Histological and Ultrastructural Study of the Chlorpyrifos-Induced Pulmonary Changes in Male Adult Albino Rat

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Abstract

**Background:** Organophosphorus compounds are insecticides used on a wide scale for protection of crops and stored food products as well as maintaining and improving the public health through the control of vector borne diseases. In 1974, the use of these insecticides caused the death of thousands of living domestic animals.

**Aim of the Work:** To detect the histological and ultrastructural changes of chlorpyrifos toxicity in the lungs of adult male albino rat.

**Material and Methods:** The present work was carried out on thirty adult male albino rats divided into three main groups; Group I [the normal control], Group II: Received 1/4LD50 of chlorpyrifos for one week, Group III: Received 1/4LD50 of chlorpyrifos for three weeks.

**Results:** The histological study of the lungs of Group II showed extensive damage with loss of normal alveolar pattern, diffuse cellular infiltration, ruptured interalveolar septa with the formation of large irregular emphysematous air spaces and interstitial hemorrhage. Electron microscopic study demonstrated pneumocytes with obvious degeneration. Their nuclei were shrunken and pyknotic; the cytoplasm was rarified with scarce degenerated organelles, the lamellar bodies were empty and there was shortening of the surface microvilli. In comparison with Group II, the histological and ultrathin sections of rats of Group III exhibited excessive collagen deposition and chronic inflammatory cell infiltrations.

**Conclusion:** This study showed that chlorpyrifos had deleterious histopathological effects on the lungs of albino rats.

**Key Words:** Lung – Chlorpyrifos – Histopathological – Oxidative stress.

Introduction

INSECTICIDES occupy a unique position among the many chemicals that man, pets and farm animals encounter daily. Accidental and/or prolonged exposure to these contaminated cause toxicity, neurological syndrome, immunosuppression, tumors, respiratory, renal, hepatic, cardiac and reproductive failures and economic losses [1]. Chlorpyrifos is a broad-spectrum organophosphate insecticide utilized extensively in agriculture and for residential pest control throughout the world under different registered trademarks [2]. The extensive use of chlorpyrifos cause health hazards to animals and human due to its persistence in the soil and crops [3]. Moreover, chlorpyrifos elicits a number of toxic effects including hepatic dysfunction, pulmonary and immunological abnormalities [4]. Organophosphorous insecticides, including chlorpyrifos, are known to enhance the production of Reactive Oxygen Species [ROS] which in turn generate oxidative stress in different tissues [5]. Additionally, this result is proved by the detection of the accumulation of lipid peroxidation products in different organs including the lung in rat studies [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.

**Material and Methods**

- **I- Experimental animals:**
  The present study was carried out on 30 male adult Sprague Dawley albino rats in the animal house of the ophthalmology institute [weighing 150-250gms]. The rats were fed on a wet diet formed from bread mixed with milk alternate with a dry diet of wheat for one day/week. 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] from 1/12/2016-31/12/2016 and was orally administrated to animals by gastric gavage [7].

Experimental design: The tested rats were allotted into 3 groups:

- Group I [normal control]: Consisted of 10 rats and were given no drug and were sacrificed after one and three weeks.
- Group II [chlorpyrifos treated group for one week]: Consisted of 10 rats and were given a daily dose of 16.4mg/kg b.w. [1/4 LD50] of chlorpyrifos by gastric gavage for one week.
- Group III [chlorpyrifos treated group for three weeks]: Consisted of 10 rats and were given a daily dose of 16.4mg/kg b.w. [1/4 LD50] of chlorpyrifos by gastric gavage for three 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 [8].

The removed lung specimens were subjected to:
- Light microscopic study using hematoxylin and Eosin and Masson’s trichrome stains [9].
- Electron microscopic study [10].
- Histomorphometric study: Imaging analyser technique for the calculation of the area % of the collagen fibres.
- Statistical study: Using the measuring field menu the area, area % and standard measuring frame of a standard area equal to 118476.6m² 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 [the normal control group]:
The light microscopic study: The lungs of the rats of the normal control group showed normal structure of the lung. Bronchioles appeared as intralobular airways that are lined by ciliated simple cuboidal epithelium surrounded by smooth muscle and fibrous connective tissue layers while the pulmonary arterioles showed muscular walls lined by squamous endothelium and surrounded by fibrous connective tissue and smooth muscle 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 (I) and (Table 1)].

Electron microscopic examination: Ultrastructural examination of the lungs of the normal control rats showed pneumocytes type 2 with the euchromatic nucleus [with the chromatin condensed at the periphery] and the perinuclear space exhibited characteristic microvilli on their luminal surface and multiple characteristic electron dense lamellar bodies showing alternating light and dark lamellae in their interior; these lamellar bodies were typically found near the luminal surface of the cell Fig. (2).

Group II (chlorpyrifos treated group for one week):
The light microscopic study: The lungs of the rats of Group [II] showed massive ruptures of the interalveolar septa in some specimens resulting in massive emphysematous bullae Fig. (3). It was found that there was massive hemorrhage accompanying the lungs of some rats of this group. The hemorrhage was intra-alveolar and inter-alveolar Fig. (4). With more magnification to exhibit the types of the cells of the inflammatory infiltrates, it was found that there was predominance of the acute inflammatory cells in the form of macrophages, neutrophils, plasma cells and eosinophils Fig. (5).

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 (I) and (Table 1)].

Electron microscopic examination: Electron microscopic examination of the lungs of rats of Group [II] showed disappearance of the alveolar spaces Fig. (6). The pneumocytes that appeared degenerated with empty lamellar bodies Fig. (7).

Group III [which received chlorpyrifos for three weeks]:
The light microscopic study: The lungs of the rats of Group [III] showed chronic inflammatory cell infiltrations in the form of macrophages, lym-
phocytes and plasma cells. Moreover, the degenerated pneumocytes with karyolitic nuclei were also observed within the infiltrates Fig. (8). With more magnification; it was observed that there was marked increase of fibroblasts around the congested capillaries inside the inter-alveolar septa Fig. (9).

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 (I) and (Table 1)].

**Electron microscopic examination:** The most characteristic feature which was demonstrated by the lungs of the rats of Group [III] was excessive deposition of collagen fibres in the interalveolar septa with the appearance of the fibroblast Fig. (10).

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>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13.89</td>
<td>0.049721</td>
<td>0.014561</td>
<td>3.5344337</td>
<td>0.0253 *</td>
</tr>
<tr>
<td>II</td>
<td>24.85</td>
<td>0.236646</td>
<td>0.094834</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>38.24</td>
<td>0.351898</td>
<td>0.17128</td>
<td></td>
<td></td>
</tr>
</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.

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] showing normal structure of the Arteriole [Ar] formed of Musculosa [M1] lined by endothelium [arrow head] and surrounded by average thickened Connective Tissue [CT 1]. The Bronchiole [Br] is lined by single layer of cuboidal epithelium [arrow] surrounded by Musculosa [M2] and average thickened connective tissue [CT2]. [H & E; X400].

**Fig. (2):** An electron micrograph of a section in the lung of the control Group [I] showing a pneumocyte type 2 demonstrating the characteristic Lamellar Bodies [LB], the euchromatic Nucleus [N] with the perinuclear space [arrow heads] and the surface microvilli [arrow] [X10000].
Fig. (3): A micrograph of a section in the lung of a rat of Group [II] showing rupture of the interalveolar septa and dilated alveoli with formation of massive emphysematous bullae [E]. [H & E; X100].

Fig. (4): A micrograph of a section in the lung of a rat of Group [II] showing massive intraalveolar and interalveolar hemorrhages [arrows]. [H & E; X400].

Fig. (5): A micrograph of a section in the lung of a rat of Group [II] showing inflammatory cellular infiltrations in the form of macrophages [M pointed by arrow], eosinophils [ES pointed by arrow] and neutrophils [Ne pointed by arrow]. Congested Capillary [C] is also seen. [H & E; X1000].
Fig. (6): An electron micrograph of a section in the lung of a rat of Group [II] showing collapsed alveolar space [arrow] between two degenerated pneumocyte type 2 [P2]. Note the Capillaries [C] and the Endothelial cell [En]. [X6000].

Fig. (7): An electron micrograph of a section in the lung of a rat of Group [II] showing degenerated pneumocyte type 2 [P2] with irregular Nucleus [N] and empty Lamellar Bodies [LB]. Note cell debris [arrow] that is present in the alveolar space [A]. [X8000].

Fig. (8): A micrograph of a section in the lung of a rat of Group [III] showing karyolitic pneumocytes [arrows], Macrophages [M], plasma cells [P] and Lymophocytes [L]. [H & E; X400].
Discussion

Chlorpyrifos had multiple effects on the target cells including generation of reactive oxygen species and induction of intracellular oxidative stress causing disruption of the normal cellular development and differentiation resulting in significant histopathological alterations in different organs including liver, lung and testes [12].

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. Some specimens also revealed ruptured interalveolar septa with the formation of large irregular air spaces denoting emphysematous changes.

Similar observations were reported by [13] who studied the effect of chlorpyrifos on the male rat lung, where the damage of the lung architecture accompanied by the presence of emphysema and inflammatory cellular infiltrations occurred after 10 days from the administration of the drug. In contrast, [14] recorded these changes in the tested animals after 28 days from giving the drug.

In the present work, lungs of the rats that received chlorpyrifos for one week showed extensive interstitial, intra-alveolar as well as intrabronchiolar hemorrhage and exudates. Hemorrhage and exudates were reported by [15] who also suggested injury of the blood-air barrier allowing leakage of RBCs in case of intra-alveolar hemorrhage.

In the current study, it appeared that lung injury induced by chlorpyrifos triggered an inflammatory
response leading to invasion of the pulmonary parenchyma by a variety of inflammatory cells including neutrophils, eosinophils, lymphocytes and plasma cells. It was also noticed that inflammatory cells were acute cells in the tested group that received chlorpyrifos for one week and chronic cells in the tested group that received chlorpyrifos for three weeks. This observation was a frequent finding reported by many research workers concerning the effect of chlorpyrifos on the lung as [16].

It was observed that the lung parenchyma of the examined rats which received chlorpyrifos for three weeks appeared with similar alterations with the lungs of the rats that received chlorpyrifos for one week except for the 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 [19] and [20]. Also, [17] demonstrated that collagen deposition and pulmonary fibrosis increased strongly up to a maximum at day 21 after chlorpyrifos exposure, the time where the rats were sacrificed in this present study. In contrary, [18] reported the pulmonary fibrosis after three months from the beginning of the drug administration.

Regarding the electronic microscopic examination of the lungs of the exposed animals, pneumocytes type 2 showed variable degrees of degenerative changes and empty lamellae bodies. Their nuclei were frequently seen irregular and lobulated with clumps of heterochromatin and might be shrunken and pyknotic or karyolytic.

The histopathological changes of pneumocytes type 2 recorded by the present study were also reported by many investigators as [19] and [20]. [21] 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 and [22] added that oxidative damage of the lung tissue appeared to be an important mechanism in the pathogenesis of lung injury.

**Conclusion:**

The present results showed that chlorpyrifos caused extensive damage to the lung.

**References**


And the abstract in Arabic:

ويهدف هذا البحث لدراسة التأثير الضار للكلوربيروفوس على الرئة. وقد تم إجراء هذه الدراسة على ثلاثين فأر أبيض بالغ والتي تم تقسيمهم إلى ثلاثة مجموعات: المجموعة الأولى (المجموعة الضابطة) والمجموعة الثانية وهي التي تم أخذ عينات من عقار الكلوربيروفوس لمدة أسبوع (وهي الرغبة التي إذا تم استخدام أقل تركيز منها يتوقع أن تقلل 50% من حيوانات التجربة التي تعمل معها) يوميا والمجموعة الثالثة وهي التي تم أخذ عينات من عقار الكلوربيروفوس لمدة ثلاثة أسابيع.

وفي ضوء نتائج هذه الدراسة فقد أمكن القول أن عقار الكلوربيروفوس تأثيرات ضارة على الرئة في الفقار الأبيض. وقد توقعت هذه النتائج وأختتم البحث بالتوصيات اللازمة فجعلنا تجربة التعرض للكلوربيروفوس ودائماً بفضل التوصيات التي أفادت إلى الآفات الوراثية وتلوث العاملين في هذا المجال وإرشادات التخزين والإشراف وإتباع الإرشادات الصحية والمهنية السليمة للتخلص من:N:Q

هذا المبدأ.