

# The Possible Protective Role of *Moringa Oleifera* and Vitamin E on Doxorubicin-Induced Cardiotoxicity in Adult Male Albino Rats: Histological and Immunohistochemical Study

Randa S. Metwally, Mohammed M. Yousef, Al-shimaa E. Abdelfatah, Rania I. El-desoky

Department of Histology and cell Biology, Faculty of Medicine Benha University, Egypt.

**Corresponding to:** Randa S. Metwally, Department of, Histology and cell Biology Faculty of Medicine Benha University, Egypt.

**Email:**

mggs8912@gmail.com

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**Abstract**

**Background:** Doxorubicin (DOX) is highly effective anti-neoplastic agent, but it has side effects including cardiotoxicity. *Moringa oleifera* and vitamin E are potent antioxidants that prevent cardiotoxicity. **Objective:** evaluate the possible protective role of *moringa oleifera* and vitamin E on doxorubicin-induced cardiotoxicity. **Materials and Methods:** Sixty-five adult male albino rats were randomly divided into five groups. Group I (control group). Group II (affected group): rats were injected intraperitoneally with a cumulative dose of 15 mg/ kg of DOX for three weeks. Group III (*moringa* group): given in a dose of (500 mg/ kg/ day) by gastric tube orally for four weeks. Group IV (vitamin E group): given in a dose of (100 mg/ kg/ day) by gastric tube orally for four weeks. Group V (*moringa* and vitamin E). Heart specimens were taken and prepared for histological, immunohistochemical and EM examination. **Results:** Group II showed disorganized, widely separated muscle fibers, cytoplasmic vacuolation, pyknosis of many cardiomyocyte nuclei, the mitochondria appeared distorted, extravasation of RBCs and inflammatory infiltrations. There was significant increase ( $P < 0.01$ ) in collagen fibers deposition and iNOS immunostaining compared with control group. Groups III and IV showed improvement of some histological microscopic changes, significant decrease ( $P < 0.01$ ) in collagen fibers deposition and iNOS immunostaining compared with group II. While group V showed histological architecture near to control group. **Conclusion:** each of *moringa oleifera* and vitamin E can ameliorate induced cardiotoxicity, but their co-administration can give better results.

**Key Words:** DOX, cardiotoxicity, *moringa*, vitamin E.

## Introduction

Chemotherapeutic drugs have adverse side effects on healthy body organs while they are treating cancer. Some of these side effects may be devastating and critical to human health status such as cardiotoxicity and heart failure<sup>1</sup>.

Doxorubicin (DOX) is well-established anti-neoplastic agents, used to treat several adult and pediatric cancers, such as leukemia, lymphomas and breast cancer. The successful use of DOX has been hampered by conventional toxicities such as hematopoietic suppression, nausea, vomiting, extravasation, and alopecia, yet the most feared side-effect is cardiotoxicity<sup>2</sup>.

The usage of medical plants e.g. *Moringa Oleifera* is beneficial as its bioactive constituents have an impact on multiple biological signaling pathways. It has several natural antioxidants compounds e.g. flavonoids, ascorbic acid, carotenoids and phenolics. The several parts of *moringa olifera* are supposed to act as cardiac and circulatory stimulants<sup>3</sup>.

Vitamin E is amongst the important non enzymatic antioxidant defense system, mainly obtained from the diet, and has a variety of biological functions, such as enzymatic activity, gene regulation, and

inhibition of platelet aggregation. Vit-E has been reported to be the most effective lipid soluble anti-oxidant that scavenges reactive oxygen species<sup>4</sup>.

## Material and methods

### Drugs and Chemicals

#### Doxorubicin (Dox):

Doxorubicin Hydrochloride 50mg vial was purchased in an orange red powder from **Sigma Chemical Co., St. Louis, MO, USA**. Each vial was dissolved in 0.5 ml normal saline (0.9% NaCl) and injected intraperitoneally with a cumulative dose of 15 mg/ kg of doxorubicin (each dose of 1 mg/ kg for 15 injection) for three weeks (5 injection per week)<sup>5</sup>.

#### *Moringa Olifera*:

*Moringa olifera* was purchased in a greenish powder from **National Research Centre, 33 El-Buhouth Street, Dokki, Cairo, Egypt**. 1 gm of MO was dissolved in 1 ml distilled water and given at a dose of (500 mg/ kg/ day) orally by gastric tube for four weeks starting *moringa* one week before the first dose of doxorubicin<sup>6</sup>.

#### Vitamin E:

Vitamin E 400 mg was purchased in the form of capsules of yellow oily material

from **Pharco Pharmaceuticals, Alexandria-Cairo Desert Rd. Km 31, Amriya, Alexandria, Egypt**. It was given at a dose of (100 mg/ kg/ day) orally by gastric tube for four weeks starting vitamin E one week before the first dose of doxorubicin<sup>7</sup>.

#### **Animals and Diet:**

Sixty-five healthy adult male albino rats of weight range (180-200) grams were purchased and housed at animal house in Moshtohor Faculty of Veterinary Medicine, Benha University. The animals were housed in cages under strict care and hygiene and received balanced diet and water. All ethical guidelines for animal handling were followed by the animal facility. The experimental protocol was approved by the Institutional Animal Care Committee of Benha University, Benha, Egypt (study no. MS.6.9.2021).

#### **Experimental Design:**

An experimental study was done from January 2022 to February 2022. After one week of housing, 65 adult male albino rats were randomly divided into five groups:

**Group I (control group; n=25):** The rats were further divided equally into 5 subgroups: subgroup Ia: 5 rats were left without any treatment. Subgroup Ib: 5 rats were injected intraperitoneally with

normal saline (0.9% NaCl) with a cumulative dose of 15 mg/ kg (Each dose 1mg/ kg for 15 injections) for three weeks (five injections per week). Subgroup Ic: 5 rats were injected intraperitoneally with normal saline as in group Ib and had received distilled water orally by gastric tube at a dose of (500 mg/ kg/ day) for four weeks. Subgroup Id: 5 rats were injected intraperitoneally with normal saline as in group Ib and had received sunflower oily solution orally by gastric tube at a dose of (100 mg/ kg/ day) for four weeks. Subgroup Ie: 5 rats were injected intraperitoneally with normal saline as in subgroup Ib and had received distilled water as in subgroup Ic and sunflower oily solution as in subgroup Id.

○ **Group II (affected group; n=10):**

Rats were injected intraperitoneally with a cumulative dose of 15 mg/ kg of doxorubicin dissolved in normal saline (0.9% NaCl) (each dose of 1 mg/ kg for 15 injections) for three weeks (5 injection per week).

○ **Group III (moringa group; n=10):**

Rats were injected with doxorubicin as in group II and treated with moringa at a dose of (500 mg/ kg/ day) orally by gastric tube for four weeks starting

moringa one week before the first dose of doxorubicin.

○ **Group IV (vitamin E group; n=10):** Rats were injected with doxorubicin as in group II and treated with vitamin E at a dose of (100 mg/ kg/ day) orally by gastric tube for four weeks starting vitamin E one week before the first dose of doxorubicin.

○ **Group V (moringa and vitamin E group; n=10):** Rats were injected with doxorubicin as in group II and treated with moringa as in group III and vitamin E as in group IV.

### **Sampling:**

The rats were anesthetized by ether and sacrificed by cervical decapitation after four weeks from the beginning of the study (24 hours from the last doxorubicin injection), then heart specimens (from the base of the left ventricle) were taken from rats of all groups. Specimens of the heart were fixed in 10% buffered formalin for 5 days. They were then processed and embedded in paraffin. Serial sections of 5–7 µm thickness were cut for ordinary, special staining and immunostaining. For electron microscopic preparation, specimens were fixed in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide.

### **Histological and Immunohistochemical Studies**

Paraffin sections of thickness (5-7 µm), mounted on glass slides for H&E stain to examine histological changes in various groups and Masson trichrome staining for demonstration of collagen fibers deposition. Other sections were mounted on +ve charged slides for immunohistochemical staining<sup>8</sup>.

Immunohistochemical staining for detection of iNOS (marker for activation of macrophage). The primary antibody used was the rabbit polyclonal antibody (Thermo Fisher Scientific, U.S.A; cat. no. NM\_000625) (PBS with 0.02% sodium azide and 50% glycerol pH 7.3). Site of the reaction was brownish color of cytoplasm<sup>9</sup>.

### **Transmission Electron Microscopic (TEM) study<sup>10</sup>**

Ultrathin sections were prepared, grids were examined and electron micrographs were taken using transmission electron microscope JOEL (JEM-100 SX, Akishima, Tokyo, Japan) in Electron Microscope unit, Tanta faculty of medicine, Tanta University.

### **Morphometric Study**

The mean area percentage for collagen fibers deposition (Masson trichrome stain) and iNOS immunostaining were quantified

in 10 images from 10 non-overlapping fields of each group rats using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA).

### **Statistical Analysis**

All the data collected from the experiment was recorded and analyzed using IBM SPSS Statistics software for Windows, Version 23 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) with Post Hoc LSD test was used to compare differences among the groups of morphometric results. In each test, the data was expressed as the mean (M) value, standard deviation (SD) and differences were considered to be significant at  $P < 0.01$ .

## **Results**

### **H & E Stain results:**

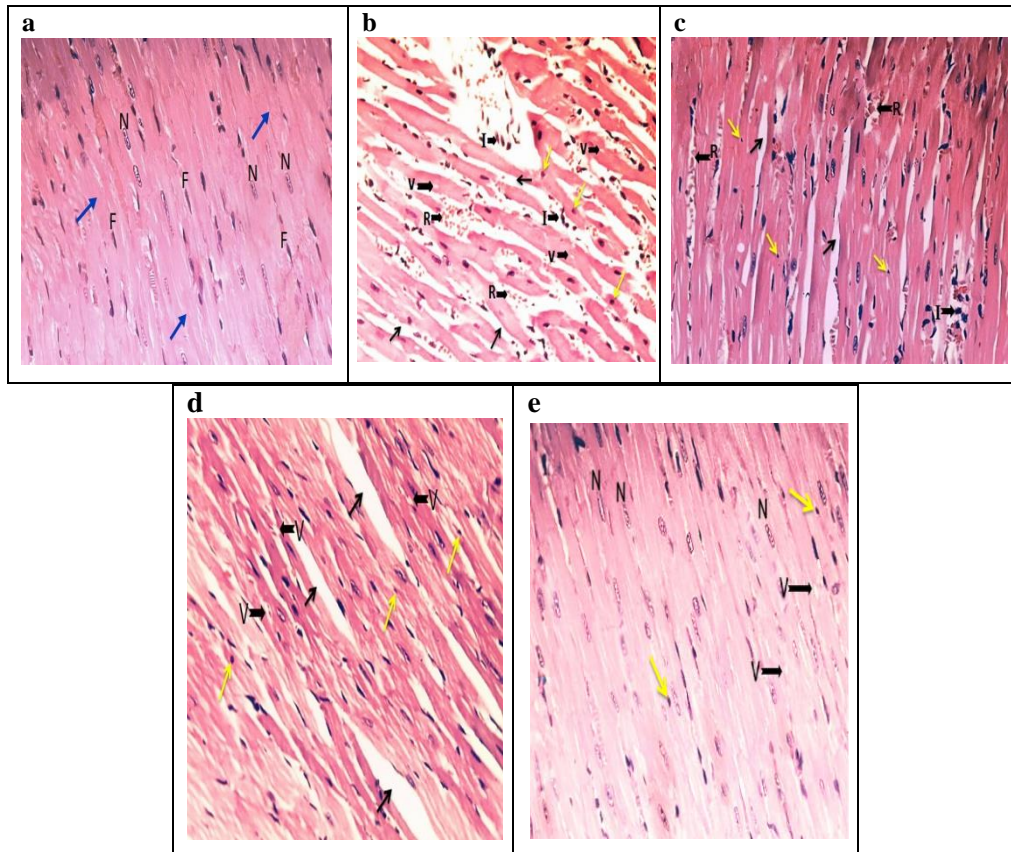
Examination of all subgroups of the group I (control group) showed similar histological architecture. Group I (control group): heart sections from group I showed normal architecture of branching and anastomosing cardiac muscle fibers. Cardiomyocytes have central oval vesicular nuclei. The endomysium connective tissue between the cardiomyocytes showed fibroblasts with flat dark nuclei (figure 1a).

Group II (affected group): heart sections from group II showed disorganized, disturbed and widely separated muscle fibers, cytoplasmic vacuolation, pyknosis of many cardiomyocyte nuclei and extravasation of red blood cells between cardiomyocytes with cellular inflammatory infiltrations (figure 1b).

Group III (moringa group): heart sections from group III showed cardiac muscle fibers slightly disorganized and separated with pyknosis in few cardiomyocytes and extravasation of red blood cells with few cellular inflammatory infiltration (figure 1c).

Group IV (vitamin E group): heart sections from group IV showed mild cardiac muscle fibers degeneration with cytoplasmic vacuolation, pyknosis in some cardiomyocytes and widely separated muscle fibers (figure 1d).

Group V (moringa and vitamin E group): heart sections from group V showed nearly normal myocardial histological architecture with vesicular centrally located nuclei and muscle fibers regularly arranged with few cytoplasmic vacuolation and pyknotic nuclei (figure 1e).

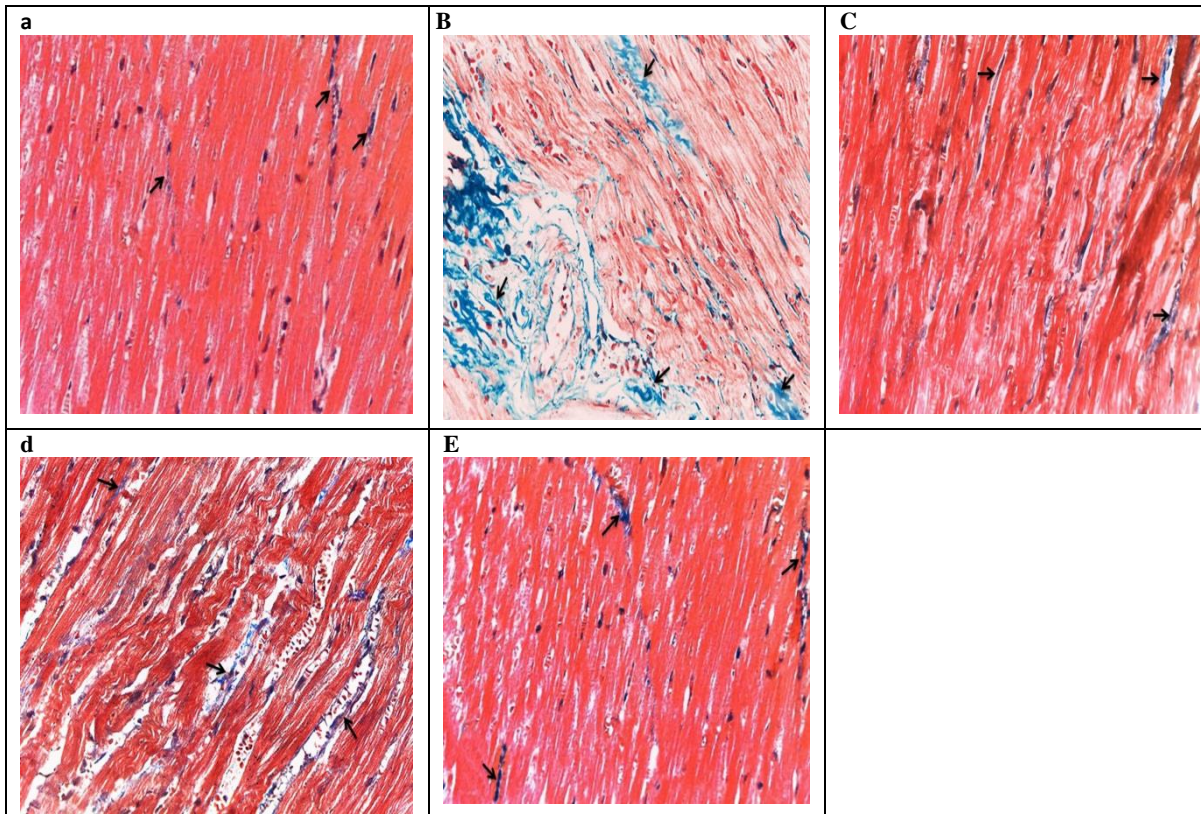


**Fig. (1):** (a) A photomicrograph of a section in the heart of the control group (group I) showing normal architecture of branching and anastomosing cardiac muscle fibers (blue arrows), cardiomyocytes with central oval vesicular nuclei (N). Flat dark nuclei of endomysium connective tissue fibroblasts (F) are noticed, (b) affected group (group II) showing disorganized, disrupted and widely separated muscle fibers (black arrows) with cytoplasmic vacuolations (V arrows) and pyknosis (yellow arrows) of many cardiomyocytes nuclei. Accumulation of red blood cells (R arrows) and inflammatory cells (I arrows) between fibers are noticed. (c) moringa group (group III) showing pyknosis (yellow arrows) in few cardiomyocytes nuclei with extravasation of red blood cells (R arrows) and few inflammatory cells (I arrows) are present in between separated fibers (black arrows), (d) vitamin E group (group IV) showing cytoplasmic vacuolations (V arrows), pyknosis (yellow arrows) in some cardiomyocytes nuclei and wide separated fibers (black arrows), (e) moringa and vitamin E group (group V) showing nearly normal histological structure with vesicular centrally located nuclei (N). Notice regularly arranged muscle fibers with few cytoplasmic vacuolations (V arrows) and pyknotic nuclei (yellow arrows). (H&E X400)

#### Masson trichrome staining results:

- Group I (control group): showed minimal collagen fibers accumulation between cardiac muscle fibers (figure 2a).
- Group II (affected group): showed marked collagen fibers accumulation around and between cardiac muscle fibers (figure 2b).
- Group III (moringa group): showed few collagen fibers accumulation between cardiac muscle fibers (figure 2c).
- Group IV (vitamin E group): showed moderate collagen fibers accumulation between cardiac muscle fibers (figure 2d).
- Group V (moringa and vitamin E group): showed minimal collagen fibers accumulation between cardiac muscle fibers (figure 2e).





**Fig. (2):** (a) A Photomicrograph of a section in the heart of the control group (group I) showing minimal collagen fibers between cardiac muscle fibers (arrows), (b) affected group (group II) showing marked collagen fibers deposition around and between cardiac muscle fibers (arrows), (c) moringa group (group III) showing few collagen fibers deposition between cardiac muscle fibers (arrows), (d) vitamin E group (group IV) showing moderate collagen fibers deposition between cardiac muscle fibers (arrows), (e) moringa and vitamin E group (group V) showing minimal collagen fibers between cardiac muscle fibers (arrows). (**Masson trichrome staining X400**).

***iNOS staining results:***

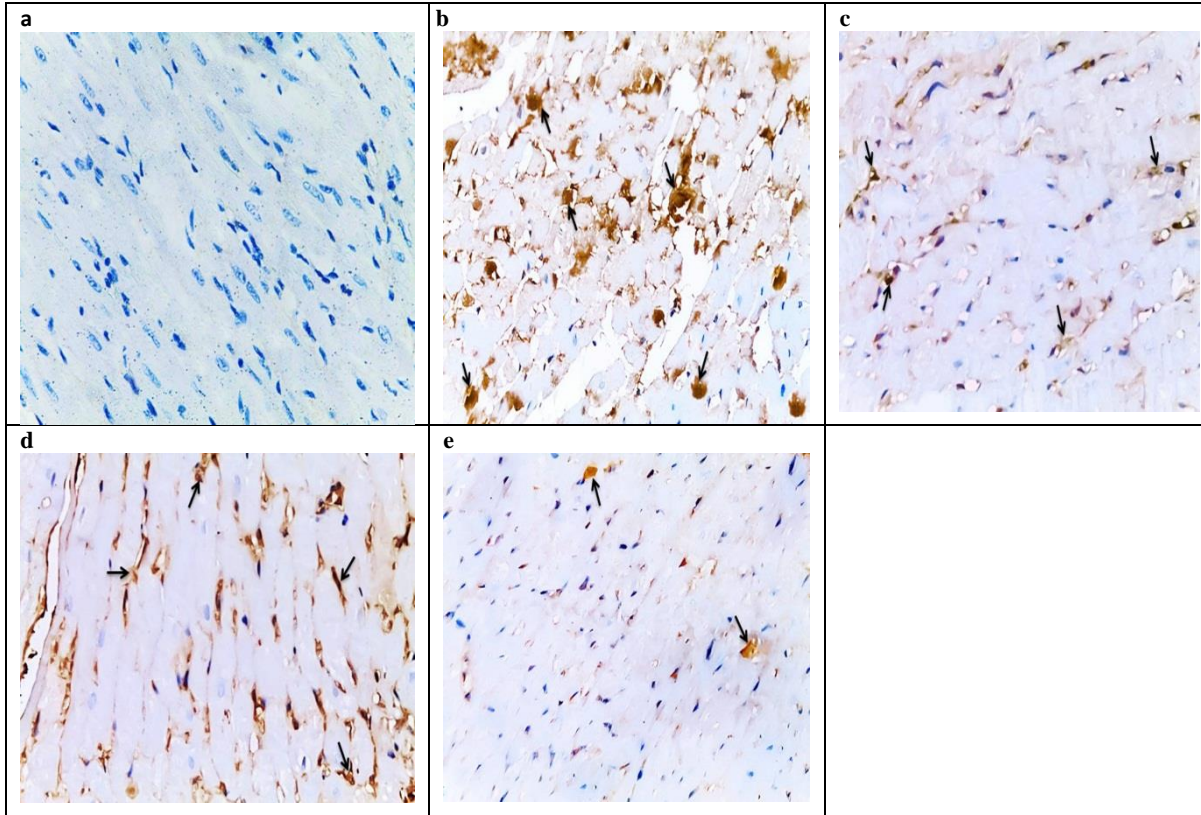
Group I (control group): showed cardiac tissue with negative immunostaining reaction for inducible nitric oxide synthase (figure 3a).

Group II (affected group): showed cardiac tissue with strong positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase (figure 3b).

Group III (moringa group): showed cardiac tissue with few positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase (figure 3c).

Group IV (vitamin E group): showed cardiac tissue with moderate positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase (figure 3d).

Group V (moringa and vitamin E group): showed cardiac tissue with minimal positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase (figure 3e).



**Fig. (3):** (a) A Photomicrograph of a section in the heart of the control group (group I) showing cardiac tissue with negative immunostaining reaction for inducible nitric oxide synthase, (b) affected group (group II) showing cardiac tissue with strong positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase (arrows), (c) moringa group (group III) showing cardiac tissue with few positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase (arrows), (d) vitamin E group (group IV) showing cardiac tissue with moderate positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase (arrows), (e) [moringa and vitamin E](#) group (group V) showing cardiac tissue with minimal positive cytoplasmic immunostaining reaction for inducible nitric oxide synthase (arrows) (**Anti iNOS, X400**)

### EM results:

Group I (control group): showed well organized myofibrils of cardiac myocytes with a transverse striation pattern in the form of alternating dark (A) and light (I) bands bisected by Z lines. The center of each A band was occupied by H zone, which was crossed by M line. The nucleus is euchromatic and the normal mitochondria with abundant cristae distributed between myofibrils (figure 4a).

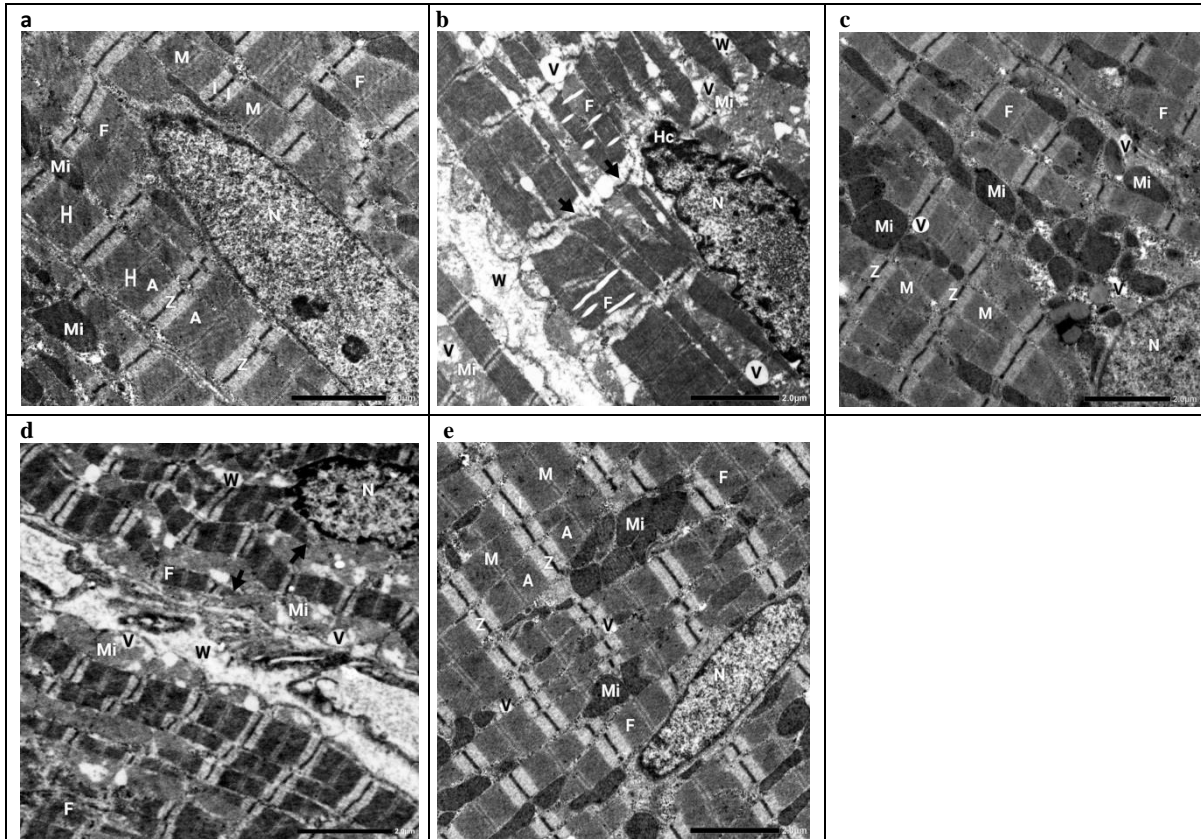
Group II (affected group): showed disorganized and disrupted myofibrils with areas of fibers loss. The mitochondria appeared distorted with vacuoles in some of them, the nucleus appeared irregular with clumps of peripheral heterochromatin and wide separation of the fibrils bundles with many vacuoles (figure 4b).

Group III (moringa group): showed mostly well organized myofibrils with apparent transverse striation pattern, Z lines and M lines. Euchromatic nucleus with mild irregular outline, near normal multiple mitochondria with different sizes and shapes distributed between myofibrils and cytoplasmic vacuoles (figure 4c).



Group IV (vitamin E group): showed disorganized myofibrils with areas of lost fibers, nucleus with irregular outline, swollen and distorted some mitochondria with vacuoles and wide separation of the fibers bundles (figure 4d).

Group V (moringa and vitamin E group): showed cardiac myocytes with well organized myofibrils with a transverse striation pattern in the form of alternating dark (A) and light (I) bands bisected by Z lines. The center of each A band was occupied by H zone, which was crossed by M line. The nucleus is euchromatic and the mitochondria appeared normal with abundant cristae and distributed between myofibrils. Few cytoplasmic vacuoles were present (figure 4e).



**Fig. (4):** (a) An electron micrograph of a section in the heart of the control group (group I) showing normal sarcomere with well organized myofibrils (F), dark band (A), light bands (I) bisected by (Z) lines. A band has (H) zone which was crossed by (M) line. Mitochondria (Mi) with abundant cristae distributed between myofibrils and oval euchromatic nucleus (N) are noticed, (b) affected group (group II) showing disorganized and disrupted myofibrils (F) with areas of lost fibrils (arrows), distorted mitochondria (Mi) with vacuoles (V) in some of them and irregular nucleus (N) with clumps of peripheral heterochromatin (Hc). Wide separation of the fibrils bundles (W) with vacuoles (V) are noticed, (c) moringa group (group III) showing well organized myofibrils (F) with transverse striation pattern, (Z) lines and (M) lines. The mitochondria (Mi) appeared with different sizes and shapes. Part of nucleus with mild irregular outline (N) and cytoplasmic vacuoles (V) were noticed, (d) vitamin E group (group IV) showing wide separated (W), disorganized myofibrils (F) with areas of lost fibrils (arrows) and swollen, elongated and distorted mitochondria (Mi) with vacuoles (V) in some of them. Nucleus with irregular outline is noticed (N). (e) moringa and vitamin E group (group V) showing sarcomere with organized myofibrils (F), dark band (A), light bands (I), (Z) lines, (M) lines, apparently normal mitochondria (Mi) and oval euchromatic nucleus (N). Cytoplasmic vacuoles are noticed (V). (TEM X4000)

**Morphometric and Statistical Results**

The mean area % and standard deviation (SD) of collagen fibers deposition and iNOS immunostaining of all groups was represented in (Tables1,2) and (Histograms1,2). There was a significant decrease ( $P<0.01$ ) in mean area % of group I, III, IV, V compared with group II and insignificant increase ( $P<0.01$ ) in mean area % of group V compared with group I.

**Table (1):** Showing the mean area %, SD of collagen fibers deposition in groups I, II, III, IV and V with comparison between all groups by Post Hoc LSD test.

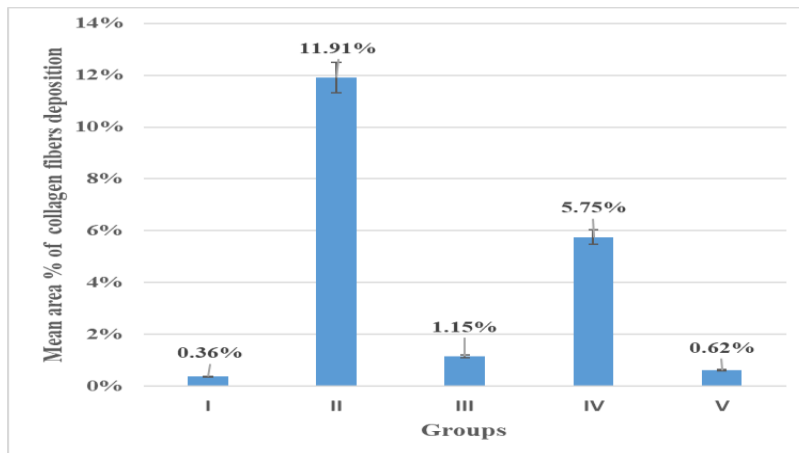
	Group I	Group II	Group III	Group IV	Group V
Mean area %	0.36%	11.92%	1.15	5.75%	0.62%
SD	0.1075	0.5817	0.2386	0.6640	0.1402
Significance at $P < 0.01$	2,3,4	1,3,4,5	1,2,4,5	1,2,3,5	2,3,4

1=sig. with group I 2=sig. with group II 3=sig. with group III 4=sig. with group IV 5=sig. with group V

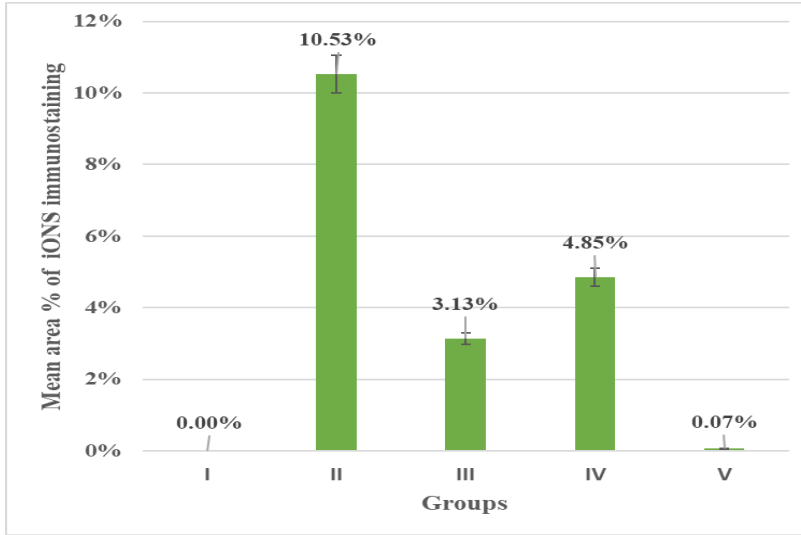
**Table (2):** Showing the mean area %, SD of iNOS immunostaining in groups I, II, III, IV and V with comparison between all groups by Post Hoc LSD test.

	Group I	Group II	Group III	Group IV	Group V
Mean area %	0%	10.53%	3.13	4.85%	0.07%
SD	0	1.1594	0.1519	0.2207	0.0291
Significance at $P < 0.01$	2,3,4	1,3,4,5	1,2,4,5	1,2,3,5	2,3,4

1=sig. with group I 2=sig. with group II 3=sig. with group III 4=sig. with group IV 5=sig. with group V



**Histogram (1):** Showing the mean area % of collagen fibers deposition in groups I, II, III, IV and V.



**Histogram (2):** Showing the mean area % of iNOS immunostaining in groups I, II, III, IV and V.

### Discussion

Cardiotoxicity is a feared side effect that may limit the clinical use of doxorubicin. It may indeed affect the quality of life and survival of patients with cancer, regardless of oncological prognosis<sup>11</sup>.

The manifestations of cardiotoxicity are chest pain that occurs as a result of myopericarditis and palpitation due to paroxysmal supraventricular tachycardia, sinus tachycardia and premature beats<sup>12</sup>.

The DOX group(Group II) showed disorganized, disturbed, atrophic and widely separated cardiac muscle fibers with vacuolated sarcoplasm, pyknotic nuclei, the mitochondria appeared distorted and extravasation of RBCs between

cardiomyocytes with cellular inflammatory infiltrations. It showed significant increase( $P < 0.01$ ) in collagen fibers deposition and iNOS immunostaining compared to control group.

These results agreed with some investigators<sup>13,14,15</sup> who reported that sections of the doxorubicin treated heart revealed the presence of myofibrillar loss, myocytes disarrangement, moderate inflammation, pyknotic nuclei and disrupted mitochondria. These findings were explained by other researchers<sup>16,17</sup> who stated that in DOX induced cardiotoxicity(DIC), the mitochondria have been identified as the main subcellular organelles injured in the

heart by doxorubicin. DOX is a cationic drug that binds with high affinity to cardiolipin (a phospholipid) forming nearly irreversible complex in the mitochondrial inner membrane. DOX disrupts the cardiolipin-protein interface, causing superoxide anion radicals formation. As a result, ROS can induce different forms of cardiomyocyte death (apoptosis or necrosis).

Some authors<sup>18</sup> reported that increased collagen fibers deposition was due to activation of MMP's (Matrix metalloprotease) like MMP-2 and MMP-9, which show toxicity in the heart by increasing collagen formation in the cardiac tissues. An increase in collagen leads to myocardium fibrosis. DOX administration increases the iNOS transcription and protein expression and induces the formation of nitrotyrosine (NT) with increased mitochondrial superoxide level in cardiac tissue. Superoxide and NT's formation is based on the peroxynitrite level because it generates potent oxidants like nitrogen dioxide and carbonates radical. It causes apoptosis of the cardiac muscle cells, decreased cardiac contractility, decreased catalase and glutathione peroxidase activity.

The moringa group (Group III) in the present study showed that cardiac muscle

fibers slightly disorganized, separated with pyknosis in few cardiomyocytes, near normal multiple mitochondria distributed between myofibrils, cytoplasmic vacuoles and extravasation of red blood cells with few cellular inflammatory infiltration. There was a significant decrease ( $P < 0.01$ ) in collagen fibers deposition and iNOS staining expression compared to group II.

These results were agreed with<sup>19,20</sup> who reported that administration of MO in cardiotoxicity induced a noticeable improvement in the histological alterations in the heart. The heart revealed soft inflammatory mononuclear collections and absence of necrosis areas. It decreased muscle fiber disruption, necrosis, focal hemorrhagic areas between the muscle bundles, mononuclear cell infiltration and myocytes swelling. MO markedly decreased fibrotic scarring as reflected by masson trichrome staining.

This significant improvement in the myocardial toxicity was explained by<sup>21,22</sup> who reported that the oral administration of MO increased the enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-reductase (GRD) as well as non enzymatic antioxidants include



vitamin A, E, C and glutathione. It reduced the lipid peroxidation products, restoring the antioxidant enzyme activities and keeping the ROS levels at acceptable cellular concentrations.

Other researchers<sup>3</sup> explained that treatment with *moringa olifera* showed normal appearance of cardiac muscle fibers with no inflammatory infiltrate to myocardium, indicating its protective effect on heart. The cardioprotective property of alkaloids, indole and vincosamide isolated from MO leaves are involved in preventing the disruption of cardiac myofibrils and thereby improving the cardiac contractile function.

The vitamin E group (group IV) in the present study showed disorganized, widely separated muscle fibers, cytoplasmic vacuolation, pyknosis in some cardiomyocytes and distorted some mitochondria with vacuoles. There was significant decrease ( $P < 0.01$ ) in collagen fibers deposition and iNOS staining expression compared to group II. These results agreed with<sup>23,24</sup> who stated that the group of mice which treated with Vit E orally showed edema in the interstitial tissue of heart, vacuolar degeneration, mild disorganization of myocardial fibers with

inflammatory cell infiltration, pyknotic nuclei, decreased intercalated discs and moderate amount of connective tissue between cardiac muscle fibers.

Other researchers<sup>25</sup> demonstrated that rats treated with vitamin E showed nearly normal architecture of cardiac muscle fibers. Cardiomyocytes revealed many vesicular nuclei, but very few ones showed irregular corrugated nuclear outline. Focal area of the disruption of the striated appearance of the myofibrils, scanty vacuolations between the cardiomyocytes and some mitochondria appeared swollen and condensed. Masson's Trichrome manifested minimal amount of collagen fibers deposition.

This improvement in the myocardial toxicity was explained by<sup>26,27</sup> reported that vitamin E is a fat soluble vitamin that is characterized by antioxidative, anti-inflammatory properties and consider an essential nutritive element. It has many biological activities including the reduction of the reactive oxygen species (ROS) toxic effect, effectively removing oxygen free radicals, modifying the damaging effect of oxidative stress, gene regulation and inhibition of platelet aggregation. Vitamin E scavenges ROS by preventing lipid peroxide (LPO) and the initiation of oxidative tissue

damage. It protects the cell membrane from lipid peroxidation which is produced by overproduction of ROS and reactive nitrogen species.

The moringa and vitamin E group (group V) in the present study showed nearly normally arranged cardiac fibers with few pyknotic nuclei and the same ultrastructure of the myocardium except for cytoplasmic vacuoles. There was significant decrease ( $P < 0.01$ ) in collagen fibers deposition and iNOS staining expression compared to group II and insignificant ( $P < 0.01$ ) in collagen fibers deposition and iNOS staining expression compared to control group.

These results agreed with the results of some researchers<sup>28,29</sup> who mentioned that the proper combination between different antioxidants showed nearly normal architecture of cardiac muscle fibers. Nearly normally arranged cardiac fibers with few inflammatory infiltration and explained by others<sup>30,31</sup> who stated that vitamin E treatment is important antioxidant in cardiotoxicity, but it does not protect completely. Several in vitro and in vivo studies have reported that the combination of vitamins with other antioxidants produces synergistic effects. The proper combination

between different antioxidants can perform a wide range of metabolic activities, free radicals scavenging, preventive actions and significant cardioprotective effects. It can repair cardiac disturbance by abrogating apoptotic signals, suppressing lipid peroxide (LPO) and protein oxidation and by improving the antioxidant defense system.

## Conclusion

This work concluded that each of moringa and vitamin E can ameliorate induced cardiotoxicity, but their co-administration can give better results.

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