Amiodarone-induced lung toxicity and the protective role of Vitamin E in adult male albino rat

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SUMMARY

Amiodarone is a highly effective anti-arrhythmic drug, used in the treatment of cardiac arrhythmias. However, it is of a limited use due its serious side effects especially lung toxicity. This study was designed to investigate the role of vitamin E in ameliorating amiodarone-induced lung toxicity in adult male albino rat. The study was carried out on 24 adult male albino rats, divided into 4 equal Groups: Group I (control), Group II (sham control), Group III (Amiodarone treated) and Group IV (Amiodarone and vitamin E treated). After the end of the experiment, the rats were sacrificed by cervical dislocation, then parts of the rat lungs of each Group were prepared for light microscopic, histochemical study, immune- histochemical and ultrastructural study. Fresh Lung sections were processed for electron microscopic study. The results revealed marked pathological alterations in the rat lungs of Group III: distortion in the pulmonary architecture, mononuclear cellular infiltration, presence of areas of consolidation with alveolar collapse, areas of emphysematous air spaces, marked degeneration of the pneumocytes and increased collagen fibers deposition and marked increase in iNOS immuno-expression. There were marked alterations in the level oxidative markers in the lung homogenates. The ultrastructural study confirmed these changes. Treatment with vitamin E in Group IV revealed noticeable improvement of the histological and ultrastructural architecture of the lung. There was marked improvement in the other parameters. The present study concluded that amiodarone has marked toxic effect on the lung of the adult male albino rat and vitamin E could partially protect the lung against such toxic effects.

Key words: Amiodarone – Lung – Vitamin E – Oxidative stress – Histology

INTRODUCTION

Amiodarone (AD) is an antiarrhythmic drug used for long-term management of various types of heart arrhythmias (Martino et al., 2001). It induced pulmonary toxicity in 5% of the treated patients. The toxicity was found to be associated with older age, long duration of treatment, cumulative dosage and preexisting lung disease (Connolly, 1999). Mahavadi et al. (2014) reported that AD resulted in thickening of alveolar septa with focal areas of fibrosis, inflammatory cellular infiltration, disruption of the alveolar epithelium and interstitial exudate.

AD-induced pulmonary toxicity is a complex and multi-factorial, involving several mechanisms including direct toxicity, hypersensitivity and elevated oxidative markers (Shimizu et al., 2003).

AD increases the production of free radicals and induces mitochondrial oxidation (Rebrova and Afanasyev, 2008). Cumulative effects of reactive oxygen species (ROS) may result in significant damage to cell structures leading to harmful effects such as lipid peroxidation and DNA damage (Aggarwal et al., 2013).

The evidence of reduction-oxidation (redox) imbalance in lung fibrosis is substantial and the rational for testing antioxidants as potential new therapeutics for lung fibrosis is appealing (Day, 2008). Several studies have documented the beneficial effects of a variety of antioxidants in amiodarone-induced pulmonary toxicity such as soybean (Abo Gazia and El Kordy, 2012).

Vitamin E has high affinity to phospholipids, cho-

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lesterol and triglycerides and it seems to be the first line of defense against lipid peroxidation of the cell membrane (Vanderveen and Vanderveen, 1990). It inhibits superoxide generation in neutrophils (Kanno et al., 1996), decreases collagenase production in fibroblasts (Ricciarelli et al., 1999) and prevents excessive oxidative radical formation at the cell membranes and mitochondrial membranes (Packer et al., 2001).

So, the aim of the present work was to investigate the possible protective role of vitamin E in ameliorating amiodarone-induced lung toxicity in adult male albino rat.

MATERIALS AND METHODS

Animals

The present study was carried out on 24 adult male Wistar albino rats, weighing 150-200 g. The rats were obtained from the Animal house, Faculty of Medicine, Cairo University. The animals were acclimatized in the laboratory for a period of two weeks before carrying out the experiment. They were allowed free access to food and water ad libitum. They were housed in separate clean cages (five rats/cage) under standard laboratory and environmental conditions approved by the Animal Use and Care Committee, Cairo University. All procedures performed in the study were in accordance with the ethical guidelines for the care and use of animals provided by Institutional Animal Care & Use Committee (ACUC) of Cairo University.

Chemicals

Amiodarone (Amiodarone Hydrochloride) was supplied by Global Napi Pharmaceutical Co., Egypt (under license of Sanofi Aventis, France), in the form of tablets. Each tablet contains 200 mg of amiodarone hydrochloride. The used tablet was dissolved in 10 ml distilled water. It was administered orally via gastric gavage in a daily therapeutic dose of 30 mg/kg B.W for 6 consecutive weeks (Kolettis et al., 2007).

Vitamin E was supplied by El Kahira Pharmaceutical Co., Egypt, in the form of capsules. Each capsule contained 400 mg vitamin E. The capsule was dissolved in 4 ml olive oil. It was administered orally via gastric gavage in a daily dose of 200 mg/kg B.W for 6 consecutive weeks (Choudhury and Jagdale, 2002).

Experimental design

The animals were randomly divided into 4 Groups (6 rats each) as follow:

Group I (control): received no medications and left to survive for 6 weeks.

Group II (sham Group): received vitamin E in a daily dose of 200 mg/kg Body Weight (BW) orally via gastric gavage for 6 weeks.

Group III (Amiodarone treated): received amiodarone in a daily dose of 30 mg/kg BW orally via gastric gavage for 6 weeks.

Group IV (Amiodarone and vitamin E treated): received amiodarone in a daily dose of 30 mg/kg BW orally via gastric gavage and vitamin E in a daily dose of 200 mg/kg BW orally via gastric gavage (given 2 hours preceding amiodarone) for 6 consecutive weeks.

By the end of experimental period, the rats were sacrificed by cervical dislocation. Then, the thoracic cage was opened by a median sternotomy incision and the lungs were extracted, prepared for light, histochemical and electron microscopic study.

Light microscopic examination

Each rat lung was cut into small pieces, fixed immediately in 10% formalin saline for 24 h. The specimens were then washed and dehydrated in ascending grades of ethanol (70%, 90% & 100%). They were cleared in xylene for two hours. Impregnation and embedding were done in soft paraffin wax at 45-50°C for three hours and in hard paraffin at 60°C for one hour. Paraffin blocks were prepared and sections of 5 μm were then stained with Hematoxylin and Eosin (H & E) (Drury and Wallington, 1980) and Masson's trichrome (Bancroft and Gamble, 2002). The specimens were examined in the Department of Pathology, Faculty of Medicine, Cairo University.

Electron microscopic examination

Fresh lung sections were trimmed into 1 mm pieces, fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer solution (pH 7.4) at 4°C and post fixed in 1% osmium tetroxide. Ultrathin sections (60-70 nm) were mounted on copper grids and counterstained with uranyl acetate and lead citrate (Hayat, 2000). Transmission electron microscopic analysis was carried out using a JEM-1400 transmission electron microscope (JEOL, Tokyo, Japan) at the Research Center, Faculty of Agriculture, Cairo University.

Histochemical analysis (Estimation of oxidative markers) in the lung tissue

Fresh lung specimens were homogenized in Tris-HCl buffer (5 mmol/L containing 2 mmol/L Ethylene-Diamine-Tetra-Acetic acid (EDTA), pH 7.4) to give a 10% (w/v) lung homogenate. The homogenates were then centrifuged at 1000 rpm for 10 min at 4°C and the supernatants were immediately used for the determination of oxidant-antioxidant status. Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Glutathione Reductase (GSH) as an index of the extent of lipid peroxidation were determined following the manufacturer's instruction provided by Biodiagnostic Company (biodiagnostic_eka@lycos.com and info@bio-diagnostic.com).

Immunohistochemical study of iNOS in the lung tissue

Sections of 5 μm in thickness were prepared from paraffin embedded tissues. Afterwards they were dewaxed in xylol and hydrated in descending grades of alcohol. They were immersed into preheated target retrieval solution to 95°C in water bath for forty minutes, and allowed to cool for twenty minutes. Sections were rinsed with phos-

phate buffered saline (PBS) for three times. Enough hydrogen peroxide H_2O_2 was applied for 5 minutes, and then rinsed with PBS. Then the sections were incubated with polyclonal rabbit anti-iNOS (dilution 1:100) for 2 hours at room temperature. Biotinylated link was added for 10 minutes, and then sections were rinsed with (PBS). The sections were incubated with streptavidin HRP solution at room temperature for 10-15 min thin sections were rinsed with (PBS). The peroxidase activity was detected using 3,3´ diaminobenzidine (DAB) kit (Dako). DAB was used as a chromogen, which is converted into a brown precipitate. Slides were rinsed in distilled water, immersed in Hematoxylin for half minute and then rinsed in tab water until blue. For negative controls slides, the sections were prepared by the same method, except that they were incubated with antibody diluents instead of the primary antibody. Brown yellow granules in cytoplasm or nuclei were recognized as positive staining for iNOS (Purcell et al.,1997).

Histomorphometric study

Morphometric study of the area % of collagen fibers deposition in the lung tissue was measured using Masson's Trichrome stained sections (x100) whereas area % of iNOS immunoexpression was measured using the immune-stained sections (x400). Five non-overlapping fields/rat paraffin block sections were examined (The total was 50 measurements/Group) and photographed using color video camera (digital camera CH-9435 DFC 290). Photographs of the above mentioned measured parameters were analyzed using Leica Qwin 500 (Imaging System, Cambridge, UK) within a frame area of 284.2168 μm^2. The green color of collagen and the brown color of immunoreaction in the analyzed photos were masked by a blue color. Morphometric study was carried out at the Image Analysis Unit, Research center, Faculty of Agriculture, Cairo University.
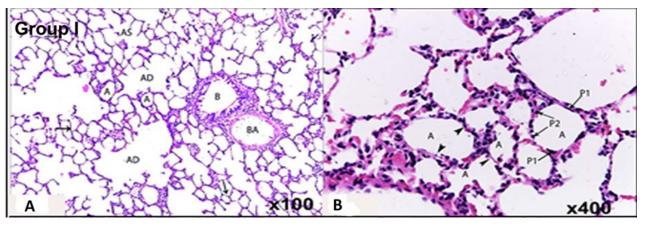
Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was performed using IBM SPSS software version 21.00 (Chicago, Illinois, USA). One-way analysis of variance (ANOVA) (data were normally distributed and variances of populations were equal), post-hoc and least significant difference (LSD) were performed for inter-Group comparison. $P > 0.05$, $P < 0.05$ and $P < 0.001$ were considered non-significant, significant and highly significant, respectively. Statistical analysis was done in the Statistical Unit, Faculty of Medicine, Cairo University.

RESULTS

Histopathological results

H&E stained sections of the rat lungs of Group I and II revealed the classic pulmonary architecture consisting of alveolar ducts, alveolar sacs and alveoli. The alveoli were lined by alveolar epithelium consisting of two types of cells; type I pneumocytes, which appeared as flat cells with flat nuclei and eosinophilic cytoplasm and type II pneumocytes, which appeared as rounded cells with rounded nuclei and eosinophilic cytoplasm. The alveoli were separated from each other by thin inter-alveolar septa. The respiratory bronchioles were lined by simple columnar epithelial cells and goblet cells and surrounded by smooth muscle layer and accompanied by bronchial arteriole (Figs. 1-A, 1-B). Group III showed distorted pulmonary architecture and thickened inter-alveolar septa with mononuclear cellular infiltration. Areas of ruptured inter-alveolar septa with large irregular emphysematous air spaces were seen. Areas of apparently normal pulmonary architecture were noticed. The pulmonary interstitial tissue was found to be invaded by a wide variety of inflammatory cells formed mainly of mononuclear cells and macrophages containing hemosiderin granules. Dilated congested blood vessels with interstitial exudates and extravasated RBCs were observed. Bronchiolar affection was evident in the form of peri-bronchial cellular infiltration and exfoliated epithelial cells (Figs. 1-C, 1-D, 1-E, 1-F).

Treatment with vitamin E in Group IV showed marked improvement of the pulmonary architecture consisting of alveolar ducts, alveolar sacs and alveoli which were lined by alveolar epithelium
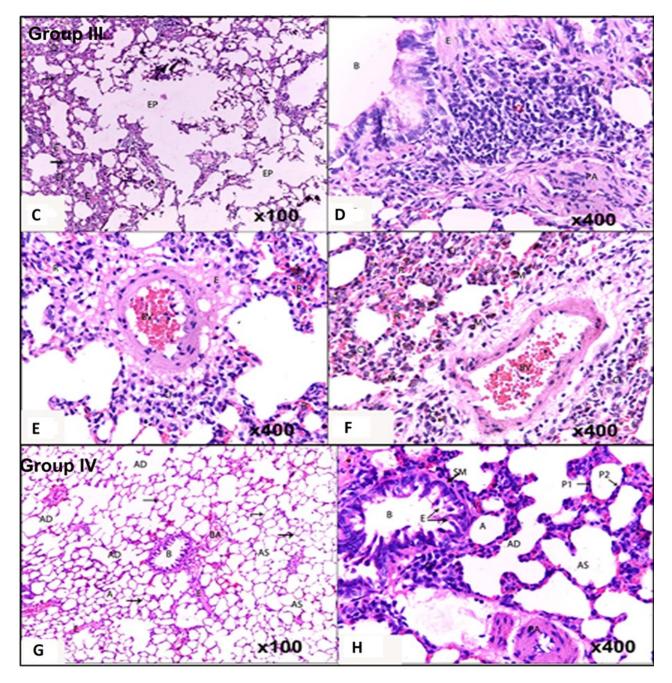

Fig 1.

Fig 1. H&E stained sections of the rat lungs of Group I showing: **(1-A)** the classic pulmonary architecture consisting of alveolar ducts (AD), alveolar sacs (AS) and alveoli (A). The alveoli are surrounded and separated from each other by thin interalveolar septa (arrows). The bronchiole (B) is accompanied by thin walled bronchial arteriole (BA) (x 100). **(1-B)** The alveoli (A) are lined by alveolar epithelium consisting of two types of cells; type I pneumocytes (P1) appeared as flat cells with flat nuclei and type II pneumocytes (P2) as rounded cells with rounded nuclei. The alveoli are separated from each other by thin interalveolar septa (arrow heads) consisting of the alveolar epithelial lining and loose connective tissue (x 400). Group III showing **(1-C)** distortion in the pulmonary architecture, thickened interalveolar septa (arrows) and mononuclear cellular infiltration (CI). Interstitial exudates (E), ruptured interalveolar septa with large irregular emphysematous air spaces (EP) are seen (x 100). **(1-D)** Marked peribronchial mononuclear cellular infiltration (CI). Thick pulmonary arteriole (PA) and interstitial exudates (E) are observed. The respiratory bronchiole (B) appeared in-between (x 400). **(1-E)** Mononuclear cellular infiltration (CI), extravasation of RBCs (R), Dilated congested blood vessel (BV) and interstitial exudates (e) are observed (x 400). **(1-F)** showing diffuse inflammatory mononuclear cellular infiltration (CI), macrophage (M) containing hemosiderin granules and extravasated RBCs (R) and engorged blood vessel (BV) (x 400). Group IV showing **(1-G)** preserved pulmonary architecture consisting of alveolar ducts (AD), alveolar sacs (AS) and alveoli (A) separated by thin interalveolar septa (arrows), normal bronchiole (B) and bronchial arteriole (BA). Minimal areas of eosinophilic exudates (E) are noticed (x 100). **(1-H)** There are preserved alveolar ducts (AD), alveolar sacs (AS) and alveoli (A) which are lined by type I (P1) and type II pneumocytes (P2). The respiratory bronchiole (B) appears normal and is lined by simple columnar epithelial cells (E) and surrounded by smooth muscle layer (SM). Thickened pulmonary arteriole (PA) and mild peribronchial cellular infiltration (CI) are observed (x 400).
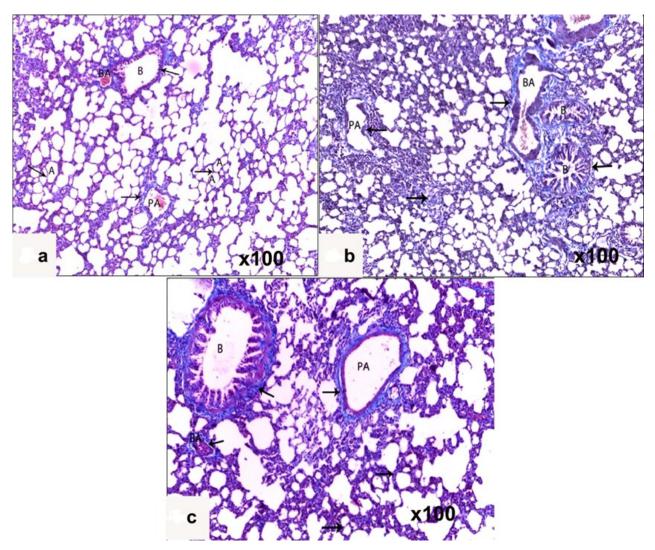
Fig 2. Masson's trichrome stained sections of a rat lung of Group I (2-A) showing minimal collagen fibers deposition (arrows) in the inter-alveolar septa, around bronchiole (B) and around the bronchial (BA) and pulmonary arterioles (PA). Group III (2-B) showing marked increase in collagen fibers deposition (arrows) in the inter-alveolar septa, around bronchioles (B) and around the pulmonary (PA) and bronchial arterioles (BA). Group IV (2-C) showing moderate increase in collagen fibers deposition (arrows). (Masson's Trichrome, x 100).

consisting of type I and type II pneumocytes. The alveoli were surrounded and separated from each other by thin inter-alveolar septa but few areas showed thickening of the inter-alveolar septa and mononuclear cellular infiltration. Few areas of ruptured inter-alveolar septa with large irregular emphysematous air spaces were seen. Respiratory bronchioles were seen lined by simple columnar epithelial cells and surrounded by smooth muscle layer. Mild peri-bronchial cellular infiltration and thickening of the wall of bronchial arterioles were observed (Figs.1-G, 1-H).

Masson's trichrome stained sections of the rat lungs of control Group showed minimal amount of collagen fibers deposition (Fig. 2-A). The lung tissues of Group III showed increased amount of collagen fibers deposition within the inter-alveolar septa, around bronchioles and around pulmonary and bronchial arterioles (Fig. 2-B). Group IV showed moderate increase in the amount of colla-

gen fibers deposition within the inter-alveolar septa, around bronchiole and around bronchial and pulmonary arterioles (Fig. 2-C).

Ultrastructural results (Fig. 3)

Electron microscopic examination of the lung tissue of Group I and II showed normal architecture; type I pneumocytes with flat nuclei and cytoplasm filled with mitochondria and rough endoplasmic reticulum. The blood-air barrier consisted of type I pneumocytes, endothelial cells and basal lamina. Type II pneumocytes appeared cuboidal with rounded nuclei and the cytoplasm contains abundant mitochondria, rough endoplasmic reticulum and many lamellar bodies (Figs. 3-A, B). Group III demonstrated marked pathological changes; dilated alveolar spaces separated by thickened inter-alveolar septa, degenerated pneumocytes and extravasation of RBCs. Type I pneumocytes have irregular and shrunken nuclei with heterogeneous distribution of their chromatin. The
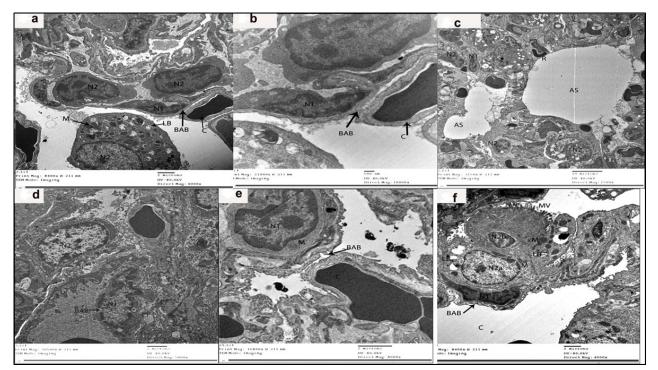
Fig 3. Electron photomicrograph of a rat lung of Group I showing (3-A) type I pneumocytes with flat nucleus (N1), type II pneumocytes with rounded nucleus (N2), characteristic lamellar bodies (LB) and mitochondria (M). Blood-air barrier (BAB) is observed consisting of endothelial cells of the blood capillary (C) and the intervening basal lamina (x 4000). (3-B) A higher magnification showing the blood-air barrier (BAB) consisting of type I pneumocytes (N1), endothelial cells of the blood capillary (3-C) and the intervening basal lamina (x 10000). Group III revealing (c) dilated alveolar spaces (AS), thickened inter-alveolar septa containing extravasated RBCs (R), degenerated type I (N1) and type II pneumocytes (N2) with irregular shrunken nuclei with heterogeneous distribution of their chromatin. (3-D) Increased collagen fibrils depositions (CF) with disrupted blood air barrier (BAB) are observed (x 1500). Group IV showing (3-E) intact blood-air barrier (BAB), type I pneumocytes (N1) and endothelial cells of the blood capillary (C) and the intervening basal lamina (x 10000). (3-F) showing normally appeared Type II pneumocytes with normal rounded nucleus (N2a), lamellar bodies (LB), mitochondria (M) and microvilli (MV). Intact blood air barrier (BAB) is noticed. (x 4000).

blood air barrier was disrupted. Type II pneumocytes showed shrunken pyknotic nuclei and empty lamellar bodies with disruption and loss of their microvilli (Figs. 3-C, D). On the other hand, Group IV revealed preservation of pulmonary architecture, normal type I pneumocytes with intact blood-air barrier. Type II pneumocytes appeared normal with rounded nucleus and abundant lamellar bodies and abundant mitochondria and rough endoplasmic reticulum with projecting microvilli (Figs. 3-E, F).

Histochemical analysis (Table 1, Fig. 5)

Mean value of Malondialdehyde (MDA)

The mean value of MDA in the lung homogenate of Groups I and II were (2.16 ± 1.42 nmol/mg proteins) and (2.46 ± 1.24 nmol/mg protein) respectively. Meanwhile, there was marked increase in the mean value of MDA level in Group III (9.21 ± 2.16 nmol/mg protein) which was statistically high significant relative to the values of Groups I and II. On the other hand, Vitamin E treatment in Group IV reversed this parameter towards the normal value (3.64 ± 3.14 nmol/mg protein) which was statistically insignificant in relation to the same values of Groups I and II.

Mean value of Superoxide Dismutase (SOD)

The mean value of SOD level in the lung homogenate of Groups I and II were (82.16 ± 1.42 U/mg protein) and (81.84 ± 2.42 U/mg protein) respectively. Meanwhile, Group III showed marked decrease in SOD level (40.24 ± 4.16 U/mg protein) which was statistically high significant relative to the values of Groups I and II. On the other hand, vitamin E treated Group showed restoration of the normal value of SOD (81.62 ± 1.28 U/mg protein) that was statistically insignificant in relation to the values of Groups I and II.

Mean value of Glutathione reductase (GSH)

The mean value of GSH level in the lung homogenate of Groups I and II were (142.21 ± 2.46 U/mg protein) and (140.18 ± 3.42 U/mg protein) respectively. Meanwhile, Group III showed marked decrease in GSH value which was (74.86 ± 2.16 U/mg protein) which was statistically high significant relative to the value of Groups I and II. On the other hand, treatment with vitamin E in Group IV reversed this parameter to the normal value (138.21 ± 4.32 U/mg proteins) which was statistically insignificant compared with the same value of Groups I and II.

Immunoexpression of iNOS in the lung tissue (Fig.

Table 1. Mean values ± SD of the oxidative markers in the lung tissue homogenates of the different experimental Groups.

Marker	Group I	Group II	Group III	Group IV
1- MDA (nmol/mg protein)				
Mean ± SD	2.16 ±1.42	2.46 ±1.24	9.21 ±2.16	3.64 ±3.14
P value			> 0.0001**	0.732
2- SOD (U/mg protein)				
Mean ± SD	82.16 ±1.42	81.84 ±2.42	40.24 ±4.16	81.62 ±1.28
P value			> 0.0001**	0.548
3- GSH (U/mg protein)				
Mean ± SD	142.21 ±2.46	140.18 ±3.42	74.86 ±2.16	138.21 ±4.32
P value			> 0.0001**	0.821 NS

* P value > 0.05 = significant; ** P value > 0.001 = high significant; NS : non-significant.
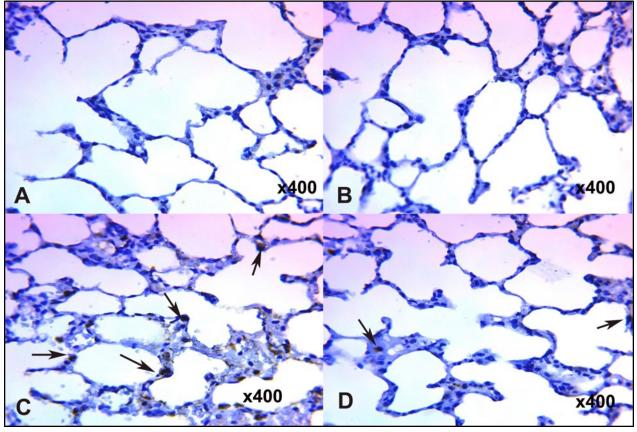

Fig 4. Immunoexpression of iNOS in the rat lung sections showing: (4-A) Negative immunoexpression of iNOS in the rat lungs of the control Group. (4-B) Negative immunoexpression in the rat lungs of sham Group. (4-C) Strong positive immunoexpression in the rat lungs of Group III. (4-D) Weak positive immunoexpression in the rat lung of Group IV (x 400).

4)
The rat lungs of the control and sham control Groups showed negative immunoexpression of iNOS (Figs. 4-A and B). Meanwhile, the rat lung of amiodarone treated Group (Group III) exhibited strong positive iNOS immunoexpression in the lung cells lining the alveoli (Fig. 4-C). On the other hand, vitamin E treated Group revealed weak positive of iNOS immunoexpression as compared with Group I, II and III (Fig.4-D).
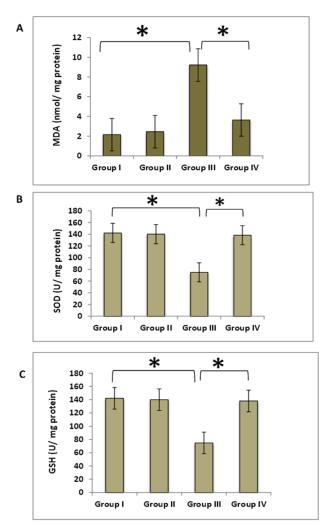
Histomorphometric analysis

Fig 5. Mean values of the oxidative markers in: MDA (5-A), SOD (5-B) and GSH (5-C) in the lung tissue homogenate of the different experimental Groups (asterisk indicates high significant value of Group III in relation to the other Groups).
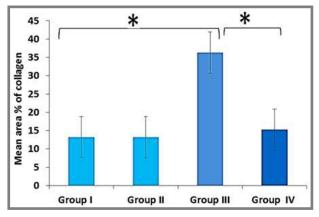

Fig 6. Relation between the mean area percent of collagen fibers deposition in the lung tissue of the different experimental Groups (asterisk indicates high significant value of Group III in relation to the other Groups).

Mean area % of collagen fibers in the lung tissue

The mean area percent of collagen fibers deposition in the lung tissue of Group III was markedly increased (36.31 ± 6.24 %) which was statistically high significant as compared with the mean area % of collagen fibers of Group I (13.24 ± 0.39). Meanwhile the mean area percent of collagen fibers of Group IV was mildly increased (15.23 ± 2.99 %) which was statistically insignificant as compared with the mean value of Group I (Table 2, Fig. 6).

Mean area % of iNOS immunoexpression in the lung tissue

The mean area percent of iNOS immunoexpression in the lung tissue of Group III was markedly increased (22.04 ± 0.62 %), which was statistically high significant as compared with the same value in Group I and Group II (0.56 ± 0.24 % and 0.65 ± 0.081) respectively. Meanwhile the mean area percent of collagen fibers of Group IV treated with vitamin E showed significant reduction in the mean area % (4.12 ± 0.18. %) when compared with Group III and statistically insignificant as compared with the mean value in Group I (Table 3, Fig. 7).

DISCUSSION

In the present study, amiodarone-treated group (Group III) showed marked degenerative change; distorted pulmonary architecture and thickened inter-alveolar septa and mononuclear cellular infiltration with areas of consolidation. These findings were in agreement with Zickri et al. (2014), who mentioned similar findings as thickening of the alveolar septa and dense cellular infiltration and extravasated RBCs. The results revealed ruptured inter-alveolar septa and formation of large emphysematous spaces, which were similar to the findings of Zaghlool et al. (2011), who observed overdistention of the alveoli with disrupted alveolar walls and cellular infiltration. Moreover, Mahavadi et al. (2014) confirmed the thickening of alveolar septa with patchy fibrosis and cellular infiltration.

The present study revealed obvious mononuclear inflammatory cellular infiltration and the presence of hemosiderin Leydig granules in the lungs of Group III. These findings were matched with the findings of Zidan (2011), who mentioned marked inflammatory polymorph cell infiltration mainly lymphocytes and macrophages. The presence of macrophages could be explained by Savani et al (2001), who suggested that macrophages engulfed the extravasated RBCs and the extruded lamellar bodies containing surfactant. Larsen et al. (2012) added that these manifestations were due to a non-specific interstitial pneumonitis predominantly composed of mononuclear cells, alveolar macrophages and fibrosis.

Moreover, peri-bronchial cellular infiltration and exfoliated epithelial cells were evident. These findings were in accordance with Abo Gazia and El-Kordy (2012), who noticed homogeneous eosinophilic interstitial exudates with perivascular and peri-bronchial cellular infiltration with sloughing of cellular debris inside the lumen of the affected alveoli and bronchioles. Moreover, this was also in agreement with Zickri et al. (2014), who observed

Table 2. Mean area % ±SD of collagen fibers deposition in the lung tissue of the different experimental Groups.

	Group I	Group II	Group III	Group IV
Mean area %	13.246	13.186	36.318	15.234
±SD	±0.398	±0.362	± 6.243	±2.998
SD error	0.31	0.24	1.78	2.4
Max	13.78	13.64	44.93	24.98
Min	12.26	12.28	30.33	18.26
P value			> 0.0001**	0.094 NS

* P value > 0.05 = significant; ** P value > 0.001 = high significant; NS : Non-significant.

Table 3. Mean area % ± SD of iNOS immunoexpression in the lung tissue in the different experimental Groups.

	Group I	Group II	Group III	Group IV
Mean area % ± SD	0.56 ± 0.24	0.65 ± 0.081	22.04 ±0.62	4.12 ± 0.18
P- value			> 0.0001**	0.062 NS

* P value < 0.05 = significant; ** P value < 0.001 = high significant; NS : Non-significant.

small bronchioles with partial obliteration of its lumen by shed epithelial cells and cellular infiltration.

The lungs of group III showed obvious congestion of the pulmonary vessels, extravasation of RBCs with interstitial exudate. These findings could be related to the direct toxic effect of amiodarone on the wall of the blood vessels. These findings were in consistent with Mahdy (2014) who noticed similar changes.

There was marked increase in collagen fibers deposition in the lung tissue of Group III. This was in accordance with Gado and Aldahmash (2013), who reported the presence of focal areas of fibrosis in the alveolar interstitial tissue.

The role of oxidative stress in the development of amiodarone-induced pulmonary toxicity was suggested by Taylor et al. (2000) and Ashrafian and Davey (2001), who attributed these findings to the massive damage to the blood air barrier and increase in free radicals generation and mitochondrial hydrogen peroxide production (Rebrova and Afanasyev, 2008). Moreover, Shimizu et al. (2003) attributed these changes to direct toxicity of amiodarone, hypersensitivity, elevated oxidant markers. Nicolescu et al. (2007) added inflammatory mediators release, phospholipidosis promotion, mitochondrial dysfunction and free radical production.

Improvement of the lung architecture in Group IV (treated with vitamin E) was coinciding with Futamara (1996), who mentioned that vitamin E could decrease AM-induced cytotoxicity in cultured pulmonary cells whereas other antioxidants were ineffective. Moreover vitamin E protection against AM-induced cytotoxicity in hamster lung cells was observed (Bolt et al., 2001). Azzi and Stocker (2000) suggested that the protective mechanism of vitamin E could be recognized primarily for its free radical scavenging and chain-breaking antioxidant properties. The authors added that vitamin E has recently gained attention for exerting several ef-
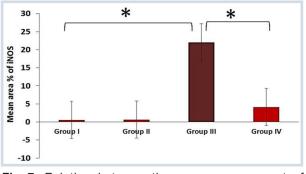
Fig 7. Relation between the mean area percent of iNOS immunoexpression in the lung tissue of the different experimental Groups (asterisk indicates high significant value of Group III in relation to the other Groups).

fects that cannot be attributed only to its antioxidant efficiency. Augustin et al. (1997) confirmed the protective effects of vitamin E against mitochondrial damage. In the present work, the decrease in fibrosis in Group IV could be explained by Cooper (2000), Sime and O'Reilly (2001) who owed that to the role of vitamin E in down-regulation of pro-inflammatory and pro-fibrotic genes expression mainly transforming growth factor (TGF)-β1, which is a crucial mediator of fibrosis. This suggestion was confirmed by Card et al. (2003) mentioned that the decrease in fibrosis was due to vitamin E down-regulation of tissue growth factor beta 1 (TGF-beta1) overexpression. Moreover, they added that in spite of the marked elevation of vitamin E in the lung mitochondrial, it did not attenuate AM-induced inhibition of mitochondrial respiration or disruption of mitochondrial membrane potential in vitro, or lung mitochondrial respiratory inhibition. They postulated that vitamin E reduces the pulmonary damage after AM administration through down-regulation of TGF-beta1 overexpression but that it does not modify AM-induced mitochondrial dysfunction.

The present study concluded that amiodarone had marked toxic effect on the lung of the adult albino rat and that vitamin E could partially protect the lung against such toxic effects. Thus, it is recommended to investigate the pulmonary functions before the use of amiodarone and throughout the course of treatment. Once, pulmonary toxicity starts to develop, shifting to another safer drug should be considered. Also, it is advisable to give vitamin E concomitantly with amiodarone to reduce its toxicity on the lung.

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