The Study of the Possible Protection of Vitamin E on Chlorpyrifos-Induced Lung Toxicity in Albino Rats


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Abstract

Background: Organophosphate [OP] insecticides are used in the agricultural and domestic pest control, accounting for 50% of the global insecticidal use. Chlorpyrifos [CPF] is one of the most widely used OP insecticides until 2000 when the United States Environmental Protection Agency restricted some of its domestic uses due to its toxicity. Many drugs including Vitamin E have been tried in combination with chlorpyrifos exposure aiming to reduce these adverse effects.

Aim of the Work: To detect the histological and ultrastructural changes of chlorpyrifos toxicity in the lungs of adult male albino rat and the possible protective role of vitamin E.

Material and Methods: The present work was carried out on sixty albino rats which were divided into three main groups; Group I: The control group which was subdivided into normal control and sham control, Group II: Chlorpyrifos-treated group which received 1/4LD50 of chlorpyrifos and Group III: chlorpyrifos with vitamin E-treated group which received 1/4LD50 of chlorpyrifos and vitamin E. All groups were further subdivided into two subgroups: Subgroup A whose rats were sacrificed after one week and Subgroup B whose rats were sacrificed after three weeks.

Results: The histological study of the lungs of Group II showed extensive damage with loss of normal alveolar pattern, diffuse cellular infiltration resulting in massive consolidation and large irregular emphysematous air space. Electron microscopic study demonstrated pneumocytes with shrunken and pyknotic nuclei. In comparison with Group II-A, the histological and ultrathin sections of rats of Group II-B exhibited excessive collagen deposition and chronic inflammatory cell infiltrations. These alterations were improved in Group III whose rats received vitamin E in combination with chlorpyrifos.

Conclusion: This study showed that chlorpyrifos had deleterious effects on the lungs of albino rats and these effects were markedly decreased with the concomitant use of vitamin E.

Key Words: Lung – Chlorpyrifos – Vitamin E – Oxidative stress.

Introduction

ORGANOPHOSPHATES [OPs] act mainly as acetylcholinesterase inhibitors [AChE] and can be an indicator of chronic toxicity of OPs [1]. Oxidative stress is one of the possible mechanisms that could be involved in the OPs toxicity [2]. Chlorpyrifos is a member of the most commonly used organophosphorus insecticide. As a result of widespread use, residues of chlorpyrifos have been detected in the air and in the crops which considered a risk for living organisms [3]. Chlorpyrifos, in particular chlorpyrifos-ethyl [CPF], resulted in deleterious effects including hepatotoxicity, pulmonary toxicity, teratogenicity, immunotoxicity as well as neurochemical and neurobehavioural alterations [4].

Vitamin E has become increasingly popular in terms of health protection because it possesses a remarkable spectrum of biochemical and pharmacological activities. Vitamin E affects the basic cell function such as growth, differentiation and apoptosis [5]. Also, it was shown to be a potent antioxidant because of its radical-scavenging activity; ability to complex heavy metal ion and to antagonize a broad spectrum of enzymes such as tyrosine protein kinase [6].

Aim of the work: The aim of the present study is to detect the histological and ultrastructural changes of chlorpyrifos toxicity in the lung of adult male albino rat. Also, to evaluate the possible protective role of vitamin E in the reduction of these pulmonary change.

Material and Methods

I- Experimental animals:

The present study was carried out on 60 male adult Sprague Dawley albino rats in the animal house of the ophthalmology institute [weighing
150-250gms] during the period from 10/12/2015 to 5/1/2016. The rats were fed on a wet diet formed from bread mixed with milk. All the rats were housed in cages, five rats/cage, under constant suitable domestic and environmental conditions.

II- Chemicals:
Chlorpyrifos: The trade name of chlorpyrifos active ingredient is chlorzan which is 48% Emulsifiable Concentrate [EC] and locally formulated in Egypt. It was obtained from the Central Agricultural Pesticide Laboratory [Dokki-Giza-Egypt] and was orally administrated to animals by gastric gavage [7].

Vitamin E: Vitamin E is present as α-tocopherol 1000mg soft capsules [EIPICO Company]. One capsule is diluted to 6.67ml of corn oil and then every ml were more diluted to 5ml of corn oil and was given to the rats at an average dose of one ml/rat [150mg/kg] by intramuscular injection as indicated [8].

- Experimental design:
The tested rats were divided into 3 groups:

  - Group I [control]: Consisted of 20 rats and were subdivided into two subgroups; each consisted of 10 rats: Subgroup A [normal control] Were given no drug and were sacrificed after one and three weeks; subgroup B [sham control]: Were given a daily dose of corn oil by intramuscular injection and were sacrificed after one and three weeks.

  - Group II [chlorpyrifos treated group]: Consisted of 20 rats and were subdivided into two subgroups; each consisted of 10 rats: Subgroup A: Were given a daily dose of 16.4mg/kg b.w. [1/4 LD50] of chlorpyrifos by gastric gavage for one week; subgroup B: Were given a daily dose of 16.4mg/kg b.w. [1/4 LD50] of chlorpyrifos by gastric gavage for 3 weeks.

  - Group III [chlorpyrifos and vitamin E treated group]: Consisted of 20 rats and were subdivided into two subgroups; each consisted of 10 rats: Subgroup A: Were given a daily dose of 16.4mg/kg b.w. [1/4 LD50] of chlorpyrifos by gastric gavage and a daily dose of 150mg/kg of vitamin E by intramuscular injection for one week; subgroup B: Were given a daily dose of 16.4mg/kg b.w. [1/4 LD50] of chlorpyrifos by gastric gavage and a daily dose of 150mg/kg of vitamin E by intramuscular injection for 3 weeks.

In each group, the rats were sacrificed by cervical decapitation at the end of the duration related to each group. The lungs were extracted and cut into several specimens.

The removed lung specimens were subjected to:

- Light microscopic study using Hematoxylin & Eosin and Masson's trichrome stains [9].
- Electron microscopic study [10].
- Imaging analyser technique for the calculation of the area % of the collagen fibres.
- Statistical study: Using the measuring field menu the area % and standard measuring frame of a standard area equal to 118476.6 m² were chosen from the parameters. In each chosen field the lung tissue was enclosed inside the standard measuring frame then the connective tissue area was masked by a green binary colour to be measured. These measurements were done using an objective lens of magnification 10, i.e. of total magnification 100. Ten readings were obtained in each specimen and their mean values were obtained. The data obtained were subjected to statistical analysis using one way analysis of variance [ANOVA] [11].

Results

Group I-A [the normal control group]:
The light microscopic examination of the lungs of the rats of the normal control group revealed normal structure of the lung tissue in the form of alveolar ducts, alveolar sacs and alveoli. Bronchioles appeared as intralobular airways that are lined by ciliated simple cuboidal epithelium surrounded by smooth muscle and fibrous connective tissue layers Fig. (1).

The statistical and histomorphometric studies showed that the amount of the collagen fibres represented about 13-15% of the whole lung tissue [Histogram 1 and (Table 1)].

Electron microscopic examination: Ultrastructural examination of the lungs of the normal control rats revealed normal alveoli separated by interalveolar septa containing blood capillaries. The blood capillaries were seen separated from the pneumocytes type 2 by the basilar lamina which is composed of the basilar membranes of both alveoli and capillaries Fig. (2).

Group I-B [sham control]:
The light microscopic examination of the lungs of the rats which received the corn oil for one and three weeks, they appeared histologically as the normal control group.

Statistically and histomorphometrically, there was an insignificant increase of the amount of the collagen fibres in relation to the normal control.
group to represent about 17-19% of the whole lung tissues [Histogram 1 and (Table 1)].

Electron microscopic examination of the lungs of the rats which received corn oil for one week and the others which received corn oil for three weeks, it was observed that they had the same ultrastructure as the normal control group.

**Group II-A [chlorpyrifos treated group for one week]:**

The light microscopic study of the lungs of the rats of Group [II-A] released diffuse alterations of the normal structure of the alveoli with disappearance of the air spaces, massive cellular inter-alveolar, periarteriolar and peribronchiorial infiltrations as well as the congested blood vessels and emphysematous bullae Fig. (3).

Statistically and histomorphometrically, there was a moderate increase of the amount of the collagen fibres in relation to the normal control group to represent about 23-25% of the whole lung tissues [Histogram 1 and (Table 1)].

**Electron microscopic examination:** Electron microscopic examination of the lungs of rats of Group [II-A] exhibited evident disturbations of the ultrastructure of the pneumocytes in the form of pyknotic nuclei and many vacuoles Fig. (4). In severely affected specimens loss of the integrity of the cell membrane was noticed where the cytoplasmic contents were seen extruded into alveolar spaces Fig. (5).

**Group II-B [which received chlorpyrifos for three weeks]:**

The light microscopic study of the lungs of the rats of Group [II-B] revealed marked thickening of the walls of the arterioles and bronchioles with marked deposition of connective tissue. There were marked destruction of the epithelium and endothelium of the bronchioles and arterioles respectively with desquamated epithelial and endothelial cells inside their lumens Figs. (6,7).

Statistically and histomorphometrically, there was a severe increase of the amount of the collagen fibres in relation to the normal control group to represent about 38-40% of the whole lung tissues [Histogram 1 and (Table 1)].

**Electron microscopic examination:** Ultrastructural examination of the lungs of rats of Group [III-A] showed partial preservation of alveolar architecture with partial restoration of the alveolar spaces Fig. (10).

**Group III-B [which received chlorpyrifos with vitamin E for three weeks]:**

The light microscopic study: The lungs of the rats of Group [III-B] showed almost complete restoration of the structure of the alveoli, marked decrease of the thickening of the walls of arterioles and bronchioles and decrease of the inflammatory cellular infiltrations and exudates Fig. (11).

Statistically and histomorphometrically, there was much less deposition of collagen fibres in relation to Group II-B to become about 24-26% of the whole lung tissues [Histogram 1 and (Table 1)].

**Electron microscopic examination of the lungs of rats of Group [III-B]:** The nuclei of pneumocytes type 2 were rounded and euchromatic. The cytoplasm comprised lamellar bodies Fig. (12).

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**Table (1): Area % values of Masson's trichrome reaction in the studied groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Area %</th>
<th>SD</th>
<th>SE</th>
<th>F ratio</th>
<th>p-value</th>
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<tr>
<td>I-A</td>
<td>13.89</td>
<td>0.049721</td>
<td>0.014561</td>
<td>2.935567</td>
<td>0.0365*</td>
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<tr>
<td>I-B</td>
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<td>0.073918</td>
<td>0.039186</td>
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<tr>
<td>II-A</td>
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<td>0.236646</td>
<td>0.094834</td>
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<tr>
<td>II-B</td>
<td>38.24</td>
<td>0.351898</td>
<td>0.17128</td>
<td></td>
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<tr>
<td>III-A</td>
<td>20.53</td>
<td>0.192788</td>
<td>0.065667</td>
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<tr>
<td>III-B</td>
<td>26.62</td>
<td>0.283593</td>
<td>0.106434</td>
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</tbody>
</table>

* : p<0.05 = Significant.
** : The F-ratio indicates the overall significance among the different animal groups. The greater the numerical value of the F-ratio, the more significance the difference is.
*** : SD = The standard deviation.
**** : SE = The standard error.
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Histogram (1): Area % of collagen fibres in the studied groups and the Standard Deviation [SD].

Fig. (1): A micrograph of a section in the lung of a rat of the control Group [I-A] showing normal architecture of the lung with patent Alveoli [A], Alveolar Sac [AS] and Alveolar Duct [AD]. Note the Arteriole [Ar] and Bronchiole [Br]. [H & E; X100].

Fig. (2): An electron micrograph of a section in the lung of the control Group [I-A] showing normal capillary [C], pneumocyte type 2 [P2] towards the alveoli and the basilar lamina [arrows] in between [X4000].

Fig. (3): A micrograph of a section in the lung of a rat of Group [II-A] showing congested Arteriole [Ar], periarteriolar and peribronchiolar inflammatory cellular infiltrations [*]. Note the emphysematous Bullae [E] and the destructed epithelium [arrow heads] of the Bronchiole [Br]. [H & E; X100].

Fig. (4): An electron micrograph of a section in the lung of a rat of Group [II-A] showing a degenerated pneumocyte type 2 with pyknotic nucleus [arrow] and large Vacuoles [V]. Note the alveolar space [A]. [X8000].
Fig. (5): An electron micrograph of a section in the lung of a rat of Group [II-A] showing severely damaged pneumocyte type 2 [P2] with large Vacuoles [V] and disrupted cell membrane with extrusion of cell organelles [arrow] into the alveolar space [A]. [X12000].

Fig. (6): A micrograph of a section in the lung of a rat of Group [II-B] showing congested Arteriole [Ar] with very thick Connective Tissue [CT] surrounded by inflammatory cell infiltrations [*]. Note the desquamated endothelial cells inside the lumen [arrow], [H & E; X400].

Fig. (7): A micrograph of a section in the lung of a rat of Group [II-B] showing congested Arteriole [Ar] with markedly thickened Connective Tissue [CT1]. Note the extensively destroyed Bronchiole [Br] with disrupted epithelium [arrow head] and desquamated epithelial cells [arrow] with surrounded inflammatory cell infiltrations [*]. [H & E; X400].

Fig. (8): An electron micrograph of a section in the lung of a rat of Group [II-B] showing degenerated pneumocytes type 2 [P2] surrounded by lymphocytes [L]. [X3000].

Fig. (9): A micrograph of a section in the lung of a rat of Group [III-A] showing marked restoration of the normal structure of the Bronchiole [Br] and the Arteriole [Ar] with less inflammatory cellular infiltrations [*]. [H & E; X400].

Fig. (10): An electron micrograph of a section in the lung of a rat of Group [III-A] showing marked restoration of blood air barrier with normal pneumocyte type 2 that demonstrated Lamellar Bodies [LB] and euchromatic Nucleus [N] with perinuclear space [arrow]. Normal Capillary [C] and Basilar Lamina [BL] are also seen. [X10000].
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Discussion

The present results showed that CPF may have properties to induce oxidative stress indicated by enhancement of Malondialdehyde [MDA] production, decrease in Glutathion [GSH] content, Glutathione peroxidase [GST] and Catalase [CAT] activities in rat tissues. The increase of free radicals and lipid peroxidation may result from the inhibition of GSH levels induced by CPF toxicity. The present findings are in agreement with other investigations indicating that accumulation of lipid peroxides has been resulted after exposure to chlorpyrifos in rat lungs \[12,13\].

Concerning the histopathological changes affecting the tested group that received chlorpyrifos for one week in comparison of the normal control group, there were extensive damage of its architecture with loss of normal alveolar pattern and massive inflammatory cellular infiltrations. Areas of consolidation and collapse were obvious. Some specimens also revealed ruptured interalveolar septa with the formation of large irregular air spaces denoting emphysematous changes.

Similar observations were reported by \[12,13\] who studied the effect of chlorpyrifos on the male rat lung that revealed damage of the lung architecture accompanied by the presence of emphysema and inflammatory cellular infiltrations.

It was observed that the lung parenchyma of the examined rats which received chlorpyrifos for three weeks showed an increase in the fibrous connective tissue deposition. Measurements of the connective tissue area percentage in the histological sections, which were carried out in this experimental group by the image analyzer showed a statistically significant \(p<0.05\) increase of the connective tissue when compared to that of the control group and in Group II-B that received chlorpyrifos for three weeks more than Group II-A that received chlorpyrifos for one week. The mentioned results of the present study are in agreement with the results reported by Li et al. \[14\] & Goel et al. \[15\].

Regarding the electronic microscopic examination of the lungs of the exposed animals, pneumocytes type 2 showed shrunken and pyknotic or karyolitic nuclei. The cytoplasm of these cells was rarified and contained many vacuoles.

The histopathological changes of pneumocytes type 2 recorded by the present study were also reported by many investigators as \[16,17\].

Khan & Kour \[18\] explained the pathogenesis of pulmonary toxicity by multiple factors, but it appeared to be initiated through the production of Reactive Oxygen Species [ROS] by the activated chlorpyrifos iron oxygen complex.

In the available literature there was a general agreement on the effect of vitamin E in suppressing chlorpyrifos-induced lung damage and the protective mechanism of vitamin E against pulmonary inflammation and fibrosis was explained by many authors as reported by Akhgari et al. \[19\].

Gultekin et al. \[20\] explained the potent anti-inflammatory action of vitamin E by being able to prevent transcription of pro-inflammatory genes including cytokines, Interleukins [IL], Granulocyte Macrophage Colony Stimulating Factor [GM-CSF] and Transforming Growth Factor [TGF].
In addition to decrease inflammation, Kalender et al. [21] suggested that vitamin E directly inhibited lung fibrosis by direct suppression of fibroblasts and decreased transcription of type 1 procollagen mRNA in the fibroblast thus suppressing collagen synthesis.

**Conclusion:**

The present results showed that chlorpyrifos caused extensive damage to the lung. Although this, it appeared that the exposure to chlorpyrifos in combination with co-administration of vitamin E was highly beneficial in lowering the pulmonary adverse effects of chlorpyrifos.

**References**

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الملخص العربي


وفي ضوء نتائج هذه الدراسة فقد أمكن القول أن لعقار الكلوروبيرفوغوفس تأثيرات ضارة على الرئة في الفار الأبيض وأن إعطاء فيتامين [E] مع الكلوروبيرفوغوفس يمكن أن يقلل من تلك التأثيرات الضارة بشكل ملحوظ. وهذه النتائج أدت إلى تطبيقات هامة في الإنسان حيث ينصح بإعطاء الأفراد الذين يتعرضون للكلوروبيرفوغوفس فيتامين [E] لحد من تأثيرات الضار على الرئة. لقد توقفت هذه النتائج وأخذت البحث بالتوصيف اللازمة وتم تجنب التعريض للكلوروبيرفوغوفس واستخدام بدائل طبيعية للفضاء على الأفلاط الزراعية وتغذية العاملين في هذا المجال وإرشادهم إلى الطرق السليمة للتخزين والاستخدام وإنتاج الإرشادات الصحية والمهنية السليمة للتخلص من تناول وزيادة هذه المبيد.