Oxidative Stress in Amiodarone-Induced Pulmonary Toxicity in Rats and the Protective Effect of L-carnitine and Vitamin C

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**ABSTRACT**

This study aims to investigate the oxidative stress role in the pathogenesis of amiodarone-induced pulmonary toxicity and to show whether antioxidants’ co-administration with amiodarone could exert any protective effect. The study was carried out on 36 Sprague-Dawley males, rats were divided into the following six equal groups, drugs were given by gastric tube every day for 7 days as follow; control group: received distilled water, amiodarone (AM) treated group: received amiodarone (100 mg/kg body weight), L-carnitine (LC) treated group: received L-carnitine (100 mg/kg body weight), vitamin C treated group: received vitamin C (1 mg/100 g body weight), amiodarone and L-carnitine treated group: received amiodarone along with LC and amiodarone and vitamin C treated group: Received amiodarone together with vitamin C. The histopathological findings showed that amiodarone disrupted lung architecture and caused inflammatory cells infiltration in addition to extensive fibrosis. Increased level of Malondialdehyde (MDA) and decreased level of catalase (CAT) in lung tissue homogenates were observed in the AM-treated group. Administration of L-carnitine and vitamin C improved the biochemical and histopathological alterations in the lungs induced by amiodarone. Vitamin C was more protective than LC as regard the histopathological changes. Antioxidants administration induced a significant decrease in MDA and an increase in CAT in lung tissue as compared with AM treated group. The oral administration of LC and vitamin C reversed the biochemical and histopathological alterations induced by AM. They may have a protective role in the AM-induced lung toxicity.

**KEYWORDS**

Oxidative stress, Amiodarone toxicity, L-Carnitine, Vitamin C.

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**Introduction**

Amiodarone (AM) is one of the best effective antiarrhythmics that is valuable in the treatment of supraventricular and ventricular arrhythmias (Zaidel, 2019). However, it is of restricted use because of its serious side effects especially lung toxicity (Gawad et al., 2018). Pulmonary and extrapulmonary toxic effects were related to a high cumulative dose of amiodarone. It has a half-life about 30-60-days and high lipid solubility (Galaly et al., 2018).

Pulmonary toxicity develops in about 15% of patients utilizing amiodarone. 10-20% of those may be manifested by respiratory failure and adult respiratory distress syndrome (ARDS) with a high rate of mortality (Schwaiblmair et al., 2010), and can lead to extreme pulmonary fibrosis (Nacca et al., 2012).

Chronic use of amiodarone is usually associated with lung toxicity which is manifested by dyspnea, cough and chest pain in the presence of radiographic interstitial infiltrates (Kaya et al., 2017). Old age is more...
at risk for the development of lung complications (Papiris et al., 2010).

Diffuse alveolar damage is a typical histopathological finding of amiodarone-induced lung injury. It is partitioned into an acute exudative phase and a late reparative phase. The exudative phase appears in the 1st week after lung injury, is characterized by alveolar and interstitial oedema and hyaline membranes. The reparative phase occurs 1-2 weeks after the exudative phase, is characterized by the proliferation of type II pneumocytes and interstitial fibrosis (Samiei et al., 2016).

Oxidative stress plays a significant role in the pathogenesis of amiodarone–induced lung toxicity (Gawad et al., 2018). Administration of antioxidants with amiodarone may have a protective effect on amiodarone toxicity. Furthermore, antioxidants may improve the antiarrhythmic action of amiodarone. Thus, co-administration of antioxidant agents with amiodarone may lead to the more broad application of amiodarone (Vereckei, 2015).

One of the final products of polyunsaturated fatty acids peroxidation in the different cells is Malondialdehyde (MDA). Its level is a well-known marker for oxidative stress. An increase in free radicals causes overproduction of MDA. Membrane lipid peroxidation can damage pulmonary endothelium (Kinnula et al., 2005).

The first line of defense against oxidative stress is the antioxidant enzymes. Catalase (CAT) and superoxide dismutase (SOD) are important antioxidant enzymes that protect the lungs against oxidative stress (Zickri et al., 2014).

L-carnitine has an important antioxidant property counteracting age-associated mitochondrial dysfunction in rats. It transports long chain fatty acids into the mitochondria for \( \beta \)-oxidation (Terruzzi et al., 2019).

Vitamin C has been described as the most important antioxidant because of its powerful antioxidant activity. It showed organ protective effects in the nervous, cardiovascular, respiratory, gastrointestinal, coagulation and immune systems in preclinical and in clinical studies (Hill et al., 2018).

This work aims to study the pulmonary toxicity of amiodarone, to study the possible mechanism associated with amiodarone-induced pulmonary toxicity in rats and the role of LC and vitamin C in ameliorating amiodarone-induced lung toxicity in Sprague-Dawley adult male albino rats.

**Material and Methods:**

This study was approved by Institutional Review Board (IRB), Faculty of Medicine, Mansoura University (code no: R/19.07.555).

**Drugs:**

- Amiodarone (amiodarone hydrochloride) 200 mg, in the form of tablets, was purchased from the company of Global Napi for pharmaceuticals products, Egypt (under license of Sanofi Aventis, France). Amiodarone solution was freshly prepared by dissolving the tablet in distilled water.
- L-carnitine (LC) 500 mg (3-hyrdoxy, 4-trimethylamino butyric acid) was obtained from Global Napi, Egypt in the form of tablets. Each tablet was dissolved in 10 ml distilled water.
- Vitamin C 100 mg/ml: was purchased from Unipharma, Egypt in the form of drops.

**Experimental animals:**

Healthy thirty six Sprague-Dawley male rats (weight, 180-220 g) received amiodarone...
(100 mg/kg/d) for 7 days by gastric intubation (Samiei et al., 2016). They were obtained from the Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University, Egypt. The animals housed in separate cages at room temperature (22 ± 4 °C) after clinical examination, with free access to water and feed.

The experimental study was performed according to the Ethics Committee of the National Research Center and in accordance with the ethical guidelines for the care and use of animals provided by the Institutional Animal Care and Use Committee of Mansoura University.

**Experimental design:**

Drugs were given daily for seven days by gastric intubation to the rats. The rats were randomly divided to six groups, each group containing 6 rats: (i) Control group: rats were given distilled water through orogastric tube; (ii) AM treated group: the rats received AM (100 mg/ kg/ body weight) (Sancar Baş et al., 2016); (iii) LC group: the rats received LC (100 mg/kg body weight) (El-Nahrawy et al., 2017). (iv) Vitamin C group: the rats received vitamin C (1 mg/100 g body weight) (Shalan et al., 2005). (v) AM+LC group: the rats received amiodarone along with LC (100 mg/kg body weight) (El-Nahrawy et al., 2017). (vi) AM+ Vitamin C: the rats received vitamin C (1 mg/100 g body weight) together with AM for (Shalan et al., 2005). The doses used in this study were calculated based on the recommended human maintenance doses.

At the end of the 8th day, the rats were sacrificed with deep anesthesia (Ketamine). The lungs were freshly taken, washed and prepared for the histopathological study and determination of malondialdehyde and catalase levels in the Lungs of experimental rats.

**Histological preparations:**

Following euthanasia, each rat lung was cut into small pieces, fixed for 24 h in 10% formalin saline. The specimens were then washed and dehydrated in ascending grades of ethanol (70%, 90% and 100%). They were cleared for 2 hours in xylene. Impregnation was done in soft paraffin wax at 45-50°C for 3 h 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 for routine pathology evaluation and Masson’s trichrome to evaluate fibrosis. The specimens were examined in the Department of Pathology, Faculty of Medicine, Mansoura University.

Hematoxylin and Eosin and Masson's trichrome stained slides were scored for pigmented macrophages and degree of inflammation. 5-point scale for lung inflammation and fibrosis was used as follows: none (0+), minimal (1+), mild (2+), moderate (3+), marked (4+), using both degree and extent.

Fibrosis was evaluated using the same grading scale, but it is applied for Masson's trichrome stained sections. Fibroblastic proliferation or collagen deposition in the interstitium of the lung is considered as fibrosis. It is dependent on the degree of proliferation or deposition. Minimal was characterized by minimal deposition, mild showed increased interstitial thickness, moderate showed definite remodeling and severe involved considerable thickening and a honeycomb appearance (Snyder-Talkington et al., 2016).

**Estimation of oxidative stress markers:**

a) Determination of malondialdehyde content in lung homogenates:

It was measured as thiobarbituric acid-reactive substances by spectrophotometer at an
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absorbance of 532 nm, by the method of Ohkawa et al. (1979) and the concentrations were expressed as nmol / 0.6 g tissue.

b) **Determination of antioxidant enzyme catalase in lung homogenates:**

It was measured by spectrophotometer according to the method of Higgins et al. (1978), via the assay of hydrogen peroxide (\( \text{H}_2\text{O}_2 \)). Catalase activity is expressed as nmol /0.6 g tissue using a molar absorbance of 43.6 for \( \text{H}_2\text{O}_2 \).

**Statistical analysis:**

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 21). Shapiro test was used to test the normality of data. Continuous variables were presented as mean ± SD (standard deviation). ANOVA test was used to compare more than 2 means.

The results were considered non-significant when the probability of error is more than 5% (\( p > 0.05 \)) and significant when the probability of error is less than 5% (\( p \leq 0.05 \)).

**Results:**

Microscopic examination of H&E stained lung sections of the control group showed the histological features of the normal lung in the form of patent alveoli, alveolar sacs and thin interalveolar septa. Alveoli were lined mainly by type I pneumocytes, few type II pneumocytes and some macrophages. Bronchioles with patent lumen surrounded by regular smooth muscle layer and lined by ciliated simple columnar epithelial cells (Figure 1a). On the other hand, lungs of AM-treated group showed extensive pulmonary inflammation destroying the bronchiolar structure (with smooth muscle wall) and lymphoid follicles formation (Figure 1b-c).

L carnitine and vitamin C groups revealed mild inflammatory cellular infiltrate mainly lymphocytes (Figure 1d-e). Treatment of AM rats with L carnitine showed moderate inflammatory cellular infiltrate mainly lymphocytes with a lymphoid follicle (Figure 1f). Amiodarone rats treated with vitamin C showed mild pulmonary interstitial inflammation (Figure 1g).
Fig. (1): Hematoxylin and Eosin staining Lung sections; a): control group: no pathological abnormalities x100 magnification. b): amiodarone group: revealed extensive pulmonary inflammation even lymphoid follicles formation (x200). c): amiodarone group: revealed extensive pulmonary inflammation with most of the bronchiolar lumen and the wall is infiltrated by neutrophils and pus cells (x400). d): L-carnitine group: revealed mild inflammatory cellular infiltrate mainly lymphocytes x100 magnification. e) Vitamin C group: revealed mild inflammatory cellular infiltrate mainly lymphocytes f): amiodarone + L-carnitine group: revealed moderate inflammatory cellular infiltrate mainly lymphocytes with lymphoid follicle formation (x100). g): amiodarone + vitamin C group: revealed mild pulmonary interstitial inflammation (x100).
Microscopic examination of Masson's trichrome stained lung sections of the control group revealed no to minimal fibrosis stained blue (Figure 2a). On the other hand, lungs of AM-treated group showed extensive pulmonary interstitial fibrosis with focal atrophy of the bronchiolar epithelium and congested blood vessels (Figure 2b).

The lungs of rats received L-carnitine revealed minimal interstitial pulmonary fibrosis (stained blue) (Figure 2c). The lungs of rats received vitamin C revealed mild pulmonary interstitial fibrosis (stained blue) and congested blood vessels (Figure 2d).

The lungs of AM rats treated with L-carnitine showed mild interstitial pulmonary fibrosis (Figure 2e.) The lungs of AM rats treated with vitamin C revealed minimal pulmonary interstitial fibrosis (stained blue) together with markedly congested blood vessels (Figure 2f). Treatment with L-carnitine and vitamin C in Groups V and VI showed marked improvement of the pulmonary architecture.

![Fig. (2): Masson's trichrome stained lung sections for evaluating fibrosis](image)
The data illustrated in table (1) demonstrates that daily oral administration of AM (100 mg/kg body weight) for 7 days in rats caused oxidative stress determined by a significant increase in MDA and a significant decrease in CAT in the lung homogenates as compared to the control group (p<0.001). L-carnitine and vitamin C administration significantly decreased MDA and increased CAT when compared to the AM-treated rats (Table1).

**Table (1):** MDA (nmol /0.6 g tissue) & CAT (nmol /0.6 g tissue) in the lung tissues among the studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol /0.6 g tissue)</th>
<th>CAT (nmol /0.6 g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>158.60±5.26</td>
<td>17.17±0.28</td>
</tr>
<tr>
<td>AM</td>
<td>295.68±5.37*^a</td>
<td>13.29±2.13*^a</td>
</tr>
<tr>
<td>L-Carn</td>
<td>249.31±58.93 *^a,b</td>
<td>16.31±0.35*^b</td>
</tr>
<tr>
<td>Vit C</td>
<td>212.50±54.32*^a</td>
<td>15.31±0.79*^a</td>
</tr>
<tr>
<td>AM + L-Carn</td>
<td>215.75±13.25 *^a,b</td>
<td>15.94±0.43 *^a,b</td>
</tr>
<tr>
<td>AM + Vit C</td>
<td>218.86±17.41 *^a,b</td>
<td>15.48±0.57 *^a,b</td>
</tr>
<tr>
<td>ANOVA test</td>
<td>10.63</td>
<td>10.41</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE (n=6) MDA: Malondialdehyde, CAT: catalase, AM: amiodarone, L-Carn: L-carnitine, Vit C: vitamin C. *p value is significant if ≤ 0.05 a: means significant difference vs. control; b: significant difference vs. AM treated group.

**Discussion:**

This study aims to investigate the pulmonary toxicity induced by amiodarone, the role of oxidative stress and the possible protective effects of L-carnitine and vitamin C as antioxidants in ameliorating amiodarone-induced lung toxicity in adult male albino rats.

In the current study, the histopathological examination of AM-treated rats’ revealed extensive pulmonary inflammation destroying the bronchiolar structure with most of the bronchiolar lumen and wall is infiltrated by neutrophils and pus cells with lymphoid follicles formation. In addition, AM-treated rats’ lung sections stained with Masson's trichrome revealed extensive pulmonary interstitial fibrosis with focal atrophy of the bronchiolar epithelium and congested blood vessels.

Administration of LC and vitamin C ameliorated the histopathological changes in the lung tissues induced by amiodarone. Vitamin C was more protective than LC as regards the histopathological changes.

Al-Shammari et al. (2016) reported that AM-treated rats showed histopathological changes in the lungs in the form of interstitial capillary dilation, granulomatous inflammation, interstitial pneumonitis and thickened alveolar walls. Moreover, amiodarone induced mononuclear cells infiltration, severe pneumocytes degeneration, areas of consolidation with alveolar collapse, emphysematous air spaces and increased
collagen fibers deposition in rats’ lungs (Gawad et al., 2018).

Hasan et al. (2016) suggested that oxidative stress plays a critical role in the pathogenesis of AM- induced lung toxicity. Increase in the production of ROS results in the oxidative damage to membrane lipids. Lipid peroxidation due to free radicals production results in damage to the pulmonary endothelium and can be measured by MDA. Catalase is one of the enzymatic antioxidant defense systems that are responsible for eliminating free radicals (Cherian et al., 2019).

In the current study, rats received amiodarone orally exhibited a marked increase in MDA and a significant decrease in CAT in the lung tissue homogenates as compared to those in the control group. These alterations in MDA and catalase levels could be attributed to oxidative stress.

These results are in agreement with Gado and Aldahmash (2013) who reported that catalase levels decreased significantly in AM-treated rats, suggesting oxidative stress mechanism in AM-induced lung toxicity.

The present experimental results revealed that rats receiving the antioxidants LC and vitamin C showed significant changes in the oxidative indices (MDA and CAT) in the lung homogenates in comparison to those obtained from the control group. Additionally, the histopathological examination of lung tissue revealed improvement in lung architecture that means antioxidants co-administered with amiodarone exert partial protective effect on amiodarone toxicity.

The results of the current study are in agreement with those obtained by El-Nahrawy et al. (2017) who reported that LC significantly increased the levels of antioxidant enzymes. Durukan et al. (2012) reported that vitamin C is effective in decreasing cellular cytotoxicity of amiodarone. Vitamin C is an enzyme cofactor and antioxidant that could inhibit oxidative stress (Viswanatha-Swamy et al., 2011).

In conclusion, amiodarone-induced pulmonary toxicity in rats following daily oral administration may be due to oxidative stress ending in severe lung toxicity as evidenced by an inflammatory reaction and severe fibrosis in lung tissues. Antioxidants LC and vitamin C could partially protect the lung against such toxic effects. Thus, co-administration of antioxidants with amiodarone may lead to the more widespread application of amiodarone.

References:


Vereckei, A. (2015): “The role of oxidative stress in amiodarone toxicity, in left ventricular dysfunction of hypertensive patients and heart failure with preserved


دور الإجهاد المؤكسد في التسمم الرئوي الناجم عن الأميودارون في الفئران والتأثير الوقائي لـ كارنيتين وفيتامين ج

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كشفت النتائج المرشدة الحالية أن الأميودارون تسبب في تغيرات ملموسة في الرئة تضمن ذلك حدوث التهاب في أنسجة الرئة بالإضافة إلى التليف الواسع. لوحظت زيادة في مستويات دلالات الأكسدة Catalase وانخفاض مستوى الكاتالاز Malondialdehyde في المجموعة التي أعطيت أميودارون.

تستخدم إل- كارنيتين وفيتامين ج مع أميودارون يقلل من مدى تلف الرئة الناجم عن الأميودارون، ويسمن من مستويات دلالات الأكسدة في الرئة. لذلك قد يكون استخدام هذه الأدوية دور في الوقاية من سمية الرئة الناجمة عن استخدام أميودارون.