Original Article

The Modulating Role of Vitamin E Against Potassium Dichromate's Cardiotoxicity in Adult Albino Rat

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ABSTRACT

Introduction: One extremely hazardous inorganic chemical reagent is potassium dichromate (PD). Despite its genotoxic, immunotoxic, and carcinogenic effects, it is nevertheless frequently employed in industrial and laboratory settings. Vitamin E is fat-soluble antioxidant and a vital element for physiological functions in humans.

Objective: To elucidate the preventive potential of vitamin E against adult rat cardiac damage produced by potassium dichromate.

Material and Methods: 30 adult male albino rats were divided into three groups. Group I served as control (group Ia: received distilled water and group Ib: received olive oil, both through intragastric tube). Group II (PD, 30 mg/kg once daily through intragastric tube for 21 days) and group III received both PD (30 mg/kg) and vitamin E (100 mg/kg) through intragastric tube once daily for 21 days after a pretreatment period with vitamin E (100 mg/kg) for 7 days. Samples of cardiac muscle were prepared for light and electron microscopic examination, immunohistochemical study by anti-connexin 43 as well as morphometric analysis.

Results: Group II showed marked degenerative changes in the cardiac muscle. there is marked loss of muscle fiber striations and fragmentation of nuclei. There is also disturbed ultrastructure of the cell and abnormal intercalated discs. All pathological signs were indicator of cardiac damage due to PD toxicity. Group III showed improvement of the histopathological alterations caused by PD and the cardiac fibers regained their normal structure. Anti-connexin 43 immunoreaction in group III appeared comparable to control against the weak positive reaction in group II. Morphometric analysis revealed statistically significant increase in myocardial thickness in group III in comparison with group II.

Conclusion: Given its antioxidant properties and protective effects on the cardiomyocyte, vitamin E is suggested as a possible intervention to lessen the deleterious consequences of PD.

Received: 09 June 2024, **Accepted:** 12 August 2024

Key Words: Antioxidant, connexin 43, potassium dichromate.

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ISSN: 1110-0559, Vol. 48, No. 3

INTRODUCTION

Cardiovascular system is one of the most important and disease vulnerable systems in the body[1]. The wall of the heart consists of three distinct layers; the pericardium, myocardium, and endocardium. The endocardium is comprised of simple squamous epithelial cells forming the inner lining of the heart chambers and valves. The subendothelial layer contains loose elastic tissue, collagen bundles, nerves and occasionally blood vessel. The pericardium is a fibrous sac that surrounds the heart. The epicardium refers to the visceral region that is in contact with the heart. It is a product of mesothelium, rich in neurovascular tissue and adipocytes. Because of this layer's good lubrication, the heart may contract more smoothly against the parietal pericardium[2].

The myocardium, which is composed primarily of connected cardiomyocytes, makes up a large portion of the heart wall. Cardiomyocytes exhibit a branching, linear arrangement in contrast to the unbranched linear appearance of muscle cells in skeletal muscle tissue. The cardiomyocytes in this highly vascularized layer have glycogen granules as an extra energy source[3,4].

Each myocyte has cylindrical shape and contains a single, centrally located nucleus where di- or multinucleated cells could be present. The myocyte is surrounded by a cell membrane known as the sarcolemma[2]. The cytoplasm of the cardiomyocyte (sarcoplasm) contains a large number of mitochondria to meet the high metabolic demand of the cells. Cardiac muscle cells contain branched fibers connected via intercalated discs that bind each cardiomyocyte to its neighboring cell. They contain gap junctions and desmosomes. The gap junctions that appear perpendicular to the heart muscle fibers, allow the cardiomyocytes to contract simultaneously and permit rapid transmission of action potentials from one cell to the other[2,3].

The striations of the cardiac muscle are shorter than those of the skeletal muscle[3]. There are repeating units of contractile fibers known as sarcomeres. This functional unit consists of thick myosin and thin actin filaments[2].

DOI: 10.21608/ejh.2024.295014.2082

The myosin and actin filaments are organized in repeating units. Bundles of myofilaments, run the length of the cell. Dense Z lines, which cross across bright regions of the cell called I bands, encircle the myofilaments on both sides[3].

One of the potentially dangerous inorganic chemical reagents is potassium dichromate (PD). It is one of the chromium hexavalent compounds that has been connected to toxicity to several organs[5]. Chemical exposure can occur by skin contact, inhalation, and accidental ingestion, particularly in children. It is utilized in tanning, painting, printing, electroplating, and glassware cleaning in addition to being an analytical reagent. Despite its carcinogenic, genotoxic, and immunotoxic effects, it is still frequently employed in industrial and laboratory settings[6,7].

Human health is seriously threatened by exposure to such a heavy metal[8,9]. Reduction of hexavalent chromium produces excess reactive oxygen species (ROS). This ultimately triggers an electrolyte-stressing mechanism[10]. Overloading with calcium and sodium causes additional harm to the mitochondria and other organelles[11]. ROS have the ability to amplify the inflammatory cascade, which is set off by pro-inflammatory chemicals and cytokines, ultimately leading to tissue damage[12]. Consequently, an imbalance in the generation and removal of ROS by the antioxidant defense system may lead to a state of oxidative stress[13,14].

Antioxidants play a significant role in biological systems. They shield cells from damage caused by free radicals and inhibit or buffer the oxidation of dangerous substances. Plant-based natural antioxidants promote general health, provide defense against illnesses like heart disease, neurological disorders, and gastrointestinal problems, and slow down the aging process[15,16].

Vitamin E is an essential micronutrient and fat-soluble antioxidant[15,17]. Although it is produced by photosynthetic organisms like plants, it is regarded as a dietary supplement that is only obtained by food[18]. It is not produced in the human body. Vegetable oils such canola, peanut, sunflower, walnut, and cereal grain oils are sources of vitamin E[15,19]. One of the most potent forms of vitamin E, alpha-tocopherol, scavenges free radicals and shields cell membranes. To guard against damage from oxide radicals, it is added to medications and dietary items[4,20]. Moreover, vitamin E has anti-inflammatory properties when consumed regularly[19,21].

Vitamin E is reported to enhance the immune system. Furthermore, research has demonstrated a protective effect against disorders that impair cognitive function, such Parkinson's and Alzheimer's. It has recently been the subject of research about its potential benefits in cancer prevention[18].

Numerous studies suggested that consuming vitamin E could prevent or postpone the development of chronic illnesses linked to ROS molecules[4]. When taken as a supplement, it has been shown to benefit certain populations, including elderly individuals with respiratory diseases and hemodialysis patients. It has been observed that vitamin E accumulates in the heart and lungs' mitochondrial and endoplasmic reticulum membranes, which are more likely to produce free radicals. While vitamin deficiencies are extremely uncommon, they can happen to adults with disorders related to fat malabsorption. In addition to liver failure and digestive tract disorders, this deficiency causes ataxia[18].

Nowadays, due of its photoprotective properties, vitamin E is one of the main components of skin creams and hair products. Its nutritional value makes it a useful addition to animal feeds for cattle and poultry, as well as for improving the flavor and appearance of meat[18]. It is a part of food packaging and preservation that extends the shelf life of canned goods. It is also employed as an antibacterial and antioxidant stabilizer. It is used for tissue regeneration and wound healing due to its capacity for cell proliferation[17].

In recent years, efforts to boost the supply of natural vitamin E through plant in *vitro* cultures have gained momentum due to the growing demand for it[18].

Therefore, the present study was carried out to examine the possible protective effect of vitamin E against the degenerative changes induced in rat myocardium due to exposure to potassium dichromate (PD).

MATERIAL AND METHODS

Chemicals and drugs

Potassium dichromate (PD) was acquired from Sigma Aldrich (St. Louis, MO, USA) and dissolved in distilled water. Vitamin E (alpha-tocopheryl acetate) was purchased from BDH, England and was diluted in olive oil.

Animals

Thirty male adult albino rats, two months old, weighing between 170 and 180 grams, were obtained from the Assiut University Faculty of Medicine's animal house. Before the experimental protocol began, rats were kept in polypropylene cages with a standard light/dark cycle and temperature of 25 °C for seven days in order to adapt them to the controlled laboratory environment. Water and food were freely available. The Committee of Animal Research Ethics at the Faculty of Medicine, Assiut University, Assiut, granted consent for this work (approval no: 04-2024-300402).

Experimental design

Three groups of ten rats each were created by random assignment:

Group I (control group): was divided into:

- Group Ia: received distilled water through intragastric tube once daily for 21 days.
- Group Ib: received olive oil through intragastric tube once daily for 21 days.

Group II (PD-treated group): received PD by intragastric tube at a dose of 30 mg/kg once daily for 21 days[22].

Group III (PD and Vitamin E treated group): received both PD (the same regimen as group II)[22] and vitamin E (100 mg/kg) by intragastric tube once daily for 21 days after a pre-treatment period with vitamin E (the same route and dose) for 7 days[23].

At the end of the experiment, rats in all studied groups were sacrificed by cervical dislocation under diethyl ether inhalation. The heart from each rat was immediately dissected out and the following techniques were used:

I- Light microscopic examination

Cardiac samples were sectioned at 5µm after being fixed in 10% buffered formalin solution and embedded in paraffin blocks for histological examination. After deparaffinization, the sections were stained with hematoxylin and eosin (H&E) to examine the architecture of the heart muscle under a light microscope[24].

II- Immunohistochemistry using anti-connexin 43 (Cx43)

The gap junction protein known as connexin 43 is unique to heart tissue and facilitates communication between cells. Samples of left ventricular heart tissue were extracted from each of the three groups under investigation, fixed in paraffin, and sectioned serially every 5 µm. After xylene was used to deparaffinize the slides and ethanol was used to rehydrate them, antigen retrieval was carried out in a pressure cooker using 0.01 M citrate buffer (pH 6.0), and the slides were then allowed to naturally cool to room temperature. The next step involved a 10-minute RT incubation in 0.3% hydrogen peroxide. The sections were blocked for 15 minutes at room temperature using goat serum. After an overnight incubation at 4 °C with a rabbit polyclonal anti-Cx43 antibody (1:1,000 dilution; Abcam, UK), the slices were treated for 30 minutes at room temperature with an HRP-conjugated goat anti-rabbit IgG antibody (AB clonal, Wuhan, China). DAB solution was used for immunodetection in accordance with the manufacturer's instructions. The slides were counterstained with Mayer's hematoxylin after being cleaned[25]. Connexin 43 positive reaction appeared as brownish coloration and nuclei appeared blue.

III- Electron microscopic examination

The cardiac specimens were prepared for ultrathin sectioning by slicing them into thin slices (approximately 1 mm3), fixing them in 4% glutaraldehyde, washing them in phosphate buffer, and post-fixing them in 1% osmium tetraoxide. The ultrathin slices (50–60 nm) were stained, inspected, and photographed using a transmission electron microscope (JEM–100 Cx11, Jeol, Assiut, Egypt) with the use of uranyl acetate and lead citrate[24].

IV- Morphometric measurements

By utilizing slices stained with hematoxylin and eosin at a magnification of X400 in various random fields, the thickness of the myocardium was evaluated in different experimental groups. Using an image analyzer computer system, an OLYMPUS DP27 digital camera coupled to an OLYMPUS CX41 light microscope and a PC using cell Sens Standard software (version 1.7), measurements were taken in ten non-overlapping fields in ten randomly selected sections.

Statistical analysis

The three experimental groups' histomorphometric measurements yielded data that was represented as mean ± SD. The data was then statistically analyzed using SPSS, version 22 (SPSS Inc., Chicago, Illinois, USA). Using Tukey's post hoc test and the analysis of variance (ANOVA) test, histograms were created and the significance between the experimental groups was compared. At p *values* ≤ 0.05, statistical significance was established.

RESULTS

Light microscopic examination using

A. H&E staining

As regard the control group Ia, a normal histological structure of cardiac muscle fibers was detected where the fibers were stained pink with fine cross striations throughout their length. Nuclei were ovoid and centrally located with regularly branched cytoplasmic network and striations. Interstitial connective tissue appeared between the muscle fibers as a clear space (Figure 1a). Those findings were in control group Ib (Figure 1b).

PD-treated rats (group II) showed disorganized histological structure of the cardiac muscle. Marked loss of muscle fiber striations as well as fragmentation of nuclei were detected. The interstitial space was increased and appeared irregular with apparent hemorrhage between the muscle fibers. (Figures 1 c,d). Marked lymphocytic infiltration and brown pigments appeared within the field (Figure 1d).

In contrast, PD and vitamin E-treated rats (group III) showed obvious improvement in the histological appearance of cardiac myocyte striations. Minimal and more regular interstitial space and blood capillaries were regular. The nuclei regained their ovoid normal shape (Figure 1e).

B. immunohistochemical staining

As regard group I, brownish areas were detected within the field among the cardiac tissue indicating increased reactivity to connexin 43 immunohistochemical stain (Figure 2a). However, group II showed marked decrease in the reactivity (Figure 2b) on the contrary to group III where a noticed improvement in reactivity was detected (Figure 2c).

Electron microscopic examination

As regard the control group (group I), normal ultrastructure of cardiac myofibrils with regular myocardial striation's architecture and intact intercalated discs were detected. Abundant mitochondria of preserved integrity were scattered between the myofibrils (Figures 3 a,d). The nucleus was regular, euchromatic with intact nuclear envelope and double nucleoli (Figure 3d).

PD-treated rats (group II) showed disturbed ultrastructure of the myocyte. Cardiac myofibrils showed disarrangement and loss of the usual striated pattern (Figure 3e). Some intercalated discs were disorganized and the cytoplasm was rarefied. Mitochondria appeared swollen, irregular and pleomorphic while some were with destructed cristae (Figures 3 b,e). The nucleus was irregular and showed clumped chromatin with absent nucleolus (Figure 3e).

As compared to group II, PD and vitamin E-treated rats (group III) showed improvement in the ultrastructure of the cell and the pattern of the myofibrils as well as the intercalated discs. The cytoplasm showed less rarefaction but some mitochondria were still swollen (Figures 3 c,f). The nucleus was oval, regular, euchromatic with intact nuclear envelope and prominent nucleolus (Figure 3f).

Morphometric measurements

The mean thickness of the myocardium was: 1498.8 ± 28.5, 931.6 ± 36.8 and 1491 ± 27.2 microns in groups I, II and III respectively. In comparison to group I, group II showed a statistically significant ($p \leq 0.05$) reduction in the myocardial thickness of the group II. However, the reduction in group III was statistically non-significant ($p > 0.05$). On the other hand, the mean thickness of myocardium in group II showed a statistically significant ($p \leq 0.05$) reduction as compared with group III (Table 1, Figure 4).

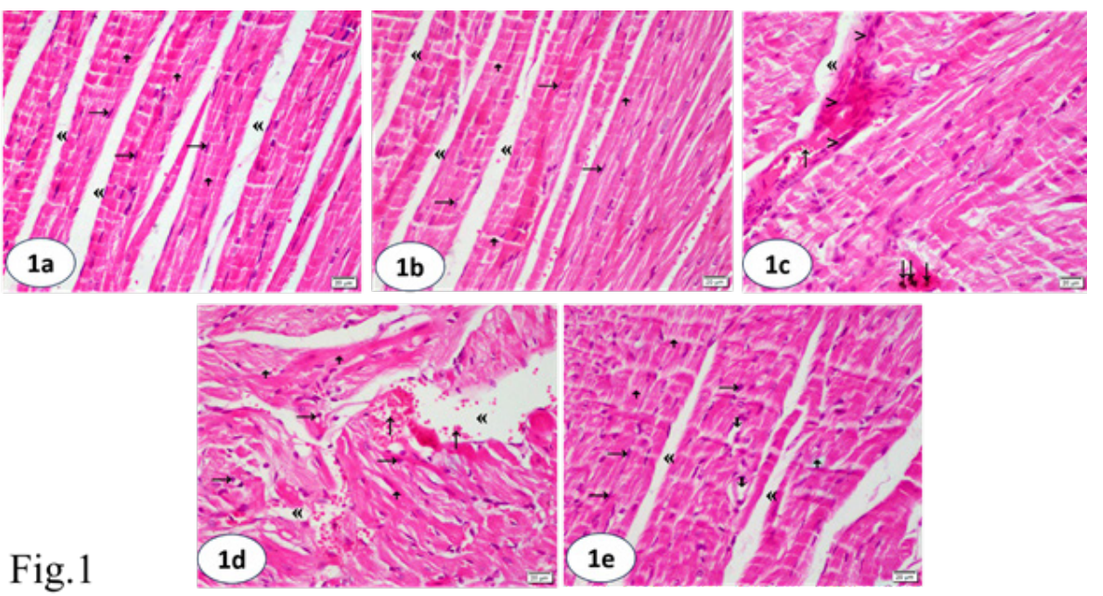

Fig.1

Fig. 1: Photomicrographs of longitudinal section of the heart of adult rats. The control group (Figs.1a&1b) shows normal histological structure of cardiac muscle fibers. The fibers are stained pink with fine cross striations throughout their length. Nuclei (→) are ovoid and centrally located with regularly branched cytoplasmic network with striations (↑). Interstitial connective tissue appears between the muscle fibers as a clear space («). PD-treated group (Figs.1c&1d) shows disorganized histological structure of cardiac muscle. Marked loss of muscle fiber striations (↑) as well as fragmentation of nuclei are detected (→). The interstitial space («) becomes irregular and increased with apparent hemorrhage (↑) between the muscle fibers. Fig.1c shows marked lymphocytic infiltration (>) as well as brown pigments (↓). PD and vitamin E-treated group (Fig.1e) shows the approximately normal histological appearance of cardiac myocyte striations (↑), minimal and more regular interstitial space («) and regular blood capillaries (↓). Nuclei (→) regain their ovoid regular shape. (H&E x400)

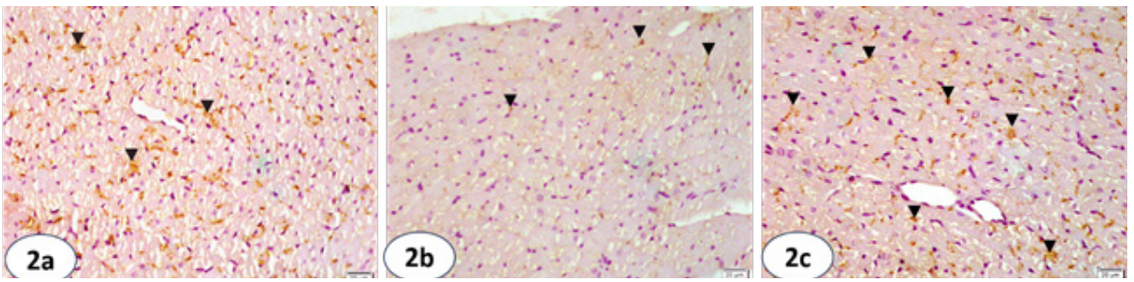

Fig. 2: Photomicrographs of connexin 43 immunohistochemical staining of the heart of adult rats. Positive areas are detected (▼) within the field among the cardiac tissue. The control group (Fig.2a) shows pronounced reaction while PD-treated group (Fig.2b) shows a noticed decrease. PD and vitamin E-treated group (Fig.2c) shows noticed improvement in immunoreaction. (Connexin 43 x400)

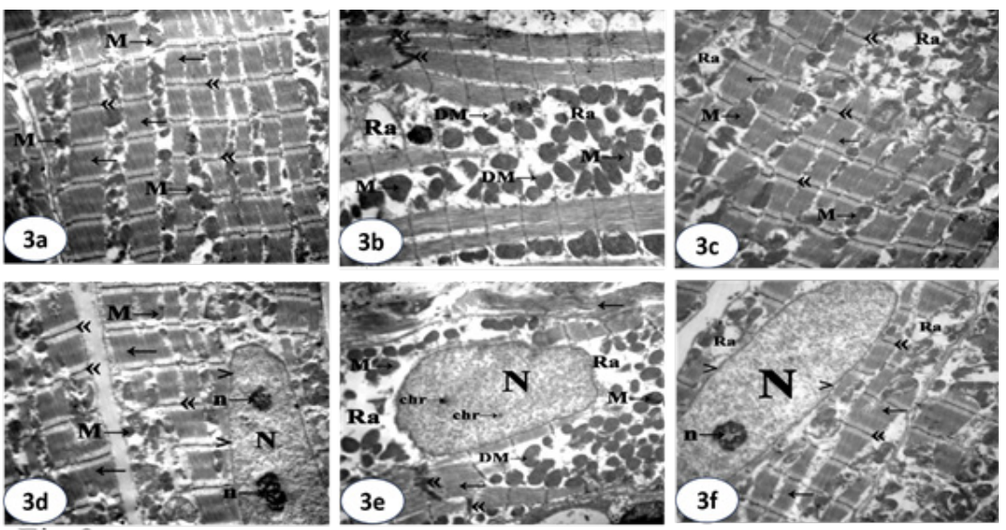

Fig. 3: Electron photomicrographs of myocardial cells of the heart of adult rats. The control group (Figs.3a&3d) shows normal ultrastructure of cardiac myofibrils (←) with regular myocardial striation's architecture as well as the intercalated discs («). Abundant mitochondria (M→) with preserved integrity are scattered between them. The nucleus (N) is regular, euchromatic with intact nuclear envelope (>) and double nucleoli (n→) (Fig.3d). PD-treated group (Figs.3b&3e) shows disturbed ultrastructure of the cell. Some intercalated discs («) are disorganized. The cytoplasm is rarefied (Ra). Mitochondria (M→) are large, swollen irregular pleomorphic while some are destructed (DM→). Cardiac myofibrils (←) show disarrangement and loss of the usual striated pattern. The nucleus (N) is irregular and shows clumped chromatin (chr→) with absent nucleolus (Fig.3e). PD and vitamin E-treated group (Figs.3c&3f) shows improvement in the ultrastructure of the cell and the pattern of the myofibrils (←) as well as the intercalated discs («). The cytoplasm shows less rarefaction (Ra) but some mitochondria (M→) are still swollen. The nucleus (N) is oval, regular, euchromatic with intact nuclear envelope (>) and prominent nucleolus (n→) (Fig.3f). (TEM x5800)

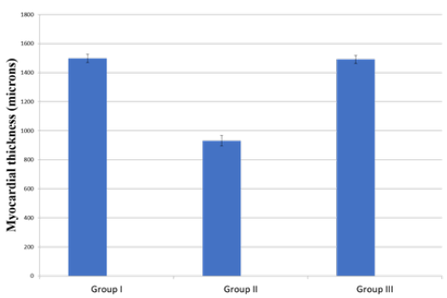

Fig.4

Fig. 4: A histogram showing the relation of the mean thickness of the myocardial thickness (mean ± SD) in the adult rat in the different studied groups. Notice that the error bars indicated the standard deviation values.

Table 1: Showing the mean thickness of the myocardium (microns) in the adult rat in the different studied groups

	Group I (Mean ± SD)	Group II (Mean ± SD)	Group III (Mean ± SD)	*P-value*1	*P-value*2	*P-value*3
Myocardial thickness	1498.8± 28.5	931.6 ± 36.8	1491 ± 27.2	0.00*	0.9 NS	0.00*

(NS) → Non-significant (p> 0.05)
(*) → Significant ($p\leq$ 0.05)
*P-value*1 compares group II to group I
*P-value*2 compares group III to group I
*P-value*3 compares group II to group III

DISCUSSION

Exposure to PD, which is now known to be an occupational carcinogen, at work or in the environment may put the lives of a great number of people in jeopardy. The current study made an effort to look into vitamin E's possible protective impact against the cardiotoxicity brought on by PD[11,26].

The light microscopic sections of group II revealed several pathological signs of cardiac muscles and these findings were in consistent with Yang *et al*.[27] and El Shoura *et al*.[11].

The observed degenerative process in the heart muscle of group II rats in this investigation was suggested by the significant loss of muscle fiber striations and the fragmented nuclei. The toxin's active metabolites, which may directly harm endothelial capillaries, may be the cause of the interstitial bleeding and the observed increase in interstitial space. According to Attia *et al*.'s hypothesis[4], this might then cause interstitial edema, bleeding, and potentially the production of microthrombi, which would further harm the myocardium.

Strong inflammatory response is triggered by cardiac damage, according to Liu *et al*.[28]. The current work's detection of cellular lymphocytic infiltration may be explained by this. Furthermore, the pigments seen inside the cells may be the released lipofuscin pigments, which are a colored consequence of intracellular catabolism gone wrong and aid in autophagy[29].

The disturbed ultrastructure of the cells in group II of the current work comes in line with the light microscopic results. In a study by Shati *et al*.[30], the rarefied cytoplasm and disordered intercalated discs were reported as indicators of heart injury.

The altered calcium homeostasis may be the cause of the enlarged mitochondria found in the present study. This disruption may then lead to an accumulation of salt inside the cell, which produces edema and organelle enlargement. Furthermore, as reported by Shati *et al*.[30], the heart muscle fibers have very low concentrations of enzymatic antioxidants, which may play a significant role in mitochondrial damage. Cellular apoptosis may occur as a result of such damage[31].

The clumped chromatin observed in the present study of group II was described in a study of Aki *et al*.[32] as the most prominent feature of apoptotic cells. Even the lack of a nucleolus may indicate that the cells' ability to produce ribosomes has been lost, which would then have an impact on protein synthesis[33].

According to Yang *et al*.[27] and El Shoura *et al*.[11], lipid peroxidation is the cause of the degenerative alterations that accompanied PD toxicity. The cardiac myofibers may sustain membrane injury as a result of this. Furthermore, it has been shown by Chaâbane *et al*.[34] that PD is able to readily cross cell membranes. Molecule oxygen is biologically reduced to reactive intermediates inside the cell, where it is further reduced to superoxide anion and hydrogen peroxide (H_2O_2). The researchers hypothesized that the resulting intermediates combine with H_2O_2 to produce hydroxyl radicals, which have a variety of harmful effects[34].

Numerous studies claim that PD causes nephrotoxicity, cardiotoxicity, and hepatotoxicity by the generation of ROS or mitochondrial damage, which results in cell death or apoptosis. Oxidative stress has been identified as one of the main causes of organ damage and degeneration associated with PD due to the overproduction of the lipid peroxidation marker and impaired action of various antioxidants[11,27].

Conversely, the treatment of vitamin E in conjunction with PD resulted in a noteworthy decrease in the symptoms of degenerative heart muscle. The cardiomyocyte histology in group III was refined to a level that seemed similar to that of group I. These findings were consistent with the potent antioxidant function of vitamin E and with the findings of Attia *et al*.[4] and Elkerdasy[35]. Attia *et al*.[4] noted that vitamin E can prevent oxidative damage and stop the chain reaction of radicals. Consequently, it may be maintaining the myocytes' functional integrity, demonstrating its protective effect against cardiotoxicity. The most potent form of vitamin E, tocopherol, is also membrane-bound in animals and is believed to have two functions: it stabilizes the membrane and functions as a significant biological antioxidant on the membrane's surface, according to Shati *et al*.[30].

The connexin is a well-known membrane protein that is an important component of gap junction channels in the intact myocardium. Connexin 43 is the most abundant one in cardiomyocytes being responsible for the communication between cardiomyocytes[36,37] and this could illustrate the increased reaction in group I. The remarkable decrease in the immune-reactivity of connexin 43 in group II as well as the marked improvement in reaction in group III, were in line with studies of Zhang *et al*.[36] and Yang *et al*.[37]. The present controversial response in the immunoreactivity

proved the destructive effect of PD and the stabilizing role of vitamin E for biological membranes.

Sinovas *et al*.[38] added that connexin fragments are found within the nucleus and that inhibition of connexins has significant effects on cell differentiation. This revealed an additional mechanism underlying the detrimental consequences of PD and the preventive benefits of vitamin E. PD suppresses connexins, but vitamin E maintains them.

The current morphometric data validated the histology findings, which point to the detrimental effects of PD and the modulatory action of vitamin E as the reasons for the decrease in cardiac thickness in group II and the improvement in group III. These results align with the observations made by Liu *et al*.[39].

CONCLUSION

The histopathological data obtained from the present study indicated that vitamin E administration offered a protection to the myocardium of adult rats against PD induced cardiac oxidative damage. adult rats' myocardium was protected from PD-induced cardiac oxidative damage by vitamin E supplementation. The study demonstrated how vitamin E can shield against harmful effects and help normal heart cells operate again.

ETHICAL STATEMENTS

This study was carried out in strict accordance with the International Guidelines for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Ethics Committee at the Faculty of Medicine, Assiut University, (Approval number: 04-2024-300402).

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدور المعدل لفيتامين هـ ضد التسمم القلبي بثنائي كرومات البوتاسيوم في الفأر الأبيض البالغ

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مقدمة: ثنائي كرومات البوتاسيوم هي مادة كيميائية غيرعضوية خطرة ولها آثارمسرطنة وسمية جينية ومناعية؛ غير أنها لا تزال تستخدم على نطاق واسع في التطبيقات المختبرية والصناعية. فيتامين " هـ " هو أحد مضادات الأكسدة القابلة للذوبان في الدهون وهو عنصر حيوي وهام للوظائف الفسيولوجية في البشر.
الهدف: توضيح القدرة الوقائية لفيتامين" هـ " ضد التسمم القلبي الذي يسببه ثنائي كرومات البوتاسيوم في الفأرالبالغ.
المواد وطرق البحث: تم تقسيم ٣٠ فأراً ذكراً بالغ إلى ثلاث مجموعات؛ وصممت المجموعة الأولى كمجموعة مرجعية، بما في ذلك (المجموعة الأولى أ : التي تلقت المياه المقطرة والمجموعة الأولى ب: التي تلقت زيت الزيتون، كلاهما عن طريق الأنبوبة المعدية)، والمجموعة الثانية تلقت (٣٠ ملجم/كجم من ثنائي كرومات البوتاسيوم مرة يومياً عن طريق الأنبوبة المعدية لمدة ٢١ يوماً) والمجموعة الثالثة التي تلقت كلاً من ثنائي كرومات البوتاسيوم (٣٠ ملجم/كجم) وفيتامين " هـ " (١٠٠ ملجم/كجم) عن طريق الأنبوبة المعدية مرة في اليوم لمدة ٢١ يوماً بعد فترة معالجة سابقة بالفيتامين (١٠٠ ملجم/كجم) لمدة ٧ أيام. وقد أُعدت عينات من عضلة القلب للفحص بالمجهرالضوئي والإلكتروني، ولدراسة الكيمياء النسيجية المناعية بواسطة مضاد الكونيكسين ٤٣ فضلاً عن التحليل المورفومتري.
النتائج: أظهرت المجموعة الثانية تغيرات انحلالية ملحوظة في بنية عضلة القلب نظراً إلى وجود خسارة ملحوظة في تخطيط الألياف العضلية وتجزئة النويات. كما أن هناك اضطراب في التركيب الدقيق للخلية والأقراص البينية ظهرت غير طبيعية. وكانت جميع العلامات المرضية مؤشراً على الضرر القلبي الناجم عن التسمم بثنائي كرومات البوتاسيوم. وأظهرت المجموعة الثالثة تحسناً في هذه التغيرات حيث استعادت ألياف القلب هيكلها الطبيعي. وقد ظهر التفاعل المناعي المضاد للكونيكسين ٤٣ في المجموعة الثالثة متماثلاً للمجموعة الأولي علي نقيض التفاعل الإيجابي الضعيف في المجموعة الثانية. وبيّن التحليل المورفومتري زيادةً ذات دلالة إحصائية في سُمك عضلة القلب في المجموعة الثالثة بالمقارنة مع المجموعة الثانية.
الاستنتاج: نتيجة لنشاطه المضاد للأكسدة وتأثيره الوقائي على عضلة القلب يوصى بأن يكون فيتامين هاء مرشحاً محتملاً للحد من الآثار الضارة لثنائي كرومات البوتاسيوم.