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Vitamin E Attenuates Cardiomyopathy Via Alleviation of Autophagic Stress

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Abstract

Introduction: Vitamin E (Vit E) is well known antioxidant. Bisphenol A (BPA), widely used industrial chemical product, is associated with increased risk of cardiac diseases. This study identifies the potential protective effect of Vit E on BPA induced cardiomyopathy through alleviation of oxidative and autophagic stress. **Materials and Methods:** Twenty –four adult male rats were used in the study. They were randomly divided into 4 groups; negative control, Vit E positive control, BPA induced cardiomyopathy, and BPA+ Vit E treated group. All substances were given orally via gastric gavage for 14 days. Rats were sacrificed and their hearts were dissected out. Serum, cardiac homogenates, and cardiac tissues were obtained for biochemical and histopathological evaluation. **Results:** There were significant increase in serum LDH and CK-MB, tissue homogenates showed elevated levels of NO and MDA and decreased level of GSH in BPA group. Immunohistopathological evaluation of autophagic mediators showed significant increase in LC3 and P62 in BPA group. On Histological examination, there was pathological alteration in BPA group compared to control group. Vit E administration showed significant improvement in cardiac enzymes and oxidative stress. Also, alleviation of autophagic process and restoration of the myocardial architecture with reduction of the fibrous tissue were observed with Vit E administration. **Conclusion:** These results demonstrate that Vit E exhibits substantial protective effects in BPA induced cardiomyopathy by attenuating inflammation, oxidative stress, and alleviation the autophagic stress though improvement of lysosomal function. Alleviation the autophagic stress by Vit E might be an important therapeutic target for cardiomyopathy.

Keywords: Cardiomyopathy; Bisphenol A; Vitamin E; oxidative stress; LC3; P62.

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1. Introduction

Cardiomyopathy is a group of progressive cardiac dysfunction lesions caused by structural changes in ventricles and damaged function of the myocardial wall. Cardiomyopathy accounts for 0.6– 3.4% of cardiovascular diseases and has recently shown an increasing trend. Generally, the etiology is obscure and may be associated with viral infection, autoimmune response, heredity, drug poisoning, and abnormal metabolism [1].

The mechanisms involved in the pathogenesis of cardiomyopathy remain poorly understood and further understanding the mechanisms underlying cardiomyopathy is still needed to provide proper therapeutic strategies [2].

BPA is of great interest because it is a high-volume industrial chemical used worldwide, with adverse health effects in multiple organ systems [3].

Several studies indicate that BPA exposure in adult populations is associated with increased risk for CV diseases. Experimental studies suggest that both acute and chronic BPA exposure could affect the physiological functioning of CV system and promote abnormal CV activities such as arrhythmias, cardiac remodeling, atherosclerosis, and altered blood pressure [4]. BPA is considered a ubiquitous environmental toxin in humans, as it is detectable in the urine of most adults and children's samples. Preclinical studies have described that BPA is able to induce oxidative stress, mitochondrial membrane depolarization, cell death, lipid peroxidation, as well as abnormal autophagy [5].

Lots of evidences indicated alterations of autophagy involved in a wide range of cardiac diseases including cardiomyopathies [6]. Autophagic stress generally refers to a relatively sustained imbalance in which the rate of autophagosome formation exceeds the rate of its degradation. Autophagic stress is related to aging, and many disease states in response to protein or organelle damage [7; 8].

Cardiomyocytes have a low level of autophagic activity under physiological conditions, but it may become important in situation of cardiac stress being has a protective role against accumulation of toxic protein aggregates [9; 10]. Autophagy participates in scavenging proteins with abnormal structures and separation of toxic protein bodies during cardiomyocyte remodeling. Moreover, efficient autophagic activity can remove the damaged mitochondria and thereby indirectly prevent excessive reactive oxygen species (ROS) production and inflammasome activation [9; 11].

Vitamin E is a non-enzymatic antioxidant naturally present in biological systems. This molecule protects the cell membrane from lipid peroxidation, which is induced by overproduction of ROS and RNS. Vit E has shown protective effects in vivo and in vitro [12].

Many published studies have described the protective role of Vit E in many diseases; however, there is no previous study demonstrated protective effect of Vit E in cardiotoxicity. Therefore, this study demonstrated the protective effects of vitamin E in bisphenol inducing cardiomyopathy by its antioxidant effect and alleviation of autophagic stress.

2. Materials and Methods

2.1 Ethical approval

This study was approved by the Intuitional Research Board (IRB) (Code number: R.21.03.1242-2021/03/07), Faculty of Medicine, Mansoura University and followed the EU and NIH guidelines for animal care.

2.2 Animals used

Adult male Sprague Dawley rats (n=24; weight, 200±40 gram) were obtained from the medical experimental research center (MERC) of faculty of medicine, Mansoura university. The animals were adapted to these conditions for at least one week before being used in the experiments and general conditions were monitored throughout the study. All accessible efforts were done to minimize the animal discomfort and the number of animals used.

2.3 Chemicals used

BPA and vitamin E were purchased from Sigma-Aldrich, (St. Louis, MO, USA). Corn oil was bought from El-Gomhorya Company for Pharmaceuticals, Mansoura, Egypt. Antibodies against LC3, P62 were obtained from Abcam, Egypt.

2.4 Study design

After a week of acclimatization, the rats were randomly divided into 4 groups (n = 6/group):

G1 (negative control group): in which rats were received oral corn oil 0.5 mL/Kg/day orally via gastric gavage.

G2 (Vit E positive control group): in which rats were received vitamin E dissolved in corn oil at a dose of 1000 mg/kg day orally via gastric gavage [11].

G3 (Bisphenol pathological group): in which rats were received BPA at dose of 250 mg/kg/day dissolved in corn oil, orally via gastric gavage [13].

G4 ((Bisphenol + Vit E group): in which rats were received oral BPA 1 hour after Vit E administration daily at previous doses.

All groups were subjected to these substances for 14 days.

2.5 Processing the specimens

Twenty-four hours after the last dose, the rats were anesthetized with sodium thiopental (120 mg/kg) intraperitoneally. Blood samples were taken to assess cardiac enzymes.

The rats were fixed on a surgery board and the upper part of the body is disinfected with 70% ethanol and ,then an incision was made at the level of xiphoid process and the anterior thoracic wall was opened bilaterally exposing the heart, a needle was inserted into the left ventricle and the renal vein was incised to allow for free circulation, then 500 ml of normal saline was immediately perfused into the circulation, then the heart from each rat was excised, washed with physiological saline and small portion of the heart was dissected, weighted and then prepared for tissue homogenate for determination of oxidative stress while, the large portion of the heart was soaked in 10% neutral formalin to be processed to prepare paraffin blocks. Sections were cut and used later for histopathological examination using with hematoxylin and eosin (H & E) and Masson stains. Also,

they were immunostained for LC3 and P62 studies [14].

2.6 Preparation of tissue homogenate

Cardiac tissues were gently blotted between the folds of a filter paper and weighed in an analytical balance. 10% of homogenate was prepared in 0.05 M phosphate buffer (pH 7) using a polytron homogenizer at 4 °C. The homogenate was centrifuged at 10,000 rpm for 20 min for removing the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant was aliquoted and stored at−80 °C for further analysis of malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione (GSH) according to manuals instructions.

2.7 Assessments of cardiac enzymes

Determination of serum cardiac creatine kinase-MB (CK-MB) and lactate dehydrogenase enzymes: CK-MB in the serum was determined using method determined by [15] using CK-MB sensitive kit purchased from cloud clone company& LDH in the serum was determined using modified method [16] using LDH sensitive kit purchased from Human company.

2.8 Assessments of cardiac oxidative stress

Tissue homogenates were obtained and centrifuged to measure the levels of malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione (GSH) by using commercial colorimetric kits from the Biodiagnostic company (Cairo, Egypt) according to the manufacturer's instructions.

2.9 Immunohistochemistry

Immunohistochemical determination of autophagic markers: LC3, P62

Endogenous peroxidases were blocked with 0.3% H2O2. Antigens retrievals were performed by heating in microwave using sodium citrate buffer (pH 6) for 20 minutes and then blocked with 5% bovine serum albumin in tris buffered saline. Sections were then incubated overnight at 4 °C with a primary antibody against LC3 and P62. The reaction was detected using ABC kit following the manufacture instructions (Abcam, Egypt). Sections were then counterstained with hematoxylin, dried and mounted with a synthetic resin medium [17].

2.10 Pathological examination

Cardiac tissues were fixed in 10% neutral buffered formaldehyde and subsequently dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax. Paraffin sections (4 µm) were cut and stained with:

- Hematoxylin and eosin (H&E): as a routine histopathological examination.
- Masson trichrome stain: for cardiac tissue fibrosis assessment.

2.11 Statistical data and analysis

The statistical package for social science (SPSS) software (version 22.0, IBM, Chicago, IL, USA) will be used for analysis of the data that will be expressed as the mean ± SD. The one-way ANOVA and T tests were used. Probability (p) value less than 0.05 (5%) will be considered as statistically significant.

3. Results

The results of this study on BPA -induced cardiomyopathy in rat model indicate protective effective of vitamin E in this model.

3.1 Body Weight

Negative control group and Vit E control group show showed highly significant increase in body weight before and after treatment ($P < 0.001$). The BPA group showed non-significant change in body weight before and after treatment. Combined Vit E and BPA group showed significant increase in body weight before and after treatment ($P < 0.004$) Figure (1).

More also, BPA group showed highly significant decrease in body weight in comparison to control and treated groups ($P < 0.001$) Table (1).

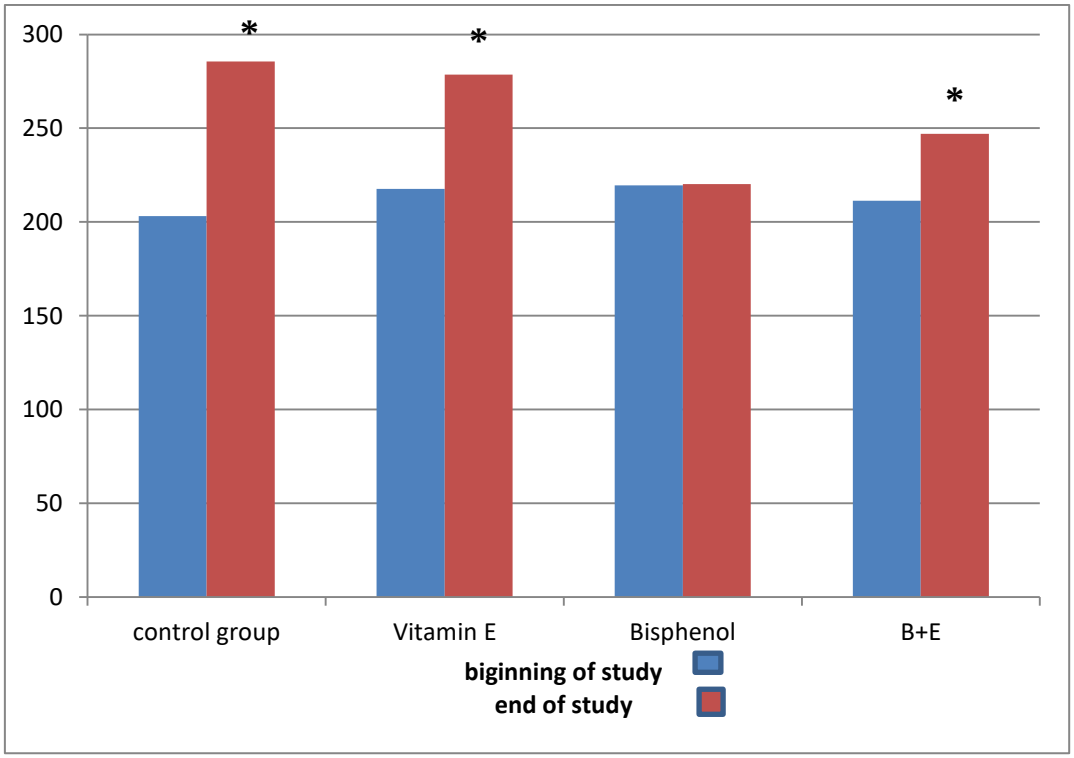

Figure 1: Body weight of experimental group beginning and end of study

*: Significance of the group after and before treatment

Table 1: Body weight (grams) within the control and the experimental groups

	C1 group (N=8)	**E1 group (N=8)**	**B1 group (N=8)**	**B1+E1 group (N=8)**
Weight at end of study	285.62 ± 4.08	278.50±10.16	220.21± 9.78	247± 11.70
P1		0.676	<0.001**	<0.001**
P2	<0.001**	<0.001**		<0.001**

N: number, SD: standard deviation, ANOVA test
** Highly statistically significant result
P1: comparison in relation to control group at end of study.
P2: comparison in relation to BPA group at end of study.

3.2 Heart Weight

Analysis of variance displayed that BPA group showed highly significant decrease in heart weight in comparison to control . The BPA + Vit E group showed a significant increase compared to BPA group (P < 0.001). Figure (2).

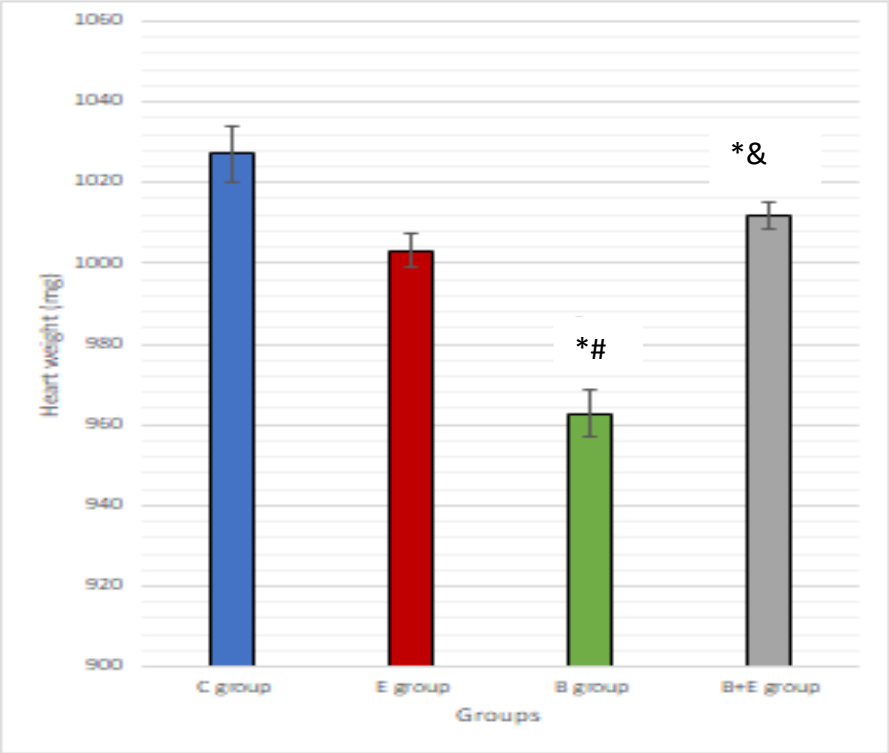

Figure 2: Effect of Vit E administration on heart weight

*: Significance in relation to control (C) group
#: Significance in relation to vit E (E) group
&: Significance in relation to BPA (B) group.

3.3 The effect of vitamin E on oxidative stress markers

To elucidate the effect of oxidative stress in the BPA induced cardiomyopathy and the ameliorative effect of vitamin E, we measured the serum level of MDA, NO and GSH. BPA induced cardiomyopathy caused a significant increase in serum MDA, NO and significant decrease in GSH concentrations compared with the examined groups ($P < 0.001$). The BPA + Vit E group showed a significant decrease in the serum MDA, NO and significant increase in GSH concentrations compared with the BPA group ($P < 0.001$). Figure (3).

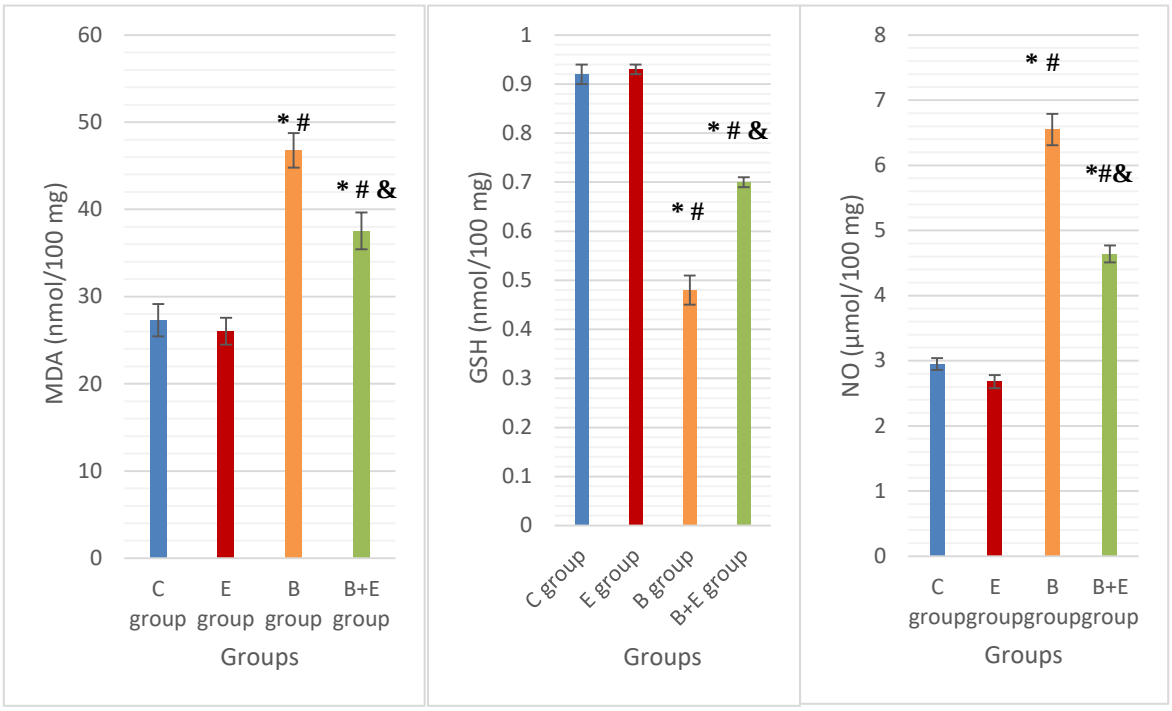

Figure 3: Effect of Vit E administration on oxidative stress markers; malondialdehyde (MDA) concentration, reduced glutathione (GSH) and nitric oxide (no).

*: Significance in relation to C group
#: Significance in relation to E group
&: Significance in relation to B group

3.4 The effect of vitamin E on cardiac biomarkers

The BPA group showed highly significant increase in serum LDH, and CK-MB activities compared to the control group ($P < 0.001$). Vit E and BPA group showed highly significant decrease in serum LDH, and CK-MB activities compared to BPA group. Figure (4).

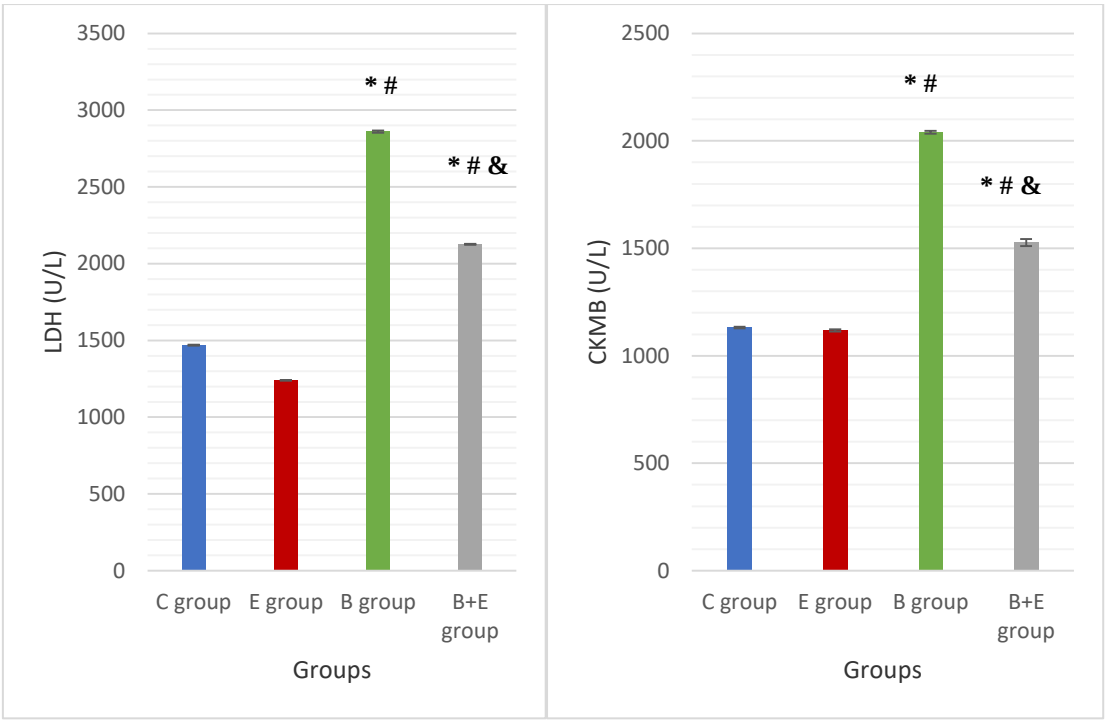

Figure 4: Effect of Vit E administration on cardiac biomarkers.

*: Significance in relation to C group

#: Significance in relation to E group

&: Significance in relation to B group

3.5 The effect of vitamin E on immunohistochemistry detection of cardiac LC3 and P62

To explore whether the cardioprotective effect of Vit E was associated with autophagy, the changes of autophagic vacuoles and autophagic substrate were studied. Myocardial sections were stained with LC3 immunostaining and quantitative scoring of autophagic vacuoles in cardiac tissue was done. Immunohistochemistry analysis of BPA group showed significant increase in LC3 immunostaining as compared with the control and Vit E treated groups. Section of a rat treated with BPA and vitamin E for 2 weeks showing significant decrease in LC3 immunostaining as compared to BPA group. Figure (5).

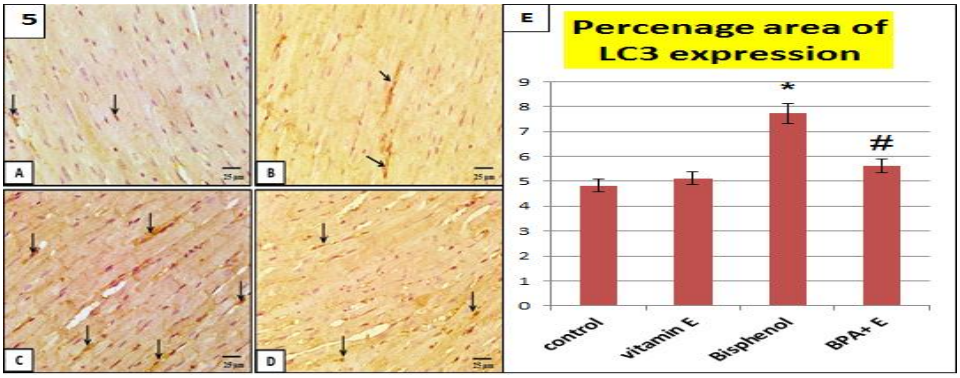

Figure 5: A photomicrograph of myocardial section stained with LC3 immunostaining **(5A):** A section of a control rat showing positive immunostaining (arrows). **(5B):** A section of a rat treated with vitamin E showing positive immunostaining (arrows). **(5C):** A section of a rat treated with BPA showing an increase in immunostaining (arrows) as compared with both control and vitamin E treated groups **(LC3 x 400). (5D):** A section of a rat treated with BPA and vitamin E showed significant decrease in LC3 immunostaining as compared with BPA group (arrows) **(LC3 X 400). (5E):** Quantitative changes of autophagic substrate **LC3** in cardiac tissue in which there is significant increase in LC3 in BPA group (*) compared to normal group and there is significant an increase in LC3 in BPA and vitamin E group (#) compared to BPA group**.**

Immunohistochemistry analysis of myocardial sections was stained with P62 immunostaining and quantitative scoring of autophagic vacuoles in cardiac tissue was done. Immunohistochemistry analysis of BPA group showed high significant increase in P62 immunostaining as compared with the control and Vit E treated groups. In contrast, section of a rat treated with BPA and Vit E showing marked an increase in P62 immunostaining as compared with BPA treated group. Figure (6).

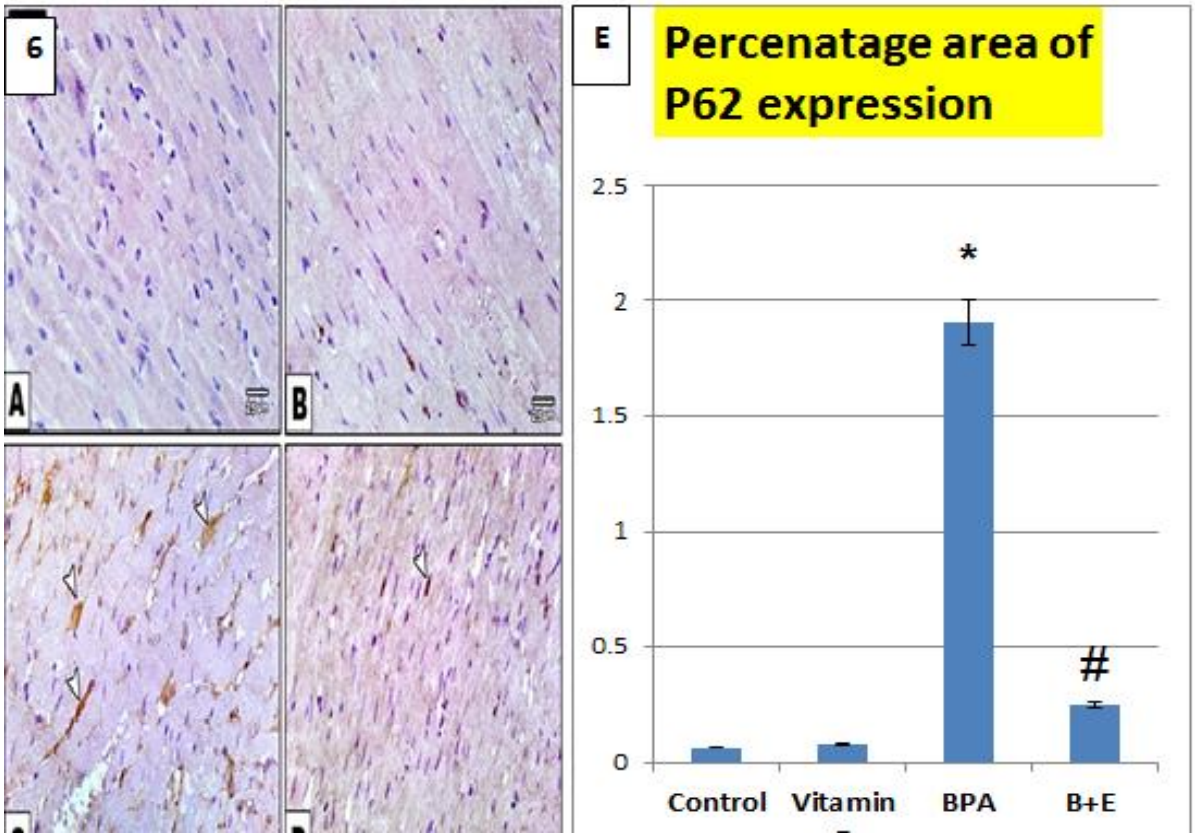

Figure 6: A photomicrograph of myocardial section stained with P62 immunostaining**. (6A):** A section of a control rat showing negative immunostaining. **(6B):** A section of a rat treated with vitamin E showing negative immunostaining. **(6C):** A section of rat treated with BPA showing an increase in immunostaining as compared with the control and vitamin E treated groups (arrow heads). **(6D):** A section of a rat treated with BPA and vitamin E showing marked reduction in P62 immunostaining as compared with BPA treated group (arrow heads) **(P62 x 400). (6E):** Quantitative changes of autophagic vacuoles and autophagy substrate **P62** in cardiac tissue in which there is significant increase in P 62 in BPA group (*) compared to normal group and there is significant reduction in P62 in BPA and vitamin E group (#) compared to BPA group**.**

3.6 The effect of vitamin E on cardiac pathology

A- Hematoxylin and eosin (H&E) stain:

Sections of control and Vit E treated rats were showing normal architecture of the myocardium. Cardiac muscle fibers show acidophilic cytoplasm with central, oval, and vesicular nuclei (Figure **7**). In contrast, heart sections of the rats treated with BPA for 2 weeks showing congested blood vessels which appeared surrounded by inflammatory cells (Figure **8**) and showed spacing between the cardiac muscle fibers in some sections. BPA and Vit E treated group showing restoration of the myocardial architecture more or less than normal. However, spacing between some of the cardiac muscle fibers still present (Figure **9**).

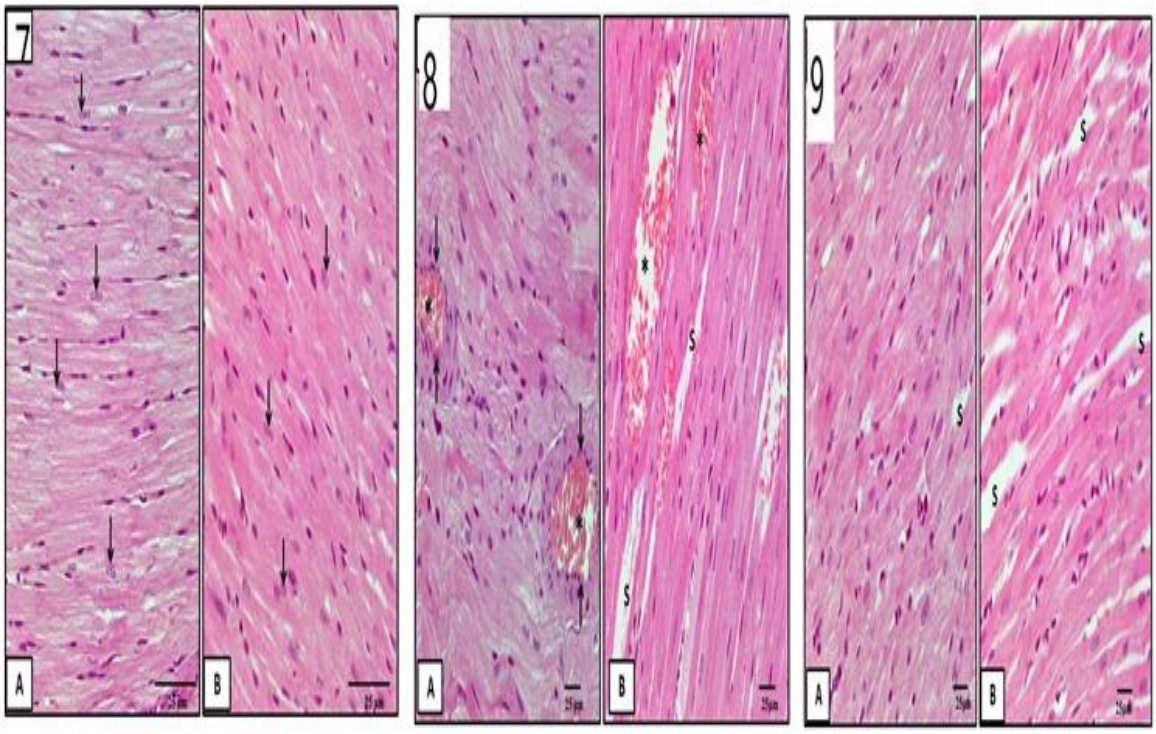

Figure 7,8,9: A photomicrograph of myocardial section, **(7a)** section of a control rat showing the normal architecture of the myocardium. Cardiac muscle fibers show acidophilic cytoplasm with central, oval, and vesicular nuclei (arrows). **(7b):** section of a rat treated with vitamin E for 2 weeks showing the normal architecture of the myocardium. Cardiac muscle fibers show acidophilic cytoplasm with central, oval, and vesicular nuclei (arrows). **(8a, 8b):** sections of rats treated with BPA showing congested blood vessels (*) which appeared surrounded by inflammatory cells (arrows). There is spacing between the cardiac muscle fibers (S). **(9a, 9b):** sections of rats treated with BPA and vitamin E for 2 weeks showing restoration of the myocardial architecture more or less than normal. However, spacing between some of the cardiac muscle fibers still present (S) **(H & E x 400).**

B- Masson's trichrome stain:

We made a pathological examination of cardiac sections stained with Masson's trichrome to assess the fraction of the cardiac tissue occupied by interstitial tissue fibers. In control group and vitamin E group there were normal distribution of the fibrous tissue in between the cardiac muscle fibers, Figure (**10a, 10b**). In contrast, heart sections of the rats treated with BPA for 2 weeks showing marked increase in the amount of fibrous tissue in between the cardiac muscle fibers, **Figure (10C).** BPA and Vit E treated group showing reduction in the amount of the fibrous tissue in between the cardiac muscle fibers, **Figure (10D).**

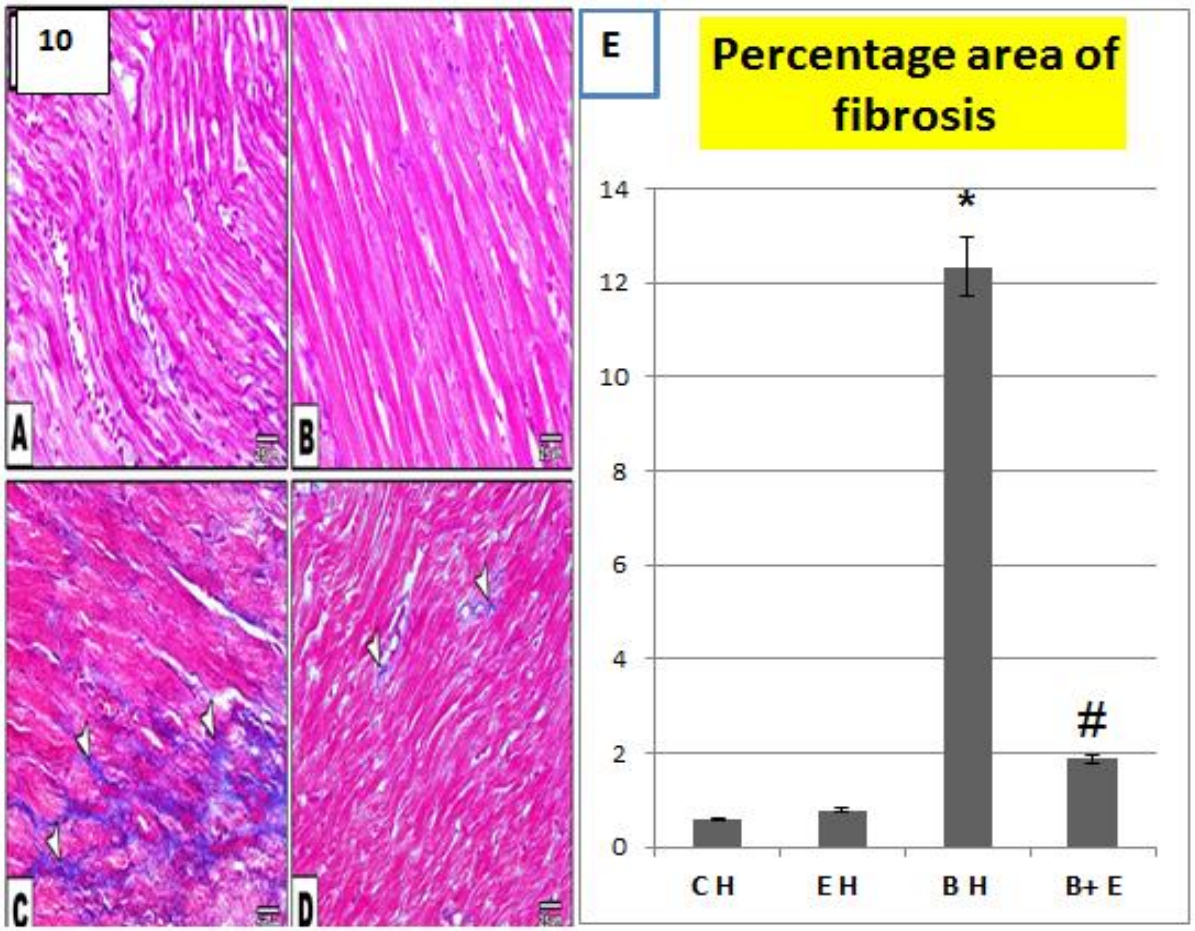

Figure 10: A photomicrograph of myocardial section **(10A):** section of a control rat showing the normal distribution of the fibrous tissue in between the cardiac muscle fibers. (**10B):** A section of a rat treated with vitamin E showing the normal distribution of the fibrous tissue in between the cardiac muscle fibers. **(10C):** A section of a rat treated with BPA showing marked increase in the amount of fibrous tissue in between the cardiac muscle fibers (arrow heads). **(10D):** A section of a rat treated with BPA and vitamin E showing reduction in the amount of the fibrous tissue in between the cardiac muscle fibers (arrow heads) **(Masson Trichrome x 400). (8E):** Quantitative changes of cardiac muscle fibers stained with Masson Trichrome (MTC) in which there is significant increase in fibrosis in BPA group (*) compared to normal group and there is significant reduction in fibrosis in BPA and vitamin E group (#) compared to BPA group**.**

4. Discussion

The incidence of CVDs could be attributed to many causes, including genetics, lifestyle, and environmental factors. BPA, a widely used chemical pollutant, has a damaging impact on the heart tissue [13]. The present study demonstrates for the first time the efficacy of Vit E in combating the undesirable health hazard of BPA on the heart. In addition to antioxidant effect of Vit E, protective effects of vitamin E as demonstrated in this research mediated by the modulation of autophagic process and improvement of mitochondrial functions

In this study, oral exposure to BPA, at 250 mg/kg/day, for 14 consecutive days affects the body weight, heart weight, cardiac enzymes, oxidative markers and autophagic process.

BPA induced a significant decrease in both body and heart weights in comparison to the control groups. Similar

result was reported in a previous study on body weight loss with BPA administration [18]. The significant differences observed between the control and BPA-treated rats with respect to body and heart weights showed that BPA precipitated oxidative stress affecting both heart and body weights. This toxic effect of BPA on the heart confirms previous reports on the toxicity of BPA [19, 20].

Considering the insufficiency of the research on the BPA-induced inflammation in the heart tissue, we also investigated the serum level of LDH and CK-MB, which could be used as suitable biomarkers for inflammation in the heart tissue. A significant increase was revealed in the serum levels of LDH, and CK-MB in BPA group compared to control groups. This agrees with Vanani and his colleagues study [13]. This result indicating that inflammation could possibly be a mechanism for BPA induced cardiac damage [21].

Elevated levels of oxidative stress markers could have a fundamental role in the cardiotoxicity induced by BPA [22, 23]. MDA, an index of lipid peroxidation, has been considered as a potential biomarker for assessing oxidative stress in tissue injuries [24]. GSH, as the most important non-enzymatic antioxidant, is the first line of defense against ROS, and tissues' depletion of GSH led to oxidative stress and consequently tissue damage [25]. BPA cause consumption of GSH probably for the sweeping of radical hydroxyls [20].

In this study, there are significant increase in oxidative markers MDA and NO and significant decrease in antioxidant GSH in BPA group compared to control groups. These finding were in harmony with Khan and his colleagues Peerapanyasut and his colleagues who found that the impact of BPA might be due to the induction of mitochondrial functional impairment and mitochondrial dynamic disturbances [26, 27].

Further instance about the pathogenesis of BPA induced cardiotoxicity, autophagic process for first time was evaluated in this study. BPA is implicated to have significant impacts on autophagy. The association between BPA exposure and autophagic process alteration in different organs, showing controversial data [28, 29].

In this study, immunohistochemical determination of LC3 and P62 level were determined. The experiments demonstrate that exposure to BPA up-regulated the levels of autophagy proteins LC3-II and p62. The level of LC3-II reflects the balance between the rates of autophagosome generation and degradation in a dynamic pathway but cannot determine autophagic flux [30]. The imbalance between autophagic induction and inefficient completion of autophagy results in autophagosomes accumulation [31]. On the other hand, P62 has been reported to act as an autophagic receptor and substrate, which binds with organelles or proteins to autophagosomes for degradation [32, 33], so P62 levels increase when autophagy is inhibited, and decrease when autophagy is activated [34]. We evaluated immune expression of p62 in cardiac tissue to determine whether the accumulation of autophagy vacuoles correlated with impaired autophagic degradation.

Although the basal levels of autophagy maintain cardiovascular homeostasis by the removal of damaged cytosolic material, the autophagic activity might be rapidly increased in response to the elevated levels of ROS and oxidative stress which caused mitochondrial and DNA damage [35].

Previous researches proposed that BPA increases the expression of LC3-II [28, 36, 37]. Guo and his colleagues explained increase in LC3 by BPA as a protective effect from mitochondrial and DNA damage [38].

Additionally, concomitant with our result, Priego and his colleagues and Song and his colleagues studies; demonstrated that BPA increases P62. Despite BPA exposure increase the formation of the autophagosome, it disrupted autophagic flux by decreasing the Autophagosome-lysosome fusion leading to increase P62 level [5,34].

To confirm the heart biochemical results, we made a pathological examination of cardiac sections stained with

hematoxylin and eosin. BPA group showed pathological changes compared to normal group. There are congested blood vessels which surrounded by inflammatory cells and showed spacing between the cardiac muscle fibers in some sections. These findings are in line with previous observations [39].

In addition, cardiac sections stained with Masson's trichrome were done to assess the fraction of the cardiac fibrous tissue. BPA group showed marked increase in the amount of fibrous tissue in between the cardiac muscle fibers.

Several studies described the capacity of BPA to induce fibrosis in different tissues [40, 41;42]. Continuous administration of BPA induced an increase of collagen deposition as well as an increase of extracellular matrix proteins such as fibronectin [5].

The data presented in this work exhibit that BPA promote cardiac inflammation and fibrosis, probably by increased oxidative stress and blockage of autophagic flux. To sum up, our work supports the idea that BPA could be an important toxic for patients with cardiac diseases and contribute to cardiotoxicity progression.

Vitamin E is a potent antioxidant and anti-inflammatory that has protective effects on many pathological conditions including atherosclerosis and associated cardiovascular complications, nonalcoholic fatty liver, and other diseases [43]. More importantly, Vit E treatment did not alter body and heart weights, and fibrosis, oxidative, inflammation and autophagic alteration in non-BPA control rats, suggesting these beneficial effects of Vit E were functional only under fibrosis, oxidative stress, and inflammation in the BPA induced cardiotoxicity.

The amelioration of the BPA induced alterations; body and cardiac weights, cardiac inflammation, oxidative stress and autophagic process; by the concomitant treatment with vitamin E shows its ability to protect the heart against BPA-induced toxicity.

The administration of Vit E along with BPA showed significant increase in weigh compared to BPA group. Moreover, combination of BPA and vitamin E in rats showed significant decrease the elevated levels LDH and CK in BPA group. These results are similar with that of Hadi and his colleagues Donia and his colleagues that showed that Vit E significantly decrease the elevated cardiac enzymes and returned this due to antioxidant and ROS scavenging properties of Vit E [44,45].

As Cardiac tissue is vulnerable to oxidative stress due to the low rates of expression of hydrophilic anti-oxidative detoxification systems [46], Vit E considerably reversed the high levels of MDA and NO induced by BPA and significantly increases GSH levels compared with the BPA group. These finding were in consistency with Badgujar and his colleagues who clarified that Vit E could decrease the lipid peroxidation, restore the antioxidant enzyme activities, and increase GSH in the cytoplasm, which is required in ROS scavenging [47]. In addition, Abdel-Daim & Abdeen mentioned that Vit E neutralizes ROS and up-regulates antioxidant enzymes protecting the cell from lipid peroxidation, DNA damage, and apoptosis [12].

In demonstration of Vit E effect on autophagic process in this study, there was no significant increase in LC3 in concomitant BPA and Vit E group suggesting non-effect of Vit E on autophagic induction and autophagosome formation. Hence, Vit E did not influence autophagic induction significantly, as assessed by LC3 immunohistochemistry in our study, suggesting that the anti-oxidative effect of Vit E does not alleviate autophagic stress by reducing the formation of autophagosomes. The study about senescence revealed that oxidative stress-induced autophagy impairment was closely associated with the reduced degradation capability of lysosomes [48]. Vakili and his colleagues study demonstrated that Vit E had significantly lower LC3

expression compared with the ovariectomy-induced osteoporosis group which explained by that autophagy-related genes can be induced by several inducers of cellular stress [49]. Generally, ROS act as the autophagy inducer in the upstream of autophagic pathway [50].

Vitamin E was reported to alleviate autophagic process by increase the enzymatic activities and the lysosomal degradation, resulting in a recovery of lysosomal-dependent autophagosome degradation [11].

In the present study, we found the balance between the autophagosome formation and degradation was disrupted by the dysfunction of lysosomal-dependent degradation pathway evident by assessment of LC3 and P62, suggesting that the accumulation of autophagic vacuoles is a sign of autophagic stress. Interestingly, Vit E could decrease the autophagic stress by activating autophagy via increasing the lysosomal degradation, resulting in recovery of lysosomal-dependent autophagosome degradation. Vitamin E does not affect the formation of autophagosomes.

On pathological evaluation, there was improvement in concomitant BPA and Vit E group compared to BPA group. There was restoration of the myocardial architecture and decrease inflammation evident by H&E stain. This is in concomitant with Fayez, & Zaafan study who reveal that vitamin E showed marked decrease in inflammatory cells infiltration in doxorubicin induced cardiotoxicity [51].

More ever concomitant BPA and Vit E group showed significant decrease in fibrosis compared to BPA group demonstrated by Masson stain. Zamin and his colleagues and Alcalá and his colleagues previously demonstrated that Vit E decrease tissue fibrosis by reducing collagen deposition as revealed in previous studies [52,53]. This can be explained that Vit E reveals non-antioxidant properties; it inhibits mitogen-activated protein kinases (MAPK) signaling pathways [54]. These MAPK (ERK, JNK, and p38), activated by ROS, modulate the synthesis of MMPs and the release of pro-inflammatory cytokines and involved in the activation of transforming growth factor-beta (TGF-β) a potent inducer of collagen synthesis [55].

5. Conclusion

Our study demonstrated that Vit E administration revealed amelioration BPA induced cardiomyopathy by alleviation of oxidative stress, inflammation and modulation of autophagic stress. Vit E reduces autophagic stress though increasing the degradation of autophagosomes without effect on its formation. We speculate that the modulating effect of Vit E on autophagic process may be associated with its attenuation of oxidative stress.

Alleviation of autophagic stress by Vit E might be an important therapeutic target for Cardiomyopathy and adjuvant administration of Vit E could be a new promising solution in the treatment of cardiac complications.

6. Conflict of interest

The authors have no conflict of interest.

7. Funding resources

None, authors funded the research completely.

8. Authors Contributions

The study was conducted at MERC, Mansoura Faculty of Medicine. Eman A. Farrag designed the study. Hend M. Hassan examined the examined cardiac tissue specimens and interpreted the histological and immunohistochemical results. Zienab Abdallah and Eman Abdelrazik established the model of the study. All the authors wrote, revised the manuscript, and approved the submitted manuscript.

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