (200 mg/kg body weight/day) as a single dose^[13].

Group IV: animals were injected CYP as in Group III with immediately consecutive administration of alpha lipoic acid orally in same dose as Group II daily for 10 consecutive days.

The rats were weighted using electronic balance then anesthetized and sacrificed on day eleven of experiment. The left ventricle of the heart of each rat was excised and rinsed using normal saline. The tissues were divided and subjected to the following studies:

I-Histological study

The cardiac specimen obtained from each animal was fixed in 10% formal saline. Paraffin blocks were obtained after specimens processing. Blocks were cut into sections of 5µm thickness to obtain slides for haematoxylin and eosin (HandE) and masson trichrome stains (MT). HandE stain used to identify the cardiac tissue general architecture while MT stain is specific for detection of collagen^[14].

II- Immunohistochemical study

Bcl2 and Bax proteins were identified by using immunohistochemical staining. 5µm slices obtained from paraffin blocks were fixed in glass slides which coated with pol-l-lysine then deparaffinized followed by rehydration then incubated in 3% hydrogen peroxide for a duration of 10–15 minutes using a humidity chamber. Ultra V Block was kept for 5 minutes.

For bcl2 immunostain, Monoclonal primary antibody against Bcl-2 was added in a dilution of (1:50) Dako, code M 0887 (Clone 124), (Dakocytomation, Copenhagen, Denmark) to sections and were incubated at 4 °C while Rabbit monoclonal antibody to Bax (EPR18284) (Dakocytomation, Copenhagen, Denmark). Washing was done by PBS and secondary immunoglobulin was used at room temperature for 40 minutes. Washing of the specimen was performed again PBS and incubation was done with streptavidin peroxidase for 10 minutes then subjected to DAB chromogen substrate application for each slide for 3 minutes. Moreover, the slides were counterstained with Mayer's hematoxylin. Normal lymphoid tissue was used as a positive control. For obtaining negative control, primary antibody step was omitted^[15].

III- Ultrastructure study

Small pieces of heart specimen (1 mm³) were prepared from each of the three groups then fixed in a mixture of paraformaldehyde (pH: 7.3) and 2.5% glutaraldehyde for overnight at 4° C. 1% Osmium tetroxide was used for another fixation. Cardiac specimens were passed through dehydrating steps using ascending grades of alcohol then subjected to propylene oxide two changes then imbedded in epon. Ultrathin sections of 60 nm thickness were obtained then painted with copper grids and stained with lead citrate and uranyl acetate^[16].

Examination of the grids were performed using transmission electron microscope (Seo-Russia) in Alexandria medical research institute.

RESULT

Light microscopic results

H and E stained cardiac sections

Group I: Control group revealed normal histological character of cardiac muscle fibers as they are longitudinally arranged with branching and anastomosing pattern. The cardiac myocytes showed central oval vesicular nuclei and acidophilic sarcoplasm. Fibroblasts were seen in the connective tissue between the cardiac muscle fibers (Figure 1).

Group II: alpha lipoic acid treated showed the same histological appearance as control group.

Group III: Cyclophosphamide treated revealed various degrees of structural changes. Some of the cardiac fibers showed deeply stained acidophilic sarcoplasm, other fibers had irregular outline and separated with focal disruption of some of them, (Figures 2,a,b). Moreover, other cardiac fibers showed areas of vacuolated sarcoplasm (Figure 2.c). Some fibers lack nuclei with pyknotic and eccentric nuclei of some cardiomyocytes (Figure 2.a). Dilated congested blood capillaries were observed (Figure 2,b) with areas of focal aggregation of mononuclear cell infiltration between cardiac fibers could be detected (Figures 2,b,c). Some of the cardiac muscle fibers were separated from each other by wide intercellular spaces which contained extravasated RBCs (Figures 2,b,c).

Group IV: Cyclophosphamide and alpha lipoic acid treated showed that most of the cardiac muscle fibers appeared nearly like that of the control group. Meanwhile, some cardiac muscles were widely separated and some nuclei were deeply stained and pyknotic (Figure 3).

Masson's trichrome (M.T) stained sections of cardiac myocytes from control group showed few collagen fibers between the cardiac muscle fibers (Figure 4,a). Sections from cyclophosphamide treated group (group III) revealed increase in the collagen fibers especially around blood vessels. Other section, the collagen fibers replace degenerated parts of cardiac muscle fibers (Figures, 4,b,c). Sections from rats of cyclophosphamide

(CP) and alpha lipoic acid (ALA) treated group (group IV) showed few collagen fibers between the cardiac muscle fibers (Figure 4, d).

Immunohistochemical results

Control group demonstrated moderate expression of Bcl-2 immunostain in myocardial fibers while weak expression was noted in CP administered group (Figures 5,a,b). In CP and lipoic acid treated group, there was strong expression of Bcl-2 in myocardial fibers (Figure 5,c).

Positive Bax immunostaining expression was observed as brown cytoplasmic staining in myocardial muscle fibers. Sections of cardiac muscle of control group demonstrated moderate immuno-positive expression in cardiac muscle fibers (Figure 6,a). Strong immunoreaction was noticed in most of cardiac muscle fibres of cyclophosphamide treated group while weak positive immunoexpression was revealed in rats treated with CP plus lipoic acid (Figures 6,b,c).

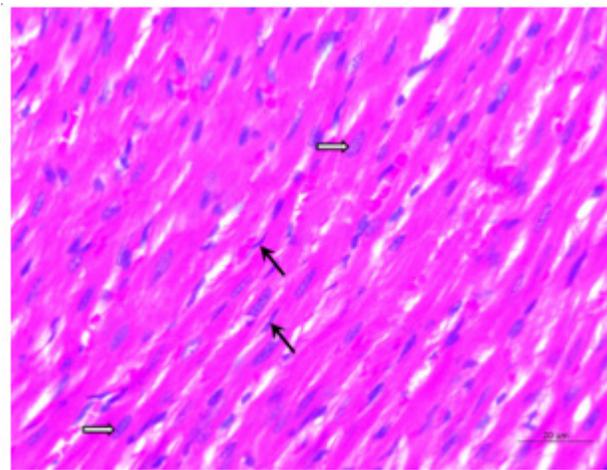
Electron microscopic results

Examination of ultrathin sections of myocardium from the control group (group I) showed that the cardiac myocyte contained single oval nucleus with dispersed euchromatin. The sarcoplasm showed regularly arranged myofibrils with transverse striation pattern in the form of alternating dark (A) and light (I) bands bisected by Z lines. The Z-lines divide the myofibrils into sarcomeres. The center of each A band was occupied by a pale H zone, which were further bisected by dark thin M line. Rows of elongated or spherical mitochondria were observed in between the myofibrils. Intact intercalated disks representing intercellular junctions between adjacent cardiac myocytes were also observed. Sarcolemma appeared invaginated at Z line to form T tubule with mitochondrial aggregation below sarcolemma. Blood vessels were present between cardiac muscle fibers (Figures 7,a,b).

Group II: alpha lipoic acid treated had the same histological criteria of control group

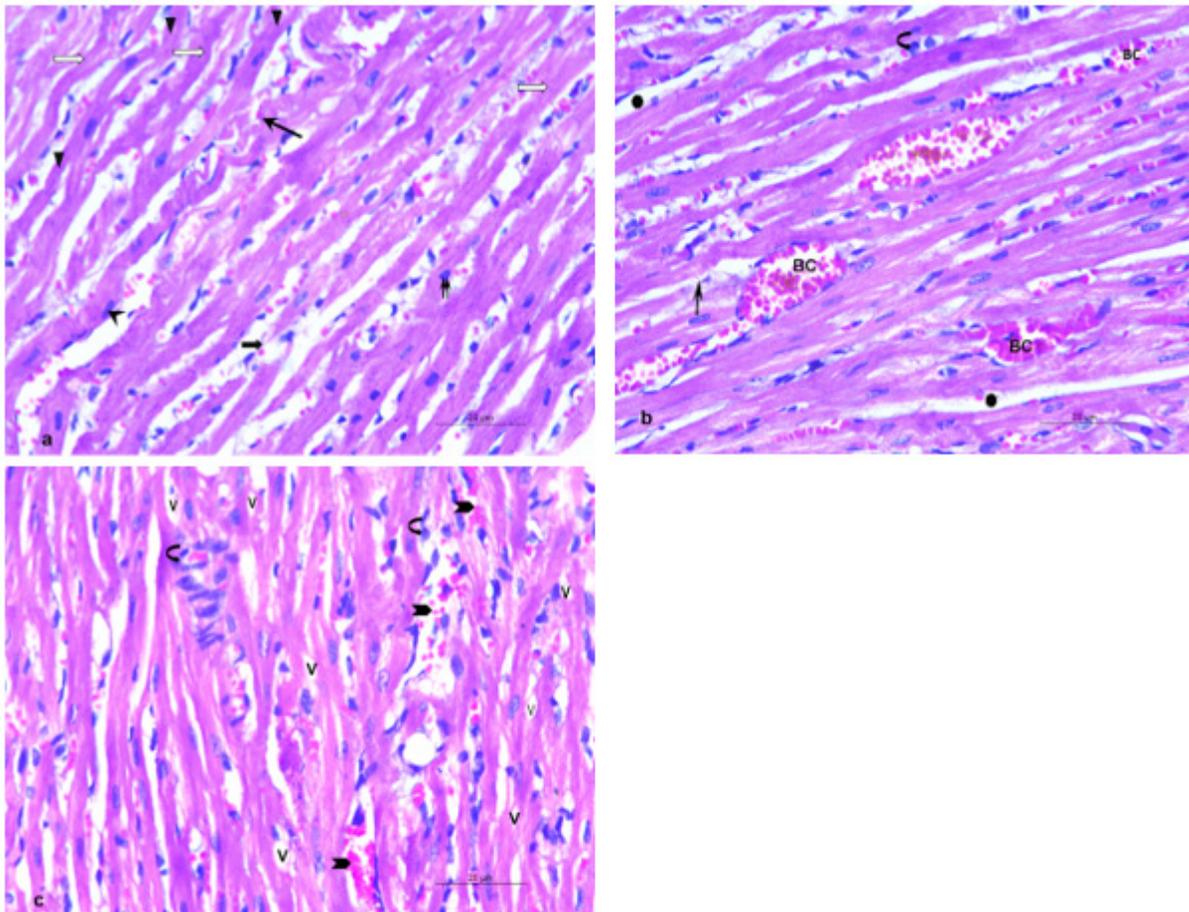
Group III: In cyclophosphamide treated group, examination of ultrathin sections of myocardium of cyclophosphamide treated group showed Some nuclei appeared irregular in shape and heterochromatic meanwhile chromatolysis was noted in others (Figure 8,b,c). Massive fragmentation and lysis of myofibrils with disorganization of their normal arrangement and interrupted Z line could be noted (Figures 8,b,c,d). The mitochondria appeared swollen and irregularly arranged with different sizes and shapes (Figure 8,c,d) with dilated SER (Figure 8,a).

Group IV: Ultrathin sections examined in cyclophosphamide (CP) and alpha lipoic acid (ALA) treated animals revealed an almost normal appearance of cardiac muscle fibers with regularly arranged myofibrils showing a normal striation pattern. However, Some nuclei of cardiomyocytes had irregular outlines (Figures 9,a,b).



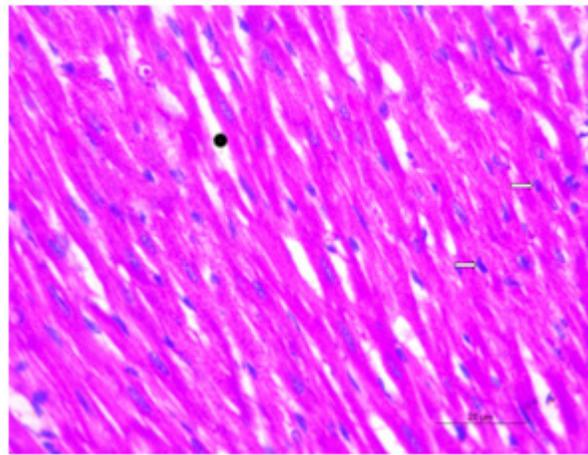
(Fig.1)

Fig. 1: A photomicrograph of a longitudinal section of control adult rat myocardium showing branching and anastomosing muscle fibers with central oval vesicular nuclei (\longleftrightarrow), and spindle-shaped connective tissue cells (\rightarrow) in the interstitial tissue. (H and E, X400)



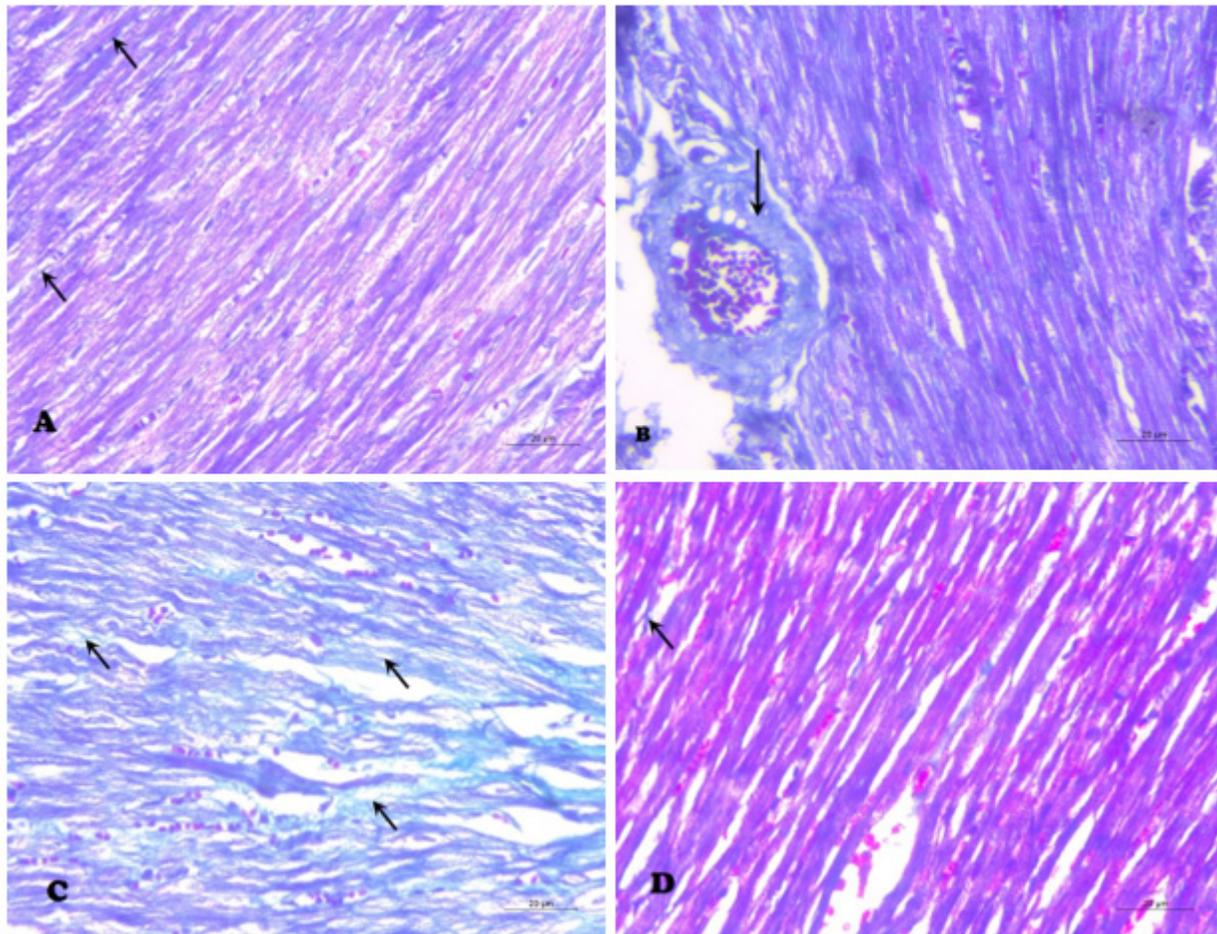
(Fig.2)

Fig 2: photomicrograph of a section in the myocardium from the cyclophosphamide-treated group (group III) showing a) Fibers with intensively eosinophilic sarcoplasm (\blacktriangledown), some of these fibers have irregular outline (\rightarrow) with discontinuity of other fibers (\blackrightarrow). Some fibers lack nuclei (\longleftrightarrow), others have small dark pyknotic (\uparrow) and some nuclei are eccentric (\blacktriangleup). b) wide separation of interstitium between muscle fibers (\bullet). The interstitium contains dilated congested capillaries (BC) and few inflammatory cells (\bullet). Some muscle fibers show degenerative changes with nuclear fragmentation (\rightarrow). c) areas of sarcoplasmic vacuolation (V), destructed fibers with inflammatory cells (\bullet), extravasated RBCs in the interstitium (\blacktriangledown). (HandE, \times 400)



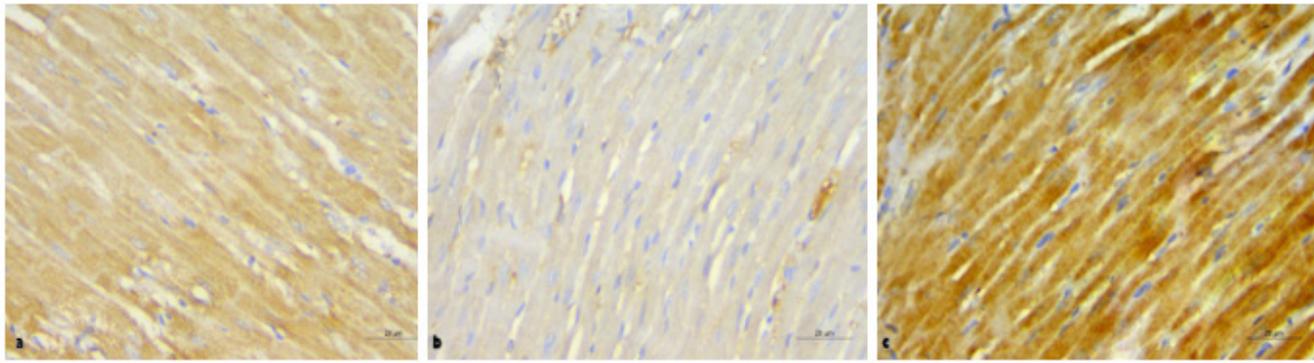
(Fig.3)

Fig. 3: photomicrograph of a section in the myocardium from the cyclophosphamide and lipoic acid-treated group (group IV) showing Some nuclei are small dark irregular pyknotic (⇐⇒), with wide interstitium(●). (HandE, × 400)



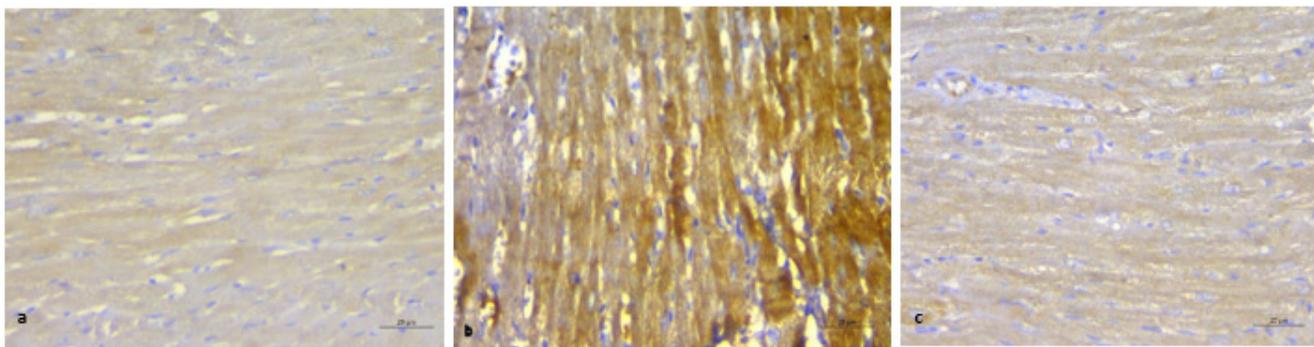
(Fig.4)

Fig. 4: photomicrograph of a longitudinal section of control adult rat myocardium showing A) few collagen fibers between the cardiac muscle fibers in control group (→). B) moderate increase in collagen fibers around the congested blood capillaries in cyclophosphamide treated group. (→).C) Focal areas of collagen in between muscle fibers others replace degenerated parts of the cardiac myocyte (→)in cyclophosphamide treated group. D) minimal amount of collagen fibers between the cardiac muscle fibers in cyclophosphamide and alpha lipoic acid treated group (→).(Masson Trichrome's X 400)



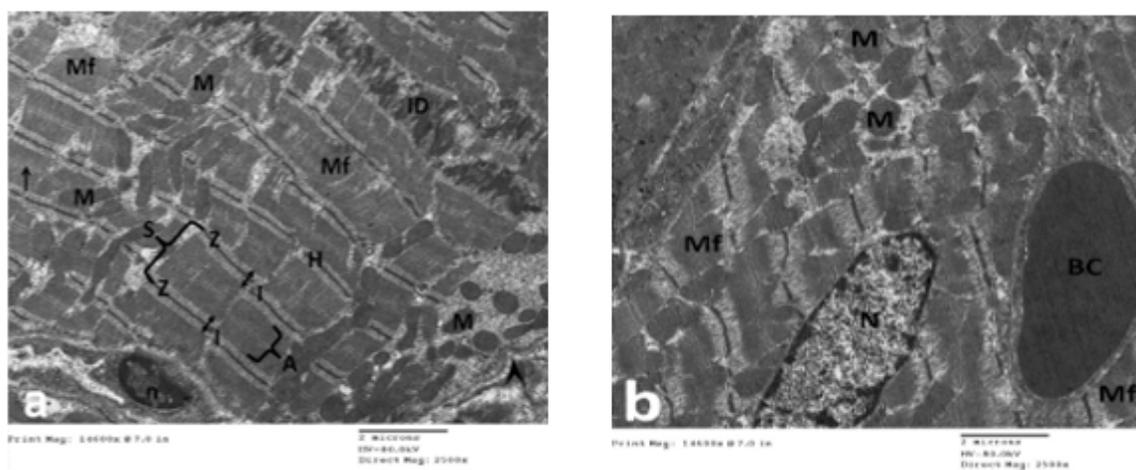
(Fig.5)

Fig. 5: photomicrograph of a longitudinal section of adult rat myocardium showing a) Control group of rat heart showing moderate Bcl-2 expression. b) Cyclophosphamide treated group of rat heart showing mild Bcl-2 expression.c) Cyclophosphamide and lipoic acid treated group showing dense Bcl-2 expression. Bcl2 immunostain (x400).



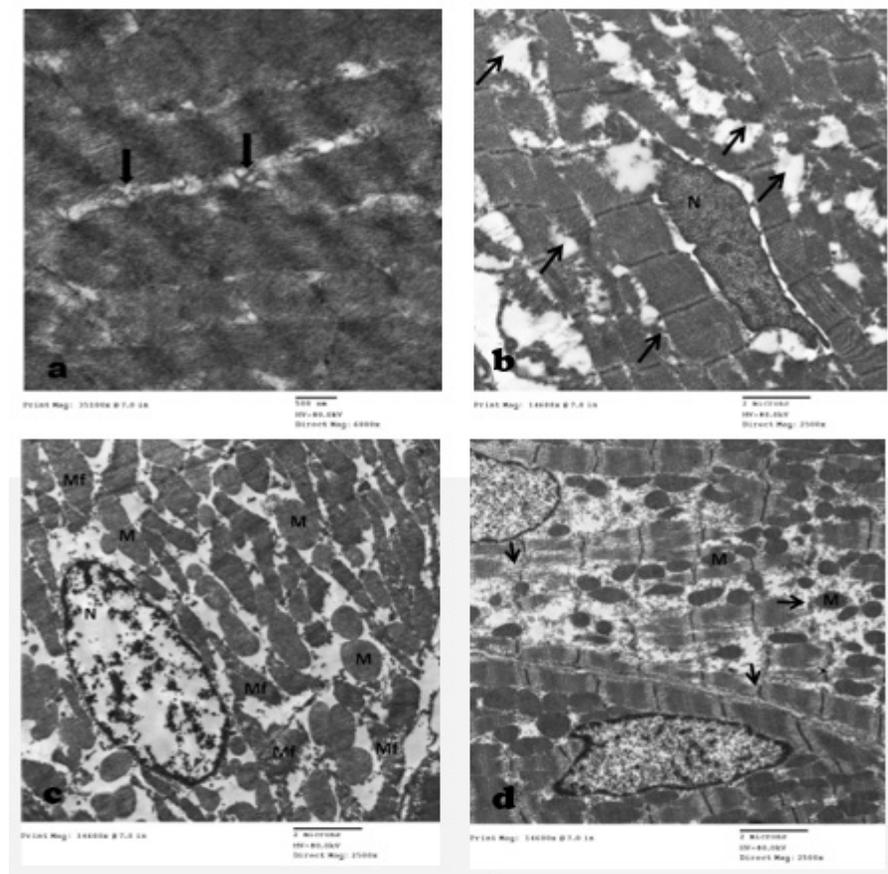
(Fig.6)

Fig. 6: photomicrograph of a longitudinal section of adult rat myocardium showing a) Control group of rat heart showing moderate Bax expression. b) Cyclophosphamide treated group of rat heart showing increased Bax expression.c) Cyclophosphamide and lipoic acid treated group showing decrease of Bax expression. Bax immunostain (x400).



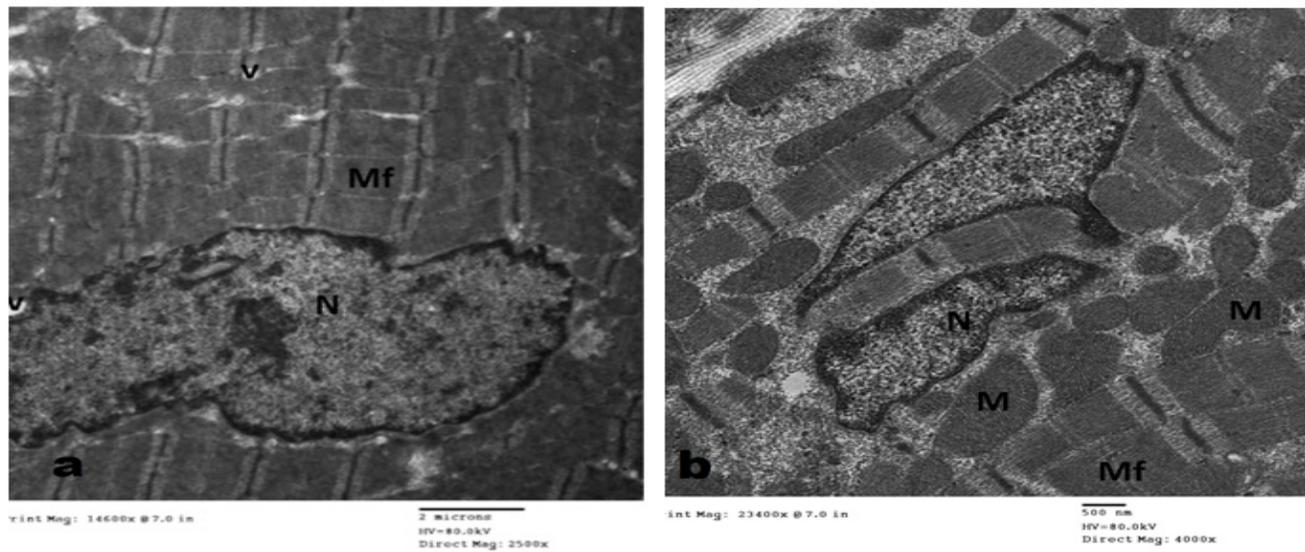
(Fig.7)

Fig. 7: An electron micrograph of rat myocardium from the control group showing a) two cardiac myocytes separated by intercalated disk (ID). Sarcolemma appears invaginated at Z line form T tubule (▲), small mitochondria are aggregated below sarcolemma. Sarcomeres are composed of dark (A) band and two hemi light bands (I). In the center of each A band a pale area called the H band (H), which is bisected by a thin M line (↑), in the center of the light band Z line present. elongated mitochondria appear between myofibrils(M). Notice: nucleus of endothelial cell of interstitium (n) b) Part of cardiac myocyte with oval euchromatic nucleus (N)surrounded by myofibrils(Mf). Mitochondria (M) are present in between the myofibrils. Notice: blood capillary (BC) in the interstitium. (a and b×14600)



(Fig.8)

Fig. 8: An electron micrograph of rat myocardium from the cyclophosphamide-treated group (group III) showing a) Sarcomere with thick Z line with dilatation of SER (█), b) Part of cytoplasm of cardiac myocyte showing focal loss of myofibrils and mitochondria. Notice: heterochromatic irregular nucleus (N). c) Nucleus of cardiac myocyte (N) with chromatolysis. Notice : disruption and loss of myofibrils (Mf), and swollen mitochondria (M). d) Part of two cardiac myocytes with focal loss myofibrils (→) with abnormal shaped small sized mitochondria (M) and interrupted Z line (↗). (a×35100) (b, c and d ×14600).



(Fig.9)

Fig. 9: An electron micrograph of rat myocardium from cyclophosphamide plus alpha lipoic acid treated group showing a) near normal structure of cardiac myocytes with normal arrangement of myofibrils (Mf) with mild nuclear irregularity (N). b) Nearly normal mitochondria. (a ×14600) (b×23400).

DISCUSSION

In this study, our aim was to elucidate the possible ameliorative role of alpha lipoic acid in cyclophosphamide induced cardiotoxicity.

Microscopic examination of cardiac muscle fibres of CYP treated animals demonstrated some wavy myocardial muscle fibres with vacuolations. The previous findings suspect CYP cardiac muscle intoxication. Same results were reported in cardiomyopathy^[17]. The wavy myofibers was explained as a result of contraction or spasm of the broken fibres^[18]. Additionally, stretching of fibres lead to striations pulling apart and destruction^[19].

Moreover, light microscopic examination of cardiac sections of cyclophosphamide treated group revealed Some cardiac muscle fibers showed patches of intensively eosinophilic sarcoplasm and devoid of nuclei and were widely separated. This was noticed in myocardial degeneration caused by administration of cisplatin^[20]. Myocytes cell loss might occur because of hydrolytic enzymes liberation from lysosomes in dying and dead cells with result of homogenous, acidophilic cytoplasm^[21].

Ultrastructural alteration in the nuclear apparatus after CYP administration was illustrated in the present study. They include irregular shaped nuclei with heterochromatin while chromatolysis was noted in others. They explained unusual nuclear shape related to cytoskeletal proteins damage^[5].

Moreover, Ultrastructural examination of CYP treated rats demonstrated irregularly arranged mitochondria and swollen with different sizes and shapes which indicate mitochondrial injury. Mitochondria is one of the main cell organelles responsible for energetic production and metabolism. Mitochondrial injury might cause decreased complexes I and II–III activity. Toxins that suppress electron transport or oxidative phosphorylation in mitochondrial cristae mostly lead to depletion of ATP with resulting swelling of mitochondrial organelles, which most probably impair the normal heart function and cardiac contractility^[22,23]

On the other hand, Focal lysis of myofibrils with disorganization of their normal arrangement and interruption of Z line were major findings in myocardium of cyclophosphamide treated rats. This was explained as secondary event to mitochondrial dysfunction which lead to imbalance in calcium uptake and loss of ATP production which are important factors in normal myofibrillar function^[24].

Furthermore, dilated endoplasmic reticulum was detected on ultrastructural examination of CYP treated group. It was resorted this dilatation as part of damage induced by CYP to various types of cells. They concluded that, sarcoplasmic reticulum reorganization is usually accompanied by cardiac muscle cytoskeleton abnormalities as well as macromolecular injury^[25].

The present results were confirmed by an increase in expression of Bax which is pro-apoptotic molecule if inserted in mitochondrial membrane induce cell apoptosis. Bax family of proteins are stimulated by caspases cleavage, protein kinase inhibition and phosphatases activation and raised intracellular PH^[26].

Moreover, the present results also revealed that CYP administration enhanced Bcl2 expression as compared to control group. Bcl-2 is anti-apoptotic molecule has a critical role in attenuation and inhibition of apoptosis^[27,28].

It was noticed that CP significantly increases the mRNA expression levels of apoptotic genes, p53 with decreases the anti-apoptotic gene Bcl2^[29].

The ratio between Bax and Bcl2 (pro and antiapoptotic) molecules represent rheostat which is responsible for mitochondrial pathway susceptibility to apoptosis^[30,31]. Presence of high level of Bcl2 induce formation and predomination of Bcl/Bax heterodimers which is responsible for protection of cell survival. Reduction of Bcl2 level stimulate Bax to form homodimers which accelerate cell death^[32,33].

In the present study, Histopathologic changes of cardiac toxicity were characterized by congestion of blood capillaries and extravasation of red blood cells (RBC) in the interstitial tissue. Similar results were recorded by other researchers who resorted RBC extravasation and myocardial oedema maybe due to impaired capillary endothelial permeability, microvascular injury or direct CYP toxicity^[34].

The presence of focal areas of mononuclear cellular infiltration was also noticed in cyclophosphamide treated group which denotes inflammation. It was found that cyclophosphamide Cardiotoxicity was characterized with marked congestion and edema, as well as severe inflammation in the cardiac muscles^[35,17].

In the present work, The moderate increase in collagen fibres deposition between cardiac muscle fibres was noted in cyclophosphamide treated group. They attributed that deposition either due to overactivity of fibroblasts to produce collagen or reduction in collagen degradation by matrix metalloproteinase^[36]. It was concluded that, myocardial fibrosis may impair the cardiomyocyte nutrition and disturb it's function^[28].

Fibroblasts represents one of the main myocardial cell populations which increased in number due to cardiac diseases and toxicity. The increased fibroblast number can be due to different mechanisms such as endothelial and epithelial cells transformation into myofibroblast and fibroblasts; increased myocardial fibroblasts replications and circulating bone marrow cells migration and transformation into fibroblast^[37,38].

Although the pathogenesis of cyclophosphamide cardotoxicity is thought to be due to low selectivity for proliferating cells, toxic metabolite generation as

acrolein as well as CYP toxic metabolite accumulation as phosphoramidate in the cardiomyocyte cytoplasm with induction of apoptosis. The molecular events underlying such toxicity still not clearly understood^[39].

The pathogenesis underlying cardiotoxicity may be due to oxidative stress, release of inflammatory endocoids mediators as nitric oxide and cytokines with activation of adenosine diphosphate-ribose^[40].

Excess ROS (reactive oxygen species) endanger the cell as it can cause many changes on protein as peptide chain fragmentation, alteration of electrical charge of proteins and oxidation of specific amino acids which lead to increased susceptibility to proteolysis by degradation by specific proteases and lysis of myofibril^[41].

Calcium plays fundamental rule in initiating apoptotic cell death. Interaction between ROS and calcium signals is bi-directional, calcium ions able to enhance mitochondrial ROS production. Mitochondrial dysfunction capable of enhancing ROS production with CYP treatment^[42,43].

Additionally, high level of calcium accompanied with drug stimulate respiratory chain activity leading to more accumulation of ROS. Moreover, oxygen species can target calcium channel in endoplasmic reticulum leading to more release of both calcium and ROS with subsequent opening of mitochondrial permeability transition pore (mPTP) causing pre-apoptotic factor release^[44].

Mitochondrial generation of ROS causes its swelling leading to breakdown of outer mitochondrial membrane causing intermembrane proteins release and enhance cell death^[45]. This is proved by increased BAX expression in this study.

Moreover, anti-apoptotic factors as Bcl2 and Bcl-XL might be inactivated by oxidative stress^[46].

However, during apoptosis a relationship was proved between calcium and bcl2 protein family as calcium activation cause suppression of anti-apoptotic bcl2 expression and enhance cell apoptosis^[47].

Cardiac fibrosis is triggered by diverse cues, including myocyte death and inflammation. Cyclophosphamide causes severe inflammation in the cardiac muscles^[48]. Inflammatory mediators stimulate myocardium fibroblasts to undergo differentiation into the myofibroblast which infiltrate the injured regions of the heart and a rise in collagen synthesis and lay down collagenous septa between myocytes^[49]. TGF β appears to be responsible for cardiac fibrosis by promoting myofibroblast formation and ECM production^[50].

Alpha lipoic acid is universal antioxidant with a prompt dual protection as it able to exert its action both intracellular and on cell membrane^[51]. Although alpha lipoic acid significantly improved cardiac muscle architecture when administered in combination with CYP medication, but still not attained the complete normal morphology. Nuclei of few cardiac muscles were irregular,

deeply stained and pyknotic. Wide intercellular spaces still present between cardiac muscle fibres. Ultrastructural study showed regularly arranged myofibrils with a normal striation pattern. However, some nuclei of cardiomyocytes had irregular outlines.

The relative architectural improvement noted in the group administered (ALA and CYP) in this study was suggested due its effective antioxidant property. It is capable of scavenge reactive oxygen species *in-vitro*^[51].

ALA able to improve the mitochondrial citric cycle with subsequent increased cycle enzyme level; ATP and glutathione with stimulation of activity of electron transport complex. ALA can improve mitochondrial dysfunction through its action in metabolism regulation, increased mitochondrial coenzyme level and protection against free radicle^[52,53].

Alpha lipoic acid acts as free radical scavenger with recycling of other cellular antioxidant. It interacts with membrane lipid resulting in maintaining cellular integrity and reduce enzymatic loss. Additionally, alpha lipoic acid able to restore ATP ases activity by restoring the cellular thiols level preventing the peroxidation of catalytically essential sulfhydryl groups in ATPases^[11].

CONFLICT OF INTEREST

There are no Conflict of interest

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المخلص العربي

دور حمض ألفا ليبويك على التسمم القلبي الناجم عن عقار السيكلوفوسفاميد في ذكور الجرذان الالبينو البالغين: دراسة هستولوجية نسيجية

منار بشندى واميمة زيدان

قسم التشريخ و الاجنة، كلية الطب البشرى، جامعة المنوفية، مصر

الخلفية: سيكلوفوسفاميد هو عقار يستخدم كعلاج كيميائي لعلاج سرطان الدم، وورم الغدد اللمفاوية والورم النخاعي. وقد ذكرت دراسات عديدة مجموعة واسعة من اعراض التسمم القلبي الناتجة عن استخدام السيكلوفوسفاميد في علاج المرضى المصابين بالسرطان. و يعد حمض ألفا ليبويك هو أحد مضادات الأكسدة الطبيعية المستخدمة على نطاق واسع للوقاية والعلاج في حالات مختلفة.

الهدف من العمل: تم إجراء هذا البحث لدراسة دور حمض ألفا ليبويك في التسمم القلبي الناجم عن عقار السيكلوفوسفاميد باستخدام الدراسات الهستولوجية النسيجية والتركيبية الدقيقة.

المواد و الطرق: تم استخدام أربع واربعون من ذكور الجرذان الالبينو البالغين حيث تم تقسيمهم الى اربع مجموعات: المجموعة الأولى: (الضابطة)، المجموعة الثانية (المعالجة بحمض ألفا ليبويك) بجرعة (٢٥ مج / كغ من وزن الجسم / يوم) تعطى عن طريق الفم، المجموعة الثالثة (المعالجة بعقار السيكلوفوسفاميد) بجرعة (٢٠٠ ملجم / كجم من وزن الجسم) حقنة واحدة يتم حقنها داخل الصفاق، المجموعة الرابعة (تم علاجها بحمض ألفا ليبويك بالإضافة إلى عقار السيكلوفوسفاميد). و قد أجريت التجربة لمدة عشرة أيام. و قد خضعت عينات العضلات القلبية للدراسة باستخدام المجهر الضوئي والالكتروني والدراسات الكيميائية المناعية.

النتائج: أظهر الفحص المجهرى لعضلة القلب للمجموعة المعالجة بعقار السيكلوفوسفاميد (المجموعة الثالثة) اضطراباً مؤثراً وفجوات فى ألياف عضلة القلب مع اختراق من الخلايا أحادية النواة بين ألياف عضلة القلب. باستخدام المجهر الالكترونى.

لوحظ تدمير اللييفات العضلية وتورم الميتوكوندريا وترتيبها بصورة غير منتظمة مع وجود توسع في الانابيب الناعمة. أظهرت بعض النويات انحلال الكروماتين وكان البعض الآخر غير متجانس مع وجود غشاء نووي للخلايا غير منتظم. تم تأكيد هذه النتائج من خلال انخفاض كبير في التفاعل الكيميائى المناعى ل Bc12. كانت المساحات بين الخلايا واسعة وتحتوى على أوعية دموية مزدحمة وموسعة بالكولاجين. استخدام حمض ألفا ليبويك يحسن بنية عضلة القلب ولكن لا يزال لم يتحقق العودة الكاملة للشكل الطبيعى للنسيج القلبي.

الاستنتاج: استخدام حمض الالف ليبويك مع عقار السيكلوفوسفاميد يخفف من إصابة القلب الناتجة التأثير السمي الضار لهذا العقار.